INSTITUTES OF THE ROYAL NETHERLANDS ACADEMY OF ARTS AND SCIENCES
PROGRESS REPORT 1981

VERHANDELINGEN DER KONINKLIJKE NEDERLANDSE AKADEMIE VAN WETENSCHAPPEN TWEEDE REEKS DEEL 79

NORTH-HOLLAND PUBLISHING COMPANY - AMSTERDAM, OXFORD, NEW YORK, 1982
Institutes of the Royal Netherlands Academy of Arts and Sciences

Progress Report 1981
CONTENTS

Research teams and participants - 1
Historical background - 2
The organization of research - 3
I. Adaptability of the nervous system in adult organisms - 5
II. Interaction of the nervous system and behavior during maturation - 9
III. Brain-endocrine interactions during maturation and adaptation - 12
IV. Development and plasticity of behavior - 19
V. Mathematical and computational aspects of neurobiology - 24
Mechanics workshop - 28
General technical service - 28
Electronics workshop - 29
Guest workers and work-visits abroad - 30
Publications - 31
Abstracts (incl. posters) - 37
Papers read (seminars, etc.) - 41
Teaching - 43
Miscellaneous - 46
Seminars given at the Institute - 48
Research teams and participants

Director: Prof. Dr. D.F. Swaab
Manager: K.E. de Roos

I. Adaptability of the nervous system in adult organisms
Dr. H.B.M. Uylings (head)
Drs. C.G. van Eden (PhD student)
Drs. M. Hofman (PhD student)
Drs. W. van Norde (TAP)
Dr. R.W.H. Verwer (coordinator histological technicum)
M. Dozy (from 1-5-1981)
P. Evers
C. de Raay

II. Interaction of the nervous system and behavior during maturation
Dr. M.A. Corner (head) (NIBR and University of Amsterdam)
Dr. R.E. Baker
Drs. H.L.M.G. Bour (PhD student)
Dr. A.M.M.C. Habets
Drs. F. van Huizen (PhD student, from 1-8-1981)
Drs. M. Mirmiran (PhD student)
Dr. H.J. Romijn (coordinator electron microscope service)
M.T. Mud
H.F. Pronker
P. Wolters

III. Brain-endocrine interactions during maturation and adaptation
Prof. Dr. D.F. Swaab (head) (NIBR and University of Amsterdam)
Dr. G.J. Boer (coordinator radio-isotope laboratory)
Dr. R.M. Buijs
Drs. E. Fliers (PhD student FUNGO/ZWO, from 1-3-1981)
Drs. J. Kruisbrink (1-2-1981/1-8-1981, AKZO; from 1-9-1981, NIBR)
Dr. F.W. van Leeuwen
Dr. C. Misra (IBRO Fellowship)
Drs. T. Öcal (ETP, until 1-7-1981)
Dr. P. Pévet (University of Amsterdam)
Dr. C.W. Pool (coordinator animal care facilities)
Drs. P.J. van der Sluis (PhD student)
Drs. G.J. de Vries (PhD student)
B. Fisser
E. de Graaf (FUNGO/University of Amsterdam, from 1-5-1981)
J.J. van Heerikhuize
A.A. Sluiter

IV. Development and plasticity of behavior
Dr. N.E. van de Poll (head)
Dr. J.P.C. de Bruin
Dr. J.G. van Oyen
Drs. J. Scholtens (alternative military service)
Drs. J. Slopsema (alternative military service, from 1-2-1981)
Dr. H.H. Swanson
S.M. de Jong-van Zanten
E.M. Verbraak
S.M. van der Zwan
V. Mathematical and computational aspects of neurobiology

Dr. H.L. Walg (head)
Dr. J. van Pelt
M. Timmerman (from 1-9-1981)

Secretariat
W. Chen-Pelt
P.J. van Nieuwkoop
J. Sels
J. van der Velden

Animal care facilities
R. Hofer
N. de Vries

Library
Drs. J. Blaauw
Drs. C. Winkler

Electronics workshop
J. Overdijk (head)
R. Nooy

Mechanical workshop
A.W. Kamstra (head)
M. Westdorp

Drawing department
H. Stoffels

Photography department
A.T. Potjer (head)
T.C. Sypkens-Potjer

General technical service
J.C. de Jong (head)
L. Tibbertsma

Household service
M.A. Scheermeijer-Beuker (head)
H.H. Barbé-Scheermeijer
C. de Haas-Joole
J.W. Pals-Cappon
M. de Vos-Harthoorn

Canteen
C. de Groot

Administration
H. Sijtsma (head)
P.A.M. van der Poel

Historical background

At a meeting of the International Association of Academics, held in Paris in 1901, the anatomist Wilhelm His proposed that research into the nervous system should be placed on an international footing. In 1904 this resulted in the formation of the International Academic Committee for Brain Research, which pointed out that 'the time is not far distant when the study of the millions of brain cells will have to be divided amongst researchers in the way that astronomers have been obliged to divide the millions of stars into various groups'.

The committee set itself the task of 'organizing a network of institutions throughout the civilized world, dedicated to the study of the structure and functions of the central organ...'. The first country to respond to this ambition was the Netherlands: on the basis of a report drawn up by Profs. C. Winkler and L. Bolk, the Royal Netherlands Academy of Arts and Sciences (KNAW) applied to the government for permission to found an institute for brain research. On June 8th, 1909, the 'Netherlands Central Institute for Brain Research' was opened in a wing of the then newly erected Department of Anatomy and Embryology of the University of Amsterdam.

The first director was Prof. C.U. Ariëns Kappers, who gained international fame with his work in the comparative anatomy of the nervous system. Much of the material used in his research, including a considerable number of human
and animal brains, is still at the institute. He regarded the study of the development of the brain as being essential to the understanding of the normal and pathological structure and function of the nervous system. That his contention is still valid is evidenced by the central place this approach occupies in the research program of the institute today.

Prof. C.U. Ariëns Kappers was succeeded in 1946 by Prof. B. Brouwer, whose principal field of study was pathological anatomy. A member of his staff, Prof. J. Drooglever-Fortuyn, introduced electrophysiology to the institute's field of research.

After the death of Prof. Brouwer in 1949, the institute was expanded and re-organized to allow for a multi-disciplinary approach to brain research. In 1952, Prof. S.T. Bok (one of the pioneers of quantitative morphological analysis of the brain, especially the cerebral cortex) was appointed director. After his retirement in 1962, he was succeeded by Prof. J. Ariëns Kappers, whose special field of study was the circumventricular organs. Under his direction, research into the structure and function of the pineal gland became an important part of the institute's work.

On the first of October, 1978, Dr. D.F. Swaab, acting director since November 1975, was appointed to be the new director. In December 1979, the office of 'extraordinary professor' of neurobiology at the University of Amsterdam was conferred upon him. The construction of new quarters for the institute within the complex of the Academic Medical Center of the University of Amsterdam is now nearing completion.

The organization of research

As of the first of January, 1977, the central research theme 'Maturation and Adaptation of the Nervous System' is being investigated by five multi-disciplinary research teams, which in the coming period will be working along the following lines:

I. Adaptability of the nervous system in adult organisms

Neurotransmitters, hormones and environmental conditions are essential for normal development of the cortex. This team orientates its research into the question as to how and to what extent these factors are influential to the development and plasticity of the cortex.

Attention is mainly directed to the effects they have on the pre-frontal cortex of rat as well as man. In rats, because of the experimental opportunities such study offers; in man, because such research is adherent to the neuropathological study of the pre-frontal cortex area in case of abnormalities in its development. This research also encompasses the study of the structural differences in the cortex between the sexes, and between the right and left hemisphere of the brain. Quantitative and qualitative light and electron microscopic techniques constitute the core of this research (whereby in collaboration with members of research team III) immunocytochemical and autoradiographic techniques are employed. The aspects of physiology and behavior are being investigated in coordination with members of research teams II and IV, while the chemical aspects are being investigated together with members of research team III.

II. Interaction of the nervous system and behavior during maturation

During the period of synapse formation in the central nervous system, rhythmical patterns of spontaneous bioelectrical activity are widespread and pronounced. The degree which these neuronal discharges contribute to the emergence of ordered networks (synaptic organization) is still very poorly understood. Research team II is approaching this problem in vivo by studying the contribution
of active (i.e., REM) sleep to brain development, in collaboration with research teams I (neuroanatomical effects), III (biochemical effects) and IV (behavioral effects). In addition, a tissue culture 'model' system allows for a more controlled manipulation of neuronal activity during development, with subsequent assays for structural and functional abnormalities. In collaboration with research team V, endeavors are being made to develop a computer model of cortical networks in tissue culture, in order to test hypotheses about the reciprocal relationship between structure and function. Techniques used by the research team include electrophysiology, electron microscopy and culturing in vitro, while quantitative morphological, immunocytochemical, behavioral and computer techniques are contributed by research teams I, III, IV and V, respectively. The two electron microscopes, that are available for use by all scientists at the institute, are supervised by this research team.

III. Brain–endocrine interactions during maturation and adaptation

Brain cells produce certain substances, such as neuropeptides, which exert an influence upon the nervous system itself as well as on the pituitary gland and, by their hormonal actions, other organs as well. An appropriate maturation process and a well adjusted functioning of the nervous system is also dependent on hormones produced elsewhere in the body. The interaction between nervous and endocrine systems is being studied during the early growth and the maturation stage of the nervous system, in relation both to parturition and to the individual's adaptation to its environment. The emphasis in this research lies on the function of neuropeptides during the above mentioned processes. Research team I is studying the morphological consequences of the effects produced by peptides on brain development. The effects of gonadal hormones on peptidergic fiber growth, and their selectivity as regards extrahypothalamic connections in culture, are being studied in coordination with research team II, as are the electrophysiological properties of peptidergic synapses. The functional implications of the extrahypothalamic pathways and their sexual dimorphism are studied in coordination with research team IV. The workgroup is engaged in the application of immunocytochemical methods, applicable to both light and electron microscopic work, along with biochemical techniques, radioimmunoassays and clinical observations.

IV. Development and plasticity of behavior

Under the influence of sex hormones during critical phases of development, changes take place in the central nervous system which may markedly affect the behavior of an individual in adult life. In the rat this sensitive period occurs around the time of birth. The brain shows growth spurts at different stages in various parts of the brain, and the periods during which sex hormones influence the central nervous system may be related to this phenomenon. Between this sensitive period, when hormones are active, and adulthood lies a long period of development during which environmental (including social) factors may interact with the organizational effects of the hormones. Sexual dimorphism of brain and behavior forms an important part of the research conducted in collaboration with other research teams, such as morphological aspects (team I), relations sleep patterns (team II) and biochemical and immunological aspects (team III). The influence of sex hormones on several aspects of adult behaviors such as sex and aggression, and some forms of learning are also being investigated. Special emphasis is placed on the possible identification and localization of associated functional differences in the nervous system, using lesion, stimulation, ingestion and implantation techniques. Since the consequences of organizational effects of sex hormones sometimes only appear under certain conditions, the relationship between hormones and specific behaviors in adult animals will also be considered.
V. Mathematical and computational aspects of neurobiology

This division, which exercises supervision over use of the computers (including a powerful, recently acquired Digital VAX 11/780) has a twofold task. On the one hand, it is involved in all computerized data processing activities; on the other hand, it approaches diverse questions arising from the central theme from a theoretical point of view. Current subjects are the description of growth of neuronal branching patterns in terms of its topological parameters (in collaboration with research team I) and the simulation of bioelectrical activities in neural networks (in collaboration with research team II).

Apart from the research data obtained by the research teams, servicing departments assistants, guest workers, students and apprentices, several other activities are mentioned in this report, such as an inventory of the papers, seminars and lectures that were presented by members of our staff.


1. Adaptability of the nervous system in adult organisms

In this project, the plasticity and recovery of the 'mature' central nervous system after retardation due to environmental perturbations during early development are being studied. The adverse conditions involved in our studies include undernutrition, neuropeptide deficiency (together with research team III), and sleep deprivation (together with research team II). Emphasis is placed on the study of (a) normal development, (b) impairment in this development and (c) the recovery potential. Neurons, dendrites and synapses are studied, both qualitatively and quantitatively, using light and electron microscopic techniques. This project is represented in the FUNGO-workgroup 'Development and Aging of the Brain and Behavior' (nr. 13-51-16).

THEME 1. EFFECTS OF NUTRITIONAL REHABILITATION AND ENVIRONMENTAL ENRICHMENT OF CEREBRAL AND CEREBELLAR CORTEX OF PREVIOUSLY UNDNERNOURISHED RATS.

In collaboration with Dr. P. McConnell (Dept. of Anatomy, University of Birmingham, U.K.) experiments were carried out to document the effects of neonatal undernutrition upon the rat brain, and to determine the extent to which deleterious abnormalities could be reversed by post-weaning refeeding and/or environmental enrichment.

The experimental conditions can be summarized as follows: three groups of rats were undernourished from birth through 30 days of age; one group then was sacrificed, whereas the other two were given nutritional rehabilitation up to 150 days post-partum (dpp). Of these two latter groups, one was housed in standard conditions, the other in an 'enriched' environment. For each of the three experimental groups there was a control group which received adequately nutrition.

The experimental conditions can be summarized as follows: three groups of rats were undernourished from birth through 30 days of age; one group then was sacrificed, whereas the other two were given nutritional rehabilitation up to 150 days post-partum (dpp). Of these two latter groups, one was housed in standard conditions, the other in an 'enriched' environment. For each of the three experimental groups there was a control group which received adequately nutrition.

A topological analysis, in which the extent and pattern of branching of cerebral dendrites was studied, showed so far the following results. Branching was assessed in Golgi-stained neurons, on the basis of the number of terminal segments. The extent of branching of multipolar, non-pyramidal dendrites was comparable in all experimental groups to the values observed in the corresponding control group. Thus, as was concluded earlier from metrical observations, under-
nourishment appears to have no influence on dendritic parameters. Likewise, no significant differences were found in the extent of dendritic branching in multipolar, non-pyramidal dendrites when the 30-day-old rats were compared with 150-day-old animals which had been housed under standard conditions. In contrast, enrichment had a stimulatory effect upon the extent of branching, in underfed as well as in control rats, such that the dendritic trees had a higher number of terminal segments.

