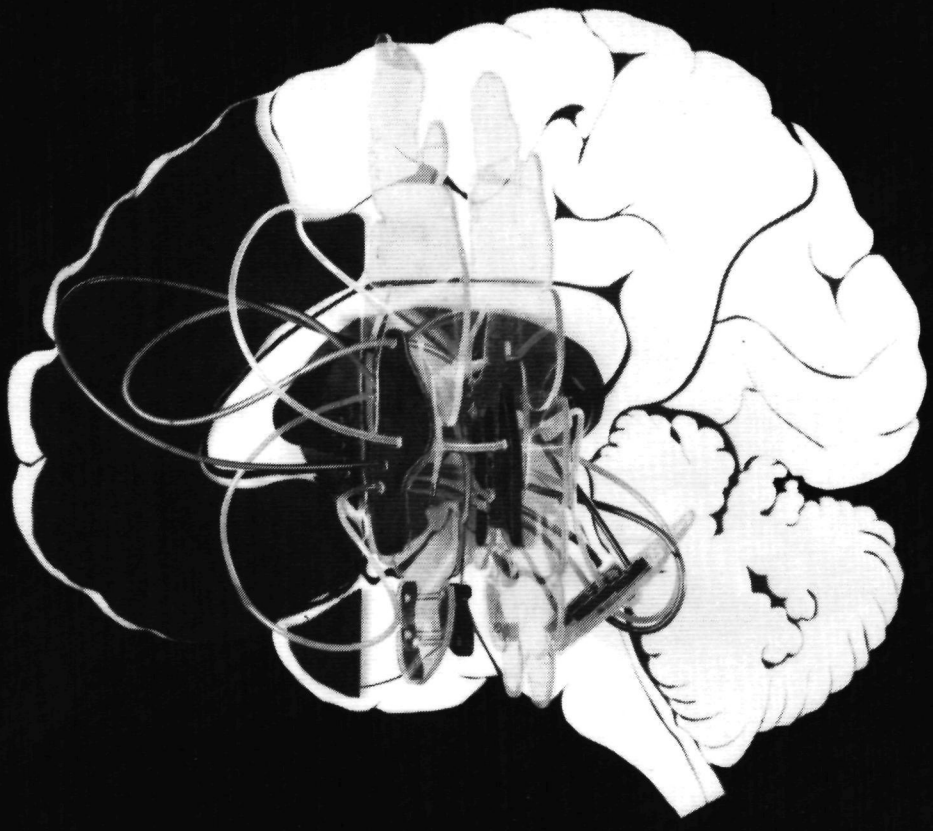
HUMAN BRAINSTEM MONOAMINERGIC STRUCTURES IN PARKINSON'S DISEASE AND ALZHEIMER'S DISEASE



A neuroanatomical and morphometric analysis

Peter van Domburg

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HUMAN BRAINSTEM MONOAMINERGIC STRUCTURES IN PARKINSON'S DISEASE AND ALZHEIMER'S DISEASE

A neuroanatomical and morphometric analysis

Een wetenschappelijke proeve op het gebied
van de geneeskunde en tandheelkunde,
in het bijzonder de geneeskunde

proefschrift

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Katholieke Universiteit te Nijmegen,
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"Qu'est-ce qu'il en sait celui-là si je suis folle?
Il est-y dans ma tête? Il y est-y dans la vôtre?
Faudrait qu'il y soye pour savoir....."

L.F.Céline, Voyage au bout de la nuit, 1932

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ABBREVIATIONS:

A8	= Area retrorubralis
Acc	= Accumbens
ACh	= Acetylcholin
AChE	= Acetylcholinesterase
AD	= Alzheimer's disease
Ai(SN)	= Subnucleus anterointermedius (substantia nigra)
al	= Ansa lenticularis
Al(SN)	= Subnucleus anterolateralis (substantia nigra)
al	= Ansa lenticularis
AM	= Amygdala
Am(SN)	= Subnucleus anteromedialis (substantia nigra)
ap	= Ansa peduncularis
APo	= Area preoptica
bci	= Brachiumcolliculus inferior
bcs	= Brachium colliculus superior
BO	= Bulbus olfactorius
Bst	= Bed nucleus of the stria terminalis
bv	= Blood vessel
C	= Nucleus caudatus
CA	= Commissura anterior
cc	= Corpus callosum
cci	= Commissura colliculi inferioris
ccs	= Commissura colliculi superioris
Cer	= Cerebellum
CGL	= Corpus geniculatum laterale
CGM	= Corpus geniculatum mediale
ChAT	= Choline acetylcholinesterase
CI	= Colliculus inferior
Cing	= Gyrus cinguli
CM	= Centrum medianum thalami
CP	= Commissura posterior
Cpg	= Corpus parabigeminum
CS	= Colliculus superior
Cs	= Nucleus centralis superior
csm	= Commissura supramamillaris
Cun	= Nucleus cuneiformis
Cx(pf)	= Cortex (prefrontal)

DA = Dopamine
DAergic = Dopaminergic
Dark = Nucleus Darkschewitschi
DBB = Diagonal band of Broca
DBH = Dopamine-ß-hydroxylase
dl = dorsolateral
DM = Nucleus dorsomedialis thalami
dm = dorsomedial
dnb = Dorsal noradrenergic bundle
dpcs = Decussatio pedunculus cerebellaris superior
dtd = Decussatio tegmenti dorsalis
dtv = Decussatio tegmenti ventralis
Ent = Entorhinal cortex
EW = Nucleus Edinger Westphal
f = Fornix
fld = Fasciculus longitudinalis dorsalis (Schütz)
flm = Fasciculus longitudinalis medialis
fmp = Fasciculus mamillaris princeps
fpc = Fibrae pontocerebellaris
Fr = Formatio reticularis
fr = Fasciculus retroflexus
fs = Fibriae strionigrales
GABA = Gamma-amino-butyric acid
Gcm = Griseum centrale mesencephali
Gcs = Griseum centrale spinalis
Gp = Griseum pontis
GPe = Globus pallidus externus
GPi = Globus pallidus internus
H = Habenula
HDB = Horizontal part of DBB
Hip = Hippocampus
HL = Nucleus habenularis lateralis
5HT = Serotonin (5-hydroxytryptophan)
Hyp = Hypothalamus
If = Nucleus interfascicularis
Ip = Nucleus interpeduncularis
ir = immunoreactivity
l = lateral
LC = Locus coeruleus

Lc = Nucleus linearis caudalis (centralis)
LH = Lateral hypothalamus
ll = Lemniscus lateralis
lm = Lemniscus medialis
lot = Tractus olfactorius lateralis
Lr = Nucleus linearis rostralis
LS = Lateral septum
L.Temp = Lobus temporalis
m = medial
M = Magnocellular
Mam = Corpus mamillare
mfb = Medial forebrain bundle
MS = Medial septum
NIII = Nucleus nervus oculomotorius
nIII = Nervus oculomotorius
NIV = Nucleus nervus trochlearis
nIV = Nervus trochlearis
NV = Nucleus tractus mesencephalicus nervus trigeminus
NVm = Nucleus motorius nervus trigeminus
NVmes = Nucleus mesencephalicus nervus trigeminus
NVs = Nucleus sensorius nervus trigeminus
NA = Noradrenaline
NAN = Nucleus annularis
NFT = Neurofibrillary tangles
Nic = Nucleus intercollicularis
NKF = Nucleus Kölliker-Fuse
Nll = Nucleus lemniscus lateralis
NMl = Nucleus lateralis, corpus mamillare
NMp = Nucleus mesencephalicus profundus
NOA = Nucleus olfactorius anterior
NPlm = Nucleus paralemniscalis
NnP = Nuclei pontis
NnPb = Nuclei parabrachiales
NPl = Nucleus parabrachialis lateralis
NPl = Nucleus paralemniscalis
NPm = Nucleus parabrachialis medialis
NPP = Nucleus peripeduncularis
NRd = Nucleus raphe dorsalis
NRp = Nucleus reticularis pontis

NRTp = Nucleus reticularis tegmenti pontis (Bechterew)
NSm = Nucleus supramamillaris
NSp = Nucleus subpeduncularis
NTld = Nucleus tegmentalis laterodorsalis (Gudden)
NTd = Nucleus tegmentalis dorsalis
NTPd = NTPP pars diffusa
NTPc = NTPP pars compacta
NTPP = Nucleus tegmenti pedunculopontinus
NTPT = Nucleus tractus pedunculus transversus
NTS = Nucleus tractus solitarius
NVest = Nucleus vestibularis
OCx = Olfactory cortex
P = Putamen
PAG = Periaqueductal grey
Pbp = Nucleus parabrachialis pigmentosus
pc = Pedunculus cerebri
pcs = Pedunculus cerebellaris superior
PD = Parkinson's disease
PF = Nucleus parafascicularis thalami
PH = Gyrus parahippocampalis
PHF = Paired helical filaments
Pir = Cortex piriformis
Pl(SN) = Subnucleus posterolateralis (substantia nigra)
pm = Pedunculus mamillaris
Pm(SN) = Subnucleus posteromedialis (substantia nigra)
PN = Nucleus paranigralis
PO = Nucleus preopticus hypothalami
Pret = Area (nucleus) pretectalis
Ps(SN) = Subnucleus posterosuperior (substantia nigra)
Pulv = Pulvinar thalami
PV = Nucleus paraventricularis hypothalami
R = Nucleus ruber
S = Septum
SC = Area subcoeruleus
SDAT = Senile dementia of the Alzheimer type
Si = Substantia innominata
SN = Substantia nigra
SNc = Substantia nigra pars compacta
SNl = Substantia nigra pars lateralis

SNr = Substantia nigra pars reticulata
SPA = Substantia perforata anterior
SP=NP = Senile (neuritic) plaques
SPP = Substantia perforata posterior
Sr = Sulcus rhinalis
SS = Somatosensory (area of nucleus caudatus)
Sth = Nucleus subthalamicus
Sulc = (peri)Sulcal cortex
Sum = Nucleus supramamillaris
T = Tectum
TH = Tyrosine hydroxylase
Th = Thalamus
tmnV = Tractus mesencephalicus nervus trigeminus
TO = Tuberculum olfactorium
to = Tractus opticus
tpr = Tractus pallidoreticularis
tpt = Tractus peduncularis transversus
ttc = Tractus tegmentalis centralis
ttes = Tractus tectospinalis
ttd = Tractus trigeminothalamicus dorsalis
ttp = Tractus tectopontinus
tts = Tractus tegmentalis superior
VA = Nucleus ventralis anterior thalami
VDB = Vertical part of DBB
VL = Nucleus ventrolateralis thalami
VM = Nucleus ventromedialis thalami
VP = Ventral pallidum
VS = Ventral striatum
VTA = Ventral tegmental area
Zi = Zona incerta

I. INTRODUCTORY CHAPTERS

A: "War ich krank? Bin ich genesen?
Und wer ist mein Arzt gewesen?
Wie vergaß ich alles das!"
B: "Jetzt erst glaub' ich dich genesen:
Denn gesund ist, wer vergaß ."

F. Nietzsche, Die Fröhliche Wissenschaft. (1886)

I.1. INTRODUCTION

Nowadays the aged people in our society are regarded as having lost a number of highly valued human attributes. Whereas cultural, social and psychological variables might influence our way of judging intellectual and motor faculties (Bahemuka 1982), great value is given to elucidate the relationship between the natural process of senescence and pathological changes in the brain. Questions whether loss of intellectual and motor abilities are inevitable features of later life and whether senile changes are the result of morphological changes in the brain, have been quite differently answered (Critchley 1956; Roth et al. 1967; Tomlinson et al. 1968,1970; Comfort 1973; Jarvik 1975; Wisniewski and Iqbal 1980; Mann and Yates 1982; Tomlinson and Corsellis 1984; Joseph et al. 1988; Mortimer 1988; Woodruff-Pak 1988). An unitary nosologic tradition about mental disorders apparently contributed to this obscurity (Horsman and van Tilburg 1984; Berrios 1985; Roth 1986; Beach 1987).

First a distinction between chronological and biological aging should be made, for it is obvious that only a proportion of old people and an unexpected proportion of rather young people are afflicted by such neuropsychiatric and physical disabilities (Kay et al.1964; Gottfries 1986). Nevertheless, epidemiological data indicate that there is a very high correlation between age and dementia (Tanja and Hofman 1985; Rocca et al. 1986; Henderson 1986), such that, at least statistically, 100 % dementia might be expected around the age of 105 years (Gottfries 1986). Likewise extrapolation of the regression line for normal age-related changes in the nigrostriatal system suggests that a clinical syndrome of parkinsonism might be reached in many individuals well over age 100 (McGeer et al.1977; Calne and Peppard 1987; Mortimer 1988).

Alzheimer's disease (AD) and Parkinson's disease (PD) as processes of pathological or degenerative involution of cognitive and motor associated structures, respectively, continue to be compared with physiologic, senile involution (Barbeau 1976; Bowen et al. 1979; Wisniewski and Iqbal 1980; Mortimer et al.1982, 1988; Calne and Langston 1983; Mann 1984; Gottfries 1986; Riederer and Kruzik 1987; Brayne and Calloway 1988). It is noteworthy that Alois Alzheimer (1907)

in his original publication "Ueber eine eigenartige Erkrankung der Himrinde" described an exclusively **presenile** disease and James Parkinson (1817) in his "Essay on the shaking palsy", explicitly excluded **cognitive** deterioration from its clinical picture.

Since long, intellectual impairment, dementia and cortical pathology, the dominant features of senile dementia of Alzheimer's type (SDAT), are also reported in PD (Ball 1882; Pollock and Hornabrook 1966; Alvord et al. 1974; Lieberman et al. 1979; Hakim and Mathieson 1979; Oppenheimer 1984; Yoshimura 1988; Cummings 1988). On the other hand extrapyramidal clinical symptoms are also found in patients with SDAT (Pearce 1974; Faden and Townsend 1976; Mölsa et al.1984,1987; Mayeux et al. 1985; Leverenz and Sumi 1986; Sagar 1987; Huff et al.1987; Fletcher and Sharpe 1988; Bakchine et al. 1989). Apparently, there is a relationship between biochemical, neuropathological and quantitative morphological changes of both cortical and subcortical areas with senile mental and motor deterioration (Roth et al. 1967; Bowen and Davison 1975; Mann et al. 1985; Roth 1986; Mountjoy 1986; Hardy et al. 1986; Riederer and Kruzik 1987); but such correlations, though statistically significant, are far from being perfect.

Since the finding that damage to the dopaminergic nigrostriatal tract accounts for many of the motor difficulties of PD (Bernheimer et al. 1973) and the successful clinical application of this concept (for reviews see Birkmayer 1987; Hornykiewicz 1988) it has become attractive to view other degenerative disorders, like AD, in terms of a selective involvement of one or several functional or neurotransmitter specific systems (Rossor 1982; Mann and Yates 1986; Perry et al. 1987; Van Hoesen and Damasio 1987; Koller 1987; Goldman-Rakic 1987a,b, 1988). Although a "cholinergic hypothesis" in AD (Davies and Maloney 1976; Whitehouse et al. 1982; Perry 1986; Höhmann et al. 1988) has been an attractive analogon of the dopaminergic model in PD (Appel 1981), the lack of clinical pharmacological applicability of this concept (Terry and Katzman 1983; Davies 1986; Sahakian 1988) stressed the need for more extensive and systematic investigation of the involvement of other, e.g. monoaminergic, neurotransmitter systems in AD related cognitive deficits (Bowen et al.1986; Whitehouse 1986b; Rossor 1988; Kaye et al. 1988). From the wealth of quantitative studies of cell counts and biochemical assays, a consensus emerged that subcortical cholinergic, noradrenergic, dopaminergic and serotonergic projections are all consistently damaged in AD (Tomlinson et al.1981; Perry et al. 1981; Bondareff et al.1982; Iversen et al.1983;

Hardy et al. 1986; Mann and Yates 1986). Eventual correlations with changes in cortical target areas would have implications for further research into etiology (Mann et al. 1985, 1986; Yamamoto and Hirano 1985a; German et al. 1987; Jellinger 1987c; Gibb et al. 1989b). As Mann and Yates (1986) stated there is an anatomical "tie up" with PD in that all the transmitter systems outside the cortex which are consistently affected in AD (nucleus basalis of Meynert, locus coeruleus, ventral tegmental area, nucleus raphe dorsalis) have diffuse cortical projections (see also Saper 1987a). Besides, like substantia nigra neuron loss in PD, cell loss in the locus coeruleus and nucleus basalis of Meynert, in AD, appeared to be topographically arranged (Marcyniuk et al. 1986, 1989) apparently associated with cortical projections that might account for the clinical symptomatology.

The aim of the present study is to show the extent of degenerative changes, like cell loss, changes of cell size and neuropathologic findings (presence and distribution of senile plaques and neurofibrillary tangles) in brainstem monoaminergic nuclei of patients of Alzheimer's disease and Parkinson's disease, as compared to the normally aged brains. Furthermore, to provide a method for the post mortem estimation of morphometric changes in these nuclei, adding to detailed knowledge of neurodegenerative features in relation to clinical symptomatology. In discussing general neuropathology of AD and PD (Ch. I.2.) special reference will be given to monoaminergic brainstem nuclei, locus coeruleus (LC), nucleus raphe dorsalis (NRd), substantia nigra (SN) and ventral tegmental area (VTA). The monoamines already have been the subject of many aging studies and it even has been suggested that changes in the related projections in particular hold the "key" to brain senescence (Samorajski 1975; Pradhan 1980; Mann and Yates 1982). Knowledge about the putative role of the neurotransmitters dopamine, noradrenaline and serotonin and their involvement in a wide variety of functions associated with human behaviour, has grown far beyond the possibilities to provide an overall review here (Hornykiewicz 1966; Mendels and Frazer 1974; Laverty 1974;
Nieuwenhuys 1985; Clark et al. 1987; Weiner and Molinoff 1989). Likewise, their involvement in different neuropsychiatric disorders like depression, cognitive and certain motor disturbances, reminiscent of the clinical signs that can be seen in age-related disease like Parkinson's disease and Alzheimer's disease, is well documented (Hornykiewicz and Kish 1986; Agid et al. 1987; Kaye et al. 1988; Chan-Palay and Asan 1989a).

Analysis of the human **mesencephalic dopaminergic cell continuum**, characterized by rather diffusely located neuromelanin containing neurons, served as a model for studying the monoaminergic brainstem nuclei in general (Ch. I.3.). Neuromelanin was used as a marker for catecholaminergic neurons, both in neuroanatomical and quantitative analysis (Bogerts 1981; Saper and Petito 1982; Bogerts et al. 1983; German et al. 1988a). Chemical characteristics of this brain pigment and some relevant features, like its possible involvement in age-related diseases, are discussed in chapter I.4. Observations on the neuromelanin pigmented substantia nigra complex, macroscopically distinguishable in the human mesencephalon (Vicq D'Azyr 1786; Foix and Nicolesco 1925; Winkler 1929; Huber et al. 1943 ; Olszewski and Baxter 1954), have quite a long historical tradition (for discussion see Keppel Hesselink 1986). Hassler (1937) suggested a detailed, cytoarchitectonically based, subdivision of the SN, to define an exact demarcation of subareas involved in parkinsonian syndromes (Hassler 1938). Nowadays dopamine-containing neurons in the mesencephalon are mostly subdivided into three groups: A8 (retrorubral area), A9 (substantia nigra proper) and A10 (ventral tegmental area) (Dahlström and Fuxe 1964), giving rise to more or less separated mesostriatal, mesolimbic and mesocortical projections (Björklund and Lindvall 1984). Evaluation of monoaminergic projections, encompassing monoamine histofluorescence (Andén et al. 1964; Ungerstedt 1971a; Fallon et al. 1978a,b; Fallon and Moore 1978a,b), immunohistochemistry (Gerfen et al.1982, 1987a,b; Gerfen 1985; Hökfelt et al. 1980) and anterograde or retrograde tracing methods (e.g. Carter and Fibiger 1977; Beckstead et al. 1979; Swanson 1982) were mostly performed on the rat brain. Data on comparative neuroanatomy (Ch.II.2.3.) therefore must precede the presentation of hodological features of the nuclei under investigation (Ch.II.2.4.). In view of the growing knowledge of neurochemical changes, some data on the chemoarchitecture will be included in the anatomical section (Ch.II.2.5.).

The pathophysiology of PD-behavioral manifestations (Ch.II.2.6.) and the localized cell loss in the SN pars compacta, have been ascribed to different transcortical loops (DeLong and Georgopoulos 1981; Cools et al. 1984b; Alexander et al. 1986; Marsden 1986). Thus the most medial part of the SN-VTA seems to be related to structures associated with cognition and cognitive disorders (Fallon et al. 1978b; Bogerts et al. 1983; Rinne et al. 1989), whereas the more laterally placed part of the SN pars compacta is most clearly involved in the motor disorders

of PD (Oppenheimer 1984; Jellinger 1987a,b; Hornykiewicz and Kish 1986; Hirsch et al. 1988). In studying the mesencephalic catecholaminergic nuclei it also seems worthwhile to pay attention to some adjacent structures in the reticular formation, that are reported to provide a minor cholinergic input to the cerebral cortex (Saper 1987a) and the possible interaction of cholinergic and catecholaminergic structures (Henderson 1987; Freeman and Gibson 1988). In order to relate neurodegenerative changes with the many available neuroanatomical data, an atlas of the human mesencephalon and rostral rhombencephalon was designed (Ch. II.2.7.2.). A brief review on the human Locus Coeruleus (CH.II.3.) and nucleus raphe dorsalis (Ch.II.4.) may complete the neuroanatomical section.

Although consensus has been reached on the clinical definition of the dementia syndrome (Consensus 1987; Schulte 1989) and AD (McKhann et al. 1984; Khachaturian 1985), there is yet the striking continuity of symptoms within the field from purely motor to exclusively cognitive dysfunctioning, which can manifest in quite divergent disease states and even physiologic aging. Current evidence suggests both AD and PD dementias to be primarily progressive cortical diseases in which cholinergic projections are involved in an "Alzheimer-like" fashion (Hakim and Mathieson 1979; Arendt 1983; Perry et al. 1985; Quinn et al. 1986; Whitehouse et al.1985,1986b). It is compelling to attend the imperfect correlation of atrophy of the nucleus basalis in PD with dementia or abnormal senile plaque formation (Candy et al.1983; Cummings 1988; Levin et al. 1989). On the other hand implications of basal ganglia dysfunction in cognitive deficits receive a growing interest (Cools et al. 1984a; Marsden 1982, 1986; Mendez et al. 1989). Apparently, dysfunction of subcortical systems, supporting cortical structures, may lead to certain kinds of intellectual impairment (Neumann 1968; Ross and Stewart 1981; Katz et al. 1987; Ruberg and Agid 1988), important to differentiate on behalf of possible therapeutic consequences (Bowen et al. 1975; Fleet et al. 1987; Rossor 1988; Levin et al. 1989).

The question arises whether PD dementia is a specific entity, determined by impaired recall, visuospatial disturbances, executive deficits, bradyphrenia, personality changes and depression (Brown and Marsden 1988; Cummings 1988; Schulte 1989). Apart from this "subcortical dementia" (see also CH. II.2.6.), as described in progressive supranuclear palsy (Steele et al. 1964; Albert et al. 1974, 1978; Dubois et al. 1988; Cummings and Benson 1988) or dementias in Huntington's disease (McHugh and Folstein 1975) and vascular disease (Caplan and

Schoene 1978; De Reuck et al. 1980; Román 1985; Fisher 1982,1989), other dementia syndromes, like that observed in Guam(Hirano et al. 1961; Elizan et al. 1966; Kwang-ming Chen and Chase 1986), MPTP-induced parkinsonism (Langston et al. 1983; Stern and Langston 1985) and those associated with prefrontal disorders (Rossor 1988; Neary et al. 1988; Gotham et al. 1988), might contribute to PD-related cognitive decline (Yoshimura 1988; Quinn et al. 1986). Such a subcortical dementia syndrome might as well account for the lack of the predominantly cortical triad of agnosia, apraxia and aphasia as is usually met with in AD (Cummings 1988; Ruberg and Agid 1988).

Morphometric analysis of neuronal elements (Ch.III.1.) variable in size and shape, is marked with specific problems (Coleman and Flood 1987). Highly variable results in cell number of e.g. human catecholaminergic brainstem nuclei (Chs.III.2, III.3.) have been reported (Pakkenberg and Brody 1965; McGeer et al. 1977; Brody 1978; Tomlinson et al. 1981; Mann et al. 1982,1983; Bondareff et al. 1982; Iversen et al. 1983; Bogerts et al. 1983; Halliday and Törk 1986; German et al. 1988a), due to methodological differences and mathematical processing. Therefore no major conclusions will be drawn from overall cell numbers based on our relatively small number of cases. Rather an attempt will be made to relate localized cell loss and neurodegenerative changes to anatomical data of previous chapters (Ch. III.5, IV). Recent developments in stereology (see Gundersen 1986) have criticized habitual sampling strategies and counting methods that might introduce unacceptable bias. Methodological considerations have to account for such items (Ch.III.5.2.). Systematic quantitative analysis might correlate localized variations to neuroanatomical and neurochemical data, providing a better distinction between pathologically and normally aged brains than is possible with any single parameter (Brizzee 1987). Some speculations as regards pathophysiological manifestations in the etiology of AD may be derived from the typical distribution pattern of neurofibrillary tangles and senile plaques within single brains (Ch. III.4.)

Recent investigations on mesolimbocortical projections, closely related to what is called the "ventral striatum" (Heimer and Wilson 1975; Alheid and Heimer 1988) and prefrontal lobe , suggest a more specific involvement of mesostriatal sub-circuitry, traditionally related to the motor system, in cognitive and limbic circuitry (Thierry et al. 1973,1977; Nauta and Domesick 1978; Simon and LeMoal 1988; Fibiger and Phillips 1988; Thierry et al. 1988; Mogenson et al. 1988). Mesolimbocortical projections, originating in the VTA, might account for

Parkinson's disease- related dementia (Javoy-Agid and Agid 1980; Torack and Morris 1988). Although the dopamine hypothesis of schizophrenia (Stevens 1973, 1979; Crow et al.1978, 1982; Nemeroff 1986) and a specific VTA-syndrome (LeMoal et al. 1976; Galey et al. 1977; Scarnati et al. 1980), still lack a firm pathophysiological basis, the available data stress the importance of a better knowledge of this dopaminergic mesolimbocortical projection neurons and their possible involvement in dementias of Alzheimer's and Parkinson's disease. Likewise noradrenergic projections have been associated with schizophrenia (see Hornykiewicz 1982b; Crow 1982) and various dementia syndromes (Chan-Palay and Asan 1989b). There is also a growing evidence of the involvement of monoaminergic central nervous system circuits in depressive states (Kostić et al. 1987; Mazure et al. 1987; Chan-Palay et al. 1989a,b). It will be of much interest to correlate quantitative findings of various monoaminergic nuclei, as well as those of other brain areas, in AD (Ch. IV).

This study forms part of a research program on morphometric parameters of different brain areas in aging and aging diseases, especially Alzheimer's disease and Parkinson's disease. Parallel studies have been performed on the neocortex (Broere 1990), the nucleus basalis of Meynert (Vogels 1990), the hippocampus (De Vries 1990) and the amygdala (Vereecken in preparation).

I.2. NOTES ON NEUROPATHOLOGY

Along with amyotrophic lateral sclerosis and the PD-ALS-dementia complex of Guam (Hirano et al. 1962, 1986), Alzheimer's disease (AD) and Parkinson's disease (PD) are prototypes of human neurological disorders characterized by occurrences of cytoskeletal pathology in specific populations of nerve cells (Wisniewski et al. 1979; Tomlinson and Corsellis 1984; Price et al. 1986). A tremendous amount of investigation has been directed to these structural changes, because they make up the essential characteristic abnormalities, and their presence serves as diagnostic criterion for these two most common types of adult-onset chronic degenerative disorders of the central nervous system (Tomlinson et al. 1970; Terry and Katzman 1983; Quinn et al. 1986). Refinements in clinical diagnosis of the mental disorders of the aged, in recent decades had their counterpart in improvements of the neuropathological classification of age-related disease, especially dementia (Mayeux et al. 1985; Flicker et al. 1985; Kellet 1987; Alafuzoff et al. 1987; Chui 1987). On the other hand, over the last decade, a number of studies have begun to address the potential overlap of neuropathological, but particularly neurochemical manifestations of these two disease states, as well as the aging process itself (for reviews Price et al. 1986; Perry et al. 1987; Hamill et al. 1988). Macroscopic and microscopic alterations occur in the brains of many old people (Tomlinson et al.1968), some of the latter being grouped under the term 'senile changes'. It has been alleged, however, that their presence in small amounts in the brains of old people is not correlated with intellectual deterioration (Tomlinson et al. 1968,1970,1984). Such senile changes include (Congophilic or argyrophilic) plaques, with enlarged neurites, Alzheimer's neurofibrillary tangles (Alzheimer 1907), granulovacuolar degeneration, Hirano bodies and Lewy bodies. Recent preliminary studies suggest most, if not all, of them to be more or less related to cytoskeletal disorganization and specific proteins normally associated with the microtubules that are responsible for rapid axonal transport in the healthy brain (Price et al. 1986; Anderton 1987; Selkoe 1989).

As regards these neuropathological changes, quantitative rather than qualitative changes form the major differentiating feature between the morphology of

the brains of normal and many demented old people (Gibson 1983; Tomlinson and Corsellis 1984; Hardy et al 1986). We are still uncertain, whether AD is a specific, discrete, qualitative disorder such as an infectious process, endogenous or exogenous toxic disorder, or biochemical deficiency, or whether it is a quantitative disorder, in which an exaggeration and acceleration of the normal aging processes occur and dementia appears when neural reserves are exhausted and compensatory mechanisms fail. Vascular lesions also play an important role in the dementing process and in some instances significant ischaemic destruction accompanies severe senile changes (Fisher 1982,1989; Terry and Katzman 1983; Hardy et al. 1986). Evidence exists on neuronal loss throughout life and it has always been stated, on subjective assessment, that neuronal loss is severe in 'senile dementia' (for reviews see Haug 1985; Coleman and Flood 1987). Likewise, the lesions in parkinsonism are multifocal, show a significant overlap with age-related changes in nigro-striatal circuitry (McGeer et al. 1977; Joseph et al. 1978; Calne and Peppard 1987) and with Alzheimer neurofibrillary changes, especially as regards postencephalitic parkinsonism (Hassler 1938; Oppenheimer 1984) the Guam Parkinson-dementia-complex (Hirano et al. 1961, 1986) and the Steele-Richardson-Olszewski syndrome (Tellez-Nagel and Wisniewski 1973; Agid et al. 1986). Even less specific for these neurodegenerative diseases are the age-related changes of accumulation of lipofuscin (Mann and Yates 1974a,b) and the decrease in the arborization of dendritic trees (see Coleman and Flood 1987). Major weight loss of the brain is usually seen only in younger patients. Because of the age dependency, most surveys of overall brain weight based on patient groups, consisting largely of elderly persons with AD (Tomlinson et al. 1980; Terry et al. 1981; Mann et al. 1985), have shown only a slight (less than 10%) but significant or a nonsignificant change in brain weight, when compared with nondemented patients of that age.

The site of predominant pathologic findings as well as their exaggeration are leading principles in neuropathological diagnosis of both AD and PD (Hirano and Zimmerman 1962; Terry and Katzman 1983; Oppenheimer 1984; Hardy et al. 1986; Quinn et al. 1986; Perry et al. 1987). In the SN of patients dying with PD gross depigmentation and microscopic depigmentation of neurons, loss of neurons, extracellular neuromelanin, 'tombstone' formation (foci of macrophages containing the rather indigestible neuromelanin pigment) and the appearance of Lewy bodies in the remaining neurons, but also in the locus coeruleus and nucleus basalis

of Meynert, are the characteristic findings (Greenfield and Bosanquet 1953; Oppenheimer 1984; Forno 1986). In idiopathic parkinsonism the loss of neurons occurs in a reasonable stereotyped pattern, with the medial cell groups being relatively spared (Oppenheimer 1984; Jellinger 1986b; Hornykiewicz 1988). Morphological changes in AD, that is, the presence of plaques and tangles, are widely distributed in neocortex, allocortex and many deep gray areas down through the pontine tegmentum, but largely exclude the basal ganglia, thalamus and substantia nigra (Terry and Katzman 1983; Tomlinson and Corsellis 1984; Ball 1982,1984). The temporal lobe cortex and hippocampus are the areas most severely affected by the increased neurofibrillary tangle formation in senile dementia due to Alzheimer's disease (Terry et al. 1981; Wilcock and Esiri 1982; Mann 1985; Hardy et al. 1986). A severe loss of the large neocortical neurons is also characteristic of the disease but few attempts have been made to exactly substantiate this (Haug 1986; Coleman and Flood 1987; Broere 1990). In demented patients without evidence of cerebrovascular disease, a highly significant correlation was found between the presence and severity of dementia and the number of neurofibrillary tangles in the cerebral cortex (Wilcock and Esiri 1982; Pearson et al. 1985). A significant but much weaker and inconstant correlation was found for the presence and severity of dementia and the number of argyrophilic plaques present in the cortex (Wilcock and Esiri 1982; Gibson 1983; Mann 1985; Pearson et al. 1985). The neurofibrillary changes are much more severe in Alzheimer's disease (usually with a younger age of onset) than in senile dementia of the Alzheimer type (SDAT), whilst senile (neuritic) plaques are most prominent in SDAT (Wisniewski and Iqbal 1980).

Autopsy criteria for the diagnosis of AD are given by Khachaturian (1985). In any cortical microscopic field encompassing 1 sq. mm (microscopic magnification x200) the number of senile or neuritic plaques and of neurofibrillary tangles should exceed two to five per field. Frontal, temporal and parietal lobes, as well as the amygdala and hippocampal formation are always sampled. For any patient between 66 and 75 years of age, the number of senile plaques (Bielschowsky silver, thioflavin S or Congo Red staining technique) must be greater than ten per field (Khachaturian 1985). In patients older than 75 years, neurofibrillary tangles may sometimes not be found in the neocortex, but the number of senile plaques should exceed 15 per microscopic field. In Parkinson's disease the autopsy criteria for the diagnosis were less strictly quantified, but the findings mentioned before, with special emphasis on the appearance of Lewy bodies in the SN and LC, served as

a safe guide for pathological diagnosis (Oppenheimer 1984). The neuropathology of parkinsonism differs from that of normal aging in being more severe (especially in the SN) and also by showing much more frequent Lewy bodies (Greenfield and Bosanquet 1953; Ohama and Ikuta 1976; Forno 1986).

In 1912 Lewy described peculiar inclusions that were spherical or elongated in shape, in the dorsal motor nucleus of the vagus and the nucleus basalis of Meynert. The term "Lewy bodies" usually implies acidophilic (occasionally polychromatophilic), single or multiple, most often spherical inclusions in the nerve cell cytoplasm of neuromelanin pigmented nerve cells. They contain mainly protein, but not carbohydrate and are therefore not stained with the periodic acid Schiff reagent, a feature that makes it easy to tell them apart from the many corpora amylacea that can be seen in the aged brain (Greenfield and Bosanquet 1953; Forno 1969,1986). Lewy bodies are found either near the nucleus (perikaryal) or within dendrites (intraneuritic) and are easily recognizable by light microscopic examination of ordinary chromatically stained sections (e.g. hematoxylin and eosin, trichrome), as intracytoplasmic, single or multiple, spherical, acidophilic or polychromatophilic masses with a dense core and a peripheral halo (Oppenheimer 1984). By electron microscopy (Duffy and Tennyson 1965) the pattern resembles a sunflower.

There is an extensive literature on the composition and meaning of Alzheimer neurofibrillary tangles (see Tomlinson and Corsellis 1984; Selkoe 1986; Iqbal et al. 1986). The aggregates may be demonstrated by various staining techniques and immunohistochemical techniques, but are not easy to see in preparations stained with heamatoxylin and eosin or in Nissl (Alzheimer 1907; Brun 1983; Tomlinson and Corsellis 1984; Price et al. 1986; Anderton et al. 1987; Défossez and Delacourte 1987). The essential change consists of the thickening and tortuosity of fibrils within the neuronal cytoplasm. The aggregates comprise double helical stacks of subunits shaped like a C, surrounded by a fuzzy coat, which can be stripped of by protease treatment. Protein sequencing and molecular cloning showed that the fragment was part of 'tau', a protein normally associated with the microtubules (Selkoe 1986,1989; Kowall and Kosik 1987). The paired helical filaments (PHF), formed by these proteins, together with non-neurofilament proteins (Selkoe 1986,1989), form highly insoluble cross-linked polymers (Tomlinson and Corsellis 1984). The configuration of the aggregates may vary according to the site and the type of nerve cell afffected, being pyramid- shaped in cortical neurons,

but more often round in subcortical nuclei (Fig.III.1.a). Pigments, such as neuromelanin or lipofuscin may also be present. Neurons in the subcortical regions are less often affected (see Chs. III. and IV.). Ishii (1966) studied the distribution in the hypothalamus (see also Saper and German 1987) and brainstem and pointed out that the vulnerable nuclear groups showed certain metabolic features in common, including a high content of monoamines and an early accumulation of brain pigments.

Senile (neuritic) plaques are discrete structures in the neuropil (see Fig.III.1.b) with an average diameter of 70 microns (Wisniewski and Terry 1973) that occur in largest numbers in the neocortex and amygdala (Wisniewski and Iqbal 1980; Tomlinson and Corsellis 1984). They were first described by Blocq and Marinesco (1892) as sclerotic plaques of microglia, but in order to emphasize degenerating small axons and dendrites noted in electron microscopic studies (Wisniewski and Terry 1973) the name neuritic plaque (NP) is more appropriate. The smallest type consists of a sharply circumscribed granular deposit of amyloid strongly fluorescent in Congo-red staining (Puchtler and Sweat 1965; Selkoe 1989). Extracellular amyloid fibrils are composed of a 4-kd peptide termed the ß-amyloid protein (A4 protein), of which the coding gene is located on human chromosome 21 (Anderton 1987; Hardy 1988). The axons and myelinated fibers in the surrounding tissue are displaced, but show little or no evidence of abnormality. The other two types of plaques are larger and are usually referred to as typical (or classical) plaques and primitive plaques (Mann 1985). The typical plaque consists of a central core of amyloid surrounded by a corona of reactive astroglial cells, microglia, macrophages, degenerative axons and occasional dendrites (Tomlinson and Corsellis 1984; Iseki et al. 1989). The primitive plaque lacks the central core of amyloid. The presence of various forms of plaques suggests that these forms represent different stages in evolution (Wisniewski and Terry 1973; Selkoe 1986; Défossez and Delacourte 1987). Plaques have also been described in animals in relation to spongiform encephalopathy, aging and viral encephalitis (see Wisniewski and Terry 1973; Pearson 1985), but they differ from those described in man by the absence of the paired helical filaments (Wisniewski and Terry 1973). In some cases of AD, as well as in individuals of advanced age, amyloid may be conspicuous in small leptomeningeal and intracortical blood vessels and stain densely with dyes such as Congo-red (Tomlinson and Corsellis 1984; Mann 1985; Bergeron et al. 1987; Yamada et al. 1988). Ultrastructurally these deposits are associated with

perithelial cells of the basal lamina and are referred to as Congophilic angiopathy. The capillaries with plaque-like degeneration were not infrequently observed in the brain stem of patients with AD (Rudelli et al. 1984; Iseki et al. 1989). Although Hirano bodies and granulovacuolar degeneration have not been searched for in our study, they also form part of the neuropathological phenomena in degenerative diseases of the aging brain, that are almost exclusively restricted to the hippocampus (Tomlinson and Corsellis 1984: Mann 1985). Hirano bodies are rod-shaped eosinophilic intra-cytoplasmic inclusions, commonly seen in the pyramidal cell layer and the stratum lacunosum of hippocampi in patients with AD (Tomlinson and Corsellis 1984; Mann 1985). Granulovacuolar degeneration refers to cytoplasmic inclusions, which consists of an electron-dense granule surrounded by a membrane-bound vacuole (Tomlinson et al. 1970; Wisniewski and Terry 1973; Mann 1985). They might also contain tubulin-like immunoreactivity (Price et al. 1986).

In neuronal perikarya, neurofibrillary tangles (NFT) consist of three populations (Défossez and Delacourte 1987): firstly strongly immunolabeled tangles, with a specific antiserum raised against PHF. Secondly, less dense tangles were weakly immunolabeled, but strongly thioflavine-stained. Thirdly "ghost tangles" which correspond to extracellular NFT, were exclusively thioflavin-stained (Défossez and Delacourte 1987). Likewise in our Congo-red histofluorescence microscopy weakly stained extracellular fibrils contrasted with well-shaped intracellular tangles. Immunolabeled neurites, thioflavin-(or Congo-red) stained neurites and transition figures can also be observed around neuritic plaques and some vessels with amyloid angiopathy (Tomlinson and Corsellis 1984; Selkoe 1986, 1989; Anderton 1987; Défossez and Delacourte 1987). But generally the cerebral amyloid of senile plaques is ultrastructurally distinct from PHF, consisting 4-8 nm wide filaments that are not wound together in pairs (Anderton 1987; Selkoe 1986, 1989). The immunohistochemical localization of immunoglobulins and other serum proteins within senile plaques, neuronal perikarya and astrocytes, suggested a leakage of blood vessels (Wisniewsky and Iqbal 1980; Mann et al. 1982; Davies 1988). Neuropeptidergic systems form another component of plaques in AD (e.g. Struble et al. 1987).

A major question for further study concerns the cellular origin and role of microvascular amyloid in the degeneration of neurites of multiple neurotransmitter specificities in AD cortex (Davies 1988). Current molecular research on

Alzheimer's disease is focussed on two major goals: identifying the locus and nature of the genetic defect or defects causing familial forms of the disease, and studying the genesis of the earliest and most specific phenotypic abnormalities in a retrograde fashion, in the hopes of arriving at a molecular alteration that is directly linked to genetic defect (for review see Kay 1986; Hardy 1988; Tanzi et al. 1989; Selkoe 1989). One of the leading hypotheses that attempts to explain the pathogenesis and progression of AD suggests a primary defect in blood brain barrier function and/or structure within the cerebral cortex, which may be the cause of the cerebral vessel amyloidosis common in many patients with AD (for review Hardy et al. 1986; Kozlowski and Nilaver 1988; Toledano-Gasca 1988) and be related to aging (Mooradian 1988). It is of interest to know that both noradrenergic (locus coeruleus) and serotonergic (raphe dorsalis) projections have been shown to be involved in the regulation of the integrity of the blood brain barrier (Chan-Palay 1976; Mann et al. 1983; Mann 1985). Neurotoxins as a possible mediator between widespread cerebral nuclei that degenerate in both AD and PD, and other neurodegenerative disorders, have been shown to provide an acceptable explanation for various manifestations as regards the pathological changes of the aged brain (Hardy et al. 1986; Saper et al. 1987; Langston 1988; Gajdusek 1988). The suspicions that NP's and NFT's must in some way be responsible for the clinical expression of AD began to give way when it was discovered that the cortex from patients with AD was severely depleted in markers for cholinergic neurons (Davies and Maloney 1976; Terry et al . 1978; Bowen and Davison 1980; Perry 1986), an observation later equated with atrophy, neurofibrillary degeneration and loss of parent cell bodies in the nucleus basalis of Meynert (Whitehouse et al. 1981). These findings were soon followed by more extensive studies on neurochemical changes (see Terry et al. 1978; Rossor 1980, 1988; Mann and Yates 1986; Procter et al. 1988). This again tempted investigators to speculate on a mono-transmitter deficit in AD (the cholinergic hypothesis) following the dopaminergic model in PD (Perry 1986; Hornykiewicz 1988). Nevertheless, the core-defect in AD remains an abnormally high degree of neurofibrillary degeneration, just like the Lewy body remains the key finding in PD brains. Integrative views on age-related neuropathological decrement of the central nervous system nuclei nowadays have to incorporate compiled data of specific genetic programs, functional roles and/or morphological connections as well as neuropathological findings within a particular area.

I.3. MATERIALS AND TECHNIQUES

I.3.1 Cytoarchitectonic analysis

For defining subareas in the substantia nigra and ventral tegmental area, and the boundaries of the locus coeruleus and nucleus raphe dorsalis as well as their topographical relationship to surrounding structures, brain stems of patients with no history of neurological disorders, varying in age between 60 years and 80 years (Table I.1.), were used. The brain material was obtained at routine autopsy and fixed by immersion in 4% buffered formaldehyde for several months. On gross examination the brains did not show major abnormalities. The brain stems (from the middle of the mamillary body through the pons) were embedded in paraffin and cut transversely, i.e. perpendicular to the longitudinal axis of the brainstem, into sections of 20 µm with a Reichert-Jung 2050 microtome. The sections were stained according to Nissl (cresylecht violet) and Klüver-Barrera (1953). In addition a horizontally sectioned brain stem was available.

As an aid for the cytoarchitectonic analysis based on the Nissl-stained sections, two brain stems were used for a *pigmentoarchitectonic* analysis (Braak 1978,1980; Braak and Braak 1986). Sections of 800 µm were cut perpendicular to the brain stem axis with the aid of a freezing microtome. The sections were oxidized with performic acid and stained with aldehydefuchsin for demonstration of lipofuscin pigment (Braak 1978). An additional, unstained, brain stem sectioned serially at 800 µm was available, in which the pattern of neuromelanin containing cells is particularly well visible. These thick sections greatly facilitate the recognition of nuclear borders and internal configuration of the substantia nigra and VTA.

The topographical relationship of the various nuclei and subnuclei in the SN,VTA, locus coeruleus and nucleus raphe dorsalis are illustrated with camera lucida drawings of eight representative sections through the human mesencephalon and three representative sections through the dorsal part of the rostral rhombencephalon (see Ch. II.2.7.). For an analysis of fiber bundles adjacent Klüver-Barrera stained sections were used. Delineation of the cell masses was performed using the following cytoarchitectural criteria: a) density and arrangement of the neuronal elements; b) size and shape of the cell bodies (see II.2.2.and table II.1.); c) aspect

and distribution of the Nissl substance, and d) presence and distribution of neuromelanin for nigral and locus coeruleus neurons, or lipofuscin for dorsal raphe neurons. Cell sizes were measured with a Zeiss-Kontron MOP-Videoplan. As nomenclature for the mesencephalic nuclei Braak and Braak's (1986) subdivision of the SN into subnuclei, and Hallıday and Törk's (1986) subdivision for the VTA nuclei, were used.

Table I.1.: Data on the brains used for quantitative morphometric analysis

Nr.	Age	Sex	Diagnosis	Cause of death / Clinical details	P-M-delay hr.	Brain weight gr.
87029	60	F	Normal	Sepsis / Decompensatio cordis	14	1162
87177	62	M	Normal	Aorta rupture / Aorto-bifurc bypass	<24	1351
86179	69	M	Normal	Trauma / Myocard infarct	50	1200
88269	80	M	Normal	Myocard infarc / Rheum arthritis	48	1475
88184	91	M	Normal	Carcinoma/ No dementia	36	1288
87068	65	F	AD	Sepsis/ 3 yrs progressively dementing	<12	865
87395	69	M	SDAT	Cachexia/ Dementia for 12 yrs (N I H)	<12	1077
88177	76	F	SDAT	Cachexia/ Dementia for 14 yrs	6	970
87368	82	M	MID	Circ insuff / Dementia for 6 yrs (only available for LC counting)	<14	1353
88221	93	F	SDAT	Pneumonia/ Dementia for 16 yrs	<12	842
89142	68	M	PD	Circ insuff /Imbecility,juvenile epilepsia*)	--	1300
89141	69	F	PD	Circ insuff /Cachexia,Depression,*) Diabetes,Hypertension	32	1370
87377	74	F	PD	Myocard infarc /2 yrs PD	8	1370
86204	79	M	PD	Cachexia/ Osteoporosis	50	1210
87062	66	M	AD+PD	Sepsis/Rectal carc.,4 yrs dementia (N I H)	<24	1136

*) Prof dr D J Ruiter, Pathological Anatomical Institute, Nijmegen

I.3.2. Quantitative analysis

For quantitative analysis of the number of neurons in the SN and VTA in aging and aging diseases both sides of the mesencephalon of 15 human brains were analysed; five control brains, four brains of patients with Alzheimer's disease (and one MID brain, only for LC countings), four brains of patients with Parkinson's disease and one brain of a patient in whom both Alzheimer's and Parkinson's disease were diagnosed (Table I.1.). Paraffin sections of 20 µm, cut perpendicular to the longitudinal axis of the brain stem were examined, from a level through the middle of the mamillary body through the caudal pole of the pons, to include the locus coeruleus. The neuropathological diagnosis was performed on paraffin embedded sections stained for Nissl, Bodian, Yamamoto and Congo red and thioflavin-S. (Puchtler and Sweat 1965; Yamamoto and Hirano 1986).

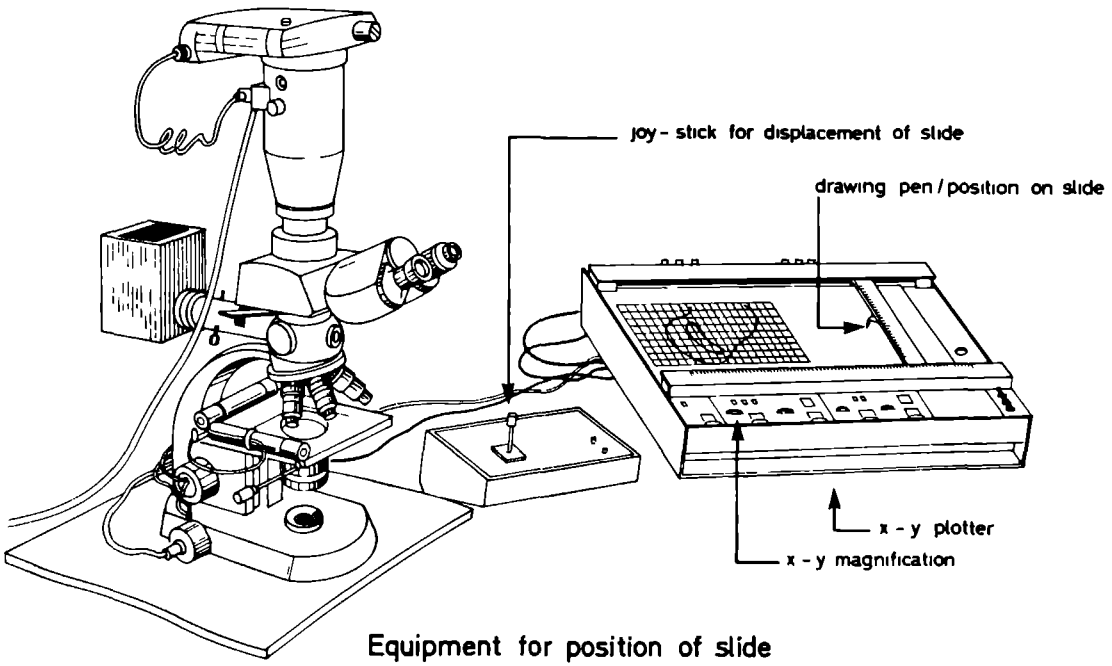


Fig. I 1
Schematic representation of the plotting equipment

The following sampling procedure was used:
1) outlines of the sections as well as of the areas containing neuromelanin pigmented neurons, or the dorsal raphe neurons, equidistant at 800 µm (each 40th section of 20 um) were drawn with an X-Y plotter (Kipp Instruments) attached to a Zeiss Standard microscope (for schematic representation of plotting equipment (Fig.I.1.).
2) in these outlines cells can be plotted in relation to a point of reference, i.e. the bottom of the cerebral aquaduct (see Fig. I.2.). The X-Y plotter is tuned in such a way that displacement of the plotter-pen across one square (1 cm^2 of the plotter table) corresponds to a displacement of the microscope stage of 1 mm^2;
3) in each square of the plotting table one counting is performed; cells were counted via an ocular grid (1x1 cm^2). In the magnification used (40x10) the counting grid corresponds to a 0,25x0,25 mm^2 fraction of a plotter section. Profiles dissected by the right and the bottom line of this grid were included, those dissected in the left/ upper part were not. So a 1/16 fraction of a plotter square is counted;
4) sampling is done in a systematic random way (Gundersen and Jensen 1987) so that no bias is introduced because of random clustering of cells. Data on the actual position of the sample studied can be obtained from the outlines on the plotting table. A comparable approach was used by Agnati and co-workers (Agnati et al. 1984a,b) and German et al. (1983);
5) according to stereological principles (Cavalieri's principle; see Mattfeldt 1987; Vogels 1990) only 8 equidistant sections of a given structure, selected randomly, provide a counted cell number with a reliability of 95 %;
6) the following elements were counted: neuromelanin-containing cells, neurons without neuromelanin, but within the same outlines, dorsal raphe neurons containing lipofuscin or those within these outlines with a visible nucleolus, and neurofibrillary tangles and senile plaques within the sections analyzed. Identification and counting of such elements was done using a X 400 magnification. Melanin deposits without cell boundaries (free neuromelanin, frequently present in aging diseases) may contribute in defining nuclear outlines, but were not counted as neurons;
7) the so counted number of neurons or other elements, e.g. senile plaques, has to be multiplied by 16 for extrapolation to the real cell number in that section and by 40 because of the fraction of sections analyzed (Fig. I.3.);
8) Correction formula: the identification and quantification of neural elements,

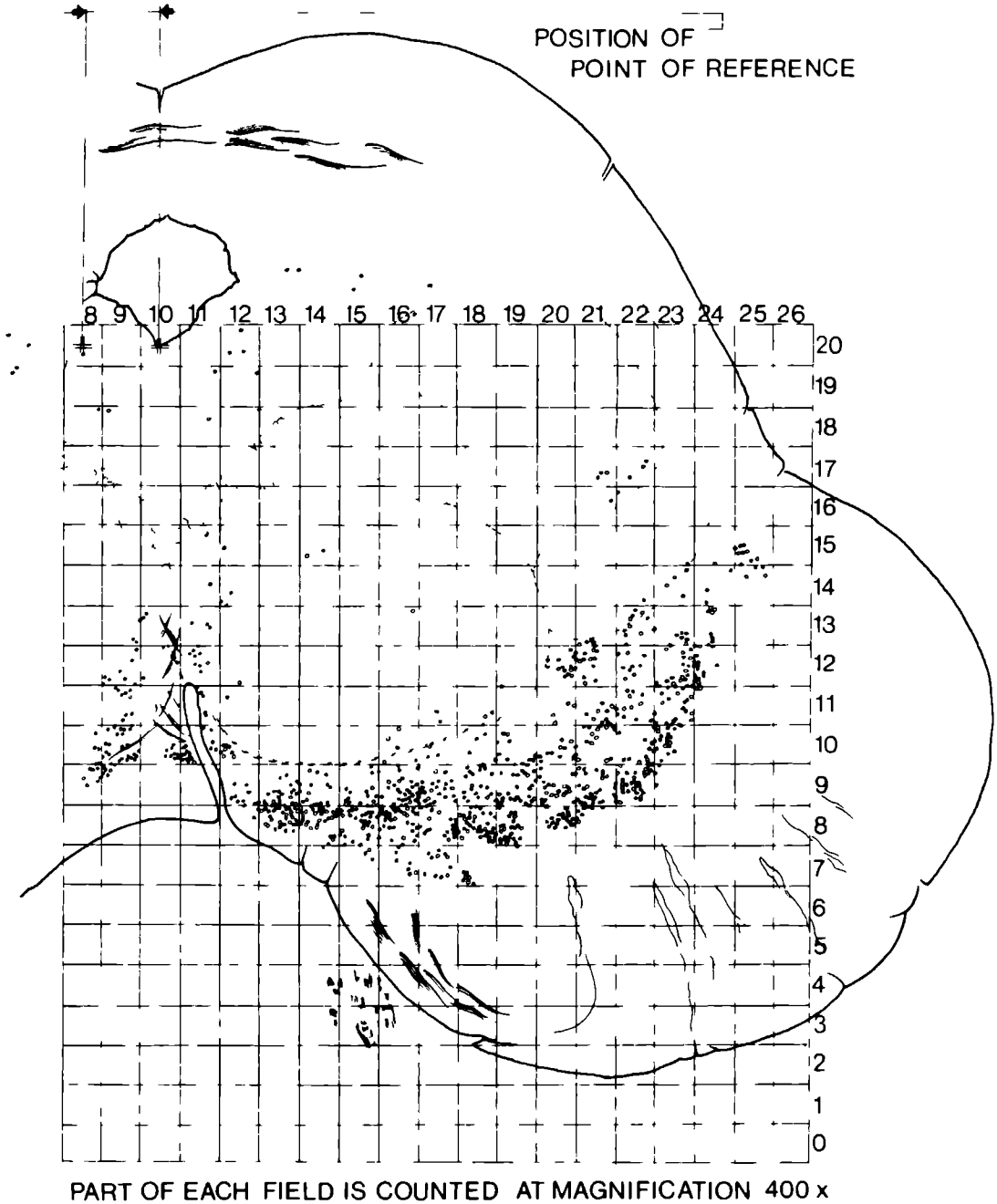


Fig I 2

Projection of the counting grid on microscopical slides

variable in size and shape, is marked with specific problems (Haug 1986; Gundersen 1986; Coleman and Flood 1987), that often introduce unacceptable bias. The actual counted cell number, multiplied with the number of sections represented as well as the fraction of the counted area (Fig. I.3.), actually is an overestimation of the real cell number. In order to correct for this bias the most used correction formula is that of Abercrombie (1946). Correction is performed by the following calculation:

$$Nr = Np \times \frac{T}{Dm + T} \quad \text{(Abercrombie 1946)}$$

In this formula:
Nr =the real cell number in the (sub)nucleus studied.
Np =the counted number.
Dm =the mean profile diameter of the counted elements.
T =the section thickness.

In order to correct for the "lost caps", not counted in routinely performed countings, Floderus (1944) suggested an adjustment to this formula, preventing an overcorrection:

$$Nr = Np \times \frac{T}{Dm + T - 2h} \quad \text{in which:}$$

2h =the diameter of the smallest detectable element ("lost cap").
This formula was used in some cases, in order to get an estimation of the real cell number of the given structure.

Profile (smallest and largest) diameters were measured for each separately quantified area (the pars compacta and the pars reticulata of the substantia nigra, ventral tegmental area and area retrorubralis, rostral and caudal locus coeruleus neurons) with the aid of a Zeiss-Kontron MOP-Videoplan. A mean cellular diameter was calculated from 25 samples of these areas. In this way possible variation due to shrinkage in different disease states or differences in tissue processing are also

corrected for these cellular elements that had to be identified by their contours. The mean cellular diameter here is calculated for an analogous spherical contour that could represent the actual profile area (D-circle). Cells without neuromelanin and thus comprising a visible nucleolus, but within the confines of the typical neuromelanin-countaining structures, were counted in the same way for optimal comparability.

The shape and size of neuropathologic findings identified in Congo red fluorescence (Puchtler 1962; Puchtler and Sweat 1965) is highly variable, to such a degree that only semiquantitative estimation is possible. Thus, no correction formula was used to calculate the "exact" number of neurofibrillary tangles and senile plaques.

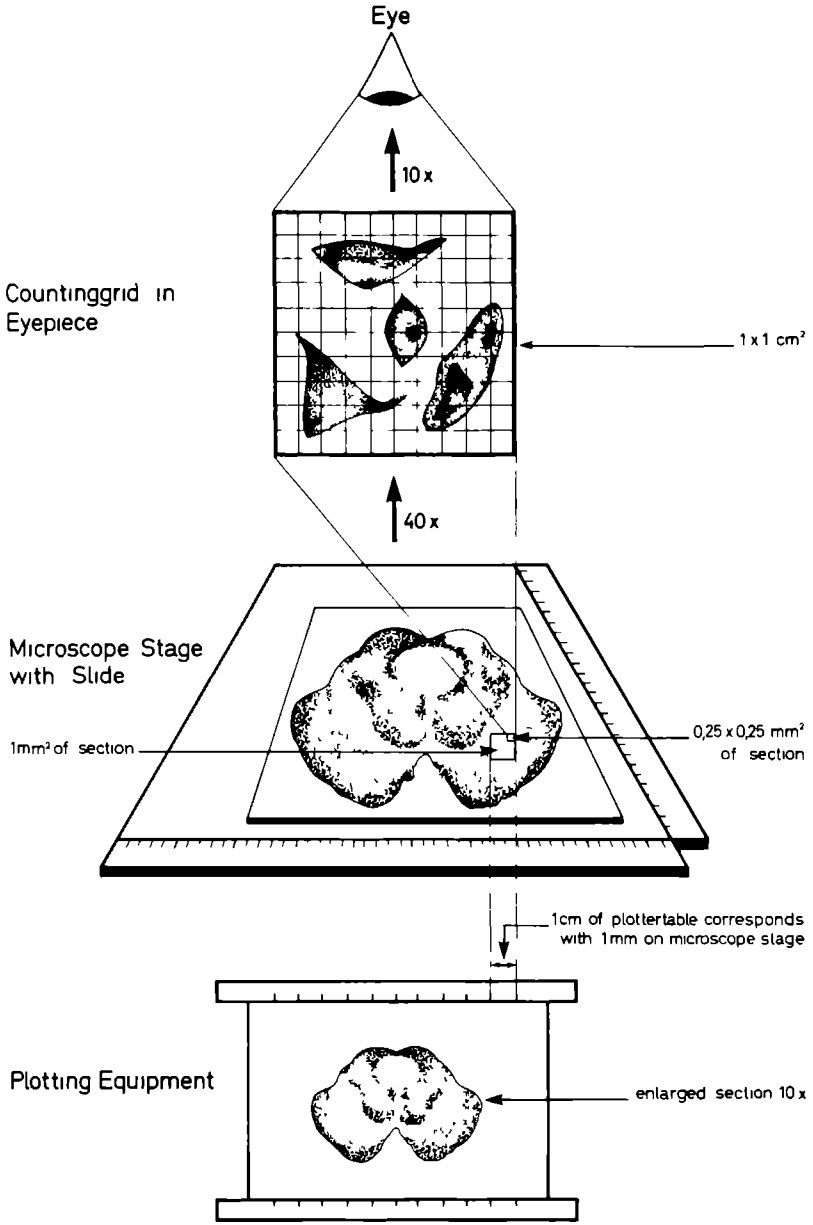


Fig I 3

Schematic representation for the calculation of real cell numbers extrapolated from factors of magnification (see text)

I.3.3. Immunohistochemical procedures

For immunohistochemical studies brain stems were obtained between two and five hours after death in cases of Alzheimer's and Parkinson's disease, and between three and 60 hours post mortem in control cases. At first, the brain stem was immediately removed, cut into slices of three to five mm and fixed in either buffered 4 % formaldehyde or formaldehyde-sublimate at 4° C for 24 hours. After dehydration the slices were embedded in paraffin wax and cut serially into sections of 10 µm on a Reichert-Jung 2050 microtome. Standard immunohistochemical procedures as described by Pearson and co-workers (Pearson et al. 1983) were used. A tyrosine hydroxylase antiserum of Bugene Technics (Allendale, USA) was used in a delution of 1/500.

More recently, however, anothjer fixative was used and the brain was perfused (see Beach et al. 1987) through the internal carotids and basilar artery (or vertebral arteries). Immediately after removal, the brain was put on crushed ice and perfused with 0.5-1 liter of buffered saline, followed by two liters of Somogyi's fixative (see Somogyi and Takagi 1982) using a peristaltic pump (Gibson; 25 r.p.m.). But also without prior perfusion-fixation better results were obtained than in the above mentioned series. After perfusion, the brain stem as a whole was fixed for a few hours in Somogyi's fixative, then cut into slices of 3-5 mm. These slices were further fixed overnight and then cut into sections of 30 µm on a freezing microtome. Standard immunohistochemical procedures using a monoclonal antibody against tyrosine hydroxylase (INC-STAR, Stillwater, Minneapolis, USA) were employed: after washing for three times 15 minutes in 0.05 M TBS (0.05M Tris with 0.7 % NaCl) at room temperature, free floating sections were immersed in 0.05 M TBS containing 1 % normal rabbit serum (NRS), 0.5 % bovine serum albumin and 0.5 % Triton X-100 for one to two hours, incubated overnight with a monoclonal antibody against TH (1:1000 in NRS), again washed for three times 30 minutes in 0.05 M TBS and then incubated for 90 minutes with the second antibody, RAM/PO (Rabbit anti-Mouse peroxidase, Dakopatts P260 Denmark) 1:90. After rinsing for three times 30 minutes in TBS the sections were stained with 0.02 % DAB, containing 0.6 % nickel-ammoniumsulphate as intensifyer (see Adams 1981) and H2O2. Sections were washed in TBS, mounted on gelatin-coated slides, dried overnight, and counterstained with Cresylviolet. In figure I.4. an example is shown of a case (a male who died of a heart attack, 81 years old) with a post mortem delay of 25.5 hours.

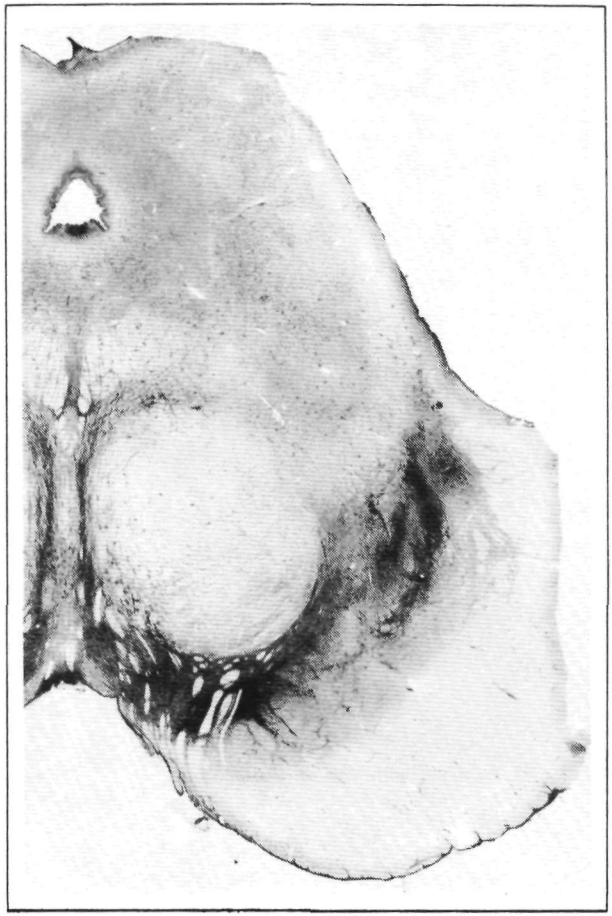


Fig. I.4
Tyrosine hydroxylase staining of the human mesencephalon (x 4)

I.3.4. Neuropathological procedures

The histological diagnosis of idiopathic Parkinson's disease was established on the basis of severe depletion of pigmented neurons in the SNc and the occurence of several hyaline inclusion bodies (Lewy 1913) in the remaining pigmented cells of this nucleus and the nucleus coeruleus (Oppenheimer 1984; see Ch. I.2). The diagnosis Alzheimer's disease was made on the basis of the recently formulated criteria by McKhann et al. (1984) and Khachaturian (1985). Paraffin embedded sections were stained for Nissl, hematoxylin eosin, a modified Bielschowsky stain (Yamamoto and Hirano 1986) and Congo red (Puchtler and Sweat 1965).

