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Strategies and Problems for Future Studies of Auditory Development

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In this paper I have been asked to outline some of the unsolved problems for the understanding of hearing development. Throughout the workshop from which the companion papers have been derived, each investigator has pointed out specific areas where more information is needed. One approach to my task would be to summarize those concerns. This might be of considerable use to young investigators in search of "an experiment". However, by the time I could describe a consensus regarding the need for specific data, the difficult task, that of defining the problem, would be accomplished. Futhermore, the more universal the consensus, the more likely we know the outcome and the more probable the experiments are already underway or completed by some aspiring graduate student and his or her manic professor. Given current publication schedules, such a summary becomes even less useful since it is likely that the results will be published before my "astute" summary of unsolved problems has been printed. (I might be further embarrassed to find the results published from my own laboratory.)

Thus, at the risk of being obscure or of restating the obvious, I will attempt to outline some areas where *more* than data is needed; where either the problem, the approach, or the fundamental principles have not been clearly identified. The reader is referred to earlier reviews (1, 2, 3, 4) for more comprehensive literature summaries.

TISSUE INTERACTIONS IN THE DEVELOPMENT OF THE INNER EAR AND CENTRAL AUDITORY PATHWAYS

Inner ear

In the past decade, advances in organ culture methodologies have allowed the growth of mammalian cochleas in vitro. A major advantage of the in vitro system is that it permits analysis of the sites of origin (fate mapping) and tissue interactions necessary for particular aspects of organogenesis. Thus, many of the conclusions regarding ontogenetic relationships between contiguous tissues which were worked out in lower vertebrates earlier in this century have been extended to mammals. More recently the application of transfilter techniques to in vitro systems has offered a means of addressing the chemical and physical constraints of the inductive interactions between the different tissue compartments (5). It is not a criticism, but a fact, that our knowledge in this area is crude at present. Interactions between major tissue compartments and their temporal constraints will soon be known. On the other hand, several of the most interesting problems of induction have not, to my knowledge, been addressed in any substantial way. For example, the local and/or long range inductive factors responsible for *differential* expression of various cell types within the organ of Corti are unknown. So are the inter- and intracellular factors that determine when and what specializations will appear on the cells. When and what regulates the microtubular structures responsible for growth of pillar cells? What regulates the growth of stereocilia and the uniform arrangement of actin filaments? And when are the orientation of stereocilia and their remarkable gradients established? Only when

descriptive developmental studies have been adequately completed and related to other developmental events in one organism can we begin testing hypotheses about the tissue interactions which normally regulate such events as cilia development. The importance of normative developmental investigations should be reemphasized at this point. Without normal developmental analyses it is impossible to understand how normal development is regulated or how aberrant factors initiate pathology. That is, without such analyses it cannot be clear whether an experimental manipulation applied during development actively creates a pathology, or if the same manipulation prevents or disrupts some tissue interaction which is needed for normal development.

One popular and seemingly fruitful approach toward understanding tissue interactions at a cellular level is the examination of mutant ears raised in vitro. This strategy employs a "genetic scalpel" to exclusively remove (in mutants) particular cell types or factors (proteins) and then examines how development proceeds in the absence of that "essence". In this way, developmental events and structures which can emerge *independent* of a missing factor can be recognized and the inductive responsibilities of the missing elements can be inferred. In practice, it must be recognized that a "genetic scalpel" is not always perfectly sharp. In addition to the cautions outlined by Steel (this Supplement) it is not always apparent precisely when and where the *primary etiology* of the mutation takes place. Is it only on the missing cell type? When was its first expression? What was the cellular chain of events leading from the original expression of the mutation to the aberrant cochlea? While such questions do not diminish the value of the genetic approach, they must eventually be addressed.

A related area of investigation concerns the origin of the cell types in the inner ear. Noden (6) has described the derivatives of neural crest in chicks, thereby confirming that the entire sensory epithelium and ganglionic structures are of placodal origin. Beyond these investigations and Ruben's (now classic) tritiated thymidine studies of birthdates (7), only scattered observations are available. Fundamental questions such as the site(s) of origin and lineage relationships of various cell types remain unexplored. When methods for growing cochlear tissues from earlier stages become available, the application of modern labeling techniques using HRP or florescent dyes to analyze cell lineages in the primitive otocyst will be feasible. Such studies will provide valuable information.