In pyramidal cells, 30 days of neonatal undernutrition had no apparent influence on the topological extent of dendritic branching. However, as the animals grew older (to 150 dpp), the dendrites had more branches. This effect was even more pronounced when the animals were housed in an enriched environment. In agreement with these topological results, a metrical analysis showed that enrichment had an enhancing effect on the outgrowth of both pyramidal and non-pyramidal multipolar dendritic trees. The dendrites of pyramidal cells from previously undernourished rats benefited the most from enriched rearing conditions. Metrical analysis, however, showed that the pyramidal dendritic tree in 30 days underfed rats had a significant smaller radial extension and terminal segments, then those in 30 days control rats.

Quantitative ultrastructural investigations were performed with the electron microscope, in order to determine the influence of undernutrition plus subsequent rehabilitation (with or without environmental enrichment) on synaptogenesis in the rat cerebellum. The major part of the synaptic structures in the molecular layer consists of contacts between parallel fibers and Purkinje cell dendritic spines. The profiles of both synapses and Purkinje cell dendritic membranes were traced using a computerized digitizing device, so that the amount of dendritic membrane surface area involved in synaptic contacts could be determined. In 30-day-old control rats, about 8% of the membrane surface of distal dendritic segments is used for making contacts with parallel fibers. The corresponding figure in 30-day-old undernourished rats is 7%. However, following rehabilitation in an enriched environment (to day 150 pp), this proportional deficit was fully restored: both in control and in refeed animals almost 9% of the dendritic surface comes to participate in synaptic contacts.

Because the total dendritic area involved in synaptic contacts is a rather global parameter, the number of synapses per unit volume was determined as well. Conventional methods for determining the numerical density of synapses are based on the assumption that synapses have a flat disk shape. However, since the synapses in the cerebellar molecular layer do not satisfy this assumption, we applied a recently developed procedure for estimating the numerical synaptic density. In this procedure the mean projected height of the synapses must first be determined from serial sections, and this parameter was then used together with the mean number of synapse profiles counted per unit test area.

At 30 dpp a reduction in the numerical density of synapses was found in the underfed animals as compared with the controls, and this deficit persisted after rehabilitation for 150 dpp in an enriched environment. This is due to the fact that there is no difference in numerical density between 30-day-old and 150-day-old rats, either in the enriched control group or in the enriched refeed animals. A preliminary estimate of the numerical density of spines of Purkinje cell suggests that some of these spines may be devoid of synaptic contacts in 30-day-old undernourished animals.

**THEME 2. THE DEVELOPMENT OF PRE-FRONTAL CORTEX IN THE RAT**

In preparation for a quantitative study of pre-frontal cortex (pfc) development, based on measurements of (a) cortical thickness and (b) volumes of cytoarchitectonic sub-areas, a study was made of the distribution of histochemical parameters as they relate to the cyto-architectonic boundaries of the pfc. A start has been made with the study of the distribution of heavy metals (Timm's
method) and acetylcholinesterase (AChE) activity. Refined staining techniques have revealed a distribution pattern in both these measures which coincides perfectly with the cyto-architectonic pfc subdivisions. With Timm’s method the heavy metals are visualized by silver deposits; in fixed vibratome and in unfixed cryostat sections most of the precipitated silver is contained in two layers, corresponding to cortical layers I-III and V, respectively. In addition to the correspondence between AChE activity and cyto-architectonic sub-areas of the pfc, an extra sub-division of AChE activity could be found in area Ald. The highest AChE activity within the pfc has been found within the pre-limbic and dorsal anterior cingulate cortex, in layers III-V.

The distribution of serotonin (5HT) containing fibers in the pfc, as demonstrated immunocytochemically with the PAP method, was fairly homogeneous, but with a somewhat higher density both in the most superficial layers and in layer V. Two types of fibers could be distinguished, the first of which consists of straight smooth fibers running perpendicular to the pial surface within the cingulate and pre-limbic cortex. The second type of fiber is fine and tortuous and possesses numerous varicosities. With respect to sub-areas in the pfc, only the dorsal part of the agranular insular cortex (Ald) received a dense innervation of 5HT fibers. The diffuse distribution of 5HT fibers over the remaining parts of the pfc did not allow the discrimination of other cyto-architectonic sub-areas. We have therefore concluded that cyto-architectonic sub-divisions of the pfc are not merely an anatomical property but may also have a functional meaning.

The quantitative study of the development of the cyto-architectonic sub-divisions from day 6 post-partum (pp), i.e., the first day that a sub-division can be discerned, through adulthood has been started. Initially we intended to use the inbred WEzob rat strain (CPB, TNO, Zeist) for our measurements, because this strain has been used for behavioral examination by research team IV. However, it appeared that the currently available animals of this strain have a disconcertingly high incidence of brain disorders. For instance, 70% of the animals showed enlarged ventricles accompanied by a severe atrophy of the optic nerves. These problems have caused a considerable delay in our research schedule, and in fact have forced us to switch to the Wistar strain.

THEME 3. STRUCTURAL DEVELOPMENT OF THE VISUAL CORTEX IN THE RAT

This study is being conducted in collaboration with Dr. J.C. Parnavelas (University of Texas at Dallas) and with research team V. Global topological analysis of basal dendrites of pyramidal cells from layer II showed that the extent of branching increases significantly between day 6 and 14 pp, after which no further changes were observed. Preliminary results have shown that basal dendrites of layer II pyramidal cells have a preference for terminal growth. Prior to day 14 the data have a more complex character, which is being examined in more detail. No changes take place in the extent of branching of non-pyramidal cell dendrites (both multipolar and bitufted) in layer IV between 10 and 90 days of post-natal life. Based on the extent of branching, a clear distinction could be made between the dendrites of multipolar and bitufted non-pyramidal cells at any given stage of development.

Measurements of the dendritic fields of large, medium and small pyramidal neurons have been carried out, except three stadia, and the analysis of the data is in progress. Furthermore, the computer programme for statistical analysis of the orientation and extent of dendritic fields has been improved, in collaboration with Prof. A. Ruiz-Marcos (Cajal Institute, Madrid) and Dr. H.L. Walg (research team V), so as to ensure the correct analysis of spatial orientation of neuronal dendritic fields.

Topologies. The topological properties of neuronal trees are important factors in the analysis of development and growth. Previously we have derived simple formulae for the determination of exact probabilities for the occurrence of various
types of dendritic trees in both the segmental and the terminal growth models. The different types were able to be ranked so as to enable the application of the Kolmogorov goodness-of-fit test for discontinuous distributions. Since the number of possible tree types can be very large, we developed a way of lumping several types into new categories, based on the number of terminal segments connected to each of the two sub-trees originating from the first branching point. Relatively few observations (about 5 trees of a given degree) are required, although for very large trees it may be difficult in practice to meet even this requirement. We therefore developed an additional procedure in which very large neuronal arborizations are lumped into only two classes, each class must theoretically contain 50% of the observations according to the growth model being tested. It was also proved that mixtures of smaller trees could be analyzed in a similar fashion in cases where only sparse observations were available (this work was carried out in collaboration with Dr. J. van Pelt of research team V).

THEME 4. THE INFLUENCE OF VASOPRESSIN DEFICIENCY ON THE RAT BRAIN

A morphometrical study of the cerebellum in vasopressin deficient HOM-DI Brattleboro rats is part of a collaboration with research team III. Overall areal measurements have revealed that there is an underdevelopment of the HOM-DI cerebellum, as compared with the HET-DI cerebellum, which is more pronounced on day 24 than on either day 12 or day 180. Examination of individual lobes showed that each lobe was affected to a different extent, with the most pronounced underdevelopment being found in sub-lobule VId. The white matter was even more strongly underdeveloped, regardless of the age of the animals, while in 24- and in 180-day-old HOM-DI rats the molecular and granular layers were proportionately reduced. Sexual dimorphic effects were demonstrated in 180-day-old animals, with males showing a more pronounced underdevelopment than females.

THEME 5. GROSS BRAIN INDICES, A MEASURE FOR ENCEPHALIZATION

The study of 'encephalization' in mammals was continued by analyzing our recently formulated index for relative brain size. The evolutionary increase in brain size relative to body weight was shown to be a discontinuous process, in which a specific number of neuronal elements appears to be added to the cerebral cortex with each step. A theory of corticalization has been developed in which the surface and volume of the cerebral cortex were divided into two hypothetical components, viz., a body-size related component and a component presumed to be associated with 'higher order' brain functions. From these results it was deduced that the number of neurons in the cerebral cortex is directly proportional to the total cortical surface, whereas the neuronal packing density is inversely proportional to cortical thickness. Based upon this outcome, and presupposing a basic uniformity in the cerebral cortex, we were able to estimate several cellular parameters (including the number of neurons and of cortical columns) from gross parameters such as brain weight and total cortical surface area. The medulla oblongata appears to be a brain structure which has an absolutely fixed relationship to body weight, i.e., contributes nothing to the process of encephalization during evolution.

Miscellaneous

a. The construction of the new semi-automatic device for the measurement of neuronal processes has reached the final phase of testing (see the reports of the mechanical and electronics workshop).

b. In collaboration with research team II, the weights of 8 parts of the CNS were determined in REM sleep-deprived and control rats (cf. research team II).
II. Interaction of the nervous system and behavior during maturation

During the period of synapse formation in the central nervous system, rhythmic patterns of spontaneous bioelectrical activity are widespread and pronounced. The degree to which these neuronal discharges contribute to the emergence of ordered networks (synaptic organization) is still very poorly understood. Our research team is approaching this problem in vivo by studying the contribution of active (i.e., REM) sleep to brain development, in collaboration with research teams I (neuro-anatomical effects), III (biochemical effects) and IV (behavioral effects). In addition, an in vitro 'model' system allows for a more controlled manipulation of neuronal activity during development, with subsequent assays for structural and functional abnormalities. A collaboration with research team V is attempting to develop a computer model of neuronal networks in tissue culture, in order to test hypotheses about the reciprocal relationship between cortical structure and function. Techniques used by the research team include electrophysiology, electron microscopy and culturing in vitro, while quantitative morphological, immunocytochemical, behavioral and computer techniques are contributed by research teams I, III, IV and V, respectively.

The research team is represented by three projects (unsubsidized) in the FUNGO-workgroup 'Development of Aging of the Nervous System and Behavior' (nrs. 1351–17, –35 and –36).

THEME 1. NEURONAL BASIS OF SLEEP/WAKE RHYTHMS AND THEIR SIGNIFICANCE

The behavioral consequences of chronic clomipramine (CPM) administration during early post-natal life were reinvestigated using a large series of male rats. Both the previously noted intensification of AS and the disturbances of masculine sexuality could be confirmed. A significant effect was once again found on open-field behavior, but this time with the experimental rather than the control animals being more active. In addition, the range of individual variations was abnormally small in the former group. These open-field results were replicated in chronic AS-deprivation experiments using, instead of CPM, either continuous rocking (pendulum) or daily administration of clonidine (CLO, an α-noradrenergic agonist). In both the CLO- and the CPM-induced AS-deprivation series (but not in the pendulum series, where the deprivation was less severe) the overall brain size was significantly reduced. This resulted from specific effects upon the cerebral cortex and medulla oblongata, paralleled by a reduction in total protein in these structures.

CLO-treated, chronically AS-deprived male rat pups were also studied for the ability of their brains to respond to extra stimulation in the post-weaning period. Confirming our earlier experiments, this 'enriched' rearing led in untreated animals to enlargement of the brain (specifically of the cerebral cortex) as compared to standard rearing conditions. In contrast, the two groups of CLO-treated rats did not differ from one another with respect to any brain region, and had a cortex weight which was significantly lower than in the corresponding untreated groups. The question of AS-deprivation in relation to sensory stimulation was pursued into the post-weaning period by injecting (female) rats daily with clonidine immediately following a 2 hr exposure to the enriched environment. The whole brain and the cerebral cortex in the enriched control group were, as expected, heavier than in the control group which was reared under standard conditions. The enriched + CLO rat brains, on the other hand, were not different from the standard-reared rats, and were significantly lighter (also the cortex) than in the untreated enriched group. AS-deprivation, therefore, appears to neutralize the growth-promoting effects of sensory stimulation during post-natal brain maturation.

Using improved chronic recording techniques in unrestrained animals, a longitudinal study was made of spontaneous action potential discharges recorded from
the medial pontine reticular formation (PRF). These preparations did not show the strong increase in selectivity of neuronal firing during active sleep (AS) which had been observed, as a function of age, in acutely implanted rat pups. The longitudinal studies thus failed to support the proposition that this change is due to an AS-specific enhancement of already active PRF neurons. In this series of experiments, in fact, the selectivity of spontaneous firing actually declined with age (up to 5 days following implantation of the electrodes). The developmental time-table thus appears to be more complex in the lower hindbrain than was first supposed. New unilateral (electrolytic) PRF lesions led to an enhancement of AS motility, but not to an increase of spontaneous neuronal discharges on the intact side during AS. It could therefore be concluded that the PRF probably does not contribute significantly to the generation of AS, although it might be involved in the characteristic inhibition of motor activity which is normally observed during this behavioral state. This hypothesis is consistent with the results of bilateral PRF lesion experiments in young rats. Not only did such animals fail to show any reduction in the amount of time spent in AS, but they also showed a dramatic intensification of twitches and other movements during AS. Even pseudo-hallucinatory ('oneiric', i.e., dream-like) behaviors such as walking, chewing, grooming and fighting were frequently noted. The brain showed electrical activity patterns which were characteristic for AS (continuous O-waves in the hippocampus, and low-voltage fast waves in the neocortex) and which were surpressed by clomipramine, together with all the oneiric behaviors, in favour of slow-wave sleep.