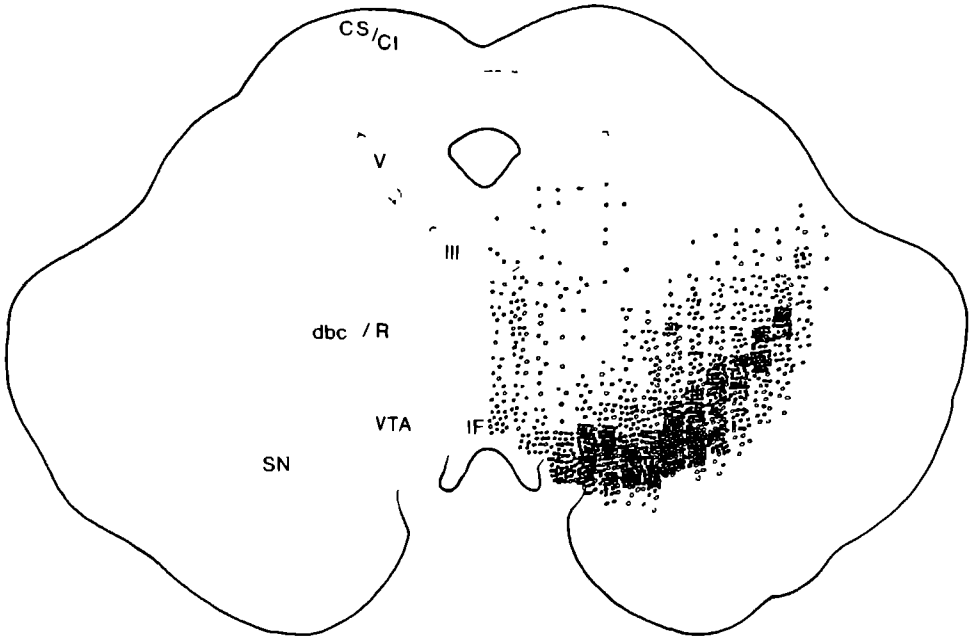


Fig 15

Neuromelanin containing cell bodies as found in the whole rostral to caudal extent of the human mesencephalon (♂, 69 yrs), plotted in one section Each point represents 500 perikaryae Brain nr. 86179

I.4. NEUROMELANIN (AS A MARKER FOR CATECHOLAMINERGIC NEURONS)

Melanin granules can be defined as complex aggregates of quinoid polymers, with several enzymes in a protein matrix (Cotzias et al. 1964; Marsden 1969; Graham 1978). There is no specific chemical test for directly demonstrating melanin (Greiner and Nicolson 1965; Lillie 1969), but it has a typical appearance, at least in the neural elements in the brain stem (Bogerts 1981), and certain histochemical criteria have been suggested (Cotzias et al. 1964; Lillie 1969; Barden 1981). The term melanin, however, is used to denote various brown and black pigments found in mammals, amphibians and plants (Cotzias et al. 1964; Greiner and Nicolson 1965; Lillie 1969; Marsden 1969; Körner and Pawelek 1982). It embraces a variety of pigments, as can be found in hair, feathers, skin, tumors, meninges and certain brainstem nuclei (Cotzias et al. 1964; Bazelon et al. 1967). The *neural* melanin has some features in common, but differs in some essential aspects (Lillie 1969; Marsden 1969,1983), especially with regard to its dependence on catecholamines as precursor (Van den Wende and Spoerlein 1963), the capacity to reduce silver solutions (Lillie 1969) and spectroscopic features (Pakkenberg 1966; Graham 1978; Marsden 1983).

Neuromelanin resembles lipofuscin (Mann and Yates 1974,b) and electron-microscopic studies suggest that neuromelanin is a partially melanized lipofuscin granule (Duffy and Tennyson 1965), whereas lipofuscin exhibited the properties of bleached neuromelanin (Marsden 1983). This last author suggested that neuromelanin consists of a core of lipofuscin, upon which melanin substances are deposited. Both pigments, like other lipopigments formed by peroxidation of polyunsaturated fatty acids and their interaction with biologically useful molecules and structures, are strongly associated with aging (Mann and Yates 1974 a; Graham 1979; Barden 1981). In contrast to lipofuscin, however, neuromelanin is restricted to a number of cell types and is suggested to have some positive, or even toxic, function (Mann and Yates 1974 b; Marsden 1981). Another feature of neuromelanin, as opposed to other melanins, is that the greatest amounts are found in the primates, where more nigral cells contain a greater number of larger and darker granules than in other mammals (Scherer 1939; Marsden 1961; Barden 1981). The

size of the granules and the amount of pigment in the neurons of the substantia nigra and locus coeruleus in the adult gorilla, are about identical to those of the corresponding regions in a 4-year-old child (Scherer 1939). The pigment, however, is dark gray-black in the child, as it is in the adult human, whereas in the gorilla it is brown (Adler 1942). Often the smallest amounts of pigment are masked in Nissl preparations by the dark color of the cytoplasm. Kastin et al. (1976) suggested that a "melanin-like" substance, similar to sepia melanin, is present in the rat brainstem, although not visible in fresh or silver-stained sections light microscopically. But, as Barden (1981) stated: "Neuromelanin granules, as they are known by the usual light and electron microscopic criteria, do not occur in rodent brain". In primates, the intensity of pigmentation increases as the relationship to man becomes closer (Marsden 1961). Mann and Yates (1974b) demonstrated that neuromelanin granules first appear in the human locus coeruleus at birth and in the substantia nigra some 18 months later. Beheim-Schwarzbach (1954) described the melanogenesis of locus coeruleus cells in comparison to other cytological features. A slower melanogenesis for the caudal part of the locus coeruleus was demonstrated, whereas in the involutionary phase free neuromelanin can be found after neuronal loss. Depigmentation, however, is typical for both the substantia nigra and locus coeruleus at old age, for the dopaminergic neurons of the substantia nigra somewhat earlier than for the noradrenergic neurons of the locus coeruleus (Mann and Yates 1974b; Graham 1979; Marsden 1983).

There is a striking resemblance in the distribution pattern of neuromelanin containing neurons and the catecholaminergic cell bodies described by Dahlström and Fuxe (1964) in the rat, as well as tyrosine hydroxylase immunoreactive neurons, as described by Pearson et al. (1983) and catecholaminergic cell bodies in the human fetus (Nobin and Björklund 1973). Therefore melanin was used as a natural marker for catecholaminergic neurons in previous studies (Bogerts 1981; Saper and Petito 1982) and may also indicate dopaminergic neurons of the mesencephalon (Van der Wende and Spoerlein 1963; Cotzias et al. 1964; Marsden 1969; Das et al. 1978). Besides, the loss of neuromelanin containing, pigmented neurons in Parkinson's disease, noted since long (Trétiakoff 1919; Foix and Nicolesco 1925; Hassler 1938) appeared to be closely related to the loss of dopamine in the striatum (Cotzias et al. 1964; Hornykiewicz 1966).

Although the correlation of neuromelanin pigmented cell bodies with tyrosine hydroxylase immunoreactive elements (Gaspar et al. 1983; Pearson et al. 1983)

may not be absolute (Barden 1971,1981; Marsden 1983) there is enough evidence that at least the demarcation of mesencephalic catecholaminergic nuclei, and especially the dopaminergic part of the substantia nigra, can be based on the presence of neuromelanin (for reviews see Bogerts 1981; Saper and Petito 1982). Neuromelanin containing catecholaminergic neurons, that also may develop Lewy bodies in Parkinson's disease, are the substantia nigra, the nucleus paranigralis, the nucleus parabrachialis pigmentosis, the nucleus tegmenti pedunculopontinus, the locus coeruleus and subcoeruleus and sympathetic ganglia (Marsden 1983; Jellinger 1987a). Other nuclei, described as neuromelanin pigmented, without however observed Lewy bodies, are: the nucleus arcuatus and the nucleus periventricularis hypothalami, the nucleus intracapsularis, the nucleus pontis centralis oralis and the nucleus retroambigualis (Barden 1981, Marsden 1983), as well as dopaminergic neurons in the retina (Cohen 1983) and area postrema (Barden 1981). Foix and Nicolesco (1925) already noted the gradual extent of neuromelanin containing neurons in the surroundings of substantia nigra and ventral tegmental area; Bazelon et al.(1967) described a column of neurons containing pigment (based on a modified Lillie silver technique), extending the entire length of the brain stem, until it ends in direct continuity with the intermediolateral gray of the spinal cord. It closely followed the course of the general visceral efferent column of the brain stem (Bannister 1971).

The potential toxicity of neuromelanin and its precursors seems to be closely related to the biochemical processes in which it is involved (Graham 1978,1979; Marsden 1983). Enzymes associated with neuromelanogenesis are: lysosomal hydrolase, tyrosinase, peroxidase, monoaminoperoxidase and enzymes of autooxidative pathways (Barden 1981). Early investigators concentrated on the conversion of tyrosine to dihydroxyphenylalanine (DOPA) and of DOPA to the colored dopaquinone, from which polymers are formed, leading to the melanochromes of the skin (and equivalents) or synthetic melanin (Körner and Pawelek 1982). The (copper dependent) enzyme tyrosinase is essential in this pathway (Lillie 1969; Bazelon et al. 1967; Graham 1978,1979). (It is of interst to know that melanotropin (MSH) causes an increase in tyrosinase activity, whereas melatonin inhibits this reaction (Cotzias et al. 1964; Greiner and Nicolson 1965; Körner and Pawelek 1982). Rainero et al.(1988) measured increased levels of αMSH-like immunoreactivity in cerebrospinal fluid of patients with Parkinson's disease). Neural melanin, however, probably is generated by auto-oxidation of catecholamines, that is their

non-enzymatic oxidation by molecular oxygen (Graham 1978; Barden 1981). Although there is little doubt that tyrosine is the precursor amino acid for the catecholamines, the intermediate stages and the enzymes responsible for the formation of neuromelanin, are different from that of other melanins (Marsden 1969). Barden (1975,1981) devised a model system to test for the possibility that neuromelanin is formed from catecholamine derived precursors. Lipofuscin became pseudoperoxidatively melanized following its impregnation with a catalyst, iron sulfide, and its exposure to a solution of DOPA and hydrogen peroxide. He commented however, that the application of metal compounds was not necessarily in correspondence with the natural situation. The same author also demonstrated neuromelanin and catecholamine in the same neuron (Barden 1975). Again, from investigations of dog hypothalamus it appeared that there was an age correlated, inverse relationship within neurons in which catecholamine histofluorescence is gradually replaced by increasing quantities of accumulation of neuromelanin. In brain tissue the enzyme tyrosine hydroxylase (TH) is responsible for L-DOPA formation, from which the catecholamines are derived, also explaining the fact that albino patients have a normally pigmented substantia nigra without skin melanin. Besides skin pigmentation does not alter in patients with Parkinson's disease, whereas substantia nigra pigmentation decreases (Barden 1981). Unless its physicochemical difference from tyrosinase (Marsden 1969) both enzymes (TH and tyrosinase) depend on the presence of metal ions for their effect (Cotzias et al. 1964). As a consequence of these biochemical pathways, several mechanisms for the typical pathophysiology of age dependent degeneration of pigmented brainstem nuclei have been proposed (Cotzias et al. 1964; Mann et al.1977; Graham 1979; Cohen 1983; Hirsch et al. 1988).

Mann and co-workers (Mann et al. 1977; Mann and Yates 1982) suggested a mechanical disruption of cellular elements by the accumulation of neuromelanin, based on the observation of "cell growth" and RNA loss with increasing neuromelanin. According to these authors, the nerve cell apparently can accommodate a certain amount of melanin but when a "saturation level" is exceeded, at about the age of 60 years, atrophy and cellular death result. The most plausible characterization of the granules then, is that of a waste product (Graham 1979). The observations of Beheim-Schwarzbach (1954) about the evolution of locus coeruleus neurons throughout the human life-cycle and the increase of neural volume noted by Graham (1979), fit well in this theory. Cell countings of Vijayashankar

and Brody (1979) in the locus coeruleus showed a maximal declination at above 60 years, corresponding with the period of depigmentation (Mann et al. 1974a,b, 1977). But apart from mechanically induced cellular damage neuromelanin and associated waste products could have cytotoxic properties (Graham 1979, Barden 1981). Cotzias et al. (1964) stressed the binding capacities of melanin granules, which accounts for the presence of metals and other toxic substances.

In line with such a proposal is the high affinity binding of N-Methyl-4-phenylpyridine (MPP+) to neuromelanin, as described by D'Amato et al. (1986,1987a), resulting in the well-known nigrostriatal degeneration caused by N-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine: MPTP (Calne and Langston 1983). The other remarkable, related phenomenon is the apparent relation of "the intensity of pigmentation to the degree of evolution of the brain" (Marsden 1969). The unique characteristic of the human brain, in this respect, apparently leads to maximal accumulation of neuromelanin, in a life span of seven decades, after which it decreases (Mann and Yates 1974b; Graham 1979) and could partly explain the specific vulnerability of the human brainstem catecholaminergic neurons to age related degeneration (Hirsch et al. 1988). As Graham (1979) stated, however, rather the by-products of catecholamine oxidation than neuromelanin itself, would be toxic. This also would explain why the dopaminergic neurons, with slower melanogenesis, would be more vulnerable than the more rapidly pigmented noradrenergic neurons of the locus coeruleus (Graham 1979; Mann and Yates 1974b).

Dopamine is present in different forms (Cohen 1983); most of the intracellular dopamine is stored within vesicles, but a considerable amount is also present in the cell cytoplasm and in the synaptic cleft, where it is catabolized by oxidative deamination by monoamineoxidase (MAO) and O-methylation by catechol-O-methyltransferase (COMT) (Graham 1978, Sourkes 1989). Apart from the accumulation of neuromelanin, such pathways lead to formation of hyperreactive free radicals (Cohen 1983, 1984). A by-product of oxidative deamination is hydrogen peroxide, a cellular toxin, often accompanied by generation of superoxide free radicals and hydroxyl free radicals, which are also cytotoxic (Cohen 1983, 1984). Normally, a variety of scavenger systems, among which the catecholamines themselves are counted (Cohen 1983, 1984), serve to prevent the excessive accumulation of these toxic agents (Perry et al.1982; Marsden 1983). Free radical products resulting from dopamine catabolism, occur more rapidly and dopamine

o-quinone is more likely to react with cellular elements than other quinones (Graham 1979), explaining why dopamine is more cytotoxic than the other amines (Graham 1978). Based on these presumptions, therapy with L-DOPA in Parkinson's disease, has a dual effect. By increasing intracellular dopamine, L-DOPA will protect against superoxide-mediated damage. But increased cellular dopamine may also spur hydrogen peroxide formation via MAO-activity (Cohen 1983, 1984).

II. NEUROANATOMICAL SECTION

"The royal road of the sensations of the body to the soul is through the corpora striata, and all determinations of the will also descend by that road.....; the Mercury of the Olympus, it announces to the soul what is happening to the body, and it bears the mandate of the soul to the body."

E. Swedenborg, Oeconomia Regni Animalis, 1740
Transl. Prof. Ramström, 1910

II.1. INTRODUCTION

The aim of this chapter is to provide a schematic view on the neuroanatomical organization of the human mesencephalon and rostral rhombencephalon, with emphasis on the monoaminergic nuclei: the substantia nigra (SN), the ventral tegmental area (VTA), the nucleus raphe dorsalis (NRd) and the locus coeruleus (LC). Monoaminergic brainstem nuclei are characterized, among others, by their intermingling and interconnectivity as regards nuclear organization (Moore 1980; Steinbusch 1981) and fiber trajectories (Lindvall and Björklund 1974; Felten and Sladek 1983). The acceptance of the monoamines as neurotransmitters in the central nervous system was a slow process (for review see Björklund and Lindvall 1984; Carlsson 1987), the real breakthrough coming with the histochemical techniques visualizing the cellular localization of the monoamines by means of the fluorescence microscope (Falck et al. 1962; Dahlström and Fuxe 1964). Similar techniques provided a means of studying ascending monoaminergic pathways of which the nigrostriatal projection (Andén et al. 1964) nowadays might be the most studied pathway in the brain (Graybiel and Ragsdale 1983). Other studies on mesotelencephalic projection systems soon followed (Andén et al. 1966a,b; Ungerstedt 1971a; Lindvall and Björklund 1974) and still form the basis of our understanding of central nervous system monoaminergic systems.

The successful application of immunohistochemistry to neurotransmitter specific systems was largely based on the fact that certain enzymes (like tyrosine hydroxylase and dopamine-ß-hydroxylase), present in the catecholaminergic neurons, are also found in the glandular cells of the adrenal medulla (Pickel et al. 1975; Hökfelt et al. 1975,1976). Because adrenaline and noradrenaline are synthesized from dopamine, various catecholaminergic neurons might share certain enzymatic features and the differentiation of noradrenergic elements from dopaminergic cells and processes is still a controversial subject. This problem was partly solved by proper lesion experiments, combined with formaldehyde fluorescence studies or identification of transmitter specific elements by their morphologic features directly studied in the microscope (see Lindvall and Björklund 1974; Berger 1977; Levitt et al. 1984; Lewis et al. 1987).

The functions of the central monoaminergic neurons and their role in neurologic and psychiatric disorders are less well understood than the morphology and synaptology of these systems. The remarkable exception of the nigrostriatal-cortical dopaminergic system, involved in extrapyramidal function and disorders, especially Parkinson's disease, might serve as a model system in studying monoaminergic brainstem nuclei in aging and aging diseases. The concept of the mesotelencephalic projection system is based on its extensively studied counterpart in the rat brain (Björklund and Lindvall 1984). Another, more recently developing, aspect is the compound organization as regards various neurochemical substances throughout the brainstem monoaminergic nuclei (for review Nieuwenhuys 1985). As a highly convergent output system (Szabo 1980a; Nauta 1986) of motor function, closely related to structures that have widespread projections to the cortex and might be involved in general levels of consciousness (Moore 1980), the anatomy of human brainstem monoaminergic nuclei deserves a thoroughly examination, for the interpretation of quantitative changes in the aged or those with neurodegenerative disease.

II.2. THE MAMMALIAN SUBSTANTIA NIGRA AND VENTRAL TEGMENTAL AREA

II.2.1. General organizational principles

Mesencephalic catecholaminergic nuclei form a rather uniformly composed cell continuum in different mammalian species, with clusters sometimes only separated by fiber tracts (Schwyn and Fox 1974; Moore and Bloom 1978) in this overall 'reticular' brain region (Divac et al. 1978; Moore 1980; Köhler and Goldstein 1984; Fallon and Loughlin 1985). Whereas the pattern of distribution of dopaminergic (DAergic) nerve-cell bodies in the rat is rather clearly organized, in cats DAergic cells are more uniformly distributed throughout the SN (Wiklund et al. 1981; Poirier et al. 1983; Parent 1986). In monkeys the pattern is even more complex and compartmentalized, such that, e.g., it is hard to distinguish DAergic from nonDAergic projection neurons, especially in ventral nigral parts (Garver and Sladek 1975; Nauta and Cole 1978; Felten and Sladek 1983; Satoh and Fibiger 1985; Arsenault et al. 1988). There is general agreement, however, in following Dahlström and Fuxe's (1964) subdivision in A9,A8 and A10 for the SN proper, the retrorubral area and the ventral tegmental area dorsomedially adjacent to the SN, respectively, defined in rats.

Different cytologic criteria lead to considerable disagreement in defining cytoarchitectural boundaries (see also Table II.1.). Apparently the spread of dopaminergic neurons into many adjacent nuclei (Dahlström and Fuxe 1964; Swanson 1982; Köhler and Goldstein 1984; Fallon and Loughlin 1985) particularly interfered with the demarcation of so-called "out-lying neurons" (Nauta et al. 1978) of the SN, predominantly found rostromedial, medial and caudal to the SN proper (Dahlström and Fuxe 1964; Felten and Sladek 1983; Parent 1986; Halliday and Törk 1986). Therefore this area dorsomedially from the SN, corresponding with Tsai's (1925) original description of the nucleus tegmentalis ventralis in the opossum, usually is referred to as ventral tegmental area (VTA). The nucleus linearis rostralis, linearis caudalis (or centralis) and nucleus interfascicularis mostly are included (Phillipson 1979a; Halliday and Törk 1986), although projections of midline structures are sometimes dealt with separately (Swanson 1982;

Fallon and Loughlin 1985). Thus the VTA, as originally defined, may be subdivided into a midline, a medial and a lateral part (Simon et al. 1976; Fallon and Moore 1978b), the latter two corresponding to nucleus paranigralis and nucleus parabrachialis pigmentosus, respectively. The nucleus parabrachialis pigmentosis originally was identified in the human brain by its neuromelanin content (Olszewski and Baxter 1954) and described in the cat by Taber (1961). Other, cytological as well as hodological data contribute to its identification in the rodent brain as well (Halliday and Törk 1986). The nucleus parabrachialis pigmentosus blends into the pars compacta of the substantia nigra and actually often is referred to as "dorsal pars compacta" (Moore and Bloom 1978; Fallon et al. 1978a,c; Hökfelt et al. 1980; Loughlin and Fallon 1984; Gerfen et al. 1987a). Szabo (1980a) identified the dorsal pars compacta with the retrorubral area A8. The nucleus paranigralis in the rat brain, together with the most medial part of the SN, is separated by its lateral counterpart by the accessory optic tract and its nucleus, more clearly developed in the rodent brain (Winkler 1929; Crosby and Woodburne 1943; Paxinos and Watson 1986).

Caudally from the nucleus ruber, dorsolaterally continuous with the SN, the area A8 is defined as a retrorubral extent of catecholaminergic neurons, continuous also with the VTA (Dahlström and Fuxe 1964; Köhler and Goldstein 1984; Fallon and Loughlin 1985; Deutch et al. 1988). The different component nuclei of A8,A9 and A10 have now also been identified in primates and man (Francois et al. 1985; Parent 1986; Arsenault et al. 1988), although there is substantial variation in their volumes and cell densities (Halliday and Törk 1986).

In rats the neurons of the pars compacta form a rather thin horizontal layer that is clearly distinguishable from the more diffusely organized reticular part (Poirier et al. 1983; Fallon and Loughlin 1985). In cats, Jimenez-Castellanos and Graybiel (1987) identified two zones within the pars compacta: a caudal densocellular zone with closely packed tyrosine hydroxylase (TH)-positive neurons and neuropil and a more rostrally located nigral zone in which TH-positive neurons are loosely arranged. Although horizontal layers and mediolateral subdivisions of the pars compacta are readily demonstrable with acetylcholinesterase histochemical staining (Jimenez-Castellanos and Graybiel 1987, 1989) there is no clear overlap with the distribution pattern of tyrosine hydroxylase-immunoreactive neurons.

From the pictures, based on a highly specific polyclonal antibody raised against dopamine-glutaraldehyde-lysyl protein conjugate, presented by Arsenault

et al. (1988), it may be concluded that the topographic distribution of SN DAergic neurons does not principally differ from that in various monkey species (see Felten and Sladek 1983; Francois et al. 1985; Satoh and Fibiger 1985) and from that in TH immunohistochemistry or the distribution pattern of neuromelanin in humans (Gaspar et al. 1983; Pearson et al. 1983). Areas of the SN similar in size and configuration have also been visualized in a horseradish peroxidase study of striatonigral connections (Smith and Parent 1986). These large oval sectors, delimitated by the DAergic cell-trabeculae in monkeys are more pronounced than in the human brain. At rostral levels the medial SN pars compacta is particularly well developed and the ventral displacement of the caudolateral pars compacta also is remarkably constant.

The VTA is much less well developed in primates and its boundaries are less well defined than in rodents (Francois et al. 1985; Arsenault et al. 1988), but these authors apparently do not include the few DAergic neurons at more dorsal levels, adjacent to the periaqueductal gray, which would greatly expand the VTA (Halliday and Törk 1986). Besides, TH immunoreactivity seems more widely distributed than the neuromelanin pigmented neurons in dorsal and lateral VTA groups in humans (Gaspar et al. 1983). The retrorubral area in the squirrel monkey does not extend as far caudally as in other primates (Tanaka et al. 1982; Arsenault et al. 1988), but it can be distinguished from the dorsal SN on cytologic grounds (Arsenault et al. 1988).

Table II.1: Cytologic characteristics used in cytoarchitecture of SN

Explanation of symbols: ++ used as criterion for classification by these authors
+ some remarks without classification
c comparable to the classification proposed here

Authors	cellular orientation	E.M. synaptic specification	dentritic morphologie	neuro-melanin content	Nissl substance	details of nucleus	cell shape	cell size
Olszewski and Baxter 1954 (human)	+			c	+		+	+
Rinvik and Grofova 1970 (cat)		++	c		+		+	+
Gulley and Wood 1971 (rat)		++	++		++	++	+	++
Schwyn and Fox 1974 (monkey)		++	c	+	+	+	+	+
Marchand et al. 1979 (monkey)			c		++	+	++	++
Beckstead et al. 1982 (cat)			+				+	++
Poirier et al. 1983 (monkey)			+		c	++	++	++
Felten and Sladek 1983 (monkey)	+	++	c	+			++	++
Francois et al. 1979,1984,1985 (monkey)			+	c	++		+	++
Halliday and Törk 1986 (human)	+	+	+	++	+		c	+
Braak and Braak 1986 (human)			++	c	c	++	c	+
Parent 1986 (human)			+				+	+
Yelnik and Francois 1987 (monkey)	c		c				++	++

II.2.2. Cytology

The pars reticulata of the SNr is generally considered as the receptive field, with a neuropil rich in fine unmyelinated fibers, whereas DAergic pars compacta neurons are the principal source of nigrostriatal projections (Poirier et al. 1983; Parent 1986). Golgi studies (Rinvik and Grofova 1970; Schwyn and Fox 1974; Juruska et al. 1977; Danner and Pfister 1982; Yelnik et al. 1987; Francois et al. 1987) show that nigral neurons give rise to long radiating dendrites with few branches. Small neurons, of which most are found in the pars reticulata (Gulley and Wood 1971), most likely are interneurons (Francois et al. 1979). Larger neurons generally have abundant cytoplasmatic organelles (Rinvik and Grofova 1970), but variations in Nissl staining pattern, size and shape are considerable. At present there is still no general consensus regarding classification of neurons of the mammalian SN (Parent 1986). In a comparative study of the substantia nigra in rats, cats and rhesus monkeys, Poirier et al.(1983) identified four types of nigral neurons with distinct cytological features: 1) compacta-type neurons, the most abundant type, characterized by large cell bodies containing unevenly distributed and intensely stained Nissl substance; 2) reticulata-type neurons with discrete Nissl bodies are triangular or round; 3) intermediary-type fusiform or triangular neurons, containing less intensely stained but more diffusely distributed Nissl substance, and 4) globular-type neurons, characterized by a high nuclear/cytoplasmic ratio, that are much smaller than the other three types. According to Domesick et al.(1983) DAergic cells are distinguishable from non-DAergic cells in non-primates by their dark appearance in Nissl preparations. In primates this DAergic character might be derived by the appearance of neuromelanin (See Ch. I.4.).

In Golgi preparations (see above), the dendritic fields of the pars compacta neurons are oriented primarily in a dorsoventral direction, while those of the pars reticulata have a rostrocaudal orientation. The most ventral region of the pars reticulata contains inverted pyramid-shaped DAergic neurons with dendritic disc-like formations oriented mostly parallel to the crus cerebri (Grofová et al. 1982(cat); Fallon and Loughlin 1985(rat)). Together with the mediolateral orientation of the most dorsal compacta and the VTA neurons, these morphological features are suggestive of a laminar organization, based on fiber orientation (Fallon et al. 1978a; Grofová et al.1982). A similar laminar pattern on hodological grounds, was suggested for rats (Gerfen et al. 1987a).

Close dendrodendritic appositions might be associated with non-synaptic dendritic release of DA (Chéramy et al. 1981; Wassef et al. 1981). Golgi electron microscopy also revealed direct synaptic contacts of striatonigral axons, suggestive of a feedback information system to the pars compacta neurons (Somogyi and Smith 1979; see also Percheron et al. 1989). Dendrites ensheathed in a meshwork of thin, beaded fibers of striatal origin, are termed "woolly fibers", as was described for ventral pallidal dendrites (Haber and Nauta 1983). The cell types, connections and neurotransmitter relationships within the SNr have led to the conclusion that it is a pallidal structure (Nauta 1979; Switzer et al. 1982; Poirier et al. 1983; Beach and McGeer 1984; Parent 1986). The tendency of dendritic processes to form long and thick bundles appears to be another characteristic of the mammalian SN (Gulley and Wood 1971; Arsenault et al. 1988).

Axons of the medial pars compacta neurons are directed dorsomedially towards the hypothalamic area of the medial forebrain bundle, whereas those located laterally were preferentially directed towards ansa lenticularis on their way to the striatum (Preston et al. 1981; Felten and Sladek 1983; Giguère et al. 1984; Fallon and Loughlin 1985). A substantial part of nigral efferents in primates is composed of myelinated fibers (Arsenault et al. 1988), whereas fine unmyelinated axons characterize DAergic and serotonergic ascending projections within the median forebrain bundle of rats. Non-DAergic, presumably GABAergic, projections stem from pars reticulata neurons and from the pars lateralis. Neurons of the "reticulata" and "lateral" types are mainly located in the ventromedial and rostrolateral parts, respectively (Giguère et al. 1984). The pars lateralis of the SN, on cytoarchitectonic, immonohistochemical and hodological grounds might be a distinct subdivision, with some "reticulata" features (Chesselet and Graybiel 1983; Francois et al. 1985; May and Hall 1986), extending in the peripeduncular nucleus. A morphologic distinct subset of nigrotectal projection neurons, apart from the general reticulata nigrotectal and nigrothalamic projection neurons, might define this area as a distinct nigral subdivision (May and Hall 1986).

In cats and monkeys, the intermingling of pars compacta neurons with those of the pars reticulata mimics a clear laminar pattern (Poirier et al.1983; Giguère et al. 1984; Francois et al. 1985; Arsenault et al. 1988). Projection neurons might also be distinguished by their pattern of collateralization, i.e. sparsely for VTA neurons and adjacent dorsal pars compacta neurons, whereas medial pars compacta neurons and pars reticulata cells might have widely branched axons (Parent 1986; Fallon

1988). Apart from differences in collateralization also electrophysiological evidence of subpopulations within the nigral projection system exists (Deniau et al. 1979; Shepard and German 1988). Nigrostriatal dopaminergic neurons display a high degree of plasticity and the capacity for regeneration (Dunnett et al.1983; Schultzberg et al. 1984; Won et al. 1989). Such discoveries have opened a new and promising field of investigation that could eventually lead to the development of new treatment strategies for severe motor disorders, resulting from the specific loss of striatal dopaminergic innervation.

A Golgi study of the VTA in rats (Phillipson 1979b) revealed that each cytoarchitectural subdivision of the VTA is characterized by differences in cell type and dendritic organization. (see also Köhler and Goldstein 1984; Halliday and Törk 1986). In general, VTA neurons tend to fall into one of two cell types (with intermediate forms): type 1 neurons, small to medium sized, with two or four primary dendrites, dividing into varicose spiny secondary dendrites, and type 2 more common laterally and having many local circuit connections. A major difference with neurons in SN compacta (see Juraska et al. 1977) appears to be that, whereas SN compacta dendrites are organized in vertical as well as horizontal planes, in the VTA no long ventrally directed dendrites were observed. Besides, according to Domesick et al. (1983) dark-staining dopaminergic cells and light-staining non-dopaminergic cells were intermingled in the VTA, rather than grouped in separate strata as they are in the SN.

II.2.3. Fiber connections of the rat substantia nigra and ventral tegmental area

Topographical principles of the mesotelencephalic projections are formulated by most authors who studied SN and VTA hodology in the rat (Ungerstedt 1971a; Carter and Fibiger 1977; Moore and Bloom 1978; Fallon and Moore 1978b; Beckstead et al. 1979; Swanson 1982), but controversion exists as to the correspondence with nigral and ventral tegmental subnuclei (for review Fallon 1988). Fig.II.1. gives a general impression, summarizing the available data in the rat brain.

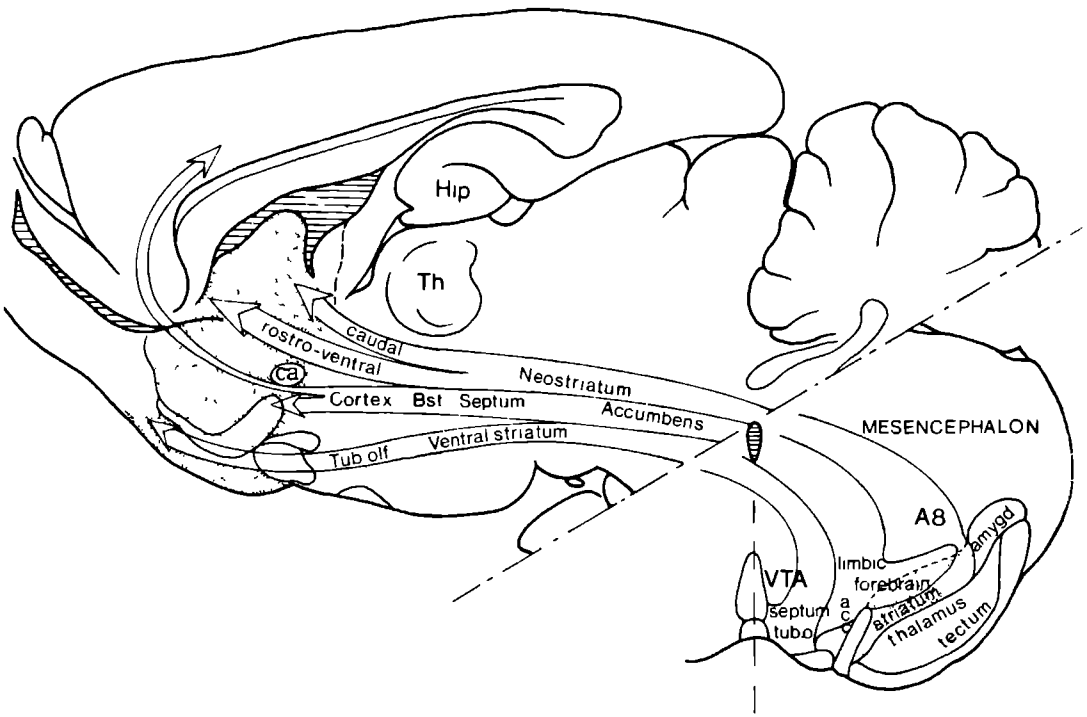


Fig II.1

General topographic principles of mesencephalic dopaminergic projections to forebrain structures in rats

Generally, VTA projection neurons overlap with the most medial and dorsal SN compacta (SNc) neurons, providing a continous "mesolimbic projection" (Björklund and Lindvall 1984; Fallon and Loughlin 1985). Mesocortical projections originate from different subnuclei, and are closely associated with the mesolimbic system (Björklund and Lindvall 1984). Thus, the **mesotelencephalic** fiber system generally is subdivided in a **mesostriatal** (Andén et al. 1964, 1966) and **mesolimbocortical** (Ungerstedt 1971a; Thierry et al. 1973) projection. The "limbic" ventral striatum (Heimer and Wilson 1975) exhibits a greater intensity of CA fluorescence in rats than the caudatoputamen (Graybiel and Ragsdale 1983). Projections from A8 are included and continuous with the other mesotelencephalic projections (Deutch et al. 1988), whereas the SN pars reticulata (SNr) has its own characteristic efferent organization to thalamus, tectum and tegmentum.

The medial forebrain bundle (mfb) is the main trajectory connecting brainstem (monoaminergic) reticular formation structures with diencephalic and telencephalic regions (Andén et al. 1966; Nieuwenhuys et al. 1982; Björklund and Lindvall 1984; Oades and Halliday 1987). The nigrostriatal pathway is located dorsolaterally from and in close association with the mfb. Diverging DA and NA pathways mostly follow classical routes to their targets, like fasciculus retroflexus, ansa peduncularis, fornix, stria terminalis and capsula externa (Nieuwenhuys et al. 1988). Within the major systems topographical principles imply an inversed dorsal to ventral relationship of projection neurons with their striatal and limbic targets; a general medial-to-lateral topography (Björklund and Lindvall 1984) and an anterior to posterior topography (see also Fallon and Moore 1978b; Beckstead et al. 1979; Gerfen et al. 1987a). Afferents connecting the limbic forebrain and midbrain follow two primary routes, a dorsal one in which the lateral habenula is an important relay (Nauta 1958; Sutherland 1982) and the ventrally located mfb. Thus, DAergic cells of the ventral tegmentum may receive converging limbic and striatal input.

Striatomesencephalic projections

The main part of the nigral afferent projections is the striatonigral projection, well established in rats, passing mainly through the comb bundle (or pencils of Wilson) towards the pars reticulata (Gerfen 1985; Fallon and Loughlin 1985). Within this projection (see also Ch. II.2.5) an inhibitory part, originating from GABAergic neurons, mainly from the lateral caudate-putamen (Araki et al. 1985), may be

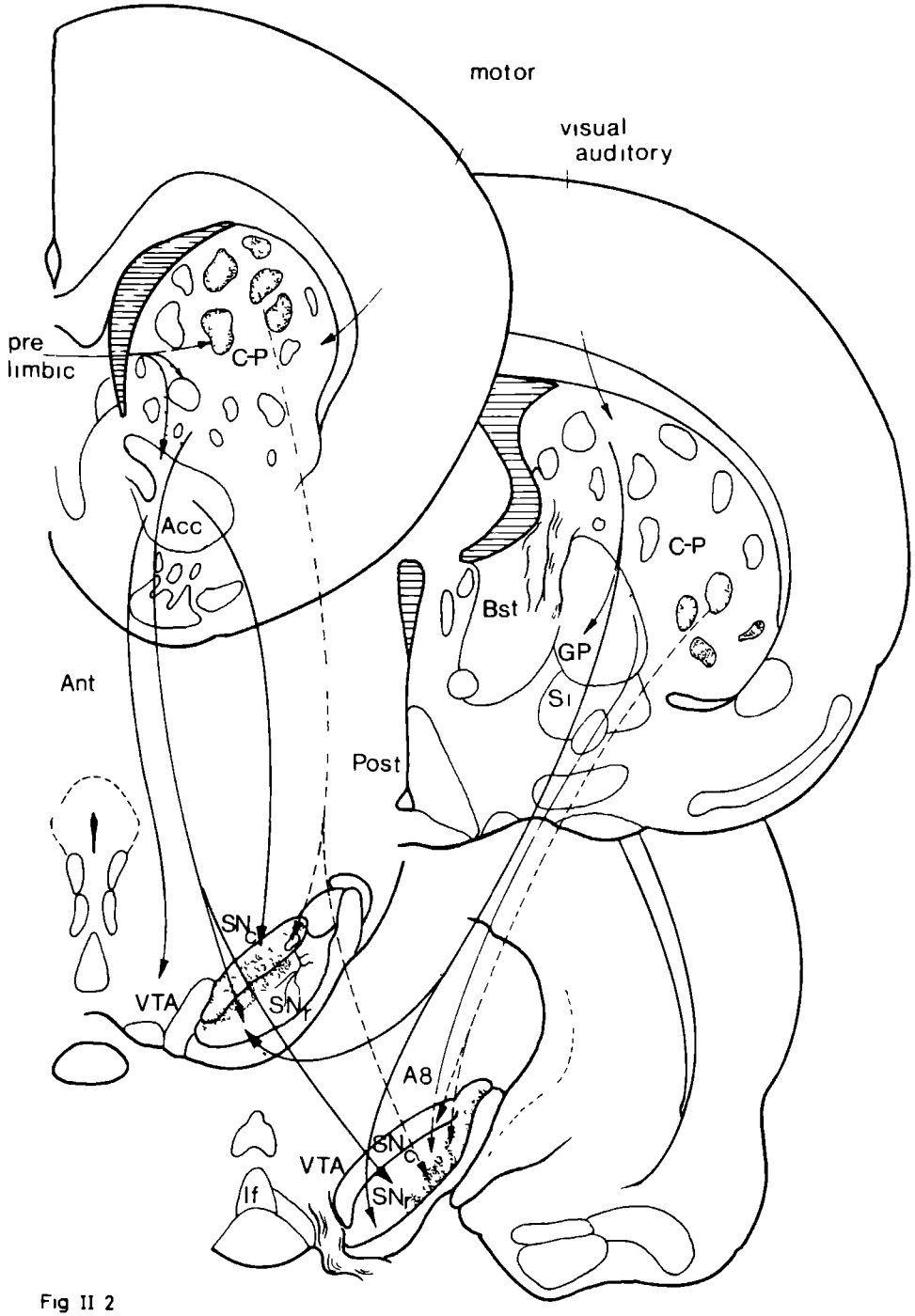


Fig II 2

The striatomesencephalic projections and their topography in rats

distinguished from an excitatory part containing substance P. The latter arises mainly from cells rostrally in the caudate-putamen and cell bodies surrounding the globus pallidus. The topographic organization of these projections (Fig.II.2.) is presumably a consequence of the differential association with neocortical and mesocortical/allocortical afferents. E.g., a caudatoputamen patch ("striosomal") and matrix compartment receive a prelimbic cortical and neocortical input, respectively (Gerfen et al. 1987b). More diffuse projections of some associational cortical areas (Heimer et al. 1982; DeLong et al. 1983a; Gerfen 1985; McGeorge and Faull 1989) might be reflected in an additional non-topographic strionigral projection (Gerfen 1985). The "striosomes", from which the latter originates, are neurochemically defined compartments, comprising an essential feature in the striatum (Graybiel and Ragsdale 1983; Graybiel 1986). In rats these patches are characterized by their richness in substance P, enkephalin-like immunoreactivity and opiate receptors, whereas the striatal matrix distinguishes itself by AChE-positive staining and also contains many somatostatin fibers (Gerfen 1985). Somatostatin-immunoreactive neurons may provide an intrinsic system linking the patch and matrix compartments (Gerfen 1985).

Dorsal matrix regions of the striatum, receiving visual cortical input, project to the ventral "nigrotectal" region of the SNr (McGeorge and Faull 1989); the lateral and central regions, related to motor cortices, project in turn to that region of the SN that gives rise to the nigrothalamic projection (Faull and Mehler 1978). Target sites are non-dopaminergic neurons and also D1-type (i.e. adenylate cyclase-linked) receptors of pars compacta dendrites (Altar and Hauser 1987). Rostrally and caudally located patches to the SN DA neurons (dotted area in Fig.II.2.) appear to converge on a single nigral locus (Gerfen 1985).

The striatonigral projection from the dorsal striatum has a counterpart in the ventral striatal complex and its distinct projections to the SN dorsal pars reticulata and medial pars compacta, VTA and A8 (Nauta et al. 1978; Haber et al. 1985; Domesick 1988). Terminals on dopaminergic nigrostriatal projection neurons have also been described (Somogyi et al. 1981a). They provide a major link between dorsal (motor) and ventral (limbic) striatum (Swanson and Cowan 1979; Nauta et al. 1978; Alexander and DeLong 1985; Gerfen 1985; Parent 1986). The uninterrupted continuum of subcortical grey matter that stretches in a paramedian zone caudalward from the septum, over the preoptic region and hypothalamus, with reciprocal ascending and descending projections within the mfb, has been named

"limbic forebrain-midbrain circuit" (Domesick 1988). The VTA belongs to the limbic midbrain area as defined by Nauta (1958), together with the ventral half of the central grey substance, dorsal and median raphe nuclei and dorsal tegmental nucleus of Gudden (see also Nauta and Haymaker 1969; Simon 1981).

Non-striatal afferents to the substantia nigra and ventral tegmental area

Apart from the massive striatonigral projections to both the SN and VTA, these structures receive a variety of non-striatal afferents, summarized in Fig. II.3. and II.4. (based mainly on data by Phillipson 1979c; Swanson 1982; Gerfen et al. 1982; Fallon and Loughlin 1985). **Cortical** projections, from neocortical, mesocortical and allocortical structures, innervate the VTA. Cortical projections to the SNr, are restricted to projections from prefrontal (area 32 of Brodmann) and motor cortices.

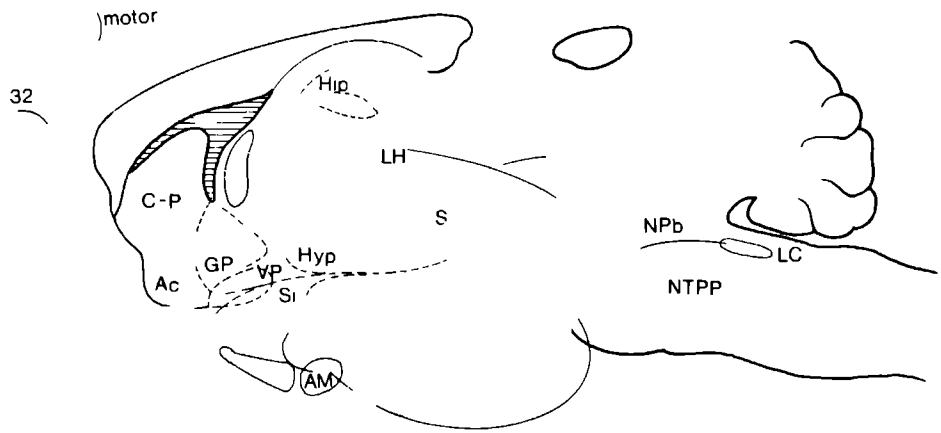


Fig. II. 3
Non - striatal afferents of the substantia nigra in rats

Dorsal pallidonigral, most likely GABAergic, fibers terminate in the pars reticulata and pars compacta of the SN. Ventral pallidonigral fibers, i.e. those of the substantia innominata and so-called "ventral pallidum" (Heimer and Wilson 1975; Alheid and Heimer 1988; Grove 1988b) together with projections from other basal

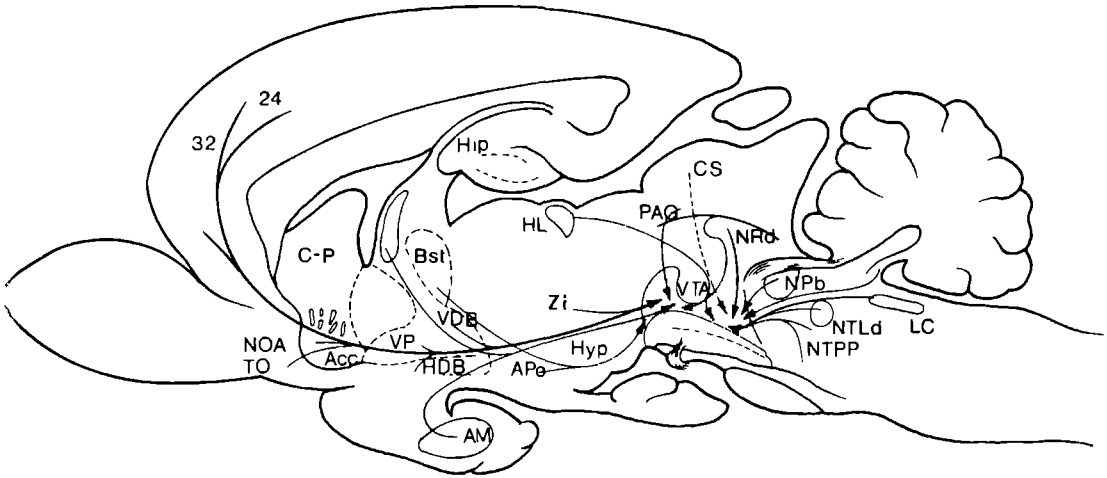


Fig II 4
Non-striatal afferents of the ventral tegmental area in rats

forebrain structures, such as the lateral septum, Broca's diagonal band nuclei and bed nucleus of the stria terminalis, appear to end mainly in the rostroventral part of the VTA (Swanson and Cowan 1979; Grove 1988b). Dorsal substantia innominata efferents have more widely distributed projections throughout the SNc and VTA, whereas more ventrally located cells mainly project on VTA and dorsal layer of the substantia nigra pars compacta (Grove 1988b). The central nucleus of the amygdala projects to the pars lateralis and the nucleus peripeduncularis; more sparsely to the VTA (Maeda and Mogenson 1981).

Diencephalic projections to the SN and VTA arise from the lateral habenula (Herkenham and Nauta 1979), subthalamic nucleus, anterior hypothalamus and medial and lateral preoptic areas (see also Simon et al. 1979). Afferents originating in several hypothalamic nuclei, follow a trajectory immediately dorsal to the SN (Nauta 1969; Nauta and Domesick 1978). Brain stem projections are found from the deep layers of the colliculus superior to the SN and serotonergic fibers from the nucleus raphe dorsalis (Dray et al. 1978); noradrenergic fibers from the locus coeruleus and cholinergic fibers from the nucleus tegmenti pedunculopontinus (Ch. II.3). Projections from parabrachial nuclei were demonstrated by Saper and Loewy (1980) and Spann and Grofova (1989). To a lesser extent, the nuclei raphe

magnus, raphe medius and raphe pontis connections with the VTA further characterize the hardly accessible interconnections of the monoaminergic systems (Pasquier et al.1977; Phillipson 1979c; Felten and Sladek 1983; Björklund and Lindvall 1984).

Mesostriatal projections

Current knowledge on the organization of the **mesostriatal** projection system in rats is summarized in Fig. II.5. (based on data by Fallon and Moore 1978b; Beckstead et al. 1979; Veening et al. 1980; Swanson 1982; Fallon and Loughlin 1985; Gerfen et al. 1987a). Mesostriatal projections arising from neurons in the SN, VTA and retrorubral area, of which 95 % is dopaminergic, are compartmentally organized in the striatum (Gerfen et al. 1987b): 1) the ventral part of the pars compacta projects to striosomes or patches. In this projection an oblique medial-to-lateral topography (Veening et al.1980) is found: medial projection neurons are located more rostrally, lateral projection neurons more caudally in the SN; 2) the dorsal tier of DAergic neurons in the pars compacta, continuous with DAergic neurons in the VTA project to more ventral parts of the dorsal striatal matrix and to the ventral striatum; 3) neurons of the pars reticulata project to the striatal matrix in a medial-to-lateral and an anterior-to-posterior topography. Many SNc neurons project throughout the whole rostrocaudal extent (Moore and Bloom 1978; Beckstead et al. 1979); 4) DAergic neurons from the retrorubral area (A8) project mainly to the dorsolateral part of the caudate-putamen. Thus, distinct dorsal and ventral sets of midbrain DAergic neurons project, respectively, to striatal matrix and patches and there is a nonDAergic mesostriatal projection to the matrix (Gerfen et al. 1987a). These patch-matrix mesostriatal DAergic systems are biochemically and developmentally distinct (Gerfen et al. 1987b). A calcium-binding protein (CaBP) is expressed in dorsal tier mesostriatal DAergic neurons, matching DAergic neurons directed to the striatal matrix. Dopaminergic neurons that do not express CaBP (in the ventral tier of the pars compacta as well as in the pars reticulata) are distributed in a pattern that matches the origin of the DAergic projections to the striatal patches. Nigrostriatal projections partly supply local collaterals to SNr, SNc and retrorubral area, whereas meso-accumbens projections likewise have a tendency to collateralize to other forebrain structures (Albanese and Minciacchi 1983; Fallon 1988). The highest DA content is found in the septal pole of the nucleus accumbens (Voorn et al. 1986; Groenewegen et al.1989) whereas

adjacent olfactory nuclei and islands of Calleja receive a dense plexus of DAergic terminals (Fallon et al. 1978, 1983)(Fig. II.7.).

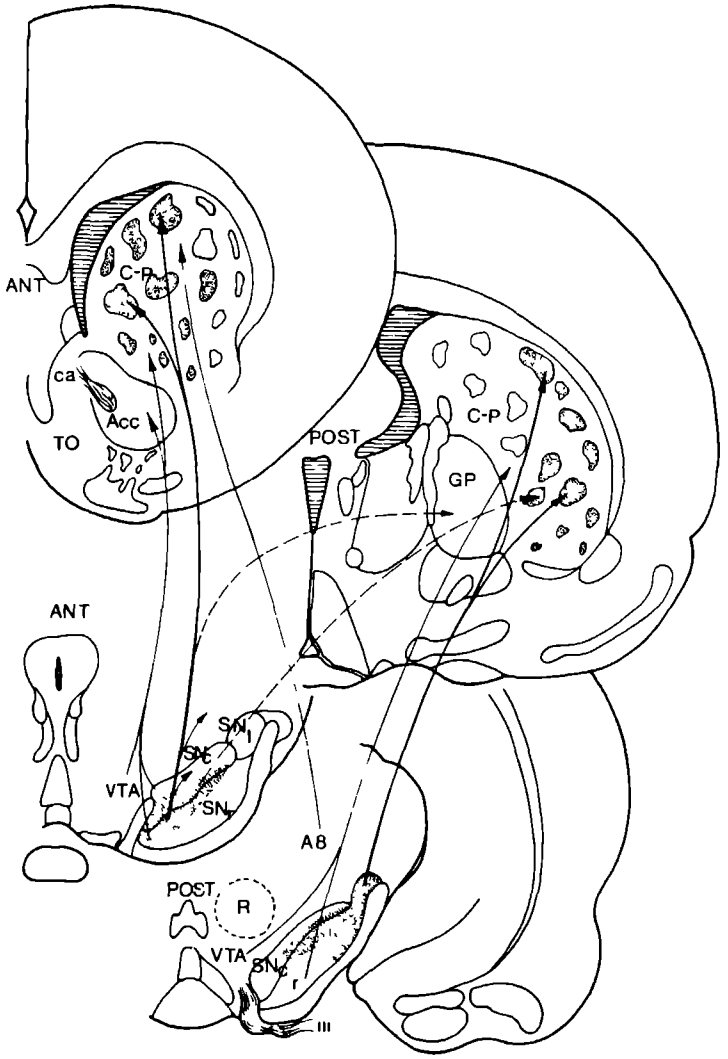


Fig II 5
Nigrostriatal projections and their topographical organization in rats

Mesolimbocortical projections

The nonDAergic component in the mesolimbocortical system is substantially larger, up to 72 % of the VTA projections to the lateral septum (Swanson 1982). The ascending projections from A10 cells are organized in a topographic fashion (Fig.II.6.), such that the neurons in the midline nuclei, nucleus linearis rostralis, nucleus linearis centralis/caudalis and nucleus interfascicularis preferentially project to the prefrontal cortex, lateral septum, lateral habenula and diagonal band of Broca, while neurons in the nuclei paranigralis and parabrachialis pigmentosis (and adjacent dorsomedial substantia nigra) project more heavily to the piriform cortex, entorhinal cortex, nucleus accumbens, olfactory tubercle and nuclei, and amygdala (Carter and Fibiger 1977; Moore and Bloom 1978; Fallon and Moore 1978b; Beckstead et al. 1979; Swanson 1982; Oades and Halliday 1987; Fallon 1988). More specific limbic projections are directed to the lateral septal nucleus (Deniau et al. 1980; Vertes 1988) and central and basolateral amygdaloid nuclei. Continuous with the DAergic projections to the amygdala are those to allocortical structures, including anterior entorhinal and piriform cortex, the former in a typical clustered pattern. Controversial hippocampal projections might stem from rostral (Segal and Landis 1974; Swanson 1982) and caudal (Simon et al. 1979) extensions of the VTA. Verney et al. (1985) described DAergic terminals ventrally in the deep layers of the subiculum.

More recently, adjuvant details on basal forebrain afferents of Broca's diagonal band/septal area (Vertes 1988) and substantia innominata, including bed nucleus of the stria terminalis (Grove 1988a) have been established. DAergic afferents of the nucleus basalis of Meynert have their origin mainly in the most lateral part of the SN (Martinez-Murillo et al. 1988).

A, partly DAergic, mesohabenular pathway (Phillipson and Griffith 1980; Simon 1981; Albanese and Minciacchi 1983) has been suggested to be an important link between limbic and extrapyramidal circuitry (Herkenham and Nauta 1977; Sutherland 1982). Minor projections to hypothalamic nuclei might be provided by branching fibers along the medial forebrain bundle (Kizer et al. 1976; Nauta and Domesick 1978; Simon et al. 1979; Lindvall and Björklund 1983). The main targets are the nuclei supraopticus and suprachiasmaticus, innervated by nonDAergic projections from the caudal VTA. DA in the nucleus subthalamicus probably stems from ascending collaterals of SNc axons that also branch to GP, on their way to the striatum (Fallon et al. 1978 ;Lindvall and Björklund 1983; Fuxe 1985).

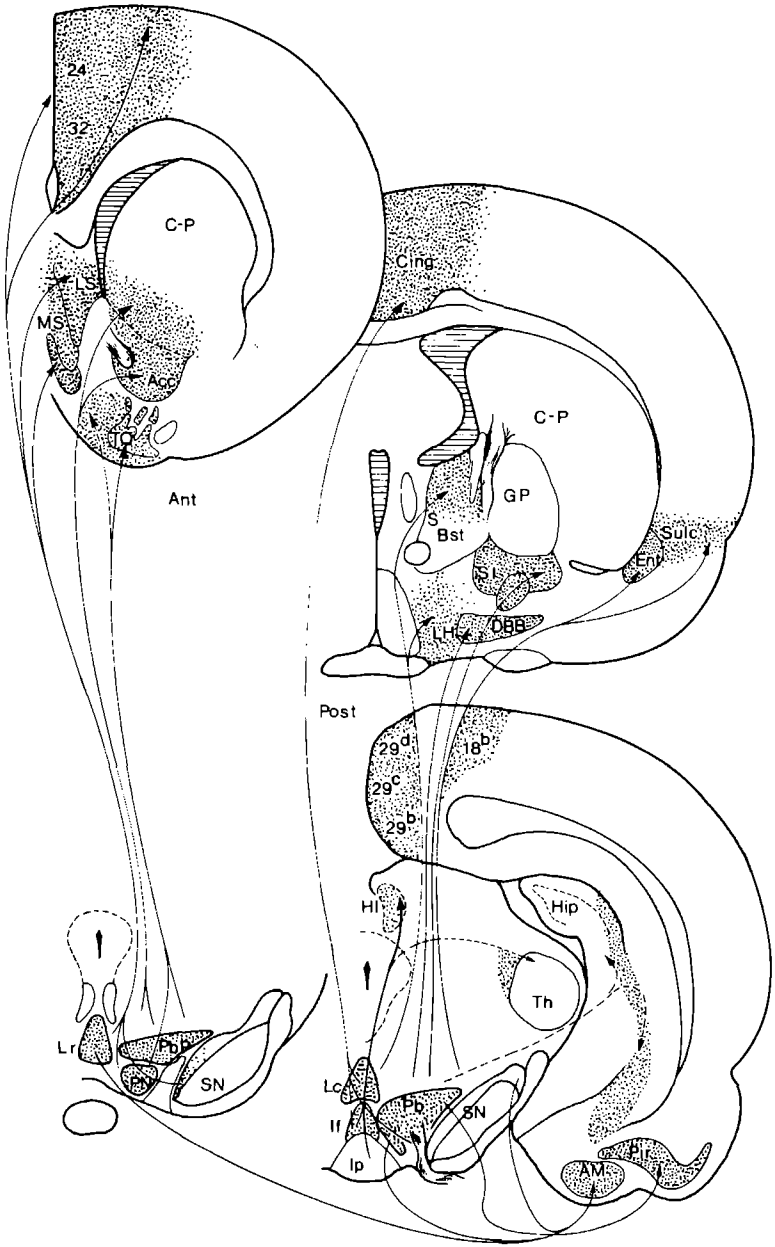


Fig. II.6

Topographical organization of ventral tegmental area efferents in rats

Mesolimbocortical projections

Neocortical projections from the mesencephalic DAergic cell continuum, first suggested by biochemical studies of Thierry et al. (1973), are now well established (for reviews see Björklund and Lindvall 1984; Oades and Halliday 1987). The DAergic innervation of the **frontal neocortex** comprises three different projection systems:

1) anteromedial,i.e. to **medial prefrontal** cortex (including Brodmann's area 32 in primates), and the basal layers of the supragenual part, predominantly arising in the medial part of A10 (Emson and Koob 1978; Lindvall et al. 1978; Beckstead et al. 1979). These projections have their origin in the VTA and the most medial SNc of which 30-40 % is DAergic (Swanson 1982).

2) suprarhinal; DAergic projections from the dorsolateral part of A10 to the deep cortical layers, dorsal to the rhinal sulcus.

3) the supragenual system, giving rise to a dense innervation of the superficial layers of the anterior cingulate cortex, arises primarely from the SN (Carter and Fibiger 1977; Lindvall et al. 1978; Emson and Koob 1978; Van Eden et al. 1987). The projection fields of DAergic afferents to the frontal neocortex coincide very closely with the areas of termination of the mediodorsal thalamic nuclear projections (Divac et al. 1978; Schmidt et al. 1982; Berger et al. 1985; Van Eden et al. 1987), usually defined as **prefrontal cortex** in various mammalian species (Divac et al. 1978). The cingulate part of the prefrontal cortex (comparable to Brodmann's area 24) is subdivided in a supragenual part (area 24a ventrally and area 24b dorsally) and a pregenual part (above area 32; Berger et al. 1985). At the level of the hippocampal commissure it adjoins the retrosplenial cortex (comparable to area 29). Projections to these areas have their origin in more laterally located cells in the SN/VTA at progressively more caudal levels (Sobel and Corbett 1984). The highest densities of cortical DAergic terminals are found in prelimbic and agranular insular areas (Van Eden et al. 1987), the lowest in the medial precentral area.

Additional projections, suggestive of a columnar organization, were demonstrated to sensorimotor area 29b,c,d and to the anterior part of area 18b i.e. secondary visual cortex (Berger et al. 1985). Terminals with a typical varicose aspect in this regions are located in superficial layers I-III and show a postnatal ingrowth later than those to some prefrontal areas (Verney et al. 1982; Kalsbeek et al.1988). Although Loughlin and Fallon (1985) suggested that the retrosplenial terminals originate from collaterals of those projecting to area 24, Berger et al. (1985) stress the different location of mesencephalic neurons projecting to prefrontal and other cortical areas.

Nigrothalamic, nigrotectal and nigrotegmental projections

The SNr gives rise to major nonDAergic projections to the thalamus, superior colliculus and mesencephalic tegmentum (Fig.II.7.). The nigrotectal, nigrothalamic and nigrostriatal cells in rats are segregated into complementary longitudinal regions of the SN and each of these populations of nigrofugal neurons have somata exhibiting a different size distribution (Faull and Mehler 1978). Nigrotectal and nigrothalamic projection neurons are generally slightly larger than DA neurons projecting to the striatum. The **nigrothalamic** projections arise from large (20-25 μm) multipolar cells in the rostrolateral and central parts of the SNr (Faull and Mehler 1978). Such nigrothalamic neurons first distribute local collaterals to both the pars reticulata and the pars compacta as well as to the midbrain reticular formation (Grofová et al. 1982; Parent 1986). Their GABAergic axons run dorsally and rostrally to reach the thalamus and topographically innervate the ventromedial nucleus. Other projections from the SNr and the VTA to the thalamus include the mediodorsal nucleus and intralaminar nuclei

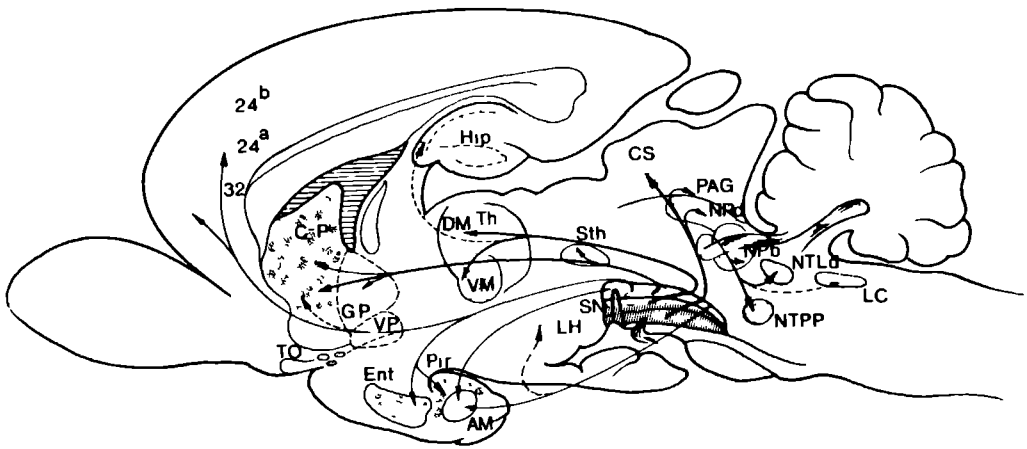


Fig. II 7

Non-striatal efferents of the rat substantia nigra

The **nigrotectal** projection arises from medium-sized cells in the ventromedial (rostrally) and ventrolateral (caudally) part of the SNr (Faull and Mehler 1978) to innervate deep layers of the superior colliculus on both sides of the brain (Wright and Arbuthnott 1980; Gerfen et al. 1982). Williams and Faull (1985) showed a more extensive distribution of nigrotectal neurons including more dorsal regions

of the rostral pars reticulata, known to contain large numbers of nigrothalamic neurons (see also Bentivoglio et al. 1979). The nigrotectal axons are thin and unmyelinated and form symmetric synapses in the superior colliculus with GABA as the inhibitory neurotransmitter (Chevalier et al. 1981; Araki et al. 1984). Of the numerous GABAergic neurons in the SNr at least some have axons which collateralize to innervate both thalamus and superior colliculus (Bentivoglio et al. 1979; Parent 1986).

Nigrotegmental projections arise throughout the SNr and the VTA and innervate the nucleus tegmenti pedunculopontinus, central grey, parabrachial nuclei, raphe nuclei, locus coeruleus and adjacent area of nucleus tegmentalis dorsalis (Simon et al. 1979; Wright and Arbuthnott 1980; Swanson 1982; Gerfen et al. 1982; Fuxe 1985). Locus coeruleus afferents might originate in the VTA and are difficult to separate from adjacent projections (Ch. II.3). The projection to the mesencephalic locomotor region (Saper and Loewy 1982; Rye et al. 1987) overlapping the nucleus tegmenti pedunculopontinus (Moon Edley and Graybiel 1983; Scarnati et al. 1989) is one possible route for the extrapyramidal motor system to influence reticulospinal neurons, innervating the spinal cord (for review Rye et al. 1987).

II.2.4. Fiber connections of the primate substantia nigra and ventral tegmental area

The more complex cellular distribution in the SN of higher mammals first of all reflects a clear separation of the striatum in nucleus caudatus and putamen, whereas these nuclei are not separated in rodents. Furthermore, patch and matrix components of the neostriatum might be reflected in nigro-striato-nigral connections differently in various species, like they are in ontogenesis (Jimenez-Castellanos and Graybiel 1987, 1989). This mosaic architecture of the striatum has been most thoroughly studied in the cat, where initially retrogradely labeled strionigral projection neurons appeared as geometrically complex fields interrupted by distinct zones of sparse labeling (Graybiel et al. 1979; Desban et al. 1989). The zones of poor labeling appear to correspond to the striosomes, striatal areas where AChE staining is characteristically weak in contrast to the AChE positive staining of the striatal matrix (Graybiel and Ragsdale 1979,1983). Such striatal zones, poor or rich in AChE activity have also been observed in the rhesus monkey and the human striatum (Graybiel and Ragsdale 1978; Smith and Parent 1986). AChE-poor zones can also be distinguished within the DAergic part of the primate SN, surrounded by an AChE-rich matrix (Jimenez-Castellanos and Graybiel 1987). Although horizontal zonations and mediolateral subdivisions of the pars compacta are readily demonstrable with AChE histochemical staining there was no clear overlap with tyrosine hydroxylase-immunoreactive neurons. Such nigral patches form only one of different mosaic-like patterns of which the typical striosomes form the most outstanding example (Malach and Graybiel 1986; Jimenez-Castellanos and Graybiel 1989). The combination of tracer and immunohistochemical studies suggests an extremely complex conceptual circuitry, hardly visualizable unless schematized.