Central nervous system

The problems of tissue interactions in the developing central auditory pathways should also be considered. While such processes are likely to follow the same principles as found in other parts of the central nervous system (8), most have not been examined in the developing auditory system. One source of tissue interactions which has been examined rather carefully is the role of input from the cochlea on the ontogeny of neurons in the brain stem auditory nuclei. Beginning with the classic investigation of Rita Levi-Montalcini (9) and followed by experiments from our laboratory (10, 11, 12) and others (13, 14), we can now infer that the presence of an intact basilar papilla (cochlea) has little or no effect on the development of brain stem centers (proliferation or initial migration of cells) until after synaptic contact and functional synapse formation between the periphery and the CNS occurs. After that time, however, an intact cochlea is required for normal cell survival, neuronal growth, and stabilization of neuronal position. How has the postsynaptic neuron changed so as to become dependent on input? What are genetic and cellular mechanisms responisble for this change in "state" and are they irreversible? These are among the many questions which have yet to be adequately addressed in any neuronal population. It is likely that the auditory system will be useful in providing answers.

TIMING OF FUNCTIONAL DEVELOPMENT

Onset and maturity of function

For the remainder of this paper, I will consider the period of functional development of the auditory system. By this I refer to the period beginning just prior to the time at which acoustic energy in the environment can produce neuronal potentials in the eighth nerve and ending at the time when the ability of the animal (or person) to perceive this signal is mature. The definition of each of these times is difficult; there are technical, conceptual and semantic problems.

Let us first consider why we want to determine the earliest time at which sound evoked activity is present. In most cases, the objective is to define structure–function relationships; that is, to determine the morphological or biochemical changes which are "responsible" for the onset of hearing and/or "responsible" for changes in perceptual attributes (thresholds frequency, selectivity, etc.). There are two apparent flaws. First, any attempt to divide the ontogeny of hearing development into discrete stages, such as before onset and after onset, is imposing artificial boundaries on a continuous process. The implication is that the underlying morpho-physiological processes are also step functions, or "triggers". The assignment of "stages", or developmental boundaries in order to relate hearing development to discrete morphological or physiological events appears to be another case of "Essentialism" (15) and the implication of a step function may actually distort the developmental process in ways that impede progress by enticing investigators to search for a "trigger". In fact, development of hearing, as measured by observing thresholds (see Ehret or Eggermont, this issue) or frequency selectivity (16, 17, 18, 19) is a continuous developmental process. Thus, an experimental strategy which consists of defining correlations between structure and function at one or two developmental time points is likely to produce results of only transient interest and, therefore, is rather unsatisfying. A more fruitful approach will be to relate the temporal aspects of various developmental functions, such as suggested in the accompanying chapters by Eggermont and Ehret.

Onset of function is defined above in terms of responses to acoustic events. This is arbitrary. Do we mean environmentally meaningful sound levels or what can ultimately be produced? There is no rational reason to define function on the basis of peripheral events. It seems obvious, but may be worth stating, that the definition of onset of function must be referenced to the object under investigation. If, for example, cells in the cochlear nucleus are of interest, the onset of sound evoked discharges in the cochlear nerve may be of little interest or importance because of its dependence on receptor and middle ear properties. Instead, the important or interesting parameter may be "spontaneous" activity (whether cochlear driven or not) of eighth nerve synapses on the cochlear nucleus neurons. This distinction becomes important when one considers that numerous investigations have attempted to draw conclusions about the relationship between peripheral activity (presumed from sound elicited responses or manipulations) and development of the central nervous system. Yet only a couple of studies have attempted to actually investigate the onset of neuronal activity (as opposed to sound elicited responses). In this context, it is interesting to recall that over two decades ago Marty and Thomas (20) showed that evoked potentials could be elicited in the cerebral cortex of kittens by electrical stimulation of the eighth nerve at birth, a time when it is difficult or impossible to record tone elicited responses. Similarly, we could record postsynaptic potentials in the chick brain stem auditory nuclei a day before tone elicited responses could be evoked (12).

Technical difficulties involved in determining the onset of auditory function can also be easily documented. There are a myriad of factors, only some of which are appreciated at any time in history or by any individual group of investigators, using any particular

methodology. These factors include: (*a*) the obvious—such as acoustic energy output of the stimulating equipment; (*b*) the subtle—such as "stimulus complexity"; (*c*) the obscure —such as temperature of the preparation; (*d*) the mathematical—such as averaging and characteristics of amplifiers and A to D converters; (*e*) the often overlooked and dramatic —such as stimulus repetition rate; (*f*) personal—such as ability and experience at working with young animals. It is only when all of these factors are optimized *for the neonate* (not for the adult) that we can hope to determine the earliest time at which sound elicited activity can be evoked. As more sensitive and efficient methodologies or new factors are discovered, the time designated as the first responses will be revised.