THEME 2. TISSUE CULTURE MODELS OF DEVELOPING STRUCTURE AND FUNCTION IN THE CEREBRAL CORTEX

Modifications of our chemically defined medium (CDM) were made which have enabled improved results to be obtained in culturing dissociated rat cerebral cortex neurons in vitro. On the basis of blood serum levels, several dose-response experiments were performed for different compounds of the CDM, in order to optimize their final concentration in the nutrient medium. Such experiments revealed that 1/10 of the original insulin and triiodo-L-thyronine concentrations, reduction of the 'Bottenstein cocktail' to one-half of its original value, and omission of corticosterone (1) visibly accelerated neuronal outgrowth, (2) selectivity promoted the outgrowth of stellate cells, (3) raised the final network density of cells and fibers, and (4) greatly reduced the occurrence of intracellular fatty inclusions. In addition, lowering of the culture temperature from 38°C to 35°C appeared to be beneficial in promoting neurite outgrowth. Despite these improvements, pyramidal cells still started to degenerate around 18 days in vitro (d.i.v.) in these dissociated cortex cultures, leaving behind a neuronal network of almost exclusively 'stellate' cells (which in turn degenerated after about four weeks). It is not yet known if these selective degeneration patterns also occur in cortical explants (i.e., non-dissociated cerebral tissues). Although the circular outgrowth zone around such explants also was seen to degenerate around 18 d.i.v., the cultures themselves stayed alive and functional for several months. Synapse counts revealed that a high numerical density was obtained already at 15 d.i.v., and was maintained until about 40 d.i.v., but then fell rapidly to a steady low level which persisted through at least 109 d.i.v. In one case, the pyramidal cells in a dissociated culture could be kept in excellent condition for at least 32 days simply by not refreshing the nutrient medium after day 5. The question now is whether such long-term survival is due to the release of neurotrophic substances by glial cells, or to the exhaustion of growth-stimulating factors in the nutrient medium (thus resulting in a stabilization of the neuronal outgrowth). A series of experiments has recently been started in an attempt to prevent selective pyramidal cell degeneration in the cultures by adding specific fatty acids and vitamins to the nutrient medium.
The ethanolic phosphotungstic acid method for selectively staining the pre-
synaptic grid has been adapted for in vitro material, on the basis of systematic
modifications of the standard procedure. Pilot studies have demonstrated the
existence of developmental changes in the number of dense projections per grid
in CDM-cultured cerebral cortex neurons. Studies involving the possible influ-
ence of bioelectric activity upon various aspects of synaptogenesis (numerical
density, pre-synaptic grid structure, and vesicles) are in progress using tet-
rodotoxin in order to block all action potential discharges during the period of
neuronal maturation in vitro.

In order to complement and extend the anatomical findings, 66 of the CDM-
grown cultures were recorded extracellually by means of saline-filled micro-
pipettes. All of the cortical explants (n=13, ages 12-109 d.i.v.) evinced spon-
taneous spike activity, half of them showing concomitant slow waves as well.
Thus, the survival of even a minimal number of synapses suffices to enable the
long-term persistence of complex activity patterns. Simultaneous recordings at
two sites in a few experiments revealed the presence of widespread synchronous
slow waves. These were associated with bursts of action potentials, the overall
rates of which often showed complex longterm fluctuations. In contrast, record-
ings made in dissociated cerebral cortex cultures (n=53, ranging from 7-41 d.i.
v.) revealed spike activity in only 33 cases, aged 11 d.i.v. and older. In these
cultures, slow wave activity was found only up to 22 d.i.v. (in 11 cases), and
was clearly related to the thickness of the reaggregates. After 18 d.i.v. the
ratio of bioelectrically active to silent cultures declined precipitously (from 19:3
to 14:16), presumably reflecting the onset of the degeneration phenomena de-
scribed above. For spike-train analysis a computer program has been developed
which estimates some of the first, second and higher-order statistical parameters
(spike-interval distribution, auto- and serial correlations, Markov ordering),
along with their respective confidence limits. Methods are also being tried out
for reliably dividing each recording into 'stationary' segments which can then
be studied separately. Of special interest are developmental trends in the serial
dependency of interspike intervals, which could reflect changes in the intensity
of functional coupling among neurons.

THEME 3. DEVELOPMENTAL MECHANISMS UNDERLYING SPECIFICITY IN THE
FORMATION OF NEURONAL INTERCONNECTIONS

Organotypic mouse spinal cord explants with attached dorsal root ganglia
(DRG) revealed that afferent fibers grow equally into the dorsal and ventral
halves of the cord, both in artificial (CDM) and in serum-containing (HSM) nu-
trient medium. Based upon electrophysiological criteria for identifying DRG
evoked responses as being monosynaptic in origin, the following conclusions
were drawn. In CDM the fibers remain largely ipsilateral but branch equally
throughout the dorsal and ventral halves, regardless of their point of entry.
This is also true for ventrally entering DRG fibers in HSM, whereas the dorsally
entering fibers become heavily concentrated within the dorsolateral area of the
explant. The electrophysiological findings were supported by a limited series of
horseradish peroxidase studies, in which stained DRG fibers were usually found
only in those areas of the cord from which monosynaptic evoked responses had
been recorded. With maturation in vitro the ventral DRG projections largely
disappeared in HSM but not in CDM. Longitudinal observations in HSM cultures
showed that this loss of ventral afferent projections was not due to cell death
among the explanted DRG neurons. Blocking all bioelectric activity by means of
xylocaine had little or no effect upon the pattern of primary afferent termination:
the degree of dorsal pathway preference and of (dorsolateral) terminal selectivity
appeared to be equal to that seen in the control (HSM) cultures.

The overall amount of polysynaptic evoked activity following DRG stimulation
was constant with age in HSM, and was unaffected by complete functional sup-
pression by means of xylocaine. CDM-grown cultures were in general more active than the HSM preparations, and showed a steady increase in this activity with age. The DRG responses were about equally distributed between dorsal and ventral, and between ipsi- and contralateral regions, both in the younger HSM explants (18–24 d.i.v.) and in the CDM series at all ages. The latter, however, showed a progressive increase in the amount of polysynaptic spread from the sites of afferent fiber terminations, eventually attaining values comparable to those found in the youngest HSM cultures. With maturation in vitro, HSM-evoked polysynaptic activities became largely restricted to the dorsal half of the cord fragment (both ipsi- and contralaterally), although this selectivity was less pronounced than for the monosynaptic responses (see above). Xylocaine-grown cultures showed a strong tendency for their complex evoked potentials to be restricted to the (ipsilateral) dorsolateral region of the explant, which suggests an important role for spontaneous bioelectric discharges in 'sculpting' interneuronal connectivity patterns.

In an attempt to see if serum-borne factors could restore selectivity to SC-DRG co-cultures grown in CDM, three experiments have been initiated. In the first of these, the effects of growth promoting hormones (corticosterone and triiodo-L-thyronine) were studied. Data from the literature show that these hormones greatly increase the number of synapses and enhance function in cortical cultures. In the second experiment chondroitin sulphate (CS, a glycosaminoglycan, or 'GAG') was added to the nutrient medium. GAGs are produced and released into the medium by fibroblasts, which are present in HSM cultures but largely absent in CDM cultures. In the third experiment galactose was added, since it is an important sugar component in glycoproteins as well as in gangliosides and cerebrosides (all of these compounds are components of nerve cell membranes, and as such may function in intercellular recognition and adhesion). Preliminary electrophysiological results show that both CS and galactose-supplemented media dramatically increase the proportion of DRG monosynaptic connections within the dorsal cord, while cultures grown in hormone-supplemented CDM showed no such selective enhancement of dorsal connections. In addition, greatly increased number of DRG-fibers occurs in the presence of both GAG- and galactose-supplemented CDM, whereas there was no apparent difference between the CDM-control and the CDM-hormone grown cultures.

III. Brain–endocrine interactions during maturation and adaptation

In this project the production and secretion of peptides by nerve cells, and their actions on the brain are being studied. Emphasis is laid on (1) vasopressinergic and oxytocinergic cells, i.e., on their sites of production, transport, release, reception and interaction with various neurotransmitter systems in the rat and human brain in relation to central processes, (2) the possible involvement of these neuropeptides in brain development and labor, and (3) their changes during aging. Furthermore, new methods are being developed to enable the study of specificity in immunocytochemistry; special attention is being paid to the pineal gland by Dr. P. Pévet. The main disciplines include immunological techniques, electrophysiology, light and electron microscopy and biochemistry, while clinical material is obtained in collaboration with various university clinics in the Netherlands and the United Kingdom. Parts of this project are represented in the FUNGO project nrs. 13-35-07, 13-35-20 and 13-51-30 (the last two with financial support).

THEME 1. THE SITES OF PRODUCTION, TRANSPORT, RELEASE AND RECEPTION OF VASOPRESSIN AND OXYTOCIN

Various hypothalamic peptides, such as vasopressin (AVP) and oxytocin

12
(OXT), influence central processes. These peptides are synthesized in the paraventricular (PVN) and supraoptic nucleus (SON), whereas the suprachiasmatic nucleus (SCN) produces AVP only. By means of immunocytochemical techniques, using purified antisera, it has been demonstrated that in addition to the 'classical' projections towards the neural lobe, massive projections from the PVN and SON innervate the limbic system (mainly AVP) and various regions in the brain stem (mainly OXT).

Immuno-electron microscopy has revealed that these peptidergic fibers terminate synaptically in different regions of the limbic system. So far these peptides do not seem to be fundamentally different, either in their anatomical localization or in their mode of action, from the classical aminergic and amino acid transmitters. Consequently, a morphological substrate has been provided for the influence of these peptides upon central processes. In order to determine whether these peptides are also able, as are true neurotransmitters, to be released following depolarizing stimuli, induced peptide release was studied in various regions of the CNS. An in vitro incubation procedure was set up, the results of which show a calcium dependent release of both AVP and OXT in the septum and in the nucleus of the tractus solitarius following treatment with either veratridine or increased potassium.

In order to get a better insight into the contribution made by the three OXT- and/or AVP-producing hypothalamic nuclei to the extrahypothalamic fiber system, the SCN was lesioned. The vasopressinergic innervation of the rat brain was then established by following the changes in the immunocytochemical localization of AVP-containing fibers at various times after lesioning. Disappearance of vasopressinergic fibers suggested that AVP-containing pathways run from the SCN towards the periventricular nucleus, the dorsomedial nucleus of the hypothalamus, and the organum vasculosum laminae terminalis. However, since vasopressinergic fibers persisted in the lateral septum, lateral habenular nucleus, amygdala, diagonal band of Broca, nucleus of the tractus solitarius, interpeduncular nucleus, and the dorsal raphe nucleus after lesioning, the SCN at best can provide only a minor fraction of the projections to these areas. Thus, in contrast to the literature including our own previous observations, these findings indicate that the SCN is not a major source for vasopressinergic pathways within the brain.

The evidence for the presence of AVP in the SCN was based solely upon findings using immunocytochemistry and radioimmunoassay (RIA). Since the presence of another, related, peptide is regularly put forward, RIA was combined with (1) gel electrophoresis, which separates protein material according to molecular weight, and (2) isoelectric gel focusing, which separates proteins and peptides according to isoelectric pH. The problem of poor solubility of some tissue components in the gel was overcome by addition of dimethyl formamide. The results of electrophoresis of SCN homogenates show that radioimmunoassayable AVP is largely restricted to the peptidogenic fraction (MW < 10,000) and has the same position as synthetic AVP in the gel. Isoelectric focusing of SCN peptide extracts revealed a single immunoreactive peak with exactly the same isoelectric position and slope in the assay as AVP.

The electrophysiological properties of vasopressinergic synapses, especially in the septum, are being studied in collaboration with research team II. A recording system was set up which enables the determination of unit activity, firing rate and (averaged) evoked potentials. Currently an iontophoretic application system is also being set up. Further electrophysiological measurements, however, are awaiting more detailed anatomical studies on the origin of the vasopressinergic pathways to the septum (see above).

A start was made in visualizing the binding sites for AVP. Slightly fixed brains are frozen, and then sectioned on a cryostat. The sections are incubated with various concentrations of tritiated AVP, kindly supplied by Organon (Oss)
and the Rudolf Magnus Institute (Utrecht). Once the optimal conditions for AVP binding have been established, a radioautographic procedure will be used which should enable us to determine the binding sites throughout the brain. In addition, immunocytochemical procedures are being tested for demonstrating peptide binding sites in general.

In order to visualize peptidergic fibers in the brains of animals that cannot be infused (e.g., because their blood is needed for assays) and the possible adaptation to human brain material, the influence of various types of immersion fixation on the staining of extra-hypothalamic fibers has been studied. It was found that the optimal fixative for the visualization of AVP- and OXT-containing fibers in the rat brain is 2.5% glutaraldehyde plus 1% paraformaldehyde fixative at pH 7.5. Using this fixative in human brains, considerably more AVP containing fibers arising from the SCN were found than with the conventional formalin fixation. However, as a result of using this new fixation procedure, penetration became an important problem, especially in the voluminous adult human brain: the center remained unfixed even after a period of 2-4 weeks. A considerable improvement was attained by dissecting small brain areas from fresh human material, followed by immersion fixation.

Since evidence has been reported for a regulatory effect of opioids on AVP and OXT release within the neural lobe of the hypophysis, the localization of enkephalin (ENK) immunoreactivity was studied (using an antibody against Leu-ENK, obtained from Prof. R. Miller of the University of Chicago, USA) at the light and electron microscopic levels. Contrary to recent claims, no co-existence of immunoreactivity could be demonstrated for ENK and either AVP or OXT within the same fibers. ENK was present only in beaded fibers, distributed throughout the neural lobe. They surround the neurohypophysial glial cells (pituicytes), and innervate them by making synaptic contacts upon their somata and processes. The immunoreaction was localized in dense core vesicles with a diameter of 100 nm and diffusely distributed over the cytoplasm. This immunoreactivity cannot be attributed to dynorphin or β-endorphin, but reactivity with other opioids cannot be excluded at present. It is postulated that pituicytes play an intermediary role in the regulation of AVP and OXT release by opioid peptides.

