ADVANCES IN SLEEP RESEARCH

Volume I

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Preface

The past 20 years have been extraordinarily productive ones in the area of sleep research. We have accumulated much observational and experimental data and have thereby elaborated many new insights and challenging concepts. However, with this explosive research effort, there is also a need for thoughtful, critical analysis. The introduction of this first volume of "Advances in Sleep Research" is an attempt to supply, in part, a forum for such critical reviews.

The field of sleep research has been a very fruitful and exciting one, to a large extent because of a multi-disciplinary approach. The meetings of the Sleep Society have provided a forum for direct intellectual communication and challenge across disciplines ranging from biochemistry, cellular neurophysiology, pharmacology and general physiology to biological rhythm research, ontogeny and phylogeny. In addition, psychological, neurologic, psychiatric and other clinical medical disciplines have been an active and important aspect of the field. In that same spirit, I have invited critical summaries and reviews across a broad spectrum of research areas from individuals directly involved in their areas of review. The papers in this first volume do not cover the full range of areas of sleep research, however, it is planned to include critical reviews of most of the important areas of sleep in subsequent volumes.

Elliot D. Weitzman, M.D.

November, 1973

ADVANCES IN SLEEP RESEARCH

Volume I

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Advances in Sleep Research, Vol. 1

CHAPTER 1

Chemical Anatomy of Brain Circuits in Relation to Sleep and Wakefulness

PETER J. MORGANE
WARREN C. STERN

I. GENERAL INTRODUCTION

There is little doubt that the dramatic emergence of biochemical neuroanatomy has resulted in a significant merging of pharmacology, physiology, and biochemistry so that a new level of understanding of the organization of the nervous system is being achieved. The study of brain pathways, many of them previously unsuspected, based on the presence of specific chemical substances, presumably transmitters elaborated by these neurons, has opened up new vistas well beyond imagination just a few years ago.

Some questions we propose to discuss in this review, among others, are whether there is approximate congruity between histofluorescence and anatomical boundaries and how brain areas, coded as to their chemical anatomy, relate to nonfluorescing nuclear areas of the brainstem (previously identified by cytoarchitectonic analyses) and to various fiber systems. Terminological problems have been a major stumbling block in this area as many reticular structures go by eponymic names and purely topographical (e.g., latero-dorsal, medio-lateral, etc.) designations. Further, the correspondences, or homologies, of well known brain areas and fiber systems with fluorescing areas have not been worked out with any degree of precision. If chemical morphology is going to help unravel brain relations and functions then we must translate it into a more precise topographic reference, relate chemical systems to other non-chemically identified areas and, perhaps, place its formations into stereotaxic coordinates so that they can be manipulated by conventional physiological techniques.

Any attempt to study the various neural systems subserving the states of vigilance must take into account the architecture of morphological substrata, comprised of diverse neural subsystems, which overlap neural fields long related to the regulation of sleep-waking cycles. The elucidation of the anatomical underpinnings subserving sleep-waking states and other

behaviors received its greatest impetus with the development of fluorescence histochemical techniques with which, for the first time, it has been possible to directly identify neural cellular assemblies and pathways on the basis of their transmitter chemistry, i.e., chemical neuroanatomy. This type of morphological analysis developed at a most opportune time since various attempts, using pharmacological and lesion approaches, were being made to correlate brain chemistry and behavior. Hence, direct visualization of transmitter substances within specific neuronal assemblies, i.e., direct visualization at a cellular level, was a significant advance. Application of histofluorescence methods allowed for the derivation of entirely new maps of the brain and identified neural pathways not previously seen by conventional anatomical studies. For example, the amine-containing neurons form systems of small diameter fibers with diffuse terminal distribution, and these do not often correspond to recognized ascending and descending pathways. Hence, there is some question whether most of these chemical pathways have ever really been seen at all by studies of normal anatomy or those using silver degeneration techniques. It is, thus, likely that the technique of fluorescence histochemistry has demonstrated neuronal cells and pathways that were previously unrecognized by all conventional methods of neuroanatomy. Nevertheless, using these techniques, various poorly understood areas of the brain, especially the reticular formation, have acquired histochemical specificity, whereas previously it had been a vast area defying orderly subdivision, and difficult to manipulate physiologically with any confidence of approaching exact structures in a repeatable fashion.

