SYMPOSIUM ON NEUROANATOMY AND NEUROPHYSIOLOGY OF THE AUDITORY SYSTEM

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Experiential Afferent Influences and Development in the Avian N. Magnocellularis and N. Laminaris

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Three lines of research have led to increasing interest in the avian auditory system. First, a large body of knowledge on bird vocalization has generated interest in relating behavioral capacities to structure and function of the auditory pathways. Second, the unique phylogenetic position of Aves as a second offshoot of reptilian lines promises to reveal valuable insights regarding the origins of the mammalian auditory system (Boord, 1969; Mehler, 1974). Finally, the avian auditory system is currently used in several laboratories as an advantageous system in which to investigate the role of experience in nervous system and behavioral ontogeny (Gottlieb, 1976; Rubel, 1978). Other contributions in this volume discuss avian phylogeny and the relationships between bird song and auditory physiology. In this paper we will consider the qualities of the avian auditory system which lend themselves to study of experiential roles in nervous system ontogeny. In so doing, we will briefly review our own investigations of brain stem auditory nuclei development in the chick.

Statement of the problem

There can be little contention with the statement that the early experience of an organism can influence its behavioral and physiological development. Although some behaviors, and perhaps some species, appear more resistant to fluctuations in the environment, either qualitative (e.g., imprinting) or quantitative (e.g., song recognition) influences of early experience have been discovered for most behaviors thoroughly investigated (Newton & Levine, 1968). Uncovering the neural mechanisms responsible for experiential modulation of behavior development has been difficult, however. While examples of morphological, biochemical, and physiological changes in the nervous system of animals reared in restricted environments are abundant (Gottlieb, 1978), the causative chain of events relating nervous system development to experiential factors is far from understood. At least one reason for this lack of mechanistic information is that there has been little attempt to operationally define, in terms that can be applied to neurons and neural systems, what is meant by "experiential modifications." For example, it is now well known that monocular deprivation of form vision will lead to dramatic changes in the distribution of ocular dominance of neurons in the cat and monkey visual cortex. However, we do not know how that manipulation of the organism's interaction with the external environment alters the cellular milieu of visual system neurons. In other words, while it is known that monocular form deprivation leads to distinct changes in the distribution of light impinging on the retinal surface, how this is translated into chronic changes in the environment of the neurons in the cerebral cortex remains unknown. Stated more generally, in order to unterstand the mechanisms underlying experiential modulation of neural development it is necessary

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to define the difference between the "normal" and the "altered" environment of the neurons under investigation. Some chronic or tonic change in the environment of the brain regions that are influenced by early experience must underlie these phenomena.

Viewed in this way, the problem of understanding how alterations in early experience influence neural ontogeny is subsumed under the general problem of tissue interactions in the developing nervous system. Specifically, during ontogeny, as a result of receptor development and synaptic formation along neuronal networks, the neuronal environment becomes subject to both phasic and tonic changes in the external environment of the organism. Modifications of "normal" experience must have differential influence on the ontogeny of neuron structure and function by producing some change, qualitative or quantitative in the afferent input to the neurons under investigation. Thus, integral to understanding the mechanisms by which the early experience of an organism influences neural development is documenting the effects of manipulating the integrity and the activity of afferents on the developmental history of a neural network.

Qualities of a model system

Given the complexity of the nervous system, the task of defining the ways in which afferent input influences the ontogeny of structure and function appears formidable. One strategy for approaching such problems has been the careful selection of a preparation which possesses characteristics particularly advantageous for analysis of the particular topic. When the characteristics of a "model system" have been clearly defined, one can survey a variety of preparations and choose the one which most closely approximates an "ideal" preparation.