**THEME 2. NEUROPEPTIDES IN DEVELOPMENT AND LABOR**

AVP and OXT were found by RIA to be present early in fetal life. The amount of AVP in the fetal brain appeared to be higher on day 14 than on the following days, a phenomenon which was not so clear for OXT. The combination of RIA with isoelectric focusing revealed a substantial amount of AVP or OXT immunoreactivity in the fetal brain, which could not be attributed to AVP or OXT themselves. This was particularly true on day 14-15, after which these compounds gradually disappeared. Whether they represent AVP or OXT precursors or are functionally distinct substances needs further investigation.

From the 12th post-natal day onwards, a marked and persistent sex difference developed with respect to the density of the immunocytochemically stained vasopressinergic fiber network in the rat lateral septum and lateral habenular nucleus. The fiber density in both areas was much greater in males than in females. Males castrated on the first post-natal day showed, when examined on the 26th day, a fiber density which was as low as that in intact female rats. In male rats castrated on post-natal day 7 a fiber density developed which was intermediate to that of normal male and female rats, while castration on the 14th post-natal day had no effect upon the development of the normal male fiber density. Testosterone in both neonatally castrated male and in normal female rats caused an AVP fiber network to develop which was similar to that seen in normal male rats. No difference was found whether the administration of testosterone took place in the first, second or third post-natal week of life. Experiments are
in progress to determine whether this sensitivity to gonadal steroids also per-
sists into adulthood.

Earlier findings on the AVP deficient Brattleboro rat revealed a retardation
of cerebellar development, which persists into adulthood and cannot be restored
at that time by AVP replacement therapy. The particular characteristics of the
stunted growth, not comparable with any known condition of underdevelopment,
suggests a role for AVP in perinatal brain development. To substantiate this
postulate, suppletion of AVP in the homozygous diabetes insipidus (HOM-DI)
Brattleboro pups was carried out between days 5 and 28 post-natally. The follow-
ing schemes were applied: (1) daily sub-cutaneous injections (i.e., discontinu-
ous and peripheral) of either Pitressin tannate or AVP suspended in oil; (2)
daily intracerebro-ventricular injections of AVP (i.e., discontinuous and central)
and (3) sub-cutaneous implantation at day 5 of Accurel tubing loaded with AVP
(i.e., continuous, peripheral release). However, none of these substitution pro-
cedures, using various dosages of AVP, were able to restore the growth deficit
of the HOM-DI brain (as measured by total cerebellar weight, protein and DNA
content at day 32). Improved Accurel technique, which gives a constant release
of AVP for at least two weeks in both pre- and post-natal periods, is currently
under investigation. In a first preliminary study to investigate the possible
necessity of Pitressin for normal brain growth at an earlier stage, Pitressin
treatment of HOM-DI females throughout pregnancy appeared to give a resto-
ration of cerebellar growth in their HOM-DI offspring. Direct fetal supplemen-
tation of AVP using the Accurel technique appears to be feasible and will now
be carried out in a larger series.

Based upon the promising results obtained by Dr. D. Gash (University of
Rochester, New York) with hypothalamic transplants containing AVP neurons in
the brain of adult HOM-DI Brattleboro rats, a start has been made with applying
this technique to 5-day-old HOM-DI rats (in collaboration with Dr. Gash). The
rationale is to develop a technique capable of counteracting their genetic de-
ficiency. Donor tissue (17-19 day fetal hypothalamic areas containing the SON
or PVN) was obtained from Wistar rats. By means of a manually manipulated
cannula, the minced and concentrated tissue was introduced at the bottom of
the IIIrd ventricle of the HOM-DI pups. Although a large number of transplants
survived and cells and fibers of the graft appeared to be immunocytochemically
positive for AVP, no longterm alleviation of the diabetes insipidus in these rats
was achieved. Growth of vasopressinergic fibers from the cells of the graft into
host brain was seen only occasionally.

The potency of Accurel (microporous polypropylene) as a matrix for continu-
ous release of peptides has been further investigated (project subsidized by
ENKA Research Institute, Obernburg, FRG). Accurel tubing, lumen-filled with
AVP or OXT and heat-sealed at both ends, was previously shown to give a con-
stant release of the hormone for several weeks if immersed in water. However,
immersion in a proteinous solution (10% swine serum), co-loading with gelatin,
or sub-cutaneous implantation in AVP-deficient Brattleboro rats all gave an
asymptotical release. The extremely high release during the first few days ap-
ppeared to be due to inhibition by proteins and/or peptides of the adsorption of
the internal surface of the Accurel tube. Accurel preparation with collodion re-
stored the long-lasting constant release of AVP best up to 50 days. The amount
released daily could be manipulated by either changing the AVP concentration or
co-loading with gelatin (which reduces adsorption). Application, in HOM-DI
Brattleboro rats, of AVP/Accurel/collodion preparations containing 22 μg of hor-
mone showed a clear reduction of diuresis during a whole month, indicating the
everogenous potency of this technique, not encountered with any other substitution
procedure. Preliminary results have already indicated that the Accurel technique
is also feasible both at fetal stages (intrauterine injection of a small-diameter
 tubing) and as a brain cannula (i.e., offering the possibility of refilling).
AVP and OXT are transported both to the human fetal pituitary and into the fetal brain itself. Since, in earlier studies, clearcut changes were observed in the peptidergic system during the course of development, a project has been started in collaboration with various obstetrical clinics, aimed at determining whether peptide levels in either the fetal or the maternal compartment reflect the stage of brain development of the human fetus. Maternal blood, amniotic fluid and umbilical cord blood were therefore collected, and the levels of AVP and OXT in these samples are currently being compared (in collaboration with research team V) using several parameters: gestational length, sex, birth weight, and head circumference. Preliminary results indicate an inverse correlation between AVP in the amniotic fluid and the percentile of head circumference. Amniotic OXT, on the other hand, appears to reflect mainly maternal OXT levels. Passage of maternal OXT into the amniotic fluid is currently indicated by rat experiments and by observations in human anencephalics. The human material provides also additional evidence for a role of fetal neurohypophyseal hormones in the course of labor.

THEME 3. VASOPRESSIN AND OXYTOCIN IN THE AGING HUMAN BRAIN

Aging is generally considered to go together with degenerative changes, e.g., weight and cell loss in the central nervous system. A study of changes occurring with age in the peptidergic neurons producing AVP and OXT has several advantages as compared to 'conventional' neurons. Using immunocytochemistry, the morphology of AVP and OXT producing cells during aging can be described precisely. Synthetic activity can be measured by determining quantitatively the distribution of thiamine–pyrophosphatase (an enzyme specific to the Golgi apparatus), while, in principle, the consequences of aging can be followed in the peripheral circulation and urine by assaying the hormones directly. In the vasoressinergic neurons of the human SON and PVN, changes were observed related to aging, i.e., decreased immunocytochemical reactivity after the 8th decade of life and lipofuscin accumulation. These cellular changes are currently being described in quantitative terms. In order to investigate vasoressinergic and oxytocinergic pathways running from the hypothalamus to limbic structures in the human brain, optimal conditions for fixation and tissue treatment were established. While investigating extra–hypothalamic pathways, special attention is being paid to the influence of age and sex.

In the rat, a continuous increase in brain weight was observed up to the oldest age group (32 months) in contrast to the aging human brain. In disagreement with reports in the literature, urine production decreased during aging; urine excretion of OXT and AVP did not decrease. At the cellular level, the neurosecretory activity in SON and PVN did not decrease significantly during aging. Current research is being directed to the question of the extent to which morphological and functional changes in the hypothalamo–neurohypophyseal system of aging rats can be usefully compared to the changes observed during aging in the human SON and PVN.

THEME 4. METHODOLOGICAL DEVELOPMENTS CONCERNING SPECIFICITY IN IMMUNOCYTOCHEMISTRY

Specificity of the immunocytochemical localization of a compound requires knowledge of the characteristics of the first antiserum. The specificity can only be investigated by (1) separating all compounds present in the tissue and (2) determining the affinity of the antiserum for each of these compounds. None of the techniques available, however, allows for a test for antibody binding to small peptides present in the tissue (MW < 2,000 D). SDS electrophoresis, which is used in these approaches cannot be applied to small peptides, nor can the immobilization techniques commonly used for proteins. To solve this problem, iso-
electric focusing on polyacrylamide gels has been chosen to separate peptides, while the focused peptides were immobilized by fixation of the gel on glutaraldehyde treated filter paper. The immobilized peptides could be stained immunocytochemically by the PAP or conjugate method. The previously described fixation method had to be reinvestigated since staining results obtained with this method for OXT were highly variable. Moreover, the sensitivity was often insufficient (10-50 ng peptide) to detect peptides from small brain areas. Finally, binding studies using tritiated AVP, angiotensin II, Met-ENK and glutathion (in collaboration with A.M. Meier, Gist-Brocades NV, Haarlem) showed that only a small fraction of the peptides (< 2%) was immobilized. Several parameters were tested: fixation time, glutaraldehyde concentration, and time of treatment, pH during fixation, and gel thickness. Of these, gel thickness proved to be the important factor: reducing the gel thickness from 0.4 to 0.2 mm improved the staining results considerably, as did the use of tetramethylbenzidine as chromogen in the peroxidase reaction. The method now has a threshold sensitivity of about 1 ng, which is within the range of the neuropeptide content in most brain areas.

Another aspect in the procedure for defining specificity in immunocytochemistry is the quantification of the reaction towards a defined tissue antigen in a model system. For this the defined antigen substrate sphere (DASS) method has been selected. The currently used peroxidase anti-peroxidase (PAP) procedure allows a more sensitive detection of the first antibody than does the indirect fluorescence method, which means that the former has to be determined with the PAP procedure instead of with immunofluorescence procedures. The DASS system has been adapted to this purpose, resulting in conditions for optimal coupling of the antigen to the Sepharose beads as well as for the spectrophotometric determination of the horseradish peroxidase (HRP) activity using o-phenylene diamine as a chromogen. However, the more potent antisera evinced some serious drawbacks in this procedure. For instance, the linearity between final absorbance and first antiserum dilution turned out to be seriously influenced by a 'Bigbee' effect (i.e., anti-AVP in a low dilution of 1/50 tested on AVP beads showed no reaction). Our conclusion was that the PAP procedure could not be used to quantify the first antibody's reactivity in such a model. Since the reactivity in each indirect immunocytochemical staining technique is largely determined by the characteristics of the second antibody used, a switch was made to an indirect HRP-conjugate staining. The same goat anti-rabbit IgG preparation as routinely used in the PAP staining procedure was coupled to HRP (in collaboration with Dr. Boorsma, Free University, Amsterdam) and all subsequent steps in the DASS procedure were reevaluated. This led to revised incubation conditions and the use of two types of controls (antisera on control beads and control antiserum on antigen-coupled beads). The validity of this model system for predicting the reactions of an antibody in a tissue section appeared to be good for anti-AVP or anti-OCTX.

THREE 5. PINEAL HORMONES

In most species there is a seasonal change in reproductive activity, such that the young are born during the season in which the probability of survival is maximal. Although in temperate zones, the length of the day is the primary environmental signal, factors such as ambient temperature, rainfall and food supply also influence the reproductive cycle of mammals. The pineal is believed to integrate information concerning day-length and other environmental factors and to transduce this information to the neuroendocrine reproductive system in order to optimize survival of the species. Chemical factors of the pineal gland involved in this process are: (a) peptide and/or proteins, and (b) indoleamines. By combining different techniques, we are attempting to identify the pineal peptides and to establish how their synthesis and release are regulated (in collabor-
ation with Drs. I. Ebels, University of Utrecht; A. Reinharz, University of Geneva; and C. Neaçsu, Institute of Cellular Biology and Pathology, Bucharest). Using immunocytochemistry and radioimmunoassay, we concluded that vasotocin (which has been regarded as the anti-gonadotropic hormone) is in fact absent in the mammalian pineal, a result which has been confirmed by other teams. A new bioassay has enabled us to describe vasotocin-like biological activity in the pineal of several mammalian species. It was demonstrated that this biological activity is probably due to a peptide possessing the same Pro-Arg-Gly (NH(2)) tripeptidic carboxy-terminal end as vasotocin. At present we are trying to purify and isolate this peptide, and to determine its physiological effect. The processes involved in the synthesis and release of the pineal proteic/peptidergic compounds were identified at the ultra-structural level some years ago, thus making it is possible to reveal, by means of electron microscopy, some of the processes implicated in regulating the availability of these substances. An in vitro culture system has been developed in order to study these mechanisms in detail. With this technique we have demonstrated that the noradrenergic innervation is implicated in the regulation of protein production, but in a species-specific fashion. This could explain the numerous contradictory results reported in the literature.

Melatonin has been (and still is), considered as the pineal hormone 'par excellence', but we have now been demonstrating that a different 5-methoxyindole, 5-methoxytryptamine (5-MT), is also able to induce gonadal atrophy in the hamster. In view of the observation that, in the mole, production of 5-methoxyindoles in the pineal is much higher than that of melatonin, this result indicates that (along with melatonin) 5-MT may well be involved in the control of reproductive function. We have also been able to show that indoleamine metabolism in the retina and in the Harderian gland is similar to that occurring in the pineal, further demonstrating that the pineal is not the only source of 5-methoxyindoles. Since (1) the 5-methoxyindole synthesizing organs (retina, Harderian gland, intestine, pineal etc.) all are organs receiving information, directly or indirectly, from the outside world (light, temperature, and food); (2) the rhythm of melatonin and 5-methoxytryptophol production by these organs is influenced by external parameters; and (3) melatonin probably controls pineal peptide synthesis, the specific function of the methoxyindoles might be to enable the integration of environmental information within the pineal.