Of particular interest, of course, is that many of these neural areas, now identifiable by transmitter histochemical approaches, are composed of regions where manipulations had previously been shown to affect the vigilance states. Hence, a knowledge of the anatomical organization of chemically coded neurons is a prerequisite for any firm elucidation of the role of brain areas in the sleep-waking cycle, and, especially, for attempting to correlate chemical changes in the brain with the vigilance states. In this review, we will concentrate on the study of those chemical systems whose organization has been charted in some detail, i.e., the monoaminergic and cholinergic systems and relate these, as precisely as possible, to neural areas where physiological studies have indicated involvement of these regions in sleep-waking activity. It should be emphasized that concentration on monoaminergic and cholinergic systems should not be taken to indicate that other putative neurotransmitters do not also play important roles. However, at present, there is limited knowledge concerning the topography of neurons containing histamine, gamma-aminobutyric acid (GABA), glycine, glutamic acid, etc. No doubt, in time, when these systems are better known, they too can be worked into a general chemical schema of control of the arousal continuum. Since the anatomical organization of chemical circuits is of paramount importance for understanding the possible role of monoamines

and acetylcholine in the regulation of sleep-waking behavior, the details of topography of these chemical systems will be discussed, especially in relation to known anatomical areas and fiber systems. It is well to point out that most of the histofluorescence tracing of chemical systems in the brain has been done in the rat, whereas the cat has been the animal most commonly used for studies of sleep-waking behavior. What is known about differences in chemical anatomy of the cat, and other species, especially the monkey, will be mentioned in the discussion below, where appropriate. This approach is obviously needed in considering the possible underlying causes of species differences in normal sleep-waking cycles and in following pharmacological and other manipulations.

II. CHEMICAL NEUROANATOMY

The important work of the Swedish group dealing with histofluorescence mapping of chemical pathways in the brain has been of inestimable value in developing correlations between morphology, physiology, brain chemistry, and behavior. This group has primarily confined themselves to studies of monoamine systems and have shown that there exist two distinctly different types of neurons which contain in their cell bodies and processes concentrations of primary catecholamine (dopamine or norepinephrine) and indoleamine (serotonin), respectively. Most importantly, they have traced axonal processes from these cell bodies and shown that these give rise to monoamine-containing synaptic terminals in which the catecholamines and serotonin are accumulated in very high concentrations. One of the most important aspects of the histofluorescence mapping technique is that it has allowed chemical neuronal systems to be traced continuously from the lower brainstem to forebrain areas, the many implications of which we will develop in detail below. Although the monoamine-containing nerve cells have a wide-spread distribution within the lower brainstem it has been difficult to systematize them on account of quite scanty knowledge as to what functional or anatomical systems they might belong. The fact that these chemical systems overlap morphological areas which, when lesioned or stimulated, affect the sleep states, has led to their direct implication in sleep-waking behavior, especially when it developed that these manipulations affecting sleep were correlated with specific changes in amine levels in brain areas to which these chemical neurons project. Obviously, there is a great necessity to precisely localize transmitter substances in the brain before endeavoring to understand such complex phenomena as sleep, arousal, sexual activity, etc., all of which are dependent on synaptic linkages whose transmitters can now be studied histochemically, physiologically, and pharmacologically. In this context, identification of the neurons responsible for the biosynthesis and storage of monoamines is clearly essential to any understanding of the functional role of these biologically active substances in the central nervous system.

As is well known, it is likely that several chemical substances are involved in synaptic transmission in the mammalian central nervous system. The Falck-Hillarp technique (1962) has demonstrated norepinephrine, dopamine, and serotonin within nerve cell bodies and terminals and the belief that these amines act as neurohumors is strengthened by observations that activation of nerve fibers leads to their release from the terminals. Since histochemical evidence suggests that discrete systems of neurons are identifiable by their content of particular amines, it seems likely that such neurohumorally homogeneous systems may well have a functional, as well as a chemical, identity. Chemical neuroanatomy makes use of the histochemical formaldehyde fluorescence method of Falck et al. (1962). This method is based on the fact that certain monoamines like dopamine, norepinephrine, and serotonin, on reaction with formaldehyde vapor, are converted to fluorescent isoquinolines (derived from dopamine and norepinephrine) or beta-carbolines (from serotonin), which can be directly visualized in a fluorescence microscope (Corrodi and Jonsson, 1967). The method is considered highly specific and sensitive and it has been calculated that only about 4×10^{-16} g of norepinephrine is necessary to obtain a reaction product visible in the microscope provided the amount is concentrated in a small volume such as in a varicosity of a nerve terminal (Jonsson, 1971). With the help of such highly sensitive and specific histochemical fluorescence methods it has been possible to study the precise cellular localization of monoamines in the nervous system as well as plot the trajectory of their processes. In the mammalian brain norepinephrine and serotonin have been found to be accumulated in high concentrations within the fine, varicose, terminal parts of the nerve fibers, with lesser concentrations in the cell bodies and axons. The axons of neurons usually have concentrations of monoamines that are too low to be seen distinctly. However, these can usually be brought out either by axotomy, following which there is a buildup of transmitters in the proximal stump of the axon, or by use of monoamine oxidase inhibitors which prevent enzymatic destruction of monoamines.