As regards the general organizational principles of mesotelencephalic projections no major species differences could be derived from literature. Therefore many of the data presented for rats might be applicable to primates also. Supplementary details, based mainly on studies in different monkey species, will be given here followed by schematized representations for the human brain. The arrows in hodological presentations represent trends in topographical principles without claiming completeness.

Striatomesencephalic projections (Fig. II.8.)

Whereas the motor cortex mainly projects to the putamen, (Künzle 1975) associative areas of the prefrontal, temporal, parietal and anterior cingulate cortices project, in the form of patches, almost exclusively to the caudate nucleus in cats and monkeys (Divac et al. 1967; Goldman-Rakic 1983; DeLong et al. 1983a; Smith and Parent 1986; Malach and Graybiel 1986). This dichotomy is reflected in **striatomesencephalic projections**: putamenal neurons project massively to the globus pallidus and much less to the SN, whereas the reverse is true for the caudate nucleus (Parent et al. 1984). A third component of striatal efferents projects to both substantia nigra and globus pallidus (Féger and Crossman 1984). The caudatonigral fibers, arising mainly in the striatal matrix (see also Besson et al. 1988), form a highly complex terminal plexus covering the entire rostral two-thirds of the pars reticulata, preferentially in its medial subdivision (Smith and Parent 1986). The extrastriosomal matrix also has efferents to the globus pallidus and pars lateralis of the substantia nigra. The putamenonigral fibers occur as more discrete fascicles confined to the dorsolateral caudal SNr. As a whole the pattern of anterograde and retrograde labeling observed in the SN after caudate injections is largely complementary to that seen after putamen injections (Parent 1986). Strionigral projections to the pars compacta terminate on clusters that constitute nigrostriatal projection neurons, suggesting a reciprocal link at this level (Smith and Parent 1986; see also Jimenez-Castellanos and Graybiel 1989). But it is unclear how the nigro-striatonigral circuit is exactly closed at the nigral end, especially with the complexity of the pars compacta dendrites in mind (Nauta and Domesick 1979; Percheron et al. 1989; Haber and Groenewegen 1989).

Comparison of data obtained in several animals suggests a consistent topographical localization of striosomes, i.e.: more numerous rostrally than caudally in the nucleus caudatus, and preferentially located more medially than in the dorsolateral quadrant (Desban et al. 1989). Striatal cell populations that innervate the SNr in cats were mainly found in clusters of the lateral two-thirds of the caudate matrix, in a rostral-to-caudal topographic relationship, partly overlapping the dorsolateral somatosensory cortical target area as described by Malach and Graybiel (1986). The intermediate SNr receives projections that originate in the entire rostrocaudal extent of the caudate nucleus; the anterior part projecting to more ventrally located SNr neurons and the posterior caudate projecting to more dorsally located SNr neurons. It will be of particular interest to determine whether patches

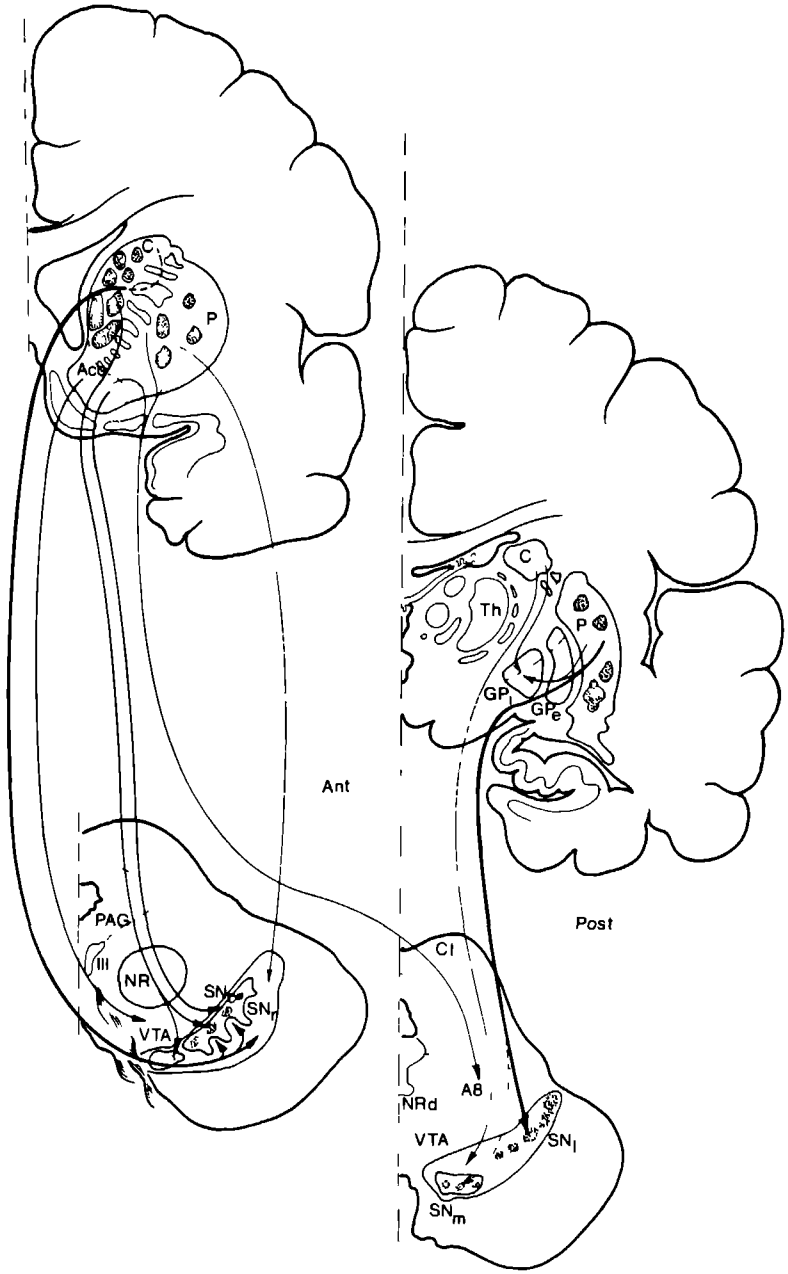


Fig II 8

Striatomesencephalic projections and their presumed topography in the human brain

labeled retrogradely from the SNr are in register or not with those labeled anterogradely by injections of tracers into the sensori and motor cortex (see also Somogyi et al. 1981a,b; Freund et al. 1984). Besson et al. (1988) suggested a relative separation, based on (striosomal) D1 and (matrix) D2 receptor containing striatal neurons projecting to medial SNc and SNr, respectively.

Data obtained from cats suggest some topographic ordening in efferents from the nucleus accumbens (Groenewegen and Russchen 1984). A medial and lateral subdivision of this ventral striatal nucleus project to VTA and SN, respectively. The retrorubral area and adjacent nucleus tegmenti pedunculopontinus are innervated by both parts of the nucleus accumbens (Groenewegen and Russchen 1984). Their findings correspond also with the inversed dorsal-to-ventral topographic relationship as described in rats (Nauta and Domesick 1979), suggestive for a limbic input via the ventral striatum and subsequently through the mesencephalic DAergic cell groups A8 and A9 to the dorsal striatum (Nauta 1978; Groenewegen and Russchen 1984).

Non-striatal afferents to the primate substantia nigra and ventral tegmental area (Fig.II.9)

The substantia nigra and adjacent tegmental pedunculopontine nucleus have traditionally been considered as final relays in descending projections of the extrapyramidal motor system, providing their input almost exclusively from the striatum, including globus pallidus, by way of the ansa lenticularis (Wilson 1914; Winkler 1929; Nauta and Mehler 1966; Nauta and Domesick 1979). A close connection of the substantia nigra with the midbrain reticular formation also was suggested for the human brain in the beginning of the century (Sano 1910, Winkler 1929). As has been demonstrated both for rats and cats, a substantial ascending input to the SN and VTA stems mainly from raphe nuclei, nucleus tegmenti pedunculopontinus and, less significant, from the locus coeruleus (see Ch.II.3 and II.4). Projections from the (cholinergic) nucleus tegmenti pedunculopontinus have been confirmed by Moon Edley and Graybiel (1983) in cats, whereas Parent and deBellefeuille (1983) noted retrograde labeling after nigral injections in monkeys, both in the (serotonergic) dorsal raphe nucleus and the tegmental pedunculopontine nucleus of primates. The latter projects to pars compacta neurons, whereas serotonergic terminals are mainly found in the pars reticulata (for review see Parent 1986). The VTA seems more specifically related to the other catecholaminergic

brainstem nuclei and the cerebellum (Oades and Halliday 1987), but interference with locus coeruleus projections to the cholinergic nucleus interpeduncularis is likely.

A substantial afferent projection to the primate SN is provided by the **nucleus subthalamicus** (Winkler 1929; Nauta and Cole 1978; Carpenter et al. 1981a,b; Parent and Smith 1987). The distribution of terminals favors the more ventral strata of the SNr, near the cerebral peduncle, avoiding regions with neuromelanin pigmented neurons (Nauta and Cole 1978; Parent et al. 1984). Similarities between the pars reticulata and the internal pallidal segment (see also Schwyn and Fox 1974; Marchand et al. 1979; Nauta 1979; Yelnik et al. 1987) are in accord with a projection of the subthalamic nucleus to both structures as well as comparable efferents of SNr and internal pallidal segment to thalamic nuclei (Parent and de Bellefeuille 1983). Whereas both globus pallidus and nucleus entopeduncularis in rats and cats project to the SN, such afferents in the primate SN exclusively stem from the external pallidal segment (Parent and deBellefeuille 1983; Percheron et al. 1984b). Furthermore, pallidonigral and subthalamonigral projections are more clearly separated in monkeys than in rats (Carpenter et al. 1981a).

It would be of interest to know more about possible diencephalic and basal forebrain projections to the VTA, as they were described in rats (Ch. II.2.3). Given the numerous retrogradely labeled neurons of large size in the substantia innominata of the monkey brain after large tracer injections in the SN (Parent and deBellefeuille 1983; see also Jones et al. 1976) a substantial afferent projection from basal forebrain structures to the "limbic midbrain area" (Nauta 1958) in primates has to be considered. This limbic midbrain area was defined in the cat as the region that has its rostral beginning in the VTA, extends caudalward along the base of the mesencephalon where it includes the interpeduncular nucleus and than arches dorsalward along the caudal aspect of the decussation of the brachium conjunctivum as the nucleus centralis superior, which in turn becomes continuous with the central grey caudal to the trochlear nucleus, including the dorsal tegmental nucleus of Gudden (Nauta 1958; see also Ch. II.3). Numerous fibers of the medial forebrain bundle, mamillo-tegmental tract and fasciculus retroflexus distribute to this area, providing at least indirect connections with limbic structures, particularly the septal-hippocampal system (see also Nieuwenhuys et al. 1982, 1988). There is evidence of lateral preoptic, lateral hypothalamic and habenular projections to the

cat VTA (Nauta 1958, Swanson 1982; Oades and Halliday 1987) although such feedback connections of the VTA might be of minor importance in higher mammals according to Oades and Halliday (1987). In his original description of these limbic-midbrain connections in cats, Nauta (1958) suggested already possible species differences primarily in the number of synaptic interruptions rather than the ultimate distribution pathways, which might explain the relative lack of such connections in tracing experiments.

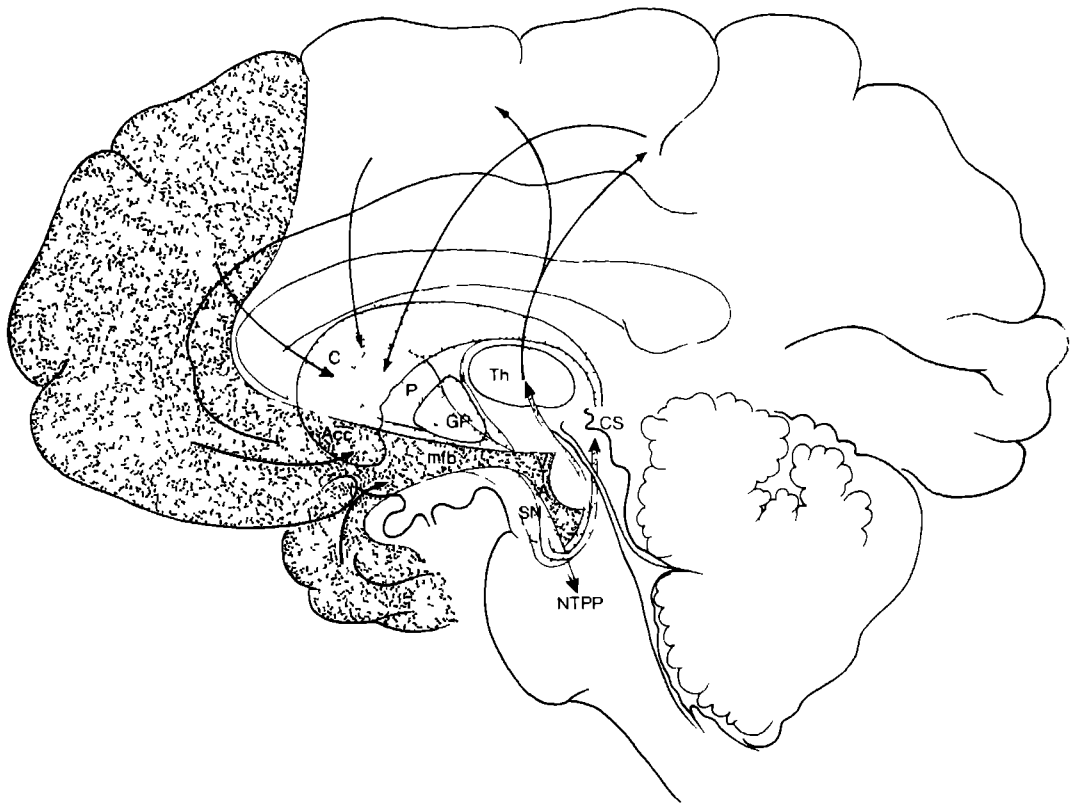


Fig II 9

Non-striatal afferents of the human substantia nigra and ventral tegmental area

Autoradiographic silver-grains where distributed throughout the neuromelanin-containing midbrain cell-continuum, with greatest density dorsolateral to the SNc, after central amygdaloid injection of anterograde tracer (Price and Amaral 1981). Labeling of the peripeduncular nucleus suggests a reciprocal connection with the amygdala as was also described for the extreme pole of the rat SN (Ch.II.2.3). Likewise, Hopkins and Holstege (1978) found retrogradely labeled cells both in the magnocellular basal nucleus and the amygdala of cats after injections in the dorsolateral nigral complex. Direct corticonigral projections generally have been considered to be of minor importance (Parent 1986). Apart from the observations in rats, corticonigral projections have been demonstrated in cats, based on structural changes after frontal lobe ablation (Usunoff et al. 1982), which remind of similar observations in the human brain (Winkler 1929). Projections from area 6 and 9 of the prefrontal cortex in monkeys (Macaca fascicularis) were suggested by Künzle (1978) but no afferents from similar cortical areas have been observed in the rhesus monkey (Selemon and Goldman-Rakic 1988). Indeed Künzle (1978) noted that labeling of the SN might originate from passing fibers to structures in the more caudally located reticular formation.

Mesostriatal projection in primates (Fig.II.10.)

Mesostriatal projections in primates, like those in rats, have their origin mainly in the DAergic pars compacta of the SN
and may be divided in dorsal-to-ventral and anterior-to-posterior subdivisions (Langer and Graybiel 1989). Although the ventral striatum, including nucleus accumbens and olfactory tubercle, is less well defined in primates (see Heimer and Wilson 1975; Alheid and Heimer 1988), the concept of its striatal character is partly based on its connectivity comprising the triad of cortical, thalamic and mesencephalic dopaminergic afferent projections (see also Heimer et al. 1982; Young et al.1984; Berendse et al. 1988). Different histochemical features are comparable to those of the dorsal striatum (Graybiel and Ragsdale 1983; Kwak et al. 1984; Haber and Watson 1985; Alheid and Heimer 1988; Groenewegen et al. 1989) and are in concordance with the pattern of DAergic terminals, although the latter proposition is mainly based on data in rats (Herkenham et al. 1984; Voorn et al. 1986; Gerfen et al. 1987a,b; Berendse et al. 1988; Groenewegen et al. 1989) and cats (Graybiel and Ragsdale 1979; 1983; Giguère et al.1984). The VTA and A8 projections to the

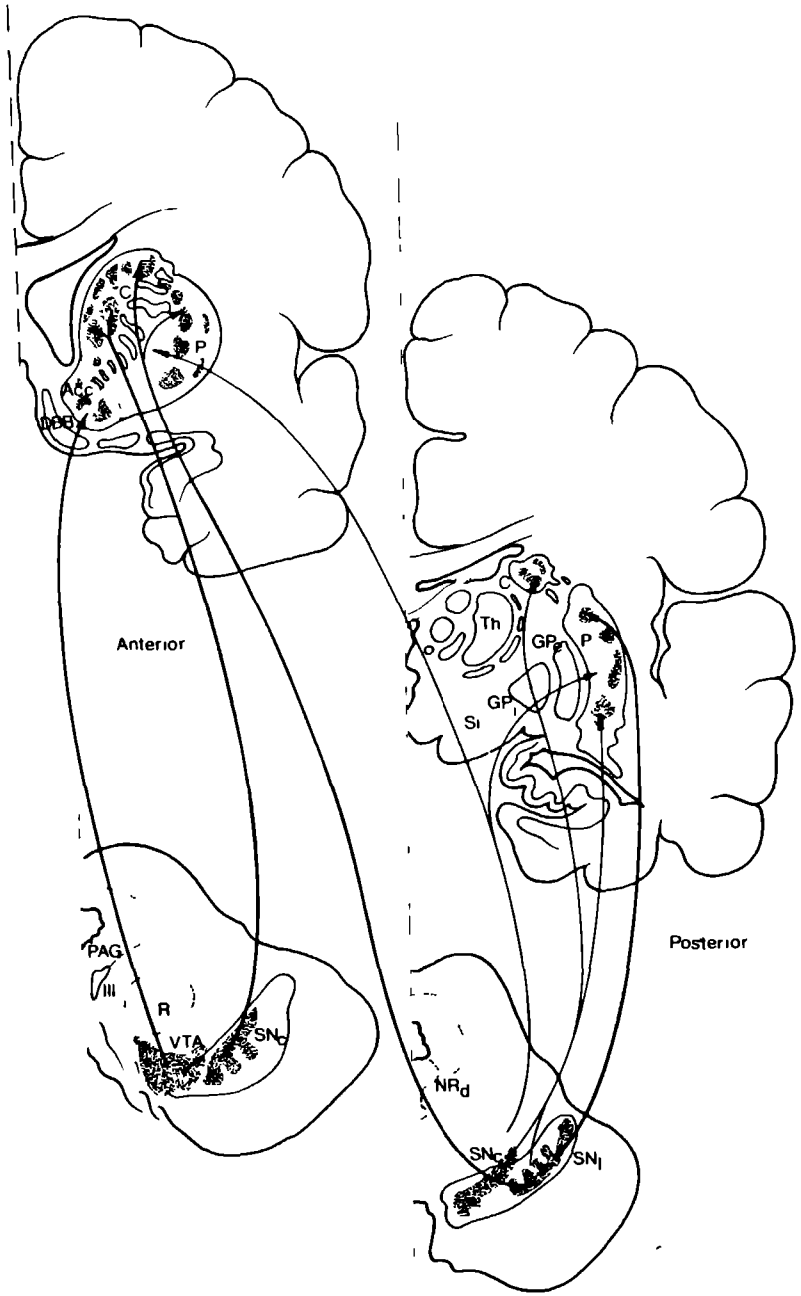


Fig II. 10

Mesostriatal projections and their presumed topography in the human brain

anterodorsal matrix compartment in primates, associated with D1 receptors (see also Ch. II.2.5.), are opposed to nigral projections of the main horizontal band of the pars compacta mainly directed to striosomes, associated with D2 receptors (Camps et al. 1989; Langer and Graybiel 1989) particularly in the rostromedial caudate nucleus (for review see Cools et al. 1989). Comparisons among D1 receptor binding site distribution, AChE staining and TH-immunoreactivity suggest similar compartmentalization principles for cats, monkeys and human brains (Besson et al. 1988; Camps et al.1989).

Generally the rostral parts of the SN pars compacta project to the striosomes of the caput nucleus caudati in a medial-to-lateral topographic pattern (Szabo 1979; Parent et al. 1983; François et al. 1984; Jimenez-Castellanos and Graybiel 1987). More caudally located nigral cells contribute to this rostral caudate innervation in its dorsolateral extension, as might be derived from studies in both monkeys and cats (Szabo 1980a,b; Beckstead 1987). These projections are directed to the striosomal system (Jimenez-Castellanos and Graybiel 1985, 1987). The latter part of the caudate nucleus corresponds with the area of substance P positive neuron clusters, described in cats (Beckstead 1987) and monkeys (Beach and McGeer 1984; Graybiel and Chesselet 1984), which also has been shown to be sensitive to D2 specific dopamine receptor ligands (Beckstead 1987; Camps et al. 1989). Axons from this caudal SN pars compacta also project densely to the caudal part of striatal patches in cats (Beckstead 1987).

The primate putamen is mainly projected on by posterior nigral regions, but clusters of caudate related nigral neurons are closely intermingled with putamen associated cells (Parent et al. 1983b). A dopaminergic projection from the internal part of the caudolateral SNc (the part most severely and consistently affected in PD), to the putamen was suggested by the preferential loss of catecholamines from the putamen in PD (Waters et al. 1988). Sometimes also pars reticulata neurons participate in this nigrostriatal projection (François et al. 1984). Ventral parts of the dorsal striatum are projected on by medially located pars compacta neurons, continuous with those of the VTA. At more caudal levels this medial part of the SN also reaches the medioventral putamen and most caudal extension of the caudate nucleus (Szabo 1980a,b; Parent et al. 1983a; François et al. 1984; Besson et al. 1988). A striking inconsistency concerning striosomal directed DAergic neurons is the fact that, generally TH-staining in the striatum is most developed in the matrix compartment (Beckstead 1987; Graybiel et al.1987; Besson et al. 1988). In

summary, these data agree with the medial-to-lateral and inversed dorsal-to-ventral topography described in rats (Ch.II.2.3.).

The patches of high density nigral terminations within the caudate nucleus interdigitate with those of thalamic afferents in cats (Beckstead 1985). Nigrostriatal projection neurons also have a complex relationship to AChE-poor zones within the SN that extend in a finger-like pattern into an AChE-rich matrix (Jimenez-Castellanos and Graybiel 1989). This latter nigral mosaic-like pattern might be related to AChE staining of different nigral afferents, added to the overall AChE immunoreactivity of nigrostriatal projection neurons. There was no overlap with the differential distribution of projections to either the caudate nucleus or putamen, like those described by Parent et al. (1983b). Thus the significance of this nigral compartmentalization and its relation to striatal inhomogenities has to be elucidated yet (Jimenez-Castellanos and Graybiel 1987, 1989).

Mesolimbocortical projections and substantia nigra efferents (Fig.II.11.)

Apart from the mesostriatal projection a significant part of DAergic axons, directed to various basal forebrain, cortical, diencephalic and brainstem structures has been demonstrated also in primates. Fig. II.11. presents an account of these projections. Like in rodents (see Swanson 1982) in primates a substantial part of the non-striatal efferents of the mesencephalic DAergic cell continuum might be nonDAergic (Björklund and Lindvall 1984; Parent 1986; Saper 1987a; Lewis et al. 1988b). It is hard to say if these projections have their origin either in the SNr or the VTA. One possibility in distinguishing these subsets is to consider the pattern of collateralization, being a typical feature of pars reticulata axons (Parent 1986) and only exceptional for VTA projections, at least in rats (Deniau et al. 1980; Swanson 1982; Sobel and Corbett 1984).

The number of nigrothalamic projection neurons, intermingling with nigrotectal neurons in primates, decreases from rostral to caudal within the pars reticulata of the SN. The reverse is true for the less abundant nigrotegmental projection neurons (for review see Parent 1986). Differences in number, intranigral distribution and somatodendritic size and shape of these projection neurons in monkeys have been suggested (Beckstead and Frankfurter 1982; Beckstead 1983; Parent et al.1983b; François et al. 1984). Projections to the medial magnocellular part of the nucleus ventralis anterior and the mediodorsal thalamic nucleus have

their origin more medially in the SN, whereas lateral parts project on remaining parts of the magnocellular portion of the ventral anterior nucleus and paralaminar, parvicellular and densocellular parts of the mediodorsal thalamic nucleus (Ilinsky et al. 1985). A topographical organization is maintained throughout the nigrothalamocortical system in primates, in such a way that lateral parts of the SN are indirectly related to more posterior regions of the frontal lobe, and the medial part of the nigrothalamocortical system is more selectively related to anterior regions of the frontal lobe (Ilinsky et al. 1985). Thus the nigral input to the various subdivisions of the mediodorsal nucleus may be organized in a manner similar to the distribution pattern of different cortical afferent systems in the striatum (Goldman-Rakic and Brown 1981; Goldman-Rakic 1983).

The source of nigrotectal projections to the intermediate gray layer of the monkey colliculus superior is a heterogenous population of pars reticulata neurons (May and Hall 1986). No distinct longitudinal zones of nigrotectal and nigrothalamic projection neurons, as have been described in rats, are found in monkeys. Retrograde tracer studies in primates (Beckstead and Frankfurter 1982; Parent et al. 1983b; François et al. 1984; May and Hall 1986) have demonstrated that nigrotectal cells generally tend to occupy a somewhat more ventrolateral position as compared to nigrothalamic and nigrotegmental projection cells. Besides, these presumed GABAergic, neurons are believed to represent a morphological distinct subpopulation (Beckstead and Frankfurter 1982; François et al. 1984; May and Hall 1986), particularly as regards the pars lateralis substantiae nigrae (see also Winkler 1929), with its typical, multi-dendritic large neurons (François et al. 1984,1987; May and Hall 1986; Yelnik et al.1987). Species differences, between monkey and rat regarding the arrangement of dendrites within the pars reticulata have been noted. The almost exclusively projection of the pars lateralis to the colliculus superior, might have caused a more restricted definition of topographic patterns of SNr neurons in rodents and cats (Faull and Mehler 1978; Beckstead et al. 1979,1982). A projection to the amygdala from this pars lateralis in cats (Meibach and Katzman 1981) might as well originate from the peripeduncular nucleus, which has been noted to receive afferents from the amygdaloid complex in the monkey (Jones et al. 1976; Price and Amaral 1981). The demarcation problem of the pars lateralis in the human brain is dealt with in Ch. II.2.7.

Although a direct inhibitory (GABAergic) SNr influence on neurons of the nucleus tegmenti pedunculopontinus has been disputed in rats (Rye et al. 1987),

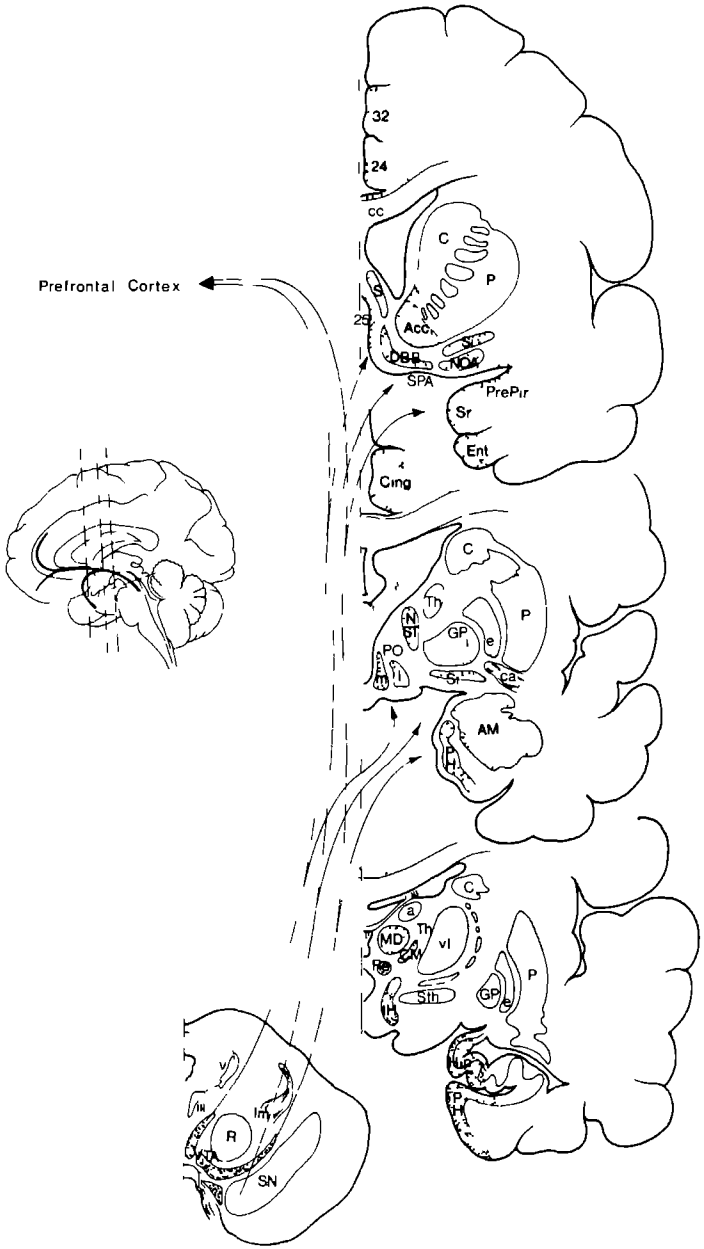


Fig II 11

Mesolimbocortical projections and their presumed topography in the human brain

there is much evidence for such a projection, at least as regards terminals within the traditionally defined boundaries of the tegmental pedunculopontine nucleus in different species (for review see Parent 1986). More caudal regions of the brainstem reticular formation also receive some input from the pars reticulata, but projections to the locus coeruleus, parabrachial regions, nucleus raphe dorsalis, cerebellum and spinal cord arise preferentially from the VTA and A8 (Oades and Halliday 1987).

There is a growing evidence of mesencephalic dopaminergic basal forebrain projections, particularly from VTA neurons, in the primates, similar to that in non-primates, but many details are lacking yet (Nobin and Björklund 1973; Russchen et al. 1985; Gaspar et al. 1985; Oades and Halliday 1987). The main terminal area of these projections is the nucleus accumbens, the only basal forebrain area containing high concentrations of all three monoamines (Gaspar et al. 1985; Garcia-Rill 1986). The "striatal" character of this projection might be reflected by its relatively high collateralization, providing branches also to lateral septum and prefrontal cortex (Oades et al. 1986). The patchy terminals in the ventral striatum are continuous with those to the bed nucleus of the stria terminalis. Likewise, they accompany the pathway through the stria terminalis (see Nobin and Björklund 1973;Felten and Sladek 1983) and many terminals in the substantia perforata anterior (Gaspar et al. 1985). Tyrosine hydroxylase immunoreactivity in the human olfactory tubercle, continuous with the islands of Calleja, nucleus olfactorius anterior and parolfactory cortex (area 25), might be more extensive in the human brain than in rodents (Gaspar et al. 1985). Part of this tyrosine hydroxylase immunoreactivity might stem, however, from a supplementary DAergic cell cluster described by Köhler et al.(1983), containing particularly local projection neurons. The typical terminal distribution pattern of granular cells, surrounded by a network of DAergic terminals (Björklund and Lindvall 1984; Gaspar et al. 1985) is similar in both rodents and primates.

Projections to the lateral septal nucleus, continuous with those of the bed nucleus of the stria terminalis, likewise might be more extensive than those described in rats (Gaspar et al. 1985). They most likely stem from midline VTA nuclei, as described in rats, but could also be collaterals of mesothalamic or mesohabenular projections (see Fuxe 1985). More diffusely spread afferents to the substantia innominata, extending in the area of the ventral pallidum and magnocellular nucleus of Meynert, have been found by Russchen et al.(1985). The functional implications of these rather ill-defined basal forebrain projections in the human

brain, including also the presumed septofimbrial nucleus (Gaspar et al. 1985), medial preoptic area,i.e. horizontal limb of Broca's diagonal band, and amygdaloid complex, have recently been reviewed by Alheid and Heimer (1988). Like in the prefrontal cortex, dopamine turnover might be particularly high here (Bannon and Roth 1983), implicating a possible underestimation of DA-like immunoreactivity in post- mortem analysis.

Fig II 12

Mesolimbocortical projections in the primate brain: inversed dorsal - to - ventral topography
Sagittal view

Mesocortical projections of the human substantia nigra were suggested already by Winkler (1929), based on observations in neuropathology in which loss of temporal and parietofrontal cortical grisea led to degeneration of specific parts of the SN complex. Degeneration of the temporal lobe led to loss of a 'pars

lateralis', whereas medial parts of the SN were lost following parieto-frontal cortical destruction.

Recent studies in cats (Scheibner and Törk 1987) and monkeys (Levitt et al. 1984; Lewis et al. 1987,1988b) as well as the human brain (Gaspar et al. 1989) demonstrated a dopaminergic input to most, if not all, cortical areas. The elaboration and specialization of the primate neocortex, as compared to that of rodents, led to a significant expansion and differentiation of monoaminergic projections, greatly exaggerating those of the medial frontal, the cingulate, insular and temporal cortical areas found in rats. Different levels of evidence, as regards dopaminergic terminals in the primate cortex, however, depend on the kind of characterization of dopamine-like elements, like the radioenzymatic determination of DA and DA/NA ratios (Björklund et al. 1978), the appearance of TH-immunoreactivity (Levitt et al. 1984; Lewis et al. 1987, 1988b) or the less ambiguously radiotopographic demonstration of various dopaminergic neuronal processes (Berger et al. 1988). Several lines of evidence indicated, however, that a highly specific anti-TH antiserum can select dopaminergic structures as well (Lewis et al. 1987). Thus, TH immunoreactivity was complementary to NA-specific dopamine-ß-hydroxylase immunoreactivity, rather than overlapping with this marker, whereas lesions of ascending NAergic fibers in the brainstem did not alter TH immunoreactivity significantly.

In contrast to the distinct TH-labeling of locus coeruleus neurons, cortical NA processes seem to have TH concentrations or TH characteristics that make them hardly recognizable in such TH immunoreactive preparations (Lewis et al. 1987; Gaspar et al. 1989).

In contrast to the rat (Björklund and Lindvall 1984; Berger et al. 1985; Descarries et al. 1987) much information is still lacking in monkeys regarding the exact mesencephalic origin and collateralization patterns of DAergic mesocortical systems. Retrograde transport studies combined with CA histofluorescence in monkeys and cats, demonstrated that the mesocortical projection arises primarily from DAergic and nonDAergic (see also Swanson 1982) neurons in the VTA and medial pars compacta (Markowitsch and Irle 1981; Porrino and Goldman-Rakic 1982; Scheibner and Törk 1987; Lewis et al. 1988a). Those of the medial SNc actually might stem from collaterals to other forebrain structures, such as striatum or septum, as was demonstrated in rats (Fallon et al. 1978b).

Following Berger et al.'s (1988) suggestion cortical DAergic innervation in

primates might be subdivided in those to granular and those to agranular cortical areas. Those granular cortical regions, in which layer IV is most prominent (primary sensory areas), had the lowest density of labeled fibers, whereas **agranular** cortical regions, such as **anterior cingulate (area 24),insula and motor cortices (area 4,6, including supplementary motor area)**, were most densely innervated, both in monkeys and humans (Lewis et al. 1987; 1988b; Berger et al. 1988; Gaspar et al. 1989). The approximate twofold denser innervation in the agranular cortices as compared with the granular **prefrontal, parietal and posterior cingulate**, appears to be at variance with previous biochemical data (Björklund et al. 1978; Levitt et al. 1984). There is general agreement as regards cortical zones that receive very few DAergic projections, including area 1,2,3, and the peristriate area 18, primary visual and auditory cortices. The distribution to such areas is limited to lamina I (Lewis et al. 1987; Berger et al. 1988). Generally, the distribution pattern coincides with cytoarchitectonic and functional boundaries. A rostrocaudal gradient might characterize the distribution of fiber density in the prefrontal cortex, increasing from area 10, 9, 8b and 6 to 4 (Lewis et al. 1987). In medial to lateral direction, over area 25 to area 24, and orbitofrontally from 10 over 11-13 to area 12 a similar gradient was noted (Levitt et al. 1984; Lewis et al. 1988b). Within the parietal lobe, again, there is roughly an increase in TH- immunoreactivity notable from the somatosensory areas to area 5 and area 7, respectively, complementary to the dopamine-ß-hydroxylase decrease over this trajectory (Morrison et al. 1982b; Lewis et al. 1987).

Although the densest DAergic innervation is observed in the motor cortex of primates, the projection-systems to the prefrontal cortical areas were most extensively studied (Goldman-Rakic and Brown 1981; Porrino and Goldman-Rakic 1982; Levitt et al. 1984). Unlike the correspondence of this area with the target area of the mediodorsal thalamic nucleus in rodents (Divac et al. 1978), such a definition would be too narrow in primates (Goldman-Rakic and Porrino 1985). Actually, DAergic projections to the prefrontal region in primates seem to parallel more closely afferents from the ventral anterior thalamic nucleus than those from the mediodorsal nucleus (Lewis et al. 1988b). Like in primates, TH-immunoreactivity in the human prefrontal cortex is generally lower than that in other cortical areas, but minor differences, like the TH optimum in area 9 of monkeys, which seems only moderately innervated in humans (Lewis et al. 1988b; Gaspar et al. 1989) might be attributable to methodological variation. The lowest levels of

TH- immunoreactivity in the prefrontal cortex were observed in areas 10 and 46, the latter including the fundus of the (monkey) sulcus principalis, similar to the pattern as regards NAergic fibers. In comparison to DAergic processes, NAergic fibers have a substantially lower overall density, exhibit less marked regional variation and have a complementary laminar pattern of distribution (Lewis et al. 1988b; Parnavelas and Papadopoulos 1989). Serotonin projections differ substantially from DAergic projections on both regional and laminar base (Lewis et al. 1987; Berger et al. 1988).

Throughout the neocortex layer IV has the lowest density of DA-like immunoreactivity. The most densely innervated areas display fibers in all laminae, whereas the moderately innervated granular cortices have a bilaminar pattern, avoiding the granular layer IV (Lewis et al. 1988b; Berger et al. 1988; Gaspar et al. 1989). In frontal and cingulate cortices most of the DAergic fibers were present in layers II and superficial III (Levitt et al. 1984) but also coursed through deep layers V and VI, thus converging with amygdaloid projections (Lewis et al;. 1988b). In very sparsely innervated regions, DAergic fibers are limited to layer I. Differences in the amount of TH immunoreactivity of layers II-IV may reflect species differences (Gaspar et al. 1989). A functional specialization is suggestive than, such that fibers preferentially innervate motor relative to sensory regions and sensory association relative to primary sensory areas (for review see Foote and Morrison 1987).

Retrograde labeling studies of the parietal (area 7,5) and primary sensory cortex (Lewis et al. 1988a) and various prefrontal cortical areas (Porrino and Goldman-Rakic 1982) revealed the origin of DAergic projections to be the mesencephalic VTA, A8 and medial SNc. Such preliminary data could not establish a clear topographical organization of the mesocortical projection system. Such a topographical organization is suggested, however, by detailed studies in cats (Markowitsch and Irle 1981; Scheibner and Törk 1987). VTA nuclei contribute differentially to various cortical projections, the nucleus paranigralis being a remarkable exception. The latter might be more specifically involved in mesolimbic projections to the ventral striatum (Szabo 1979; Scheibner and Törk 1987). Projections to the entorhinal cortex and hippocampus mainly stem from the nuclei interfascicularis, linearis centralis and parabrachialis pigmentosis. The nucleus linearis rostralis, particularly well identifiable in cats (see also Halliday and Törk 1986), has the best developed cortical projections, especially to dorsolateral and

cortical areas. The nucleus interfascicularis might be related to the lateral habenula (Scheibner and Törk 1987), although a "contamination" with the projection of the nucleus interpeduncularis is likely.

Similarly as in rats, the most densely innervated cortical area in cats is the prefrontal cortex, converging with projections of the mediodorsal thalamic nucleus (Markowitsch and Irle 1981). This might explain the more widespread retrograde labeling observed by Porrino and Goldman-Rakic (1982) in monkeys, including the paranigral nucleus and medial SN. The contribution of SN cells to the mesocortical projection, thus particularly is directed to prefrontal and anterior cingulate cortices, as might be derived from various animal experiments (Carter and Fibiger 1977; Björklund et al. 1978; Porrino and Goldman-Rakic 1982; Bannon and Roth 1983). Apart from the well established nigral pars lateralis projections to the amygdala, dopaminergic projections to the primate temporal lobe (Björklund et al. 1978) are controversial yet, especially as regards a hippocampal innervation.

II.2.5. Chemoarchitecture of the substantia nigra and ventral tegmental area

The distribution pattern of dopamine in the ventral mesencephalon was first studied in the rat (Baertler and Rosengren 1959) as was its relation to nigro-neostriatal projections (Andén et al. 1964, 1966a,b). Stepwise the knowledge on dopaminergic projections evolved and ten years later Berger (1977) examined the catecholaminergic innervation pattern in adult human cortex. This search was stimulated by the current hypothesis concerning the role of dopaminergic mechanisms in complex behaviour patterns in the rat as well as in certain human psychiatric disorders such as schizophrenia and some forms of dementia (Kety 1959; Berger 1977) and by the exciting demonstration of the close correlation of nigrostriatal dopaminergic dysfunction with Parkinsons's disease (Bernheimer et al. 1973). Nobin and Björklund's (1973) histofluorescence findings on the topography of monoamine neuron systems, including mesencephalic catecholaminergic neurons in 3-4 month old fetuses were for a long time the only reliable data available on the DAergic mesencephalic cell continuum in the human brain. Usually central monoamine neurons were identified at the light microscopic level with the formaldehyde fluorescence technique of Falck and Hillarp (Carlsson et al. 1962; Falck et al.1962) by which the putative monoamine transmitters themselves, i.e. dopamine, noradrenaline and 5-hydroxytryptamine, were directly visualized in the microscope. Immunohistochemistry utilizing antibodies to tyrosine hydroxylase (TH) in conjuction with the peroxidase-antiperoxidase technique can now be successfully applied to identify catecholaminergic neurons, not only in frozen sections (Gaspar et al.1983; Waters et al. 1988) but also in formalin fixed, paraffin embedded human adult postmortem brain (Pearson et al. 1979, 1983a). The same holds true for peptides, as will be reviewed briefly here.

Even dopamine receptors can now be reliably studied in the human brain (Camps et al. 1989; Cortés et al. 1989). The two types of dopamine receptors D1 and D2 (the main binding site for neuroleptics) mediate different responses to dopaminergic action in the striatum (Seeman and Grigoriadis 1980; Niznik 1987; Cools et al. 1989). The patchy distribution of receptor binding both in cats and human striatum, is reminiscent of the uneven terminal pattern of the nigrostriatal projections (Besson et al. 1988; Camps et al. 1989; Langer and Graybiel 1989). Nevertheless, the vast majority of both D1 and D2 receptors, most thoroughly

studied in cats (see Beckstead 1988), was located on intrinsic striatal neurons. Indeed, in Parkinson's disease as well after experimental selective destruction of DAergic neurons, the densities of both types of receptors were unchanged (Pierot et al. 1988; Beckstead 1988), which might explain the response to dopamine (agonist) therapy. Within the SN D2 receptors are primarily located on DAergic neurons of the lateral pars compacta and their dendrites, whereas D1 receptors, predominant in the medial pars reticulata, might be associated with striatonigral neurons (Beckstead 1988; Camps et al. 1989). Relatively low levels of both receptor subtypes were present in the VTA, unlike the situation in rats (Dubois et al. 1986). The ventral striatum, particularly the islands of Calleja, present another important binding site for dopamine (Camps et al. 1989). The rostro-dorsal part of the caudate nucleus, in which the matrix compartment and D1 receptors are relatively abundant, corresponds to a substance P-rich area both in cats and primates (see Beckstead 1987, 1988) which has been shown to be responsible for a dense substance P innervation of the internal part of the globus pallidus and SN (see also Fig.II.13.). Met-enkephalin cell bodies have a minor predominance in central and ventral portions of the caput nucleus caudates (Beckstead and Kersey 1985), a region that is characterized by the highest density of striosomes (Graybiel and Ragsdale 1983) and somewhat more D2 receptors (Camus et al. 1986; Cools et al. 1989). This region projects to the ventral pallidum and ventral parts of the SN pars reticulata (Beckstead and Cruz 1986). **Dopamine** may exert its influence at various sites with different functional consequences. Dopamine is also released within the SN from dendrites, influencing neighbouring DAergic cells as well as GABAergic nigrothalamic and nigrotectal neurons, by means of dendrodendritic and dendro-axonic contacts (Ruffieux and Schultz 1980; Wassef et al. 1981). In contrast to dendritic DA of the SN, vesicular DA represents the functional part in the nigrostriatal axons. DA autoreceptors within the SN are of the D2-type, i.e. those not linked to adenylate cyclase or adenylate cyclase inhibiting (Morelli et al. 1988). As yet there is no general consensus even as to wether DA is excitatory or inhibitory active in the human striatum, nor do we fully understand the mechanisms through which L-DOPA produces benefit in patients afflicted with Parkinson's disease (Graybiel and Ragsdale 1983; Hornykiewicz and Kish 1987).

γ-Aminobutyric acid (GABA), mostly indicated by its metabolic enzyme immunoreactive glutamic acid decarboxylase (GAD), is the neurotransmittor of the pars reticulata projection neurons (DiChiara et al. 1979; Chevalier et al. 1981;

Araki et al. 1984) and recently specifically has been demonstrated to be involved in the nigrotectal projection in cats (Ficalora and Mize 1989). GAD immunoreactive cells in the monkey SN are particularly abundant in the dorsolateral part of the pars reticulata, where they lie within a dense neuropil of GABA-containing striatal axon terminals (Parent 1986). GABAergic neurons from the nucleus accumbens project to the rostromedial part of the SN and possibly also the VTA (Walaas and Fonnum 1980).

The appearance of **substance P** in the human SN has been thoroughly studied (Emson et al. 1980; Cuello et al. 1981; Del Fiacco et al. 1984; Beach and McGeer 1984; Mai et al. 1986). The SNr contains the highest concentration of GABA and substance P in the brain, which attests to the density of these parallel striatonigral pathways. A comparative study of Inagaki and Parent (1984) showed differences in the distribution pattern of substance P-like immunoreactivity, which might reflect the increasing complexity of the striatonigral terminal fields in primates relative to non-primates. Because substance P antigenicity is preserved in routinely fixed, paraffine embedded archival materials, Mai et al. (1986) succeeded in tracing substance P **pathways** also. It has become clear that the striopallidal and strionigral tracts, carrying the majority of substance P fibers, give rise to a dense plexus of "woolly fibers" throughout the pars reticulata (Beach and McGeer 1984), confirming the "pallidal" character of this nigral part. The cells of origin are preferentially located anterolaterally in the striatum (Cuello et al. 1981; Glowinski et al. 1982), exerting a tonic facilitatory influence on DAergic neurons. In the human substantia nigra substance P-like material within the pars compacta appears to be arranged in strands, often parallel to blood vessels, most apparently in the subnucleus anteromedialis and posterolateralis (Mai et al. 1986). In the dorsal tier both substance P-like immunoreactivity and enkephalin-positivity are conspiciously lacking (Haber and Groenewegen 1989).

Like substance P, the distribution pattern of enkephalin-like immunoreactivity in the human substantia nigra complex is well established now (Emson et al. 1980; Cuello et al. 1981; Gaspar et al. 1983; Bouras et al. 1984; Haber and Groenewegen 1989). Both these peptides ,most likely are associated with striatonigral projections (Cuello et al. 1981; Sandyk 1985). Fibres with enkephalin-like immunoreactive varicosities were observed in many areas of the human mesencephalon (Bouras et al. 1984) and sometimes in the direct vicinity of neuromelanin pigmented cells. In the pars reticulata the terminal pattern resembles that of

substance P, i.e. boutons all over perikarya and along dendrites; but the distribution pattern is less uniformely (Gaspar et al. 1983), with a maximal density of enkephalin-like immunoreactivity in the medial part (Haber and Groenewegen 1989), covering also the area of the nucleus paranigralis. There appears to be an increasing overlap between peptidergic afferents and TH-positive elements along the evolutionary lines (Inagaki and Parent 1984; Haber and Elde 1982; Waters et al. 1988), but TH-positive clusters, although keeping with peptidergic boundaries, do not overlap unequivocally (Haber and Groenewegen 1989).

A third strionigral pathway, supplying the pars reticulata with **dynorphin** has been described in rats (Fallon and Loughlin 1985) and cats (Chesselet and Graybiel 1983), suggesting again an evolutionary trend. The differential distributions of enkephalin and dynorphin in the SN of cats might underline the identity of a separate pars lateralis, corresponding with low dynorphin-like immunoreactivity in the most rostro-lateral part of the SN (Chesselet and Graybiel 1983). In humans the substance P, met-enkephalin and dynorphin fibers largely avoided the region of the caudolateral pigmented cells but also the nucleus paranigralis (Waters et al. 1988).

Neurons containing both DA and **cholecystokinin (CCK)** have been associated primarily with VTA efferents of the midline structures and the rostro-lateral part of the rat SN (Hökfelt et al. 1980). These areas are known to contain projection neurons to the caudal-medial nucleus accumbens and central amygdaloid nucleus, respectively. Other basal forebrain structures that received such combined CCK-DA input were the bed nucleus of the stria terminalis, the lateral septal nucleus, olfactory tubercle, ventral thalamus, diagonal band of Broca and basal lateral hypothalamus. Thus CCK seems more specifically related to mesolimbic projections, whereas neostriatal CCK might originate mainly from descending cortical projections (Zaborski et al. 1985). Seroogy et al. (1988) described a population of TH-immunoreactive neurons that contained both CCK and **neurotensin.** Neurotensin might be found in VTA DAergic neurons, but more often in fibers, like those ascending from locus coeruleus, nucleus raphe dorsalis and parabrachial nuclei (Kalivas 1985). The same counts for **somatostatin**-immunoreactivity detected in these mesencephalic areas (Beal and Martin 1984). The facilitatory effect of neurotensin on DAergic neurons is opposite to the effect of DA on its autoreceptors (Kalivas 1985).

Serotonergic perikaryae have been described in the VTA nuclei surrounding

the interpeduncular nucleus, oriented perpendicular to the midline and cytologically different from those of the B9 raphe group (Steinbusch 1981). Serotonin immunoreactive fibers and terminals are abundant in the rostral part of the SNr and the pars lateralis of rats. Likewise both choline-acetyltransferase-positive (cholinergic) cells and fibers are present, the former probably ectopically located neurons of the pontomesencephalic tegmental cholinergic system (Gould and Butcher 1986; Henderson et al. 1987). These projections particularly provide the medial third of the SN with a cholinergic input Beninato et al. 1988). Acetylcholinesterase in the SN of rats (Lehmann and Fibiger 1978) and cats (Jimenez-Castellanos and Graybiel 1985) is a less specific marker, the exact meaning of which has to be elucidated yet. Noradrenaline-like (dopamine-ß-hydroxylase) immunoreactivity is restricted to fibers passing the VTA on their way to the medial forebrain bundle (Fallon and Loughlin 1985).

II.2.6. A model of nigral organization. Functional and pathophysiological considerations of basal ganglia dysfunction.

II.2.6.1. The complex relationship of the substantia nigra with the striatum (fig II.13)

From previous chapters it will be clear that the dopaminergic cell continuum in the mesencephalon has extensive reciprocal connections with the striatal complex, its medial and dorsal part also with the prefrontal cortex and basal forebrain structures. A dense dopaminergic innervation, i.e. the mesostriatal projection, is characteristic for the entire striatal complex including the caudate nucleus, the putamen, the nucleus accumbens and the olfactory tubercle. This mesostriatal projection is compartmentally organized with distinct sets of DAergic neurons projecting to striosomes and matrix, respectively, suggesting specialized channels directed at dopaminergic modulation of sensorimotor and integrative processing in the striatal matrix and limbic-related mechanisms represented in the striosomal system (Langer and Graybiel 1989). The exact functional significance of the mosaïc-like organization of both SN and striatum and their complex interrelationship is an important topic of present-day investigations (Jimenez-Castellanos and Graybiel 1989; Albin et al. 1989; Graybiel 1989). Part of the mechanism provided by this neurochemically defined differentiation may involve presynaptic variation in enzymatic regulation of dopamine content in and out of striosomes (Graybiel et al. 1987). Apart from this chemical compartmentalization, caudate-related, putamen and ventral striatum-related projections appear to be partly separated in primates and cats (Smith and Parent 1986; Beckstead and Cruz 1986). This has partly been explained because of the caudate nucleus and putamen being transected by the internal capsule and the dorsal striatum being separated from its ventral counterpart by the deep olfactory radiation (Nauta 1979, 1986). Within the dorsal striatum nigral axons synapse with dendritic spines and cell bodies of spiny, mostly cholinergic and GABAergic, neurons (Graybiel and Ragdsdale 1979; Carpenter 1986; Gerfen 1988), preferentially inhibitory (see Marsden 1982) but possibly partly excitatory (see Carpenter 1986). The idea that DA has variable effects is supported by clinical experience with DA antagonists and agonists and their effects (Clark et al. 1985; Bunney 1988; Albin et al. 1989). Spiny target neurons are the

constituent of the two striatal compartments: striosomes and matrix (Bolam et al. 1988). Tyrosine hydroxylase positive fibers within the striatum are mainly found in the matrix compartment. More proximal non-immunoreactive boutons on dendritic spines of striatal cells probably originate from cortico-striate glutamate terminals, suggesting a modulatory role of dopamine on the flow of corticostrio-nigral information (Bolam et al. 1981, 1988; Penney and Young 1983; Parent 1986; Gerfen 1988).

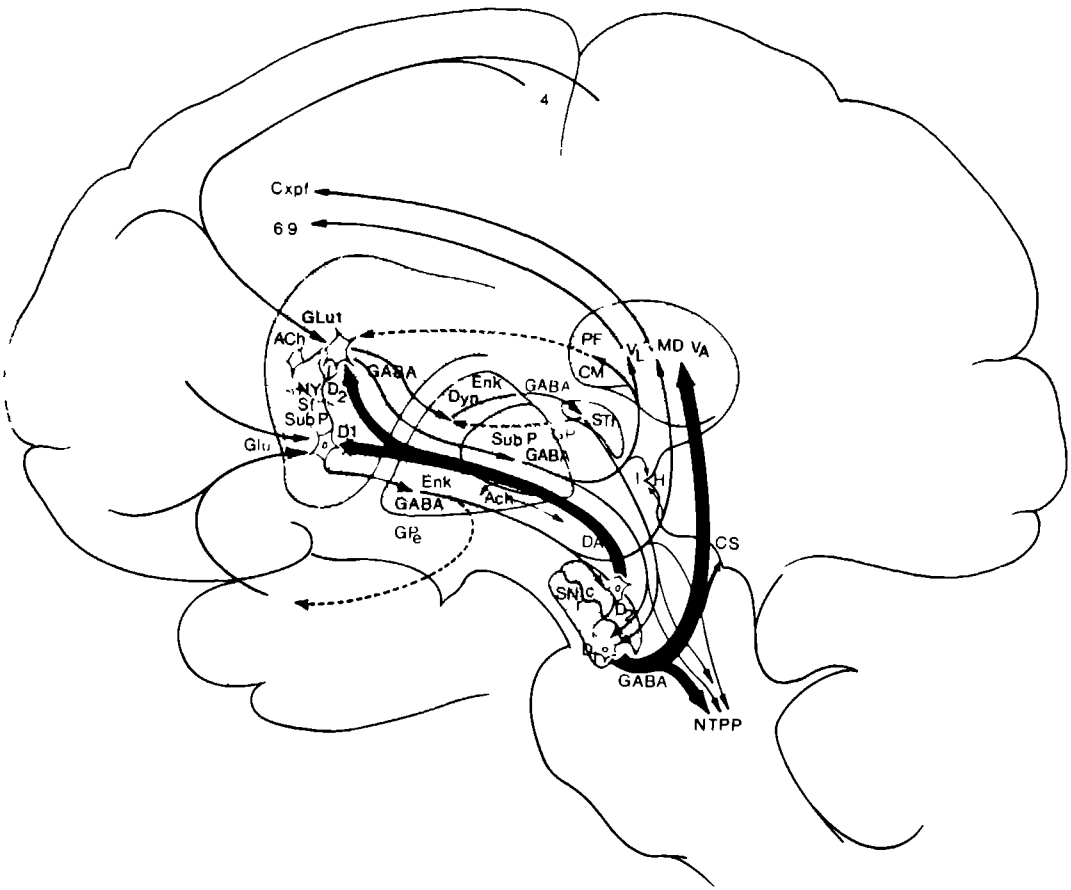


Fig II 13

Relationship of the substantia nigra to the basal ganglia Extrapyramidal circuitry

Striatal projections to the SN/globus pallidus internus (or entopeduncular nucleus in rodents) can be distinguished from striatal projections to the globus pallidus externus (Fig.II.13), on anatomical, immunohistochemical and functional grounds (Beach and McGeer 1984; Féger and Crossman 1984; Beckstead and Cruz 1986; Parent et al. 1984). This subdivision is supported by autoradiographic binding studies of dopamine receptors (Beckstead 1988; Camps et al. 1989; Cortés et al. 1989). Projections from the striosomes go mainly back to the SN pars compacta. Matrix neurons containing substance P mainly project upon the globus pallidus internus and SN pars reticulata, while those containing enkephalins project mainly upon the globus pallidus externus (Graybiel and Ragsdale 1983; Beckstead and Kersey 1985; Haber and Watson 1985). Both segments of the globus pallidus also receive GABAergic fibers and co-localization of transmitters has been described (Graybiel and Ragsdale 1983; Carpenter 1986). Dynorphin projections seem to be directed to the internal segment (nucleus entopeduncularis) in cats and rats (Beckstead and Cruz 1986) but in the human GP to the external segment (Haber and Watson 1985). The two striatal compartments may be linked functionally by interneurons that contain both somatostatin and neuropeptide Y (Gerfen 1984; Chesselet and Graybiel 1986) and possibly also by dendrites that cross the borders between striosomes and matrix (Bolam et al. 1988). Whereas substance P concentration in the SN pars reticulata has been shown to be decreased in Parkinson's disease (Waters et al. 1988) no changes in the external part of the globus pallidus levels of enkephalin have been reported. Projections of the globus pallidus pars externus only reach the SN and globus pallidus internus via the subthalamic nucleus, where glutamate might also be a neurotransmitter (Carpenter 1986; Beckstead and Cruz 1986).

Within the SN striatal afferents largely terminate on dendrites and neurons in the pars reticula transforming information to thalamic nuclei, the superior colliculus and the mesencephalic reticular formation, including the nucleus tegmenti pedunculopontinus (Fig.II.13.). The main function of the striatal efferent activity appears to be inhibition of tonically active GABAergic neurons in the pars reticulata and globus pallidus internus. Thus, the net effect of cortical excitation is disinhibition of structures innervated by globus pallidus and SN (Graybiel 1986; Swerdlow and Koob 1987). Restricted lesions of areas of the striatum will lead to behavioral deficits similar to those produced by damage to the areas of cerebral cortex projections to that region of the striatum (Marsden 1982).

The multipotential, collateralized, output organization of the SN pars reticulata (see Ch.II.2.4.) appears to differ markedly from that of the pars compacta, but is remarkably similar to that of the internal pallidum, which is the other major output structure of the basal ganglia (Graybiel and Ragsdale 1979; Parent et al. 1983b; Nauta 1986; Yelnik et al. 1987). Presumed GABAergic projections from the primate SNr to the thalamus, are organized in such a way that the medial SN projects to the medial magnocellular subdivision of the nucleus ventralis anterior and nucleus mediodorsalis thalami, projecting in turn to the most anterior regions of the frontal lobe (Ilinsky et al. 1985; Giguère and Goldman-Rakic 1988). The lateral SN pars reticula projects to the more lateral and more posterior parts of the ventral anterior magnocellular nucleus and paralaminar, parvicellular and densocellular subdivisions of the mediodorsal nucleus and intermediate layers of the colliculus superior (Graybiel 1978; Beckstead 1983; Francois et al. 1984; Ilinsky et al. 1985). The latter thalamic nuclei also project back to more posterior regions of the frontal lobe, like frontal eye fields and areas of the premotor cortex (Ilinsky et al. 1985; Goldman-Rakic and Porrino 1985).

Hypokinesia and rigidity, such as in Parkinson's disease, are hypothesized to result from a complex series of changes resulting in an increase in basal ganglia output, particularly to the ventrolateral thalamic nucleus (Narabayashi 1983). The ventrolateral thalamic nucleus has been known since long to be related to tremor generation (Lee 1987). Hyperkinetic disorders generally are postulated to result from impairment of striatal neurons projecting to the lateral globus pallidus and related subthalamic nucleus dysfunction (Wilson 1925; Marsden and Fahn 1987). All these abnormal movements share some common pharmacology in that the abnormal movements are suppressed by the administration of dopamine D2 receptor antagonists and exacerbated by DA agonists. D2 receptor antagonists seem to potentiate the activity of striatal neurons projecting to the globus pallidus externus, as measured by an increase in enkephalin levels, whereas anticholinergics antagonize this DAergic effect (for review see Albin et al. 1989).

II.2.6.2. Functional aspects of mesencephalic dopaminergic projections.

A key role played by the nigrostriatal projection is shown indirectly after unilateral 6-hydroxydopamine induced destruction of this dopaminergic pathway in rodents, with resulting biochemical asymmetry of the striatum (Ungerstedt 1971b). Within one to two days after lesioning, administration of DA agonist drugs, such as apomorphine, provoke circling behavior away from the side of lesion. This circling is prevented by lesions of the SN pars reticulata (Leigh et al. 1983). The GABAergic striatonigral projection appears to be critical for the mediation of this circling and GABA agonists, such as muscimol or baclofen, cause contralateral circling (for review see DeLong and Georgopoulos 1981). This circling comprises a purely (locomotor) activation component, mediated mainly via the nucleus accumbens (Reavill et al. 1981; Oades et al. 1986; Cools 1986), whereas postural asymmetry (i.e. stereotypic head and tail deviation to the side of injection) is predominantly a function of striatal asymmetry and its output. The nigro-tegmental projection, directed to the so-called "angular-complex", a reticular formation structure adjacent to the periaquaductal gray (Reavill et al. 1981; Leigh et al. 1983) is crucial in the mediation of this effect. This projection is very likely to be involved also in muscular rigidity or stereotyped behaviour, as can be seen in Parkinson's disease (Narabayashi 1983; Leigh et al. 1983; Ellenbroek et al. 1984). Rotational behaviour might be conditioned to environmental stimuli (Fibiger and Phillips 1974; Carey 1986; Casas et al. 1989) suggesting a role of the nigrostriatal projection in the generation of movements in the absence of sensory or mnemonic guidance (Cools et al. 1984b; Vrijmoed-De Vries and Cools 1986). The nigrostriatal dopamine system, rather than conveying highly specific information about movement in the striatum, seems to exert a more tonic modulatory effect upon the striatum (DeLong and Georgopoulos 1981; Marsden 1982; Cools et al. 1984b). Likewise, the VTA seems to modify (primarily inhibit) nucleus accumbens mediated activity, depending on environmental circumstances, like time of day and novelty of perception (Galey et al. 1977; Oades et al. 1986; Cools 1986; Van den Bos and Cools 1989). It has been known for a long time that different DA-antagonist sensitive mechanisms in the nucleus accumbens and caudate-putamen may mediate hyperactivity and stereotyped behaviour, respectively, in rats (Costall et al. 1977). In the lateral part of the VTA projection neurons, adjoining the very medial part of the dorsal SN pars compacta, somewhat more non-DA cells have

been found (Wang 1981a), together with a somewhat lower DA turnover in comparison with its medial counterpart projection (Agnati et al. 1980). Besides, based on electrophysiological experiments (Wang 1981 a,b), type I DA neurons with fine unmyelinated axons can be distinguished from non-DA, more heterogeneous, type II cells. Type I neurons show a markedly reduced firing rate following intravenous injection of apomorphine, amphetamine or the iontophoretic application of DA and GABA (Fuxe et al. 1977; Wang 1981 b; Bannon and Roth 1983).

A depressant effect of systematical administration of amphetamine at low doses on DA neurons of the SNc and VTA is primarily mediated through the striatonigral feedback pathway; at high doses its effect may be mediated by DA autoreceptors as well (Aghajanian et al. 1977; Bannon and Roth 1983; Thierry et al. 1988). Apart from its effect on the nigrostriatal and mesolimbic projections (Iversen and Koob 1977), amphetamine might also influence norepinephrine projections (Aghajanian et al. 1977; Fuxe et al. 1977; Wang et al. 1981a,b; Thierry et al. 1988) for the induction of stereotyped behavior in animals. Stressful situations were found to activate selectively the mesocortical DA neurons as indicated by the enhanced rate of DA utilization (Thierry et al. 1976; Antelman et al. 1988; Dunn 1988; Roth et al. 1988). Delineation of the various output links subserving elements of circling behaviour and stereotypy could throw light upon clinical observations which indicate that different symptoms and signs in Parkinson's disease are related to different basal ganglia output pathways.

II.2.6.3. The extrapyramidal system (Fig.II.13.)

It is clear then, that nigral organization as well as the effects of dopaminergic substances are essentially determined by a close relationship of the SN to the basal ganglia, the organization of which is primarily determined by cortical afferents. The definition of the basal ganglia is more extensive on anatomical grounds as is usually adopted clinically. But much about basal ganglia circuitry remains unknown yet and some of the results obtained in primate experiments are inconsistent with findings in rats and cats. As proposed by Nauta (1979, 1986), referring to Heimer and Wilson's (1985) study, a striatal input zone nowadays can be expanded to include the nucleus accumbens septi and parts of the olfactory tubercle. The classical output zone, including external and internal pallidal segment, can now be expanded to include the nondopaminergic part of the SN and

a part of the globus pallidus, extending ventral to the anterior commissure and into the olfactory tubercle (see also Young et al. 1984; Parent 1986). Whereas the ventral striatum is continuous with the amygdaloid complex (Alheid and Heimer 1988), this "ventral pallidum" is part of the substantia innominata, including the nucleus basalis magnocellularis of Meynert (Hedreen et al. 1984; Carpenter 1986; McGeer and McGeer 1987). The cholinergic neurons of the nucleus basalis are much more intimately intermingled with neurons of the globus pallidus in rats and cats than in primates (Mesulam et al. 1983a, 1984; Parent 1986). The functional significance of such intermingling and possible connectivity of limbic and basal ganglia neuronal elements at pallidal levels remains to be investigated. The subthalamic nucleus, globus pallidus and SNr all send fibers to the nucleus tegmenti pedunculopontinus, as one of the major final output nuclei (Garcia-Rill 1986; Nieuwenhuys et al. 1988). Adjuvant sub-circuits apparently play a key role in the regulation of motor function (Armstrong 1986; Jellinger 1988; Albin et al. 1989).

The first evidence of striatal function was obtained after bilateral destruction in rabbits, resulting in an irresistible drive to run forward, whereas electrical stimulation of the corpus striatum elicited tonic-clonic movements of the contralateral extremities, even after the motor cortex had been destroyed (for reviews see Wilson 1914; Hassler 1979). Because animals were able to run, even after bilateral interruption of the pyramidal tract, the name 'extrapyramidal motor pathways' was introduced and made very nearly synonymous with the basal ganglia and their efferent connections by convention (Nauta and Domesick 1979). Whereas unilateral limited acute pallidal lesions have no clear effects on untrained monkeys (DeLong and Georgopoulos 1981), bilateral extensive lesions in monkeys (Denny-Brown and Yanagisawa 1976) and humans (Jellinger 1986a; Laplane et al. 1989) resulted in global akinesia, inactive posture and emotional stolidity.