Miscellaneous

A better understanding of the morphology and topography of astrocytes in the normal human brain is a prerequisite for the study of the development and differentiation of brain tumors. In collaboration with Prof. F.C. Stam and Drs. W. Kamphorst (Free University, Amsterdam) a study was initiated to use antibodies raised to glial fibrillary acid protein (GFAP), a marker for astrocytes. The morphology and distribution of GFAP-containing glial cells was studied immunocytochemically using the PAP technique in normal brains from patients of 15 to 91 years of age. Fetal and neonatal brains are currently under investigation. A morphological continuum was found, ranging from GFAP-negative glial cells, via slightly GFAP-positive gracile cells, to strongly positive large cells with the classical appearance of fibrous astrocytes. In Bergmann cells, only the radial fibers were stained (except in those brains where occasional GFAP-positive cell bodies were found). In the sub-pial layers, sclerotic GFAP-positive cells were present; bipolar glial cells were seen in the tangle of the choroid plexus, while a few positive cells, sometimes with positive processes were found in the ependyma (tanyocytes). GFAP-positive neurons and their axons were sometimes found to stain in the visual cortex, thalamus and pallidum; the basket fibers around cerebellar Purkinje cells were also stained on occasion. In the 91-year-old brain, a clearcut increase in the number of GFAP-positive glial cells was
found in almost all areas, whereas in the younger age group no age-dependent changes were observed. The thickness of the GFAP positive sub-pial and subependymal glial layers did not change with age. However, the number of positive glial cells in the molecular layer of the cerebrum was distinctly larger in the older age group. In the cortices of the older brains a GFAP-positive glial corona was found around senile plaques, and only in these cortices were stained glial cells with the classical aspect of protoplasmatic astrocytes observed. In the final stage of neurofibrillar degenerations, positive glial fibers seem to grow between the remains of parallel neurofibrils.

As in previous years, the radioimmunoassays for vasopressin, vasotocin, oxytocin and α-MSH have been applied to a large number of investigations of members of our group itself, as well as to collaborative studies with investigators from other laboratories.

The project with Dr. C.F. Goodfellow (University of Leeds, UK) on human labor was continued. The effect of epidural analgesia upon oxytocin release during the second stage of human labor was studied by radioimmunoassay of paired peripheral blood samples taken at full cervical dilatation and crowning. Ten normal primigravidae with, and ten without epidurals were compared. A significant increase in oxytocin was found in normal controls, but an increase was absent in epidurals. Forceps deliveries were required more frequently in epidural patients, and were associated with lower oxytocin levels. Since distension of the lower birth canal and stimulation of pelvic autonomic nerves leads to oxytocin release, these differences can be attributed to the lumbar epidural block.

In collaboration with Dr. A. Tielen (Medical Physics Institute, TNO, Utrecht), hippocampi of female guinea pigs were stained immunocytochemically with anti-leu-enkephalin serum; several new loci were found to contain immunopositive fibers.

A study was started with Dr. F.T. Russchen (Free University, Amsterdam) on the distribution of various peptides within the amygdala of the cat and monkey. Together with Drs. P. Schot and J. Wijdenes (Free University, Amsterdam), incubations were performed on pond snail neurons, using various antisera, while various antibodies and PAP were prepared with the latter.

With Dr. P. Grimmelmikhuy sensing (Max Planck Institut, Heidelberg), peptide assays on hydr as are being performed.

Together with Dr. P. Barth (Free University, Amsterdam) collodion-embedded cerebelli of children that died after severe epileptic insults were stained with anti-glutamic acid decarboxylase (GAD). Immunoreactive GAD was found to be present mainly surrounding the Purkinje cells: most probably in basket cell fibers.

In cooperation with Dr. I.W. Henderson (University of Sheffield, UK) the occurrence of hydronephrosis within the Brattleboro rat strain is currently under investigation.

IV. Development and plasticity of behavior

Research in this project is concerned with the study of development of social behavior (aggressive and sexual responses), and of emotional and learning aspects of behavior. All these modes (which in adulthood generally exhibit clearcut sex differences in the rat and many other species) are being studied in relation to the lasting consequences of gonadal hormones and environmental factors, acting during pre- and early post-natal development on the neural substrates involved in their regulation.

During 1981 this work continued along three lines: (1) analysis of motivational aspects of sexual and aggressive behavior patterns, (2) sex differences in avoidance learning and emotionality, and (3) the neural substrate underlying
aggressive and sexual behaviors. Moreover, an increasing interest in the study of behavior on the part of other research teams at the institute has stimulated several conjoint projects. (viz., effects of underfeeding and enriched environment on behavioral development with research team I, and behavioral effects of REM sleep deprivation with research team II). Parts of this project are represented in FUNGO (‘Development and aging of brain and behavior’ and ‘Behavioural Mechanisms’), BION (‘Ethology’), and PSYCHONOMY (‘Comparative and Physiological Psychology’). Behavioral research on sexual motivation in rats was subsidized by ZWO (PSYCHONOMY, nr. 15-25-09). During 1982 a 3-year subsidized project will be started on aggressive behavior, also subsidized by PSYCHONOMY, together with the University of Swansea (on a grant from the European Training Programme for Brain and Behaviour).

THEME 1. ANALYSIS OF MOTIVATIONAL ASPECTS OF SEXUAL AND AGGRESSIVE BEHAVIOR

Hormonal factors which determine masculine sexual responses have been studied almost exclusively in tests against females artificially brought into behavioral oestrus by means of ovarian hormones. Similarly, females were tested for feminine responses with males of proven sexual vigour and activity. This combined use of partners with a high stimulus quality, together with observations restricted to interactions between the two animals, is not appropriate to the study of sexual motivation. Therefore, tests and methods of behavioral observations were used for the measurement of motivational aspects of sexual behavior, specifically measuring attractiveness (semi open-field test), proceptive behavior (acts which increase sexual activity in the partner, thus showing in animals the urge to engage in sexual behavior) and sexual reward (measured in a Y-maze).

Earlier results of tests in which proceptive behavior, attractiveness and receptivity in female rats were studied indicated that the attractiveness of testosterone (TP-) and oestrogen + progesterone (EB-P)-treated females was significantly higher than that of EB- or OIL-treated females. These first two groups were also more proceptive towards a male in the Y-maze apparatus. The same behavioral measures were studied with respect to neonatally castrated males, androgenized females, normal males, and females treated in adulthood with EB, TP or OIL. No differences were detected in attractiveness of any of these groups to females. None of the groups showed any consistent preference in the Y-maze for sexually active males or females. Definite influences of treatment with gonadal hormones in adulthood could be established only with respect to masculine sexual activity, with TP-treated animals invariably being more active than EB-, and EB- more active than OIL-treated animals. No differences could be established on the basis of perinatal hormonal manipulation. These results, together with those of earlier experiments showing frequent masculine sexual responses in females born in all female litters, indicate that mounting responses are a general behavioral characteristic of both males and females, not dependent upon perinatal exposure to testosterone.

The project on sexual motivation of rats was continued, with the support from a research grant from ZWO. In collaboration with Dr. B. Meyerson (University of Uppsala) attractiveness of neonatally castrated males and neonatally androgenized females was studied in a semi open-field situation. In this test the orientation of an animal towards an experimental animal is measured, thus giving an indication of its attractiveness. Intact male rats older than 50 days oriented themselves preferentially towards neonatally castrated male rats of the same age, as compared to sham-operated controls, and did not prefer oestrus females to androgenized females. Interpretation of the data on motivation during sexual interaction requires a more sophisticated analysis of the behavioral repertoire (especially proceptive and general orienting behaviors). Various procedures were tried in an attempt to create an optimum test situation. One of these consisted of strap-
ping the male into a harness, thereby restricting its range, and giving the female maximum freedom of movement and choice of intimate contact.

As in most mammalian species, male rats are generally assumed to show more intra-specific fighting than do females. This phenomenon is usually associated with the 'organizing' and 'activating' effects of male gonadal hormones. This hypothesis was tested in males and females of the S3 rat strain: experimental procedures consisted of gonadectomizing the animals at least 5 weeks prior to experimentation, and then treating them with male or female gonadal hormones. Following a 24-hour isolation period, pairs are tested for aggressive interactions during a given period. The hormonal status of both animals is varied systematically, thus enabling an analysis of activating and provoking (i.e., mediated via the opponent) effects of the compounds investigated. Oestrogen and testosterone were equally effective 'activators' in males, but only testosterone was effective as an activating factor in females. Since oestrogen may have stimulated feminine sexual responses (with inhibition of aggression as a secondary effect) a subsequent experiment investigated the effects of oestrogen combined with dihydrotestosterone, a hormonal combination which suppresses feminine sexual behavior. Females injected with these two hormones indeed showed high levels of aggression, in fact equal to the males level. Dihydrotestosterone alone activated aggression in males, but was less effective in females.

These results indicating a central effect of dihydrotestosterone, are not in agreement with earlier studies (which were interpreted on the basis of exclusively peripheral effects of this hormone). In those studies, activating effects of testosterone were thought to result from oestrogenic effects upon the brain, together with peripheral effects of dihydrotestosterone (aromatization hypothesis). Further experiments along this line, designed (in collaboration with Dr. N. Bowden, University of Swansea, UK) to specifically test the aromatization hypothesis with respect to aggression in male and female rats, are in progress.

THEME 2. AVERSIVELY MOTIVATED LEARNING AND EMOTIONALITY IN RATS

Last year, the investigations of sexual dimorphism in the reactions to novel and aversive stimuli in rats (and its modification by gonadal hormones) concentrated on analysis of results from an experiment on effects of the neonatal presence or absence of gonadal hormones upon several behaviors in later life. Furthermore, the data from an experiment on the effects of agonistic experience on subsequent behavior in TP- and OIL-treated female and male rats were analyzed. The results of these experiments, along with previous data, were compiled in a thesis (Van Oyen, Amsterdam, 1981). In addition, the importance of sexual dimorphism in pituitary-adrenal function for the sex difference in the reactions to novel and aversive stimuli was investigated in a series of experiments in which male and female rats were treated with either Dexamethasone or saline, and subsequently tested on open field, active and passive avoidance behavior. With the supervisory cooperation of Dr. Boer (research team III) and Dr. De Kloet (RMI, University of Utrecht) a radioimmunoassay for the measurement of corticosterone levels was started.

Analysis of the influence of gonadal hormones upon reactions to novel and aversive stimuli revealed that sex differences in open-field behavior (ambulation, rearing, defecation) can be changed by neonatal hormonal manipulations. Neonatally unoperated males, as well as females that were treated with testosterone on the day of birth, showed lower levels of ambulatory activity, less rearing and more defecation in adulthood than did untreated females or neonatally castrated males. In contrast, the presence or absence of testosterone in adulthood hardly affected open-field behavior, but was a critical variable in the manifestation of sex differences in passive avoidance. Thus, no sex differences in passive avoidance were observed in animals gonadectomized in either adulthood or at prepuberal age unless they were subsequently treated with testosterone. However, the
effects of testosterone depend upon the sex of the animal, another finding which points strongly to the importance of gonadal hormonal conditions during early development.

Similarities with the above mentioned testosterone (activation) experiments, showing sex differences in passive avoidance behavior, were found in an experiment dealing with the consequences of being in an aggressive encounter. Whereas, in confrontations with more aggressive opponents, TP-treated males showed stronger inhibition of both aggression and approach responses, TP-treated females showed less inhibition than OIL-treated animals. Similar sex-dependent hormone effects also became apparent in confrontations between animals that had previously won or lost in an aggressive encounter. These results support our hypothesis that sex differences in reactions to aversive stimuli are physiologically related to sex differences in reactions to agonistic experiences.

The consistent presence of behavioral differences between male and female rats, as concerns their reactions to novel and aversive stimuli, suggests the existence of a sexually dimorphic neural or neuroendocrine system that is responsible for these differences. The pituitary-adrenal system was considered a likely candidate because of its known sexual dimorphism (higher levels of ACTH and corticosterone in females) and its involvement in these very same behavioral reactions. A series of experiments, however, showed that administration of dexamethasone, in a dose that effectively blocked pituitary-adrenal activity, failed to affect the sex differences in the tested behavioral reactions. It did, however, provide some indications that similarity during passive avoidance behavior between the hormonal state of the animal on the learning and retention trials was a critical variable in the performance of male but not female rats. The phenomenon of state-dependent learning will be further investigated in cooperation with W. Koek (University of Utrecht).

THEME 3. THE NEURAL SUBSTRATE UNDERLYING AGGRESSIVE AND SEXUAL BEHAVIOR

A. Function and development of the prefrontal cortex (pfc)

Following experiments in which the behavioral consequences of thermal pfc lesions were observed a new approach was followed to study the functions of the pfc (defined as the cortical projection area of the nucleus dorsomedialis thalami). Studies using terminal degeneration techniques have demonstrated a spatial separation of the rat pfc into two sub-areas, medial and orbitofrontal. In the last few years it has become apparent that the dopaminergic system projects to precisely these two regions in the frontal lobe. Since various studies have provided evidence that dopamine is important for the functioning of the pfc, we have directed one of our research plans towards answering the following two questions:

1. Is the rich innervation of the pfc by dopaminergic fibers, as witnessed in qualitative fluorescence studies, accompanied by correspondingly high levels of endogenous dopamine?
2. Can the behavioral deficits resulting from thermal lesioning of the orbitofrontal pfc (i.e., increased aggressiveness in a social-agonistic context, increased locomotor activity in an open-field) be mimicked when the manipulation is restricted to the depletion of dopamine in the orbitofrontal pfc?

This research was performed in collaboration with Dr. J. van der Gugten (University of Utrecht), where radiometric methods are available for determining concentrations of DA and NA in minute samples of brain tissue.