A final problem with attempts to relate onset of function to single morpho-physiological events is that there is now strong evidence that no single event (or simple set of events) triggers the beginning of auditory function (1, 21). In the past century, most structures of the cochlea have been related to onset of function and, thereby, assigned the role of the "trigger". The implicit or explicit implication is that other structural and/or morphological events are temporal coincidences (e.g. see 22). However, the remarkable fact is that during the period of cochlear development, when responses to sound can first be recorded, the structures at any level of the cochlea appear to be nearly synchronized in their developmental time course providing the structural, biochemical, and physiological substrates for functional ontogeny. A few examples are: (*a*) mechanical thinning of basilar membrane and loss of major blood vessels, loss of Kölliker's organ by degeneration of the tall columnar cells to form an inner spiral sulcus, loss of marginal pillars connecting tectorial membrane to the first row of Hensen's cells, growth of pillar cells; or (*b*) neural-development of endocochlear potential, maturation of cilia structure, formation of synapses, and myelination of ganglion cell bodies and distal processes. It has recently been observed that important middle ear and central nervous system changes occur during the same time period.

The apparent ontogenetic synchrony of a large number of seemingly unrelated processes suggest to this author an active regulatory process. It is unlikely to involve a systemic hormone such as thyroxin, since cochlear differentiation spreads as a wave outward from the basal or mid-basal region of the cochlea. Possibly there is some local process controlling the *rate* of development of many diverse tissues within the cochlea. What is responsible, and how such the regulation occurs, is completely unknown at present. Understanding the temporal regulation of cochlear differentiation remains virgin territory for important research contributions.

When is the auditory system mature? Some of the same criticisms noted above regarding onset of function can be applied toward designating a time when "development is finished". It is important to note that different auditory abilities mature at different rates. One must, therefore, be cautious in assigning a time when audition is adultlike on the basis of one parameter such as threshold. It is well known that other parameters, such as those reflecting temporal processing, take much longer to mature.

Gradients: their meaning and their regulation

One of the first observations on development of the cochlea was that there is a gradient of differentiation. Differentiation of most elements begins in the first half of the basal turn and progresses in both directions. The apex matures last. This general principle has been documented in numerous species of birds and mammals with respect to a large variety of cell types and maturational processes. While exceptions probably exist, and are worthy of investigation, the overwhelming number of observations documenting the existence of this general gradient cannot be overlooked. It is beginning to appear that when the spatio-temporal pattern of development is examined in brain stem auditory centers, a similar pattern

emerges. In the chick, for example, we have noted a basal (projection area) to apical (projection area) sequence in terms of cytoarchitectonic development, dendritic maturation in n. laminaris, dentritic absorbtion in n. magnocellularis and functional development of n. magnocellularis (19, 23, 12, Young & Rubel, unpublished observations). In mammals, Romand & Romand (24) showed a similar gradient of myelination for kitten eighth nerve. Sweitzer & Cant (25) have documented the same pattern with respect to eighth nerve penetration of the dorsal cochlear nucleus in the hamster.

Thus, there seems to be a spatio-temporal pattern of development which is pervasive across virtually all vertebrates with elongated cochleas (basilar papillae). Further research aimed at documenting this pattern or its exceptions in the cochlea or in the nervous system will be of value. Of much greater importance, however, will be research addressed at understanding the gradient itself. Although it appears to be a universal principle of cochlear ontogeny, we remain totally naive as to how it is established, how it is regulated in the developing organism, or its importance for the establishment of adult structure and function.

HOW DOES THE DEVELOPING COCHLEA WORK?

Much developmental research is aimed at showing how the immature auditory system differs structurally and functionally from that of the adult. Yet we often make the assumption that major principles of cochlear organization and information processing do not differ. Developmental investigations aimed at elucidating the emergence of these major operating principles present a challenge for future investigation. Translated into practical terms, this means that the developmental events and mechanisms underlying the fundamental principles of cochlear function remain largely unknown. A few examples are considered below.

Place principle.

The brilliant contribution of von Békésy, showing that there is an orderly sequence of frequency selectivity along the length of the basilar membrane (the place principle) is one of the foundations of modern hearing science. While species differ radically in the frequency range to which they are responsive, it has generally been assumed that the frequency organization of an individual is relatively constant throughout its life span. A paradox between the basal to apical development of the inner ear (discussed above) and the generally low to high frequency ontogeny of frequency sensitivity (see 1) led us to question this assumption and examine the representation of frequency along the basilar membrane and in the brain stem nuclei during hearing development.

Two experiments showing that there is a systematic shift in the frequency code along the basilar membrane during hearing development were recently reported (26, 27). In the first experiment, high intensity pure tones were used to produce localized damage to the basilar papilla (cochlea) of chicks at three different ages. There was a systematic developmental shift in the position of damage produced by each of the acoustic stimuli. With each frequency the region of damage shifted apically as a function of age of the animal at the time of sound exposure. In the second study, we examined the tonotopic organization of second and third order neurons in the brain stem auditory nuclei of embryonic and hatchling chickens using microelectrode mapping procedures. Again, the results indicate that the frequency organization of the cochlea is shifting during development such that regions responsive to high frequencies in adults are maximally sensitive to much lower frequencies in neonatal animals of the same species. As the basal region becomes respon-

sive to high frequencies, progressively apical regions become tuned to the lower frequencies.