A "model system" for the analysis of environmental influences on neural development should possess the following qualities:

1. Genetic homogeneity. Given that there is an interaction of genetic and environmental factors in virtually all developmental events, it is highly desirable to use animals of similar genetic constitution.
2. Homogeneity of the embryonic environment. It is also well known that variations in factors such as circulating maternal hormones, temperature, etc., can have both quantitative and qualitative influences on neural and behavioral ontogeny which may differentially interact with experimentally-induced modifications of afferent input.
3. Simple structural and functional organization. Ideally, it is desirable to have only one or two inputs to a small set of neurons, each of which could be structurally and functionally defined and each of which could be quantitatively manipulated along a continuum from no afferent input to supranormal afferent input. In addition, the neuronal pool should be sufficiently limited and well defined that both structural and functional identification of any subset in possible throughout development.
4. Access to the preparation throughout development. It should be possible to manipulate the quantity or quality of afferent input at any stage of development and assess the functional and structural consequencies of such manipulations. Ideally, it would be desirable to be able to perform manipulations of afferent activity both in vivo and in vitro while the neuronal system is developing.

5. Direct access through peripheral receptor system. Since variations in the sensory environment can produce neuronal and behavioral modifications during development, it would be desirable if definable variations in afferent activity could be produced through these biologically relevant pathways.
6. Behavioral analog. To insure that the dimension along which afferent input is being manipulated in order to produce neuronal modifications is "biologically meaningful," it is desirable to determine if similar manipulations will produce functional changes in the organism's behavior.

Avian brain stem auditory pathways

The above considerations led us to the avian brain stem auditory system. Nucleus magnocellularis (NM) and nucleus laminaris (NL), which comprise 2nd- and 3rd-order auditory neurons, respectively, appear to be uniquely suited to investigations of the role of afferents in neuronal development and maintenance. Our initial investigations (RUBEL & PARKS, 1975; PARKS & RUBEL, 1975 and 1978) were primarily intended to provide detailed structural and functional information on the organization of this system. We then described the normal morphological development of NM and NL at the light microscope level (RUBEL et al., 1976). We are currently extending these observations with additional neurophysiological and morphological analyses of normal organization and development (SMITH & RUBEL, 1977; JACKSON et al., 1978). Finally, we have begun to describe how modifications of afferent input can alter the development and maintenance of the normal neuronal elements in this system (PARKS & ROBERTSON, 1976; PARKS, 1978; JACKSON & RUBEL, 1976; BENES et al., 1977; PARKS & RUBEL, 1977). In the following account we will briefly describe the results of some of these investigations. Our emphasis will be to demonstrate how the characteristics of this neural system make it appropriate for further analysis of the roles that afferent information may play in the ontogeny of neural structure and function.

The major avian brain stem auditory pathways, first described in detail by CAJAL (1908), are schematically shown in Figure 1. Axons of the cochlear ganglion cells enter the dorsolateral quadrant of the medulla and bifurcate into medial and lateral bundles. The lateral axon branches terminate in a cochleotopic manner in nucleus angularis (NA), which is composed of mixed cell types lying in the dorsolateral angle of the medulla. The medial branches of the VIIIth nerve fibers course along the floor of the fourth ventricle (IV) to terminate in NM. NM is a well-defined cluster of 3800—4200 large round or ovoid cell bodies which are either devoid of dendrites or have short, bushy, rudimentary dendrites. The cells are organized into dorsoventral columns with VIIIth nerve afferents coursing between the columns and terminating on the cell bodies as large endbulbs. Another input to the NM cells is of unknown origin, but may derive from axon collaterals of other NM cells (JACKSON et al., 1978). As shown in Figure 1, NL in chickens is composed of a discrete monolayer sheet of 1200—1600 cell bodies with bipolar dendritic orientations. NL cells receive binaural, spatially-segregated innervation from the magnocellular nuclei; axons from NM pass in the uncrossed dorsal cochlear tract to innervate the dorsal dendrites and cell bodies of the ipsilateral NL, and decussate in the crossed dorsal cochlear tract to innervate the ventral dendrites and cell bodies of the contralateral NL. No other inputs to NL have been observed. This

relatively simple structural organization, the accessibility of avian embryos for neurophysiological studies or peripheral manipulations, and the possibilities for precise control over the acoustic environment of embryos and hatchlings has warranted further investigation of the functional organization of NM and NL.