Ad 1. This question could be answered affirmatively. The analysis of brain tissue punched in the orbitofrontal pfc, and medial pfc, and in the dorsolateral frontal cortex demonstrated a heterogeneous distribution of dopamine within the frontal lobe. Endogenous dopamine levels in the two pfc sub-areas exceeded that
of the (non-pre)frontal region by a factor 3-4. In contrast, the distribution of noradrenaline over these three cortical regions is quite homegenous.

An unexpected outcome of these measurements was the lateralization of dopamine levels in the medial pfc, with the left hemisphere exceeding the right by ca. 30%. Ad 2. In order to answer the second question, the neurotoxin 6-OHDA was administered locally into the orbitofrontal pfc, while protecting the noradrenergic neurons by injecting dimethylpropamin (i.p.) about half an hour earlier.

Radiometric analysis following the behavioral tests showed that endogenous dopamine levels had been reduced to ca. 30% of the control level. The reduction in noradrenaline levels was much less (80-85% of control). Despite the satisfactory biochemical outcome, neither locomotor activity nor agonistic behavior appeared to be influenced by the severe reduction in dopamine content. Unless the residual 30% is sufficient for 'normal' functioning, we must conclude (pending replication and extension of the behavioral test results) that cortical dopamine is not involved in behavioral regulation.

B. Perinatal androgens and sexual dimorphism of the brain

The establishment of sexual dimorphism by the action of androgens on the developing brain was investigated using graded doses of testosterone (newborn female rats received 0, 2.5, 5 or 10 μg testosterone propionate: TP). It proved possible to fractionate the response obtained, thus creating a range of sexual aberrations. The mean age and weight at vaginal opening were the same in all groups but perforation was incomplete with the larger doses. At 7 weeks, cornified smears were seen daily (continuous vaginal oestrus) in 0, 33, 72 and 92% of animals treated with increasing doses of TP. All females with regular oestrus cycles showed the full range of female sexual behavior only on the day of vaginal oestrus. In contrast, sexual receptivity in females with irregular cycles occurred on consecutive days, irrespective of the vaginal smear. The level of receptivity was low, however, and at no time did these females show proceptive, i.e., soliciting, behavior towards the male partner (on the contrary, they easily became aggressive). When confronted with stimulus oestrus females, androgenized females frequently mounted, in contrast with normal females, but this male-like behavior was seen only in individuals which had previously shown female responses to male partners. Androgenized females thus differed from males in that stimulation of mounting was not associated with suppression of lordosis responses under appropriate circumstances. At ovariectomy, all androgenized females (except four cases of the lowest dose of TP) had anovulatory ovaries. A single dose of oestrogen and progesterone, followed by chronic testosterone treatment, evoked the same sexual responses in given individuals as had been shown before ovariectomy. The responses of androgenized Wistar females to the more aggressive S3 females in agonistic encounter tests will be evaluated to ascertain whether or not also the sexually dimorphic responses to being defeated are programmed by perinatal endocrine influences.

BEHAVIORAL EXPERIMENTS IN COLLABORATION WITH OTHER RESEARCH TEAMS

In collaboration with research team I experiments were carried out to see whether or not deleterious effects of pre-weaning undernourishment can be reversed by post-weaning environmental enrichment. Sex differences in response to undernutrition and/or environmental stimulation were also investigated. Severe undernourishment during suckling, followed by several months of refeeding, had no effect in behavioral tests concerned with reactions to novel or aversive stimuli, but, in tests for sexual behavior, males that had been malnourished during infancy were more active than well-fed controls. Differential post-weaning environments produced corresponding differences in all behavioral measures,
irrespective of the previous feeding conditions. Enriched animals were more active and exploratory, and less fearful, than were those which had been housed in standard cages. Females were more active and exploratory than males. Nutritional deprivation resulted in a permanent deficit in body weight, accompanied by a proportionally smaller deficit in brain weight. Enrichment increased brain weight in females only.

Further experiments were aimed at investigating the interaction between pre-weaning handling and post-weaning housing. Male and female rat pups were handled from birth to 30 days and then placed in groups of 10 in an enriched cage or in pairs or alone in a small cage. The handling procedure seemed to have been stressful since it led to increased adrenal weights at weaning. One of the most striking effects was on sexual maturation of females; vaginal opening was accelerated by handling, irrespective of housing conditions. Moreover, isolation rearing advanced, while enrichment rearing retarded vaginal opening (in comparison with paired controls) in both handled and unhandled animals. Handled animals showed more ambulation and less defecation in the open-field test, and exhibited more effective masculine sexual behavior. In general, handling accentuated sex differences, and enhanced sensitivity to differential rearing, while at the same time decreasing individual differences within a given treatment group.

Together with research team II, the behavioral consequences in adulthood of chronic deprivation of active sleep, by means of pharmacological agents injected during post-natal development, were further investigated (see research team II).

A pilot study to investigate sex differences in diurnal sleep patterns in rats was undertaken in cooperation with research team II. Adult male and female rats were gonadectomized, and electrodes were implanted in the occipital cortex together with an EMG electrode in the neck muscle. Sleep patterns were recorded on four subsequent days. On the second (baseline) day no sex differences were found. However, oestrogen injected on the third day drastically suppressed active sleep in males, but not in females. This surprising preliminary outcome, in contrast to literature data, but so far based on only a few animals, will be further tested using larger groups.

V. Mathematical and computational aspects of neurobiology

The primary responsibilities of this research team consist in (1) the management of the institute’s data-processing equipment and procedures, and (2) the development of new experimental support methods, including systems design, analysis and computer programming. In addition, the research team has a scientific task: theoretical aspects of the outgrowth of individual neurons, and network connectivity in relation to bioelectrical activity are elaborated in cooperation with research teams I and II.

INSTRUMENTATION

Since April the institute possesses a Digital Equipment Corp. VAX 11/780 with 512 Kb of memory, 67 Mb disk storage, a plotting line printer and a high-quality printer, a tape-drive, and also a Laboratory Peripheral Accelerator sub-system with A/D conversion inputs and two parallel digital I/O interfaces. A number of interactive terminals are connected to this computer. The second system is a Perkin Elmer (Interdata) model 70 with 64 Kb of core memory, printer, card reader, papertape reader, console, 10 Mb disk drive, magnetic tape transport, plotter and several terminals, dedicated to experiments. The real-time, on-line experiments are still connected to this system. The third computer, an IBM 1130 with 16 K words, card and papertape equip-
ment, disks, printer and graphic console has been used exclusively for statistical computations with earlier developed software packages.

CONSEQUENCES OF THE INTRODUCTION OF THE VAX 11/780

Much time had to be spent in becoming familiar with the new computer system. Several courses about programming features, system management and Operating Systems Internals were passed, the extensive documentation had to be worked through, and a system management policy had to be established. Furthermore, word processing was still to be introduced, a start was made to get the Laboratory Peripheral Accelerator operational, and procedures and utilities needed to be written. The development of a user library with matrix operations, general mathematical sub-routines, graphic and plot software and (interactive) free format input modules (terminal or card image from tape) has been initiated. The same applies to the development of a tape-routine library, necessary for reading data tapes from the Interdata on the VAX in a way that corresponds optimally to the approach used so far (non-labelled binary tapes, with no record management formatting).

Several conversions from the Interdata and the IBM were started, while much time was also required to become familiar with the broad spectrum of possible (hardware) trouble situations. The essential knowledge was mainly acquired by assisting the DEC support engineers during their actions. The conclusion from the experience obtained is that adequate system management is almost a full time job.

PROJECTS AND CONVERSIONS

1. In collaboration with research team I: Computer-controlled dendrite microscopy.

   a) Analysis of orientation of dendritic fields
   The program ACRON, which computes a matrix neuron from a single group of nerve cells, has been changed to give a more readable output and to compute the total projected surface. The program COMPDENS, which computes the matrix neurons of two groups of nerve cells (including their difference in orientation, as introduced by Dr. Ruiz-Marcos (Cajal Institute, Madrid), has been considerably modified so far to make possible statistical inferences at any desirable significance level.

   b) Metrical analysis
   The statistical characterization of the population of metrical parameters by means of Kolmogorov-Smirnov one-sample test procedures was completed on the Interdata and converted to the VAX. Populations can now be fitted to normal, semi-normal, and log-normal distributions: moreover, an iterative fitting procedure for gamma-distributions was realized. The metrical framework program LMVERW was partially converted to the VAX, as far as the analysis and statistics sections were concerned. Work on graphic representation of cells and data-base manipulations will be carried out soon. To enable the data-tapes to be read on the VAX, the Interdata version of this program had to be adapted to produce a record format suitable for the DIGITAL machine.

   c) Topological analysis of branching patterns (MICTOP, MANTOP)
   The topological analysis of branching patterns, and the implementation into computer programs have reached an operational state. Theoretical work has concerned: the ordering of branching pattern, and their identification by unique numbers and/or several codes; computation of the exact probabilities of occurrence of these patterns under two extreme growth hypotheses (viz., terminal and segmental growth); the construction of frequency distributions and procedures for appropriately lumping frequency classes; and the investigation of
the most appropriate statistical tests. The software now enables the user to easily handle the observed branching patterns (e.g., of dendritic trees from neuronal tissue) and to extract the essential information concerning topological characteristics from the empirical observations. Hypothesis testing in connection with the two growth models can be carried out routinely. For these tests, the Kolmogorov goodness-of-fit test for discrete distributions is used, since it utilizes the most information from the probability distributions, and imposes the least restrictions on the required number of classes and observations per class (this work has been performed in collaboration with Dr. R.W.H. Verwer of research team I).

2. In collaboration with research team II
a) Automatic sleep-detector project
An elementary program for statistical evaluation of sleep-detector output was developed. Acquisition can be done from either paper-tape or disk, while a number of periods of variable length can be sequentially analyzed, independent of the length of paper-tape used. A truth-table performs the decision and classification, sleep-stage counting, and sleep-stage period computations. Consecutively, basal statistics such as distribution-moment calculations and histogram representation are given.

b) Development of bioelectric activity in neuronal networks
Some work has been done on the distributed model of a neuronal network. Refinements with respect to the shapes of the post-synaptic potentials have been incorporated, and the modes of presentation of the electrical behavior of the network during simulation of physiological activity have been extended. Spike trains from single units can be monitored in this model, and interspike interval histograms calculated.

c) Analysis of spike trains and bursts
Support has been given to the development of a program package by research team II in which serial and autocorrelogram, interval histograms, interval spectrum and spectrum of counts, Markov-order estimation (according to Nakahama) dependency estimations, tests for stationarity and Poisson processes have been implemented, so as to be able to represent the results graphically. Furthermore, a program is currently being developed for giving statistical characterization of neuronal bursting patterns, for which program the pattern recognition paradigm is in the process of being elaborated.

3. In collaboration with research team III
a) Radioimmunoassays
The RIA-analysis program was converted to the VAX, but since paper-tape can not longer be the input medium, a suitable replacement medium had to be found. Because direct registration on the VAX without a backup medium is undesirable, the best solution appeared to be a local registration medium that can be interrogated like a remote terminal. Hence, two floppy disks were installed, and reading procedures are being developed.

b) Correlation analysis on clinical data pertaining to birth
To be able to draw conclusions with respect to the relationship between fetal brain development and peptide levels in the fetal and maternal compartments, parameters such as gestation length, birth weight, head circumference, placental weight, and data on labor were scored, together with the measured peptide levels from different sources (maternal blood, amniotic fluid and umbilical cord blood) by research team III in collaboration with obstetric clinics. Thereafter, a program was developed for creating a data-base from these items. Records can be inserted, mutated and deleted. The fundamentals of the program
are as follows: from each record which meets certain criteria (selection keys), 1-5 items can be extracted so as to form groups for unrelated or related sample analysis. The related sample analysis (restricted at this stage to 2 groups) now yields correlation coefficients and least squares polynomial fitting, including graphic output. Correlation analysis can also be carried out automatically between all applicable parameters. The method has revealed a number of unexpec ted relationships between, among other things, peptide levels in the different compartments. Partial correlation and factor analysis will be introduced next, in order to obtain an irreducible parameter space.

c) Correlation analysis on clinical data pertaining to aging of the human brain
A method has been developed for a number of parameters which are assumed to reflect the degree of aging of the human brain. Parameters such as brain weight and surface area of vasopressinergic or oxytocinergic cells in both the supra-optic (SON) and the paraventricular (PVN) nucleus were registered, together with other patient-related data. The problem is more heterogeneous than the one mentioned above, since the data-base contains separate record entries for SON and PVN measurements. Exploitation of this information system is in a pre- liminary stage.

4. In collaboration with research team IV

a) Skinner-box package
Both the acquisition and the analysis program were adapted to enable manipulation with the Interdata tape unit. Furthermore, the analysis package was extended with a section of code which makes possible cumulative box matrices from individual animals. This code also uses virtual storage in order to process the data from an unlimited number of animals (at the cost of execution time). Basic statistical parameters from cumulative event data of both rows and columns are given. In the future, data which have been reduced using this method will serve as input for more advanced statistical analyses and decision table routines.

b) Analysis of keyboard-scored behavior (ABEDA)
The analysis part of the program has been converted to the VAX, while the acquisition part is still running on the Interdata. The program has been extended with routines for cycle analysis, in which the test is subdivided into parts defined by the occurrence of predetermined "initial" and "final" behavioral items. Additionally, routines have been written for building multidimensional data arrays, and for inserting or extracting lower dimensional sub-arrays from it. This offers a great flexibility in handling all data from experiments, and in selecting appropriate groups for further statistical testing. The statistical package within ABEDA has been reorganised and parametrized in such a way that particular tests can easily be selected during analysis-time, and new tests incorporated. A special programme (MANSTAT) has been written, which enables the use of these statistical tests on data entered via the terminal.