Marsden (1982) formulated the hypothesis that the basal ganglia are responsible for the automatic execution of learned motor plan, restricting basal ganglia function exclusively to motor control. His attention was mainly directed to basal ganglia output, in which the striatum might 'call-up' standard motor programs, like postural reflexes, whereas the perceptual processes involved in the decision to move might be independent of striatal function (see also Sheridan et al. 1987). But the lesion studies of Denny-Brown and Yanagisawa (1976) and Hassler (1978) had already shown that the basal ganglia not only directed attention to a

particular object in the environment, but also inhibit other distracting sensory input (Penney and Young 1983). Likewise, proprioceptive feedback is not normal in Parkinson's disease (Marsden 1982), which made Cools et al. (1984a,b), mainly based on pharmacologic animal experiments, suggest a role of the basal ganglia in esthablishing new programs appropriate to available stimuli. According to these authors (for review see Cools et al. 1989) the SN pars reticulata transforms the striatonigral information stream, reducing the degree of freedom in programming motor behaviour by adding information from proprioceptive, exteroceptive and interoceptive input. Confirmation of a SN gating mechanism has been gained recently in rats (Schneider 1984; Cools et al. 1984b; Gale 1985; Baumeister et al. 1988). Dopaminergic output of both SN and VTA might influence the cortico-striato-nigral information stream both at striatal and subcortical level, without altering essentially the function of the neuronal system (loop) that is regulated (Penney and Young 1983; Simon and LeMoal 1988; Miller and DeLong 1988; Weinberger et al. 1988). Evidence for processing of sensory information by the basal ganglia has been reviewed by Schneider (1984) and Swerdlow and Koob (1987). The dependence of motor function on the behavioral and environmental set in patients with Parkinson's disease is very well in accordance with clinical experience (Marsden 1982; Evarts et al. 1984).

Both anatomical organization and clinical evidence in Parkinson's disease and Huntington's disease (Penney and Young 1983; Schneider 1984; Mayeux et al. 1984; Cummings and Benson 1984; Phillips and Carr 1987) support views that the dorsal striatum is also involved in several types of cognitive activities. In such cases all subdivisions of the caudate nucleus are more affected than the putamen, suggesting that also the pattern of SN cell loss might differ according to prevailing motor or cognitive symptomatology (Kish et al. 1988; Hornykiewicz and Kish 1986; Gilbert et al. 1988). The indisputable effectiveness of DA blocking agents in schizophrenia (Sayed and Garrison 1983; Nemeroff 1986; Bunney 1988) and of catecholamine potentiating drugs in depression (Frazer and Mendels 1977; Swerdlow and Koob 1987; Taylor et al. 1988) not only represents over- and underactivity of the target amines, but could also reflect the absence of opposing or potentiating elements (Clark et al. 1985). E.g. the many similarities in the actions of neurotensin and neuroleptic drugs (Nemeroff 1986; Bisette and Nemeroff 1988) and the presumed role of CCK in their therapeutic effect (Wang 1988) raised the question of their potential involvement in schizophrenia. Likewise, the relative overactivity

of dopamine in the striatum in Huntington's chorea might result from atrophy of opposing systems, like GABAergic (Bruyn and Went 1986). Cognitive deficits characteristically found in this illness are of the prefrontal type, suggesting secondary effects of caudate degeneration on prefrontal function (Taylor et al. 1988; Weinberger et al. 1988). Likewise, abnormally high levels of DA transmission or DA receptor overactivity in the striatum, nowadays measurable by positron emission tomography (Wang et al. 1986; Laplane et al. 1989) may be involved in schizophrenia (Matthysse 1977; Crow et al. 1978; Nemeroff 1986; Swerdlow and Koob 1987; Weinberger et al. 1988).

Nowadays basal ganglia disorders should better be understood as the result of deficiency of several neurotransmitter metabolites due to chains of enzymatic error in transstriatal circuitry and not only of dopamine deficiency. The highly variable symptomatology in parkinsonian syndromes of all etiologies might be determined by the degree and pattern of nigral cell loss, determining the degree and pattern of DA loss in striatal nuclei and changes in the interacting non-DA systems (Hornykiewicz and Kish 1986; Lee 1987). Cholinergic neurons promote the positive feedback loops, whereas dopaminergic neurons inhibit the circuit. The traditional "cholinergic-dopaminergic balance" of the clinical and pharmacological literature will be found to subsume a variety of systems (Graybiel 1989). Cholinergic mechanisms of the basal ganglia are not any longer confined to intrinsic striatal neurons. Other cholinergic systems, including those originating in the midbrain and basal forebrain, may influence striatal mechanisms indirectly (Garcia-Rill 1986; Lee et al. 1988; Graybiel 1989). Because of differences in the DA-related and ACh-related activities in the striosomal and matrix system, it should be possible for target drugs to influence selectively specific DAergic or cholinergic subsystems (Fuxe et al. 1977; Besson et aL. 1988; Graybiel 1989).As regards responses to DA agonists and antagonists the two types of receptors, D1 and D2 (Seeman and Grigoriadis 1980; Niznik 1987), show a patchy distribution both in cats (Beckstead 1988; Langer and Graybiel 1989) and human (Besson et al. 1988; Camps et al. 1989; Cortés et al. 1989), reminiscent of the uneven nigrostriatal terminal pattern. Whereas, D2 receptors are the main binding site for neuroleptics, the existence of both types of DA receptors in limbic structures and the prefrontal cortex (Thierry et al. 1988) may account for many of the newly found "basal ganglia"-symptomatology after pharmacologic manipulation of DAergic projections.

II.2.6.4. Functionally segregated extrapyramidal circuits.

Based on older silver impregnation studies (see Nauta and Domesick 1979 for review), it was generally believed that the basal ganglia integrated inputs from widespread cortical areas funneling this information to the motor cortex via the ventrolateral thalamus. The extrapyramidal motor function of the basal ganglia was supposed to be determined by several minor feedback circuits such as: Striatum-globus pallidus-thalamus-striatum, globus pallidus-nucleus subthalamicus-globus pallidus, striatum-substantia nigra-striatum and cortex-striatum-substantia nigra-thalamus-cortex (Graybiel and Ragsdale 1979; Carpenter 1981; Jellinger 1986a, 1988; Nieuwenhuys et al. 1988). More recent data, mainly based on autoradiographic tracing methods, however, suggest the existence of several parallel, but segregated, cortico-subcortical circuits in which inputs to the basal ganglia tend to remain anatomically and functionally segregated throughout the basal ganglia-thalamocortical circuitry (Alexander et al. 1986). As Kemp and Powell (1970) proposed, any subdivision of corticostriate projections is related to the functions of the cortical area under consideration and not upon their size.

Despite morphological characteristics suggesting anatomical convergence from cortex to striatum to the successively smaller regions of pallidum and SN (Percheron et al. 1984a,b, 1989), single cell recording studies (DeLong et al. 1983b, 1985) show that there is relatively little physiological convergence between inputs from various cortical areas. In a sense, the concept of funneling is retained, because a single cortical area receives information derived from the processing of inputs from several cortical areas through the basal ganglia, whereas connected associational cortex areas may project to one similar striatal area (Selemon and Goldman-Rakic 1988; Goldman-Rakic 1987). Besides, apart from such feedback loops, the existence of feedforward circuitry also has to be kept in mind (Groves 1983; Cools et al. 1984a,b; Armstrong 1986) especially as regards pathophysiological manifestations in various defect states, like motor (Garcia-Rill 1986), cognitive (Simon et al. 1980; Schneider 1984; Agid et al. 1987) affective (Stevens 1973; Swerdlow and Koob 1987; Taylor et al. 1988) and oculomotor (Hikosaka and Wurtz 1983a,b; Scheel-Krüger 1983, 1986; Wise 1984; Faull et al. 1986) disturbances. The general view emerging from these findings is that transstriatal processing permits neurochemically specialized channels to be established in the basal ganglia. This parallel circuitry offers another framework for the interpretation

of such complicated adverse effects of anti-dopaminergic medication like tardive dyskinesia (e.g. Baldessarini 1980; Groves 1983; Fibiger and Lloyd 1984), presumed cognitive changes after dopaminergic medication (Fibiger and Phillips 1974; Blin et al. 1988; Hietanen and Teräväinen 1988) and dyskinesias following treatment of Parkinson's disease (Chase et al. 1973; 1988).

The entire cerebral cortex projects onto the striatum in a highly ordered arrangement, of which figure II.14. gives an account.

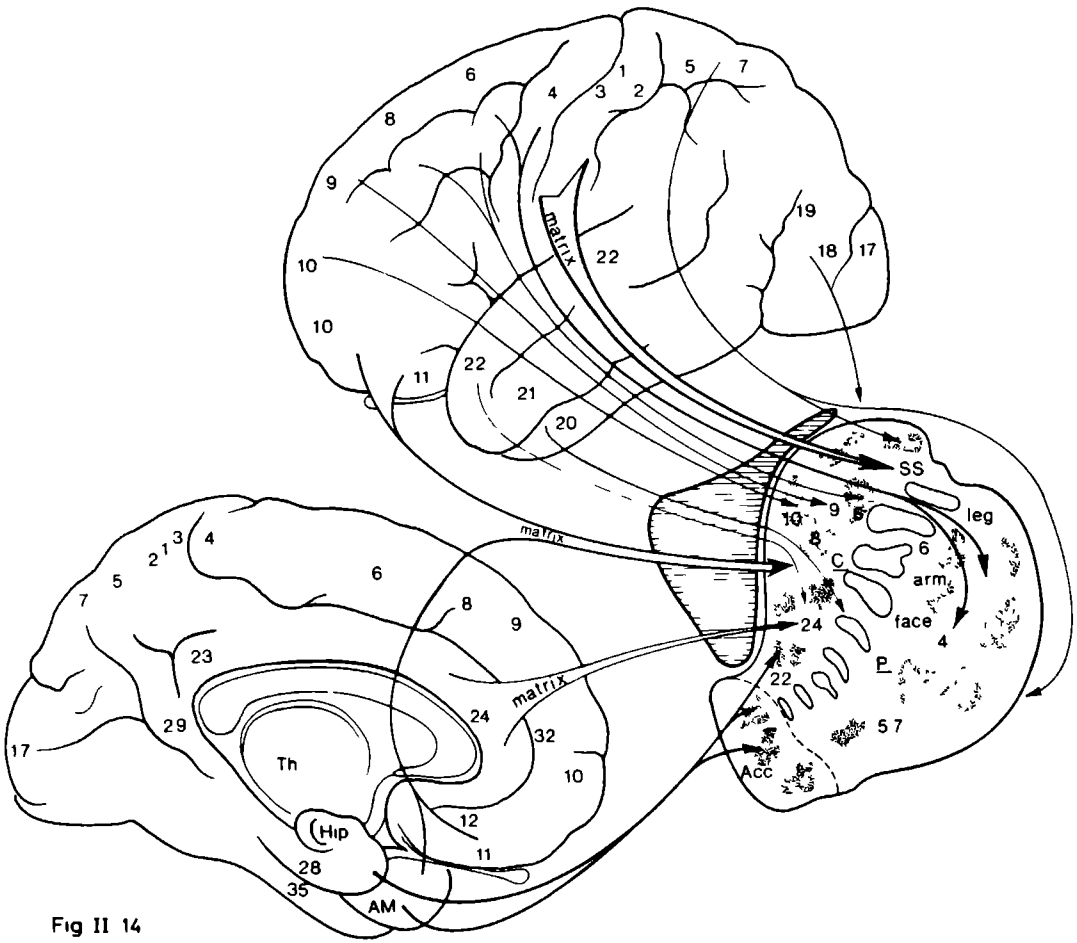


Fig II 14

Corticostriatal projections and their presumed topography in the human brain

Somatosensory and motor cortices of various species innervate specific dorsolateral and central parts of the nucleus caudatus and putamen matrix, respectively (Kemp and Powell 1970; Selemon and Goldman-Rakic 1985; Malach and Graybiel 1986; McGeorge and Faull 1989), which in turn project preferentially to that region of the SN giving rise to the nigrothalamic projection. In primates the dorsolateral part of the caudate nucleus matrix is a typical somatosensory recipient area, related to areas 1, 2 and 3 of Brodmann (Kemp and Powell 1970; Künzle 1977), whereas the putamenal matrix is particularly projected on by the motor cortex in a somatotopic order (Künzle 1975; DeLong et al. 1983; Percheron et al. 1984). These target areas are continuous, or rather interdigitating, with those of the posterior parietal cortex (area 7 and 5 of Brodmann) and the premotor area 6 (Künzle 1978; Ragsdale and Graybiel 1981; Selemon and Goldman-Rakic 1985). Terminals of the posterior parietal cortex are also observed in ventral parts of the caudal putamen (Selemon and Goldman-Rakic 1985). Projections of the supplementary motor cortex (area 9) have been described to terminate both in patch and matrix compartments (Ragsdale and Graybiel 1984; Selemon and Goldman-Rakic 1985; 1988). Indeed, part of Brodmann's area 9 has been indicated as typical prefrontal (Goldman and Nauta 1977; Künzle 1978) related to somewhat more medially placed caudate striosomes (Graybiel 1984). More frontally located cortical areas (dorsomedial and dorsolateral prefrontal areas i.e. parts of area 9 and 10 of Brodmann) have their target sites on progressively more medially located striosomes of the caudate nucleus (Goldman and Nauta 1977; Künzle 1978; Selemon and Goldman-Rakic 1985). These projections are continuous with those of areas 8 (frontal eye fields) and 6 of Brodmann (Künzle 1978; Selemon and Goldman-Rakic 1985). Projections of cortical areas 6 (premotor), 7, 8 (frontal eye fields) and 9 (supplementary motor) apparently are convergent and project to adjacent longitudinal domains of the neostriatum mediating many aspects of spatial function (Selemon and Goldman-Rakic 1988). They are terminated on themselves by the mediodorsal thalamic nucleus (Goldman-Rakic 1985). Medial and lateral orbitofrontal cortices (belonging to areas 10, 11 and 12 of Brodmann) project to central regions of the caudate matrix (Graybiel 1984, Selemon and Goldman-Rakic 1985), together with projections of the anterior cingulate cortex (area 24) and a minor basomedial amygdaloid projection (Ragsdale and Graybiel 1988). Limbic regions, particulary the basolateral amygdaloid nuclear complex, allocortical (entorhinal and piriform) and archicortical (hippocampal) areas project to striosomes of the

ventromedial caudate nucleus and to the ventral striatum, particularly the medial part of the nucleus accumbens (mainly based on data fron rats, see: Swanson and Cowan 1979; Graybiel 1984; Nauta 1986; McGeorge and Faull 1989). Thus, the basolateral amygdala, like the hippocampus, serves as the hub of a circuit that constitutes the limbic system at the level of cortex and thalamus (Nauta 1958, 1986; Ragsdale and Graybiel 1988). The principal of ventral prefrontal areas projecting to more medial parts of the caudate nucleus than do dorsal prefrontal areas is also reflected in the topography of temporal lobe projections to the striatum (Van Hoesen et al. 1981).

In conclusion, these findings seem to implicate the matrix in functions related to somatosensory and motor processing, whereas the striosomal system seems more closely related to conditional and affective components of behaviour (see also McGeorge and Faull 1989). The matrix might also be expected to be the primary target-site of visual and auditory cortices projecting to dorsomedial parts of the striatum in rats and cats (Faull et al. 1986; McGeorge and Faull 1989; Graybiel 1989).

The largest flow of information through the basal ganglia is comprised by the 'motor loop', which is the circuit most directly involved in the control of movement (Penney and Young 1983). Related basal ganglia inputs are derived from precentral motor, premotor, supplementary motor and postcentral somatosensory areas (including area 5) defined in the human cortex. Striatal terminations are mainly directed to the matrix of the dorsolateral caudate nucleus and putamen in a somatotopical way, such that the 'face' area has a terminal labeling located mainly within the ventromedial putamen (Fig.II.14.) Likewise in rats, ventrolateral striatal ('putamen' in higher mammals) lesions increased the reaction times and produced impairments in the execution of both orofacial and forelimb movements (Pisa 1988) and putamenal neurons, but not caudate neurons (in primates) fire in relation to specific physical parameters of movement (Crutcher and DeLong 1984). Outputs from the striatum group together in the bundles known as Wilson's pencils and project topographically to the caudo-ventral part of both segments of the globus pallidus (mainly related to limb movement) and caudolateral SN pars reticulata associated with orofacial movements. Neuronal activity in the putamen and globus pallidus has been shown to be related to specific aspects of limb movements, including direction amplitude (or velocity) and load (Alexander et al. 1986). Cells of the centrolateral SN pars reticulata were related to licking and chewing (DeLong

et al. 1983, 1985); those of the lateral portions were related to eye movements (Hikosaka and Wurtz 1983b), whereas the internal segment of the globus pallidus, mainly contains cells related to movements of extremities and other body parts (Evarts et al. 1984; DeLong et al. 1985). The final stage is from the internal segment of the globus pallidus and the SNr to the ventrolateral thalamus, closely associated with fiber bundles of the capsula interna. The major output of these thalamic nuclei is to the supplementary motor area (Schell and Strick 1984), which apparently is the final destination of this extrapyramidal motor loop (fig.II.15.).

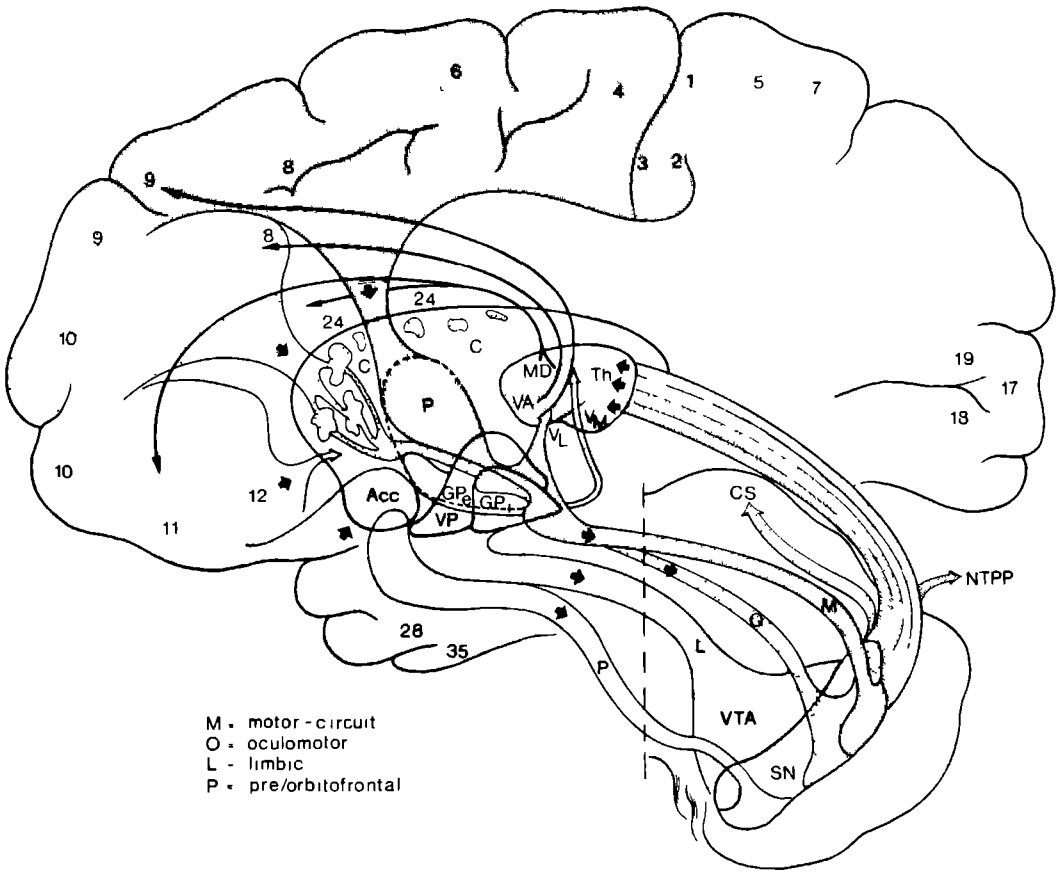


Fig. II. 15

Four main "loops" composing basal ganglia circuitry

The phenomenon of most basal ganglia cells with sensory input responding also electrophysiologically to movement, implies a convergence of sensory and motor input from different cortical areas (DeLong et al. 1985; Malach and Graybiel 1986)

An 'oculomotor loop' is associated with cortical areas known to be involved in the control of eye movements, i.e. area 8 (frontal eye fields), dorsolateral prefrontal areas 9 and 10 and posterior parietal cortex 7. Related projections from the body of the caudate nucleus (fig.II.15.) are mainly directed to the most ventral and rostrolateral parts of the SN pars reticulata, which in turn send collaterals to thalamus and colliculus superior. (Scheel-Krüger 1983, 1986; François et al. 1984). This loop is closed by projections from the ventral anterior thalamic nucleus onto the frontal eye fields (Alexander et al. 1986), focussing inputs from several areas back onto a more limited region of the cerebral cortex. Alterations in part of this circuit produce saccadic distractability (Bruce and Goldberg 1984) a symptom which is gaining growing interest in the study of basal ganglia dysfunction, like Parkinson's disease and Huntington's disease (Evarts et al. 1984; Albin et al. 1989). Various 'association' circuits, originating in prefrontal areas 9, 10, 11, 12 and posterior parietal cortex, with feedback projections to prefrontal, particularly orbitofrontal, areas may play a role in the mediation of behaviours other than simple motor (Alexander et al. 1986). A final loop running parallel to these motor loops is the 'limbic loop' of the ventral striatopallidal pathway. Although anatomical and physiological substrates of this limbic loop have been extensively studied in non-primate mammals (Haber et al. 1985; Oades and Halliday 1987) the functional role of this circuitry in human clinical conditions is clearly speculative.

II.2.6.5. Mesolimbocortical circuitry.

Whereas the SN and dorsal striatum are relatively large and differentiated in man, the nucleus accumbens and limbic midbrain area are large in rats and cats but small in man (Nauta 1958; Heimer and Wilson 1975; Carpenter 1981; see Fig II.16.) The meaning of this evolutionary trend in terms of the importance of DA for cortical function is unknown (Weinberger et al. 1988). The vast majority of cortical efferents received by the ventral striatum orginates from allocortical (including hippocampus, amygdala, entorhinal and perirhinal cortices) structures (Heimer and Wilson 1975; Swanson and Cowan 1979; Nauta et al. 1978; Kelley et al. 1982; Haber et al. 1985; Voorn et al. 1986) continuous with projections from other

associational cortical areas. Just as the major efferent projection from the dorsal striatum is to the dorsal pallidum so the nucleus accumbens sends a majority of its GABAergic containing spiny efferents into the ventral pallidum, as demonstrated for rats and cats (Mogenson 1983; Young et al. 1984) continuous with projections to VTA, medial SN, A8, hypothalamic areas, central grey and nucleus tegmenti pedunculopontinus (Heimer et al. 1982; Mogenson 1983; Groenewegen and Russchen 1984). More differentiated presumed projections of enkephalin-, dynorphyn- and substance P-containing fibers from the striatum to the globus pallidus and adjacent basal forebrain structures of the human brain have been described by

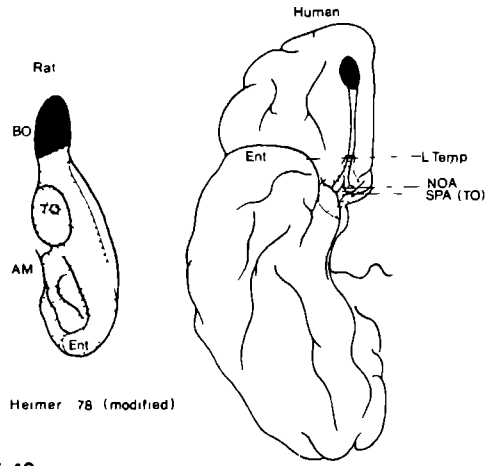
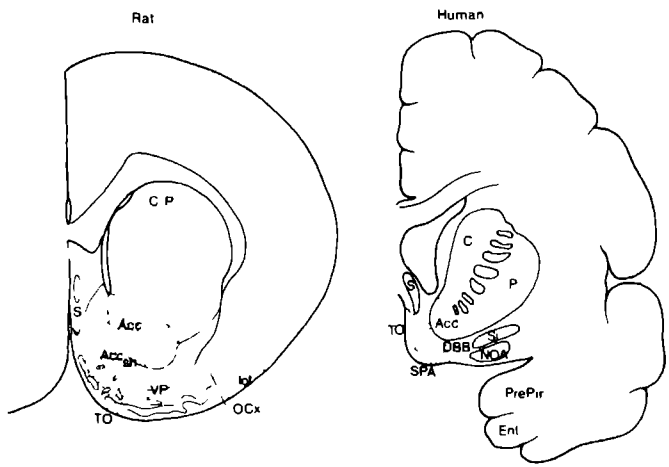


Fig II. 16
Comparison of the olfactory projection area and ventral striatal region between rats and human



Haber and Watson (1985), raising the question of which area in the primate forebrain is homologous with the ventral pallidum in rats. Projections from the ventral pallidum and rostrodorsal part of the SN pars reticulata reach the mediodorsal thalamic nucleus in different species (Carpenter et al. 1976; Beckstead and Frankfurter 1982; Young et al. 1984), which in turn maintains reciprocal excitatory connections with the cingulate (area 24), frontal principal sulcal (in monkeys) and medial prefrontal cortices (Giguère and Goldman-Rakic 1988). This positive feedback circuit, much like that proposed for the striatum- SNr- ventral thalamus-neocortex interactions, receives modifying DAergic midbrain influence from VTA projections (see also Penney and Young 1983). It has become clear that this system is in many ways qualitatively distinct from the better-characterized nigrostriatal dopamine system. The mesolimbic projection is characterized by co-localization of dopamine with neurotransmitter substances like CCK and neurotensin (Hökfelt et al. 1980, 1984; Zaborsky et al. 1985; Nemeroff 1988; Phillips et al. 1988) that might have important implications for human pathophysiology at the interface between the limbic (motivation) and motor system (Mogenson et al. 1988; Wang 1988; Alheid and Heimer 1988). A dorsal-ventral "switch", based on limbic and non-limbic portions and dorsoventral topography of DAergic mesostriatal projection systems suggests a limbic-to-motor polarity in the striato-nigral-striatal circuitry (Nauta and Domesick 1978; Groenewegen and Russchen 1984; Carpenter 1986; Nauta 1986; see also Fig. III.15).

Whereas the ventral pallidum and adjacent substantia innominata cells are under inhibitory control of striatal GABAergic output cells, a sequential GABA-to-GABA (ventral) striatal - ventral pallidal - dorsomedial thalamic circuit is also capable of exciting the corticothalamic circuitry through disinhibition, promoted by limbic cortical and VTA afferents (see van Rossum et al. 1977; Penney and Young 1983; Swerdlow and Koob 1987; Mogenson et al. 1988). These afferents provide a means to disrupt or change ongoing informational cognitive and emotional processes, thereby allowing for the initiation of new processes (Groves 1983; Flowers and Robertson 1985; Oades 1985). But the complexity of these interactions is raised by the finding of more differentiated presumed peptidergic (particularly enkephalin-, dynorphin- and substance P) mediated influences within the human brain (Kalivas 1985; Haber and Watson 1985; Swerdlow and Koob 1987; Kalivas et al. 1988). Likewise the uneven distribution of dopaminergic terminals in the ventral striatum has been shown to be parallelled by the uneven

parallelled by the uneven distribution of the neuropeptides enkephalin, substance P and dynorphin, as well as ChAT and GABA-like immunoreactivity (Graybiel and Ragsdale 1983; Kwak et al. 1984; Voorn et al. 1986; Bisette and Nemeroff 1988; Groenewegen et al. 1989). Considering the extent of experimentation on mesencephalic dopaminergic systems in recent years and the complexity of their interactions with telencephalic structures it is not surprising that they have been focussed as a 'final common pathway' for a wide variety of psychiatric disorders (Stevens 1973; Tassin et al. 1977; Bunney 1977; Javoy-Agid and Agid 1980; Bannon and Roth 1983; Hardy et al. 1986; Sandyk 1986; Swerdlow and Koob 1987; Kalivas and Nemeroff 1988; Thierry et al. 1988; Rinne et al. 1989). Characteristic of this "final common pathway" is the regulation of DA receptors in the nucleus accumbens and dorsal striatum through its effects on the cortico-subcortical glutamatergic pathways (Penney and Young 1983; Bannon and Roth 1983; Glowinsky et al. 1984, 1988), crucial for the passage of properly delimited limbic-cortical information (Glowinsky et al. 1984; Hornykiewicz and Kish 1986; Mogenson et al. 1988). Much like traditional "motor-output" of the striatum, this information might be transformed at each hierarchically lower output level into information that becomes more and more related to the presence and context of environmental and internal stimuli (Cools et al. 1984b; Gelissen and Cools 1986).

Just like parkinsonian hypokinesia, due to the consistently impaired execution phase of movements (Marsden 1982) and rigidity (Narabayashi 1983) was associated with nigrostriatal destruction (Bernheimer et al. 1973), there is a growing clinical evidence now of characteristic changes in cognitive processing associated with impairment of presumed mesolimbic projections (Tassin et al. 1977; LeMoal et al. 1977; Crow et al. 1978; Schneider 1984; Swerdlow and Koob 1987; Alheid and Heimer 1988; Glowinsky et al. 1988). As Birkmayer et al. (1983) stated: "Bradyphrenia is the psychic correlate to akinesia". It is most thoroughly documented in progressive supranuclear palsy and Parkinson's disease (Taylor et al. 1988; Dubois et al. 1988), in which cognitive processing is slower than in control subjects, matched for the speed of motor activity (Rogers 1986).Bradyphrenia is uncharacteristic of cortical dysfunction and is to be distinguished from organic dementia (Rogers 1986; Cummings and Benson 1988). There is no deficit in the cristalline intelligence, but the patient is more easily distracted and his mental performance is very dependant on mood (Birkmayer et al. 1983). This association of motor-related and motivational or attention-directing ascending projections

through mesostriatal circuitry is also consolidated by the close relationship of these ascending dopaminergic projections to the isodendritic core of the reticular formation (Ramón-Moliner and Nauta 1966; Matthyse 1977; Hassler 1978; Moore 1980; Rossor 1981; Agid et al. 1986; Nieuwenhuys et al. 1988). The interaction between motor-related and motivational (mesolimbic) ascending basal ganglia modifying projections has been suggested especially as regards certain nodal points, like the VTA, nucleus accumbens, lateral habenula and possibly nucleus tegmenti pedunculopontinus (Iversen and Koob 1977; Nauta 1979, 1986; Mogenson et al. 1988; Sutherland 1982; Armstrong 1986; Parent 1986; Garcia-Rill 1986; Oades and Halliday 1987).

The second important target site of VTA projections is the prefrontal cortex (Thierry et al. 1976, 1988; Divac et al. 1978; Bannon and Roth 1983; Weinberger et al. 1988). Characteristically the mesocortical projection is less sensitive to DA synthesis inhibition through autoreceptor stimulation (Bannon and Roth 1983; Sayed and Garrison 1983; Glowinsky et al. 1988) and has been reported to develop less tolerance to chronic neuroleptic administration (Glowi[illegible] et al. 1984; Bunney 1988; Hunt et al. 1988). Several preclinical studies underline the influence of the prefrontal cortex on subcortical systems (for reviews see Nauta 1971; Leviel et al. 1984; Goldman-Rakic 1987). Frontal lobe dysfunction in patients with Parkinson's disease has gained much interest (Taylor et al. 1986, 1988; Miller and DeLong 1988; Weinberger et al. 1988). One of the most consistent deficits observed in parkinsonian subjects is a failure to perform card sorting tasks, such as the Wisconsin Card Sorting Task, normally (Taylor et al. 1986), reflecting a deficit in the manipulation of mental sets (Growdon and Corkin 1986; Phillips and Carr 1987; Cummings and Benson 1988). The presence of various frontal-lobe related deficits in both Parkinson's disease and Schizophrenia (Matthysse 1977; Crow et al. 1978; Lees 1985; Taylor et al. 1986, 1988; Weinberger et al. 1988) reminiscent of disturbances found in dysregulation of mesolimbocortical circuitry in animal experiments, are most satisfactorily explained by abnormal activity within the association circuits of the basal ganglia (Crow et al. 1976; Miller and DeLong 1988; Laplane et al. 1989; Rinne et al. 1989). The notion that these mainly attention-related cognitive deficits might arise from a dysruption of dynamic interactions between DAergic terminal regions in the nucleus accumbens, prefrontal cortex and amygdala is a particularly evolving concept and deserves proper study (Clark et al. 1985; Sandyk 1986; Swerdlow and Koob 1987).

A core clinical syndrome of parkinsonian symptomatology, progressive dementia and behavioral disturbances (especially depression and mutism) has been suggested to be associated with the specific mesolimbocortical circuitry (Torack and Morris 1986, 1988). Such a 'mesolimbocortical dementia' is strongly reminiscent of the controversial 'subcortical dementia'. Subcortical dementia is a clinical syndrome -the cardinal features of which are those identified by Albert et al. (1974)- characterized by slowness of mental processing, forgetfulness, impaired cognition, apathy and disturbances of mood (Cummings and Benson 1984, 1988; Freedman and Albert 1985; Brown and Marsden 1988). Similar to motor disturbances in Parkinson's disease, neuropsychological mechanisms of subcortical dementia were thought to involve deficits in timing and activation (Freedman and Albert 1985; Cummings and Benson 1988). Essentially this can be considered as an impaired ability to manipulate acquired knowledge. The syndrome contrasts with dementia of the Alzheimer type in which primary cortical involvement produces aphasia, combined recall and recognition deficits. The most typical disorder in which this subcortical dementia syndrome has been described is progressive supranuclear palsy (Steele et al. 1964; Albert 1974, 1978; Agid et al. 1986; Dubois et al. 1988; Goulding et al. 1989) in which the primary site of lesion is still controversial. Similarities with the characteristic slowing of cognitive processing, mentioned before (see also Habib and Poncet 1988), are significant. The pathologic changes are concentrated in the globus pallidus, subthalamic nucleus, red nucleus, SN and rostral brainstem (Steele et al. 1964, 1988). At the actual state of knowledge it is not possible to relate these changes to one of the specific loops described before, but it is tempting to speculate on the possible involvement of the oculomotor and closely related limbic loop. But 'subcortical dementia' is primarily a clinical and not an anatomical concept (Cummings and Benson 1984; Freedman and Albert 1985; Schulte 1989). Retrospectively it occurs in diseases where various pathological changes involve primarily deep gray matter structures, like thalamus, basal ganglia and related brainstem nuclei, but also hemispheric white matter tracts (Cummings and Benson 1988). Whereas subcortical dementia primarily is a disease of ascending subcortical projections, with relative sparing of the cerebral cortex, dementia of the Alzheimer's type only secondary seems to affect subcortical structures like nucleus basalis (Vogels 1990) and VTA (this thesis). The development of neurofibrillary tangles in the perforant pathway, in the absence of senile plaques, as described by Torack and Morris (1986, 1988) in their 'mesolim-

bocortical dementia' cases, might be related to diminished dopaminergic input. The VTA abnormalities are always associated with similar cell loss and gliosis in other subcortical nuclei, notably the LC, NRd, basal ganglia and nucleus basalis. Disorders manifesting subcortical dementia are progressive supranuclear palsy, Huntington's disease, Parkinson's disease, Wilson's disease, spinocerebellar degeneration, idiopathic basal ganglia calcifications, the lacunar state and the dementia syndrome of depression (Cummings and Benson 1984, 1988). Various vascular and inflammatory conditions involving the basal ganglia and thalamus, like multiple sclerosis and the AIDS-dementia complex (Nielsen et al. 1984; Navia et al. 1986; Diederich et al. 1988; Brew et al. 1988a,b) may lead to a comparable dementia syndrome. Whitehouse (1986b), reviewing clinical neuropathological and neurochemical studies, found little support for classifying dementias into "cortical" and "subcortical" types.

Phillips et al. (1987) described a patient with apathy and loss of volition, anterograde amnesia and altered arousal after repair of a ruptured anterior communicating aneurysm. Postmortem examination of the brain revealed bilateral destruction of the septal gray, nucleus accumbens and of the nucleus of Broca's diagonal band, inferior portions of the anterior limb of the internal capsule and globus pallidus. This patient's septal lesion might have destroyed many connections of the medial forebrain bundle, contributing to the altered state of arousal (Phillips et al. 1987). Similar case studies have recently been provided by LaPlane et al. (1989) and Katz et al. (1989). Infarcts due to disruption of the perforating branches of the proximal anterior cerebral and communicating arteries might underly some cases of the subcortical arteriosclerotic encephalopathy of Binswanger's diseases (Caplan 1978, 1985; Zeumer et al. 1982; Fisher 1982,1989) in which slowness of mental processing is the predominant sign (Cummings and Benson 1984) and frontal lobe symptomes seem to predominate (Ishii et al. 1986). Ross and Stewart (1981) described a patient with devastating akinesia after surgical removal of a tumor from the anterior hypothalamic area, who lacked other parkinsonism features like tremor and rigidity, and who improved on treatment with lergotrile and bromocriptine. They concluded that akinetic mutism, which is distinct from rigidity and tremor of parkinsonian patients, may relate to a loss of dopamine modulation of the anterior cingulate cortex or other corticolimbic structures. Such reports are reminiscent of those in older literature referring to defects in the ascending reticular activating system (Dale and Lowe 1958; Neumann and Cohn

1967; Neumann 1968; Fisman 1975). Selective involvement of the nucleus basalis of Meynert may cause disturbances in memory and arousal, with otherwise preserved intellect (Mesulam et al. 1983b; Phillips et al. 1987). Indeed the basal nucleus also has been suggested to represent a telencephalic extension of the reticular formation of the brainstem (Mesulam et al. 1983a,b, 1984; Mann et al. 1984a; Parent et al. 1986).

Compared with other diseases in which dementia occurs, Parkinson's is distinct because of the lack of significant language impairment or agnosia (Freedman and Albert 1985; Lees 1985). Since the strongest predictors of the overall performance on the mini-mental state evolution in Parkinson's disease were bradykinesia and rigidity (see Ball 1984) a distinct parallel between clinical indications of dysfunction of the basal ganglia and the intellectual impairment might be drawn (Mortimer et al. 1982; Girotti et al. 1988). No association has been observed between the presence of cortical atrophy and the mental function, whereas ventricular enlargement on CT scans is no unusual finding (Growdon and Gorkin 1986). The degenerating neurons in AD often contain neurofibrillary tangles (Ch.III.), whereas those in Parkinson's disease or 'mesolimbocortical dementia' contain Lewy bodies (Greenfield and Bosanquet 1953; Forno 1986; Sima et al. 1986; Gibb 1989a). Although the same structures appear to be involved in both diseases, the varying pattern of neuronal degeneration suggest that their pathogenesis is not the same. Future studies must confirm the presumptive dopaminergic deficit and examine other terminal sites, including nucleus accumbens, septal nuclei and the olfactory tubercle, for their association with clinical manifestations. In Parkinson's disease the VTA may be overlooked because the usual pattern of cell loss involves the dorsolateral rather than the ventromedial region of the SN (see Morphometric section, Ch.III.). Neuron loss in the VTA and medial SN could be primary in Parkinson's disease and 'mesolimbocortical dementia', and secondary in AD (Hardy et al. 1986; Mann et al. 1987).

The general view evolving from this chapter might be summarized as follows:

1. Basal ganglia circuitry, or the extrapyramidal system, is composed of several 'loops' in relative balance and with opposing neurochemical forces including hyper or hypo-activity.

2. Whereas different circuits are closely related to different functional aspects of striatal function, ascending (reciprocal) dopaminergic projections serve to modify a descending cortico-striato-thalamic information stream.

3. Although a clear overlap within these anatomical trajectories is stressed by most authors, generally **medially placed SN and VTA** projections are characteristically connected with **cognition** and **motivational** aspects, whereas **laterally** placed dopaminergic neurons of the SNc are characteristically involved in the extrapyramidal **motor** circuit.

4. Because of its involvement in cognitive and emotional processing, the mesolimbocortical projection is very likely to be associated with the typical 'subcortical' type of dementia, the features of which mainly concern attention deficits and manipulation of available knowledge.

5. It is tempting to speculate on dementia of the Alzheimer type to be primarily a cortical disease (with secondary involvement of subcortical structures, including eventually mesolimbocortical structures) and of subcortical dementia being primarily caused by dysfunction of basal forebrain, basal ganglia and thalamic nuclei and their projections, with only secondary cortical involvement.

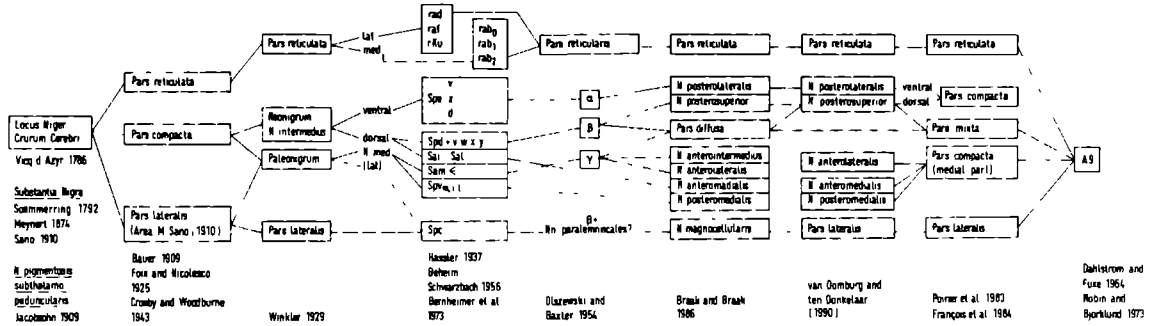


Table II. 2a

A summary of the historically grown nomenclature of the human substantia nigra area

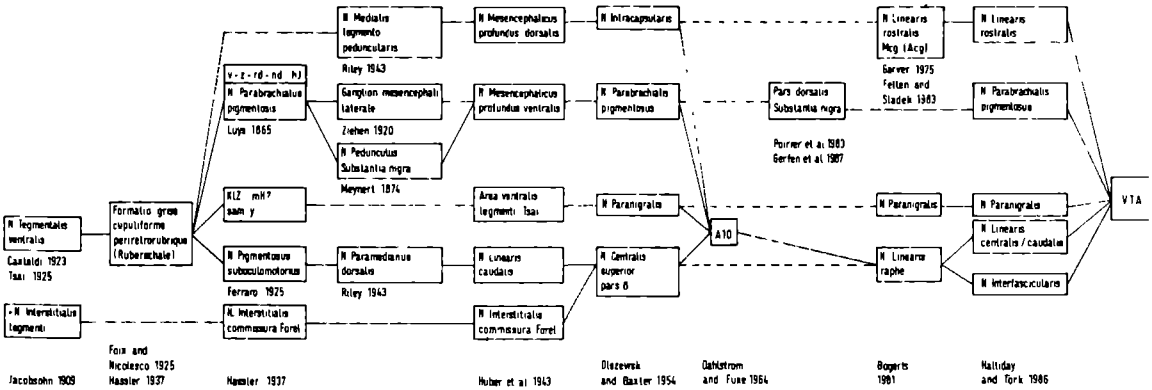


Table II.2b

A summary of the historically grown nomenclature of the human ventral tegmental area

II.2.7. An atlas of the human mesencephalon and rostral rhombencephalon

II.2.7.1. Introduction

Based on the visualization of neuromelanin-containing neurons, the substantia nigra can easily be distinguished macroscopically in the human brainstem in its rostral part (locus niger crurum cerebri, Vicq d'Azyr 1786). The substantia nigra is generally subdivided into a cell-dense pars compacta and a cell-sparse pars reticulata, as first described by Cajal(1909), based on Nissl-stained sections. A pars lateralis extends from the lateral border of A9 towards the dorsolaterally situated corpus geniculatum mediale (Bauer 1909),described as area M by Sano (1910). Various subdivisions for the human substantia nigra as well as adjacent structures, such as the ventral tegmental area, have been suggested. The historically grown differences in nomenclature are shown in table II.2.a,b. Criteria used for subdivision variably stressed cytological (Poirier et al. 1983; Braak and Braak 1986), hodological (Francois et al. 1985; Parent 1986) and biochemical (Wassef et al.1981; Waters et al.1988) data, leading to a sometimes rather confusing nomenclature. Several atlasses with subdivisions of mesencephalic catecholaminergic grisea in the human brain are available (Foix and Nicolesco 1925; Winkler 1929; Hassler 1937; Olszewski and Baxter 1954; Riley 1943; Crosby et al.1962; Nieuwenhuys et al.1988). The classic work of Foix and Nicolesco (1925) is of special value, because it includes the situation in aging and Parkinson's disease. The same holds for the detailed studies of Hassler (1937,1938), although his extensive description of pars compacta subnuclei is hardly applicable. Olszewski and Baxter's atlas (1954) gives a clear view of Nissl-stained sections, mostly referred to in literature. Riley's atlas (1943), based on Klüver-Barrera stained sections presents additional information on fiber tracts, also in horizontal and transversal sections. The terminology used for (sub)nuclei in the human substantia nigra and the ventral tegmental area, basically follows Braak and Braak's (1986) and Halliday and Törk's (1986) studies, respectively. Neuromelanin containing neurons can be found throughout the mesencephalon (Foix and Nicolesco 1925; Felten and Sladek 1983; Bogerts 1981; Saper and Petito 1982), dorsally bordering the deep layers of the superior colliculus and medially through different midline

structures. There is a continuity with pigmented neurons in the locus coeruleus and subcoeruleus caudally and the nucleus raphe dorsalis medially (Bogerts 1981). Because of the close association with nucleus ruber neurons, Foix and Nicolesco (1925) introduced the term "formation cupuliforme peri-retro-rubrique" which reaches its greatest extension at the caudal pole of the nucleus ruber.

Dahlström and Fuxe's (1964) terminology in the rat brain for this cell continuum, based on the Falck and Hillarp histofluorescence technique: i.e. A9 for the substantia nigra, A10 for adjacent neurons mainly related to the nucleus tegmentalis ventralis of Tsai (1925) and A8 for a retrorubral cell cluster, is generally accepted and overlaps with the topography of melanin-containing cells and catecholaminergic cells in the human brain (Nobin and Björklund 1973). Closely related are various structures/nuclei of the "deep tegmental gray" (Crosby et al.1962), such as the nucleus tegmenti pedunculopontinus, the nucleus centralis superior and the nucleus tegmentalis laterodorsalis (Gudden), structures that also could be involved in Alzheimer's disease and Parkinson's disease (Ishii 1966; Yamada and Mehraein 1977; German et al.1987; Jellinger 1988; Ohm and Braak 1988; Zweig et al.1988). A functional relationship with concepts as that of the formatio reticularis is considered since long, in spite of the limited knowledge about such an entity (Winkler 1929; Crosby et al.1962; Nauta 1979; Moore 1980). From a pathophysiologic point of view it would be important to know if neuropathological findings really spread through these subcortical nuclei (Neumann 1968; Saper et al. 1987) on which some etiological considerations about Alzheimer's disease are based (Rossor 1981).

There is no general consensus concerning cytological criteria on which a cytoarchitectonic subdivision could be based in the human substantia nigra (Parent 1986). In their fundamental work, Foix and Nicolesco (1925) presented general characteristics of cell body and neural processes, concerning nigral as well as perirubral pigmented neurons. For subdivisions, however, they only referred to other classical studies. On the other extreme, Hassler(1937) gave a detailed subdivision of the substantia nigra, without describing cytological details (only naming some differences in cell size). Olszewski and Baxter (1954) paired topographical data with cytological details. Hassler's approach was followed and extended with neuropathological data, by e.g. Beheim-Schwarzbach (1956) and Bernheimer et al. (1973). Although these authors gave important information about localized cell-loss and pathologic changes, they do not provide cytological

information on which a subdivision of nigral cells could be based.

More recently, Braak and Braak (1986) made an attempt to correlate cytological features to a cytoarchitonic subdivision of the substantia nigra. The subdivision of the ventral tegmental area as proposed by Halliday and Törk (1986), offers less details about cytologic features, although some typical aspects are referred to. A subdivision in nigro-striatal projection neurons is mainly based on functional considerations (Shephard and German 1988). Within the substantia nigra pars reticulata nerve cells are of different size and shape and are often devoid of neuromelanin granules, but may contain considerable amounts of lipofuscin pigment (Barden 1981). They are scattered among dendritic processes (Braak and Braak 1986), probably originating from pars compacta neurons (Rinvik and Grofova 1970). A further subdivision of the pars reticulata cannot be proposed on the basis of pigmentoarchitectonic analyses (Braak and Braak 1986). The same authors further distinguish three general types of nerve cells on the basis of soma size and shape, features of the cellular processes and the pigmentation. Type I neurons are mainly found in the pars compacta and pars diffusa. Their mean greatest diameter is about 50 μm. This medium-sized to large cell body, fusiform or less often polygonal, gives rise to two to six radiating dendrites, which occasionally branch off, one of the dendrites extending into the pars reticulata. Only neurons of the "pars diffusa" portion may send dendrites into the perirubral formation. Thorny appendages are richly present, but only a sparse number of spines are generally encountered along the dendrites. The axon arises from cell bodies or from proximal portions of primary dendrites, devoid of melanin granules. Type I neurons contain numerous elongated patches of intensely stained Nissl bodies and various amounts of neuromelanin granules. Nissl bodies as well as the nucleus are usually eccentrically located (Braak and Braak 1986). In literature the typical pars compacta projection neurons are generally identified, in different species, as medium sized neurons with long, relatively unbranched dendrites. Apart from their spread in the pars compacta, portions of at least one dendritic process extend ventrally among the fibers of the cerebral peduncle (Winkler 1929). Other portions may spread medially into the ventral tegmental area, or dorsally into the retrorubral area. Their axons may ascend laterally towards the corpus striatum or medially into the median forebrain bundle (Cajal 1909; Winkler 1929).

Type II neuron, with mean maximal diameter about 34 μm., are mainly found within the pars reticulata and pars diffusa. These cells can be either

multipolar or typical elongated, depending on the number of primary dendrites. Only the distant portion of these may bear some clusters of spines. Transparant Golgi impregnations counterstained for pigment showed the type II neurons to be devoid of neuromelanin deposits and often even devoid of lipofuscin pigment. In the Nissl stained material this aspect is less well clear, because of the intensely stained Nissl substance sharply contrasting to the pallid ground cytoplasm. Probably these cells correspond with the subclass C 1 of Marchand et al. (1979) in which they described the typical "Tigroid " in the monkey substantia nigra. These basophilic patches, more uniformly distributed around the nucleus, do not extend into the dendrites, a feature that helps to distinguish between type I and type II neurons (Braak and Braak 1986). The nuclear membrane shows numerous small infoldings, as were also described in the rat (Schwyn and Fox 1974) and the monkey (Poirier et al.1983; see also table II.1). Not all pars reticulata neurons comply with these features and details on the pars lateralis neurons are not given, in contrast to the clearly defined subset of pars lateralis neurons in the monkey (Marchand et al. 1979; Poirier et al.1983; Francois et al.1984, 1985).

Type III neurons, probably interneurons (Francois et al.1979), are generally smaller, with mean diameter about 16 µm. The few dendrites are devoid of spiny appendages and have only few branches, contrary to the axon. Several filiform processes are described with small varicose swellings. Type III neurons contain lipofuscin granules that are smaller than neuromelanin granules and stain intensely with aldehydefuchscin. Interneurons are more thoroughly described in animal species (Francois et al. 1979; Braak and Braak 1986).

Because of the great variation in soma size, also stressed in literature (Rinvik and Grofova 1970; Schwyn and Fox 1974; Domesick et al.1983; Parent 1986; Halliday and Törk 1986; Braak and Braak 1986) cell body size is not a reliable criterion for subdivision. Neurons of various subnuclei of the pars compacta have many features in common and are considered local variations of a single neuronal type (Braak and Braak 1986). Other characteristics have to be used (see table II.1). The nucleus parabrachialis pigmentosis, e.g., is hard to distinguish from the substantia nigra pars compacta dorsally unless special attention is paid to the mediolaterally orientation of these relatively small neurons (see also Olszewski and Baxter 1954; Halliday and Törk 1986). The nucleus paranigralis is continuous with the subnucleus anteromedialis but its neurons are more stellate, smaller and directed towards the more dorsomedially located interpeduncular nucleus. The

nucleus interfascicularis contains small round cells (Halliday and Törk 1986). Neuromelanin containing neurons of the midline structures (nucleus linearis rostralis and caudalis) are often oriented ventrodorsally and are topographically clearly isolated from other ventral tegmental nuclei. They are the most variable in size and shape (Halliday and Törk 1986). Generally the ventral tegmental area, like the pars reticulata, contains relatively more neurons without neuromelanin pigment, and catecholaminergic cells may be underestimated relative to TH-immunoreactivity especially in its lateral and dorsal parts (Gaspar et al. 1983). Various cell types can be found here (Olszewski and Baxter 1954; Halliday and Törk 1986), many of which contain considerable amounts of lipofuscin (Barden 1981). Cellular orientation generally follows morphological characteristics of surrounding structures, like the lemniscus medialis, root of the oculomotor nerve, fasciculus retroflexus and nucleus ruber. The distinction between dopaminergic and non-dopaminergic neurons as proposed for the non-human substantia nigra (Poirier et al.1983; Domesick et al. 1983) finds its counterpart in the human brain in neuromelanin containing neurons (Type I of Braak and Braak 1986) vs. unpigmented neurons (Hornykiewicz 1966; Bogerts 1981; Saper and Petito 1982). Morphometric analysis of different pigmento-architectonic areas might correspond with that of TH-studies (Pearson et al. 1979,1983a; Hirsch et al. 1988). Another important correspondence with studies on different non-primate species (see Ch.II.2.2.) is the similarity of pars reticulata neurons with those of the globus pallidus internus (Marchand et al.1979; Nauta et al.1978; Nauta 1979; Yelnik et al. 1984,1987; Huber 1985; Parent 1986). A complementary association between substantia nigra pars compacta and external pallidum is only hypothetical (Nauta 1979; Alexander et al. 1986:"dorsal pallidum").

II.2.7.2. Cytoarchitectonic subdivisions

The human substantia nigra and ventral tegmental area cover the entire length of the mesencephalon. Their rostral border is found at a plane connecting commissura posterior dorsally, and corpus mamillare, ventrally (see Fig.II.17.). The caudal border is present at a plane, parallel to the former, right behind the colliculus inferior, passing through the pons. In defining subnuclei, as well as area-size, comparison with data in literature (Winkler 1929; Hassler 1937; Braak and Braak 1986) is often difficult, because of the varying plane of section and of the

arbitrarily way of taking subdivisions from various atlasses. The use of "frontal" sections (e.g. Hassler 1937) i.e. perpendicular to the intercommissural axis (the line connecting commissura posterior and commissura anterior) resulted in a terminology in which rather *anteriorly* located subnuclei are named *"postero"* - lateral, which we will nevertheless follow for reasons of consistency. The description of a substantia nigra in an area as given by Braak and Braak (1986): "...from the latitude of the oculomotor nucleus to the caudal pole of the mamillary bodies" can also be explained in this way. Our sections were chosen perpendicular to the axis

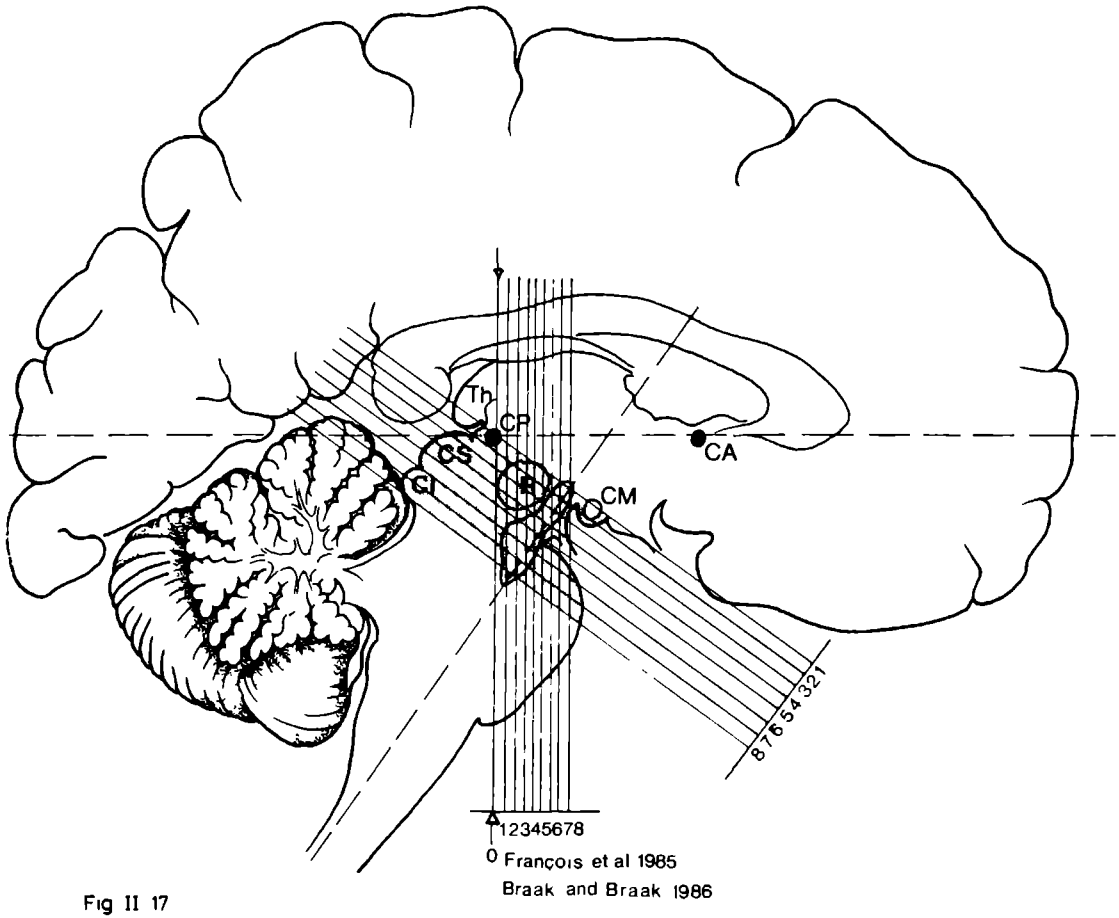
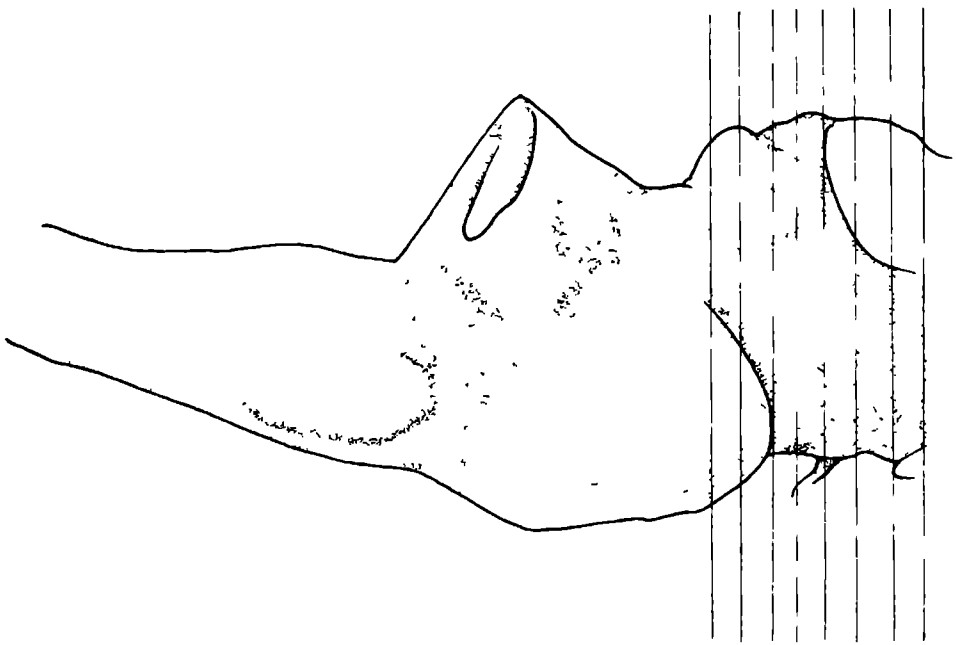


Fig II 17

Level of transection and angle of transection compared with similar studies in literature

of the brainstem, as is usually done in an atlas of the human brainstem (Olszewski and Baxter 1954; Nicuwenhuys et al.1988). Following François et al.(1985) we chose eight levels, which will be discussed subsequently. Because of the slanting position of the substantia nigra in relation to the intercommissural line, the crossing points of their transections with the substantia nigra can also be used in chosing our levels of transverse sections (Fig.II.17.).

Some variation exists in the position of different nuclei in relation to each other. Together with only seemingly subdivisions due to fiber course (Rinvik and Grofová 1970) these facts prompt to limited classification into subnuclei, contrary to Hassler's (1937)approach (Braak and Braak 1986). For the same reason it is hard to define whether the most rostral extension of the neuromelanin containing cell clusters is occupied by the ventral tegmental area (Crosby et al. 1943,1962), the substantia nigra pars reticulata (Hassler 1937; Berman 1968; Schwyn and Fox 1974; François et al.1984) or the substantia nigra pars compacta.