Björklund, et al. (1971a) have emphasized that fluorescence histochemistry of biogenic monoamines is a very sensitive, precise, and versatile method which has proved extremely useful not only for morphological studies but also in pharmacological and physiological research. However, a critical point in all these studies is the actual identification of the fluorogenic compound, i.e., the compound that yields fluorescence after formaldahyde gas treatment. Thus, final identification of a fluorogenic compound requires a direct characterization of its fluorophore. For this reason microspectrofluorimetry is now considered indispensible for confirmatory identifications in monoamine histochemistry. This is discussed in more detail with reference to several fluorescing systems of neurons identified by the Björkland group (1971a) and Aghajanian and Asher (1971) in the section immediately following the review of chemical circuits and areas.

III. THE CONCEPT OF THE RETICULAR CORE SYSTEM (FORMATION) OF THE BRAINSTEM

To understand the immense significance currently attributed to the reticular formation in the total activity of the brain requires a precise description of its finer structural peculiarities and an exact as possible definition of this core complex as an anatomical and physiological concept. At present there is no overall agreement even on the criteria for determining which brain structures are to be considered as part of this system. However, in this section several of these possible criteria will be discussed and the concept of the reticular formation will be extended considerably beyond its classical confines.

As is well know, it has been customary to epitomize neuroanatomical data as a series of circuits that presumably represent those lines of communication, cell sequences, and synapses over which information is most likely to flow, i.e., in one sense the pathway becomes the message.It is necessary now to look at the profusion of neurochemically discrete subsystems that have recently been identified in the reticular core of the brainstem in relation to classical anatomical areas described and defined by older anatomical techniques. The Golgi method, as far as is known, is entirely silent on the chemical nature of the neural ground, but has been the method *par excellence* for unraveling many of the intricacies of the reticular fields. Degeneration methods, such as the classical Nauta procedure and its variations (Fink-Heimer method, etc.), and studies of normal material (myelin stains, cell stains, etc.), have all been of more limited, albeit complementary, value in this regard. Recently, radioautographic techniques have been applied to study the reticular areas since fibers of passage do not confound the interpretations when this method is used. Naturally, combinations of all of these methods, each with its own unique advantages, offer the best opportunity for shedding light on the organization of the reticular formation. Thus, modern anatomical redefinition of the reticular formation, in terms of Golgi analysis, dendritic pattern resolution, and, especially, the newer histofluorescence chemical codings and delineation of chemical systems, has provided a structural substrate within which an otherwise diverse mass of neurophysiological data may now be better assessed. The concept of the "isodendritic core" (see below) has led to an expansion of the older view of the reticular formation so that it now extends from the reticular core and spinal cord to the basal telencephalon and, perhaps, even into the cerebral cortex. Also, the work of the Scheibels (1970) has clearly indicated a bilaminar projection of reticular axons into the forebrain with the ventral lamella, or extrathalamic by-pass (via the zona incerta), terminating within the septal area and basal forebrain zones. This will be discussed further below.