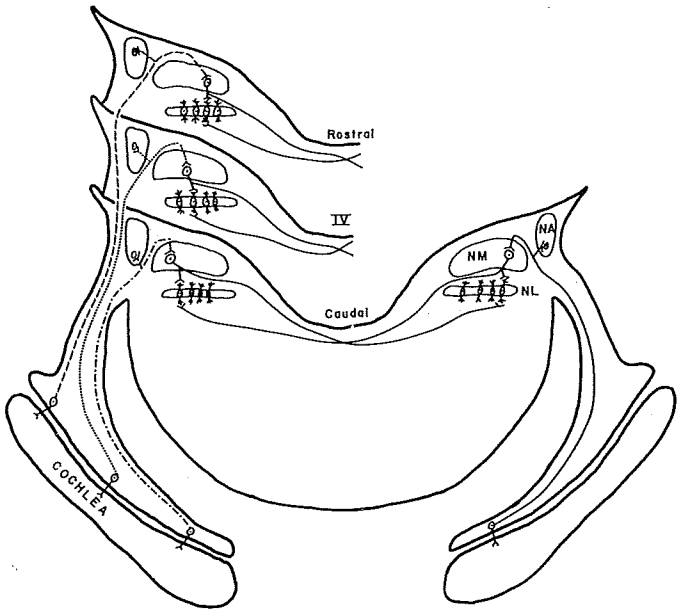

FIGURE 1. Schematic drawing showing the projections from the cochlea via the cochlear ganglion to n. angularis (NA) and n. magnocellularis (NM); and the spatially segregated, bilateral projections from n. magnocellularis to n. laminaris (NL). IV—4th ventricle (from RUBEL, SMITH & MILLER, 1976).

Neurophysiological examination of these nuclei and analysis of the projections from NM to NL revealed the following properties. NM cells respond only to a narrow range of frequencies played to the ipsilateral ear. The cells display sharp excitatory tuning curves, primary-type response histograms, and always have a definable characteristic frequency. Auditory nerve stimulation evokes only exitatory postsynaptic potentials with little convergence of auditory nerve fibers on individual NM cells. The cells form isofrequency columns, in which all cells respond to a similar characteristic frequency; the physiologically-defined columnar organization corresponds to anatomically observed cell columns. NL cells are binaurally activated by acoustic stimuli and usually show similar characteristic frequencies and thresholds to stimulation of each ear. That is, NL neurons are maximally activated by the same frequency range applied to either ear. In other respects, extracellular responses in NL are similar to NM neurons. Intracellular recordings display graded EPSP's of long duration. The organization of neurons in both nuclei is characterized by a highly stereotyped tonotopic organization; cells maximally responsive to low frequencies are situated in the caudolateral aspect of each nucleus and higher frequencies activate cells at progressively rostromedial positions. This tonotopic organization is sufficiently stereotyped that quantitative analyses

of the relationships between the position of a cell in each nucleus and its characteristic frequency allow accurate prediction of this functional characteristic (within 200—400 Hz) from positional information alone. The utility of this property is that it allows independent prediction of the normal characteristic frequency of a neuron, which can be compared to results obtained as a function of manipulations of a organism's acoustic environment.

By making small lesions in NM through tungsten microelectrodes after recording the characteristic frequency of cells in the lesioned area, and by both intracellular and extracellular injections of horseradish peroxidase, we have been able to further delineate the organization of the bilateral projections from NM to NL. There is a precise tonotopic and topographic projection from each region of NM to the dorsal side of ipsilateral NL cells and the ventral aspect of neurons in the corresponding position on the other side of the medulla. Furthermore, Golgi-stained tissue has revealed that the dendrites of NL cells are confined to the glia-free margin surrounding the cellular lamina and are qualitatively and quantitatively similar on the two sides of NL in any frequency region of the nucleus. These results allow precise delineation of the receptive cell surfaces of NL neurons as well as unusually precise specification of the source and functional properties of input to each dendritic surface of the neurons.