Figs II 18 - 25

Transections through the human mesencephalon from rostral to caudal, based on Nissl and Kluver - Barrera stained sections of brains of people >60 yrs

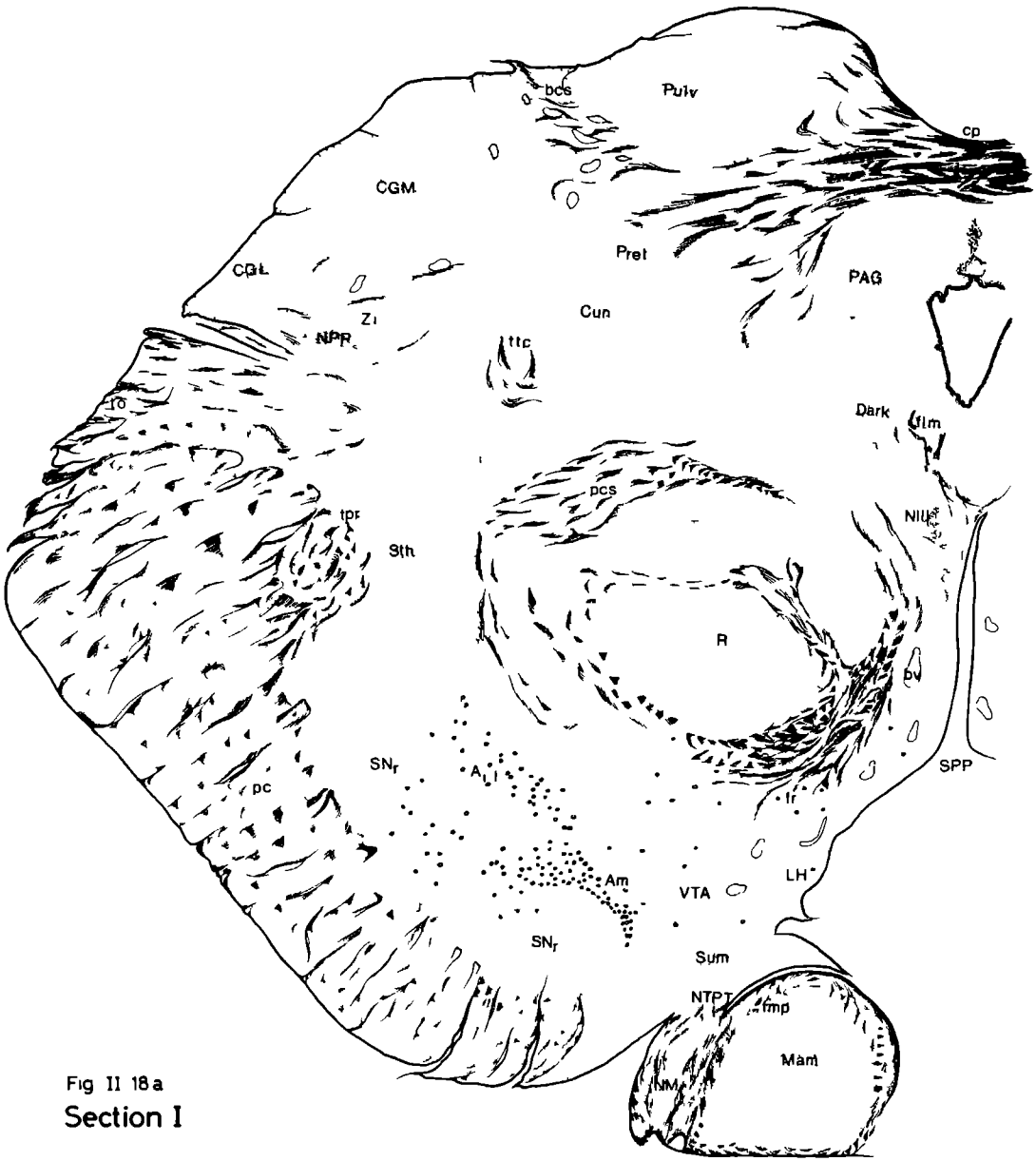
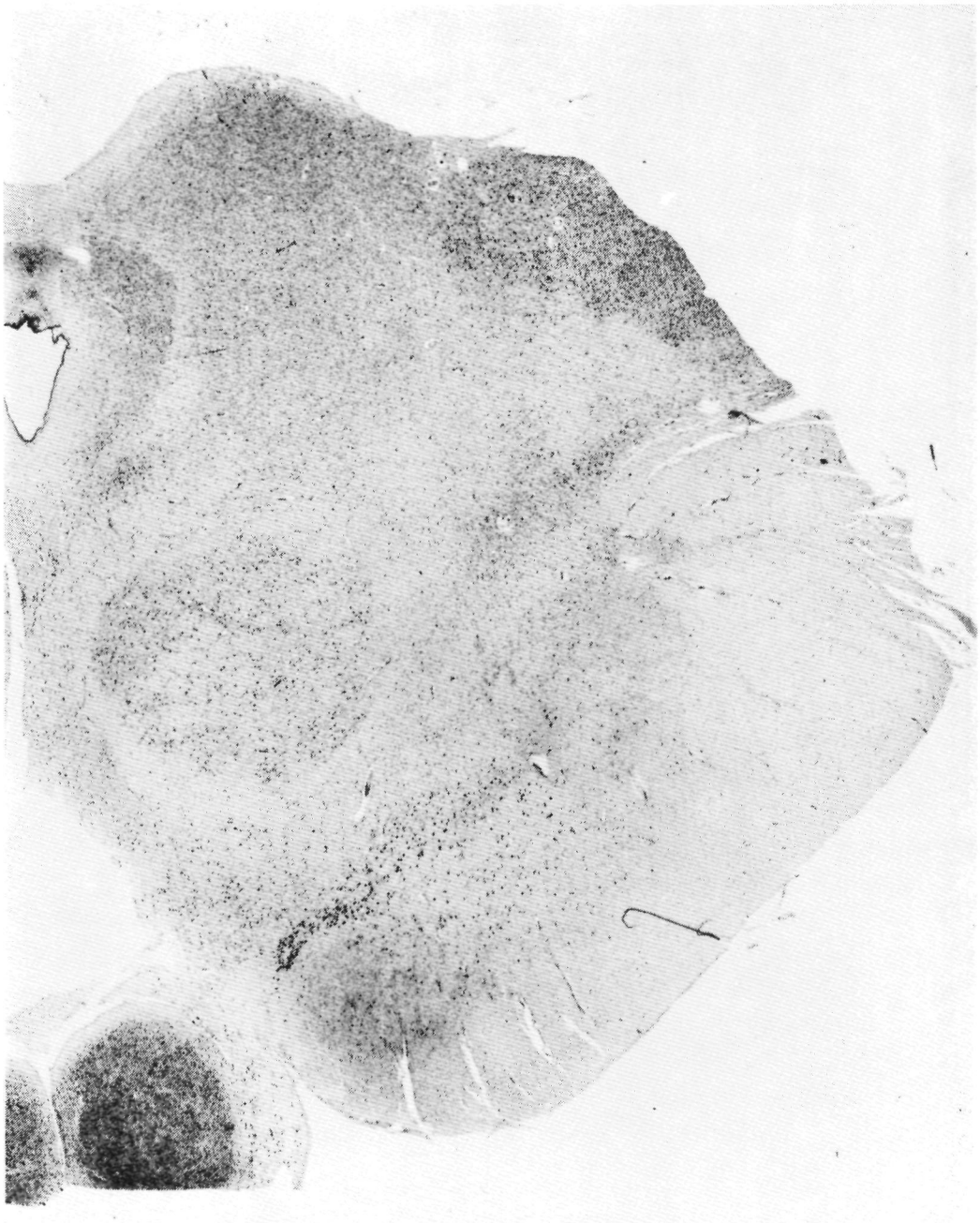


Fig II 18a

Section I

Fig. II. 18b

Microscopical section similar to Fig. 18a Nissl,(x9)



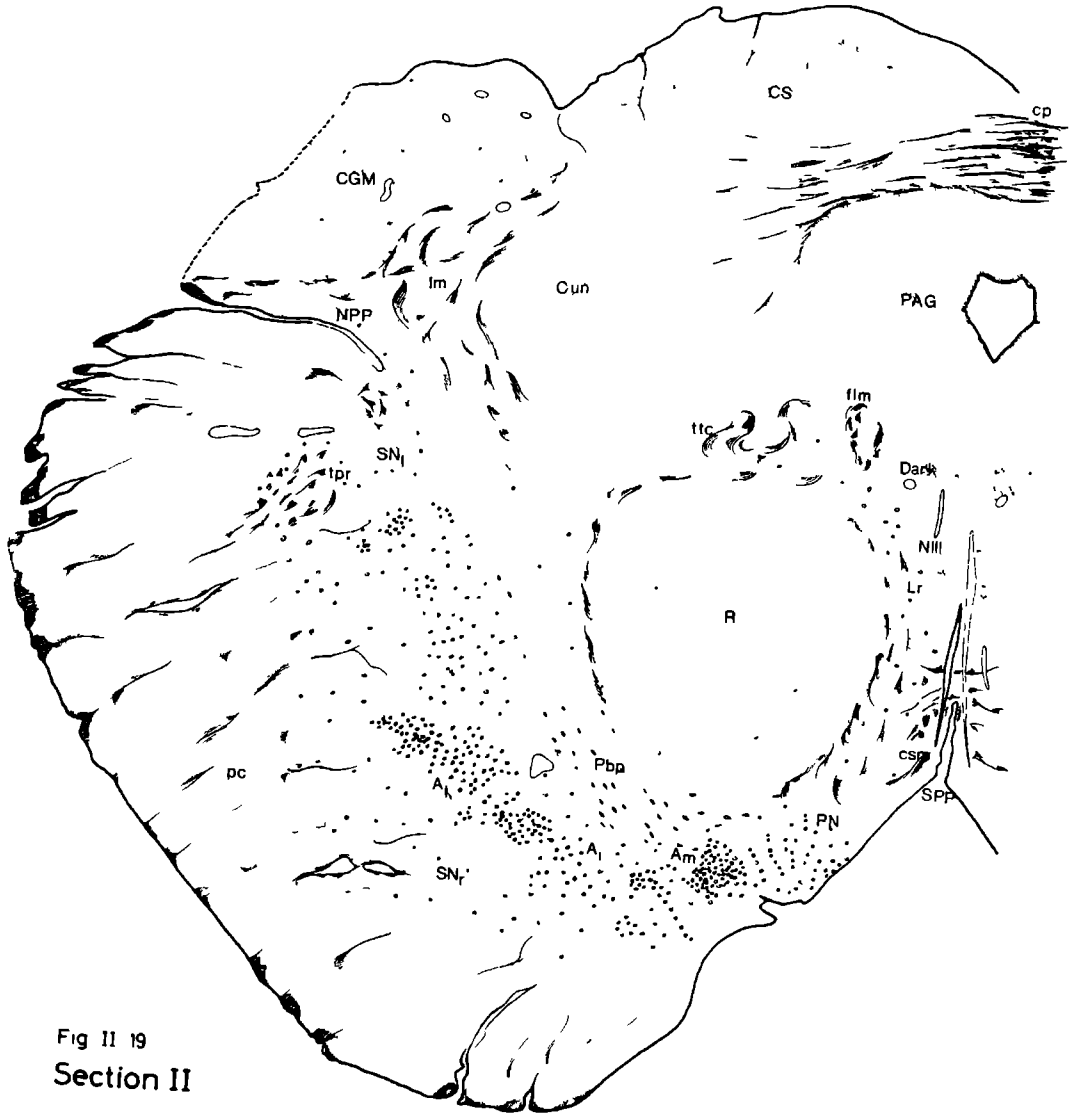


Fig II 19

Section II

Following François et al.'s (1985) description for the monkey brain, eight levels through the human brainstem will be discussed. (for reference in the human brain: Talairach and Tournoux 1988). In the first section (Fig.II.18.), through the commissura posterior and the corpus mamillare, a small cluster of neuromelanin containing cells is found at the ventromedial border of the nucleus subthalamicus, surrounded by neuromelanin-sparse neurons of the *pars reticulata* (**rab**, Hassler 1937). This cluster represents the rostral tip of the *subnucleus anteromedialis* (**Sam**, Hassler 1937) of the *pars compacta*. Between this cell group and the corpus mamillare some, densely stained, neuron clusters can be seen. Because of the presence of neurofibrillary degeneration here (see Ch.III) in Alzheimer's disease brains, it seems worthwile to define these nuclei. Crosby et al.(1962) noted that the nucleus lateralis of the corpus mamillare often is not well developed in man, just like the nucleus of the tractus peduncularis transversus (Riley 1943). The latter nucleus is comparable to the nucleus of the accessory optic tract (Sano 1910; Poirier et al.1983) and nucleus opticus tegmenti as defined in the opossum (Tsai 1925). Hassler (1937) defined a nucleus intercalatus of Ingram, apparently as part of the nucleus lateralis of the corpus mamillare (Crosby et al.1962). Likewise ill-defined are the supramamillary and the interstitial nucleus of the pedunculus mamillare (Crosby et al.1962; Poirier et al.1983), at the place where Tsai (1925) defined his *nucleus tegmentalis ventralis*. Actually, the dorsal part of this area is considered as a transition zone between the caudal hypothalamic border and the ventral tegmental area, part of Nauta's (1958) "limbic midbrain area" (extending upto the nucleus centralis superior,more caudally) and "area densa" in the atlas of Riley (1943).

At slightly more caudal levels (Section II, Fig.II.19.),a *nucleus linearis rostralis* can be distinguished, based on the presence of neuromelanin in the midline. From this level on, such pigmented neurons are rather diffusely spread throughout the midline structures, up to the raphe nuclei at the pontine level. From here on a rather well developed substantia nigra can be observed. Hassler(1937) divided the anteromedial pars compacta (**Sam**) into four subdivisions (α, β, γ, δ) of which γ probably is identical with the nucleus paranigralis. Again, his antero-intermediary nucleus is subdivided into three divisions (**m,z,l**) and laterally bordered by the anterolateral nucleus (**Sai,Sal**). At this rostral level we define two clearly distinguishable subnuclei of the anterior pars compacta: *subnucleus anteromedialis* (**Am**) and *subnucleus anterolateralis*, or *anterointermediolateralis*,

Fig II 20a
Section III

Fig. II. 20b
Microscopical section similar to Fig. 20a (Klüver-Barrera (x9)

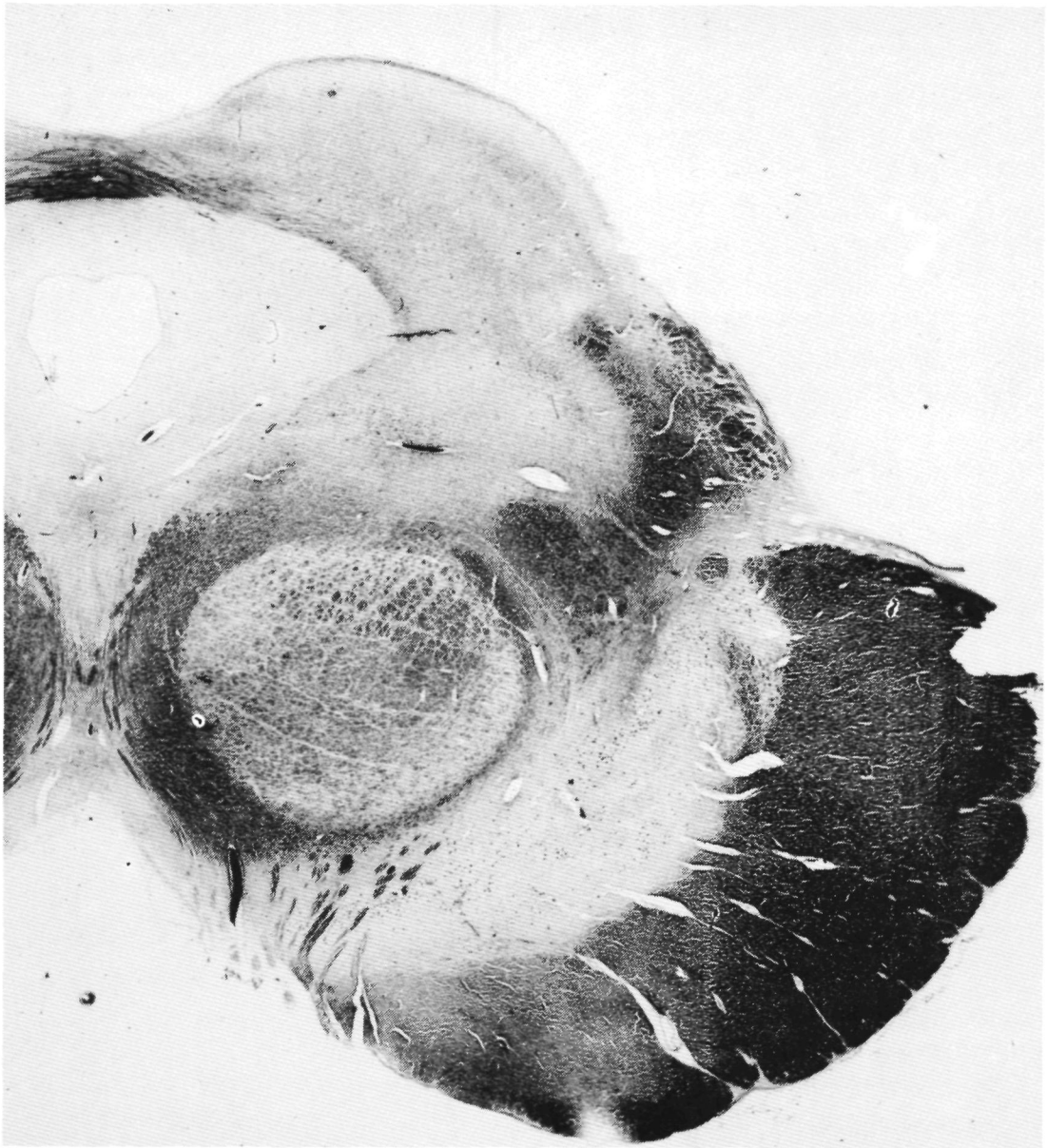


Fig II 21
Section IV

(Ai-l). A subnucleus anterolateralis is particularly distinct in horizontal sections. Following Braak and Braak's (1986) description the latter subnucleus can be divided in an intermediary and a lateral part. A further subdivision, based on the finger-like extensions in ventral direction, does not seem practical. The ventral tegmental area is well developed now, constituted by the *nuclei linearis rostralis, paranigralis* and *parabrachialis pigmentosus* (Halliday and Törk 1986), taken together by Hassler (1937) as the putamen nuclei rubri ("Ruberschale").
The nigral subnuclei are surrounded by neurons and fibers of the *pars reticulata,* mainly between the densely neuromelanin containing compacta neurons and pedunculus cerebri. Laterally the pars reticulata contains the *pars lateralis,* the latter showing slightly more pigmented and some larger neurons. The pars lateralis extends into the nucleus peripeduncularis (Olszewski and Baxter 1954).

At the level of the rostral border of the oculomotor nerve, about 1 mm. behind the caudal pole of the corpus mamillare (section III,Fig.II.20.) the ventral tegmental area is penetrated by oculomotor nerve fibers, partly masking its existence. A separate nucleus linearis intermedius (Crosby et al.1943) could therefore not be demarcated, although some neuromelanin containing neurons can be found. The substantia nigra pars compacta now clearly can be divided into a *subnucleus anteromedialis, antero(intermedio) lateralis* (part of pars γ of Olszewski and Baxter 1954) and *posterolateralis.* The latter subnucleus (pars α of Olszewski and Baxter 1954) is located more ventrolaterally (Braak and Braak 1986) The *pars lateralis* is well developed now in the most fibrous region bordering the *pars reticulata* (Poirier et al.1983; Giguère and Marchand 1983; Francois et al.1985). The impression based on our material confirms that of Yelnik and co-workers (1987), based on dendritic arborizations, that this part actually belongs to the pars reticulata (see also Schwyn and Fox 1974).
In older comparative studies it was already noted that this extension, between the cerebral peduncle and the corpus geniculatum mediale, is particularly well-developed in the primate brain (Crosby et al.1943; Francois et al. 1984). Part of the *posterolateral* and especially *posterosuperior* subnuclei represent the "intermediate" cell group, as described by several authors (Bauer 1909; Winkler 1929; Crosby et al.1943; Riley 1943; Poirier et al.1983). They correspond to pars α and ß as distinguished by Olszewski and Baxter (1954).
Hassler (1937) defined various parts of the posterolateral subnucleus: **Spz** and **Spe**, the latter being subdivided again in parts **v,z,d**. His **Spd** with its two parts,

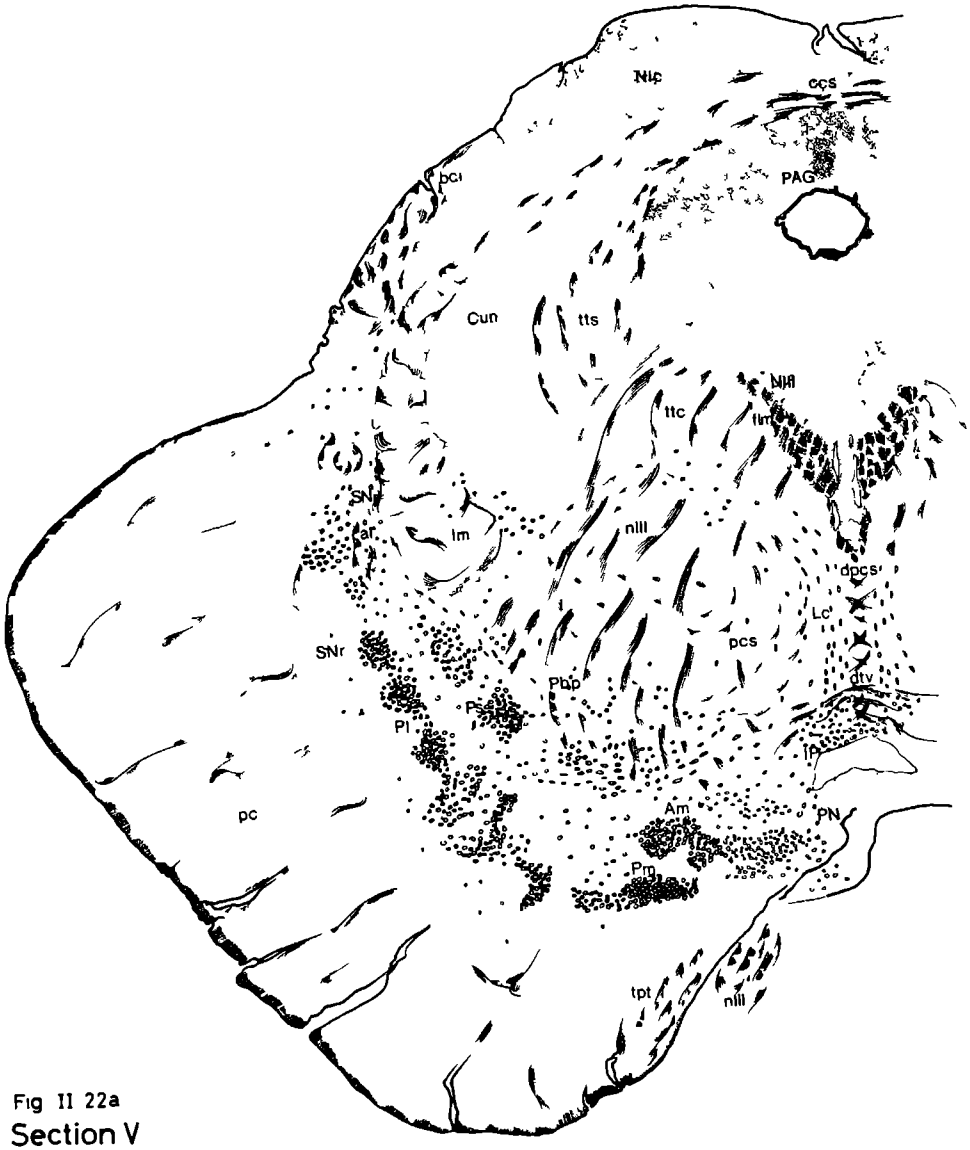
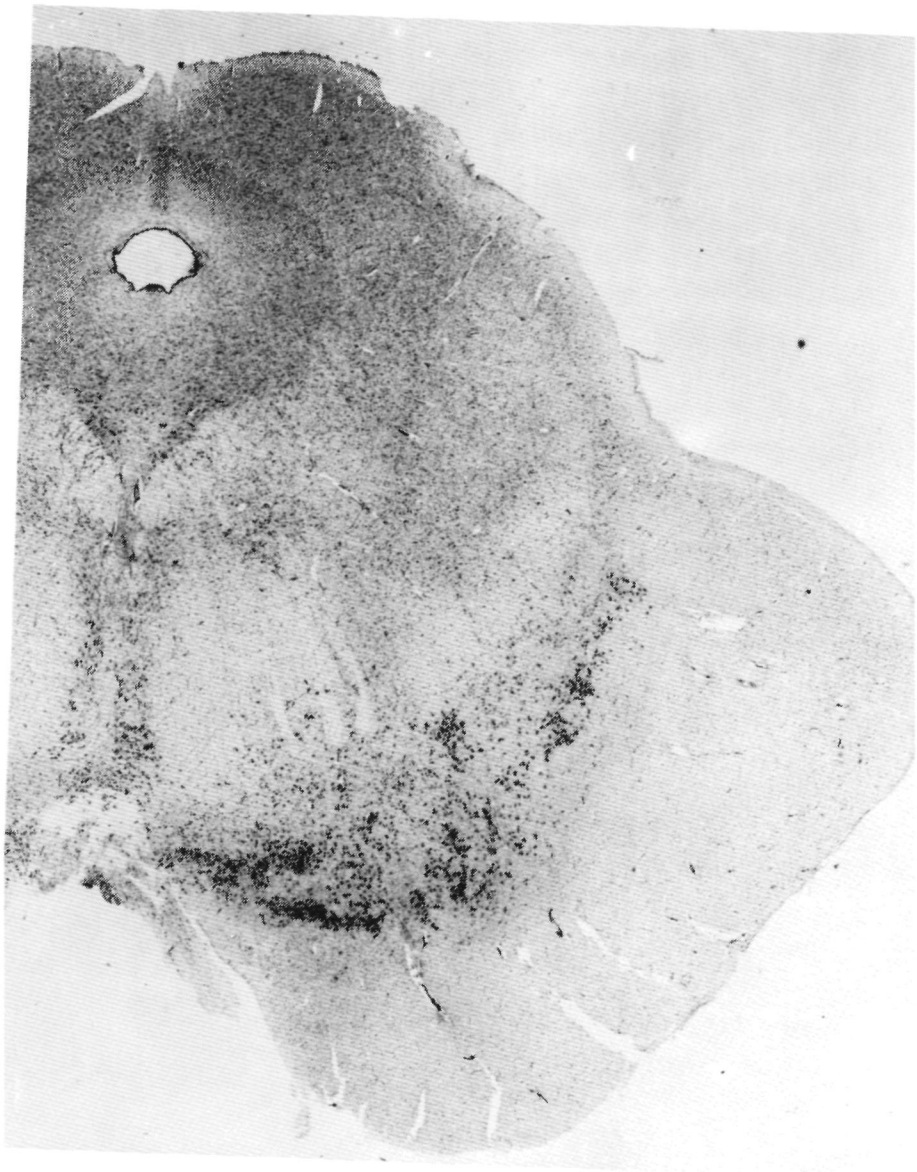


Fig II 22a
Section V

Fig. II. 22b

Microscopical section similar to Fig. 22a Nissl (x 9)



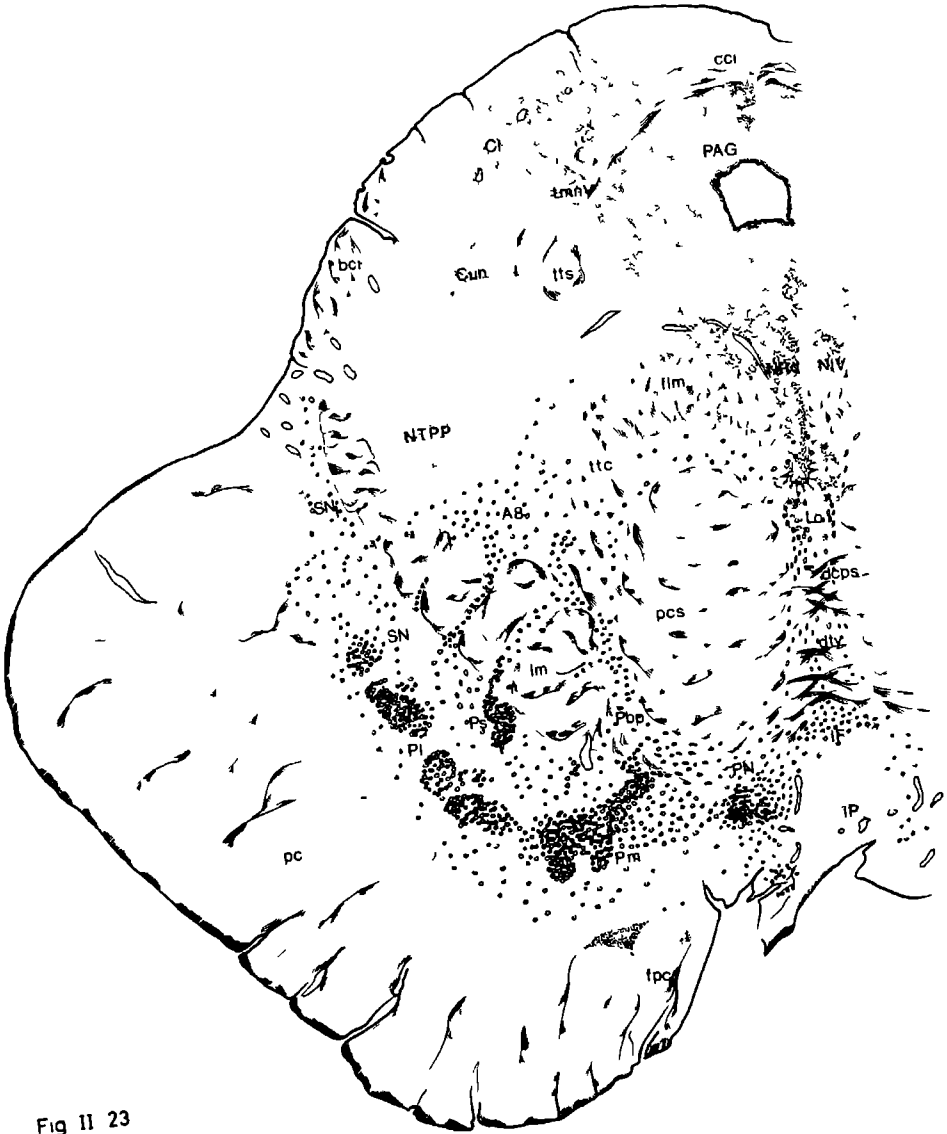


Fig 11 23
Section VI

corresponds to our subnucleus posterosuperior.

At the caudal pole of the nucleus ruber (section IV,Fig.II.21.) fibers of the oculomotor nerve leave the bulk of the midbrain tegmentum. Oculomotor nerve fibers pass through the most medial part of the pars compacta, while the lateral parts of the pars compacta (subnucleus posterolateralis and posterosuperior) are shifted to a more ventral position. The pars lateralis is well developed now. A separate subnucleus, like Braak and Braak's (1986) nucleus magnocellularis, corresponding to **Spcd** of Hassler (1937) can not be defined because of the rather diffusely spread neurons in this fibrous area (Schwyn and Fox 1974).

The subnucleus posterosuperior occupies part of, what Braak and Braak (1986) called "pars diffusa", and Francois et al. (1984) the "pars mixta". Like the "stratum intermedium" (Sano 1910) such names provide no further information on the reticulata-like surroundings of the pars compacta subnuclei. Therefore the whole nigral extension surrounding pars compacta subnuclei is termed pars reticulata in our investigation. The dorsally adjoining *nucleus parabrachialis pigmentosus,* however, should be distinguished from this nigral extension, since it belongs to the *ventral tegmental area* (Halliday and Törk 1986), and not to a "pars dorsalis of the substantia nigra" (Poirier et al.1983). At this level, however, a clear border between substantia nigra and ventral tegmental area is hard to define, unless an imaginary connection line between the more clearly demarcated medial and lateral parts is supposed. Ventral tegmental and midline nuclei are largely replaced by descending oculomotor fibers.

About one millimeter behind the nucleus ruber (section V, Fig.II.22) the ventral tegmental area shows its most typical configuration (Halliday and Törk 1986). In the midline neuromelanin-containing neurons are still sparse and a separate nucleus linearis intermedius (Crosby et al.1962) or linearis centralis (Berman 1968; Halliday and Tork 1986) is hardly distinguishable in our material.

Following Phillipson (1979a,b), who made a detailed study of this region in the rat (see also Ch.II.3), the cell cluster dorsally adjacent to the nucleus interpeduncularis is named *nucleus interfascicularis.* In sections cut perpendicular to the axis of the brainstem, however, this nucleus is found somewhat more caudally than between both fasciculi retroflexi. This area is continuous with the *nucleus paranigralis* (Hassler's **Sam,** γ) and the medial parts of the pars compacta. At this level still an anteromedial subnucleus can be distinguished, but also the beginning of the *subnucleus posteromedialis.*

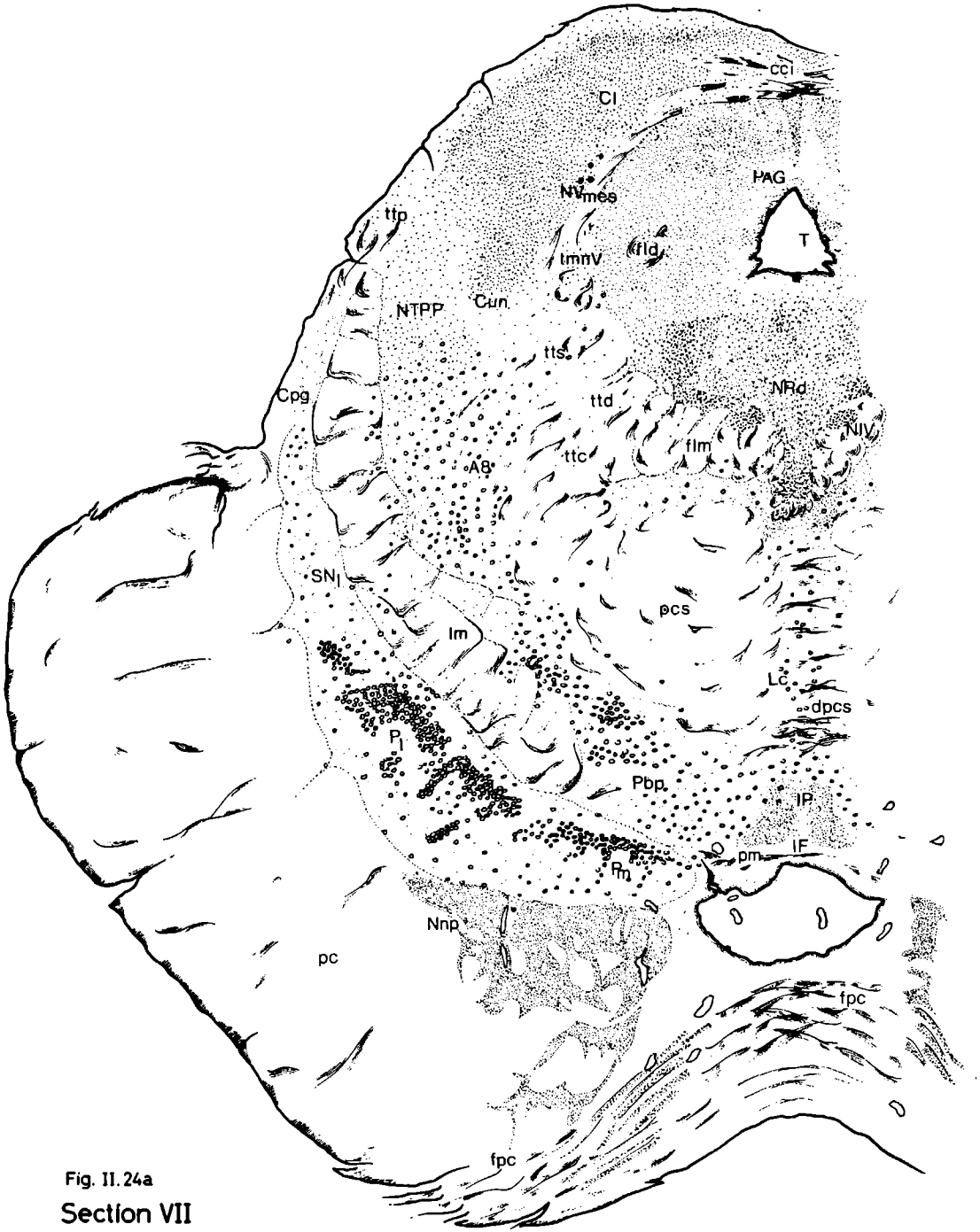


Fig. II.24a

Section VII

Fig. II. 24b

Microscopical section simular to Fig. 24a Nissl (x9)

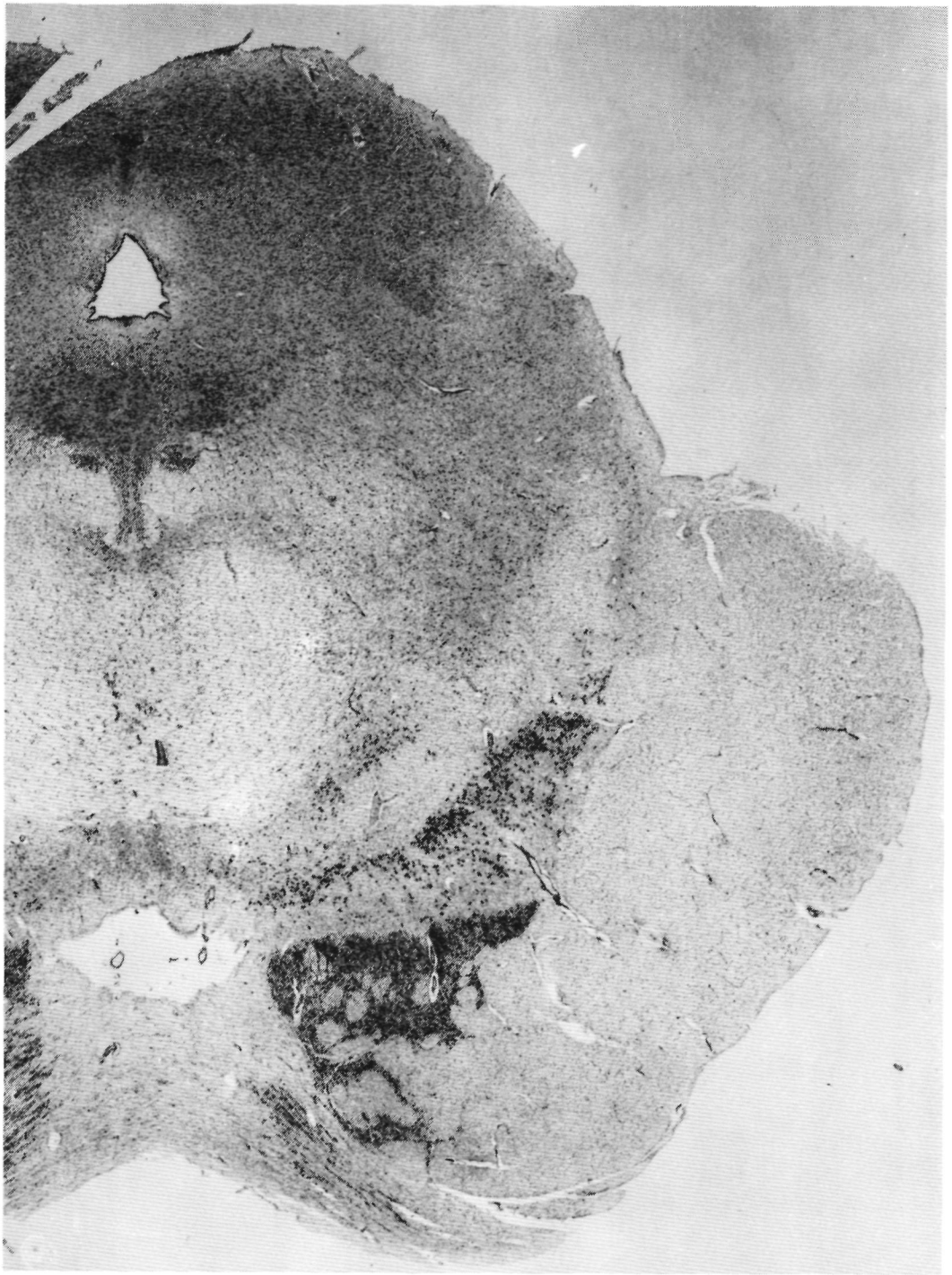


Fig. II .25

Section VIII

The subnucleus posteromedialis is composed of here by less densely packed neurons in the ventral part of the pars compacta, again subdivided by Hassler (1937) in three parts: **Spvm**, **Spvi** and **Spvl**.

Well over one millimeter behind the caudal pole of the nucleus ruber a subdivision of the pars compacta into a posteromedial and a posterolateral subnucleus becomes more pronounced (sections VI and VII, Figs.II.23 and 24). At this level the *nucleus paranigralis* is well developed, forming a continuum of substantia nigra and ventral tegmental area, but clearly distinguishable on cytologic grounds. Its perikarya are smaller, mostly oriented in dorsomedial direction and with somewhat less neuromelanin granules. Hassler (1937) defined an area **mH** here, also describing the continuity of midline structures, substantia nigra and the retrorubral extension of neuromelanin containing nerve cells. The latter area **sIII** or suboculomotor part of nigral cells (Hassler 1937) represents area A8, (Table I) following the generally accepted nomenclature of Dahlström and Fuxe (1964). Bordering, and slightly overlapping, the lateral extension of the substantia nigra the nucleus tegmenti pedunculopontinus is found (Olszewski and Baxter 1954; Beckstead and Frankfurter 1982; Nieuwenhuys et al.1988).

In the midline the *nucleus linearis caudalis* (Halliday and Törk 1986) can be distinguished, continuous with the *nucleus interfascicularis*. There is a marked spread of neuromelanin containing nerve cells into surrounding nuclei. Thus, pigmented neurons also can be found in the nucleus raphe dorsalis, the nucleus tegmenti pedunculopontinus and the nucleus laterodorsalis tegmenti of Gudden (Bazelon et al. 1967).

At the level of the colliculus inferior the nucleus raphe dorsalis is well developed and typically wing-shaped, hooking around the nucleus nervi trochlearis (section VII, Fig.II.24). It displaces the ventral tegmental area in the midline, whereas more laterally area A8 is expanding. Actually, at this level, apart from the nigral subnucleus posteromedialis and posterolateralis, hardly any demarcated subnucleus within the neuromelanin containing cell continuum can be defined. Hassler (1937) described distinct paralemniscal subnuclei (**hJ**) not distinguishable in our material. Possibly his paralemniscal nuclei are represented by the nucleus parabrachialis medialis, described by Olszewski and Baxter (1954) and the pigmented nucleus subpeduncularis, described by Ohm and Braak (1988:see also Table II.2b)

Because of the growing interest on the mesencephalic locomotor region (Armstrong 1986; Garcia-Rill 1986,Garcia-Rill and Skinner 1988) and the lack of

detailed anatomical demarcation of this area (Nieuwenhuys et al. 1988) a more detailed cytoarchictectonic map of the mesencephalic-pontine transition area would be of need for defining various structures in this region. It is remarkable that catecholaminergic neurons seem to spread throughout the grisea that form part of the ill-defined centrencephalic system (Crosby et al.1962) or reticular formation (Rámon-Moliner 1975) as well as to the mesencephalic cholinergic nuclei as defined in the rat (Mesulam et al. 1989).

In the most caudal section (section VIII, Fig.II.25), about ten millimeters caudal to the mamillary bodies, four main clusters of pigmented neurons can generally be distinguished: the caudal tip of the nucleus linearis caudalis, the nucleus parabrachialis pigmentosus and the subnucleus posteromedialis and posterolateralis of the pars compacta. Only a few intermingling pars reticulata neurons can be seen. Hassler (1937) distinguished a separated medial part (**Spcg**), which in our classification is part of the subnucleus posteromedialis. The nucleus linearis caudalis is bordered dorsally by the nucleus centralis superior (Olszewski and Baxter 1954; Nieuwenhuys et al.1988). The nucleus raphe dorsalis extends towards the other, pontine, raphe nuclei. The nucleus tegmenti pedunculopontinus can be divided in a pars compacta and a pars dissipatus. The most rostrally situated neurons of the locus coeruleus can sometimes be seen at this level, continuous with the mesencephalic catecholaminergic grisea. In the Yakovlev collection, studied by Bogerts (1981) this area is clearly defined as A7, likewise extending into the pedunculopontine nucleus and the nucleus cuneiformis.

II. 2.7.3. Discussion

Although an extensive literature on neuroanatomical and comparative aspects of the substantia nigra, ventral tegmental area and adjacent catecholaminergic structures exists, many topographical inconsistencies nowadays lead to confusion about new histochemical, functional and pathophysiological concepts. Our purpose is to relate quantitative and neuropathologic findings to well-defined subnuclei. The nomenclature is primarily based on recent studies by Braak and Braak (1986) and Halliday and Törk (1986), whereas references to older literature are necessary to resolve inconsistencies originating from the use of different standard drawings, based on different planes of section (François et al.1984).

Apart from the variation among the smallest of Hassler's (1937) subareas, not consistent in localization (Braak and Braak 1986), some general topographical principles can be defined. In the broadest sense there is a border-line between two parts of the pars compacta, running diagonally from rostro-ventro-medial to caudo-dorso-lateral (Hassler 1937).

This dual subdivision seems to be confirmed also by hodological principles (Ch.II.2.4.) and histochemical data (Ch.II.2.5). The medial pars compacta, again, can be subdivided in a medial to lateral direction, whereas the lateral pars compacta roughly tends to a composition in dorsal and ventral layers. The cell-sparse pars reticulata is not subdivided, unless different suggestions in literature (Winkler 1929; Hassler 1937; Poirier et al.1983; Table II.2a). Winkler's (1929) intermediate nucleus corresponds to most of our antero-intermedio-lateral and posterosuperior subnuclei,partly extending into the pars reticulata, His medial nucleus also covers the area of the nucleus paranigralis. The stratum intermedium (Meynert 1874; Foix and Nicolesco 1925) however, belongs to the fibrous pars reticulata, which has led to some confusion about the indication "intermediate part" (François et al.1985), sometimes defined as a separate nucleus intermedius (Riley 1943). The pars lateralis substantiae nigrae (Sano 1910) is the most fibrous part (Poirier et al.1983), with neurons provided with a dendritic pattern like the pars reticulata (Rinvik and Grofová 1970; Schwyn and Fox 1974; Yelnik et al.1987). It therefore can be considered as a lateral extension of the pars reticulata. In primates this lateral part is an important source of nigrotectal projection neurons (François et al.1984), somewhat larger in size and often rather heavily pigmented. The nucleus magnocellularis (Braak and Braak 1986) is included in our nucleus posterolateralis, but

it is not ruled out that the larger pars lateralis cell bodies also raised the suggestion of a separated entity. Different cell types are intermingled here, however, and the smaller ones extend towards the rostro-dorsally located corpus geniculatum mediale (Crosby and Woodburne 1943; Crosby et al. 1962). More caudally such neurons are scattered into the region of the nucleus paralemniscalis and the nucleus tegmenti pedunculopontinus. Following Fix (1980) a separate nucleus subpeduncularis was distinguished here by Ohm and Braak (1988).

Another nigral part, in which different neural types are intermingled (pars mixta of François et al.1985) is located dorsally of our subnucleus posterolateralis, surrounding the subnucleus posterosuperior (Braak and Braak 1986). It was defined as compacta like (or "dorsal pars compacta") by several authors (Crosby and Woodburne 1943; Poirier et al.1983; Graybiel and Ragsdale 1983; François et al.1984,1985; Gerfen 1987a).

Several less dense cell-clusters of which the subnucleus posterosuperior is the best recognizable are intermingled here with afferent and efferent fibers (Sano 1910; Crosby and Woodburne 1943; Nauta and Cole 1978; Parent 1986).

Therefore, we considered the most cell-sparse part (pars diffusa of Braak and Braak 1986) as part of the pars reticulata. It is continuous with the nucleus parabrachialis pigmentosus and this part of the ventral tegmental area probably has been misinterpreted as part of the substantia nigra by several authors (for discussion: François et al.1985).Different descriptions of the area of maximal cell loss in Parkinson's disease, like the "lateral portion" (Bogerts et al.1983; Hornykiewicz and Kish 1986), a "mid-portion" (Beheim-Schwarzbach 1956; Forno 1966), the "caudal portion" (Forno 1966) and the "ventral portion" (Foix and Nicolesco 1925; German et al.1988b), and the "neonigrum" (Winkler 1929; Riley 1943), all refer to what we call the subnucleus posterolateralis and posterosuperior of the pars compacta.

The midline structures, or linear nuclei, seem to be the most variable in size and shape,whereas packing density and other criteria (including cell number; see table III.17) sometimes show marked variation among different species (Halliday and Törk 1986). On the other hand a medial part of the ventral tegmental area is most consistently included in the ventral tegmental area in studies on the pathology of this entity (Bogerts et al. 1983; Oades and Halliday 1987; German et al. 1987; Gibb et al. 1989b). A subnucleus linearis centralis, or intermedius, often defined to indicate the transition zone between the nucleus linearis rostralis and nucleus linearis caudalis, could not be distinguished as a separate entity in our material.

Since this nucleus is well developed in the cat (Taber 1961, Berman 1968), this probably made Poirier et al. (1983) state that the "rostral linear" nucleus is unique for the cat brain. Rather the nucleus linearis rostralis is defined from the area between the nuclei of the oculomotor nerve and the nucleus ruber along its medial border in a dorsal to ventral extent. Only few pigmented neurons spread caudally into the area of the nucleus interfascicularis. Based on the appearance of neuromelanin containing cells, our subdivision seems to be most appropriate, also avoiding ill-defined entities like "pars ventralis of the nucleus supratrochlearis" (Olszewski and Baxter 1954; Bogerts et al. 1983) and "nucleus niger suboculomotorius" (Hassler 1937; Bogerts et al. 1983). It may be concluded than that Tsai's original nucleus tegmentalis ventralis mainly covers our nucleus linearis rostralis, whereas A10, as defined by Dahlström and Fuxe (1964) mostly is represented by the nucleus paranigralis and nucleus interfascicularis. These subdivisions are separated by fibers of the oculomotor nerve, passing partly through the midline structures. In its largest extent, however, the medial part of the ventral tegmental area seems to be differently developed in the human brain in comparison to other species (Crosby and Woodburne 1943; Halliday and Törk 1986; see also Rinne et al. 1989).

There is general agreement about the subdivision of the whole mesencephalic catecholaminergic, neuromelanin containing, cell continuum into three parts, A8, A9 and A10, according to Dahlström and Fuxe (1964). This entity, again, forms part of the monoamine neuron system (Hillarp et al.1966), of which Moore (1980), referring to the concept of the reticular formation, stated: "These are neuron systems not classified by the conventional criteria,that is nuclear organization, neuronal morphology and differential connections, but rather on the basis of the neurotransmitter produced by neurons comprising them". Neuromelanin containing cells spread into surrounding regions, like cholinergic nuclei tegmentalis laterodorsalis of Gudden and tegmenti pedunculopontinus, as well as pontine parabrachial nuclei (Olszewski and Baxter 1954; Bazelon et al. 1967; Marsden 1983). Next there is a continuity with the neuromelanin containing noradrenergic cells of locus coeruleus and subcoeruleus, as well as a spread into the serotonergic nuclei centralis superior and raphe dorsalis. Rostrally, neuromelanin containing neurons spread into the lateral hypothalamic zone, zona incerta and the nucleus peripedunclularis. Of particular interest is the localization of pathologic findings in these transition areas (see Ch.III). In view of our findings, the substantia nigra and, more particularly, the ventral tegmental area, have a more diffuse topography than

generally assumed. The remarkable continuity with such entities as the ascending cholinergic reticular system (Shute and Lewis 1967), the isodendritic core of the brainstem (Ramón-Moliner 1975) or the reticular formation (Hobson 1980), as well as the more recently proposed "paracores" (Nieuwenhuys 1985) are worth considering.

The overlap in distribution of cholinergic and catecholaminergic neurons has been described in the ferret (Henderson 1987) and cat (Jones and Beaudet 1987). ChAT-positive cells were described in the caudal pars reticulata in the rat, possibly of ponto-mesencephalo-tegmental origin (Gould and Butcher 1986). There has even been proposed a functional opposition of these neurochemical defined systems at ponto-mesencephalic level (Garcia-Rill and Skinner 1988). Interaction of dopaminergic and cholinergic signals (see McGeer and McGeer 1984; Niijima and Yoshida 1988), as has been proposed since long for the striatum (Glowinski et al. 1980), may well have implications for pathophysiological concepts (McGeer and McGeer 1984; Jellinger 1988b; Freeman and Gibson 1988). Future research will deliver many more details on hodological, histochemical, cytological (dendritic patterns) and functional aspects of the overlap of catecholaminergic neuromelanin containing nuclei with surrounding structures.

II.3. NEUROANATOMY OF THE LOCUS COERULEUS

II.3.1. General morphology

The largest accumulation of noradrenergic neurons in the human brainstem is a small cluster of blue-black stained cells in the dorsolateral pontine tegmentum, close to the fourth ventricle, surrounding the mesencephalic tract of the nervus trigeminus (Kemper et al. 1987; Nieuwenhuys et al. 1988). Because of its typical appearance caused by neuromelanin pigment, it was first identified in the human brainstem and named "locus coeruleus" (for review see Russell 1955). In non-primate species, where neuromelanin either is not present or can not be seen light microscopically, it was often considered as part of the adjacent trigeminal nuclear complex or related to the nucleus tegmentalis dorsalis (Crosby and Woodburne 1943; Russell 1955). Identification as one of the main catecholaminergic brainstem areas followed with the formaldehyde induced fluorescence technique of Falck et al.(1962). The initial rat brain mapping studies were done in rats by Dahlström and Fuxe (1964), referring to the locus coeruleus (LC) as area A6. Ungerstedt (1971a) provided data on the most important projections in his classic paper on monoaminergic projections. Similar studies on catecholaminergic neurons in the human fetal brain were done by Nobin and Björklund (1973) and Olson et al. (1973), whereas Hubbard and DiCarlo (1973) mapped these fluorescent structures in the monkey brain and Jones and Moore (1974), among others, in the cat.

After an initial spectrofluorimetric study by Farley and Hornykiewicz (1977), the noradrenergic character of the locus coeruleus is well-established now, by immunohistochemical staining of the rate-limiting enzyme dopamine-ß-hydroxylase (DBH), in the rodent (Swanson 1976; Grzanna and Molliver 1980; Vincent 1988) and human (Hartman 1974; Kemper et al. 1987; Chan-Palay and Asan 1989a) brain stem. Several authors also reported acetylcholinesterase (AChE) containing perikarya in the LC (Palkovits and Jacobowitz 1974; Albanese and Butcher 1980; Satoh and Fibiger 1985), morphologically indistinguishable from noradrenergic cells. It even has been stated that all noradrenergic cells also are AChE-immunoreactive (Kimura and Maeda 1982). A cholinergic input, similar to

that of the nigral CA neurons (Lehman and Fibiger 1978; Satoh and Fibiger 1985) is likely therefore. Indolamin neurons within or adjacent to the LC have been observed in the monkey brain (Schofield and Everitt 1981; Sladek et al. 1982; Felten and Sladek 1983) and cat (Léger and Hernandez-Nicaise 1980). Further histochemical characteristics of this particular histochemically heterogenous area were reviewed by Nieuwenhuys (1985). Although lacking light microscopically visible pigment in most non-primate species, perikarya are always identifiable in routinely stained preparations, whereas its dense vascularization is another outstanding uniform feature (Russell 1955; Foote et al. 1983). Blood vessels appeared directly appositted to perikarya and dendrites in the monkey brain (Amaral and Sinnamon 1977; Foote et al 1983; Felten and Sladek 1983). The close proximity of noradrenergic dendrites to the ependym led Demirjian et al.(1976) to suggest a possible neurosecretory or cerebrospinal fluid sensing function. A related noradrenaline cell cluster in the area postrema of the human brain (Kemper et al. 1987) likewise is located strategically. Felten and Sladek (1983) noted that zones of direct neuronal-vascular apposition without astrocyte interposition in monoaminergic nuclei appear to be a primate phenomenon.

In their paper on LC functions, Amaral and Sinnamon (1977) concluded that there are important parallels with the functions of the sympathetic ganglia. Generally noradrenaline projections have been implicated in a number of physiological and behavioral functions, as well as in neurological disorders (Van Dongen 1980; Foote et al. 1983; Iversen et al. 1983; Tomonaga 1983; Mann et al 1983). The traditional view of a "pneumotaxic centre" (Russell 1955) certainly is outdated now and might eventually be applied to the adjacent parabrachial region with the nucleus of Kölliker-Fuse (Nieuwenhuys et al. 1988). Variable results from physiologic experiments at least partly were due to inconsistencies of demarcation. Similar inconsistencies might lead to variable results in quantitative studies (Swanson 1976). Cell-countings concerning the LC in aging and aging diseases are mostly restricted to the neuromelanin-containing neurons (Brody 1978; Vijayashankar and Brody 1979; Bondareff et al. 1982;1986; Mann et al. 1982; 1983; Iversen et al.1983; Marcyniuk et al. 1986a,b; 1989). As regards the possibility of selective loss of melanin-containing neurons with aging (Mann and Yates 1974a,b; German et al. 1988a; Chan-Palay and Asan 1989a; Marcyniuk et al. 1989) attention must be paid also to the less well defined unpigmented neurons within the pigmento-architectonically or immunohistochemically defined borders. Topo-

graphic principles, mainly derived from animal studies, might account for the distribution of neuropathologic findings and cell loss (Marcyniuk et al. 1986a,b,1989; German et al. 1988a; Chan-Palay and Asan 1989a,b), but interspecies differences of the general distribution of the catecholaminergic neurons in the pontine tegmentum caution for oversimplification of conclusions.

The phylogenetic development of the LC, like that of the catecholamine nuclei in general, is proposed to parallel the elaboration of its cortical target areas, providing a remarkable low number of neurons innervating the extensive cortical and subcortical areas (Moore and Bloom 1978; Foote et al. 1983; Satoh and Fibiger 1985). A distinct LC, which projects to the forebrain, is not found until the reptilian and avian classes and Crosby and Woodburne (1943) continued to reduce both the nucleus LC and the nucleus laterodorsalis tegmenti to one common nucleus, even in man. Whereas the avian LC contains few hundreds of neurons, the LC in rats is composed of about 1600 cells (Swanson 1976; Loughlin et al. 1986a), in cats about 9150 (Foote et al. 1983), the monkey (subspecies Macaca) about 6500-8250 and the human with counted numbers between 10.000 to 65.000 cells (Brody 1978; Vijayashankar and Brody 1979; Bondareff et al. 1982,1986; German et al. 1988a; Baker et al. 1988, 1989; Chan-Palay and Asan 1989a). General organizational principles are recognizable in different species, but the merging of ventrolateral CA clusters with each other and with those of dorsal groups (especially LC) may reflect a more specific primate organizational feature (Felten and Sladek 1983).

Three groups, described separately by Dahlström and Fuxe (1964) in the rat (areas A4, A6 and A7) were therefore considered together as one group in both monkey (Hubbard and DiCarlo 1973) and cat (Jones and Moore 1974), where they are most loosely arranged (Berman 1968). Otherwise areas A4 and A7 are dealt with separately in primates (Felten and Sladek 1983; Demirjian et al. 1976) and human brain (Braak 1970; Bogerts 1981; Saper and Petito 1982; Pearson et al. 1983a). These areas also seem to have increased in relative size as compared to other mammals (Demirjian et al. 1976; Felten and Sladek 1983). The most compact appearance of LC (A6) cells is seen in the rat brain, consisting of a dense accumulation of uniform appearance, ventrally bordered by a less dense smaller "subcoeruleus" area, of slightly larger cells (Swanson 1976; Grzanna and Molliver 1980; Loughlin et al. 1986b). This A6 complex is easily distinguishable from the more caudoventrally located area A7 of Dahlström and Fuxe (1964). In all species the LC has its largest dorsal-to-ventral extension in the middle one-third, diminis-

hing in size again caudally (Russell 1955; Jones and Moore 1974; Demirjian et al.1976; Loughlin et al. 1986; Fig.II.26).

Confusion exists, however, as regards the identification of the nucleus subcoeruleus. In the human brain it was often situated where Dahlström and Fuxe (1964) defined A7 (Olszewski and Baxter 1954; Russell 1955; Taber 1961; Berman 1968; Nobin and Björklund 1973; Bogerts 1981; Kemper et al. 1987), whereas "A7" was ascribed to the sparse rostroventral extension, continuous with A8 (Amaral and Sinnamon 1977; Léger and Hernandez-Nicaise 1980; Bogerts 1981; Nieuwenhuys et al. 1988). Mapping studies in primates (Bogerts 1981; Tanaka et al. 1982; Felten and Sladek 1983; Satoh and Fibiger 1985) are often based on frontal sections, in which the primate brainstem is cut obliquely (see also Fig.II.17). This explains, at least partly, the seemingly rostral localization of A7, originally described "....at a level of the caudal third of the griseum pontis..." (Dahlström and Fuxe 1964). Indeed, CA labeling in the rostral deep tegmentum of the primate brain is confined to fibers and only sparsely distributed neuromelanin-containing neurons can be seen here (Pearson et al. 1983a; Kemper et al. 1987). Because the distribution pattern of CA neurons in the primate brain is best comparable to that in the rat in comparison to other non-primates (Tanaka et al. 1982) it seems appropriate to follow Dahlström and Fuxe's (1964) momenclature. Thus the term "subcoeruleus" is applied to the ventrally extending, slightly larger neurons of the LC, throughout its rostral-to-caudal extent (Taber 1961; German and Bowden 1975; Schofield and Everitt 1981; Pearson et al. 1983a; Felten and Sladek 1983). Area A7 is continous with the caudal extent of subcoeruleus neurons, but its smaller cells are identifiable by their ventrolateral-to-dorsomedial orientation, partly interspersed between fibers of the pedunculus cerebellaris superior (Taber 1961; Garver and Sladek 1975; Saper and Petito 1982; Felten and Sladek 1983; Pearson et al. 1983a). In the cat brain (Léger and Hernandez-Nicaise 1980) the LC-α corresponds with the subcoeruleus described here. An overlap with parabrachial nuclei has been described in several species (Russell 1955; Jones and Moore 1974; German and Bowden 1975; Demirjian et al. 1976; Tanaka et al. 1982; Felten and Sladek 1983; Satoh and Fibiger 1985). Other parabrachial nuclei (Fix 1980; Saper and Loewy 1980; Ohm and Braak 1988) are less well distinguishable from the reticular formation as discussed in the next section.

LC neurons are mostly described as oval or spindle shaped, with cytoplasm rich in organelles, the larger neurons having a somewhat more rounded appearance.

Several attemps to classify cellular subtypes have been performed, but only Loughlin et al. (1986) gave data on correlations with other topographic principles. Summarizing the literature on cytologic features of non-primate LC (Taber 1961; Jones and Moore 1974; Swanson 1976; Léger and Herdandez-Nicaise 1980; Groves and Wilson 1980; Loughlin et al. 1986) medium-sized multipolar and fusiform cells may be distinguished from small, stellate, neurons. Of the former 90% are catecholaminergic (Swanson 1976), with typical fluorescent appearance; the latter are probably interneurons as described by Gulley and Wood (1971), with an "isodendritic" appearance (Ramón-Moliner and Nauta 1966; Groves and Wilson 1980). In Golgi impregnated material, medium-to-large sized cells have long dendrites, that branch once or twice and extend outside the nucleus proper (Swanson 1976; Léger and Hernandez-Nicaise 1980). The same authors describe appendages on both dendrites and somata, in the rat and the cat. The orientation of dendritic fields is maximally developed in medioventral direction, reaching into the adjacent pontine central gray. Disc-shaped dendritic fields, parallel to the anterior-posterior axis of the brainstem, with predominantly longitudinal axo-dendritic synaptic configuration are described in the rat brain (Groves and Wilson 1980). Together with the subdivision in a "compacta" and a "reticulata" part (Russell 1955) and the evidence of dendrodendritic and dendrosomatic contacts between LC neurons (Groves and Wilson 1980; Foote et al. 1983; Felten and Sladek 1983) such characteristics remind us of those described in the SN (Ch.II.2; Yelnik et al. 1987).

The existence of NA "autoreceptors" on LC perikarya may be one mechanism by which adjacent regions interact (Mason and Fibiger 1979). There is substantial evidence that noradrenaline as well as the enzyme required for its manifacture, reside in LC dendrites, and is released there as a consequence of impuls activity (Groves and Wilson 1980; Foote et al. 1983).

The LC noradrenergic neurons in the human brain are less densely packed than in other species and oriented more along a horizontal plane (see Chan-Palay and Asan 1989a; Baker et al. 1989). Like in the rat, in the rostral pole of the LC larger neurons predominate, round to oval multipolar with radiating dendrites and, relatively small, eccentric nucleus. From our Nissl-stained sections (Fig.II.26.) the spread of melanin-containing neurons into adjacent nuclei is obvious. Rostrally, in the deep tegmentum, the caudal extension of A10 midline structures can be seen, whereas ventrolaterally small neurons of area A8 provide a continuity of dopami-

nergic and noradrenergic structures. The nucleus subcoeruleus, with slightly larger neurons, mainly developed from halfway to caudal, is continuous with A7 and A5, the latter adjacent to the olivary complex. Like in the animal brain, area A7 is located at caudal levels, latero-ventrally, showing its typical neuronal orientation. For the purpose of counting, neuromelanin can be used as a marker of noradrenaline, as was demonstrated quantitatively by Iversen et al. (1983) and Baker et al. (1989), but critized by others (Chan-Palay and Asan 1989a,b).

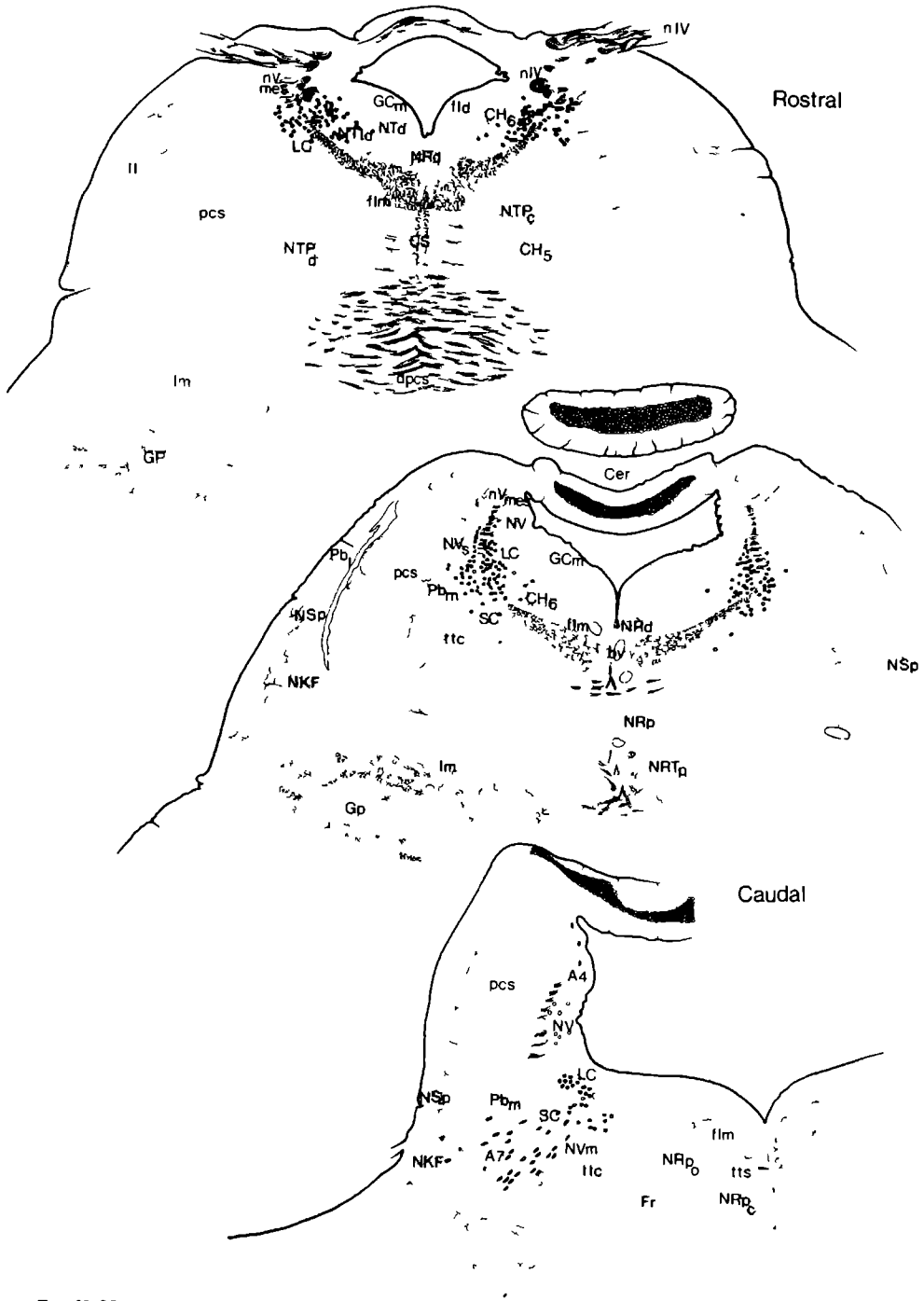


Fig II 26

Transections of the human rhombencephalon (dorsal part), demonstrating cytoarchitecture of the locus coeruleus

II.3.2. Adjacent reticular formation structures

Because of the close approximation of different rather ill-defined nuclei in the pontine tegmentum and the spread of neuropathological changes in AD and PD in this area (Ch.III), some notes on these reticular formation structures are of need. Both cholinergic and monoaminergic structures have been brought in relation to such psychologically defined phenomena, like affecting the cortical EEG and levels of consciousness and the induction of locomotion in decerebrate animals (e.g. Armstrong 1986; Leichnetz et al. 1989). Historically the problem is reflected by the search for a brainstem "relay" centre in descending motor-projections of the basal ganglia (Wilson 1914,1925; Nauta and Mehler 1966) and, otherwise, the need of a chemoarchitectonically defined substrate for the "ascending reticular activating system" (ARAS; Shute and Lewis 1967; Neumann 1968; Moore 1980; Robbins 1984).

The reticular formation is characterized by neurons of uniform morphology with extensive dendritic overlapping (Ramón-Moliner and Nauta 1966; Ramón-Moliner 1975; Hobson 1980) taking part in an "isodendritic core" which displays very little histological variation throughout the whole extent of the brainstem and basal forebrain (Ramón-Moliner 1975; Jones and Yang 1985). This core was more specifically associated with ascending cholinergic projections from the pontine tegmentum by Shute and Lewis (1967) and more recently with AD pathophysiology (Rossor 1981; McGeer and McGeer 1987). Remaining uncertainties about the organization of the reticular formation, and more particularly with the brainstem pontine tegmentum, have to do with its possible ties to the corpus striatum and monoaminergic nuclei, with unconventional nuclear organization, neural morphology and connections (Pasquier et al. 1977; Nauta 1979; Butcher and Woolf 1984; Newman 1985; Garcia-Rill 1986; Rye et al. 1987; Mori et al. 1987).

Cholinergic structures of the pontine reticular formation were separately analysed by Mesulam and coworkers in the rat and monkey (1983a, 1984) and subdivided in two main groups, Ch5 and Ch6. Cytoarchitectonic demarcation of the main related nuclei, nucleus laterodorsalis tegmenti (NTld; Ch6) and nucleus tegmenti pedunculopontinus (NTPP; Ch5), however, stems from early work on the human brain (Jacobsohn 1909; Crosby and Woodburne 1943; Olszewski and Baxter 1954). Overlap with the adjacent nuclei cuneiformis, parabrachiales and monoaminergic nuclei, particularly noted in the non-human brain, has led to a

confusing terminology (Palkovits and Jacobowitz 1974; Saper and Loewy 1980; Moon-Edley and Graybiel 1983; Rye et al. 1987). Combined studies on cholinergic and catecholaminergic markers solved many of the demarcation problems in different animal species (Palkovits and Jacobowitz 1974; Kimura and Maeda 1982; Satoh and Fibiger 1985; Jones and Beaudet 1987; Henderson 1987; DeLima and Singer 1987; Vincent 1988) and, more recently, also in the human brain (Mesulam et al. 1989). The distribution of reticular formation cholinergic systems in the human brain follows a general plan of organization similar to that described in various animal species (Mizukawa et al. 1986; Mesulam et al. 1989). The cytoarchitectonic subdivision of the human pontine tegmental cholinergic neurons in a limbic-associated part (Ch6) and an extrapyramidal part (Ch5) has no firm hodological basis in the primate yet (Satoh and Fibiger 1986).