These results are of interest for several reasons. First, they provide a mechanism to explain heretofore conflicting results regarding development of the auditory system in a variety of birds and mammals. Secondly, they suggest that the place code is not fixed, but changes during the development of hearing, and continues to change after thresholds to low and midrange frequencies are adultlike. One wonders if it also changes at other times in the life span, for example, during periods of stress or during aging.

There is now considerable evidence that developmental changes in the place code are similar in mammals (3), yet several important problems remain to be investigated. For example, what is responsible for these changes? Are they strictly due to mechanical changes in basilar membrane motion as a function of mass and stiffness of the elements, or are neuronal changes involved? What is happening at the level of individual hair cells? Are they changing the frequency to which they are selectively tuned and are there concomitant structural changes—for example, in stereocilia? Finally, what is the time course of these changes in human babies? Does it occur entirely before birth or include the postnatal period?

Intensity coding, frequency coding and temporal coding

It is generally assumed that the intensity of a stimulus is represented by amplitude and breadth of basilar membrane motion, and therefore, by the summed change in activity in the auditory nerve. It has been recognized that young animals have a much more limited dynamic range and the shapes of input-output functions are often different from those of adults. Is this merely because they are less sensitive or do the principles of intensity coding differ as a function of development? In this regard it is interesting to note that Moore and his colleagues have shown that neonatal rate-intensity functions do not parallel adult functions.

Similar questions arise regarding frequency specificity and with respect to temporal coding phenomena such as phase locking or the ability to follow rapidly presented stimuli. In most cases adult values seem to mature well after absolute thresholds reach mature levels. Temporal correlations between these events and structural changes in innervation of inner and outer hair cells by afferent and efferent processes have led Pujol and others to derive important hypotheses about the role of these structures for the development of mature function. For example, the competition and redistribution of synapses on outer hair cells (see Pujol, this supplement) is likely to have important functional consequences. However, as noted by Pujol (and above), there are a large number of temporally related structural changes and are likely to be an equivalent number of biochemical changes (e.g. in transmitters) occurring over roughly the same period. Although such temporal correlations are provocative, they are not sufficient for drawing firm conclusions. Other research strategies must eventually be used. One such strategy that may deserve more attention is that of comparing the result of similar manipulations in animals of differing ages. For example, there are experimental (and eventually genetic) ways of eliminating particular elements in young and old animals. If such elements are assuming different functional roles as a function of age, then the immediate functional effects of their elimination should differ over a similar time course as the temporal correlations noted above. Some caveats to this strategy are noted in a later section.

WHAT DOES THE BABY HEAR?

There is overwhelming evidence that the human baby is capable of hearing *in utero* (see Granier-Deferre et al., this issue). By 26–28 weeks, the human cochlea has achieved a

developmental status comparable to that found in other mammals when responses to sound can be readily evoked. At about this time, physiological and behavioral reactions to loud sounds have also been reported. Thus the normal human baby is capable of hearing for at least the last 2 1/2 to 3 months before birth. Two of the most important challenges facing hearing science are the design and execution of experiments to determine: (*a*) what the human baby is hearing; and (*b*) what function this serves for the developing auditory system and the infant as a whole.

In utero sound environment

There are now several studies that have measured the sound environment that surrounds a baby *in utero*. While the results differ in detail, all agree that there is considerable noise from internal factors (e.g. maternal heart beat, swallowing, etc.) as well as rather good transmission of low frequency environmental sounds. While further experiments are needed which evaluate the frequency-intensity characteristics of intrinsic sounds and the spectral properties of transmitted environmental sounds, it now appears that the human baby is continually exposed to audible levels of low frequency sounds.

Transfer functions

It is now quite clear that the human baby's auditory system is capable of hearing both intrinsic sounds and sounds from the mother's external environment. Thus stimuli of sufficient intensity and appropriate frequency are present for what psychologists might consider an adequate, if not enriched, *in utero* sound environment. As a result of these facts and the literature on experiential influences on brain development (see below), there has been considerable speculation about the role of fetal auditory experience in brain development and perceptual development. An implicit assumption is that peripheral auditory structures obey the same general principles and have similar functions in fetal and postnatal humans. The laboratories of Saunders, Popelka and others, have made excellent progress toward understanding postnatal ontogenetic changes in middle ear function in animals and man. Application of this work to fetal hearing is again based on the assumption that these structures have similar functions and operating principles in the human fetus. These assumptions are probably incorrect.

The human fetus cannot be considered a terrestrial organism nor a terrestrial organism living under water. For the purpose of understanding hearing in the human fetus, it must be considered an aquatic vertebrate. Thus, principles underlying the conductive aspects of sound transmission are likely to be quite different—possibly more like those of fish than those of a terrestrial mammal. In the 7–9-month-old fetus, both the external ear and middle ear are filled with amnionic fluid. Since the soft tissues are probably at nearly the same specific gravity as the fluid medium, I expect that the tympanic membrane will be relatively transparent to sound. If this is the case, then pressure waves in the medium will not be transformed into motion of the tympanic membrane and subsequent amplification by the ossicular chain. Thus, the role of middle ear structures in the fetus may be qualitatively different than in the newborn.