The reticular formation of the lower brainstem is most logically the point

of initial discussion of the neural substrata of the vigilance states. Classically, this area has been most associated with the arousal continuum dating from the experiments of Moruzzi and Magoun (1949), and before. Thus, in our discussion we will first define and delimit it and discuss its special characteristics before attempting to parcellate it into neurocytological elements whose processes we will trace into the basal forebrain and cerebral cortex. There is little question, then, that the beginnings of analysis of the multiple circuitries concerned with sleep-waking behavior should logically be the reticular formation. As Ramón-Moliner and Nauta (1966) have pointed out, one can accept neither the amalgamation of a number of physiological properties on the basis of an assumed unitary morphology nor, conversely, an assembling of anatomical regions on the basis of an assumed common physiological role. It has for some time been assumed that the generalized dendritic pattern is a cardinal identifying characteristic of the reticular formation. Of course, one can question whether dendritic peculiarities, alone or in combination with other histological properties, can be regarded as valid criteria in the conceptualization of the reticular formation. Ramón-Moliner and Nauta have proposed the term "isodendritic core" to define the vast region which extends throughout the brainstem and spinal cord, forming a matrix in which other cell groups, with more specialized dendritic features, lie embedded. The generalized dendritic patterns are usually found in cells having polygonal cell bodies, with irregularly dispersed Nissl bodies. In general, it appears to be that cytological polymorphism is one of the very striking features of the isodendritic core of the brainstem. Further, Mannen (1960) has stressed that a close commingling of passing fibers and dendrites can be considered a major feature of the core of the lower brainstem. Ramón-Moliner and Nauta have reiterated, however, that this is by no means a peculiar feature of that lower brainstem core, since the deeper layers of the cerebral cortex, reticular nucleus of the thalamus, subthalamus, zona incerta, and certain intralaminar nuclei of the thalamus are examples of the same type of histological configuration. Of course, if these areas are all considered as forebrain extensions of the reticular core, then Mannen's criterion can be accepted. In this same vein, Nauta and Haymaker (1969) have pointed out that the septal area, especially its medial, relatively magnocellular, component also exhibits a dendritic configuration and relationship of dendrites to passing fiber bundles entirely comparable to those found in the brainstem reticular formation and hypothalamus.

Leontovich and Zhukova (1963) include within the reticular formation the raphé nuclei, the central gray, the nucleus tegmenti pontis of Bechterew, nucleus of the solitary tract, vestibular nuclei, the entire substantia nigra, the parafascicular and reticular thalamic nuclei, the nucleus ventralis anterior, nucleus anteromedialis, nucleus paracentralis, nucleus centralis medialis, nucleus centralis lateralis, nuclear medialis dorsalis, nucleus medialis ventralis, the midline and commissural nuclei, the lateral habenular nucleus,

the whole subthalamus or ventral thalamus (zona incerta), the ventral part of the lateral geniculate nucleus, the fields of Forel, parts of the hypothalamus and preoptic area as well as the pallidum, the area diagonalis with its anterior pole extending into the septal area (bed nucleus of anterior commissure), stria terminalis and substantia innominata. All are brain regions characterized by afferent connections of heterogeneous origin. Thus, according to this concept, the reticular formation stretches as an uninterrupted cell column throughout the brainstem and extends to the diencephalon and the basal regions of the telencephalon and, perhaps, beyond.

From classical anatomy it has long been known that the lateral zone of the hypothalamus is directly continuous with the ventral tegmental area of Tsai, an ill defined cell territory in turn laterally continuous with the so called deep tegmental nucleus (Gudden), which extends lateralward over the substantia nigra. Further, the medial and periventricular zones of the hypothalamus—in particular, the posterior hypothalamic nucleus—merge with the periaqueductal gray substance of the midbrain. Hence, the hypothalamus is considered as part of a neural continuum or extension of the reticular core from the midbrain forward. Whether or not one chooses to designate the forebrain continuum, along with the remainder of the brainstem tegmentum, as *reticular formation,* depends on the interpretation given to the latter term. From a histological point of view, it is justifiable to consider the hypothalamus as highly comparable to those mesencephalic and bulbar regions which are held to represent brainstem reticular formation. Thus, the hypothalamus shares with the major part of the bulbar and mesencephalic tegmentum the following characteristics: 1) neurons of the isodendritic type, i.e., having long, rectilinear and sparsely branching dendrites; 2) widely overlapping dendritic fields; and 3) a free mingling of dendrites with fascicles of axons in transit. If the reticular formation implies heterogeneity of afferent connections, then certainly the hypothalamus and its extensions into the forebrain would fall under this categorization. It might be well to emphasize that the medial forebrain bundle, the principal forebrain fiber system in this complex, is composed, in part, of relatively short neural links but the system is also pervaded with longer axonal conduction routes (Valverde, 1965).

In this review we would stress that the hypothalamus is directly continuous with the vast "nonspecific" neuronal apparatus of the brainstem reticular formation and should be considered a direct extension of it. Nauta and Haymaker (1969) point out that the hypothalamus could, in fact, be regarded as the ventro-medial part of a more generalized "nonspecific" diencephalic apparatus which extends the mesencephalic reticular formation rostralwards and encompasses, in addition, the so called nonspecific thalmic cell group and the subthalamic region. From this it can be seen that the subthalamus is also regarded as a direct rostral extension of the mesencephalic tegmentum.

Some examples of morphological specifics are of interest here relative to characteristics of the reticular formation, especially its relation to the raphé