A continuous band of choline-acetyltransferase (ChAT)- positive neurons extends from the caudolateral part of the SN to midpontine levels, adjacent to the LC complex. ChAT-positive, large, multipolar neurons of the NTPP present themselves within cross-sections that also include the A6 through A9 CA cell groups in the rat (Rye et al. 1987) and are traversed by CA axons. In agreement with the traditional view of the isodendritic core (Ramón-Moliner 1975) the dendritic arborization of NTPP neurons are comparable in morphology to the majority of reticular neurons (Rye et al. 1987). Projections on the SNr, although disputed in the rat (Rye et al. 1987), have a firm physiologic as well as neuroanatomical basis (Carter and Fibiger 1977; McGeer and McGeer 1984; Moon-Edley and Graybiel 1983; Clarke et al. 1987; Niijama and Yoshida 1988; Scarnati et al 1989). Based on connections as analysed in cats, Moon-Edley and Graybiel (1983), concluded that efferents of the NTPP that follow the pattern characteristic of a wider "extrapyramidal" region (Rye et al. 1987) of the midbrain tegmentum confirm the concept of combined reticular and basal ganglia-like structures.

In Nissl stained sections of the human brain, multipolar and darkly stained neurons of the NTPP can be seen, extending from the caudal pole of the SN subnucleus posterolateralis, in close association with the ascending limb of the superior cerebellar peduncle (Fig.II.26). Neuromelanin containing neurons are scattered through this cholinergic area (Ch5) of the NTPP (Foix and Nicolesco 1925; Olszewski and Baxter 1954), mostly belonging to the adjacent area A8. A pars compacta of the NTPP is situated at more caudal levels, dorsomedially (Olszewski and Baxter 1954), corresponding to the compact sector of Ch5 as

described by Mesulam et al. (1989). An accumulation of caudolaterally placed neuromelanin containing neurons, ventrolaterally bordering the pedunculus cerebellaris superior, was named nucleus subpeduncularis by Ohm and Braak (1988), probably corresponding to the nucleus X of Fix (1980). No clear accumulation of pigmented neurons could be identified here in our material, but small darkly stained neurons in this region, intermingled with some small pigmented neurons, might correspond with this nucleus subpeduncularis (Fig.II.26). The scattered neuromelanin containing neurons might be derived from the adjacent subcoeruleus and area A7. Remarkably enough again a cholinergic structure is identified here, slightly rostrally from this area, indicated Ch8 by Mesulam et al. (1989), mainly confined to the corpus parabigeminum. The latter can be identified as a lenticular collection of relatively small and hyperchronic neurons, flanked medially by the lateral lemniscus and dorsally by the inferior colliculus (Nieuwenhuys et al. 1988).

Although Ch5 and Ch6 are only relatively separated by the fasciculus longitudinalis medialis, a separate laterodorsal tegmental nucleus (NTld) is separately defined in the ventrolateral part of the pontine periventricular gray, immediately rostral to the locus coeruleus and lateral to the dorsal tegmental nucleus of Gudden. The location of this nucleus in the human brain (Crosby and Woodburne 1943; Nieuwenhuys et al. 1988) corresponds with that in the monkey (Mesulam et al. 1984; Satoh and Fibiger 1985,1986), the rat (Kimura and Maeda 1982; Mesulam et al. 1983a; Vincent et al. 1983; Newman 1985) and the cat (Jones and Beaudet 1987; Leichnetz et al.1989). In the latter it might be homologous with the nucleus annularis of Taber (1961). In Nissl stained sections it can be distinguished from the surrounding grey matter by the presence of roundish relatively hyperchromic cells that are scattered among smaller cells of the periaqueductal and periventricular system (Fig.II.26), resembling the multipolar neurons of the NTPP pars compacta. Because the central grey caudal to the trochlear nucleus becomes an exceedingly complex region (Mesulam et al. 1989), it is hard to define exact boundaries of this nucleus and the neighbouring dorsal tegmental nucleus of Gudden. Not only intermingling with locus coeruleus neurons occurs, but also with the medial longitudinal fasciculus and rostrally with NRd neurons. Olszewski and Baxter (1954) do not indicate a separate nucleus within the boundaries of the nucleus supratrochlearis, where Riley (1943) located the nucleus tegmentalis dorsalis accessorius. In the atlas of Nieuwenhuys et al. (1988) it is primarily functionally defined, in relation to visceral afferent pathways. The cell number is relatively low

in comparison to the findings of ChAT-positive cells as described in the cat (Kimura and Maeda 1982; Jones and Beaudet 1987) and monkey (Mesulam et al. 1984; Satoh and Fibiger 1985, 1986). In their immunohistochemical study on cholinergic neurons in the human brainstem Mizukawa et al (1986) do not mention a clearly developed nucleus at this site. But their material was mainly based on patients dying with AD and a possible cell-lose can not be ruled out.

In the rat brain noradrenergic projections of the LC and cholinergic projections of the NTld to forebrain structures are closely related (Shute and Lewis 1967; Jones and Yang 1985; Satoh and Fibiger 1986) making the interpretation of the many related physiologic findings rather difficult (Nieuwenhuys et al. 1988; Leichnetz et al. 1989). Of particular interest are direct projections to the cerebral cortex and limbic structures (Vincent et al. 1983; Jones and Beaudet 1987; Nieuwenhuys et al. 1988; Mesulam et al. 1984, 1989). Apart from this common feature with basal forbrain cholinergic nuclei, Mesulam et al. (1989) discuss differences in cytochemical features and the marked heterogeneity of primary transmitter in these areas, as was also suggested for the rat by Nieuwenhuys (1985). In contrast to e.g. striatal cholinergic perikarya, which are predominantly local circuit neurons (Graybiel and Ragsdale 1983; Graybiel 1989) the cholinergic neurons of the isodendritic core, like the noradrenergic projections, bridge long distances to thalamus and cortex. Both cholinergic and catecholaminergic neurons of the dorsolateral pontine tegmentum may be among the most important brainstem influence on forebrain and cortical activity (Jones and Yang 1985). The presence of varicosities in the ascent of cholinergic fibers through the midbrain tegmentum is suggestive of synaptic termination (Jones and Friedman 1983; Rye et al. 1987). Besides, by combined AChE and fluorescence histochemistry, it has been demonstrated that the AChE-positive neurons that have no CHAT immunoreactivity, are mostly, if not exclusively catecholaminergic (Satoh and Fibiger 1985), suggestive of cholinoception of CA neurons (Kimura and Maeda 1982). An interaction of these systems, both at the level of the brainstem (Henderson 1987) and Cortex (Oades 1985; Parnavelas and Papadopoulos 1989) might be an important factor in the influence on cortical activity.

II.3.3. Fiber connections of the locus coeruleus

Given the extensive distribution of LC projections (Fig.II.27), it is not surprising that the literature on this subject is massive. As techniques for visualizing NA efferents became more sensitive and specific, the impression of a single CA projection bundle and scattered CA terminals in the lisencephalic rat neocortex (Fuxe et al. 1965; Andén et al. 1966) evolved to the separation of three different ascending NA tracts, with much more dense and specifically organized dopamine-ß-hydroxylase (DBH) immunoreactivity throughout the six layers of the entire primate cortex (Morrison et al. 1982a; Levitt et al. 1984; Saper 1987a). The data available now, about cortical terminal patterns, effects of localized lesions (Morrison et al. 1981) and possible topographical organization of LC projection neurons, might well contribute to the interpretation of neuropathological findings of this nucleus in aging diseases.

The extensive phylogenetic development of the neocortex strongly suggests that, apart from general principles, specific anatomical and functional features of the coeruleo-cortical projections may have also become increasingly specialized (Amaral and Sinnamon 1977; Clark 1979; Morrison et al. 1982a; Foote et al. 1983; Saper 1987a). Presumably, each LC neuron sustains a highly divergent axon that innervates a large terminal field, e.g. from the entire frontal to occipital neocortex (Morrison et al. 1981; Foote et al. 1983). NA projections are similar to cholinergic cortical projections therefore, in providing diffuse cortical influence with widespread axonal distribution, contrary to DA neurons constituting but discrete systems (Moore and Bloom 1979; Satoh and Fibiger 1986; Lewis et al. 1987).
Generally speaking, the ascending and descending NA projection neurons are comprised by two major subdivisions, originally defined in the rat, where they are most clearly separated (Ungerstedt 1971a; Lindvall and Björklund 1974; Moore and Card 1984). An application of this subdivision to the cat (Jones and Friedman 1983; Nakazato 1987) and primate brain (Tanaka et al.1982; Felten and Sladek 1983; Kitahama et al. 1988) historically led to confusing nomenclature (Moore and Bloom 1979; Jones and Friedman 1983). The LC system (A6 and A4) projects mainly to spinal cord, cerebellum, thalamus and cerebral cortex with few reciprocal cortical afferents ("open loops"; Segal 1980a,b), whereas the lateral tegmental system (A1, A2, A3, A5, A7) projects reciprocally to the spinal cord, brainstem, hypothalamus and basal forebrain (Clark 1979; Moore and Card 1984). As unique

component of the lateral reticular formation the long catecholaminergic bundle serves to interconnect the multiple adjacent CA neurons along its course, especially in the mammalian brain (Levitt and Moore 1980a; Jones and Friedman 1983; Felten and Sladek 1983; Nieuwenhuys et al. 1988) Thus, e.g., somatomotor or discrete visceromotor nuclei are innervated by lateral tegmental NA groups, suggesting that these adjuvant NA cells may have a general function in regulation of autonomic, visceral and neuroendocrine activity (Levitt and Moore 1980a). Otherwise, the LC, including subcoeruleus, also innervates primary sensory areas, such as the cochlear nuclei, and pontine reticular formation nuclei.
Summarizing the literature a schematic representation of pathways and terminal areas is given in Fig.II.27, indicating the most likely situation in the human brain. Ascending LC axons have their initial course in three major pathways, that appear to be rather uniform in different species:

1. The dorsal noradrenergic bundle (dnb) travelling ventrolaterally from the periventricular/aquaductal grey, originating mainly from the locus coeruleus. This appears to be a rather universal tract phylogenetically, branching to numerous mesenphalic and diencephalic grisea, following the dorsal part of the mfb (Ungerstedt 1971a; Lindvall and Björklund 1974; Garver and Sladek 1976; Nieuwenhuys et al. 1982; Jones and Friedman 1983; Jones and Yang 1985; Astier et al. 1987; Kitahama et al. 1988). LC fibers projecting to the cerebellum frequently send collaterals to the forebrain via this dorsal noradrenergic bundle (Saper 1987a). Authors studying monoamine histofluorescence (Nobin and Björklund 1973; Garver and Sladek 1976; Felten and Sladek 1983) observed a higher amount of monoamines prenatally, which apparently disappeared with age. This projection is also associated with the "Acg" (that is the nucleus linearis rostralis in our nomenclature) as described in the monkey (Garver and Sladek 1976; Felten and Sladek 1983).

2. The ventral noradrenergic pathway
According to Ungerstedt (1971a) the cell groups A1, A2, A5 and A7 (Dahlström and Fuxe 1964) give rise to this ascending fiber system, ascending through the reticular core (see above). The terminal areas of this bundle include: the ventrolateral part of the periaqueductal grey in the mesencephalon and the hypothalamus, especially periventricular, infundibular, supraoptic and paraventricular nuclei.

Telencephalic terminal areas include the area preoptica and bed nucleus of the stria terminalis (Nieuwenhuys et al. 1988). Descending bulbospinal fibers terminate in the ventral horn, intermediolateral nucleus and substantia gelatinosa.

3. The dorsal longitudinal fasciculus of Schütz is, much like the mfb, a composite system consisting of thin ascending and descending fibers. It occupies over its entire length a periventricular position and synaptical interruption may occure at e.g. the dorsolateral tegmental nucleus of Gudden. LC projections provide only a minor contribution to this fiber system, interconnecting important autonomic centers of the hypothalamus and lower medulla oblongata.

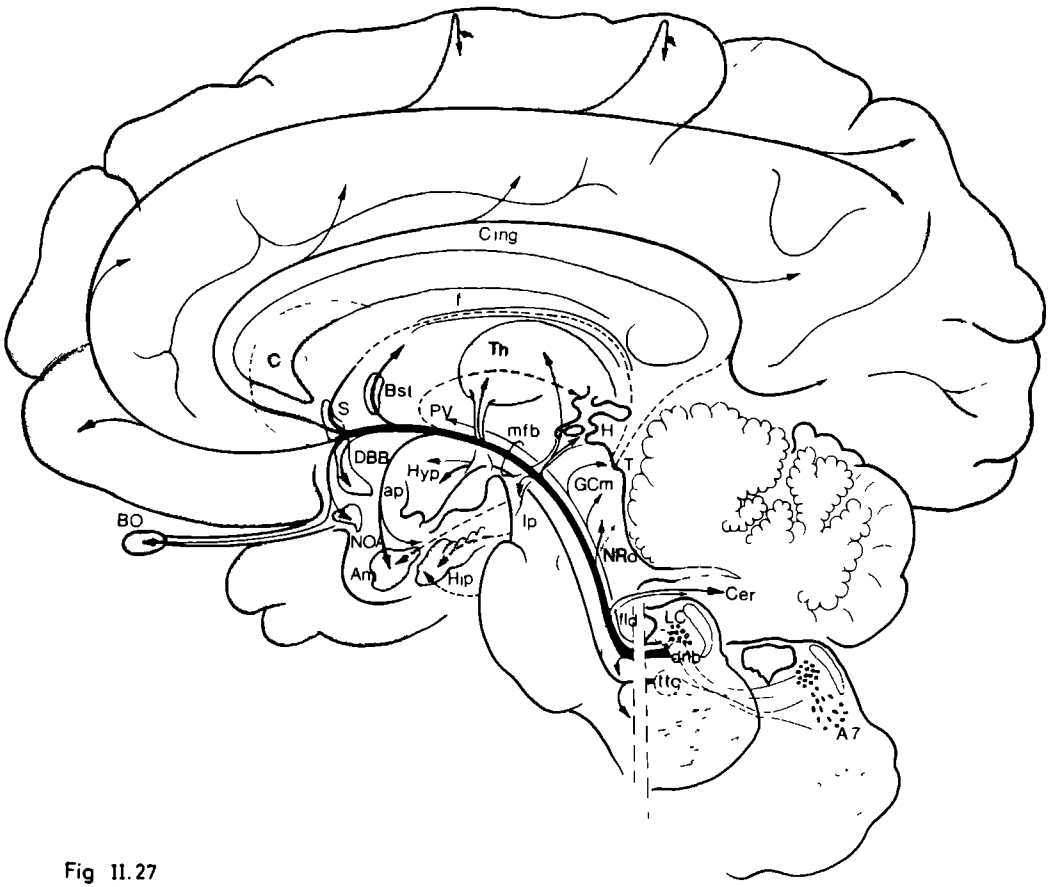


Fig 11.27
Efferent projections of the human locus coeruleus, rostral and caudal parts

Unlike their absence in reviews mentioned before, projections to the rostral caudate-putamen in the rat (Mason and Fibiger 1979) and primate (Parent et al. 1983) suggestive of NA-DA interaction at this level, are included here. The coeruleo-cortical system in the primate brain embodies organizational principles that contrast with those of the classical thalamocortical or cortico-cortical systems, which are primarily organized in a modular fashion and terminate in narrow radial columns with minimal arborization in the horizontal plane (Morrison et al. 1981, 1982a; Levitt et al. 1984; Saper 1987a). The highest NA concentrations have been observed in the cingulate, frontal and insular cortices, followed by visual, auditory and sensorimotor cortices respectively (Moore and Card 1984; Saper 1987a). The dorsolateral prefrontal cortex of the primate brain, however, shows a relatively sparse innervation as compared to other cortical areas (Morrison et al. 1982a; Levitt et al. 1984; Lewis and Morrison 1989). Regional differences in the prefrontal cortex of monkey species were demonstrated by Lewis and Morrison (1989). Unless regional variation in density, the laminar pattern was rather similar across cytoarchitectonic areas. The particular dense CA innervation around sulcal invaginations in all cerebral lobes might reflect a specialization in gyrencephalic brains (Levitt et al. 1984).

Differences between rat and monkey also exist as regards an additional lateral trajectory in the latter, following the amygdalofugal pathway to the temporal lobe and possibly also innervating more posterior cortical regions (Morrison et al. 1982a; Levitt et al. 1984). Whereas the cingulum bundle, mainly provides the medial cortex, a third route follows the ventral caudate and capsula externa, through the cortex in an anteroposterior direction (Morrison et al. 1981; Saper 1987a) in different species. Axons course through cortical layer VI, until reaching the terminal field where they turn to the pial surface and branche in a grid-like pattern. In rats they mainly innervate layers I-V, in a laminar configuration consistent especially throughout the lateral neocortex (Saper 1987a). The density of NA terminals is greatest in layers I,IV and VI (Moore and Card 1984).

In cats and monkeys axons are mainly directed towards layer II and III (Levitt et al. 1984), but substantial regional variation exists in terminal pattern as well as fiber orientation, especially in the medial cortex (Morrison et al. 1982a; Foote et al. 1983; Lewis and Morrison 1989). Thus, DBH-immunoreactive synaptic junctions in dorsolateral prefrontal and somatosensory cortices were mainly found in layers IV and V, whereas DBH immunoreactivity in layers I, II, III and VI stem

from passing fibers, running paralell to the pial surface in layer VI (Morrison et al. 1982a). The innervation pattern of the primary visual cortex differs fundamentally in that layer IV is the least densely innervated and the NA innervation is primarily directed towards the pyramidal cells of layer V and VI (Morrison et al. 1982a). The highest density of NA innervation surrounding the central sulcus of the primate has a less clear geometric pattern, although some laminar complementarity with dopamine projections might be expected (Morrison et al. 1982a).

A striking complementrary relationship of NA and serotonin innervation patterns of the primary visual cortex in the monkey brain (Morrison et al. 1982b) suggests a high degree of specificity of both monoamine projections. A similar complementary laminar distribution of DA and NA patterns, hardly distinguishable in histofluorescence preparations, has been demonstrated in the anterior cingulate cortex (Lewis et al. 1988b; Lewis and Morrison 1989). Such cortical areas apparently show more marked laminar differentiation, in comparison to the lateral neocortex. The small cortical field that lies at the juncture of cingulate and prefrontal cortex shows a confluence of DA, NA and serotonin projections, suggesting a key role in the convergence of subcortical inputs to both limbic and frontal cortices (Porrino and Goldman-Rakic 1982; Levitt et al. 1984). The exact meaning of these divergent monoamine projections remains to be elucidated yet (Saper 1987a).

Like other cortical NA afferents, hippocampal NA by far mostly stems from LC projections, as demonstrated both neuroanatomically and electrophysiologically (Moore and Bloom 1979; Segal 1980a,b; Foote et al. 1983). The densest projections are directed to the hilus of the fascia dentata, stratum lucidum of CA3 and the molecular layer of the subiculum, close to the entorhinal cortex. This innervation reaches the hippocampal formation from the ansa peduncularis-ventral amygdaloid bundle, the fornix and the cingulum, espectively (Segal 1980a,b; Moore and Card 1984; Nieuwenhuys et al. 1988).

The question whether LC neurons are spatially clustered according to their targets (and possibly reciprocal afferents) can only be answered according to the most rudimentary principles. Apparent discrepancies in the literature are partly due to differences in terminology and methods (Loughlin et al. 1986a). Ascending LC projections might interfere also with cholinergic projections, as has been described

for the cat (Nakazato 1987) and more caudoventrally placed parabrachial projections in the rat (Saper and Loewy 1980). Besides many LC cells can be doubly labelled after injections at widespread target sites (Waterhouse et al. 1983; Foote et al. 1983; Loughlin et al. 1986a,b; Saper 1987a). The terminology used here corresponds with that of most studies in rats (e.g. Mason and Fibiger 1979; Waterhouse et al. 1983; Loughlin et al. 1986a,b). The best documented (both by tracer studies and electrophysiological) topographical relationship is that of ventrally placed LC and subcoeruleus neurons projecting to the spinal cord in rats (Nygren and Olson 1977; Mason and Fibiger 1979; Moore and Bloom 1979; Loughlin et al. 1986a), cats (Nakazato 1987) and monkeys (Foote et al. 1983). Other targets of ventrally placed neurons are the caudate nucleus and cerebellum (Mason and Fibiger 1979; Foote et al. 1983; Loughlin et al. 1986a,b). Another well-documented projection is that of the dorsally placed cells to the hippocampus and septum, preferentially in the caudal half (Mason and Fibiger 1979; Segal 1980a,b; Loughlin et al. 1986a,b). Thalamic projections have their origin throughout the LC, but cortical projections preferentially originate from dorsal layers (Mason and Fibiger 1979; Loughlin et al. 1986a; Nakazato 1987). For an individual neuron within the central, compact LC, the probability of projecting to a given critical target varies along the dorsal-ventral gradient, as regards occipital, sensorimotor and frontal projections, respectively (Waterhouse et al. 1983). Findings in the cat (Nakazato 1987) and rat LC (Waterhouse et al. 1983) differ somewhat, in that in cats ascending cortical projections are mainly found in the rostral part of the LC and preferentially midway-to-caudally in the rat.
In conclusion, spatial preferences of LC projection neurons do not represent complete segregation, whereas possible interspecies variation needs further documentation. It may be said, however, that dorsally placed neurons preferentially project to cortical targets, hippocampus and septum, and ventrally placed neurons preferentially descend or reach no further than subcortical structures.

In contrast to the knowledge of the physiology, pharmacology and efferents of the LC system,little is know about neural inputs that regulate its activity (Foote et al. 1983; Aston-Jones et al 1986). Many of the afferents suggested in literature need further confirmation (Aston-Jones et al. 1986). The bulk of afferents arises from medullary nuclei: nucleus prepositus hypoglossi and nucleus paragigantocellularis (Guyenet and Young 1987), whereas other substantial sources of LC

afferents are paraventricular hypothalamic nuclei (Swanson 1987) and intermediary grey of the spinal cord (Aston-Jones et al. 1986). The other afferent projections that have been described (Foote et al. 1983; Moore and Card 1984) might have been directed to adjacent parabrachial nuclei that have similar routes of projection as the LC (Saper and Loewy 1980; see also Newman 1985). The spread of dendritic ramifications far beyond the cell body region of LC (Ch.II.3.1.) may contribute to more widespread afferent inputs, as was already demonstrated for hypothalamic projections (Mason and Fibiger 1979). Afferents from nuclei in close proximity of the LC could not be excluded by Aston-Jones et al. (1986).

Anterograde tracers, injected into the VTA produced labeled fibers entering the LC, whereas injections into the SNc did not (Beckstead et al. 1979; Swanson 1982). Details on prefrontal cortical afferents in the primate brain are provided by Arnsten and Goldman-Rakic (1984), who noted densest terminals at rostral levels of the LC, originating mainly in dorsolateral and dorsomedial prefrontal areas. They therefore suggested **that NA and serotonin containing cells, which innervate widespread areas of cortex, receive a selective prefrontal cortical projection, whereas the dopamine containing cells, which innervate more restricted areas of cortex receive afferents from widespread cortical regions.** Histochemical studies showed adrenaline, serotonin, substance P, enkephalin and neurotensin containing terminals within the boundaries of the LC (Moore and Card 1984; Arnsten and Goldman-Rakic 1984).

Fig.II.28. gives an account of the extrapolated afferent projections in the human brain (Foote et al. 1983; Moore and Card 1984; Nieuwenhuys et al. 1988), with dotted lines for more controversial projections (Aston-Jones et al. 1986).

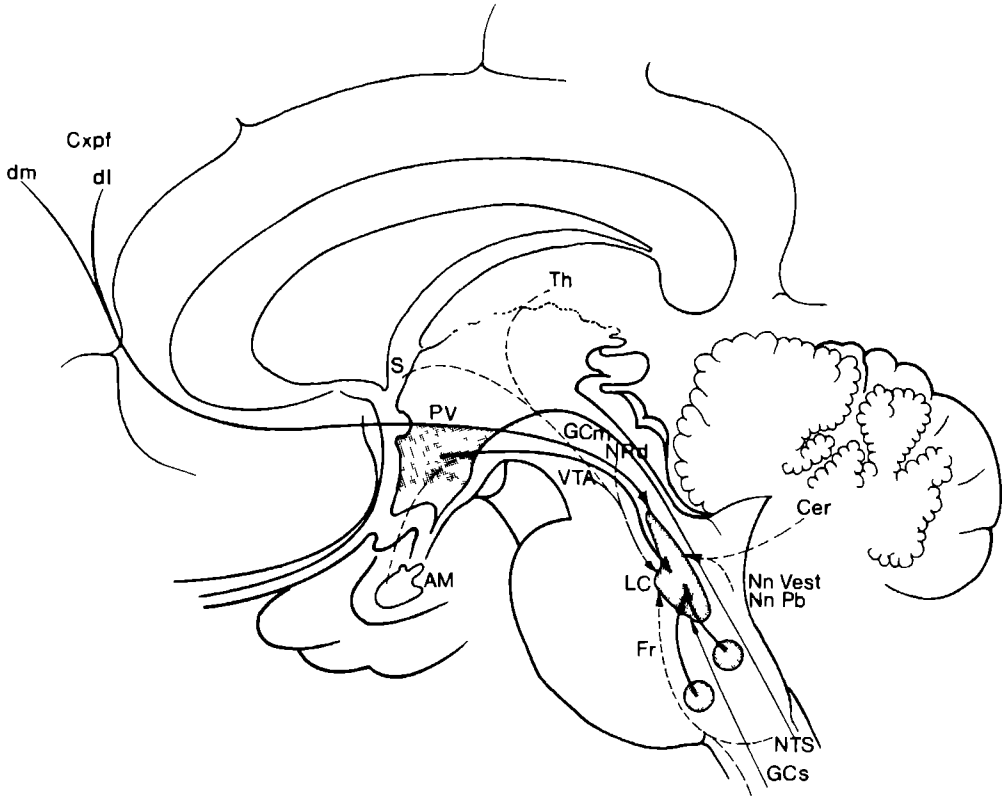


Fig II 28

Afferent projections of the human locus coeruleus

II.4. NEUROANATOMY OF THE NUCLEUS RAPHE DORSALIS

II.4.1. General morphology of the human nucleus raphe dorsalis (NRd)

As was noted already for the other monoaminergic structures, the phylogenetic advance and enlargement of the cerebral cortex also led to a considerable increase in the extent and volume of the raphe nuclei (Hubbard and DiCarlo 1974b; Parent 1981: Sladek et al. 1982: Felten and Sladek 1983; Ohm et al. 1989). Although the term "raphe" characterizes the position close to the midline, nowadays it is most commonly associated with serotonin- histochemistry. Raphe nuclei and serotonergic cell groups do not overlap 100 % (see Felten and Sladek 1983) and Dahlström and Fuxe (1964) introduced a new classification of the indolamine-containing cells in the rat brain stem, i.e., nine groups designated B1 through B9. The distribution pattern and characteristics of the serotonin groups are essentially similar in the rat, cat, monkey and human brain (Dahlström and Fuxe 1964; Hubbard and DiCarlo 1974b; Wiklund et al. 1981; Felten and Sladek 1983; Steinbusch and Nieuwenhuys 1983), but there is no generally accepted nomenclature of the related raphe nuclei in different mammalian species (Braak 1970). Generally an oral and a caudal group is distinguished, the former including a central and a dorsal portion. The central portion is indicated as nucleus centralis superior (Olszewski and Baxter 1954; Taber 1960) or nucleus raphe medianus (Crosby and Woodburne 1943; Conrad et al. 1974; Azmitia and Segal 1978). Caudal raphe nuclei are located throughout the length of the medulla oblongata (Taber 1960; Riley 1943; Braak 1970).

In the monkey (Sladek et al. 1982) and cat (Léger and Wiklund 1982) brains their is a "lateralization" and a further degree of mixing of serotonin with other monoaminergic groups in the brainstem (see also Léger and Wiklund 1982; Felten and Sladek 1983). Steinbusch and Nieuwenhuys (1983) already described serotonin-immunoreactivity in the SN and VTA and actually noted that most of the serotonergic elements might be found outside the boundaries of the raphe nuclei in

the rat brain. The serotonin group is the first to appear in ontogenesis (Parent 1981) and the few serotonin-positive neurons situated in the reticular formation of rats might be neurons that during ontogenesis did not complete their migration toward the raphe region (Steinbusch and Nieuwenhuys 1983). In the cat the ratio between serotonergic and non-serotonergic neurons in the nucleus raphe dorsalis is 70 % (Wiklund et al. 1981), whereas in rats only one-third of the NRd neurons seems to be serotonergic (Descarries et al. 1986). The intermingling of different monaminergic elements had been noted to be of particular significance in quantitative analysis of age-related changes in specific serotonergic neuron systems in the mammalian brain (Sladek et al. 1982).

Data regarding architectonical and neuronal details of the human nucleus raphe dorsalis (NRd), corresponding roughly with cell group B7 of Dahlström and Fuxe (1964), mainly stem from Olszewski and Baxter (1954), Braak (1970) and Ohm et al. (1989). Serotonergic neurons within the raphe nuclei have no special structural characteristics that make identification possible in Nissl stained sections. In the human brain the strongly lipofuscin-pigmented nuclei of the raphe system are particularly well recognizable in the PFAAF staining of Braak (1980), that was also used by Ohm et al. (1989) for description of cytologic details. The dorsal raphe nucleus extends for a distance of 20-25 mm from the level of the oculomotor nucleus to the level of the caudal pole of the locus coeruleus (Braak 1970; see als Figs.II.24,II.25.). It is continuous with the nucleus raphe linearis rostralis and nucleus interfascicularis of the VTA, based on pigmento-architecture. The nucleus raphe centralis in the nomenclature of Braak (1970; see als Ohm et al. 1989) greatly corresponds with nucleus linearis centralis and caudalis of the VTA, as defined by Halliday and Tork (1986) and followed by us (Fig.II.24.). Midline raphe nuclei and midline VTA nuclei are continuous, in the pontine tegmentum passing to the nucleus centralis superior and nucleus reticularis tegmenti of Bechterew (Olszewski and Baxter 1954; Braak 1970; Ohm et al. 1989) The NRd has its greatest extent and densest cellular area dorsomedially bordering the trochlear nucleus (nucleus supratrochlearis pars dorsalis of Olszewski and Baxter 1954). Midway from this wing-like formation an unpaired intercalate subnucleus (Ohm et al. 1989) had a cellular density and lipofuscin pigmentation that make it hardly distinguishable from the surrounding central mesencephalic grey substance. The small neurons within this subnucleus might be DAergic (Descarries et al. 1986) as has been suggested also for the cat (Wiklund et al. 1981; Léger and Wiklund 1982) and

monkey (Tanaka et al. 1982). Few neuromelanin-containing neurons could be found here in the human brain (see also Saper and Petito 1982). A possible corresponding portion of the dorsal raphe nucleus in other species displays either a lesser number or a different kind of serotonin-containing neurons (Hubbard and DiCarlo 1974b; Molliver 1987). Both parts contain a high amount of small neurons (Ohm et al. 1989). At more caudal levels a similar diffuse transition with the nucleus laterodorsalis tegementi stresses the need of histochemical techniques for the exact identification of NRd neurons.

Histochemical studies in the rat brain (for review see Steinbusch and Nieuwenhuys 1983; Weissman et al. 1987) and anti-serotonin-immunohistochemistry in the human fetus (Takahashi et al. 1986) confirm the preponderance of serotonin containing neurons in the traditionally defined boundaries of the NRd, extending into the VTA and locus coeruleus complex. Within the cytoarchitectonically defined NRd of the human brain Ohm et al. (1989) distinguished five neuronal types:

1) Large ovoid to polygonal neurons with much lipofuscin; 2) Likewise cells, displaying more dust-fine and faintly stained pigment granules; 3) Medium-sized, ovoid to polygonal neurons with loosely distributed, small pigment granules; 4) Small ovoid neurons devoid of pigment or with few intensely stained granules; 5) Small spindle shaped nerve cells with various amount of intensely stained lipofuscin granules.

Golgi-Cox studies in monkeys (Felten and Sladek 1983) revealed the presence of small clusters of dendrites oriented vertically in the midline, tangentially around the circumferential edge of the fasciculus longitudinalis medialis that also vertically or obliquely spread into the latter. These bundles consisted mainly of primary or secondary dendrites of NRd neurons, although shafts of fourth ventricular tanycytes and dendrites of the dorsal tegmental nucleus cells also were present. Similar observations in rats have been reviewed by Steinbusch and Nieuwenhuys (1983). Like other monoaminergic nuclei, serotonerigc neurons in primates provided zones of direct neuronal vascular apposition, as demonstrated electronmicroscopically (Chan-Palay 1976; Felten and Sladek 1983). The attachment of serotonergic dendrites to blood capillaries and the supraependymal plexus on the ventricular surface (Richards et al. 1981; Steinbusch and Nieuwenhuys 1983). derived from NRd and nucleus centralis superior neurons, might have implications for AD pathophysiologic considerations (Hardy et al. 1986).

II.4.2 Fiber connections of the nucleus raphe dorsalis

By far the most data on ascending projections of the NRd have been obtained from studies in rats (for review Nieuwenhuys et al. 1988). The pattern of terminals generally might be derived from studies on cortical serotonergic fiber distribution in different species (see Steinbusch 1981; Köhler and Steinbusch 1982; Morrison et al. 1982b; Takeuchi and Sano 1983; Stuart et al. 1986; Molliver 1987; Mulligan and Törk 1987, 1988; DeLima et al. 1988), but these terminals might originate from other raphe nuclei as well, especially raphe medianus, i.e. nucleus centralis superior (see Köhler and Steinbusch 1982; Molliver 1987; Vertes and Martin 1988). Besides, projections from basal forebrain serotonergic neurons have to be excluded, as was already discussed concerning lesion experiments by Andén et al. (1966). Specific ascending pathways of the NRd have been studied preferentially with tritiated aminoacids in rats (Conrad et al. 1974; Azmitia and Segal 1978) and cats (Bobillier et al. 1976), but some detailed topographical principles could also be obtained from retrograde labeling studies (Tohyama etal. 1980; O'Hearn and Molliver 1984; Waterhouse et al. 1986).

Apart from serotonergic fibers, NRd efferents containing other neurotransmitters, like substance P, thyrotropin-releasing hormone and methionine-enkephaline (for review see Steinbusch and Nieuwenhuys 1983), have been shown to originate from raphe nuclei in rats. The meaning of a particularly large number of peptides in the NRd has to be elucidated yet. Because of the lack of data on NRd efferents in primates, such fiber systems in the human brain, are mainly based on hodological data of the cat brain (Bobillier et al. 1976) and different studies on rats (Nieuwenhuys et al. 1988). The highly branched fibers of the NRd together innervate virtually the whole central nervous system, thus comprising the most exentisive central neuronal network yet described. From the study of Felten and Sladek (1983) on monoamine pathways in monkeys it can be concluded that descending projections in primates, like those of non-primates, are mostly derived from more caudally located raphe nuclei. There are extensive connections with the reticular formation (Steinbusch and Nieuwenhuys 1983) and retrogradely labeled brainstem nuclei including nucleus laterodorsalis tegmenti, locus coeruleus, nuclei raphe pontis and raphe magnus, nucleus reticularis tegmenti pontis and substantia grisea pontis (Tohyama et al. 1980; Leichnetz et al. 1989).

In all mammalian species studied, ascending fibers travel mainly in a paramedian zone through the midbrain ventral tegmental area (Andén et al. 1966b; Ungerstedt 1971a; Azmitia and Segal 1978; Bobillier et al. 1976; Felten and Sladek 1983), were serotonergic axon terminals form axodendritic contacts with both DAergic and non-DAergic cells (Hervé et al. 1987). At the level of the mesencephalic junction, the main bundle progresses ventrally and laterally to the mamillary bodies, but adjacent nucleus interpeduncularis and SNc are more specifically projected on by the nucleus centralis superior (Bobillier et al. 1976). After entering the medial forebrain bundle, NRd axons course similar to the other diffuse cortical projection systems, i.e., a lateral system through the substantia innominata to the external capsule and a medial pathway continuing rostrally through the septum. (Saper 1987a). The latter divides into a branch that runs back through the fornix to the hippocampal formation and another branch that runs over the genu of the corpus callosum and into the frontal cortex and cingulate bundle (see also Tohyama et al. 1980). Whereas projections from the nucleus centralis superior primarily contribute to the medial pathway (see also Felten and Sladek 1983), fibers of the NRd contribute to both projections, similar to the coeruleo-cortical projection system (Saper 1987a). A much smaller dorsal ascending serotonergic pathway courses within the fasciculus longitudinalis dorsalis of Schütz and a ventrolateral bundle follows the fasciculus longitudinalis medialis before joining the ventral ascending system in the VTA, giving off fibers to the SN (Bobillier et al. 1976; Azmitia and Segal 1978). Few fibers were seen in the SNc of the rat, cat and monkey, but in the SNr these fibers were densely and uniformely distributed in the rat and cat; the pars lateralis seems to contain more serotonergic fibers in higher mammals, suggesting an essential role of serotonin in extrapyramidal circuitry (Mori et al. 1987).

Fibers to the medial habenular nucleus and various thalamic centres follow the habenulo-interpeduncular tract, but also the stria medullaris. At more rostral levels fibers course through the ansa peduncularis to the amygdaloid complex and follow the capsula interna to the striatum. DAergic neurons of the NRd projecting to the striatum probably are continous with mesostriatal projections of the VTA and were observed mainly in a ventral subdivision, bordering the nucleus linearis caudalis (Parent et al. 1983a; Descarries et al. 1986). The ratio of DAergic to serotonergic fibers projecting to the striatum can not be determined with the current state of kwowledge (see also Steinbusch et al. 1981). The concentration of serotonin-immunoreactive fibers in the neostriatum was high in ventral, medial and caudal

parts and especially the area bounded by the globus pallidus, in rats, cats and monkeys (Mori et al. 1985), decreasing progressively from the caudal to the rostral plane. In both cat and monkey the distribution pattern of serotonergic fibers is more inhomogeneous, especially in the caput nucleus caudatus (Mori et al. 1985). Likewise a serotonergic fiber distribution pattern in the rat and cat was more uniformely, whereas the lateral pallidal segment in monkeys showed more sparse immunoreactivity. Projections to both dorsal and ventral striatum have their origin mainly in NRd boudaries, as was demonstrated in cats (Bobillier et al. 1976) and rats (Steinbusch et al. 1981; Steinbusch and Nieuwenhuys 1983). Projections to the nucleus accumbens, probably following the external capsule (Nieuwenhuys et al. 1988) have been regarded to be involved in the modulatory mechanism of nociception (for ref. Li et al. 1989).

Hypothalamic centres that contain NRd terminals include dorsomedial, ventromedial and infundibular nuclei, but also anterior and lateral hypothalamic areas, medial and lateral preoptic areas are innervated by NRd efferents (Nieuwenhuys et al 1988). The nucleus suprachiasmaticus might be reached by a specific dorsal raphe arcuate tract sending axon terminals also to the ventral corpus geniculatum laterale, as described by Azmitia and Segal (1978). The medial and lateral septal nuclei are reached through Broca's diagonal band and fibers passing the septum course to caudal hippocampal subiculum and cornu ammonis, as well as entorhinal cortex. The latter projection might originate preferentially from the nucleus centralis superior. The molecular layer of the gyrus dentatus is mainly reached by fibers of the perforant pathway or fornix, originating specifically from NRd neurons (Azmitia and Segal 1978). A high density of serotonin radio-ligand receptor binding was noted in area CA1 and the molecular layer of the dentate gyrus, whereas minimal labeling occured in the pyramidal cell layer of the CA2/CA3 region (Stuart et al. 1986). These data suggest primarily a role for the nucleus centralis superior in hippocampal innervation, as was also suggested by Köhler and Steinbusch (1982).

Most of the areas described also contained serotonin receptors in the monkey brain (Stuart et al. 1986), correlating particularly well with the distribution as reported in cortical and thalamic targets. As regards diencephalic nuclei the highest receptor binding was observed in the ventral lateral geniculate nucleus, paraventricular nucleus and parafascicular/central nuclei (Stuart et al. 1986). The relative absence of radio-ligand labeling in the hypothalamus, despite high levels of

endogeneous serotonin, is indicative of either serotonin cell bodies without serotonin receptors or a different subtype of serotonin receptors on hypothalamic cells.

Within the NRd a topographic ordering exists with respect to rostrocaudally aligned terminal fields in the ipsilateral cortex of rats (Tohyama et al. 1980; O'Hearn and Molliver 1984; Waterhouse et al. 1986). The rostral part of the NRd projects preferentially to reticular formation, SN and thalamic nuclei, shifting laterally to ventral parts of the caudate-putamen to enter the capsula externa to piriform and entorhinal cortices; the caudal part also provides descending axons and fibers innervating hypothalamic nuclei, ascending in the mfb more medially to occupy Broca's diagonal band and entering the cingulum (Tohyama et al. 1980), thus overlapping allocortical projections of the nucleus centralis superior, like those to the hippocampus (Köhler and Steinbusch 1982). Projections to isocortical areas arise mainly from the middle three fifths of the dorsal raphe nucleus (see also Saper 1987a for review).

Neurons projecting to the motor, sensorimotor and visual cortical areas were concentrated in dorsal intermediate and ventral portions of the NRd, respectively (Waterhouse et al. 1986). Some overlap in hypothalamic with sensorimotor cortex projections and caudate-putamen with both motor and sensorimotor cortical projections has been suggested, whereas spinal cord projection neurons overlap with the position of retrogradely labeled NRd neurons of the occipital cortex (Waterhouse et al. 1986). In primates putamen-labeled cells tend to occupy a more lateral position relative to caudate-labeled cells of the NRd (Parent et al. 1983). A substantial number of NRd neurons provide common input to neocortical and cerebellar areas that receive afferent information related to the same sensory or motor function (Waterhouse et al. 1986).

The greatest density of serotonin innervation in the monkey cortex is in the **primary sensory areas**, particularly the visual cortex (Morrison et al. 1982b; Takeuchi and Sano 1983; DeLima et al. 1988), with the most fibers distributed in **layer IV**, complementary to the densest NAergic fiber distribution in layers III, V and VI. Apparently NRd projections converge with geniculo-cortical input in spiny stellate cells of layer IV (DeLima et al. 1988). Generally, however, labeled axons and boutons are scattered diffusely, throughout the six layers of the cerebral cortex (Takeuchi and Sano 1983; Saper 1987a), whereas highest levels of radio-ligand binding to serotonin receptors were observed in cortical layers I-II (Stuart et al.

1986). Like NAergic afferents, long non-varicose "tract-fibers" where noted preferentially in layers I and VI, in both rats and cats (Takeuchi and Sano 1983; Mulligan and Törk 1988). Only minor differences have been noted between innervation patterns of other neocortical areas. In the primary motor cortex (area 4 of Brodmann) the overall number of immunoreactive fibers was small, particularly in layers IV and V, whereas the pattern in sensory and secondary visual cortex (i.e. area 18) roughly corresponded with that of the primary visual cortex. The basket-like terminal pattern as described in the cat (Mulligan and Törk 1988), resembling that formed by enkephalin, substance P and tyrosine-hydroxylase containing axons in septum and frontal cortex of rats, might be species-specific and thus represents no generalized innervation pattern (Mulligan and Törk 1988).

Although afferents to the NRd originate in far less widespread regions, they provide an important pathway in limbic-midbrain projections, as was suggested by Nauta (1958). The final relay in a massive hippocampal-raphe conduction route is formed by the interpeduncular nucleus, which provides a substantial part of NRd afferents (Groenewegen et al. 1986). A similar role is performed by the lateral habenular nucleus (Herkenham and Nauta 1977,1979), integrating the raphe nuclei into a typical limbic circuitry (Nieuwenhuys et al. 1988). The only neocortical region which projects directly to the mesencephalic raphe nuclei is the prefrontal cortex (Arnsten and Goldman-Rakic 1984).

III. MORPHOMETRIC SECTION

"Man wird bemerken können, daß ein guter Kopf nur desto-mehr Kunst anwendet, je weniger Data vor ihm liegen; daß er, gleichsam seine Herrschaft zu zeigen, selbst aus den vorliegenden Datis nur wenige Günstlinge herauswählt, die ihm nicht geradezu widersprechen, und daß er die feindseligen zuletzt so zu verwickeln, zu umspinnen und beiseite zu bringen weiß , daß wirklichnunmehr das Ganze nicht mehr einer freiwirkenden Republik, sondern einem despotischen Hofe ähnlich wird."

J.W.Goethe, Zur Naturwissenschaft, 1793

III.1. INTRODUCTORY REMARKS

In this chapter the results of the morphometric analysis will be presented. The problems concerning the sampling procedure will be discussed later (Ch.III.5.1.), but criteria as to what actually is counted will be dealt with here briefly. Essentially the following data have to be obtained:

1. Cellular size: Necessary not only for the determination of the correction formula (Ch.I.3.), but also as an indication of possible age-related changes after age 60. Differences in cellular shape may be derived from differences in largest diameter (Dm=D-max) as compared to spherical diameter (Dc=D-circle) and cellular surface (A=area). All numbers represent micrometers (µm). As regards the nucleus raphe dorsalis (NRd) no data of cellular size were collected. The cell number of the NRd is based on the counting of nucleoli, and no correction formula for splitted elements has to be calculated.

2. Number of cells counted: The absolute cell-number is determined, taking sections throughout the antero-posterior extent of the nucleus and possible tissue shrinkage, will not influence counting results. Counted numbers are multiplied by a factor to correct for the part of the whole structure that is counted and a correction formula as presented in chapter I.3.2. The data presented in the following tables are 'real cell numbers'; i.e. without limitations that are statistically quantifiable. They might be compared with any other 'real cell number' supplied by other quantitative studies. Where the mean cell number of different samples has been calculated usual *statistical procedures for calculation of standard deviation (SD) and significance were evaluated with Student's t-test.* Cytological criteria as to the incorporation of cellular elements are based on the data supplied in the anatomical section. Neuromelanin pigmented neurons are always identifiable, but cells without neuromelanin pigment sometimes are hard to distinguish from glia. Practice indicated neurons smaller than about 10 µm (mostly of typical dark spherical appearance) to belong to glial elements. Therefore, neuronal elements smaller than half a square of the counting grid (i.e. smaller than 12,5µm) are not included, unless they contained neuromelanin or other characteristic features of the nuclei under consideration.

3. Semi-quantitative data on senile plaques and neurofibrillary tangles. Typical examples of neurofibrillary tangles and senile plaques as can be found in the human brainstem, are shown in Fig.III.1. Criteria as on the identification of these neurodegenerative changes are descriptive, but no doubt representative. Comparison with criteria (i.e. photomicrographs) of similar changes in the neocortex (Broere 1990), the nucleus basalis magnocellularis of Meynert (Vogels 1990), the hippocampus (De Vries 1990) and the amygdala (Vereecken in preparation) revealed common descriptive features of the neurodegenerative changes as can be seen in various brain areas. One minor difference might be small variation in outer shape of neurofibrillary tangles, seemingly depending on the shape and size of cellular elements in the area studied. Thus, whereas cortical tangles are typically pyramidal- shaped (Broere 1990) those in various brainstem nuclei have a more spherical or polygonal appearance (Fig.III.1.). Generally the sizes of plaques and tangles differ to a large extent. Splitted parts of these elements often can not be recognized and only the most typical examples can be counted. It is often not possible to distinguish tangentially sectioned blood vessels and their fibrillary Congo red fluorescence from transsected neurofibrillary tangles. In cases of doubt such elements were not counted (for discussion see Ch.I.2.). Because of these inaccuracies as regards 'real numbers', the counting of neurodegerative changes will be named 'semi-quantitative'.

4. Within the graphical representations various points have been interconnected, without claiming a continuity of data, but for reasons of clearness.

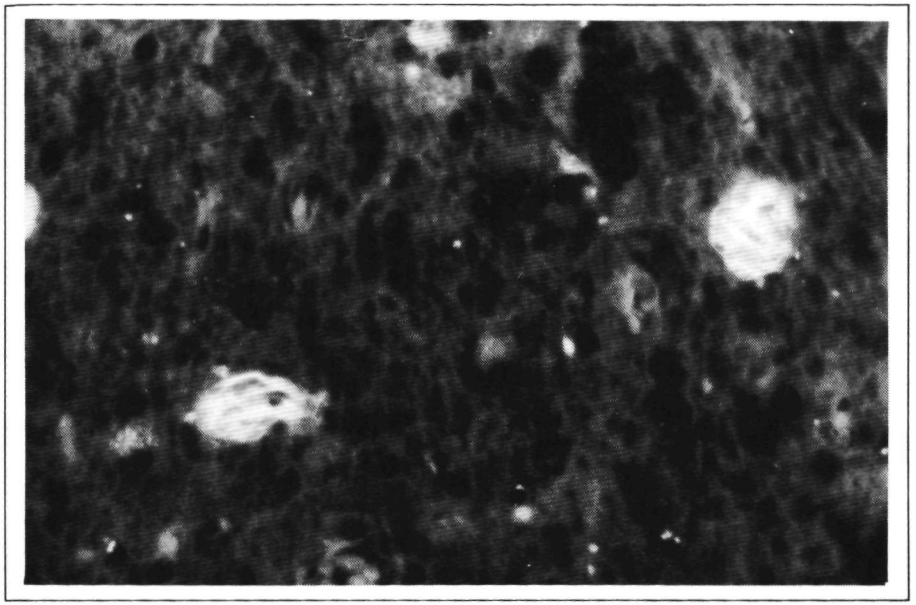


Fig. III. 1a
Neurofibrillary tangles of ventral tegmental midline structures. Congo-red (x 400)

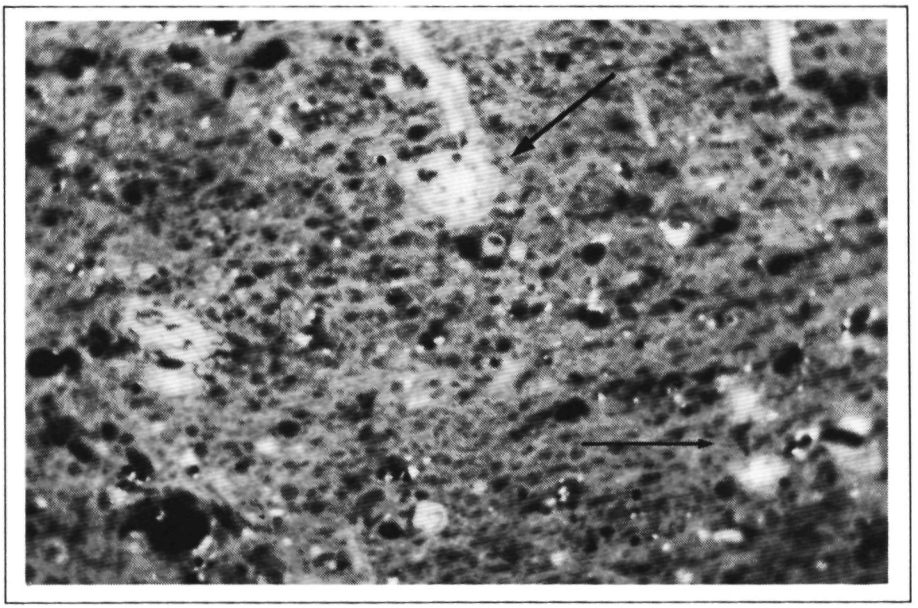


Fig. III. 1b
Neuritic plaques of mesencephalic tegmentum. Congo-red (x 400)

Abbreviations:

D-circle	= spherical diameter
D-max	= size of greatest cellular diameter
Area	= cellular surface on transsection
M+	= neuromelanin-containing neurons
M-	= neurons without neuromelanin pigment
SNmed	= medial part of the substantia nigra i.e. subnuclei Am, Ai and Pm
SNlat	= lateral part of the substantia nigra i.e. subnuclei Al, Pl, Pm and Ps
LC	= locus coeruleus
NRd	= nucleus raphe dorsalis
NFT's	= neurofibrillary tangles
SP's	= senile plaques = neuritic plaques
SD	= standard deviation
AD	= Alzheimer's disease
PD	= Parkinson's disease
C	= control cases
NS	= no significant difference (statistically)
R	= right (side of brain stem)
L	= left
TH-ir	= tyrosine hydroxylase-immunoreactive neurons
DBH-ir	= dopamine-ß-hydroxylase-immunoreative neurons
t	= t value necessary for the students t-test, calculated as

$$t = \frac{/ \bar{x} - \mu /}{s / \sqrt{n}}$$ in which:

$\bar{x}$ = mean number of cells counted in a certain area in Alzheimer or Parkinson brains

μ = mean number of cells counted in the same defined sub(region) in control brains, representing the normally expected cell number in controls

s = variance of mean values of cell counts in the relevant (sub)region in Alzheimer or Parkinson brains

n = number of Alzheimer or Parkinson brains used to calculate x and s

III.2. CELLULAR SIZES AND NUMBERS OF THE SUBSTANTIA NIGRA AND VENTRAL TEGMENTAL AREA

In order to correctly quantify neuron numbers cellular sizes have to be obtained, for the correction formula of Abercrombie (1946). Each time 25 samples were analysed, using a Kontron MOP-Videoplan equipment. Tables on substantia nigra/ ventral tegmental area, locus coeruleus and nucleus raphe dorsalis will be presented separately.

TABLE III.1. Cellular sizes substantia nigra and ventral tegmental area (μm)

		Melanin containing neurons						Neurons without neuromelanin			
Age (nr)	Diagnosis	Unit	SNmed. (μm)	SNlat.	SNret.	VTA	A8	SNc	SNr	VTA	A8
60	Normal	D-circle	40	26	25	23	24	23	21	18	21
(87029)		D-max	40	39.5	43.5	34	39	37	36	30	36
		Area	4004	4214	4420	3231	3520	3186	2705	1919	2624
62	Normal	D-circle	27	31	30	25	26.5	26		23.5	
(87177)		D-max	43.5	48	44	40.5	40	44		40	
		Area	4480	5780	5300	3700	4100	3900		3250	
69	Normal	D-circle	28	27	26.5	25	25	25		22	
(86179)		D-max	39	39	36	40	40	40		37.5	
		Area	4650	4500	4300	3800	3800	3650		3050	
91	Normal	D-circle	29	29	32	25	22	26.5		24	
(88184)		D-max	43	44	50	39.5	34	42		39	
		Area	5200	6200	3700	2900	4300			3400	
65	AD	D-circle	21	25	25	20	24	23		24	
(87068)		D-max	28	39	41	32	37.5	38		38	
		Area	2600	3900	3800	2500	3500	3300		3300	
93	SDAT	D-circle	23.5	17	26	20	20	24	22	20	20
(88221)		D-max	34.5	39.5	38	34	31	40.5	40	36	36
		Area	3373	4568	3915	2314	2423	3450	3103	2517	2517
79	PD	D-circle	24	25	28	20.5	24	24	22	20	22
(86204)		D-max	35	39	42	34	34	44	40	36	35
		Area									

TABLE III.2. Mean cellular sizes of SN and VTA

	SN(M+)	VTA(+)	t	significance
mean D-circle	29.2 ± 3.6	24.5 ± 0.9	5.2	p < 0.2
mean D-max	42.4 ± 3.8	38.5 ± 2.6	1.5	p = 0.10
± SD				
	SN(M-)	VTA(M-)	t	significance
mean D-circle	24.3 ± 2.0	21.9 ± 2.4	1	NS
mean D-max	39.8 ± 3.0	36.6 ± 3.9	0.8	NS
± SD	SNmed(M+)	SNlat(M+)	t	significance
mean D-circle	31 ± 5.2	28 ± 1.9	1.58	NS (p<0.2)
± SD	VTA(M+)	A8(M+)	t	significance
mean D-circle	24.5 ± 0.9	24.4 ± 2.8	1.58	NS
mean D-max	38.5 ± 2.6	38.3 ± 2.5	1.58	NS
± SD	SN(M+)	SN(M-)	t	significance
mean D-circle	29.2 ± 3.6	24.3 ± 2.0	2.45	p < 0.1
mean D-max	42.4 ± 3.8	39.8 ± 3.0	0.8	NS
± SD	VTA(M+)	VTA(M-)	t	significance
mean D-circle	24.5 ± 0.9	21.9 ± 2.4	1.08	NS
mean D-max	38.5 ± 2.6	36.6 ± 3.9	0.5	NS
± SD				

Table III.1. gives an account of the results of the data from Kontron MOP-Videoplan measurements. These data are summarized in **table III.2.**, providing mean profile sizes, on which some conclusions can be drawn:

1. The mean spherical diameter of neuromelanin pigmented nigral cells (**29.2 ± 3.6** μm) does not differ significantly between various nigral subnuclei, but is significantly larger than that of VTA neurons (**24,5 ± 0.9 μm**). The mean spherical diameter of VTA cells is comparable to that of A8 cells, both as regards neuromelanin containing cells and those without neuromelanin.
2. No significant change of cellular sizes related to age could be detected.
3. The mean spherical diameter of nigral (24.3 ± 2.0 μm) and VTA (21.9 ± 2.4 μm) cells without neuromelanin pigment also showed no age-related change.
4. Similar results hold for largest profile size (D-max) and cellular surface (Area), although the difference between neuromelanin-containing nigral cells and those without pigment (mean **D-max 42.4 ± 3.8 μm** and **39.8 ± 3.0 μm**, respectively is not significant now.

VTA cells without neuromelanin pigment (D-max = 36.6 ± 3.9 μm) are smaller than those of the SN (D-max = 39.8 ± 3.0 μm), but because of the high variability in cell sizes, this difference has no statistical relevance.

Data from the foregoing tables may be visualized as follows:

Fig III 2

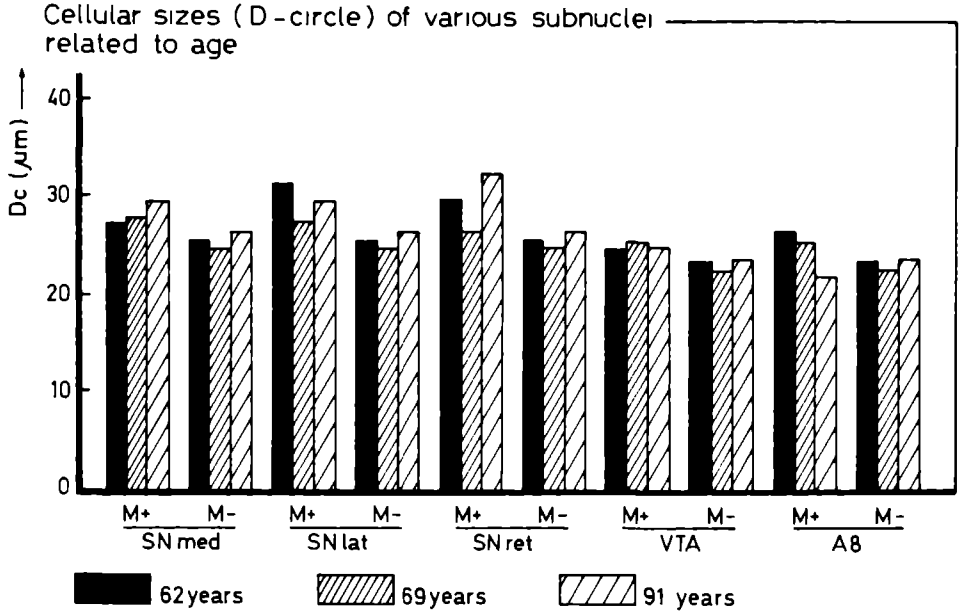
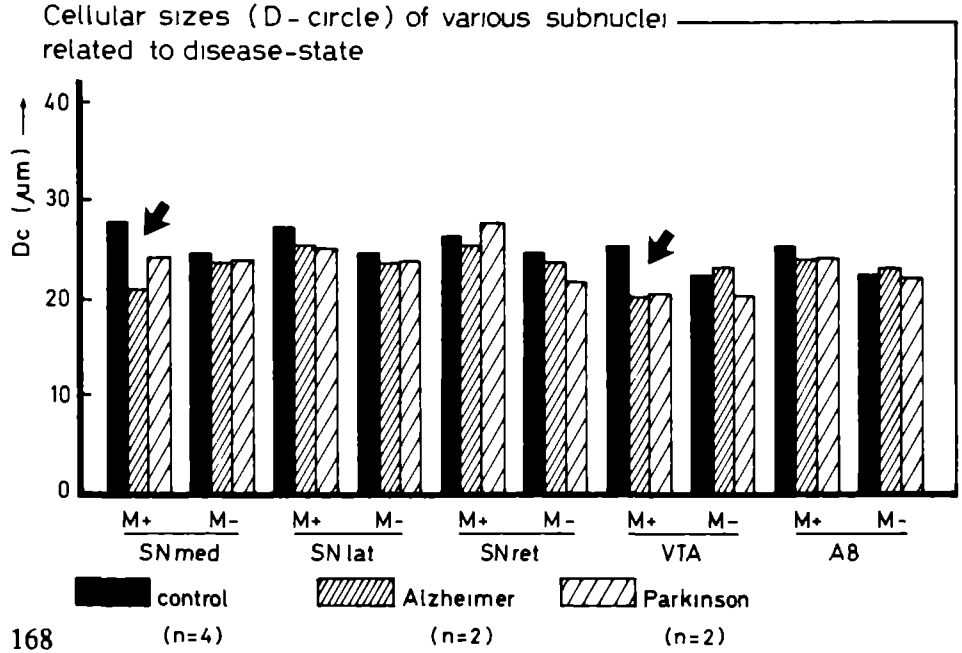


Fig III 3.



Whereas figure III.2 provides information on the distribution of cellular sizes among control cases of various age, figure III.3 gives information on such findings as regards patients with AD and PD, as compared with controls.

Figure III.3 clearly shows that, generally speaking, no important changes in spherical diameters occur in various disease states. The drop in spherical diameter of neuromelanin pigmented cells of the VTA in both AD and PD, however, is significant. Therefore, this change is incorporated in the correction performed on quantitative estimation of cell numbers. Likewise the drop in spherical diameter of the medial part of the SN, especially in AD, deserves attention (and is also incorporated in further calculations).

It may be stated that generally there seems to be a trend of neurons to shrink in AD and PD, but this trend is not uniform and without statistical significance. For mean largest and mean cellular surface of **all** subnuclei, this trend has grown to a significant level as regards neuromelanin pigmented cells. This is shown in table III.3.

Table III.3. Differences of mean cellular sizes in disease-states (n=4) as compared to controls (n=4)

Disease state	Subnucleus	Mean size (μm)	Variable	t	Significance
Controls	SNmed(M+)	31 0 ± 5.2	D-circle		
AD/SDAT		22 3 ± 1 3		6 7	p < 0 10
Controls	TA(M+)	24 5 ± 0 9	D-circle		
AD/SDAT		2 0 ± 0		5	p < 0 10
Controls	VTA(M-)	21 9 ± 2 4	D-circle		
AD/SDAT		22 0 ± 2 0		0 05	NS
Controls	SN(M+)	42 4 ± 3 8	D-max		
AD/SDAT/PD		37 3 ± 4 0		1 28	p = 0 10
Controls	VTA(M+)	38 5 ± 2 0	D-max		
AD/SDAT/PD		33 3 ± 0 9		5 7	p < 0 01
Controls	VTA(M-)	36 6 ± 5 1	D-max		
AD/SDAT/PD		36 6 ± 0 9			NS
Controls	SN(M+)	4846 ± 644	Area		
AD/SDAT		3693 ± 601		1 9	p = 0 05
Controls	VTA(M+)	3608 ± 250	Area		
AD/SDAT		2407 ± 93		12 9	p = 0 025
Controls	VTA(M-)	2905 ± 576	Area		
AD/SDAT		2909 ± 392			NS

Data on cellular sizes were incorporated in the calculation of real cell numbers, with the aid of Abercrombie's (1946) correction formula

Table III.4. shows corrected neuron numbers as quantified for the three main parts of the SN (medial, lateral and pars reticulata), the VTA and the retrorubral area (A8). In this table the left and right sides of the brainstem are separately quantified.

Table III.4. Neuron numbers substantia nigra and ventral tegmental area in controls, AD and PD brains and corrected according to Abercrombie (1946)

Age	Nr.	M=neuromel.	L/R	SN med	SN lat	SN ret	VTA	A8	SN tot
Controls									
60	87029	M	R	74510	76546	47293	46781	11695	198349
		non-M	R	10430	12363	43645	29868	21111	66439
		M	L	61245	80984	36707	39584	7047	178935
		non-M	L	8046	11038	33182	45120	11355	52267
62	87177	M	R	95396	72941	39044	58776	9596	207380
		non-M	R	19073	13835	57995	85792	37424	90902
		M	L	78179	107331	40419	46481	12295	225929
		non-M	L	16391	22665	48727	58148	46381	87783
69	86179*	M	R	62938	103726	35195	61774	9596	201859
		non-M	R	14901	26198	74735	128370	31667	115834
80	88269*	M	R	58705	116761	58291	69571	9596	233756
		non-M	R	14603	25903	72045	101997	48300	112551
91	88184	M	R	58987	102616	43718	53978	8996	205322
		non-M	R	19371	25020	77426	120109	58216	121817
		M	L	63221	75714	34645	77068	15893	173579
		non-M	L	9238	10302	44841	116296	54057	64382
AD brains									
65	87068	M	R	65897	82053	38611	21503	3056	186560
		non-M	R	9355	11467	28026	20070	9721	48848
		M	L	71970	107364	48264	30168	11002	227598
		non-M	L	11769	22029	31377	26656	10349	65175
69	87395	M	R	66857	94554	49062	41850	3172	210474
		non-M	R	9752	20284	58840	59872	21324	88876
		M	L	82589	85766	42208	38891	8724	210563
		non-M	L	22625	13653	44228	48800	31167	80506
76	88177	M	R	67415	106808	43721	40117	12224	217944
		non-M	R	14485	16899	46000	48607	43276	77383
		M	L	56483	89563	28391	39475	14058	174436
		non-M	L	4526	13579	42649	52684	48607	60755
93	88221	M	R	46158	82887	30378	27601	4890	159423
		non-M	R	27460	34401	58185	62092	28224	120046
		M	L	46158	73152	25551	31452	4278	144862
		non-M	L	14786	21123	50569	76831	13798	86479

* Left side incomplete. Neuron number of right side also applied for left side when necessary.

Age	Nr.	M=Neuromel.	L/R	SN med	SN lat	Sn ret	VTA	A8	SN tot
PD brains									
68	89142	M	R	22800	14700	11286	26320	4848	48786
		non-M	R	20672	26752	60800			108224
		M	L	36000	21462	10602	31584	4242	96494
		non-M	L	31008	34960	54400			119968
69	89141	M	R	10500	7938	4743	34545	5454	23181
		non-M	R	21584	14896	34880			71360
		M	L	10800	14994	4185	6580	3636	29979
		non-M	L	25232	16112	16000			57344
74	87377	M	R	23216	11334	17993	23118	2350	52542
		non-M	R	5506	13765	68182	58104	28248	87453
		M	L	10834	7556	10584	25299	392	28974
		non-M	L	5506	11012	64815	67788	42372	81333
79	86204	M	R	35085	35028	10321	35492	3633	80435
		non-M	R	15791	30063	49259	77761	34520	95114
		M	L	25170	35150	25613	30437	5155	85933
		non-M	L	8972	9362	28827	34857	18454	47161
PD+AD brain									
66	87062	M	R	48367	49869	20462	29224	783	118698
		non-M	R	17304	14158	52610	39176	21601	84072
		M	L	24764	37780	25048	31405	5092	87592
		non-M	L	9045	9439	30724	37416	18694	49208

Graphical representations of these data are shown in figures III.4. ad III.5. in which neuron numbers of three groups have been arranged. Neuron numbers of both sides of the brainstem are taken together in these representations. Cases of AD and SDAT are taken together and referred to as "dementia". Figure III.5. is a visualization of the graphical representation in figure III.4.

Fig III 4.