This is not to be taken as a suggestion that middle ear structures are irrelevant for fetal hearing or that there is no amplification mechanism or that the human fetus is not hearing. Quite the contrary, many hypotheses are available from a consideration of fish hearing. For example, the ossicles may be responsible for an inertial lag that results in a pressure differential at the oval and round window. My purpose here is not to propose mechanisms. It is to emphasize that without knowledge of the middle ear transfer function in the fetus, it is virtually impossible to estimate what the human baby is hearing *in utero*. One approach toward solving this problem involves simultaneous recording of cochlear microphonic or

N_1 responses and *in utero* sound pressure. Such studies, carried out using a large ungulate (such as sheep, in which the fetus also hears *in utero*), will provide a very important and lasting contribution toward our understanding of human fetal hearing.

At this time, laboratories throughout the world are investigating the physiology of neurons at each level of the developing auditory system (see Moore, and Ehret, this supplement). These attempts to determine the developmental course of the transfer functions within each part of the central auditory pathways (usually considered the development of coding) will also be of considerable importance. Certainly the maturation of coding within the auditory pathways must be related to cochlear changes and to changes in the microcircuitry of the neuronal connections; and it must somehow underlie the ontogeny of hearing. Yet, to actually relate developmental changes in the physiological response of neurons to the ontogeny of hearing perception remains difficult for several important reasons. First, for most animals, including humans, we have neither adequate data nor methodologies for evaluating perceptual development, especially beyond estimation of thresholds for sound detection (see Ehret and Weir chapters and 28). Much more work is needed to develop reliable methodologies for perceptual testing in developing animals and humans. In my opinion, suppression measures such as we have used (18, 29) or reflex inhibition procedures (30, 31) hold considerable promise. The second difficulty facing attempts to relate neuronal response properties to perceptual ontogeny involves our general lack of adequate algorithms for understanding the neural basis of behavior. What are the appropriate criteria for concluding that a change in the coding properties of neurons are responsible for the development of perceptual ability?

Prenatal testing

Is prenatal testing of hearing important or even desirable? As noted above, at this time it is not clear *what* the baby is hearing *in utero*. It is also not clear why it is hearing. On the other hand, development is an energy intensive process and a functioning auditory system must use considerable metabolic fuel. Furthermore, both clinical and experimental investigations suggest an important role of early hearing for normal maturation. Thus, on at least teleological grounds, we are prone to conclude that prenatal hearing serves an important role in normal development. That role can only be conjecture at this time.

ROLE(S) OF "EXPERIENCE" IN AUDITORY SYSTEM DEVELOPMENT

Current interest in the relationships between experiential events, central nervous system development, perceptual development and language is tremendous. The research problems are of unmistakable importance for clinical and sociological reasons, for understanding the fundamental factors that influence brain development and as possible clues to the mechanisms of learning and memory. There are many indications that experience does play important roles in auditory system ontogeny. For example, consider our inability to hear subtle (sometimes not-so-subtle) differences in a foreign language or distortions in the speech patterns of most congenitally hearing-impaired individuals. In addition, there is vast and important literature concerning the neural consequences of visual deprivation, various reports of language disturbance or retardation following chronic otitis media, and the opinion of most professionals that early intervention with amplification or bone vibration is important for congenitally deaf babies. Unfortunately, and despite this intense interest, there are only a handful of well controlled studies examining the neural or behavioral effects of auditory deprivation. The purpose of this section is not to review this literature nor is there a need to stimulate interest. Instead, the current status of the experimental literature will be summarized from the point of view of this author. In this way, I hope to indicate areas in need of further documentation, areas of overinterpreta-

tion, and some of the pitfalls we have encountered in the past 8 years while attempting to carry out such studies.

Afferent influences on the auditory system

Removal of afferent input to the central nervous system from the cochlea has an immediate and profound effect on neurons in the brain stem auditory regions, particularly in young animals. This is easily demonstrated by removing the cochlea or severely damaging the hair cells followed by physiological or anatomical analysis of neurons in the auditory pathways. For example, in rodents immediate effects of cochlear removal on glucose metabolism and on synaptic morphology have been shown; long term effects on neuronal survival, cell size, dendritic morphology, axonal pathways and response properties to stimulation of the opposite ear have been indicated. The chicken brain stem auditory system also provides an excellent preparation for such studies. We have eliminated afferent input from the cochlea to the brain by a variety of manipulations including receptor removal, high intensity sound damage or ototoxic drug damage. Immediate effects on glucose uptake, succinate dehydrogenase activity and protein synthesis have been shown (3). Long term effects include transneuronal degeneration, neuronal atrophy, dendritic size changes and sprouting of axonal projections. These changes seem to begin immediately. For example, even these "long term changes" can be seen within 2–4 days, and alterations in the size of individual dendritic trees can be recognized within 2 hours of the time that their synaptic input has been silenced. While some of these changes may be due to the elimination of "trophic substances", their rapidity suggests that presynaptic activity is also playing an important role in the regulation of postsynaptic neuronal structure.

