THE FIBRE-CONNECTIONS OF THE FOREBRAIN

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VAN GORCUM

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A CRITICAL REVIEW OF THE HODOLOGY OF THE TELEN- AND DIENCEPHALON WITH THE ADJACENT MESENCEPHALON, ESPECIALLY WITH REGARD TO THE BASAL GANGLIA, BASED ON ACUTE AND CHRONIC EXPERIMENTS IN THE RAT

by

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THE FIBRE-CONNECTIONS OF THE FOREBRAIN

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ABBREVIATED CONTENTS

CHAPTER I - GENERAL INTRODUCTION

During the previous hundred and fifty years, the mammalian forebrain has been the subject of numerous investigations, which have produced an immense number of partly exact, partly doubtfull and partly spurious data. Because of the very contradictory information in the literature, it was judged useless to add another paper to the long list of publications, another set of opinions to all opinions already in existence.

It is an ominous sign in Neuro-anatomy that in many publications, to advocate some connection, earlier studies, even very old ones also describing it, are cited, whereas a much larger number of other investigations denying the same connection are neglected. As objectionable are studies based on old-fashioned methods such as the Marchi-, and Weigert-Pal techniques, which already long have been shown inadequate and misleading, while moreover yet we have much more appropriate techniques at our service as the methods of Nauta-Gygax and of Hägquist.

As will appear in the present study, it is hardly feasible to "invent" a connection in the forebrain that has not yet been described. Although it seems incredible, even in the most recent textbooks of Neuroanatomy, presented to our students of medicine, connections invented as long as forty years ago and frequently denied since, can be found.

As will be shown by this publication, the majority of contradictions in the literature find their origin in misinterpretations, especially of fibres of passage. When the publications based on staining techniques of which the insufficiency has been proved are eliminated, together with publications of which the results cannot be controlled by lack of a complete survey of the lesions and their resulting degeneration, Neuro-anatomy might be liberated from the heavy burden of worthless historical material. It must be realized however, that in studying small parts of the central nervous system, as is usual, misinterpretations are apt to be made, because it mostly appears that the authors neglected most fibres of passage that trouble their patterns of degeneration.

The present study endeavored to investigate both the afferent and efferent connections of the corpus striatum of the rat which never have been studied completely with experimental methods before. It, however, has outgrown its limits considerably, because the lesions made to study these connections, although not entirely appropriate to study all connections of the telen-, dien- and a large part of the mesencephalon, yet offered so many data about other connections and especially about fibres of passage that it seemed justified to compare these findings with those from the literature.

To this purpose about seven hundred publications were consulted of which two hundred had to be rejected as being of no use. The five hundred remaining and most frequently cited publications were analysed and the connections described, together about 1100, grouped according to their nuclei of origin. As far as possible these "efferent connections" have been controlled with the help of the present material. Very often it was unfeasible either to confirm or to deny their existence, but in such cases often neither the authors had succeeded in eliminating certain troubling factors and a renewed investigation is necessary.

The final purpose of this publication is to give some idea of what can be considered as certain in the subject studied and what has still to be investigated or substantiated.

To complete the review of a certain nuclear mass or fibre bundle, a list of its afferent connections is added which are dealt with elsewhere in detail in their role of efferent connections.

It is realised that this publication has to be considered as a book of reference rather than a treatise on the connections considered. To that purpose at the head of every page the numbers of the chapter and the nucleus of origin, the letter indicating either the afferent (A) or the efferent (B) connection and the number of the connection (e.g. IV 1 B 10) are given.

The results are discussed and summarized briefly either with the nucleus, the structure or at the end of the chapter.

CHAPTER II - MATERIAL AND METHODS

The albino rat was chosen as the experimental animal, especially because its corpus striatum never before has been investigated with experimental methods. According to the stereotaxic atlas of de Groot (1959), which was used, rats 200-300 gm. in body weight were used.

Because it was realized from the beginning that the split fasciculi of the internal capsule would be a nuisance in studying the efferents of the corpus striatum, a way had to be found to eliminate them. To this purpose a series of frontal cortical ablations was made to determine the period, required for the complete disappearance of the degenerating fibres. These animals and their corresponding surviving times are:

No.:			
	H. 5187 – 1 day	H. 5109 – 22 days	H. 5131 – 46 days
	H. 5188 – 2 days	H. 5111 – 24 days	V 1 – 2 months
	H. 5189 – 3 days	H. 5113 – 26 days	V 2 – 3 months
	H. 5190 – 4 days	H. 5115 – 28 days	V 3 – 4 months
	H. 5191 – 5 days	H. 5117 – 30 days	V 4 – 5 months
	H. 5192 – 6 days	H. 5119 – 32 days	V 5 – 6 months
	H. 5037 – 7 days	H. 5121 – 34 days	V 6 – 7 months
	H. 5038 – 10 days	H. 5123 – 36 days	V 7 – 10 months and 14 days
	H. 5040 – 13 days	H. 5125 – 38 days	V 8 – 14 months
	H. 5043 – 16 days	H. 5127 – 40 days	V 9 – 17 months
	H. 5107 – 19 days	H. 5129 – 42 days	

At the same time 37 complete hemidecortications were made to be reoperated when the final surviving time should be known. Although the precaution was taken to begin with very young animals, at the time of reoperation only sixteen (the numbers CT 1 - CT 16) had survived, the rest having died of old age. (See about this subject: Griffith and Farris 1942).

After the optimal surviving time for the staining method of Nauta-Gygax in the rat proved to be from 5-7 days, also a series of acute experiments was performed of which 75 are considered in this publication.

THE OPERATIONS

All operations were carried out under aether-anesthesia, with the rat fixed in an appropriate stereotaxic head holder. Two kinds of operations were done.

Ablation-experiments. After baring the skull, in the small ablations a bur-hole was

made with a dental drill, and in the hemidecortications with a small circular saw a bone-flap was cut out rostrally, caudally and laterally and subsequently opened along the sutura sagittalis. The cortical surface exposed was removed by means of superficial suction. After that, the bone flap was replaced and some spongostan applicated over it. The musculus temporalis, when loosened, then was sutured to the contralateral one and the wound was closed.

Although it may not have been necessary, in general the operations were procured under sterile precautions.

Stereotaxic experiments. The stereotaxic operations were carried out with the Horsley-Clark apparatus according to the data provided by de Groot. Access to the brain was made by a small bur-hole. To adjust the electrodes most securely, use was made of an operation-microscope. Electrolytic lesions were applicated by means of the coagulator of Wyss.

In the case of the long-standing cortical ablations, the procedure was identical, excepted that some slight corrections, made by microscope, were required because of the deplacement of structures after the cortical removal.

THE PREPARATION OF THE BRAINS

After perfusion of the animal under deep anesthesia with 10% formaline, the calvaria was removed, and the head replaced in the stereotaxic head holder. In this position the brains were either sectioned transversely in the planes A 11, – and A 0, – or parasagittally, in order to obtain all microscopical sections in the same way as in the atlas of de Groot. The part of the brain acquired was fixed in neutral formaline from 2 weeks till 6 months.

Frozen sections were made 22 μ thick, which were stained according to the method of Nauta-Gygax (1954) with some slight modifications:

The sections were stained in general in a ratio of 1:10 amounting to about 40 sections by the experiment. The sections were divided over four perforated cups, that were placed in larger cups with the different liquids, in the following way.

1. Briefly rinse in aqua dest.
2. Soak sections for 30 minutes in aethanol 10%.
3. Rinse briefly in aqua dest.
4. Soak sections in 0.5% phosphomolybdic acid for 30 minutes.
5. Without washing to 0.1% $KMnO_4$ for 4-10 min. (generally about 6). The perforated cups have to be moved continually.
6. Rinse briefly in aqua dest.
7. Decolorize in equal parts of 1% hydroquinone and 1% oxalic acid till slightly green.
8. Rinse thoroughly in 3 cups of distilled water.
9. Soak sections for 90 minutes in a 3% solution of silver nitrate, in the dark.
10. Rinse two times in aqua dest. Proceed with only one cup at the time.
11. Transfer the cup with sections for 90 seconds to a constant quantity (about 30 cc) of Laidlaw's solution, moving the cup continually.
12. Dry the outer surface of the cup with filter paper and let it leak out a moment in order to prevent too much of the Laidlaw's solution to mix with the next.
13. Transfer the cup to the reducing fluid for 1 minute, moving continually.
14. Rinse briefly in aqua dest.
15. Pass sections during 15 seconds through 1% Na-thiosulfate.

16. Rinse again in three changes of distilled water.
17. Pass the separate sections through subsequent aethanol 70% and 90%, in order to prevent the sections being damaged by them glipping through the holes of the cup.
18. Mount the sections through carbolxyline and apply the cover glass with neutral synthetic resin.

With this modification, more or less standardised results were obtained, especially for the most finest fibres. When making a large stock of Laidlaw's solution and determining the number of drops of ammonia to be added to the quantity of this solution used for every cup to obtain the wanted colour, it proved to be feasible to obtain the same colour throughout all series treated with this stock. Especially point 12 has to be paid attention to, because even a small quantity of Laidlaw's solution mixing with the reducing solution, darkens the colour considerably.