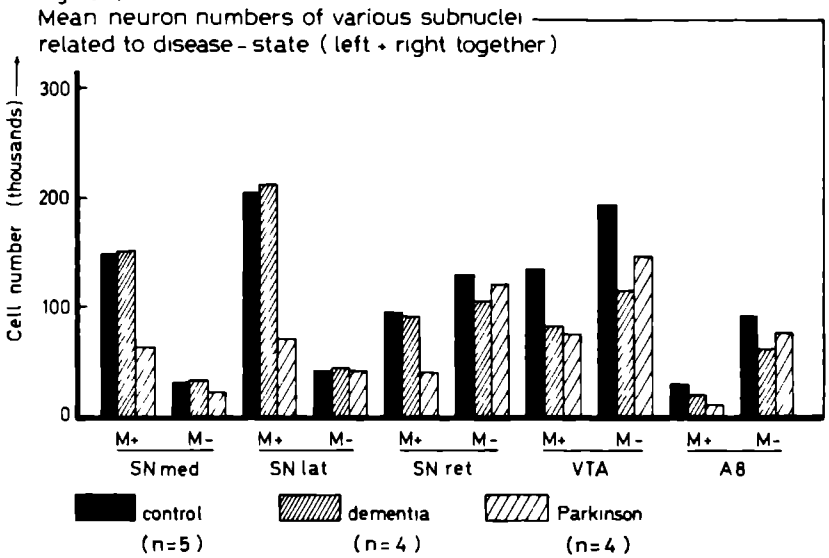
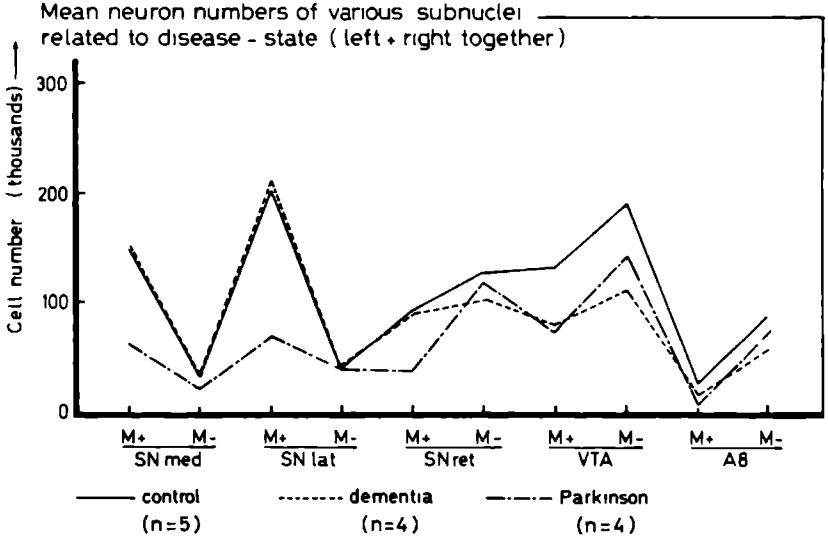


Fig III.5.



The conclusions that may be drawn from these data are summarized in the tables III.5 and III.6. Mean cell numbers of controls and PD cases, respectively, have been calculated and compared with each other and with AD/SDAT cases ("AD" in table III.6.). For discussion see Ch.III.5.2.

Table III.5. Mean cell numbers of various nigral subnuclei in PD (n=4) as compared to controls (n=5)

Subnucleus	Disease state	Neuron number	% cell loss
SNmed(M+)	Control	69,147	
	PD	21,800	68%
SNmed(M-)	Control	14,006	
	PD	16,784	NS
SNlat(M+)	Control	92,077	
	PD	18,520	80%
SNlat(M-)	Control	18,416	
	PD	19,615	NS
SNret(M+)	Control	41,914	
	PD	11,916	72%
SNret(M-)	Control	56,574	
	PD	47,145	17% (NS; p >> 0.2)

Table III.6. Mean cell numbers of VTA, SNtot and A8: differences of AD (n=4) and PD (n=4) as related to controls (n=5).

Subnucleus	Disease state	Neuron number	% cell loss	t	significance
VTA(M+)	Control	56,752 ± 11,799			
	PD	26,672 ± 8,634	53 %	3.48	p = 0.005
	AD	33,882 ± 8,019	40.5%	2.85	p < 0.025
VTA(M-)	Control	91,606 ± 29,764			
	PD	59,628 ± 13,235	35.0%	2.42	p < 0.025
	AD	49,452 ± 17,366	47.1%	2.43	p < 0.025
SN-total(M+)	Control	203,139± 19,248			
	PD	55,791 ± 26,650	73 %	5.52	p < 0.0005
	AD	191,483 ± 27,941	6 %	0.40	NS
SN-total(M-)	Control	88,997 ± 24,569			
	PD	83,495 ± 28,624	6 %	0.19	NS
	AD	78,509 ± 20,773	12 %	0.50	NS
A8(M+)	Control	10,589 ± 2,508			
	PD	3,714 ± 1,564	65 %	4.40	p < 0.0025
	AD	7,672 ± 4,101	28 %	0.71	NS; p >> 0.20
A8(M-)	Control	38,564 ± 15,313			
	PD	30,899 ± 8,756	20 %	0.88	NS; p >> 0.20
	AD	25,808 ± 15,888	33 %	0.80	NS; p >> 0.20

The **conclusions** that might be drawn from these latter tables are as follows:
1. These data firstly show the relatively greater standard deviation of neurons without neuromelanin pigment, especially as regards area A8. Therefore substantial numerical differences may be not-statistically-significant.
Next, the medial part of the SN appears to contain the greatest relative abundance of neuromelanin-containing neurons, as compared to overall cell number.

2. Both medial and lateral parts of the SN show a significant loss of neurons in Parkinson's disease (68% and 80%, respectively). No significant changes in cell number of (M-)neurons have been measured, unless **a slight increase in non-pigmented neurons in PD.**

3.As regards the pars reticulata there is a significant loss of pigmented neurons in PD (72%), but not in AD.

4. A significant loss of neurons of the **VTA**, both pigmented and those without neuromelanin, is apparent both in PD and AD. The most remarkable is the **loss of cells without neuromelanin in the VTA of Alzheimer's disease patients.**

5. Less significant are the differences concerning neuron loss of A8 and SN-total, although again a significant loss of unpigmented cells may be registered for A8 in AD-cases.

6. Neuron numbers of the various subnuclei in the case of combined AD and PD are within the realm of what might be expected from the data mentioned before. I.e., a significant loss of both pigmented cells and those without neuromelanin.

7. There appears to be no change of cell number related to age, neither in controls nor in disease cases (Fig.III.6.). Both controls and AD-cases show no clear trend among the highly variable data. Because no significant change of cell number in the SN for AD relative to controls was noted, controls and AD cases could be taken together. This new set of samples, providing a greater number of cases, can be correlated to age again (Fig.III.7.). For this figure the mean cell number of the left and the right SN, respectively, were taken in each case. Cellular sizes that might be expected related to age are represented by the hatched area.

Fig III 6 a
Cell numbers of various subnuclei in control cases related to age

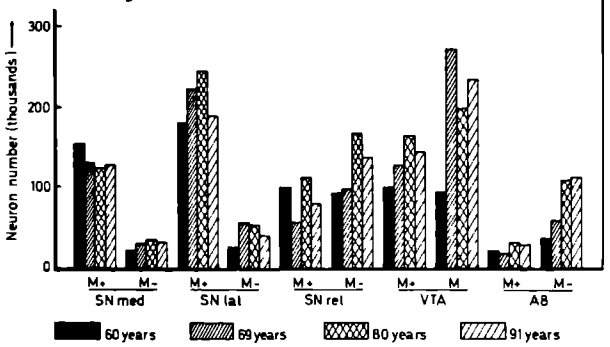


Fig III 6 b
Cell numbers of various subnuclei in dementia related to age

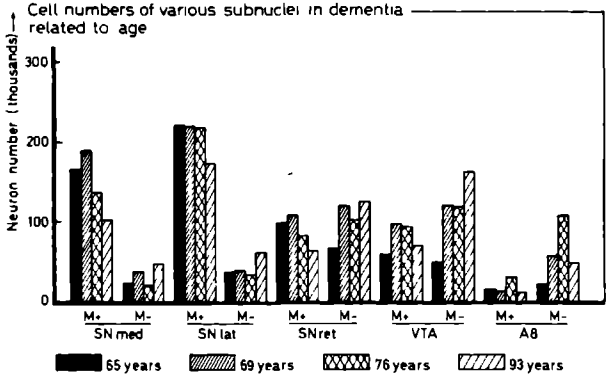
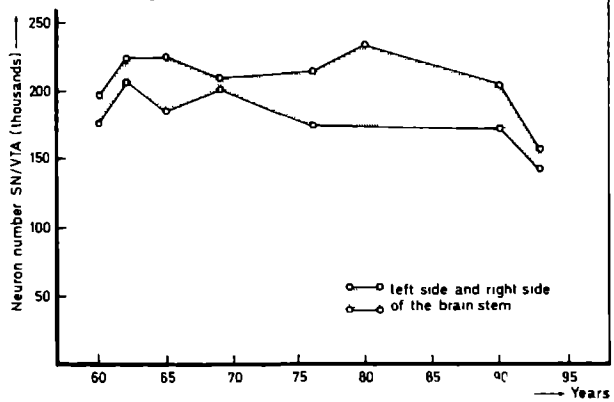


Fig III 7
Number of SN neurons including both controls and AD-cases as related to age (neuromelanin-containing cells)



III.3. CELLULAR SIZES AND COUNTING RESULTS OF THE LOCUS COERULEUS AND NUCLEUS RAPHE DORSALIS.

Table III.7. gives an account of cellular sizes of the locus coeruleus i.e. greatest section diameter (D-max) and calculated spherical diameter (D-circle) for several cases. Data were obtained similar to the procedure used for the SN and VTA. Again, these data are of need to correct for possible cellular shrinkage and counting errors as a result of splitted elements.

Table III.7. Cellular sizes locus coeruleus

Nr.	Age	Diagnosis	Variable (μm)	Rostral M+	Rostral M-	Caudal M+	Caudal M-
87029	60	C	Dm	47	51	52.8	52.8
87177	62	C	Dm	61	58.5	59.4	52.2
88221	93	SDAT	Dm	47.4	39.4	61.2	57
87062	66	AD/PD	Dm	45	50.4	47	48
86204	79	PD	Dm	44.2	44	49.2	45.6
87029	60	C	Dc	36.8	31	35.6	34.4
87177	62	C	Dc	45.4	33	37.4	31.4
88221	93	SDAT	Dc	36.4	25.6	40.6	32.4
87062	66	AD/PD	Dc	32.2	26.4	33.8	26.2
86204	79	PD	Dc	32.8	25.6	33.2	24.2

Table III.8. summarizes data on cellular sizes, averaged for controls, AD and PD patients respectively.

1. Normally, various cellular sizes may be seen throughout the locus coeruleus. The greatest range of section diameters, however, is seen in the rostral part, especially as regards neuromelanin containing neurons. Generally, neurons without pigment were somewhat smaller than those with neuromelanin.

Likewise, variation in spherical diameters rostrally is somewhat greater than caudally and rostrally the greatest neuromelanin containing neurons have been found.

2. There appears to be a global **shrinkage** of cellular elements, both pigmented and cells without neuromelanin, in cases of AD, SDAT and PD.

No changes of cellular size related to age in controls or disease cases was demonstrable.

3. The most pertinent finding is a **general reduction of cellular size throughout the locus coeruleus in Parkinson's disease**, either isolated or in combination with AD.

This **reduction in cellular size** only concerned the **rostral part in the case of SDAT**. Neuromelanin pigmented cells were reduced to 77% of their normal size, whereas neurons without neuromelanin showed a shrinkage to 81% of their original size.

Table III.8.

Variable	Disease state	Part of LC	µm ± SD	t	significance
Mean D-max	Control (n=2)	Rostral(M+)	54,0		
	AD, PD (n=3)		45,5 ± 2,2	3,86	$p < 0,025$
Mean D-max	Control (n=2)	Rostral(M-)	54,8		
	AD, PD (n=3)		44,6 ± 4,3	2,36	$0,10 < p < 0,05$
Mean D-max	Control (n=2)	Caudal(M+)	56,1		
	AD, PD (n=3)		52,5 ± 6,2	0,58	NS
Mean D-max	Control (n=2)	Caudal(M-)	52,5		
	AD, PD (n=3)		50,2 ± 4,9	0,47	NS
Mean D-circle	Control (n=2)	Rostral(M+)	41,1		
	AD, PD (n=3)		33,8 ± 1,85	3,9	$p < 0,025$
Mean D-circle	Control (n=2)	Rostral(M-)	32		
	AD, PD (n=3)		25,9 ± 0,38	16	$p < 0,0025$
Mean D-circle	Control (n=2)	Caudal(M+)	36,5		
	AD, PD (n=3)		35,9 ± 3,42	0,18	NS
Mean D-circle	Control (n=2)	Caudal(M-)	32,9		
	AD, PD (n=3)		27,6 ± 3,49	1,5	NS; $p > 0,1$

Table III.9. gives the counting of neuron numbers in the locus coeruleus. The interpretation of these results is visualized in figures III.8.a,b. The total cell number is calculated, again, after correction (Abercrombie, 1946), using cellular sizes as mentioned before.

Table III.9. Counting results Locus coeruleus

Age	Brain nr.	Left(M+)	Right(M+)	Left(M-)	Right(M-)
Controls					
60	87029	19313	18662	8296	11224
62	87177	22357	29729	18056	14884
69	86179	17143	17577	16348	13908
80	88269	19488	16212	16530	16910
91	88184	19313	19964	15128	11712
Dementia					
65 (AD)	87068	4380	5037	1315	1052
69 (SDAT)	87395	7152	8046	3520	4928
76 (SDAT)	88177	4167	5037	6312	5260
82 (MID)	87368	12483	5694	4734	3945
93 (SDAT)	88221	3723	5475	3945	3682
PD brains					
68	89142	21296	19602	15427	15427
69	89141	10406	6050	8866	9398
74	87377	10132	6854	2192	2929
79	86204	5324	10406	4576	5434
PD/AD brain					
66	87062	5066	8344	3520	4224

Table III.10. Concluding data on locus coeruleus countings (mean cell numbers) and reduction of cell number (%) with statistical significance (n=5)

Disease state		Mean cell number	% cell loss	t	significance
Control	(M+)	19.976 ± 3.627			
	(M-)	14.300 ± 2.889			
Dementia	(M+)	6.119 ± 2.466	69,4 %	5,6	$p < 0,0005$
	(M-)	3.869 ± 1.562	73,0 %	6,68	$p < 0,0005$
Parkinson's	(M+)	11.259 ± 5.644	44,6 %	1,5	$p < 0,10$
disease	(M-)	8.031 ± 4.883	44,0 %	1,28	NS ($p > 0,10$)

These data suggest that:

1. There is a significant loss of cells in the LC both in dementia and PD, somewhat more pronounced in Dementia. Indeed, one of the PD cases showed a cell number within the range of controls, whereas another PD case had an extreme loss of cells, beyond the mean of dementia cases.
2. No significant age-related changes were found. As regards neuromelanin containing neurons a trend of smaller cell numbers in older brains can not be excluded.
3. The most striking result, again, is **the loss of neurons whithout neuromelanin pigment in Dementia**, which certainly not would have been detected if only pigmented cells had been counted.
4. An additional reduction of neuron number among cells with D-max smaller than 12,5 μm (smaller undefined neuronal elements) has been calculated both in dementia (40%) and PD (48%).
5. From the plotting table sheets a more detailed image of regional cell loss can be obtained. Calculation of anterior-posterior differences revealed a global cell loss of 70% throughout the rostro-caudal extent. This was different for neurons without neuromelanin: a cell loss of 55% for the caudal half contrasts with that of 80% in the rostral half of the locus coeruleus in dementia cases. In PD the uniform rostro-caudal gradient (44% loss both of pigmented cells and neurons without neuromelanin) remained.
6. Among dementia cases, the greatest cell number has been counted in a MID brain (male, 82 yrs.).The counting conclusions, mentioned before, do not change significantly if controls and PD cases are compared with dementia of Alzheimer's type.
Table III.11. provides data on cell numbers, based on the counting of nucleoli,

Table III.11. provides data on cell numbers, based on the counting of nucleoli, within the nucleus raphe dorsalis. These data are summarized in table III.12, providing mean cell numbers. The rostral part is defined as one-third of the total anterior-posterior length.

Table III.11. Neuron numbers in the nucleus raphe dorsalis for controls, AD and PD cases.

Age	Brain nr.	Neuron number	Rostral one-third
Controls			
60	87029	385,280	163,200
62	87177	443,520	321,280
69	86179	433,280	273,280
80	88269	677,120	378,240
91	88184	663,680	341,760
AD brains			
65	87068	227,200	129,280
69	87395	189,440	96,640
76	88177	434,560	221,440
93	88221	403,840	228,480
PD brains			
68	89142	481,920	272,000
69	89141	661,120	380,160
74	87377	440,960	240,640
79	86204	689,280	371,840
AD+PD brain			
66	87062	245,760	123,520

Table III.12. Mean cell numbers of nucleus raphe dorsalis and cell loss related to controls

Disease state	(sub)nucleus	Neuron number ± SD	% loss	t	significance
Control (n=5)	NRd	520,576 ± 160,000			
Control	Rostral part	295,552 ± 74,360			
AD (n=4)	NRd	313,760 ± 125,000	39.7 %	1.94	$0.05 < p < 0.10$
AD	Rostral part	168,960 ± 67,714	43 %	1.87	$0.05 < p < 0.10$
PD (n=4)	NRd	568,320 ± 127,000	NS		NS
PD	Rostral part	316,160 ± 60,929	NS		NS

Fig III 8 a
Neuron number of locus coeruleus related to age
(neuromelanin containing cells)

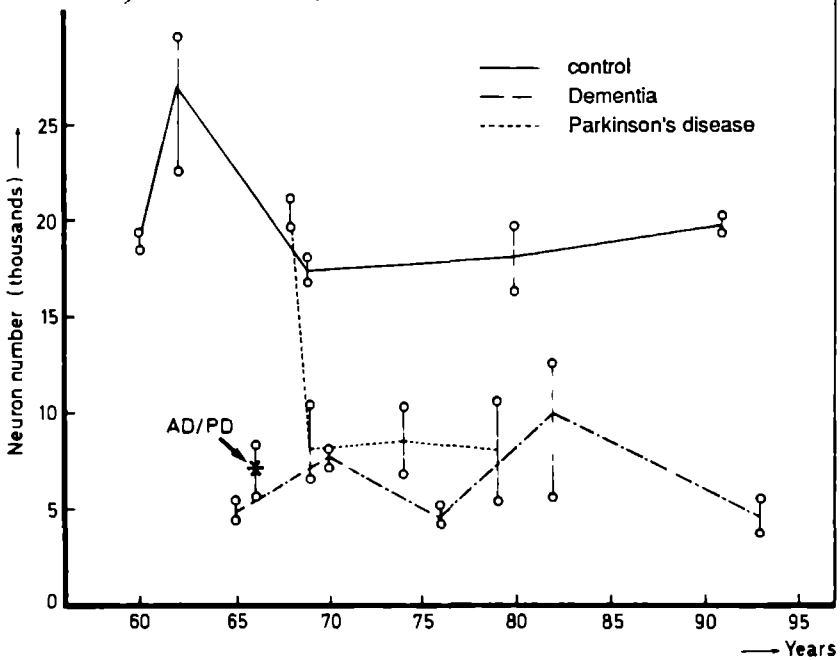
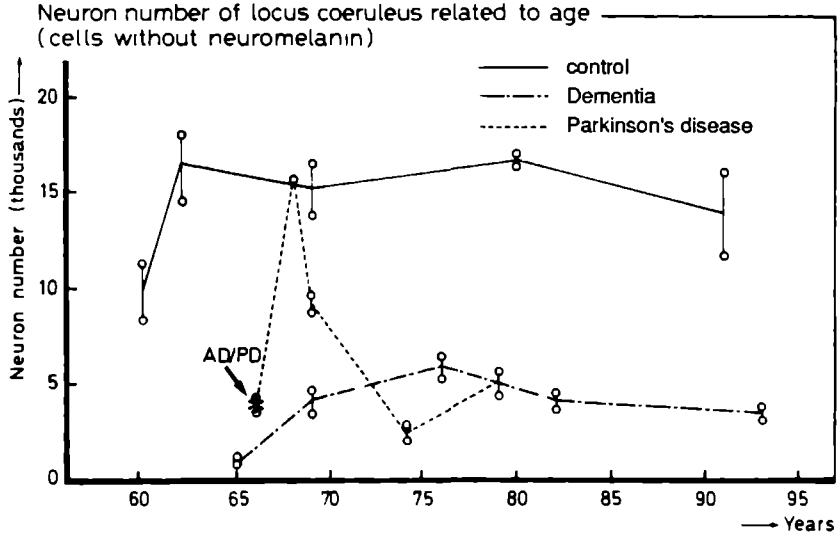


Fig III 8 b
Neuron number of locus coeruleus related to age
(cells without neuromelanin)



Each point represents left side and right side, respectively, in a single case (see tabel III 9)

These data are visualized in figures III.9.a and b. The conclusions that might be drawn:

1. The mean neuron number of the **nucleus raphe dorsalis**, in its largest extent, is **520,576 ± 160,000** cells. No significant change of this cell number was shown in PD cases.
2. There is a significant loss of NRd cells in cases of **Alzheimer's disease (39,7%)**, somewhat more pronounced in the rostral one-third of the nucleus (43%), although this difference is not statistically significant.
3. It is remarkable that there is a trend of increasing cell number with age and certainly no age-related cell-loss, both in controls and in AD cases.

Fig III 9 a
Neuron number of nucleus raphe dorsalis related to age

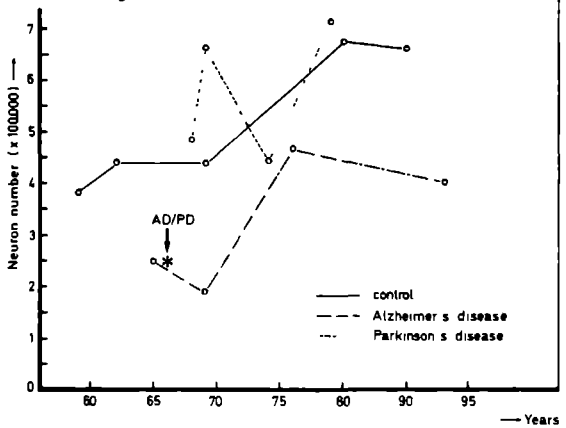
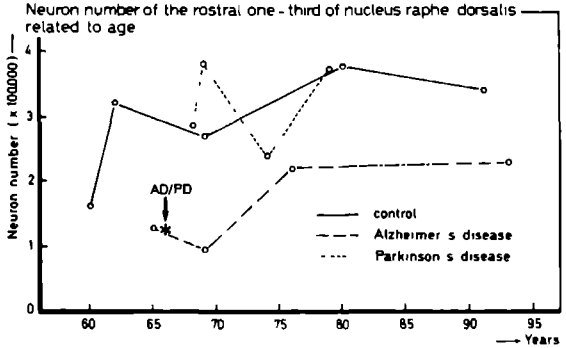


Fig III 9 b
Neuron number of the rostral one - third of nucleus raphe dorsalis related to age



Each point represents the neuron number of a single case (see tabel III.11)

III.4. DISTRIBUTION AND SEMIQUANTITATIVE CALCULATION OF NEUROFIBRILLARY TANGLES AND SENILE PLAQUES

Figures III.10.a,b and III.11.a,b give an account of the typical distribution of neurodegenerative findings in a case of Alzheimer's disease. The boundaries of the monoaminergic nuclei, as defined in the neuroanatomical section (section II), are by no means respected by these findings. Nevertheless, an attempt was made to quantify the number of neurofibrillary tangles and senile plaques, within the monoaminergic nuclei under consideration. Nuclear boundaries were defined by using plotter sheets, available after quantification of Nissl-stained sections, as a guide.

Fig. 10a and 10b

Distribution of neurofibrillary tangles (Congo red fluorescence) in the human mesencephalon and rostral rhombencephalon in Alzheimer's disease

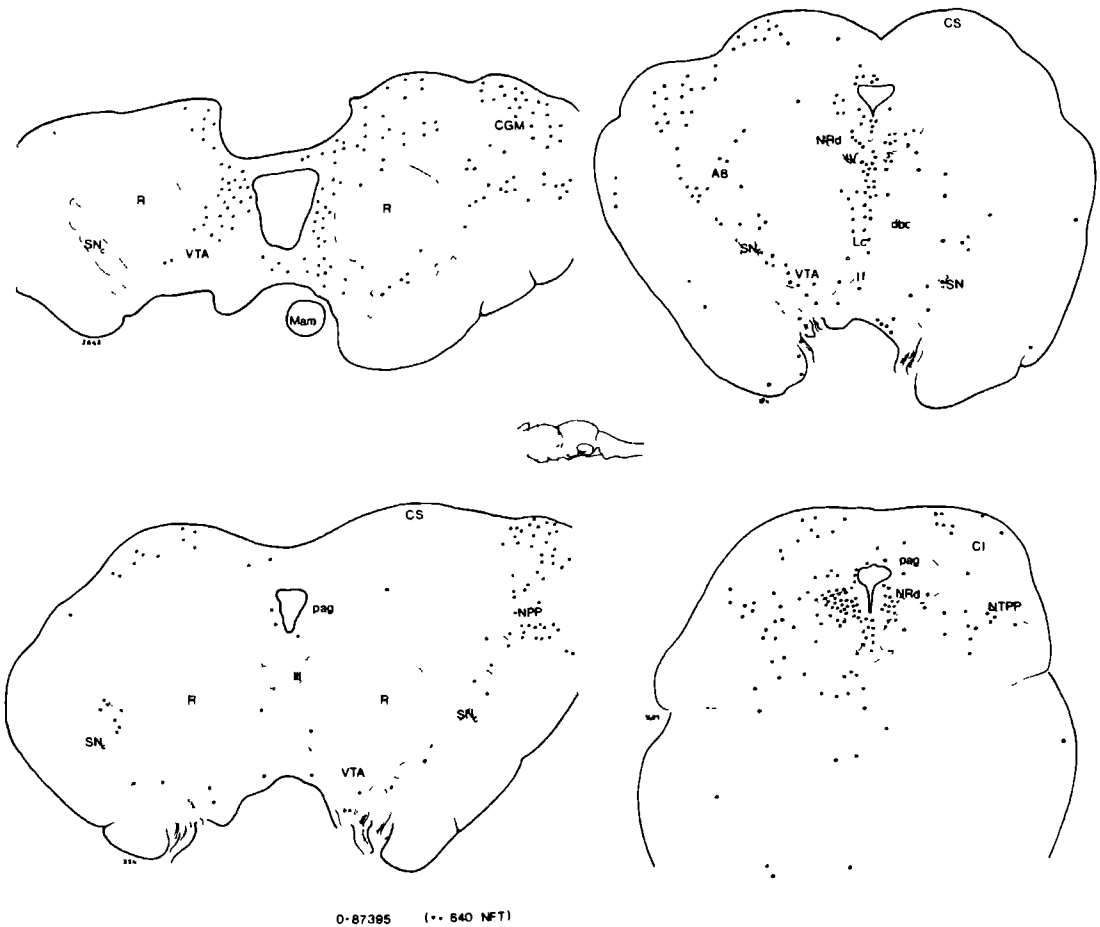
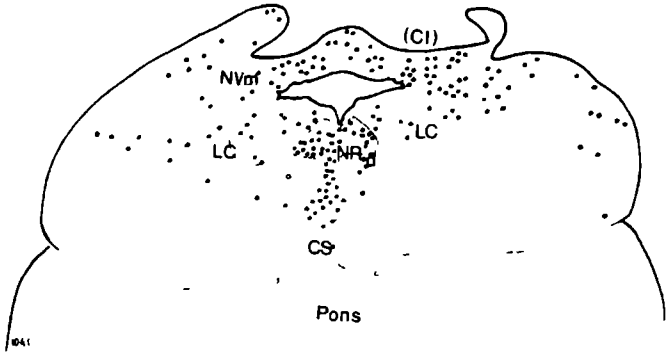
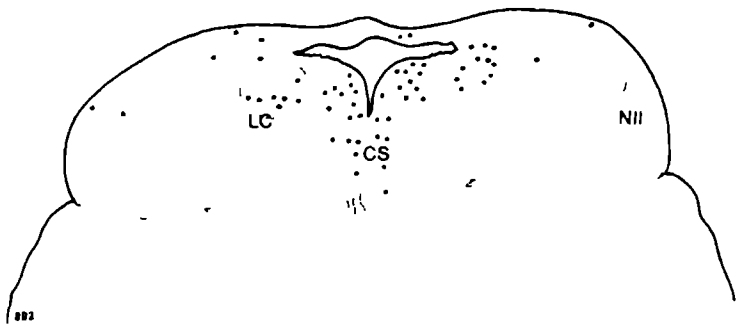
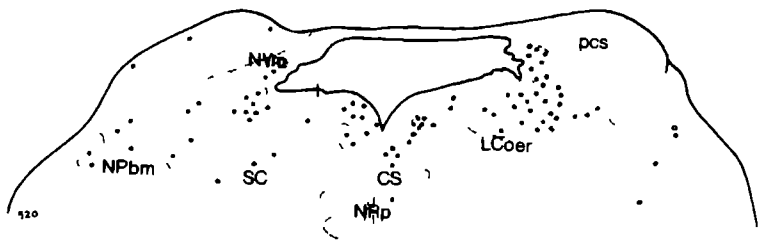


Fig. 10a







0-87395 (-- 640 NFT)

Fig 10b

Fig 11a and 11b

Distribution of senile plaques (Congo red fluorescence) in the human mesencephalon and rostral rhombencephalon in Alzheimer's disease

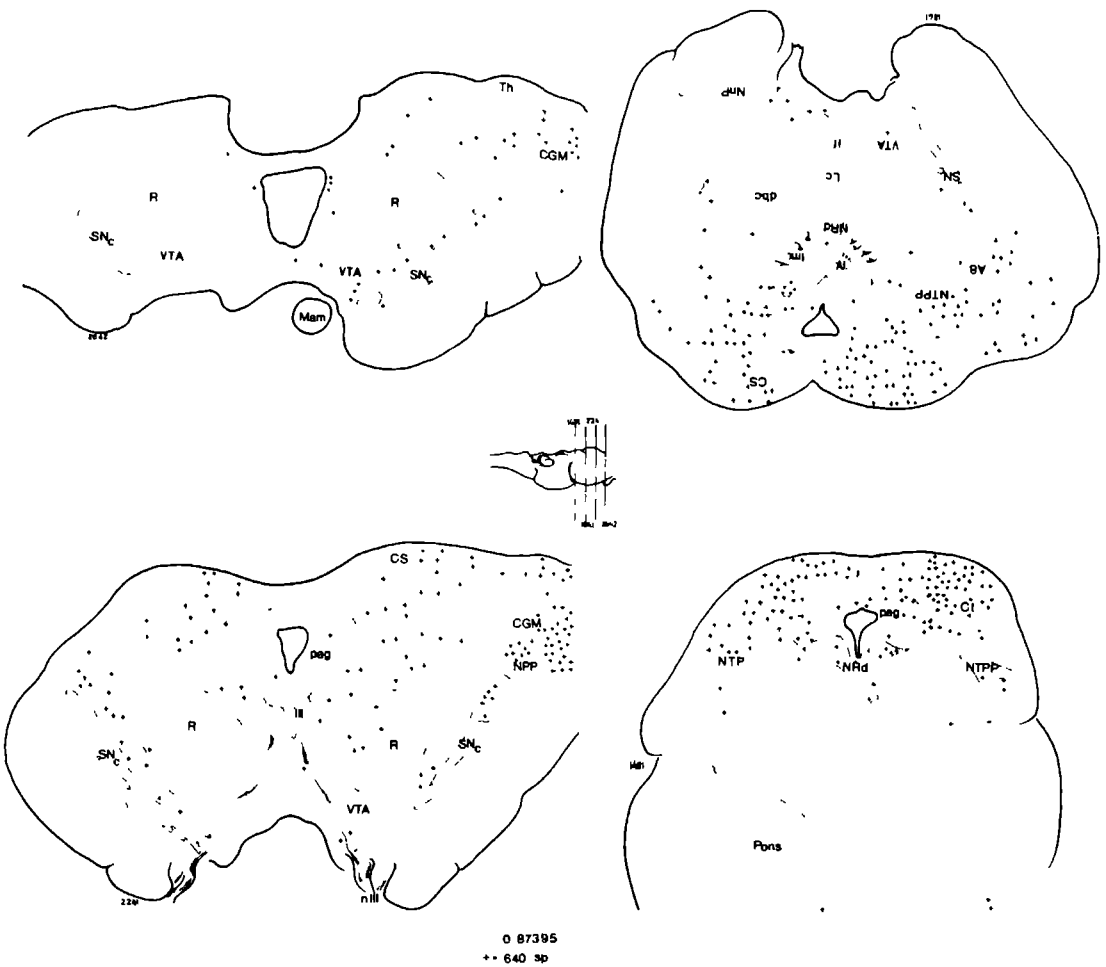


Fig 11a

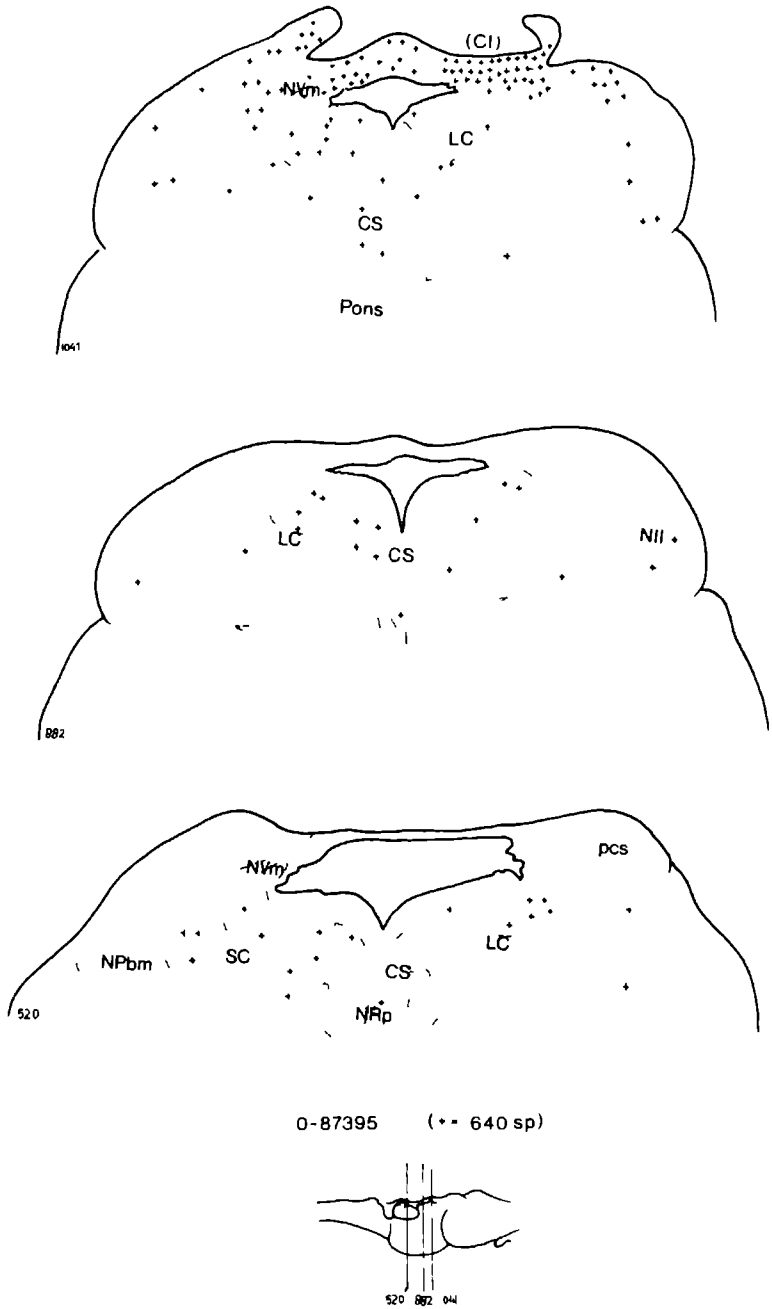

(CI)
NVm
LC
CS
Pons
1041
LC
CS
NII
882
NVm
pcs
LC
NPbm
SC
CS
NRp
520
0-87395 (•• 640 sp)
520 882 044

Fig 11b

Table III.13. presents data on semi-quantitative estimation of neurofibrillary tangles in AD brains, left and right side taken together. The counted numbers, as noted here, have to be quantified with x 16 and x 40, for the estimation of real numbers.

Table III.13. Numbers of neurofibrillary tangles, without correction, as counted for 1/40 of the rostro-caudal extent (bilaterally)

Age	Disease	Brain nr.	SNmed	SNlat	VTA	NRd	LC
65	AD	87068	2	18	50	178	19
66	AD+PD	87062	14	15	46	81	37
69	SDAT	87395	12	24	114	93	50
76	SDAT	88177	6	16	95	147	42
93	SDAT	88221	4	8	64	74	16
Mean ± SD			7.6 ± 4.6	14.2 ± 6.1	74 ± 26.5	111.6 ± 40.8	32.8 ± 12.9
Extrapolation and calculation for 40 sections			SNtot = 13.952		47.360	73.344	20.992

These estimated numbers of neurofibrillary tangles may be compared with cell losses using the following data.

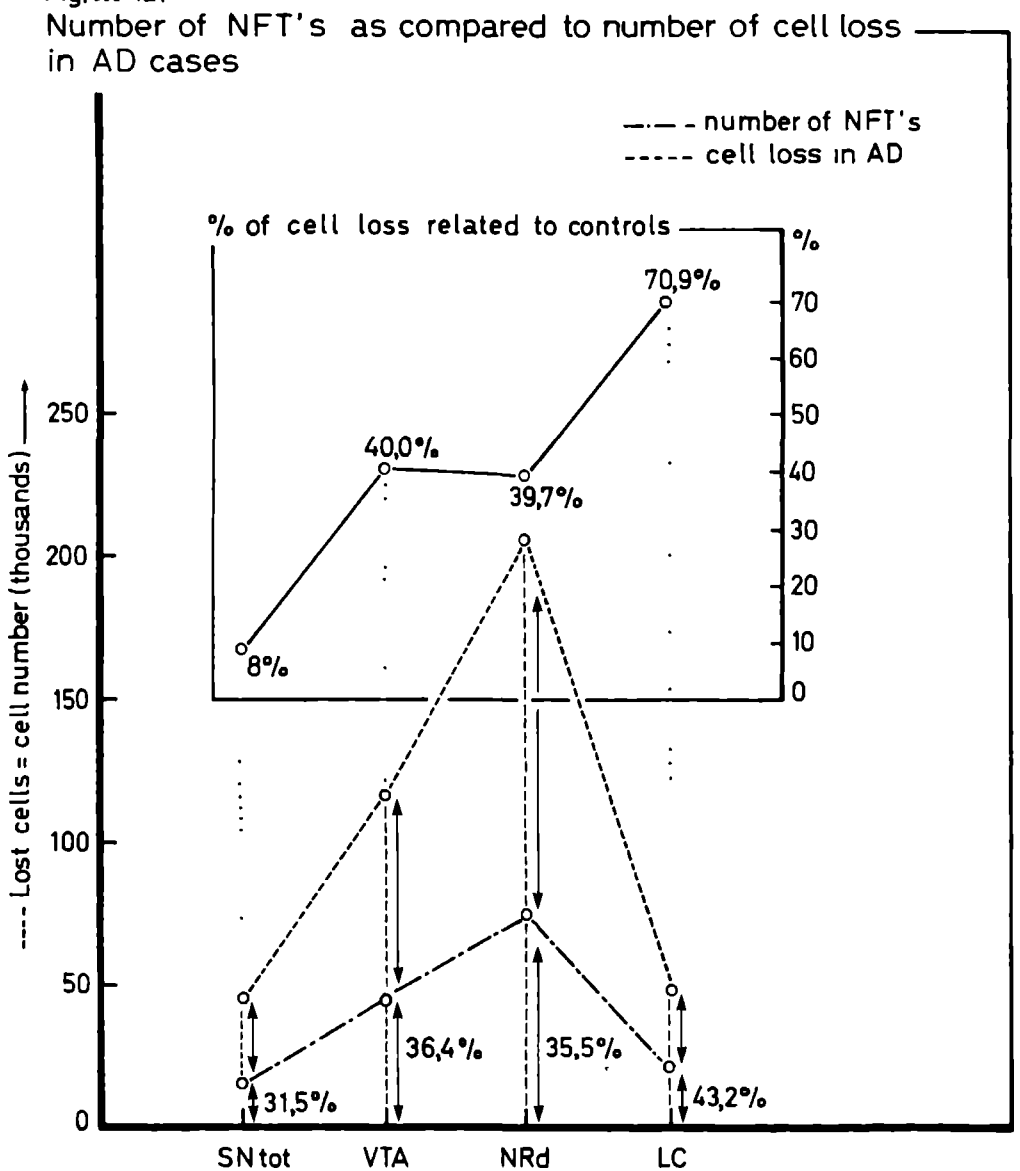
1. Number of neurons in control cases, both M+ and M-, on both sides of the brains.
2. Number of lost neurons in AD, both M+ and M-, bilaterally for the various nuclei.
3. Percentage of cell loss of AD cases as compared to controls.

Data of 1, 2, and 3. are as follows:

Neuron numbers of both sides:	SNtot	VTA	NRd	LC
in: Controls (M+/M-) (n=5)	584.272	284.928	520.576	68.552
AD brains (M+/M-) (n=4)	539.984	166.667	313.760	19.976
Cell loss in AD	44.288	118.261	206.816	48.576
Number of NFT's as compared to number of lost cells	31.5 %	40 %	35.5 %	43.2 %

In conclusion, the number of neurofibrillary tangles as a percentage of the number of lost cells in AD cases seems to be rather uniform, as illustrated in fig. III.12.

Fig. III 12.



The number of senile plaques as estimated for brains of patients dying with Alzheimer's disease is shown in table III.14.

Table III.14. Estimation of the number of senile plaques in monoaminergic nuclei SN, VTA, NRd and LC in Alzheimer's disease

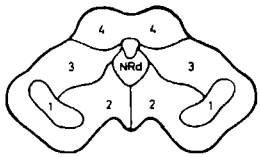
Age	Brain nr.	SNmed	SNlat	VTA	NRd	LC
65	87068	640	--	1.920	--	3.200
66	87062	1.280	3.840	4.480	3.200	7.680
69	87395	1.920	6.400	18.560	9.600	14.080
76	88177	--	5.120	10.240	4.480	2.560
93	88221	--	--	640	1.280	1.280

Senile plaques in the mesencephalon, both of control cases and of Parkinson's disease, were exceptional. Generally, it can be said that no senile plaques occurred in any of the monoaminergic brainstem nuclei in cases without Alzheimer's disease. Besides, the estimated number of senile plaques in cases with AD was significantly smaller than the number of neurofibrillary tangles and their greatest concentration was seen in the inferior colliculus and adjacent central grey.

Table III.15. gives an estimation of the number of neurofibrillary tangles that occurred in the brains of aged control patients. Neurofibrillary tangles now seemed to be diffusely spread, rather than occurring exactly within defined nuclear boundaries. Therefore the mesencephalon and rostral rhombencephalon has been subdivided into five areas and the number of tangles is noted for each distinguished area.

Table III.15. Neurofibrillary tangles within various areas of control brains and brains with Parkinson's disease. (For estimation of real number: multiplication with x 640)

Age	Brain number	State	Area 1	Area 2	Area 3	Area 4	NRd	LC
60	87029	C	-	4	-	-	9	6
62	87177	C	4	7	2	6	4	4
69	86179	C	2	4	2	3	2	-
80	88269	C	2	4	1	1	8	-
91	88184	C	-	1	-	3	2	4
74	87377	PD	7	8	4	12	11	4
79	86204	PD	2	6	-	1	5	2



1~Substantia Nigra
2~Ventral Tegmental Area
3~Reticular Formation structures
4~Tectum

It is important to note that the number of neurofibrillary tangles in the areas 3 and 4, as defined in table III.15, possibly have been underscored, since these areas were not within the scope of primary interest. The conclusion, that there seems to be a relative abundance of NFT's within areas 1 and 2 of control brains, has no validity therefore. It is remarkable, however, that both SN and VTA of the aged brain at least show some neurofibrillary degeneration.

The greatest number of NFT's occured in the brain of a patient who died with Parkinson's disease (case number 87377) in which a remarkable degree of **congophilic angiopathy** was seen. In such a case it is not always possible to differentiate between tangentially sectioned (fibrillary) vascular endothelian structures and transected neurofibrillary tangles. The number of neurofibrillary tangles as indicated here, will rather be an underestimation than an overestimation, because no fibrillary structures that could not be identified with certainty were included. The table (Table III.15) clearly shows that no clear correlation of quantitative neurofibrillary degeneration related to age could be demonstrated for the mesencephalon and rostral rhombencephalon.

III.5. GENERAL CONCLUSIONS AND DISCUSSION OF THE MORPHOMETRIC SECTION

III.5.1. Critical evaluation of the counting method.

Our counting method had to be easily adoptable with a minimum of bias, in order to provide a method for routine examination on different brainstem structures. Besides an exact location of the counted elements has to be assured. Recent developments in stereology (Gundersen 1986) have forced observers to consider different approaches in the counting and calculation of neural elements.This is especially true for the morphometric analysis in aging and aging disease, which is characterized by specific problems, as reviewed by Coleman and Flood (1987). Like immunohistochemical stained cellular elements and fluorescent profiles, the neuromelanin containing cells can easily be detected by a comparable computerized plotting system (German et al.1983,1988a; Agnati and Fuxe 1984a,b; Hirsch et al.1988). Differences in transmitter expression, caused by variations in tissue processing, age and post-mortem delay, as well as the yet undefined interaction between transmitter depletion and cell loss (Coleman and Flood 1987), arque in favour of the application of neuromelanin as a marker in cell countings. On the other hand melanin obscures other cytologic markers, such as nuclei and nucleoli (Pakkenberg and Brody 1965; Bogerts et al.1983) that could otherwise help to identify various elements. Therefore the rather large neuromelanin containing profiles, variable in size and shape, in order to be quantified demand correction by calculation (Abercrombie 1946; Bolender 1982). The correction formula as proposed by Abercrombie (1946), and used in several studies on the human catecholaminergic nuclei (Bondareff 1982; Bondareff and Mountjoy 1986; Halliday and Törk 1986; German et al.1988a) leads to some overcorrection of the counting error, especially for a relatively large mean neuron diameter with much variance ("lost caps", Haug 1986). Therefore Floderus' formula (Floderus 1944) might be used, compensating for this phenomenon, by taking into account the height of the "lost caps" (see presumptions nr. 5). Still some presumptions have to

be made and some biassing factors could not be excluded completely for practical reasons:

1. Quantification of CA neurons identified by their pigmentation presents some interpretative problems (Bogerts 1981; Saper and Petito 1982; Marsden 1983). Even though it is accepted that pigmented neurons may have at one time produced either DA or NA (see Ch. I.4.) the question remains whether all CA-producing neurons necessarily contain pigment. Therefore investigation in age-related and pathological degenerative changes in CA nuclei necessarily also has to include non-neuromelanin-pigmented neurons.

2. For a standard morphometric analysis of the substantia nigra and the ventral tegmental area it is useful to have eight equidistant sections for extrapolation to the real cell-number, after systematic random sampling, with a reliability of 95 % following Cavalieri's principle (Mattveldt 1987; see also Vogels 1990). Our atlas provides a matrix for this procedure. Some variation exists, however, in the position of different (sub)nuclei, for which this matrix does not provide sufficient information. But this variation is minimal for the different categories: substantia nigra medialis, substantia nigra lateralis, substantia nigra pars reticularis, ventral tegmental area and area retrorubralis. Each of these entities is sampled in more than eight sections (with the exception of the area retrorubralis). The selection of still more and smaller entities (i.e. subnuclei), for comparison with each other, would be of interest from an anatomical point of view, but had to be ruled out from a methodological point of view. Indeed, the reliability of countings on area A8 is significantly smaller and no conclusions will be drawn from countings of this area.

3. The neuromelanin-containing neurons have to be considered as spherical elements in order to make Floderus' and Abercrombie's correction formula (Floderus 1944; Abercrombie 1946; Haug 1986) suitable. The Kontron MOP-Videoplan calculates a "D-circle", representing the diameter of an equivalent spherical particle. The effect of these size and shape variations is not to great, as has been indicated by Gittes and Bolender (1987).

4. The same counts, to an unacceptable degree, for neurofibrillary tangles and senile plaques, of which the profiles are in no way exactly defined. Therefore counting results of neuropathologic elements in this study have to be defined "semi-quantitative".

5. Because variation in cellular sizes, although not always significant on statistical grounds, still might affect the counting results, some control calculations were

done, using Floderus' correction formula (Table III.16. and Fig. III.13.). Because of the introduction of 'lost caps' the outcome provides greater cell numbers, closer to reality. But in relation to each other the data do not change significantly as can be seen in figure III.13. Abercrombies correction formula is more generally used in literature and is therefore chosen as a standard in our calculations.
'2h' in Floderus' formula (Floderus 1944; see chapter I.3.2.) is the height of a lost cap, derived from its spherical diameter (for discussion Haug 1986; Albers et al. 1988). The numerical value of D-circle of a lost cap roughly corresponds with '2h' and is used in our calculations.
In conclusion, comparison of these data clearly shows that the use of either (uncomplicated) correction according to Abercrombie (1946) or (complicated) correction according to Floderus (1944) introduces no significant variation (compare Fig. III.13. with Fig. III.6. and III.7.).
6. Of the "lost caps", the smallest elements identified, it is impossible to trace their origin to either neuromelanin containing cells or other cells, when they do not contain neuromelanin. This, however, does not lead to much bias because the size of these elements was in the same range for the different categories.
7. In order to make the counting method uniform throughout the analysed structures, also the neurons without neuromelanin were counted as cellular profiles, although their nucleoli could well be identified. The comparability between the different quantified categories, however, is of importance, in order to justify general conclusions (Ch.IV.).
8. The choice of Congo-red fluorescence for identification and quantification of neurofibrillary tangles and senile plaques (Puchtler and Sweat 1962,1965) was based on a pilot study, comparing different ways of tissue processing and staining procedures. Congo-red fluorescence appeared to demonstrate optimally neurofibrillary changes and senile plaques, compared with Thioflavin S and Silver staining according to Gallyas (1971). It is now recognized that the Bodian stain does not reveal the density of neurofibrillary tangles and senile plaques seen in material stained with the Bielschowsky silver method, thioflavin S or Congo-red (Yamamoto and Hirano 1986). Diffuse plaque-like structures (Selkoe 1989; see also Vogels 1990), which are not detected by the conventional stains for amyloid, Congo-red and thioflavin S, were sometimes seen in Congo-red fluorescence, but were not counted as SP's (see also Fig. III. 1).
9. Perivascular fibrillary deposits or amyloid is difficult to distinguish from real

cellular neurofibrillary changes (see also Selkoe 1989). Mostly the appearance of blood cells or recognition of endothelial cells could determine the origin of the fluorescent material. Besides, lipofuscin has fluorescence properties (Barden 1981), although its granular appearance made it easy distinguishable from neurofibrillary tangles and senile plaques. The Congo-red fluorescence of lipofuscine could aid in differentiation of this age-dependent cellular pigment from neuromelanin granules, which do not excite fluorescence under the mercury arc lamp illumination of Congo-red stained sections.

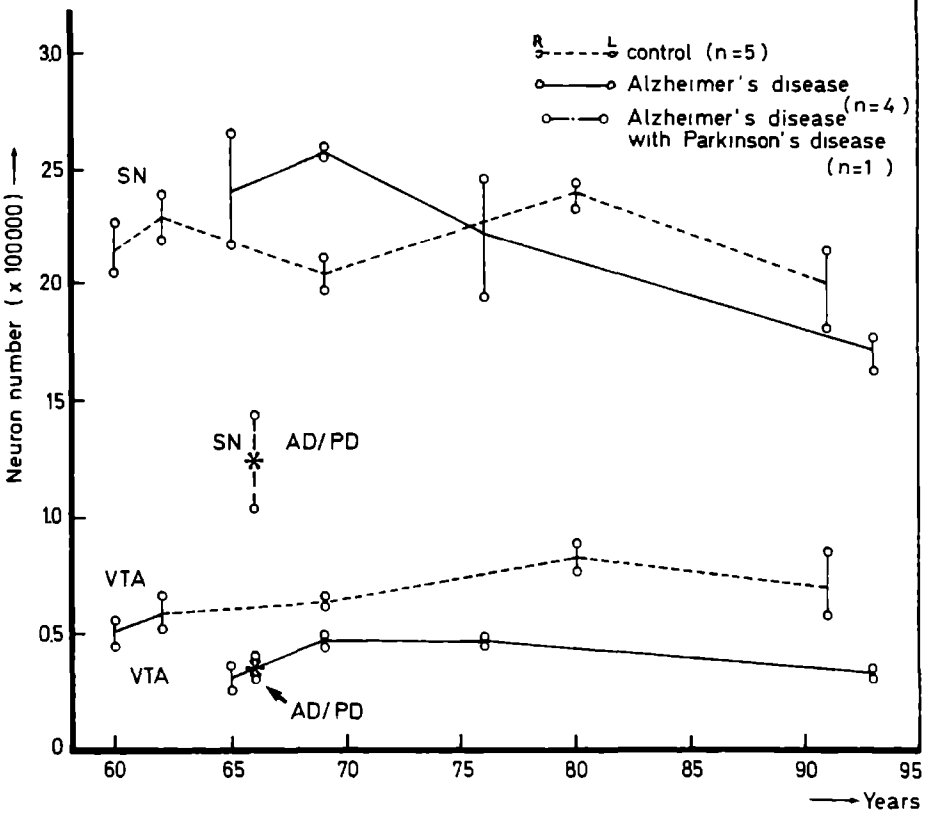
Table III.16. Neuron numbers of various subnuclei, corrected according to Floderus (1944)

Brain Nr.	Side L/R	M+/M-	SNmed	SNlat	SNret	VTA	A8
87029	R	M+	84,696	87,957	55,928	53,826	13,172
(C;60 yr)		M-	12,388	14,087	52,451	37,256	23,980
	L	M+	69,617	93,056	43,409	45,545	7,937
		M-	9,557	12,577	39,877	56,281	12,898
87177	R	M+	104,590	74,642	41,656	64,608	10,097
(C;62 yr)		M-	20,601	15,129	62,447	91,840	39,797
	L	M+	85,714	109,835	43,123	51,093	12,937
		M-	17,704	24,786	52,468	62,247	49,322
86179	R	M+	68,126	115,729	28,399	66,758	10,370
(C;69 yr)		M-	16,482	29,337	50,763	141,431	34,658
	L	M+	63,238	107,684	26,506	61,573	7,454
		M-	15,493	27,360	47,137	130,228	24,855
88269	R	M+	61,393	124,262	58,777	76,213	11,326
(C;80 yr)		M-	16,550	29,722	81,399	101,289	47,647
	L	M+	63,459	120,425	51,846	88,368	20,529
		M-	19,252	24,318	85,114	97,818	60,268
88184	R	M+	61,688	109,209	44,083	59,131	10,618
(C;91 yr)		M-	21,954	28,709	87,478	119,275	57,429
	L	M+	66,116	80,579	34,934	84,426	18,759
		M-	10,470	11,821	50,663	115,488	53,327
Mean neuron		M+	72,864	102,338	42,866	65,154	12,320
number (n=5)		M-	16,045	21,785	60,980	95,315	40,418

Brain Nr	Side L/R	M+/M-	SNmed	SNlat	SNret	VTA	A8
87068	R	M+	78,843	95,600	44,073	24,813	3,366
(AD;65 yr)		M-	10,620	13,018	31,517	21,693	10,507
	L	M+	86,109	125,090	55,091	34,812	12,117
		M-	13,361	25,008	35,285	28,811	11,185
87395	R	M+	84,915	115,180	58,232	50,772	3,602
(AD/SDAT)		M-	11,527	23,977	68,704	66,364	23,636
(69 yr)	L	M+	104,895	104,476	50,097	47,182	9,906
		M-	26,744	16,138	51,643	54,091	34,545
88177	R	M+	75,246	119,208	49,571	47,204	14,814
(AD/SDAT)		M-	16,212	18,914	53,055	57,182	50,911
(76 yr)	L	M+	63,044	99,961	32,189	46,448	i7,036
		M-	5,066	15,199	49,190	61,978	57,182
88221	R	M+	51,520	92,510	34,442	32,476	5,925
(SDAT;93 yr)		M-	30,736	38,504	67,109	73,046	33,203
	L	M+	51,520	81,645	28,970	37,008	5,185
		M-	16,550	23,643	58,325	90,385	16,232
Mean neuron		M+	74,512	104,209	44,083	40,089	8,994
number(n=4)		M-	16,352	174,401	51,854	56,694	29,675
87062	R	M+	62,438	56,520	24,834	34,361	901
(AD+PD)		M-	20,288	16,600	57,637	40,455	23,636
(66 yr)	L	M+	31,968	42,818	30,401	36,925	5,854
		M-	10,605	11,066	33,660	38,636	20,455

10. Statistical analysis of differences between controls and cases with AD or PD was done according to student's t-test. The presumption has to be made that cell numbers are "normally ditributed" among age categories and that disease cases are "sampled" in comparison to the mean of the standard population.
These presumption oblige to consider the levels of significance with restriction.
11. Floredus' (1944) and Abercrombies (1946) correction cannot rule out all bias in the determination of exact cell numbers, in material that has to be sectioned (Haug 1986; Williams and Rakic 1988).
But, evidently, the method's practicality has outweighed its problems and indeed it is most often used in literature on our subject. The use of a dissector (Gundersen 1986) is preferable on theoretical grounds, but its practicle application met with many difficulties, of which the indefinite recognition of cellular particles and the tracing of exactly similar areas in sectioned brainstem material, were the most important.

Fig. III.13.
Neuron numbers of SN and VTA as related to age. Correction according to Floderus (1944). (Neuromelanin containing neurons)



Each point represents left side and right side, respectively of a single case

III.5.2. Discussion of the counting results.

No doubt that the pathophysiological backgrounds of aging and aging diseases need be considered within the larger context of changes in glia, dendrites, axons, synapses, extracellular space, blood vessels as well as within the context of changes in neurons. But the estimation of neuron numbers and the quantification of neuropathologic findings provides a relative uniformly applicable method for grading one essential aspect. The relative few studies on exact numbers of nerve cells in the human and animal nervous system, however, seem to be related to difficulties inherent in such quantitative preparations (Hanley 1974; Brody 1978; Haug 1986; Coleman and Flood 1987). For a comparative analysis of age-related changes among species, however, data on neuron numbers and size, although highly variable, are most complete (Flood and Coleman 1988). As suggested by these authors (Coleman and Flood 1987), however, multivariate descriptors of brain morphology and chemistry, in a number of regions, are of need in order to provide more accurate distinctions between AD and normal, than is possible with any single parameter currently in wide use. The condition of various regions to be analysed has been settled more or less by the fact that other regions of the brain used for this study have been analysed in parallel studies (Broere 1990, Vogels 1990, De Vries 1990). On the other hand, the number of cases studied here is too small to draw far-reaching conclusions. **The value of our data rather is gathered from the systematic analysis of single cases, as regards main representative nuclei of monoaminergic brainstem systems, throughout their neuroanatomical extent.** A similar approach, although by far less complete, has been carried out by Mann and co-workers (Mann et al.1984a,b) and Zweig and co-workers (Zweig et al. 1988). Like most of the studies that quantify age related changes these authors measured either densities of neurons or number of neurons in representative sections, rather than total numbers of neurons. For matters of comparison, however, such semi-quantitative data have been referred to in the tables that follow. **Actually the first conclusion that might be drawn is, that variations in cellular sizes and numbers of various monoaminergic brainstem nuclei are such that major conclusions about cell loss related to aging and age-related disease should be based on large series, analysed following a systematic sampling procedure throughout the nucleus.**

The close correlation between the distribution of pathologic changes, the degree of cell loss and pre-mortem clinical assesment of disease-state show that these morphometric changes are greatly exacerbated in AD and PD, although an overlap with the findings in normally aged people is remarkable, especially as regards the eldest (Tomlinson et al. 1970; Alvord et al. 1974; McGeer et al. 1977; Mann et al. 1984 b; Jellinger 1987c). This general statement also is applicable to most of the data in our study, especially when neuron countings are corrected for tissue shrinkage and cellular shrinkage. The cell loss of the VTA in AD, e.g., was also counted without correction and the difference between normally aged and AD cases was much more pronounced then. On the other hand, because of the limited number of cases available, only the most profound effects may be detectable (Coleman and Flood 1987; Williams and Rakic 1988) and statistically significant differences between mean values of the quantitative data have a particular value in this respect (see Fig. III.6. and III.7. on the correlation between cell number and age for the substantia nigra). The many questions that have been raised in the literature as regards the importance of morphometric changes also involve the regional distribution of cell loss within a given nucleus (Zweig et al. 1988; Marcyniuk et al. 1989; Chan-Palay and Asan 1989a,b), the relation of cellular size to vulnerability of cell survival (Mann et al.1984a; Curcio and Kemper 1984) and the importance of parameters like neuromelanin (Mann and Yates 1974a,b; Hirsch et al. 1988;) and changes in monoamine levels (Cross et al. 1983; Mann and Yates 1986; Kaye et al. 1988a; Reinikainen et al. 1988). It will be clear that it is hardly possible to compare all these parameters in relation to data from literature although tables III.17-III.19 allow comparison for most of them with the references cited.

Table III.17. Some data from literature on *cell numbers* of substantia nigra and ventral tegmental area, in several species

Authors	Substantia Nigra	Ventral Tegmental Area	Correction	Species
Pakkenberg and Brody 1965	(M+) 1029/section (M-) 387/section		no	human
Mann et al. 1984/1987	494.8 ± 17.8/section	28.8 ± 2.2/section	no (nucleoli)	human
Tabaton et al. 1985	1,000 - 1,100/section		no	human
McGeer et al. 1977	450,000-150,000 (age dependent)		no	human
Bogerts et al. 1983	(M+) 300,000 (part of VTA)	(M+) 5,000	(Haug 1976)	human
Halliday and Törk 1986	436,000	690,000	Abercrombie	human
Hirsch et al. 1988	213,186 ± 5,451	32,314	no	human
Poirier et al. 1983	73,508 ± 5,583		no	monkey
Halliday and Törk 1986	71,000	47,000	Abercrombie	monkey
German et al. 1988	61,883 ± 7,170	14,110 ± 1,333	Abercrombie	monkey
Percheron et al. 1989	116,000 (SNc) 18,000 (SNl) 36,000 (SNr)		Abercrombie	Macaca mulatta
Van Domburg 1990	(M+) 203,139 ± 19,248	(M+) 56,752 ± 11,799	Abercrombie	human
	(M-) 88,997 ± 24,569	(M-) 91,606 ± 29,764	Abercrombie	human
Van Domburg 1990	(M+) 218,068	(M+) 65,154	Floderus	human
	(M-) 98,810	(M-) 95,315	Floderus	human

Total neuron numbers of the normal SN and VTA are mainly derived from neuroanatomical investigations not directly involved with neuropathology (Table III.17.). The rate of neuronal decrement with age found in several studies (Riederer and Wuketich 1976; McGeer et al. 1977,1978; Mann et al. 1984a; Riederer and Kruzik 1987; Calne and Peppard 1987), ranged from continuous loss of about 40% throughout life to a decline of 35% that begins mainly after the age of 65 (Mann et al. 1984a). Such substantial cell loss could not be confirmed in our study, but it neither could be excluded because of the small number of cases studied (Table III.6. and III.7.). The degree of cell loss in the SN is highly variable and could not be correlated with degrees of dementia (see also Gaspar and Gray 1984) or Parkinsonism (Pakkenberg and Brody 1965). The overall relation to Parkinson's disease, however, is highly significant, especially as regards the lateral part of the SN pars compacta (see also Oppenheimer 1984; Hornykiewicz and Kish 1986; Jellinger 1986b, 1987a; Hirsch et al. 1988), where cell loss was about 80% from that of age-matched controls. Secondly, within each cell group of the PD brains, there is a greater relative sparing of non-pigmented than of neuromelanin-containing neurons, as was also shown by several other investigators (Foix and Nicolesco 1925; Pakkenberg and Brody 1965; Mann and Yates 1986; Hirsch et al. 1988). Extra neuronal pigment and extremely weakly stained pigmented cells were more numerous in disease cases, particularly PD. This evidence suggest a selective vulnerability of the neuromelanin-pigmented subpopulation of dopamine-containing mesencephalic neurons in PD (see also Chapter I.4.). Likewise, a more or less differential cell loss of unpigmented neurons was seen in AD/SDAT cases. Our results as regards nigral cell loss in AD/SDAT (6% of neuromelanin-pigmented neurons and 12% of unpigmented cells; both not significant) agrees well with data from literature (Mann 1985; Chui et al. 1986; Mann and Yates 1986; Jellinger 1987b; Gibb et al. 1989b).

A matter of interest is whether cognitive impairment might be related to cell loss in the medial part of the SN (Bogerts et al. 1983; Mann 1985; Rinne et al. 1989; Gibb et al. 1989b). Such findings have a rather firm neuroanatomical basis (see Chapter II.2.6.), although other (non-anatomical) factors might be involved (see Javoy-Agid and Agid 1980; Hamill et al. 1988). Generally, the pathological basis for intellectual impairment associated with PD is controversial (Hakim and Mathieson, 1979; Bogerts et al. 1983; Gaspar and Gray 1984; Chui et al. 1986; Rinne et al. 1989). Our material could not settle this question. Patient number

87377 had the lowest neuron number of the medial part of the SN (73% cell loss) and showed also the most extensive neurofibrillary changes. The second most affected was the SN pars medialis of patient number 89141, who had a psychiatric history of major depression. Patient number 87062, who had combined AD and PD, had a relatively well-preserved medial part of the SN (40% total cell loss) and patient number 89142 (with 34% cell loss in the medial part of the SN) was deeply imbecile from youth on and only had mild symptoms of Parkinson's disease. Such findings suggest that primary cortical degeneration might be independent from primary subcortical pathology. Regrettably, no exact information on the degree and manifestation of the parkinsonian syndrome of these patients are available. Neuronal loss in the ventral tegmental area has been reported both in PD (Javoy-Agid and Agid 1980; Bogerts et al. 1983; Uhl et al 1985; Jellinger 1987a,b; Hirsch et al. 1988) and AD (Mann et al. 1987; Gibb et al. 1989b). We not only could confirm these data, but also found more specified loss of neuromelanin-pigmented cells in PD (53%) and primary loss of neurons without neuromelanin pigment (47% cell loss) in AD cases. The overall cell loss in the VTA was comparable for both AD and PD cases.