In summary, there is ample evidence, at least in young animals, that afferent innervation exerts a powerful effect on the development, maintenance and metabolism of postsynaptic neurons. While the cellular mechanisms responsible for this influence are not known, at least some of the effects appear regulated by the afferent synaptic activity.

Experiential influences on auditory system

Various experimental methods have been used to produce a conductive hearing loss in animals or to deprive them of sound exposure during development. These have included raising the animals in sound proof rooms with avocal mothers, removing the anlagen of the external ear causing an atresia, using an ear plug of known acoustic properties, suturing the ear canal closed, and rearing the animals with white noise or clicks.

Although they are not usually stated explicitly, the objectives of such manipulations seem to fall into 3 categories. *First*, in some cases the aim is to remove or distort the informational acoustic properties and determine if there are any observable abnormalities in the CNS. *Second*, some studies endeavor to disrupt the normal symmetry of neuronal activity from the two ears by performing monaural manipulations and then determine if there are asymmetries in the central nervous system. Assuming that the manipulations do not result in damage to the cochlea (which may not be an entirely safe assumption in all cases—see below), the interpretations based on the above objectives are reasonably straightforward. A severe conductive loss or masking probably does eliminate most or all informational acoustic features and, barring adaptation to the treatment, monaural occlusion probably disrupts the normal bilateral symmetry of activity. Thus, conclusions regarding the necessity of acoustic information or bilateral symmetry for normal neuronal development are warranted. Such a conclusion may be sufficient for some experimental or clinical objectives.

At a mechanistic level, however, neuronal changes due to an altered acoustic environ-

ment must be due to a change in the normal amount or pattern of activity in auditory nerve fibers and a resultant change in the neurons under consideration. Thus, the *third* objective of environmental manipulation experiments is to determine how neuronal activity influences the development of postsynaptic neurons. Here the immediate effects of the manipulations, their long term effects, and the neuronal mechanisms responsible for postsynaptic neuronal changes are not nearly as straightforward. Consider some of the following questions for which we simply do not have answers at the present time. It is often assumed that a conductive hearing loss produces an immediate and lasting reduction in neuronal activity. Is this so? Does it depend on the ambient noise in the environment? Will the auditory system adapt so as to maintain some mean "spontaneous level"? Can the manipulations alter the reception of internal sounds or bone conducted sounds? Will other inputs to the neurons under investigation be altered and how will this affect activity levels? While these and other important questions about the nature of the manipulations remain to be answered, there are indications that they do warrant serious concern and detailed examination. For example, in a recent study the acoustic environment was very well controlled and a calibrated, monaural 40 dB, flat conductive hearing loss was produced (32). A careful analysis of dendritic alterations revealed unexpected differences between cells as a function of their tonotopic position (best frequency). In our opinion, the most parsimonious interpretation of these results was that a flat conductive loss produced very different effects on the ongoing neural activity from the low frequency and the high frequency regions of the cochlea.

Another concern about environmental manipulation studies is the possibility of sensorineural involvement, either directly or secondarily. Most experiments simply have not rigorously examined this possibility. Furthermore, it is not certain what criteria are sufficient for concluding that sensorinerual damage of cochlear or ganglion cell origin has not been produced. In most cases, the assertion of a pure conductive loss is based on the nature of the procedure initially used to manipulate acoustic input. However, it is entirely possible that prolonged deprivation or masking causes receptor damage, either through a direct action or some stimulation-dependent mechanism. It is somewhat surprising that clinically we have well established procedures for determining if a hearing loss is purely conductive. Yet these same criteria have not been applied to experimental animals, even though it is quite possible to determine evoked potential thresholds to bone conduction. It seems that this should be a minimum requirement for such investigations unless reversible procedures are used (see 18, 33). Clearly, more sophisticated and multiple criteria for the analysis of cochlear integrity should be developed.

A word of caution is also in order regarding the use of pure-tone or narrow band rearing conditions. If, as suggested earlier, the response properties of neurons are changing during development, the specificity of this manipulation may be less than expected. Issues of environmental control and receptor integrity have recently become of considerable concern. Recent attempts in our own laboratory to replicate the results of acoustic deprivation studies have been disappointing. For example, in one very large study we raised animals with a 40–50 dB monaural hearing loss and examined cells in the cochlear nucleus 15–45 days later. When the deprivation procedure (removal of the ossicle) produced no detectable sensorineural hearing loss as determined by bone conduction thresholds, we observed no changes in cell size in the cochlear nucleus. On the other hand, when ossicle removal was accompanied by an oval window fistula, changes in cell size were reliably obtained (Tucci and Rubel, unpublished observations). In addition, careful comparison of our results with those from other studies indicates that changes in neuron size following complete destruction of the cochlea or hair cell damage are comparable to those seen following "deprivation".