STATISTICS
A large number of statistical programs were converted from the Interdata to the VAX. Examples of the parametric statistics currently available are: Student-t test, analysis of variance for 2 factors and unequal group size, data-screening and distribution fitting procedures. The available non-parametric tests are: Mann-Whitney, Kruskal-Wallis one-way analysis of variance, and the k-sample Chi-square method. Input is accepted from either terminal or tape (card image format). In addition, the least squares polynomial fit-and-plot program PLOTXY was converted and adapted for reading data produced by other project software, e.g., the correlation analysis system described above (see research team III).
Mechanics workshop

An enumeration of the major projects constructed by this department is outlined below. Electronic equipment for these projects was developed by the institute's electronics workshop.

For research team I
The semi-automatic measuring system for neuronal processes with a refined scanning system, as described in the 1980 Progress Report, has been completed: measuring accuracy tests and operation efficacy tests are now being carried out.

For research team II
a. Two motorized rocking apparatuses were built for the study of chronic REM sleep deprivation in infant rats. A large box, containing the nursing mother rat and her pups, can be rocked continuously: the angle of excursion is variable from 10 to a maximum of 30°, while the period of a full rotation is variable from 1 to 20 seconds.

b. A rewind apparatus was designed and built in order to make possible the reuse of the carbon paper rolls which form the basis of the writing system employed in 'Swarzer' polygraph machines.

c. Two Faraday cages were constructed, equipped with a built-in food and water supply, for making long-term registrations of sleep and wakefulness patterns in rats.

d. An 8-channel mercury-filled rotating contact was constructed in order to enable multiple electrophysiological recordings to be made simultaneously in unrestrained animals.

e. A microdive apparatus was designed and built for use with a DAVID KOPF stereotaxic animal holder. The driving system is a motorized one, and allows for descending as well as ascending steps of less than a micron. For further details, see below (Electronics Workshop).

For research team IV
Automatic semi-open field cages: Attractivity of incentive animals, and sexual preferences can be measured automatically in two teflon arenas of 80 by 80 cm square, surrounded by walls of 35 cm in height, and having two openings located opposite each or. The weight of the animals forces down a plate opening directly across from two experimental steel boxes, containing the incentive animals. This closes an electric circuit, by means of which the frequency of visits is automatically counted, and their respective durations recorded. The recording time itself can be pre-set at variable lengths of time, up to 99 minutes.

Miscellaneous
In addition to the routine repair and maintenance work, there was construction of such accessories as:

(a) chassis and front panels for apparatus being constructed by the electronics workshop;

(b) production of storage trays made of syntic fabrics, and used for storing small tubes;

(c) modifications on a wide variety of equipment (microscopes, centrifuges, gamma-counters, micromanipulators, microscissors, micropipettes, microtomes, microdrivers and photographic equipment).

General technical service
As in previous years, this department has devoted most of its time in assisting in the design and manufacture of new laboratory plant- and equipment unattainable as yet at laboratory supply stores (e.g., aggression cages) and/or in the adaptation of existing laboratory equipment to meet the researchers' requirements (e.g., skinner boxes).
While awaiting the transfer from our temporary buildings to our future 'permanent' quarters (which are nearing completion) the scope of this department's duties has also included the repair and maintenance of: 1. the buildings; 2. the electricity, heating, gas and water supplies, including water drainage and sewage; 3. the fixed installations, such as air conditioning, refrigeration and cooling units, the auxiliary generator, the integrated alarm system, various compressors, the toxic fumes disposal unit, the isotope laboratory, and the radioactive material storage cupboard. This part of their duties has become increasingly extensive and time-consuming as a result of the necessity for finding short-term, less expensive solutions to breakdowns, leakages, and a multitude of other exigencies arising from failing foundations. In addition to the control administration of alcohol supplies, this department has taken charge of the gas cylinders required by various research teams at the institute laboratories.

**Electronics workshop**

A description of some of the larger projects developed by this department is outlined below. The mechanical parts of all apparatus were built by the institute's mechanical workshop (see above).

**For research team II**

EEG amplifier: A variety of biological data in connection with REM sleep experiments are now being recorded by a prototype solid-state EEG amplifier which has been developed by this department. This has enabled us to start with the revision of 13 of the old 'Swarzer' vacuum-tube EEG amplifiers which have been in use up till now.

DC power supply: Self-contained independent power supplies have been constructed to replace the batteries in several 'Grass' amplifiers; it is now possible, therefore, to continuously carry out 24-hour sleep/waking registration recordings without any interruptions for having to recharge batteries, or for having to change amplifiers.

Syringe microdrive: In experiments by which neurotoxins or similar compounds are injected stereotaxically into a particular area of the brain, it is often required that a microliter quantity be administered gradually from a micro-syringe. This has proved necessary in experiments in which kainic acid or 6-hydroxydopamine was administered to the prefrontal cortex, in order to selectively destroy cell bodies or cause a depletion of dopamine. Such a syringe microdrive has been designed and constructed in close cooperation with the mechanics workshop. It consists of a small electromotor which, at a calibrated and adjustable speed, pushes down the plunger of a micro-syringe and which thus allows the administration of a predetermined volume, within a predetermined time-span. The whole device is small enough to be mounted on the arm of a stereotaxic apparatus.

**For research team IV**

Keyboards for practising behavioral observing: Two keyboards were constructed as accessories to a device currently in use for making behavioral observations. By pressing the keys, sound signals of a different pitch for each of the 19 keys are sent to stereophonic earphones, the right-ear channel of which connected to one of the boards, the left-ear channel to the other. This setup enables two observers to practise behavioral observing techniques, and to simultaneously monitor recordings on both boards (thereby obtaining greater accuracy and reproducibility in scoring).
Guest workers and work-visits abroad

Baker, R.E. at Department of Biological Sciences, Purdue Univ., Indiana (USA), July 9-11.

Boer, G.J. at Dr. D.M. Gash, Dept. of Anatomy, Medical Center, Univ. of Rochester, N.Y. (USA), September 8-18; at Dr. M. Rietort, Lab. de Physiologie du Développement, Collège de France, Paris (France), November 30-December 1; and at Dr. D. Greiber, Patentwesen, ENKA AG, Wuppertal (W-Germany), December 17.

Bowden, N. (guest worker from the Univ. of Swansea, UK) for research on the 'aromatization hypothesis' in aggression in rats (research team IV).

De Jonge, F. at Dept. of Medical Pharmacology, Univ. of Uppsala (Sweden), September 1980-March 1981.

Haldar-Misra, C. (guest worker from the Varanase Univ., India) for the ultrastructural study of the pineal gland of mammals in organ culture, September 1980-December 1981 (research team III).

Jirikowski, G. (guest worker from the Univ. of Ulm, FRG) to learn various immunocytochemical procedures, October 12-23 (research team III).

Mountford, L. (guest worker from the Univ. of Oxford, UK) to learn the radioimmunoassay for oxytocin and vasopressin, October 5-12 (research team III).

Ocal, T. (guest worker from the Univ. of Istanbul, Turkey) for the ultrastructural study in the pineal gland in the mouse 'eyeless', May 1980-May 1981 (research team III).

Pévet, P. at Dr. D.C. Klein, Lab. of Developmental Neurobiology, National Institute of Child Health and Human Development, Bethesda (USA), January 22; Dr. Ch. Ralph, Dept. of Zoology and Entomology, Colorado State Univ. (USA), January 23-24; Drs. M.K. Vaughan and R.J. Reiter, Dept. of Anatomy, Univ. of Texas at San Antonio (USA), January 26-27; Dr. B. Benson, Dept. of Anatomy, Univ. of Arizona (USA), January 28-29; Dr. B. Vivien-Roels, Lab. de Zoologie et Embryologie, Univ. de Strasbourg (France), October 4-5; and Dr. C. Chariñ, Lab. de Physiologie, Univ. de Lyon (France), November 12-13.

Swaab, D.F. at Dr. M. Castel, Dept. of Zoology, Hebrew Univ. of Jerusalem (Israel), August 12; and Dr. G. Schmitt, Dépt. de Physiologie, Univ. de Strasbourg (France), November 2-4.

Swanson, H.H. at Dept. of Child Health, Univ. of Manchester (UK), February 28; MRC Neuroendocrinology Unit, Newcastle-upon-Tyne (UK), February 29; Dr. B. Payman, Dept. of Dermatology, Univ. of Newcastle (UK), February 29; Dr. A.P. Payne, Dept. of Anatomy, Univ. of Glasgow (UK), February 29; Dept. of Anatomy, Univ. of Ottawa (Canada), March 13; Dept. of Anatomy, McGill Univ. (Canada), March 16; Dr. T.C. Anand Kumar, Experimental Biology Unit, AI1-India Institute of Medical Science, New Delhi (India), July 10-17; Prof. C.J. Dominic, Dept. of Zoology, Benares Hindu Univ., Varanasi (India), July 27-29; and Dr. A.R. Sheth, Institute for Research on Reproduction, Bombay (India), August 11-12.

Parnavelas, J.G. (guest worker from the Univ. of Texas at Dallas, USA) for the morphological study of neuronal development in the visual cortex of rats, September 2-4 (research team I).
Publications


Balemans, M.G.M., P. Pévet, W.C. Legerstee and E. Nevo - Melatonin and 5-methoxytryptophol synthesis in the pineal, the retina and the Harderian gland of the mole rat (Spalax ehrenbergi) and in the pineal of the mouse 'eyeless'. J. Neural Transm. 49, 247-255.


De Vries, G.J., R.M. Buijs and D.F. Swaab - Ontogeny of the vasopressinergic neurons of the suprachiasmatic nucleus and their extrahypothalamic projections in the rat brain - presence of a sex difference in the lateral septum. Brain Res. 218, 67-78.


Van Oyen, H.G., S.M. van der Zwan, N.E. van de Poll and H.L. Walg - Punishment of food rewarded lever holding in male and female rats. Physiol Behav. 26, 1037-1040.


Vivien-Roels, B., P. Pévet, J.M. Guerne, F.C. Holder, A. Meiniel, J. Dogterom and R.M. Buijs - On the presence of arginine vasotocin (AVT) in the pineal organ


Abstracts (incl. posters)


Bour, H.L. and M.A. Corner - State related activity of pontine reticular neurons in developing rats. Proc. 22nd Dutch Federation Meeting, Utrecht, 57.


Corner, M.A. and M. Mirmiran - Strategies for studying the role of active sleep in brain development, and some results from pharmacological deprivation experiments. Sleep Res. 9, 100.


Habets, A. - Chemical transmission and aspects of normal development in tissue culture. 12th Internat. Summer School of Brain Res. 'Chemical Transmission in the Brain', Amsterdam, August 30.


Papers read (seminars, etc.)
See also 'abstracts' and 'teaching' sections.


Boer, G.J. - Vasopressin release from Accurel in vitro and in vivo, AKZO Research Center, Arnhem, June 2; Neuropeptides and brain development: the Brattleboro rat as a model. Dept. of Anatomy, Univ. of Rochester, New York City (USA), September 15.

Bour, H.L. - Activiteit van neuronen in de pontine reticulaire formatie tijdens de slaap-waak cyclus, na laesies in de contralaterale hersenstam. FUNGO workgroup 'Development and Aging of the Nervous System and Behavior', Amsterdam, November 6.


De Bruin, J.P.C. - Prefrontale corticale lesies en agressie. BION research team 'Ethology', Utrecht, March 17; De rol van de praefrontale cortex in sociaal gedrag; in hoeverre is dopamine daarbij betrokken? Dept. of Pharmacol., Free Univ., Amsterdam, April 6; Veranderingen in agonistisch gedrag na letseling van de orbitofrontale cortex van de rat. FUNGO Workgroup, 'Development and Aging of the Nervous System and Behavior', Amsterdam, November 6.


Haldar-Misra, C. - Pineal and reproduction. WHO Workshop, 'Endocrine Infertility', Inst. for Res. in Reprod., Bombay (India), December 7-12.

Hofman, M.A. - Eenfalisatie bij zoogdieren. Verhaert Meeting of Dutch Morphologists, Amsterdam, March 27.

Mirmiran, M. - Sleep patterns in developing rats during exposure to an 'enriched' environment. Dept. of Comp. and Physiol. Psychol., Univ. of Nijmegen, October 9.

Pévet, P. - Peptides in the mammalian pineal gland. Dept. of Anatomy, Univ. of Texas at San Antonio (USA), January 27; Is vasotocin a pineal peptide? Dept. of Anatomy, Univ. of Arizona at Tucson (USA), January 29; Pinea and reproduction. A new approach. 2nd Netherlands Pineal Day, Amsterdam, May 14; La glande pinéale. Structure et innervation. Univ. of Lyon (France), November 13.


Slopsema, J.S. - Locale toediening van 6-OHDA in de orbitofrontal PFC; veranderingen in catecholamine-gehaltes en gedragsconsequenties. Dept. of Psychophysiol. at Univ. of Utrecht, December 2.


TEACHING

a. Students

Best, W. (pharmacy student, Univ. of Amsterdam): 'The influence of castration and testosterone suppletion on the development of a sex difference in the vasopressinergic innervation of the brain' (research team III).

Blits, P. (medical student, Univ. of Amsterdam): Kainic acid lesions of the orbitofrontal cortex of the rat'; 'Evaluation of the effect of test duration in open-field experiments' (research team IV).

Dikkeboom, R. (biology student, Free Univ., Amsterdam): 'Immunoreactivity for GAD and alpha-MSH in the cerebellum of the rat. Comparison of the localisation by means of immuno-electron microscopy on ultrathin cryokit sections and the protein-A gold technique' (research team III, in collaboration with Dr. J.W. Slot, Univ. of Utrecht).

Guldenaar, S.E.F. (biology student, Univ. of Utrecht): 'Exohypothalamic vasopressinergic and oxytocinergic innervation of the human brain (research team III).

Heinsbroek, R. (biology student, Univ. of Amsterdam): 'Dexamethason and the reaction on novel aversive stimulation in females' (research team IV).


Kerkhoven, J. (biology student, Univ. of Amsterdam): 'Immuno-electron microscopy of vasopressin and oxytocin in the rat and human brain' (research team III).
Kleiss, M. (medical student, Univ. of Amsterdam): 'Innervation of spinal cord explants by spinal ganglia in hormone enriched serum-free medium' (research team II).