The first major morphogenetic event to be considered in the analysis of any neural region is the developmental stage and duration of time during which cellular proliferation occurs. Cumulative labeling with ^{3}H-thymidine can be used to determine the time at which cellular proliferation has ceased and the occurrence of heavy labeling is a good indicator that the time of isotope injection was during one of the final mitotic cycles. Our analysis of the brain stem auditory nuclei indicated that there is no overlap in the final period of DNA synsthesis between NM and NL. The majority of NM neurons leave the mitotic cycle between 48 and 72 hours of incubation, while the final division of NL cells occurs at about 84—100 hours of incubation. Both cell groups are produced in the region of the rhombic lip, and when first recognized in the 5—6-day embryo they overlap throughout most of their rostrocaudal and mediolateral extent. It is therefore reasonable to hypothesize that both cell groups are produces by a single progenitor population, with NM cells being formed first and migrating away from mother cells. Then, in a second wave of mitotic activity, the final population of NL cells is formed. This temporal sequence of cellular proliferation, coupled with the fact that NL cells come to lie directly ventral to NM, suggests that the cells may interact during their proliferative or migratory phases. Although there is little direct evidence for this hypothesis, we have observed that, at around 5—7 days of incubation, cells labeled at the same time as those of NL can be observed streaming through the magnocellular nucleus. The occurrence and possible functions of such interactions may be particularly susceptible to investigation in this system and may represent the first interaction between neurons which are destined to be functionally connected.

Following proliferation and migration, these cell groups develop their characteristic morphologies over the period from 9 to 15 days of incubation. By 9 days of incubation both cell groups can be easily recognized, although neither has assumed its mature cytoarchitectural or positional characteristics. At this stage, both nuclei are composed of relatively undifferentiated, densely-packed cell bodies and there is no apparent subnuclear organization. In silver-impregnated tissue, it is apparent that the major afferent

axons to both nuclei are present, although preliminary electron microscopic examination has revealed no synapses. Between days 9 and 13 major morphogenetic changes occur in both nuclei. The cells of NM are displaced medially, cell density diminishes, cell size increases, and the cells become aligned in their characteristic columnar organization. The most striking changes occur in NL, where, from the undifferentiated cluster of cells seen at days 8—9, a precisely defined monocellular or bicellular lamina, with a uniform glia-free margin, emerges by day 13. This change begins in the rostromedial portion of the nucleus between days 9 and 11 and is completed in the caudolateral portion by around day 14.

Changes in cell number occur in both nuclei concurrently with these morphogenetic events. Whereas NM loses only a few cells (0—20 %) and any cell death appears to occur between embryonic days 11 and 13, cell death in NL is much more extensive (about 70—80 %) and takes place over a considerably longer period (days 9—15). These changes in cell number are of interest for a number of reasons. First, while there are large differences in the amount and duration of cell death, the period of maximal cell loss in both nuclei is between 11 and 13 days of incubation. Since proliferation of NM and NL is separated by about 24—36 hours, it appears that whatever factors regulate cell death (see Levi-Montalcini, 1949; Cowan, 1973; Hamburger, 1975) may serve to bring the two nuclei into ontogenetic synchrony. Of possible importance in this regard is the fact that both physiological and behavioral studies indicate that the onset of afferent function is closely correlated with normal cell death. Current work on functional ontogeny in vitro will be of great importance for further understanding of this relationship.

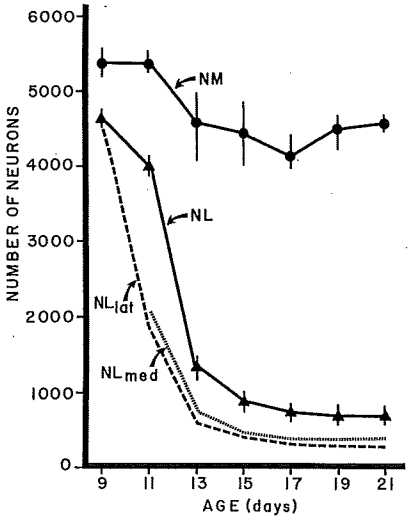

Figure 2. Cell counts in n. magnocellularis and n. laminaris from 9 days to hatching. Points are means for each time and vertical bars show ranges. Lines showing counts for the divisions of n. laminaris are mean values. NM, n. magnocellularis; NL, n. laminaris (total); NL lat., lateral division of n. laminaris; NL med., medial division of n. laminaris (from Rubel, Smith & Miller, 1976).