Neurofibrillary changes in both SN and VTA have been described since long in AD patients (Hirano and Zimmerman 1962; Ishii 1966; Yamada and Mehraein 1977; Hunter 1985; German et al. 1987; Gibb et al. 1989b) or patients with dementia (Gaspar and Gray 1984; Hunter 1985; Chui et al. 1986; Yoshimura 1988). As Gibb and co-workers recently concluded (Gibb et al. 1989b), mild degenerative changes accompanied by tangles in the SN and VTA are common in AD, but severe cell loss in the SN is rare. The distribution pattern of NFT's throughout the mesencephalon and rostral rhombencephalon in our cases (Fig. III.10. and III.11.) corresponds very well with that described by others (Hirano and Zimmerman 1962; Ishii 1966; Tomlinson and Corsellis 1984). The same counts for the destribution pattern of senile plaques (Iseki et al. 1989; Ohm and Braak 1989). The prevalence of such neurodegenerative changes in the VTA is significantly higher than that in the SN, parallel to the difference in total cell loss in AD cases. Generally, the distribution pattern of neurodegenerative changes, although preferentially restricted to the most cell-rich areas, was throughout the mesencephalon, without keeping to strict nuclear boundaries. Indeed somewhat more NFT's e.g., were found at the most rostral boundaries of the VTA (limbic midbrain area or hypothalamic-ventral tegmental transition zone) and the most lateral border of the SN ('nucleus

peripeduncularis' and, particularly, the area belonging to the corpus geniculatum mediale). At places where blood vessels are most frquently seen, like the substantia perforata posterior and near the fourth ventricle and aqueduct, relatively many neurofibrillary changes were found, sometimes rather undefinable and not of the typical 'tangle' appearance. Such changes (particularly for brain number 87377, f,74 yr,PD) were very similar to the congophilic angiopathy as described in AD (Tomlinson and Corsellis 1984; Selkoe 1989).

Locus coeruleus

Table III.18.a. Some data from literature on neuron numbers of the human locus coeruleus

Authors	Neuron number	Elements counted	Correction	Remarks
Brody 1978	18,950 - 9,310	neurons with nucleoli/M+	no	age-dependent
Vijayashankar and Brody 1979	13,378 ± 2,145	neurons with nucleoli/M+	no	mean number 60-89 years
Perry et al. 1981	66 4 ± 6 4/section 20 µm		no	LC might be about 20-25 sections
Mann et al. 1983	120 - 50/ section 20µm	nucleoli	no	age-dependent
	98.3 ± 4.3/mm^2	M+ cells/ nucleoli	no	
Tomonaga 1983	17,630/2 - 11,515/2	neurons with nuclei/M+	no	age-dependent
Iversen et al	9,642 ± 465	M+ cells	no	no significant
1983	9,426 ± 765	DBH-ir cells	no	difference M+/ DBH-ir
Bondareff et al. 1982	9,551 - 16,427	total neurons	Abercrombie	
Mountjoy and Bondareff 1986	9,500 - 17,000	total neurons		
German et al 1988	23,000 - 8,953	M+ cells	Abercrombie modified	
Baker et al. 1989	53,500/2	TH-ir/neurons	no	
Marcyniuk et al. 1989	49.7 ± 45 - 196 0 ± 12 5/section (both sides!)	M+ neurons	no	
Chan-Palay and Asan 1989a	33,130 - 19,740	TH-ir cells	no	
Van Domburg 1990	19,976 ± 3,627	M+ cells	Abercrombie	n=5
	14,300 ± 2,889	cells	Abercrombie	

The locus coeruleus is the most extensively studied catecholaminergic nucleus in aging and aging diseases, mainly for two reasons. Diffuse cortical projections have been demonstrated both in rodents and primates (see Ch.II.3.3.) and reduced levels of noradrenaline in LC projection fields have been showed both in PD and AD (Farley and Hornykiewicz 1977; Adolfsson et al. 1979; Scatton et al. 1983; Gottfries et al. 1983; Agid et al. 1987). A relationship between noradrenergic denervation and dementia, comparable to that of ACh and the nucleus basalis of Meynert, has been suggested both in AD and PD (Alvord et al. 1974; Perry et al. 1981; Bondareff et al. 1982; Mann and Yates 1983, 1986; Gaspar and Gray 1984; Mann et al. 1985; Cash et al. 1987). Secondly, the locus coeruleus was one of the first subcortical nuclei to be associated with normal aging, and suitable for the estimation of neuron numbers related to age (Brody 1978; Tomlinson et al. 1981). Nowadays computer assisted reconstruction has been shown to be a good aid in the rapid morphometric analysis of this brainstem nucleus (German et al. 1988a; Baker et al. 1989; Chan-Palay and Asan 1989a,b). Such reconstruction e.g. revealed changes in the rostro-caudal length of the nucleus, varying between controls (14.9 ± 1.4 mm), SDAT-cases (13 ± 2.2 mm) and PD-cases (12.4 ± 5 mm), whereas marked changes in neuronal morphology could be quantified (Chan-Palay and Asan 1989a,b). Counting of neuromelanin pigmented cells in the human LC (Tomlinson et al. 1981; Bondareff et al. 1982; Mann et al. 1982, 1983, 1984a; German et al. 1988a) seemed accurately to reflect the number of CA cells (Baker et al. 1989) and direct comparison could be made between studies in which TH-immunoreactivity cells were counted (see Table III.18.). It remains to be settled whether pigmentation is associated only with noradrenaline-containing neurons and whether neuronal cell loss is the only cause of depigmentation of the LC (Cash et al. 1987). Quantitative assessment of immunocytochemically identified CA neurons of the LC has been described in various studies now (Iversen et al. 1983; Baker et al. 1989; Chan-Palay and Asan 1989a,b). As can be seen from table III.18. the number of immunoreactive neurons, ranging from 9,426 DBH-positive cells (Iversen et al. 1983) to 33,130 TH-immunoreactive (Chan-Palay and Asan 1989a,b) cells roughly corresponds with our number of neuromelanin-pigmented neurons and total number of neurons, after correction for cellular sizes. A reduction in soma size occurs in cells of all morphological classes in adult brains (German et al. 1988a; Chan-Palay and Asan 1989a). We measured a mean D-max of 54,4 µm and a mean D-circle of 35,6 µm, comparable to the mean soma size of TH-ir cells of

37,4 µm of Baker and co-workers (Baker et al. 1989). Shrinkage of cells preferentially occurred in AD and PD cases and was surmised by swelling and dendritic arbor reduction of individual neurons (Chan-Palay and Asan 1989b), probably due to disintegration of the cytoskeleton (see also Mann 1985). The importance of determining both D-cirle and D-max as well as soma areas is clearly demonstrated by such findings.

Although cell loss and neurofibrillary changes in the LC have been most extensively documented in AD/SDAT (for reviews Coleman and Flood 1987; Chan-Palay and Asan 1989a,b) such findings have been known since long to be characteristic of PD also (Greenfield and Bosanquet 1953; Beheim-Schwarzbach 1952,1954). Cell loss in both disease states and in aging shows substantial variation as is shown in table III.18.b. The greatest cell loss, noted by Chan-Palay and Asan (1989a) was seen in a patient with chronic depression. The degree of LC degeneration was found to be extremely variable, especially in PD cases, ranging from 77% (AD-case 88177) to 0% (PD-case 89142), for neuromelanin containing cells.

Table III.18.b. Degree of cell loss in the human locus coeruleus: data from literature

% Neuron Loss	Disease/Age	Authors
24.6 - 87.5 (TH+)	SDAT	Chan-Palay and Asan 1989b
30.9 (TH+)	PD	Chan-Palay and Asan 1989b
48.3 (TH+)	PD-dementia	Chan-Palay and Asan 1989b
30 - 50 % (M+)	old age	Vijayashankirand Brody 1979
50 - 80 %	AD/SDAT	Tomlinson et al. 1981
37.5 - 70 %	AD/SDAT	Chui et al. 1981
70 - 87 %	PD	Chui et al. 1981
60.3 %	AD/SDAT	Jellinger 1987b
40 %	old age	Tomonaga 1983
31 - 73 %	SDAT	Tomonaga 1983
90 %	PD	Tomonaga 1983
72 - 85 %	PD	Jellinger 1987a
79 %	PD	Mann et al. 1983
40 %	old age	Mann et al. 1984a
50 - 79 %	AD/SDAT	Mann et al. 1982, 1983, 1986
55 % (TH+)	PD	Hirsch et al. 1988
56 % (M+)	dementia	Bondareff and Mountjoy 1986
60 %	SDAT	Iversen et al. 1983
58.3 %	AD	Perry et al. 1981
ca. 30 %	old age	Marcyniuk et al. 1989
32-47 %	PD/AD	Zweig et al. 1989b
69.4 %	AD(M+)	Van Domburg 1990
73.0 %	AD(M-)	Van Domburg 1990
44.6 %	PD(M+)	Van Domburg 1990
44.0 %	PD(M-)	Van Domburg 1990

Our PD-case (nr. 87377, age 74 yr.), with the most extreme cell loss in the LC (both sides) of PD cases, was the same as the one that showed relatively abundant neurofibrillary changes in the SN, VTA and NRd. Normally a highly significant correlation coefficient for the left and right sides of the brainstem nuclei locus coeruleus has been noted (German et al. 1988a; Baker et al. 1989) but laterality differences in neuron numbers of disease cases are substantial (see Chan-Palay and Asan 1989b). Our results contrasted with those of the latter authors in that we found more severe degenerative changes and cell loss in SDAT cases as compared to PD cases (see also Cash et al. 1987). Topographical arrangement of cell loss in the LC of AD cases (Marcyniuk et al. 1986; Chan-Palay and Asan 1989b) could be confirmed by us only for the preferential loss of cells without neuromelanin

pigment in the rostral part of the LC. Such findings might very well be correlated with neuropathological changes in the projection areas of these neurons in the neocortex (Mann et al. 1985; Emson and Lindvall 1986) but also, to neurochemical characteristics, like appearance or absence of neuromelanin (Mann and Yates 1974b; Cash et al. 1987). Both demented and non-demented parkinsonians show reduced levels of NA in LC projection fields, like cerebral cortex and hippocampus (Scatton et al. 1983), whereas neuronal loss in these cases is uniformly distributed throughout the nucleus. In mentally normal old people cell loss is also uniformly distributed throughout the whole nucleus (Marcyniuk et al. 1989). Whereas neuromelanin might be a risk factor in cellular decrement in PD and aging (Hirsch et al. 1988), in AD neuromelanin containing neurons were relatively more preserved. Because depigmentation was not related to the loss of noradrenaline, Cash and co-workers (Cash et al. 1987) concluded that the correspondence between catecholamine- and pigment-containing cells, which seems to hold for DA cells in the ventral mesencephalon, may not be true of NA neurons in the LC.

Neurofibrillary changes (NFT's and SP's) associated with LC cell loss have attracted much attention and have been described by many authors (Behcim-Schwarzbach 1954; Ishii 1966; Yamada and Mehraein 1977; Forno 1978; Mann et al. 1982; Gaspar and Gray 1984; Tomlinson and Corsellis 1984; German et al. 1987). Neurofibrillary degeneration seems to be age-related (Tomonaga 1983; Mann et al. 1984a) and significantly more abundant in AD than in PD and aged controls. The number of nerve cells showing neurofibrillary changes generally is only roughly estimated and comparison among data from literature is hardly possible. The mean senile plaque count per optical field of the temporal cortex correlated with changes in DBH and CAT in normal and demented cases (Perry et al. 1981) and with cell loss of the LC (Tomlinson et al. 1981; Gaspar and Gray 1984; Mann et al. 1985; Marcyniuk et al. 1986a,b). But dementia selectively associated with PD, i.e. of the subcortical type, correlated with severe neuronal loss in subcortical nuclei, without significant numbers of plaques or tangles in the hippocampus and cerebral cortex (Gaspar and Gray 1984; Chui et al. 1986).

Table III.19. Some data from literature on neuron numbers of the human nucleus raphe dorsalis

Authors	Neuron number	Elements counted	Correction	Remarks
Curcio and Kemper 1984	526,9 / 7.40 mm^2 = 71 / mm^2	total nucleoli	Abercrombie	
	21 / mm^2	nucleoli > 3.51 ± 0.22 µm	Abercrombie	
Yamamoto and Hirano 1985a,b	10.6 ± 3.4 / mm^2	neurons > 25µm nucleoli	no	
Zweig et al. 1988	103.2 - 114.5/ section			sections 12µm thickness
Van Domburg 1990	520,576 ± 160,000	nucleoli	no	

Although biochemical studies of the serotonergic system in aging AD and PD have created controversy (Robinson et al. 1972; Gottfries et al. 1973, 1983; Adolfsson et al. 1979; Benton et al. 1982; Mann and Yates 1986; McGeer and McGeer 1987; D'Amoto et al. 1987b; Reinikainen et al. 1988a) some morphometric studies of the NRd (Curcio and Kemper 1984; Yamamoto and Hirano 1985a,b; Jellinger 1987b) have called attention to this nucleus as another possible important site of abnormality especially in Alzheimer's disease. No change of serotonergic activity in cortical areas of aging rhesus monkeys has been detected (Goldman-Rakic and Brown 1981). The fact that no data on the total cell number of the dorsal raphe nucleus are available may be related to the fact that there is no clear-cut border between the NRd and the surrounding periaqueductal gray matter defined in literature. There is no natural marker at hand in the NRd, like neuromelanin in catecholaminergic nuclei. Most NRd cells contain lipofuscin (Ohm et al. 1989) that is, however, distributed throughout the brain (Mann and Yates 1974a,b; Braak

1980). Neuron numbers are usually defined as neuronal densities or neurons per section without definite volumetric data. The mean cell density of neurons in controls ranged from about 8 per mm^2 (Curcio and Kemper 1984; corrected according to Abercrombie) to 10.6 ± 3.4 per mm^2 (Yamamoto and Hirano 1985a,b). Zweig et al.(1988) noted 103.2 - 114.5 neurons per section of 12 •m thickness at three levels.

Different views on the extent of the exact area captured by the nucleus raphe dorsalis also follow from data on its rostro-caudal length, ranging from 4-5 mm (Törk and Hornung 1986) to 20-25 mm (Ohm et al. 1989). The boundaries of the NRd as defined in our study are such that (part of) the nucleus centralis superior, as defined by others, might be involved. The total cell number we counted, based on the number of nucleolated cells with lipofuscin, but without restriction to neuronal sizes, ranged from 385,280 to 677,120. Remarkably enough an age-related increase, both in controls and AD/SDAT brains was noted. Such an increase perhaps might be explained by shrinkage, and consequently higher densities, of nucleoli (Mann et al. 1984a; Curcio and Kemper 1984), without appropriate correction for this bias. No significant cell loss was detected in the NRd of PD-brains, wheras AD-brains showed about 40 % cell loss throughout the rostral-caudal extent.Neuronal loss of the NRd in AD/SDAT, as registered in literature, ranged from 77 % (Yamamoto and Hirano 1985a) to no evidence of overall neuronal loss (Curcio and Kemper 1984). Jellinger (1987a,c) also noted a neuronal decrement of 44.5 % in PD-cases. Cell loss both in AD and PD seems to be preferentially restricted to large polygonal neurons (Curcio and Kemper 1984; Yamamoto and Hirano 1985a,b; Jellinger 1987a; Zweig et al. 1988). In AD/SDAT an average of 2.25 % of the total NRd population contained NFT's according to Curcio and Kemper (1984), compared with 0.35 % in aged controls. We counted about 14 % neurofibrillary degenerated cells in AD/SDAT, compared with 2.6 % in control cases of similar age. Ishii (1966) observed in six cases with SDAT, with a mean age of 75 yrs. a substantially greater number of NFT's in the NRd, noting in four of six brains that over 50 % of the neurons contained NFT's. Yamamoto and Hirano (1985a) noted a 39-fold increase in NFT number of AD/SDAT-cases as compared to controls. They also saw few to several senile plaques in the NRd in all cases of AD, with or without amyloid core, less common than in the dorsal part of the periaqueductal grey and inferior colliculus (see also Yamada and Mehracin 1977). Although neuronal loss within the nucleus centralis superior could not be

confirmed separately in our study, these cells also clearly showed NFT's, of importance concerning their projection to the hippocampus and other brain regions severly affected in AD.

Adjacent brainstem nuclei

The tegmental pedunculopontine nucleus, pars compacta (NTPP), a putative cholinergic nucleus in the dorsolateral mesencephalic reticular formation, has been shown to suffer significant neuronal loss in PD, AD/SDAT and progressive supranuclear palsy, with frequent occurence of Lewy bodies and NFT's (Hirsch et al. 1987; Zweig et al. 1989a; Mufson et al. 1988; Jellinger 1988). Although such findings have been disputed (Woolf et al. 1989), we also saw clear accumulation of neurofibrillary degeneration within the area of the tegmental pedunculopontine nucleus (Fig. III.10. and III.11.). Both types of cytoskeletal lesion were infrequent in controls (see also Jellinger 1987b) and the degenerative changes apparently are more selectively affected in progressive supranuclear palsy and PD than in AD/SDAT, suggesting a stronger correlation of degeneration with functional aspects than with biochemical characteristics (Jellinger 1987c). Likewise, the dorsal tegmental nucleus, another cholinergic brainstem nucleus, has been shown to be involved in Alzheimer's disease neuropathology (Mann et al. 1985). Such changes are continuous with those of adjacent auditory brainstem nuclei (Ohm and Braak 1989) and nucleus of Edinger-Westphal (Hirano and Zimmerman 1962; Hunter 1985). It is not at all clear whether NFT's and SP's within such brainstem tegmental nuclei are confined to either nuclear boundariess or histochemically defined cell areas. Indeed, to our experience, NFT's and SP's were found very often in transition zones, like that of caudal hypothalamus-VTA; LC-NRd; NRd-NLTd; NTPP-CI; SN-CGM; VTA-corpus mamillare and so on.

IV GENERAL DISCUSSION

"De ouderdom is voor het leven als het laatste bedrijf van een toneelstuk.
Wij moeten trachten het einde ervan te zien vooraleer we het beu zijn, vooral wanneer wij het risico lopen er een walg van te krijgen."

Cicero, De ouderdom; ca. 50 v. Chr.
Vert. H. Beek, E De Ridder, 1949

Early and accurate diagnosis of Alzheimer's disease has a major impact on the progress of research on dementia and its eventual therapies. To address the problems involved in diagnosing AD in its earliest stage and to differentiate it from normal aging and age-related diseases, a research program was started by a "joint venture" of the Departments of Anatomy/Embryology and Neurology, emphasizing the importance of longterm studies based on standardized procedures and careful controls. An inventory of the most important brain areas involved in the dementia syndrome, as well as standardization of procedures for these brain areas, were developed. Because various brain areas present their own characteristic difficulties as regards morphometric analysis, different projects were directed at the neocortex (Broere 1990), the hippocampus (De Vries 1990), the nucleus basalis of Meynert (Vogels 1990) and the amygdala (Vereecken, in progress). The present study sought to provide detailed information about morphometric changes and procedural difficulties of the brainstem monoaminergic nuclei: Substantia Nigra (SN), Ventral Tegmental Area (VTA), Locus Coeruleus (LC) and Nucleus Raphe dorsalis (NRd). In future research cases also have to be closely observed during prolonged hospitalization in order to properly document the relationship between these monoaminergic systems and the degree and specific type of dementia.

Generally, highly variable amounts of neurons and age-related cell loss are found (Coleman and Flood 1987; Williams and Herrup 1988), suggesting that cell loss in the brain can be quite variable, but also that quantitative morphometric analysis represents a host of problems which may cloud the interpretation of many studies. The determination of cell numbers in subcortical structures is less uncertain than it is in the cerebral cortex, because of the usually relatively precise boundaries of most structures studied, as well as their generally more limited extent. On the other hand various monoaminergic brainstem nuclei appeared not at all uniformly defined in literature as regards their exact boundaries, their possible subnuclei and their connections with other brain areas in the primate brain. For these reasons a substantial part of our work has been devoted to the development of a practicable counting procedure (adapted to modern stereological principles) and extensive neuroanatomical examination of the brainstem monoaminergic nuclei under consideration. We are well aware that much about pathophysiology in aging diseases can not be captured by morphometry alone, no matter how carefully done (Saper 1987b).

While the clinical criteria of primary degenerative dementia are widely used

(McKhann et al. 1984; Khachaturian 1985; Schulte 1989) follow-up studies with neuropathological examination are rare and often retrospective (Todorov et al. 1975; Tierney et al. 1988; Chui 1989). The final diagnosis of AD still requires neuropathological confirmation (Todorov et al. 1975; Blessed 1980; Terry and Katzman 1983; Tomlinson and Corsellis 1984; Khachaturian 1985; Kellett 1987) either by biopsy or autopsy specimen. But clinical aids for differentiation (like the scales of Hoehn and Yahr (1967) and Webster (1968) in PD) are becoming increasingly important (Folstein et al. 1975; Sulkava et al. 1983; Flicker et al. 1985; Reisberg et al. 1985; Mortimer et al. 1987; Tierney et al. 1988; Gibb 1989a). A tissue bank, housing material representative of the full range of neurologic, clinical, pathologic and other data pertinent to the study of AD (Khachaturian 1985) seems to be a hardly realizable idea, although in our study support was provided by the Dutch Brain Bank. More detailed information, however, was available for patients followed at our own University hospital or local nursing homes, and undergoing autopsy at the University Institute of Pathological Anatomy.

The relationship between clinical findings and pathological phenomena has been controversial since long, both as regards Alzheimer-like neurofibrillary changes and "parkinsonian" Lewy bodies (Tomlinson et al. 1970; Wilcock and Esiri 1982; Sulkava et al. 1983; Ball 1984; Ulrich et al. 1986; Jellinger 1987b; Crystal et al. 1988). Whereas Lewy bodies might be primary findings of cortical pathology in some dementia cases (Sima et al. 1986; Kosaka et al. 1988; Gibb et al. 1989a), some 'motor-diseases' like PD (Alvord et al. 1974; Gibb 1989b), amyotrophic lateral sclerosis (Meyers et al. 1974) and progressive supranuclear palsy (Agid et al. 1986; Jellinger 1988) show significant neurofibrillary changes in brainstem nuclei. Our case of PD, number 87377, who had no clinical signs of dementia, showed substantial neurofibrillary changes throughout the mesencephalon, reminiscent of the 'idiopathic tangle type of Parkinsonism' as described by Alvord et al. (1974). Similar findings were registered in the nucleus basalis (see Vogels 1990) of this patient. Non-demented subjects with such Alzheimer pathology might have 'preclinical' AD, but numerous cortical plaques also have been seen in some elderly subjects who never developed clinical dementia (Terry et al. 1981; Wilcock and Esiri 1982; Crystal et al. 1988). Likewise, various brain diseases as well as the aging proces per se may be responsible for the typical dementia syndrome (Sulkava et al. 1983; Chui et al. 1985,1989; Ulrich et al. 1986; Gottfries 1986). Recent immunocytochemical analysis of brain tissue from pa-

tients with AD and Down's syndrome suggest that the deposition of ß-amyloid protein in a nonfibrillary ('preamyloid') form may represent earliest, age-related, Alzheimer-like abnormalities, diffusely spread over various brain areas (Hardy 1988; Selkoe 1989). The significance of this overlap becomes more clear when the association between quantifiable measures of neuropathological changes in the brain after death is examined. This subject is particularly stressed here as regards the dementia syndromes of Alzheimer and cognitive decline related to Parkinson's disease (Jellinger 1987b; Gibb 1989b). The brainstem is unique in harbouring nuclei in close association that might be typically involved in at least one of both diseases. Indeed a remarkable overlap in cellular loss of the VTA and Locus Coeruleus for both diseases stands against a more selective involvement of the SN and the NRd in PD and AD, respectively (see Ch. III.; Jellinger 1987b,c; Hamill et al. 1988; Gibb et al. 1989b). The concept of quantitative rather than qualitative change (or the principle of 'threshold effects', widely appreciated in the field of neuropsychiatry) as the major differentiating feature in aging and age-related diseases (for discussion: Tomlinson et al. 1970; McGeer and Mc Geer 1984; Coleman and Flood 1987; Brizee 1987; Brody 1987; Saper 1987b; Williams and Herrup 1988) thus only holds for certain nuclei, in certain cases.

In PD decrease of dopamine and homovanillic acid in the SN and striopallidum correlated significantly with the loss of nigral neurons (Bernheimer et al. 1973; Jellinger 1986c, 1987a; Javoy-Agid et al. 1984; Morgan and Finch 1988). In Alzheimer's disease the activity of the cholinergic enzyme choline acetyltransferase did correlate more significantly with specific aspects of the Alzheimer disease process than did the noradrenergic system (Perry et al. 1981; Mann and Yates 1986). Yet, the concept of specific mono-transmitter-related states had to be abandoned: A (biochemical) overlap of various disease states as well as the aging proces itself is significant (Rossor 1982; Perry et al. 1985; Quinn et al. 1986; Gottfries 1986). The ChAT activity in demented parkinsonian patients is severely reduced (Perry et al. 1985,1987), whereas both noradrenergic and the mesolimbic dopaminergic projections are affected in AD as well as PD (Javoy-Agid et al. 1981; Lees and Smith 1983; Mann and Yates 1986; Quinn et al. 1986; Cash et al. 1987; Hamill et al. 1988; Gibb et al. 1989b). But, the (histochemical) meaning of the substantial cell loss of neurons without neuromelanin pigment within the VTA (47 % in our cases) has to be elucidated yet. It corresponds with the loss of neuromelanin-containing, presumed dopaminergic, neurons within this area in PD (50

% cell loss in our cases). Likewise, the loss of neurons without pigment in the LC in AD cases (73 % cell loss) was nearly similar to that of pigmented SN pars lateralis neurons in PD (80 %). But in this case pigmented cells of the LC in AD cases also showed substantial decrement (69,4 % cell loss). It will be of much importance to relate such findings to more detailed immunohistochemical analysis, in future research.

Generally, in spite of age-related declines, single chemical markers do not fit very well with specific disease states, nor do changes in enzymatic activity correlate well with the extent of cell loss in the respective nuclei (Mann et al. 1986). Uneven, mosaic-like plaque distribution and morphology in the striatum and globus pallidus, with plaque aggregation near and within certain fiber tracts (Rudelli et al. 1984), likewise, was more suggestive of a relation with projection-systems. Therefore a better approach might be the analysis of functional systems, in which different nuclei are interconnected and various transmitters might be responsible for a rather sensitive equilibrium of quantitative as well as qualitative factors. E.g., it is difficult to conceive of a pathogenetic event acting directly on dopaminergic cell bodies, that would severely affect those of the VTA, while sparing those of the SN. What seems more likely is that the primary damage to these neurons occurs within other parts of their neuroanatomical circuitry, with reduction in nuclear volume (atrophy), neurofibrillary degeneration and loss of perikarya following as secondary retrograde or anterograde changes. There is growing evidence now of multisystem atrophies, providing subtle variation in clinical symptomatology, to which specific therapeutical approaches might be, at least theoretically, thinkable (e.g. Torch et al. 1977; Jellinger 1986a; Koller 1987; Quinn 1989; Gibb 1989a). The well known variability in therapeutic approaches of PD, might be a characteristic example (for review Birkmayer 1987).

Dysfunction of at-risk neurons is associated with several types of cytoskeletal pathology (Tomlinson and Corsellis 1984; Mann 1985; Price et al.1986; Kowall and Kosik 1987; Selkoe 1989). We tried to relate cell loss and neurofibrillary degeneration in AD/SDAT cases to neuroanatomical data on hodology of the various monoaminergic nuclei. First of all the highly variable results as regards normal cell number and pathologic cell loss (see Ch. III.) might well correlate with differences in regional influences based on connectivity. Such topographic loss, however, needs far more detailed analysis of a much greater number of cases. Modern computer-assisted analysis, based on quantifications of immunohistoche-

mically marked elements or on the sampling of neuromelanin-containing cells (Agnati et al.1984a,b; German et al. 1983,1988a; Hirsch et al. 1988; Baker et al. 1989; Chan-Palay and Asan 1989a,b) must serve to further confirm such presumptive parallels. The differential cell loss of the lateral and medial parts of the SN in PD and the rather selective involvement of ventral tegmental area cells without neuromelanin pigment in AD, are the most representative examples of localized cell loss that might reflect the existence of functional loops throughout basal ganglia and cortical circuitry (Ch. II.2.6.). Comparable findings on subsystems based on LC connectivity are evolving (Marcyniuk et al. 1986a,b,1989). Alterations in neurotransmitter markers, although partially overlapping in AD and PD, might appear to be highly localized, following selective involvement within neuronal populations of both subcortical and cortical structures (Emson and Lindvall 1986; Price et al.1986; Mann and Yates 1986; Procter et al.1988; Whitehouse 1989). Expanding the neurochemical inventory of the systems affected and spared, to e.g. the highly characteristically neurochemically defined striatal circuitry (Graybiel and Ragsdale 1983; Graybiel 1989; Jimenez-Castellanos and Graybiel 1989), might solve many questions on parallels and differences within the spectrum ranging from typical motor disease to exclusively cognitive decline. Besides, interactions between parallel cholinergic and catecholaminergic subsystems (Terry and Davies 1980; Jones and Friedman 1983; Henderson 1987; Graybiel 1989; Sara 1989) in which the nucleus tegmenti pedunculopontinus (see Ch. II.3.2.), nucleus basalis of Meynert and ventral pallidum (Alheid and Heimer 1988; Cools et al. 1989) might play an important role, throw new light on side-effects of dopaminergic or anti-cholinergic medication.

Neuronal loss in AD corrected for tissue shrinkage and comprising both neuromelanin-containing and unpigmented catecholaminergic neurons had a remarkable relationship with neurofibrillary degeneration. For all monoaminergic nuclei we calculated the number of NFT's to be about one-third of the cell loss (Fig. III.12.). Thus, the larger brainstem nuclei (like SN and NRd) might show a substantial number of NFT's correlating with a relatively small percentage of neuronal decrement of such larger brain nuclei. Together with the very marked shrinkage of cortical volume, accompanied by a considerable loss of projection neurons in our AD/SDAT cases (see also Broere 1990) these data suggest an independent and parallel course of degenerative changes, respectively, in PD and AD/SDAT, similar to that which has been proposed by various authors (for reviews

Pearson et al. 1985; Saper et al. 1985, 1987; Mann and Yates 1986; Jellinger 1987b). Therefore, we follow the hypothesis on anatomical correlates of the distribution of the pathological changes in cortical and subcortical structures in Alzheimer's disease (Rudelli et al. 1984; Pearson et al. 1985; Saper et al. 1985, 1987b; Mann and Yates 1986; Hardy et al. 1986). The basis of the apparent selectivity might be two-fold. Firstly, there is a chronic attack on nerve endings and secondly, those neurons with long projections (including the dopaminergic and serotonergic, but most particularly cholinergic and noradrenergic systems) are the least able to withstand such an attack. They therefore show corresponding degeneration in Alzheimer's disease. Typical signs of Parkinsonism have been shown since long to be primarily related to cell loss in the SN (mainly lateral) pars compacta and locus coeruleus (Hassler 1938; Greenfield and Bosanquet 1953; Hornykiewicz and Kish 1986; Jellinger 1986b), whereas secondary cell loss in the substantia innominata, locus coeruleus and cerebral cortex more closely correlated with signs of dementia in PD patients (Alvord et al. 1974; Hakim and Mathieson 1979; Whitehouse et al. 1981; Ball 1984; Perry et al. 1985; Korczyn et al. 1986; Gibb 1989b).

Neurofibrillary transformation in subcortical nuclei, then, might be an age-related essential feature of neurons projecting to the cerebral cortex (Wisniewski and Iqbal 1980; German et al. 1987), secondary to the structural changes in the affected brain. Such structural changes might follow anatomical pathways (Torch et al. 1977; Emson and Lindvall 1986), following e.g. an infectious event (Ball 1982; Roberts 1986; Saper et al. 1987) or otherwise a toxin or autoimmune response (for reviews see Toledano-Gasca 1988; Kozlowski and Nilawar 1988; Barker and Cahn 1988). Uptake of a "neurotoxin" at affected terminals and retrograde transport to perikarya causes neurofibrillary tangles to be formed; their accumulation leads to perikaryal changes culminating in cell death and cell loss (Hardy et al. 1986; Saper et al. 1987; German et al. 1987). As shown by one of our colleagues (see Vogels 1990; see also Arendt et al. 1985) degenerative findings in our AD/SDAT cases correlated with changes in some basal forebrain nuclei of the same brain. The cholinergic, noradrenergic and serotonergic neurons that have been implicated in the pathophysiology of AD have their cell bodies in the so-called 'isodendritic' core system in the brain (Rossor 1981; Cadet 1984, 1988) in which failing of compensatory, dendritic growth, like that of hippocampal neurons, might play a substantial role (Coleman and Flood 1986, 1987). The presence of a topographical organization within these cortical projection systems (Goldman-

Rakic and Brown 1981; Saper 1987a; Parnavelas and Papadopoulos 1989) suggests that the specific pattern of cognitive deficits in a patient with AD may reflect the distribution of the cell loss within the nucleus basalis area and/or monoaminergic brainstem nuclei.

Senile plaques have been shown to be associated with degeneration at the site of axon terminals (see Tomlinson and Corsellis 1984; Pearson et al. 1985; Price et al.1986). We found relatively few plaques within the area of the nucleus raphe dorsalis, the locus coeruleus and the nucleus tegmenti pedunculopontinus, i.e. those nuclei with relatively few cortical afferents. Instead relatively many plaques have been found at the dorsal border of the SN/VTA and especially at its most lateral site and in the nucleus peripeduncularis. These latter areas have been shown to receive substantial afferent projections from basal forebrain nuclei, paralimbic regions and the amygdala, areas that have been associated with AD degeneration. The highest density of senile plaques has been found in the midbrain roof and pretectal area. Corticotectal fibers arise from portions of the frontal, temporal, parietal and occipital lobes (Carpenter 1985; Goldman-Rakic 1987b). Besides, the superior colliculi are laminated resembling the cerebral cortex in their organization, and are also closely related to reticular formation and catecholamine-containing neurons (Carpenter 1985). Our investigation was not directed at relating exactly the distribution of the senile plaques to the organization of the superior and inferior colliculus, but the global wealth of amyloid plaques within tectal confines is in agreement with the aforementioned concept. Significant loss of neurons in the tegmental pedunculopontine nucleus, pars compacta (NTPP), a putative cholinergic nucleus of the brainstem, has been demonstrated in progressive supranuclear palsy, PD and combined PD/AD, with frequent occurrence of Lewy bodies and NFT's (Zweig et al.1989a; Jellinger 1987b,1988). Although we noted substantial neurofibrillary degeneration in AD/SDAT cases, cell number was not quantified in our study. Putative cholinergic neurons of the NTPP might be more selectively affected in PD and much less in AD/SDAT cases as can be expected from its more close relationship to the basal ganglia and ascending reticular formation than to the cerebral cortex (Vertes and Martin 1988; Spann and Grofová 1989).

The neuropathological changes in the cortex associated with AD are not uniformly distributed. The medial temporal cortex and the amygdala are most severely affected; parieto-temporal and certain prefrontal association areas are also involved, but there is relatively little pathology in the primary sensory areas and in

the motor cortex (Brun 1983; Pearson et al.1985; Van Hoesen and Damasio 1987; Broere 1990). Aside from the areal distribution of the pathology, the microscopic distribution, particularly of neurofibrillary tangles, within affected neocortical areas supports the concept of a relationship between the disease process and cortical connectivity (Pearson et al.1985). The areal distribution, with a progression of severity from the medial temporal cortex back towards the primary sensory areas, might very well be correlated to connectivity with subcortical nuclei (Ch. II.2.,II.3.,II.4.), although the exact cortical distribution of terminals of projection neurons needs definitive light- and electron-microscopic confirmation. Neurofibrillary tangles in the neocortex are particularly abundant in the infragranular and supragranular pyramidal cells of layers III and V and show a patchy distribution (Pearson et al.1985). This patchy distribution is not random and resembles that of the pyramidal cells that give rise to the corticocortical association fibers (Pearson et al.1985), but also of the preferentially prefrontal projection sites of brainstem nuclei (Emson and Lindvall 1986; Goldman-Rakic 1987b,1988). In the principal sulcus of the monkey prefrontal cortex, e.g., CA-containing axons are most concentrated in upper layer III and in the deep layers V and VI and are more sparse in other layers (Levitt et al. 1984; Goldman-Rakic 1987b). It is likely, therefore, that neurofibrillary tangles appear in projection neurons that send their axons to pathologically changed cortical areas (German et al.1987). Cortical projections of the nucleus raphe dorsalis are mainly complementary to those of the catecholaminergic nuclei LC, SN and VTA (Molliver 1987; Berger et al. 1988), i.e. particularly to layer IV and preferentially to the primary sensory cortex (Takeuchi and Sano 1983). But serotonergic fibers innervate all subportions of the hippocampal formation (Emson and Lindvall 1986; Molliver 1987), that might explain the abundance of NFT's within the NRd. There is a remarkable small proportion of senile plaques as compared to neurofibrillary tangles to be found in the NRd (SP/NFT=0.25) as might be derived from our tables III.13. and III.14. This quotient was substantially greater for the LC (SP/NFT=1.37) and VTA (SP/NFT=0.75), which might correspond roughly to the extent of **afferentiation** from prefrontal cortical structures and the formation of amyloid plaques adjacent to cortical terminals. In the SN, where neurodegenerative changes are less well pronounced, a remarkable high proportion of SP's as compared to NFT's (SP/NFT=13.76) has been found, suggesting rather strong secondary degeneration as compared to relatively few primary cytoskeletal changes (NFT's) of cortical projection neurons. In PD

neuronal loss of cholinergic and monoaminergic subcortical nuclei is not significantly related with neurofibrillary degeneration (Hakim and Mathieson 1979; Mann and Yates 1983; Ball 1984; Lees 1985; Perry et al.1985; Cummings 1988). Likewise, damage to the nucleus basalis of Meynert and other subcortical nuclei has been found without impressive cortical neuronal loss or AD pathology (Gaspar and Gray 1984; Nakano and Hirano 1984; Jellinger 1986b,c,1987b).

In our cases Alzheimer neurofibrillary tangles occurred in the SN of nonparkinsonian patiens with about the same frequency as in parkinsonians and with about the same frequency as did Lewy bodies. The somewhat higher frequency of NFT's in the LC might be explained by the fact that they also occur much more commonly in elderly people, increasing linearly up to the age of 90 years (Alvord et al. 1974) and a combination of SDAT with PD will be no uncommon finding in the very old (Zweig et al. 1989b). Indeed the number of NFT's and SP's in our case 87062 (combined PD and AD) was similar to that found in patients with AD/SDAT.

In summary, in the dementia of PD (and perhaps also that of progressive supranuclear palsy and Parkinsonism-dementia of Guam) the same subcortical structures as those in AD (i.e. nucleus basalis, locus coeruleus and perhaps also dorsal raphe) show neuropathological changes in addition to SN degeneration, although only aspecific cortical pathology has been identified as yet in these disorders. Such changes illustrate the concept of "subcortical dementia" (Cummings and Benson 1984; Chui 1989) in which the clinical deficit stems from a failure to sufficiently activate the cortex rather than as a result of primary cortical degeneration with secondary subcortical involvement. Such a view is very well in agreement with data on the functional importance of subcortical-to-cortical (especially monoaminergic) projections (Saper et al. 1987; Foote and Morrison 1987; Goldman-Rakic 1988; Gaspar et al. 1989; Parnavelas and Papadopoulos 1989). This distinction can also be recognized clinically since the early prominent aphasia, amnesia, agnosia and apraxia of AD (typifying cortical dementias) is largely replaced in "purely" subcortical dementias, by changes in mood, arousal, attention and motivation, that may underly the cognitive deficits within such patients. The concept that a failure of subcortical systems using specific neurotransmitters underlies a subpopulation of patients suffering the dementia syndrome is attractive because of its obvious therapeutic implications (Fleet et al. 1987; Gotham et al. 1988).

Both AD and PD show preponderance for specific neuronal systems in

several different regions of the brain. We do not understand why these diseases involve those neurons while sparing other cells groups. But the relation between cell loss and neurofibrillary degeneration (Fig.III.12) and the severity of pathologic involvement in different areas (Rossor 1982; Wilcock and Esiri 1982; Mann 1985; Perry et al.1987; Jellinger 1987b) support the suggestion that the disease process in both PD and AD may extend along the connecting fibers (Appel 1981; Pearson et al.1985; Saper et al. 1987). The dopaminergic cell continuum of the mesencephalon might be subdivided in a part mainly related to cognitive and motivational functions (VTA) and a more purely motor part (SNc), both related to a common output part (SNret). A similar subdivision within the noradrenergic locus coeruleus is getting growing interest, its dorsal/rostral part being mainly involved in ascending cognition-related projections, whereas more diffuse ventrally and caudally placed neurons might have motor and output-related functions. It is tempting to speculate on a similar subdivision within the raphe system, its pathology being also associated with both motor and cognitive decline, but data on this subject are preliminary yet. A systematic methodological approach in the study of pathologic changes in major brainstem circuitry of the basal ganglia and the brainstem monoaminergic nuclei, might contribute to our understanding of their functional role in human cognition and motor function, as well as the very old question as regards a possible overlap in motor and cognitive processing.

V. SUMMARY AND CONCLUSIONS

"Die waarlijk allerzotste lieden hebben gemeend met zoveel slapeloze uren en zoveel inspanning zich een of andere reputatie te moeten verwerven, het meest zinloze wat er bestaat. Maar ondertussen hebt U aan de Zotheid toch maar al dat voortreffelijk gerief in het leven te danken; en wat het allerleukste is, u profiteert van andermans gekheid!"

Erasmus: Laus Stultitiae, 1508
Vert. A.J.Hiensch, 1974

Dopamine, noradrenaline (both catecholamines) and serotonin are monoaminergic neurotransmitter substances and, together with acetylcholine, make up the most essential neurotransmitters of the human central nervous system. They seem to be responsible for specific nervous system functions that are typically susceptible to age-related decline. A substantial loss of acetylcholine is characteristic for the dementia of Alzheimer, whereas the extrapyramidal signs in Parkinson's disease are related to a loss of dopamine. Both monoamines and acetylcholine are produced in subcortical nuclei that have widespread projections on forebrain structures and the cerebral cortex. An interconnection of such projections is suggested by the concept of the "reticular formation", suggesting similarities in their pathophysiological manifestations and associated diseases. The main sources of monoamines are three brain stem nuclei: 1.substantia nigra (dopamine); 2. locus coeruleus (noradrenaline) and 3. nucleus raphe dorsalis (serotonin). The most important source of acetylcholine is spread over a complex of basal forebrain magnocellular structures, known as the nucleus basalis of Meynert. This nucleus and its relation to Alzheimer's disease, has been thoroughly examined in recent decades, and a pathophysiological concept, which reminds of the "dopamine - model" in Parkinson's disease, has been suggested. Elaboration of other monoaminergic nuclei has been less comprehensive, but changes related to aging disease, especially dementia, have been stated for all monoaminergic structures. We investigated a possible relationship of the substantia nigra, locus coeruleus and nucleus raphe dorsalis with degeneration of both Alzheimer's disease and Parkinson's disease. Loss of cells and neurofibrillary changes (as described by Alzheimer in 1907 in cortical areas) might substantiate such degeneration. The age-related decline of nigro-striatal projections, characteristic in Parkinson's disease, might serve as a model.

Dopaminergic cells of the human mesencephalon show a dark-brown appearance, because of a natural pigment: neuromelanin. Accumulation of neuromelanin-pigment is related to catecholamine break-down and characterizes both substantia nigra and locus coeruleus neurons. A substantial reduction of neuromelanin-pigmented neurons can be found in Parkinson's disease, being most pronounced in the lateral pars compacta of the substantia nigra. This "subnucleus", therefore, might be considered to be most typically involved in motor functions of the extrapyramidal system. The distribution of tyrosine hydroxylase-immunoreactivity (the enzymatic marker of catecholamines), appears to overlap with neurome-

lanin pigment distribution. Therefore, cellular decrement of catecholaminergic brainstem areas, mostly is denoted as a loss of neuromelanin-pigmented cells, generally quantified only for one or a few sections. Another restriction, which might introduce substantial bias in counting results, is the variability in the shape of neuromelanin-pigmented cells. Stereological principles might account for such bias, as well as for pathological tissue shrinkage. Therefore Floderus' (1944) and Abercrombies (1946) correction formulas were applied, necessitating exact data on cellular shape and sizes in different (disease) states. The nucleus raphe dorsalis neurons show an identifiable nucleolus, which might serve as an unequivocal counting element. A similar approach, i.e. counting of nucleoli, has been performed in parallel studies (see Ch. IV), on nucleus basalis, hippocampus, amygdala and neocortex. Neurofibrillary tangles and senile plaques, the characteristic manifestations of Alzheimer neurofibrillary degeneration, again, are highly variable in size and shape. Morphometric data on these neuropathological elements are only semi-quantitative.

Based on modern chemical neuroanatomy some traditional concepts have to be questioned: 1. specific brain nuclei have been associated with a single neurotransmitter; 2. single neurotransmitters have been related to specific (motor or cognitive) functions of the central nervous system. Apart from dopamine and acetylcholine other neurotransmitters, like serotonin and noradrenaline might be involved in Parkinson's disease and Alzheimer's disease, respectively. Neurodegenerative changes (like senile plaques, neurofibrillary tangles and Lewy bodies) appear also to be more widespread throughout the brain, although their high numbers in certain brain areas still applies as a neuropathological hallmark. Special attention has been directed to neuroanatomical data in order to solve some major questions: 1. is there an overlap in Alzheimer's disease and Parkinson's disease as regards certain parts of monoaminergic structures ? 2.is there a topographical distribution of cell loss and neurodegenerative findings, which accounts for clinical symptoms (motor and cognitive) in both disease states ? 3. are common (topographical) morphometric findings indicative of certain common pathophysiological principles and their clinical manifestation, especially cognitive disturbances ? 4. is there a correlation with changes in other brain areas functioning within the circuit under consideration ? The concept of a purely motor-related dopaminergic substantia nigra, e.g., had to be substituted for a mesencephalic dopaminergic cell-continuum. The ventral tegmental area, situated dorsally and medially to the pars

compacta of the substantia nigra, appears to be a more diffusely spread part of this continuum, projecting on forebrain and cortical structures and related to cognitive and motivational aspects of behaviour in animal experiments. Many details on both nigrostriatal (motor) and mesolimbocortical ("motivational") loops have been derived from animal studies, especially rats. Extrapolation of this circuitry to the human brain has been aimed for, with the aid of the many data available from tracing studies in the literature on primates.

A correlation of these neuroanatomical data with morphometric analysis of post-mortem (human) material, might serve to relate certain functions more particularly to certain areas of the monoaminergic brain stem nuclei. Cell number and neuropathological changes might serve, then, to estimate the involvement of certain circuits in cognitive disturbances of Alzheimer's disease and extrapyramidal functioning in parkinsonism. Morphometric analysis of routinely stained Nissl and Congo-red preparations in future research has to confirm these suggestions on the involvement of well-defined circuits in specific aspects of both disease states. Our study (primarily designed as an inventarization) is only of limited value in proving statistically significant changes in cell number as regards different subnuclei in Alzheimer's disease, because of the small number of cases and the lack of clinical follow-up. Some important suggestions on pathophysiology, however, can be stated based on combined neuroanatomical and morphometric analysis. Besides, a contribution to the relevance of various manifestations of the dementia syndrome, e.g. the so-called "subcortical dementia" (treatable on theoretical grounds by monoaminergic or cholinergic substances), might be derived.

Our findings, based on countings and neuropathological investigations, of a small number of cases, might be summarized as follows:

1. Cell los of dopaminergic cells in the lateral part of the substantia nigra pars compacta in Parkinson's disease is about 80% and is the greatest cell loss measured for monoaminergic structures in both disease states. In Alzheimer's disease a statistically significant reduction in cellular size can be seen in the medial part of the substantia nigra. As a whole the substantia nigra shows a significant cellular decrement only in Parkinson's disease, as compared to Alzheimer's disease and the aging process itself, when changes of cellar size are taken into account.
2. A remarkable loss of cells without neuromelanin pigment can be seen in the ventral tegmental area in Alzheimer's disease cases (47%). These neurons need

further neurochemical characterization. Catecholaminergic (pigmented) ventral tegmental cells show a reduction of about 40% in Alzheimer's disease and about 50% in Parkinson's disease cases.

3. The retrorubral area (A8), a less well defined component of mesencephalic catecholaminergic neurons, extending dorsally from the caudal substantia nigra, also shows a reduction in cell number, both in Parkinson's disease and Alzheimer's disease cases.

4. As regards the locus coeruleus there is a significant shrinkage of cells, preferentially in its rostral part (particularly associated with cortical projections). Corrected cell-loss of the locus coeruleus is about 70% in Alzheimer's disease and about 44% in Parkinson's disease, both concerning pigmented cells and neurons without neuromelanin.

5. The nucleus raphe dorsalis only shows a significant cell loss in Alzheimer's disease cases (40%).

6. Generally stated, the number of neurofibrillary tangles in the monoaminergic nuclei is about one-third of the number of lost cells in Alzheimer's disease.

7. Only few senile (neuritic) plaques can be found in mesencephalic structures. The pattern of distribution, like that of neurofibrillary tangles, is more diffusely spread, without obeying cellular boundaries. Relatively many neurofibrillary changes have been found in the corpus geniculatum mediale, superior and inferior colliculus, nucleus peripeduncularis, nucleus tegmenti pedunculopontinus and in the most caudal part of the lateral hypothalamic area.

8. The pattern of distribution of tangles and plaques in our study corresponds with the pattern that might be expected according to the concept of Pearson and co-workers. They (Pearson et al. 1985) stated that neurofibrillary changes occur in perikaryae of neurons that project to certain cortical areas "at risk", in relation to aging. Senile plaques might be transformations at the terminal site of cortical afferent fibers in the neuropil, preferentially in the neighbourhood of blood-vessels.

9. Primary changes in Alzheimer's disease appear in the cerebral cortex, with only secondarily, anterograde or retregrade, involvement of subcortical structures. Primary pathological changes in Parkinson's disease, however, appear in subcortical nuclei (especially substantia nigra) and their projections. Basal forebrain and cortical areas are only secondarily involved, then.

Based on these relationship an overlap in degenerated structures is very well

possible, which might account for the overlap in clinical symptomatology, described in several studies.

10. "Subcortical dementia" is a syndrome of, at least theoretical, importance, referring to primary degeneration of subcortical structures, with secondarily involvement of cognition-related (cortical) structures. Transsynaptic (transaxonal) transport of neorotoxines, along (e.g. monoaminergic) trajectories susceptible to aging and neural decrement, might play an important role. Modern investigations, based on such concepts, might shed more light on certain kinds of dementia that are hard to understand, like dementia in Parkinson's disease and dementia following encephalitis. Selective neuron loss of monoaminergic brainstem structures, related to topographical principles of their projections, might provide an additional clue to such considerations in current neuroscience.

SAMENVATTING EN CONCLUSIES

Dopamine en noradrenaline (de catecholaminen) vormen tesamen met serotonine de monoaminerge neurotransmitters en zijn met acetylcholine de meest bekende transmitter substanties van het centrale zenuwstelsel. Al vele jaren wordt aangenomen dat deze stoffen specifieke funkties vervullen, die met name bij de normale veroudering een achteruitgang vertonen. In pathologische zin zou vervolgens een uitgesproken verlies aan acetylcholine optreden bij de dementie van het type Alzheimer, terwijl de extrapiramidale dysfunktie bij de ziekte van Parkinson gerelateerd is aan een verlies van dopamine. Zowel de drie monoamines als het acetylcholine worden geproduceerd in subcorticale kernen en van daaruit getransporteerd naar wijd verbreide voorhersenstrukturen en de cortex cerebri. De betreffende projekties lijken met elkaar verweven, zoals traditioneel in het concept van de 'formatio reticularis' aangegeven, en het ligt voor de hand de pathofysiologie van deze verschillende projekties en hun oorsprongskernen, als ook de daaraan verbonden ziektebeelden, met elkaar te vergelijken. De drie monoaminen worden met name geproduceerd in hersenstam kernen: 1. de substantia nigra (dopamine); 2. de locus coeruleus (noradrenaline) en 3. de nucleus raphe dorsalis (serotonine). De meest bekende cholinerge kern, de nucleus basalis van Meynert, is een magnocellulair complex in de basale voorhersenen. De laatste decennia is veel onderzoek gedaan naar veranderingen in de nucleus basalis bij de ziekte van Alzheimer, in de hoop een vergelijkbaar pathofysiologisch model te kunnen ontwikkelen als dat voor de ziekte van Parkinson betreffende de substantia nigra en bijhorende dopaminerge projekties op het striatum. De andere monoaminerge strukturen zijn daarbij minder uitvoerig onderzocht. Wel is waarschijnlijk gemaakt dat alle monoaminerge strukturen veranderingen vertonen als gevolg van pathologische veroudering, in de zin van dementie van het Alzheimer type of anderszins, zoals dementie bij parkinsonisme. In het licht van deze kennis hebben wij ons de vraag gesteld of nu de substantia nigra, locus coeruleus en nucleus raphe dorsalis betrokken zouden kunnen zijn bij zowel de degeneratie van de ziekte van Alzheimer als bij die van de ziekte van Parkinson. Daartoe zou eventueel celverlies en de typische neurofibrillaire veranderingen (zoals in 1907 door Alzheimer

beschreven voor corticale gebieden) gemeten moeten kunnen worden. De typische leeftijdsafhankelijke nigro-striatale degeneratie, zoals beschreven bij de ziekte van Parkinson, zou daarbij model kunnen staan.

Voordeel van deze dopaminerge struktuur in het mesencephalon is ook dat zij een 'natuurlijke cel-marker' vertoont, namelijk het neuromelanine. Dit is een donkerbruin pigment, als afbraak produkt van catecholaminen aanwezig in de substantia nigra en locus coeruleus, en karakteristiek goeddeels ontbrekend in de hersenstam van patiënten met de ziekte van Parkinson. Het grootste verlies aan neuromelanine-houdende cellen bij parkinsonisme wordt beschreven voor het laterale deel van de substantia nigra pars compacta en deze "subnucleus" wordt daarom beschouwd als het meest met extrapiramidale bewegingsstoornissen verbonden dopaminerge kerngebied. Gebleken is dat neuromelanine-houdende cellen redelijk overeenkomen met tyrosine hydroxylase (de enzymatische marker voor catecholaminerge strukturen)-houdende cellen. In de literatuur wordt celverlies van de catecholaminerge hersenstam kernen dan ook vaak aangegeven als verlies van aantal neuromelanine houdende cellen, doorgaans echter slechts voor enkele doorsneden. Een beperking daarbij is bovendien de onregelmatige configuratie van gepigmenteerde cellen, waarbij men niet weet of men met hele cellen of met cel-delen te maken heeft en daardoor tot onjuiste telresultaten komt. Bovendien zijn faktoren als pathologische weefsel of cel-krimp van invloed op de morfometrische resultaten als men willekeurig gevormde neurale elementen kwantificeert. Derhalve is bij de ontwikkelde telmethode rekening gehouden met deze zogenaamde stereologische principes en worden correctie formules volgens Floderus (1944) en Abercrombie (1946) toegepast. Cellen van de nucleus raphe dorsalis zijn herkenbaar aan een niet-splijtbare nucleolus en kunnen derhalve zonder correctie formules worden gekwantificeerd (net als b.v. cellen van de nucleus basalis, hippocampus en cortex in parallel lopende projekten). Neurofibrillaire elementen, "neurofibrillary tangles"(NFT) en "senile plaques"(SP), zijn weer wisselend van vorm en grootte en bieden opnieuw problemen als het gaat om exacte kwantificering in verschillende moeilijk begrensbare hersen-arealen. Derhalve is de morfometrie van deze elementen altijd slechts semi-kwantitatief.

In het licht van de huidige kennis van de chemische neuroanatomie neemt men steeds meer afstand van het oude concept, dat: 1.specifieke strukturen gekarakteriseerd kunnen worden door enkelvoudige neurotransmitters; 2. enkelvoudige neurotransmitters gecorreleerd zijn aan specifieke (motorische of cognitieve)

funkties. Zo is inmiddels gebleken dat bij de ziekte van Parkinson naast dopamine ook acetylcholine, noradrenaline en andere chemische substanties betrokken zijn en dat verlies aan cognitieve funkties bij de ziekte van Alzheimer niet enkel gecorreleerd is aan verlies van cholinerge projekties. Tevens blijken neurodegeneratieve verschijnselen (senile plaques en neurofibrillary tangles en Lewy bodies) meer diffuus verdeeld over het cerebrum en ook niet beperkt tot een ziektebeeld, hoewel ze nog steeds als karakteristieke pathologisch kenmerken gelden. Speciale aandacht is dan ook geschonken aan de neuroanatomie van de te onderzoeken strukturen om de volgende vragen te kunnen inventariseren: 1. Is er inderdaad een zekere overlap in de ziekte van Alzheimer en de ziekte van Parkinson, betreffende de pathologie monoaminerge strukturen dan wel gedeelten van deze stukturen? 2. Is de topografische verdeling van celverlies en neurodegeneratieve verschijnselen zodanig dat dit een herkenbaar substraat vormt voor de klinische symptomatologie bij deze ziekte (m.n. motorisch vs. cognitief)? 3. Zijn er op basis van de overlap in morfometrische bevindingen van bepaalde (sub)arealen conclusies te trekken t.a.v. de pathofysiologie van beide ziektebeelden en vooral ook betreffende symptomen die bij beide ziektebeelden worden aangetroffen, m.n. cognitieve funktiestoornissen? 4. Is de neuroanatomische verdeling van de degeneratieve verschijnselen zodanig dat deze correleert met andere hersengebieden die binnen een betreffend circuit funktioneren? Zo kan bv. de substantia nigra niet meer opgevat worden in traditionele zin, maar moet het mesencephale dopaminerge celcontinuum uitgebreid worden met een zgn. ventraal tegmentaal areaal (VTA), dat, i.t.t. de substantia nigra, meer gerelateerd is aan voorhersenstrukturen en cognitieve en motivationele gedragscomponenten. Dit gebied strekt zich uit juist mediaal en dorsaal van de substantia nigra pars compacta. Daarmee is het tevens een gebied waar overlap in de pathologie van de ziekte van Parkinson en de ziekte van Alzheimer verwacht kan worden, mits inderdaad de corticale projekties in verband gebracht mogen worden met cognitieve funkties en striatale projekties met motorische funkties. Reeds vele details t.a.v. de anatomie en fysiologie der monoaminerge hersenstam kernen zijn bekend, maar meestal gebaseerd op onderzoek aan de hersenen van de rat. Zeer veel aandacht is gegeven aan de extrapolatie van deze gegevens naar het menselijk brein, waarbij m.n. de literatuur m.b.t. onderzoek aan primaten is gerefereerd.

Juist het relateren van deze kennis aan exacte morfometrische analyse van post-mortem materiaal kan suggesties t.a.v. de funkties der verschillende kernarealen op hun waarde toetsen. Het aantal cellen en neuropathologische bevindin-

gen in een belangrijke schakel (in dit geval een monoaminerge hersenstam kern) uit een circuit dient als maat voor de betrokkenheid van dat circuit bij (cognitieve) stoornissen van het Alzheimer type of (extrapiramidale) verschijnselen van parkinsonisme. De morfometrische methode ter snelle analyse van Nissl en Congorood aangekleurd obductie materiaal schept dan de mogelijkheid om gedetaileerde klinische observatie te relateren aan gedetailcerde post-mortem analyse.
Als inventarisatie is ons onderzoek nog niet geschikt gebleken om veel harde gegevens omtrent subtiele morphometrische veranderingen in subarealen bij de ziekte van Alzheimer te leveren.
De resultaten van onderzoek aan ons klein aantal breinen suggereren echter wel enige verwachtingen ten aanzien van de pathofysiologie van de betreffende verouderingsziekten en hun neuroanatomisch substraat.
Daarmee zou vooral een bijdrage geleverd kunnen worden aan de discussie rond verschillende vormen van het dementie-syndroom, zoals dat van een zgn. 'subcorticale dementie', die *theoretisch* voor medicamenteuze behandeling (suppletie van monoaminerge of cholinerge substanties) in aanmerking kan komen.

De resultaten van de celtellingen en het neuropathologisch onderzoek zouden dan als volgt kunnen worden samengevat:

1. Celverlies van dopaminerge cellen in het laterale deel van de substantia nigra pars compacta bij de ziekte van Parkinson bedraagt ca. 80% en is tevens het grootste celverlies zoals binnen monoaminerge strukturen kan worden gevonden bij de genoemde ziektebeelden. Bij de ziekte van Alzheimer is er in het mediale deel van de substantia nigra een statistisch significante reductie in cel grootte. De substantia nigra als geheel toont alleen bij de ziekte van Parkinson een significant celverlies. Er is rekening gehouden met veranderingen in cel-grootte, hoewel die veranderingen meestal niet statisch significant zijn, bij een der ziektes of bij het verouderingsproces zelf.
2. Opmerkelijk is een celverlies van niet-neuromelanine-houdende cellen in de area tegmentalis ventralis (VTA) bij de ziekte van Alzheimer (47%). Deze cellen zijn nog niet goed cytochemisch gekarakteriseerd. De catecholaminerge cellen van de VTA vertonen een reductie van 50% bij de ziekte van Parkinson en 40% bij de ziekte van Alzheimer.
3. De area retrorubralis (A8), een minder scherp gedefinieerde uitbreiding dorsaal van de substantia nigra, vertoont eveneens celverlies zowel bij de ziekte van Parkinson als bij de ziekte van Alzheimer.

4. Ook t.a.v. de locus coeruleus moet rekening gehouden worden met cel-krimp, waarbij opvalt dat deze in het rostrale deel (het meest gerelateerd aan corticale projecties) het sterkst is. Het (gecorrigeerde) celverlies van de locus coeruleus is ca. 70% bij de ziekte van Alzheimer en 44% bij de ziekte van Parkinson, zowel voor melanine-houdende als ongepigmenteerde cellen.

5.De nucleus raphe dorsalis vertoont alleen bij de ziekte van Alzheimer een significant celverlies (40%).

6. Algemeen gesteld blijkt het aantal neurofibrillaire tangles in alle drie de monoaminerge kernen steeds ongeveer 1/3 deel te bedragen van het aantal verloren cellen, bij de ziekte van Alzheimer.

7. Seniele (neuritische) plaques worden slechts weinig aangetroffen binnen de monoaminerge strukturen. Het verdelingspatroon van seniele plaques, maar ook dat van neurofibrillaire tangles, houdt zich relatief slecht aan de begrenzingen van monoaminerge kernen. Er worden m.n. ook relatief veel neurodegeneratieve verschijnselen gezien in corpus geniculatum mediale, tectum mesencephali, nucleus peripeduncularis, nucleus tegmentipedunculopontinus en caudale uitloper van de laterale hypothalamus.

8. Het verdelingspatroon van tangles en plaques is zodanig dat het concept van Pearson en medewerkers (Pearson et al. 1985) kan worden uitgebreid voor hersenstamstrukturen. Daarbij geldt dat neurofibrillaire tangles degeneratieve veranderingen zijn, binnen cellen die op 'gevoelige' corticale arealen projecteren, en gerelateerd aan veroudering. Seniele plaques zouden ontstaan ter plaatse van terminalia van corticale afferenten in het neuro-pileem, vooral ook in sterk gevasculariseerde gebieden.

9. De primaire pathologie bij de dementie van het Alzheimer-type is daarbij corticaal, met secundaire anterograde en retrograde subcorticale degeneratie. De primaire pathologie bij de ziekte van Parkinson is subcorticaal, met eventuele secundaire degeneratie van basale voorhersenen en corticale strukturen. T.g.v. deze 'verdeling' en de relatie daarvan met veroudering, ontstaat er een zekere overlap in beide ziektebeelden.

10. Het begrip 'subcorticale dementie' is, tenminste theoretisch, van waarde. Het verwijst naar primaire degeneratie van subcorticale strukturen of van de bijbehorende projecties op de cortex. Transsynaptisch transport van neurotoxinen langs sterk vertakkende, gevoelige projecties (zoals die der monoaminerge kernen) zou daarbij een rol kunnen spelen. Dit model verdient aandacht binnen het huidige

onderzoek naar slecht begrepen dementiële beelden, m.n. die welke gekoppeld zijn aan de ziekte van Parkinson, maar ook b.v. bij de encephalitiden. Selektief celverlies binnen monoaminerge kernen, gerelateerd aan de topografie van hun projecties, heeft daartoe duidelijk aanknopingspunten geboden.

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Curriculum Vitae

Peter van Domburg werd geboren op 8 november 1954 te Maastricht. Na het behalen van het diploma Atheneum B aan het St.Bernardinus College te Heerlen (1973), volgde hij aldaar ook de opleiding tot werktuigbouwkundig ing., waarvoor het diploma werd behaald in 1977. Vervolgens werd gekozen voor de opleiding Medicijnen aan de Fakulteit der Geneeskunde en Tandheelkunde te Nijmegen, waar het doctoraal examen werd behaald in 1982 en het arts-examen werd afgelegd in 1984. Gedurende enkele maanden in 1985 werd medegewerkt aan een projekt betreffende de chemoarchitectuur van het humane brein, o.l.v. prof. dr. R. Nieuwenhuys. Naast enkele klinische stages in China (acupunctuur, 1983) en West-Duitsland (psychosomatiek, Klinik Lahnhöhe, 1985), alsmede een assistentschap longziekten (Dekkerswald, Nijmegen, 1986), werd de opleiding tot huisarts gevolgd en afgesloten met een certificaat in oktober 1986. Daarop volgde de aanstelling als promovendus binnen het zgn. B1-segment van de vakgroep Anatomie en Embryologie in samenwerking met het Research-Laboratorium voor Morfologische Neurologie in het Instituut voor Neurologie, ten laste van een subsidie verkregen van de JANIVO Stichting. Aansluitend werd begonnen (per 1 september 1989) met de opleiding tot neuroloog.

STELLINGEN

I.

"It is essential, in discussing the physiology of the corpus striatum, to think anatomically".
S.A.K.Wilson, 1914

II.

Het onderscheid tussen corticale en subcorticale dementie is zinvol.

III.

"Eine statistische Korrelation führt zu einer Frage, noch nicht zu einer Erkenntnis der Art der Beziehung."
K. Jaspers, Allgemeine Psychopathologie, 6° druk,1953

IV.

Het veelvuldig gebruik van de zinsnede "Het zou wellicht zo kunnen zijn dat....." maakt een proefschrift extra dik en Poppers' falsifieerbaarheids-theorie overbodig.

V.

"Het feit, dat het postmortaal onderzoek van de hersenen van een aan dementie overleden patient de ene keer uitgesproken organisch-structurele veranderingen der hersenen en een andere keer nauwelijks hersen-stoornissen aan het licht brengt, is slechts verklaarbaar vanuit de opvatting, dat de geaardheid van de gemeenschap en van de cultuurwereld, waarmee het individu verbonden is, in hoge mate medebepalend zijn voor een al dan niet optreden van een dementeringsproces."
J.J.G.Prick, 1971

VI.

Waardevrije wetenschap is als waardeloze liefde.

VII.

Voor de ziekte van Parkinson geldt: Waar een wil is is géén weg.

VIII.

De totale hoeveelheid domheid onder gezonde mensen overtreft verre het verlies aan intellectuele funkties bij gedementeerden.

IX.

De stelling "Do more less well" (Gundersen HJG and Österby R (1981) J Microscopy 121: 65-73) is van toepassing op strikt morfometrisch onderzoek, maar moet worden omgekeerd bij de neuro-anatomische interpretatie van de bevindingen.

X.

De reden om het humane brein met dat van een rat te vergelijken dient enkel van praktisch neuro-anatomische aard te zijn.

XI.

"The political party which will have the foresight and humanity to introduce into its platform an article advocating and supporting the longer and better use of the human brain will offer a worthy issue to its electorate."
M. Critchley, 1956

XII.

Het nerveus-functionele klachtenpatroon is de meest therapie-resistente besmetting. Het in cultuur verkregen agens heet "Stigma".

XIII.

Het verschil tussen de anthroposofische en de academische geneeskunde zit in de balans tussen goede wil en Goodwill; bij beide is deze aan een kant overbelast.

XIV.

Publiceren is als vreemdgaan; er is altijd iemand bij betrokken die het níet leuk vindt.

XV.

Nietzsche dementeerde en God was dood; beide breinen zijn niet meegeteld.