The intense interest and clinical importance of this area of investigation can not be overstated. We are facing a worldwide effort to increase and improve early intervention procedures for use with hearing impaired children. The results of well controlled experiential manipulation studies can provide valuable information on the timing and procedures for optimal outcomes. Thus major emphasis should be placed on studies which endeavor:

1. To determine the short term and long term effects of various forms of "environmental manipulations" on levels and patterns of neural activity; and
2. To determine how neuronal structure and function are regulated by the amount and pattern of presynaptic activity.

Experiential influences on behavior

Behavior is often more difficult to study than sensory organs or the brain. This is especially true when applied to development; the reasons are simple. If careful, most of our analytical procedures and gadgets can be applied to developing tissues. But adequate methods to study the sensory and perceptual abilities of humans and young animals with changing motor patterns and learning abilities must be invented. Many of the difficulties are discussed in other chapters in this issue (see contributions by Ehret and by Weir) and in a recent book (28). Nowhere are these difficulties more apparent than when we examine what is known about experiential influences on perceptual development.

Two approaches can be noted. It is clear that they should be related, but at this time the nature of this relationship is obscure. One is the sensory-psychophysical approach; the other is the naturalistic approach. The former (e.g. see 18, 29) tends to use parametric variations of simple, artificial stimuli in order to ascertain the effects of experience on the ability to resolve and selectively respond to various acoustic parameters. The naturalistic approach, on the other hand, tends to use natural stimuli and the species-typical behavior patterns which they evoke; the goal being to discover how the species-typical perceptual abilities and behavior patterns are influenced by an organism's interaction with its evironment. This approach is exemplified (and almost defined by) the extensive research program on the development of species-typical perception of maternal vocalizations in ducklings by Gottlieb (see summary in 28). Obviously, the sensory abilities must provide building blocks for species-typical perceptual behaviors, but the relationship between these approaches and early experience has not been documented in any organism as yet.

Other factors

There are likely to be a host of other factors important for understanding experiential influences on the auditory system. Some are likely to be age, genetical background, nutrition, and hormones. Important clinical concerns such as the reversibility of deprivation effects also remain largely uninvestigated at this time. However, until adequate models are developed and the fundamental rules governing normal and abnormal development are discovered, these issues are of secondary importance.

CRITICAL PERIODS: IDENTIFICATION VS. UNDERSTANDING THEIR BIOLOGICAL BASIS

Embryological use of the term "critical period" refers to a restricted developmental time when a particular tissue interaction must occur in order for a subsequent developmental event or structure to emerge. The "critical periods" discussed below (and by Uziel, this issue) do not fit this definition. First, they represent a graded phenomenon that is likely to be qualitatively different from the inductive events referred to by geneticists. For this reason, they are more appropriately referred to as "sensitive periods" or periods of

supranormal sensitivity. Second, in the formal sense, a critical period refers to a specific tissue interaction. In the experiments discussed below, a specific manipulation was performed at different times of development. In most cases it is not clear that identical tissue interactions take place. For example, consider aminoglycoside ototoxicity (see chapter by Uziel). If we consider the organism as a single black box, then there is a "critical period" for ototoxicity since the same dosage will have differential effects as a function of age. However, if we consider the cochlea or the hair cell as the tissue compartment (black box), then we must provide evidence that the levels of aminoglycoside reaching this compartment (the manipulation) was similar across ages before drawing conclusions about differential sensitivity. While clinical implications may not be affected by such a distinction, it becomes critical when we attempt to understand the mechanisms underlying differential susceptibility of young organisms to aminoglycoside ototoxicity.

Development of the cochlea

Periods of increased susceptibility to ototoxic aminoglycosides and noise trauma have been defined in rodents and cats (34; Uziel, this issue). While there seems to be general agreement that the beginning of susceptibility corresponds to functional development (for aminoglycosides) and to the attainment of near adult thresholds (for noise trauma), there does not seem to be agreement regarding the termination of such periods. In fact, Henry (35) has recently concluded that the period of increased susceptibility depends on the frequency at which thresholds are evaluated, as well as on species and age. Furthermore, evidence published to date indicates that sensitive periods for drug and noise ototoxicity do not correspond.