Besides the silver-impregnated sections, for purpose of orientation of every experiment also a series of sections was stained with the method of Klüver and/or Nissl on frozen sections.

To eliminate misinterpretations by inhibition of degenerated fibres (in point 7) in general from the same experiment several series were made with different inhibition times. By inhibiting two of the four perforated cups of a series somewhat longer or shorter than the others, this gradation also could be obtained.

THE STUDY AND PRESENTATION OF THE EXPERIMENTS

Because it proved awkward, from most publications, to form an idea about the extent of the lesions, and interruption of fibres of passage that trouble the pattern of degeneration, the lesions will be presented in their entire extent, together with needle track(s) and cortical damage. To this purpose from a standard parafine series, sections were drawn at constant intervals with a drawing apparatus, and the extent of the lesions filled in under the microscope. In order to make matters not too complicated the (often arbitrary) limits of most nuclei were not depicted, but they can be found by comparison with the schemas presenting them.

For presentation of the patterns of degeneration from the same standard series 14 characteristic planes were depicted. These planes are chosen in a way as to represent every structure at least once. The filling in of the degeneration patterns in these schemas has been facilitated and shortened very much by the use of a phase-contrast condensor which made it possible with a X-100 magnification to distinguish more than with a X-400 magnification with the ordinary light microscopy. Although of course a continual controle with the aid of the latter is required, this method had another advantage of showing clearly the predominating fibre-directions amidst an intricate mashwork of degenerated fibres. This with stronger magnifications is rather difficult, while very fine and scattered fibres, easily missed with a light-microscopy alone, were very distinct in the sections studied this way.

THE LONGSTANDING EXPERIMENTS

About the experiments carried out to determine the time of appearance and disappearance of degeneration some remarks still have to be made. One day after the operation some fine and scarce fragments were observed in the immediate surroundings of the lesion. The second day, a moderate very fine fragmentation could be detected, extending somewhat farther from the

lesion, together with swollen fibres. The third day showed a much more extensive degeneration, reaching more caudally, but still very finely fragmented. After four days, the pattern of degeneration was rather complete, even the fine cortico-striate fibres being present, although the whole is still very fine, and the final density is not yet reached.

From five till seven days an optimal pattern of degeneration is present, although the fragments become coarser with increasing time, while on the other hand the very fine collateral and preterminal degeneration already seems to decrease somewhat.

After longer periods, the degenerated fragments gradually increase in size and eventually form large black clods.

From about 13 days on infiltrations with compound granular cells begin to appear in the corpus striatum and in the degenerated parts of the ipsilateral thalamus, accompanied by many small holes.

In one month the pattern consistently shows an extensive coarse degeneration without fine fibres, and heavy infiltration with compound granular cells in corpus striatum and thalamus.

After two months, the majority of the large cells of the nucleus reticularis and nucleus ventralis of the thalamus have disappeared, and after three months the corpus striatum and thalamus begin to show large, somewhat gliotic masses, staining in a peculiar homogeneous way, while the infiltrations tend to disappear.

After 6 months, we observe a retrograde transsynaptic degeneration in the medial lemniscus, probably owing to the disappearance of ganglion cells and to gliosis in the nucleus ventralis thalami. This degeneration decussates at the caudal level of the nucleus interpeduncularis and can be traced contralaterally to the trigeminal nucleus princeps. The non-trigeminal part of the lemniscus however does not show such a retrograde degeneration.

With a ten months survival period, the mammillo-thalamic tract and the fornix show a small number of degenerated fibres. In both structures the degeneration increases with advancing time, to show after 18 months, when the primary degeneration has almost completely disappeared a heavily degenerated mammillo-thalamic tract and columna fornicis. It has to be emphasized that the hippocampus, fimbria and fornix are not damaged at all by the lesions and neither show atrophical changes. Among the anterior thalamic nuclei only the nucleus anteroventralis in its ventrolateral part has largely disappeared by retrograde cellular degeneration, whereas the rest of the anterior complex is quite normal. The same holds for the corpus mammillare that neither did show cellular changes. Even when we attribute a part of the degeneration in the mammillo-thalamic tract to the part of the nucleus anteroventralis degenerated, the majority of its fibres distributing over the normal nucleus anteromedialis, anterodorsalis and dorsomedial part of the nucleus anteroventralis, cannot be accounted for to have come to degeneration in this way.

Already Tarasewitsch (1902 man) – and according to him also Bischoff – describes the same features in a during eight years hemiplegic patient with a cortical atrophy. He attributed the degeneration of the mammillo-thalamic tract to its interruption by a sclerotic plaque in his sections. He pretended to find atrophical changes in the corpus mammillare, combined with strong atrophy of the pedunculus mammillaris. This latter atrophy as well as that of the fornix, the author was unable to explain.