Kok, T. (biology student, Free Univ., Amsterdam): 'Vasopressin identification in the rat SCN' (research team III).

Koster, A.B. (medical student, Univ. of Amsterdam): 'Nephrogenic diabetes insipidus in mice' (research team III).

Kragten, R. (biology student, Univ. of Amsterdam): 'Effect of vasopressin suppletion on the developing diabetes insipidus Brattleboro rat' (research team III).

Partiman, R. (medical student, Univ. of Utrecht): 'Physiological studies on the involvement of pontine reticular neurons in the generation of active (REM) sleep in infant rats' (research team II); 'Development of vasopressinergic and oxytocinergic neurons in the human brain' (research team III).

Schlüter, N. (biology student, Free Univ., Amsterdam): 'Fetal hypothalamic transplantations in the third ventricle of Brattleboro rats during early postnatal development' (research team III).

Schwaggermann, H. (medical student, Univ. of Amsterdam): 'REM-sleep deprivation: instrumental procedures in developing rats' (research team II).

Van den Dungen, H. (biology student, Univ. of Utrecht): 'Immuno-cytochemical localization of vasotocin and isotocin in the brain of the rainbow trout, and their demonstration in immersion-fixed rat and human brains' (research team III).

Van Dongen, A.M.J. (biology student, Univ. of Utrecht): 'Electrophysiological aspects of neuronal maturation in tissue cultures of fetal rat cerebral cortex' (research team II).

Van der Tocht, C. (medical student, Univ. of Amsterdam): 'Theoretical aspects of development of bioelectrical activity in neuronal tissue cultures' (research team V).

Voorn, P. (biology student, Free Univ., Amsterdam): 'Vasopressin and oxytocin synapses in the medulla oblongata' (research team III).

b. Lectures and Theses

Boer, G.J. - Lectures on 'Hersenfuncties en de betekenis van neuropeptiden' en 'Toepassingsmogelijkheden en gevaren van neuropeptiden'. Post-academic course 'Nieuwe ontwikkelingen in de neuroendocrinologie', Free Univ., Amsterdam, October 3.

Corner, M.A. - Integrated lectures 'The physiological basis of higher nervous processes', Univ. of Amsterdam, Dept. of Clin. Psychol., November and December.


Pool, C.W. - Lecture on 'Specificity in immunocytochemistry', Boerhaave course for post-graduate medical teaching, 'Immunoperoxidase Techniques', Leyden, June 3.


(Switzerland), January 6; Integrated lectures on neuroendocrine aspects of the menstrual cycle, Univ. of Amsterdam, Med. School, January 12 and February 2; Post-academic course on Biological Psychiatry, 'Aging and dementia', Univ. of Amsterdam, Med. School, January 19; Integrated lecture on 'Development of the brain', Univ. of Amsterdam, Med. School, January 29; Co-referent for PhD thesis of M.J. van der Horn, Univ. of Amsterdam, February 12; Promotor for PhD thesis of H.G. van Oyen, Univ. of Utrecht, June 5; Committee member for PhD thesis of M.F. Schutte, Univ. of Amsterdam, July 2; Committee member for PhD thesis of P.W.J. Peters, Univ. of Utrecht, October 13; Refereeravond Verloskunde en Gynaecologie, Wilhelmina Gasthuis, Amsterdam: 'The influence of medicines on fetal brain development', February 23; Lecturer at Univ. of Amsterdam, Med. School: 'Some disturbances of brain development', October 15, and 'Formatio reticularis and monoaminergic systems', December 10; Practical course 'Microscopic neurohistology of the central nervous system', Univ. of Amsterdam, Med. School (together with H.B.M. Uylings), October 20–30.

Swanson, H.H. - Lectures on 'The sociobiology of the gerbil', course in animal behavior, Univ. of Amsterdam, Dept. of Biology, March 18 and November 18; External examiner for PhD thesis of M. Ostermeyer, Queen's Univ., Belfast (UK), September 21.

Uylings, H.B.M., together with D.F. Swaab - Practical course 'Microscopic neurohistology of the central nervous system', Univ. of Amsterdam, Med. School, October 20–30.

Van de Poll, N.E. - Integrated lectures 'Hormones and reproductive behavior', Univ. of Utrecht, Dept. of Physiol. Psychol., April-May; Committee member for PhD thesis of H.G. van Oyen, Univ. of Utrecht, June 5.

Van Leeuwen, F.W. - Lecture on 'Immuno-electron microscopy', at Boerhaave course for post-graduate medical teaching, 'Immunoperoxidase Techniques', Leyden, June 3.

c. 12th International Summer School of Brain Research: 'Chemical Transmission in the Brain'

The 12th International Summer School for Brain Research was held from August 31 to September 4 in the building of the Royal Netherlands Academy of Arts and Sciences in Amsterdam. This post-graduate course was organized by R.M. Buijs, P. Pévet and D.F. Swaab around the theme 'Chemical Transmission in the Brain on the Role of Amines, Amino-acids and Peptides', not so much to answer to the question of whether or not one ought to call a substance a neurohormone, neurotransmitter, or neuromodulator, but rather to gain a better insight into the similarities and differences among the three types of transmitters, with respect to their distribution, release, action and function. By means of lectures and extensive discussions a start was made, on the basis of a comparison of the many different properties of these substances, towards a better understanding of the questions that we have to ask ourselves about our brains. The proceedings are to be published in the Progress in Brain Research series. The series were presented, in the Academy building, by a teaching staff of 21 internationally celebrated scientists, while two public lectures were held in the 'Lutherse Kerk' (the Aula of the University of Amsterdam). The lecturers were: G.W. Bruijn (Leyden, The Netherlands), R.M. Buijs (Amsterdam, The Netherlands), L.L. Butcher (Los Angeles, USA), V. Chan-Palay (Boston, USA), T.J. Crow (London, UK), A.C. Cuello (Oxford, UK), D. de Wied (Utrecht, The Netherlands), J.J. Dreifuss (Geneva, Switzerland), S.B. Dunnett (Cambridge, UK), W.H. Gispen (Utrecht, The Netherlands), F.A. Henn (Iowa City, USA), O. Hornykiewics (Vienna, Austria), J. Joosse (Amsterdam, The Netherlands), G.W. Kreutzberg (Munich, FRG), F.H. Lopes da Silva (Utrecht, The Netherlands),
A. Mulder (Amsterdam, The Netherlands), J.M. Palacios (Baltimore, USA), P. Schubert (Munich, FRG), D.G. Smyth (London, UK), L. Sokoloff (Bethesda, USA), D.F. Swaab (Amsterdam, The Netherlands), U. Ungerstedt (Stockholm, Sweden), H. van der Loos (Lausanne, Switzerland), and W. Ziegglansberger (Munich, FRG).

The 100 participants not only contributed to the discussions, but also presented their work during four poster sessions. On one morning, the participants visited the Netherlands Institute for Brain Research, where a series of posters and demonstrations had been organized by Dr. J. van Pelt.

This Summer School was supported financially by among others, the C.N. van den Houten Fund, the European Training Programme in Brain and Behaviour Research, and the Dr. Saal van Zwanenburg Foundation, Leyden.

Miscellaneous


Boer, G.J. - project leader 'Vasopressin release from Accural in vitro and in vivo' (in cooperation with, and sponsored by ENKA Research Institute, Obernburg, FRG); supervisor of the radioisotope laboratory of the NIBR; chairman of the organizing committee of the VIIIth Dutch-British Endocrine Meeting, 1982; member of the board of the Dutch Society of Endocrinology; organizer of the NIBR seminar programme (until August); advisor for articles in 'Hersen en Onderzoek' (Dienst Wetenschapsvoorzichtig, Amsterdam).


De Bruin, J.P.C. - project leader in BION workgroup, 'Ethology'; project leader in FUNGO workgroup, 'Development and Aging of the Nervous System and Behavior'; secretary of BION workgroup, 'Ethology'; organizer of the Autumn Conference, BION workgroup, 'Ethology'.

Habets, A.M.M.C. - project leader in FUNGO workgroup, 'Development and Aging of the Nervous System and Behavior'; chairman of the session on 'Action of Neurotransmitters', at the 12th Internat. Summer School of Brain Res., September 2.

Pévet, P. - project leader of FUNGO workgroup, 'Regulatie van de hypofysie functie'; secretary-treasurer of the European Pineal Study Group; organizer of the 2nd Neth. Pineal Day, Amsterdam, May 14; member of the advisory committee for the NATO Advanced Course, 'The Pineal Gland and its Endocrine Role', Erice (Italy), June 21-July 2; editor of EPSG-Newsletter, and 'The Pineal Organ: Photobiology-Biochronometry-Endocrinology'; external referee for project grant application from 'Fonds National Suisse de la Recherche Scientifique'; referee for Cell

Romijn, H.J. - organizer of a workshop, 'Kweken in serum-vrij medium', for the tissue culture workgroup of the Ned. Ver. v. Celbiol., Rijswijk, November 5; photographic contributions to the Handbuch der Mikroskopische Anatomie des Menschen VI/7 'The Pineal Organ'; external referee for a research grant proposal to the National Science Foundation, Washington, D.C. (USA).


Swanson, H.H. - committee member of Eur. Brain and Behav. Soc.; member advisory council Internat. Soc. Psychoneuro-endocrinol.; member of ethics committee of Internat. Soc. for Res. on Aggression; external advisor on project grant in Psychobiology for National Science Foundation, Washington, D.C. (USA); referee for Anim. Behav. (4x), J. Endocrin. (1x), Physiol. Behav. (3x); consultant for 'The Behavioral Brain Sciences'.


Van Leeuwen, F.W. - organizer of a theoretical/practical day for the workshop, 'Immuno–electron microscopy' of the Neth. Soc. for Cell Biol.; organizer of the 2nd EMBO course, 'Immunocytochemistry and its Applications in Brain Research', to be held at the NIBR from May 24-28; referee for J. Neurosci. Meth. (1x).


Van de Poll, N.E. - project leader in FUNGO workgroup, 'Hersen en Gedrag', and in Psychonomy workgroup, 'Vergelijkende en fysiologische Psychologie'; advisor for the Psychonomy project, 'Hormonal regulation of sexual behavior in the male stumptail Macaca'; referee for grant application to the 'Instituut voor Epilepsiebestrijding'; advisor for articles in 'Hersen en Onderzoek' and 'De letter W' (Dienst Wetenschapsvoorlichting, Amsterdam); member of organizing committee of the Eur. ISRA Conference, Strasbourg, September 1982; referee for Behav. Proc. (2x) and J. Endocrin. (1x).

Van Oyen, H.G. - member of the advisory board for the Psychonomy project, 'Genetic analysis of succession discrimination learning'; referee for Peptides (1x).


Walp, H.L. - member of the editorial board of DECUS Holland Bulletin.
Seminars given at the institute
(organization: Dr. G.J. Boer and Dr. R.W.H. Verwer)

January 21 - Dr. J. Mos (Laboratorium voor Farmacologie, Rijksuniversiteit Leiden): In vivo voltametrie: methode om endogene en exogene stoffen in de hersenen te meten.

January 27 - Dr. C.V. Howard (Dept. of Anatomy, Univ. of Liverpool, UK): Neuroanatomical techniques: a parametric method of estimating the numerical density of neurones, with particular reference to the determination of the separate numerical densities of \( \alpha \) - and \( \gamma \)-motoneurones (a double 'tomato salad' problem).

February 18 - Prof. J. Voogd and Dr. H.K.P. Feierabend (Anatomisch Embryologisch Laboratorium, Rijksuniversiteit Leiden): Anatomisch en embryologisch onderzoek naar de longitudinale indeling van het cerebellum.

March 13 - Dr. R. Glaser (Institute of Scientific Information, Philadelphia, USA): Use and misuse of Science Citation Index.

March 25 - Dr. P.W. Nathanielsz (Dept. of Obstetrics and Gynaecology, Univ. of California, Los Angeles, USA): Low-grade uterine muscular activity throughout gestation and its effects on fetal development, sleep state and fetal breathing.

April 8 - Dr. F. Slijper (Afd. Kinderpsychiatrie, Sophia Kinderziekenhuis, Erasmus Universiteit Rotterdam): Psychosociale ontwikkeling van meisjes met het androgenitaal syndroom.


May 20 - Dr. H. Rigter (Centraal Zenuwstelsel-Pharmacologie, Organon BV, Oss): Medisch-biologisch onderzoek van hersenveroudering. Algemene aspecten, en gedragsonderzoek bij ratten.

June 17 - Dr. C. Heyting (Antoni van Leeuwenhoekhuis, Nederlands Kankerinstituut, Amsterdam): Herstel van carcinogeen geïnduceerde DNA schade in ontwikkelende ratteherkans.

August 5 - Dr. R.F. Mervis (Dept. of Pathology, Ohio State Univ., Columbus, USA): Morphological aspects of brain aging and dementia: Influence of dietary choline on dendritic spines and behavior in aging mice.

August 17 - Dr. D. Adams (Dept. of Psychology, Wesleyan Univ., Connecticut, USA): Motivational mechanisms and their neural substrate.

September 21 - Dr. R.J. Reiter (Dept. of Anatomy, Univ. of Texas, San Antonio, USA): The pineal gland: its biochemistry and physiological interaction with the reproductive system.

September 22 - Dr. G. Innocenti (Institut d'Anatomie, Univ. de Lausanne, Switzerland): Inter-hemispheric communication/reshaping of callosal connections.

November 2 - Dr. T.C.A. Kumar (Dept. of Anatomy, All India Institute of Medical Sciences, New Delhi, India): Evaluation of nasal spray steroidal contraception on the reproductive neuroendocrine function in Rhesus monkeys.

December 9 - Dr. J. Hilgers (Antoni van Leeuwenhoekhuis, Nederlands Kankerinstituut, Amsterdam): Voor- en nadelen van de techniek monoclonale antilichamen.