The volumetric analyses of NM and NL also indicate a surprising amount of developmental synchrony in the enlargement of cell bodies and neuropil areas which occurs after the period of cell death. Since it is quite likely that functional connection are established by day 15, it will be important to understand the role which afferent activity may play in the regulation of this parameter as well. It is therefore advantageous that

the period of rapid cellular and neuropil expansions can be temporally distinguished from that of cell death.

Summarizing the ontogenetic information, NM and NL display the following characteristics: i) the neurons of NM and NL go through their final phase of DNA synthesis over restricted and temporally separated periods, which allows independent labeling of either neuronal population; ii) the period of cell death does not overlap with the proliferative period; iii) the alignment of NL cells is temporally correlated with, and possibly results from, cell death; iv) cell loss occurs over a defined time period, has a definite spatial gradient and, in the case of NL, is quite extensive; and v) volumetric changes in cell bodies and neuropil regions occur in a period when functional connections are established and these changes occur following the time of maximal normal cell loss. These factors, in conjunction with the information on normal structural and functional connections, the access for experimentation afforded by the avian embryo, and the potential for controlling both the pre- and post-hatch acoustic environment suggest that these brain stem auditory pathways will serve as an excellent preparation for investigations of cell interactions in the developing nervous system.

Our first attempts at manipulating the cellular environment have used direct deafferentation. In one series of investigations (PARKS & ROBERTSON, 1976; PARKS, 1978; JACKSON & RUBEL, 1976) the effects of removing the VIIIth nerve afferents either prior to synaptogenesis (otocyst removal) or well after it (at hatching, three months, or three years) have been studied in NM. In agreement with LEVI-MONTALCINI (1949), it was found that removal of the otocyst had little or no effect on NM cell number or cell size until after 11 days of incubation, after which time both the amount and period of cell death were greatly enhanced. By 19 days of incubation, the deafferented NM had 40—50 % fewer cells than the normal population. Surprisingly, this effect was not age dependent; cochlea removal in hatchling, three-month-old, or adult chickens resulted in a similar amount of transneuronal cell loss in NM.

A second investigation (BENES et al., 1977) examined the degree to which transneuronal changes are specific to the postsynaptic membrane surface to which the input has been removed. By deafferenting one side of the NL cells it was possible to compare the deafferented dendritic surface with the other dendritic region of the same neurons, which had their normal input intact. An EM morphometric analysis revealed rapid and complete degeneration of the deafferented dendrites, while the opposite dendrites of these neurons retained their normal qualitative and quantitative characteristics.

While the total elimination of afferents to a neuron certainly interrupts more cellular processes than do changes in the pattern of synaptic activation, at least a portion of the deafferentation syndrome is probably due to the elimination of synaptic activity. Thus, the examination of developmental changes produced by total removal of afferents will yield a catalog of events, each of which can be systematically evaluated upon progressively more subtle manipulation of afferent activity. Furthermore, deafferentation results may form one end of a continuum relating the quality or quantity of afferent input to the integrity of neural structure and function. Most importantly, the avian auditory system will be valuable for testing of this and other hypotheses regarding the role of afferent activity in neural development. By controlling the sound environment it is possible to specify systematic variations in the activity impinging on known neuronal elements at any time after the receptor becomes functionally active. Furthermore, the

entire system as shown in Figure 1 is contained in a 1 mm slab of tissue and preliminary analysis (JACKSON et al., 1978) indicates that it will remain viable in vitro over long periods. This quality may allow direct experimental control (via electrical stimulation) of the amount and pattern of activity in each auditory nerve at any stage of development.

Finally, with regard to the desirability of behavioral analogs, it should be noted that the ontogeny of auditory perceptual behavior can be readily studied in avian embryos and hatchlings (GOTTLIEB, 1970; JACKSON & RUBEL, 1978) and auditory deprivation can have marked effects on these behavioral processes (e.g., GOTTLIEB, 1976; KERR et al., 1978).

While the above studies do not answer most questions of how the structural and the functional aspects of afferent input regulate neuronal ontogeny, we feel the avian brain stem auditory pathway still provides an excellent model for further investigation. It is hoped that progressively more subtle manipulation of afferent activity and increasingly sensitive measure of neuronal structure and function in this system will yield a more thorough understanding of how an organism's external environment regulates the ontogeny of neural networks.

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