It is astonishing that 62 years later this interesting question has not been reinvestigated and still is as incomprehensible as in 1902. Although neither a degenerated pedunculus mammillaris, an atrophied corpus mammillare (possibly a question of time) nor a direct interruption

of the tractus mammillothalamicus were present in the present material, the problem has not changed. The most plausible explanation seems to be a functional atrophy, since after hemidecortication the circuit: hippocampus – fornix – corpus mammillare – tractus mammillothalamicus – nucleus anterior thalami-cortex cerebri, seems to have lost most if not all of its significance.

It will be clear that in the longstanding experiments of the present material we will always find degeneration in the limbic system as well in the medial lemniscus. Moreover not all very coarse fragments of the primary lesion have disappeared at 18 month. The pattern of "degeneration" and gliotic masses as seen in not reoperated animals, is presented in the scheme CT 1, 2, 3. In the schemes of the reoperated animals it has been omitted.

LIST OF ABBREVIATIONS

AAA	area amygdaloidea anterior
ABL	nucleus amygdaloideus basalis pars lateralis
ABM	nucleus amygdaloideus basalis pars medialis
ac	aquaductus cerebri (Sylvius)
ACB	nucleus accumbens septi
ACE	nucleus amygdaloideus centralis
ACO	nucleus amygdaloideus corticalis
AD	nucleus anterodorsalis thalami
AHA	area hypothalamica anterior
AHL	area hypothalamica lateralis
AL	nucleus amygdaloideus lateralis
AM	nucleus anteromedialis thalami
AME	nucleus amygdaloideus medialis
ARH	nucleus arcuatus hypothalami
AV	nucleus anteroventralis thalami
bc	brachium conjunctivum
BCA	nucleus proprius commissurae anterioris (bed nucleus)
bcs	brachium colliculi superioris
BO	bulbus olfactorius
bp	brachium pontis
BST	nucleus proprius striae terminalis (bed nucleus)
C	nucleus interstitialis of Cajal
caa	commissura anterior (crus anterior)
cat	commissura anterior (pars intertemporalis)
cc	corpus callosum
ce	capsula externa
CGL	corpus geniculatum laterale
CGM	corpus geniculatum mediale
chi	commissura hippocampi (commissura fornicis)
cha	commissura habenularis
ci	capsula interna
cin	cingulum
CI	colliculus inferior
cic	commissura colliculi inferioris
CLA	claustrum
CL	corpus Luysii (nucleus subthalamicus)
Cl	nucleus centralis lateralis thalami
Cm	nucleus centralis (medialis) thalami
cho	chiasma opticum
COC	cortex cerebri
cp	commissura posterior
CPU	nucleus caudatus / putamen = striatum
CS	colliculus superior
csc	commissura colliculi superioris
cso	commissurae supraopticae
CT	nucleus centralis tegmenti (Bechterew)
D	nucleus of Darkschewitsch
DBB	gyrus diagonalis (diagonal band of Broca) and its bed nucleus
dbc	decussatio brachiorum conjunctivum
DMH	nucleus dorsomedialis hypothalami
drs	decussatio tracti rubrospinalis
DT	nucleus dorsalis tegmenti
dtd	decussatio tegmenti dorsalis (Meynert)
dtv	decussatio tegmenti ventralis (Forel)
ENT	cortex entorhinalis
EP	nucleus entopeduncularis
fi	fimbria hippocampi
fl	fornix longus
flm	fasciculus longitudinalis medialis
fr	fissura rhinalis
frm	fasciculus retroflexus of Meynert
fx	fornix
GB	ganglion basale of Meynert
GC	substantia grisea centralis
GP	globus pallidus
HL	nucleus habenularis lateralis
HM	nucleus habenularis medialis
HPC	hippocampus (cornu ammonis)
IAD	nucleus commissurae interanterodorsalis
ICL	nucleus amygdaloideus intercalatus
IP	nucleus interpeduncularis
IV	nucleus interventralis
ll	lemniscus lateralis
lm	lemniscus medialis
lme	lamina medullaris externa
LS	nucleus lateralis septi
LT	nucleus lateralis thalami
LTP	nucleus lateralis thalami pars posterior
m	foramen interventriculare (Monro)
MAM	corpus mammillare
MD	nucleus mediodorsalis thalami
mfb	medial forebrain bundle (fasciculus medialis telencephali)
ML	nucleus mammillaris lateralis
MM	nucleus mammillaris medialis