The clinical importance of these phenomena certainly warrant further attention. Clearly, the results to date suggest that care and frequent monitoring of auditory function in neonates exposed to noise and/or aminoglycosides is important. Yet the apparent variability of these results across species indicates that direct prediction of the period of hypersusceptibility in humans is not possible at this time. Nor can we predict the shape of the susceptibility function in any animal as a function of both age and treatment level. In order to remedy this situation, large scale parametric studies in an animal model are needed. Such data will provide the information needed to set up appropriate clinical evaluation procedures. In addition, some method to estimate the relevant periods in humans must be determined.

It is worth stressing that the demonstration of a sensitive period or its identification in humans is not an end in itself. While important, it is a restatement of the time relationship and, by itself, tells us nothing about the mechanisms underlying susceptibility to noise or drug damage. Identification does, however, provide a valuable approach to the general problem of ototoxicity. Identification of the biological substrates underlying differential sensitivity may provide important clues toward understanding both the mechanisms of toxicity and factors responsible for the *relative* immunity before and after the sensitive periods. The major challenge facing investigators in this area is identification of the biological differences underlying differential susceptibility. Their identification will allow us to predict comparable periods in humans and may provide important clues toward prevention of ototoxicity in the future.

Central nervous system

Developmental differences in the response of the central nervous system to peripheral manipulations also have been noted by many investigators. Both deprivation and peripheral ablation appear to have a greater effect on young animals than adults. Once again, however, little additional information is available; the nature of these differences is not

known. For example, it has been shown that in mice, gerbils and chicks removal of the cochlea in young animals results in severe transneuronal degeneration, whereas there is little or no change in adults. It has been assumed that this differential susceptibility corresponded to developmental changes in the auditory system, per se. Recent results from our laboratory have prompted a reconsideration of this assumption. In the chick, the auditory system is very mature at hatching and adult-like in every way we have evaluated by 10–15 days after hatching. However, cochlea removal in embryos, 1-week-old and 6-week-old chicks caused similar transneuronal changes (cell loss, cell atrophy, changes in SDH activity and decreased protein synthesis) whereas there were essentially no effects from the same manipulation in 1-year-old chickens (3). Thus the sensitive period does not seem to correspond to any features specific to auditory system development. Possibly, general systemic factors such as circulating hormones are causing differential susceptibility in young animals. Again, further experiments aimed at determining whether there is differential sensitivity as a function of age will be of little interest: however, attempts to define the biological mechanisms underlying such phenomena will have vast importance for both clinical practice and fundamental neurobiology.

Hearing and language

One objective often cited for examining the age dependent effects of peripheral manipulations on the nervous system is to understand the mechanisms responsible for "critical periods of hearing and language development". While there seems to be a consensus that such periods exist, hard facts regarding their nature are difficult to find. That is, it has proven difficult to derive parametric information on *what* aspects of perceptual and language development are dependent on early experience and on the nature of such experiential components (see again the experimental work of Gottlieb and the literature on bird song). Certainly, without precise information about these behavioral effects of early auditory experience, we have little hope of understanding the underlying neuronal mechanisms. Even then, the precise strategies for establishing a causal relationship are far from elementary.

In summary, we have established that there are several "sensitive periods" in auditory development. Over the next decade additional examples of age dependent differential sensitivity will likely be discovered. Parametric data on these phenomena and their reversibility will have important application for the design and establishment of intervention programs. A more difficult objective is to understand the cellular nature of the differential sensitivities. What, in the complex internal environment of the developing organism, causes it to be more susceptible to noise, aminoglycosides or peripheral manipulations and, of even greater importance, what is responsible for the relative immunity of the mature organism?

CONCLUSIONS

Investigations of auditory system ontogeny provide information concerning the regulatory events responsible for normal development. They can also elucidate the mechanism of pathology and, by providing a natural experiment, are a powerful way of elucidating normal structure–function relationships. There are, of course, many practical problems (such as communicating their importance to other clinicians and to parents, and developing simple screening procedures) that have not been considered. Instead, I have tried to outline some of the fundamental information that we lack, and consider some of our current research strategies.

The clinical importance of obtaining answers to fundamental problems in hearing

development and pathology cannot be overstated. In the past half century, progress in engineering, electronics, biochemistry, genetics, physiology and pharmacology have provided hearing scientists with an amazing armament of tools. Thus, we can record from or stimulate tissues in almost any manner desired, we can image small parts of the nervous system and study ionic composition at a micron-by-micron level, and we make virtually any compounds and apply them locally or systemically. These techniques are only going to improve. In my opinion, the technology is currently ahead of knowledge. Future advancements in otology must be based on a greater understanding of the mechanisms responsible for normal hearing function and the fundamentals of pathophysiological conditions. Fundamental studies of hearing development in well-conceived animal models and human babies will provide some of these principles. When this happens, advancement toward national and international goals of improved medical effectiveness will dovetail with the goals of natural science. As we come to understand normal function and pathophysiology, we will be able to utilize available technology for the effective treatment of hearing disorders.

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