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Commentary

Choosing Axonal Real Estate: Location, Location, Location

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In this issue of *The Journal of Comparative Neurology* (pages 6–27), Leake, Snyder, and Hradek examine the sequence of events whereby axons from spiral ganglion cells establish precise tonotopic maps in the three subdivisions of the cochlear nucleus (CN) of the kitten. The careful, quantitative work of this group provides some important new insights about the formation of sensory maps in the developing brain. Three take-home lessons are to be learned. First, comparison of this work with studies on the development of retinotopic maps make it unclear whether similar developmental sequences of events occur. If the events are similar, then we might hope to find similar mechanisms. If the sequences of events are dissimilar, then it is highly unlikely that the developmental principles and mechanisms will be the same. Second, single auditory nerve axons must form three distinct and separate tonotopic maps with three different target cell groups (divisions of the cochlear nuclei) that have quite different cytoarchitectures. It is of interest to ask if the process has the same characteristics in each region. The answer appears to be yes, although small differences in timing may occur. Finally, their careful quantitation allows an evaluation of the *relative* contributions of initial specificity and subsequent growth vs. overproduction and activity-dependent (or activity-independent) pruning of terminal arbors. Here the lesson appears clear: neighbor relations are established early and maintained.

Topographic maps are a fundamental organizing feature of the nervous system. In most sensory systems, the neighbor relationships of receptor cells in the sensory epithelium are preserved in a series of projections into the central nervous system, and throughout both ascending and descending pathways. Similarly, efferent projections have motor maps related to muscles, and spinal cord motor pools are ordered with topography relative to muscles. Because these maps were first discovered during the middle of the past century, two questions have dominated much of developmental neurobiology.

1. Are there common sequences of events that characterize the formation of topographic maps?
2. Are there common developmental principles and molecular mechanisms that are used to set up and maintain topographic maps in different sensory and motor systems and in different species?

Empirical research on these issues has focused on: (1) developmental changes, or lack thereof, in the specificity of the maps; (2) the roles of afferent input from the periphery in the development and maintenance of the maps; and (3) molecular cues that establish precise synaptic connections.

The best-studied topographic map, the retinotectal map, has been examined in a variety of vertebrates, including amphibians, birds, fish, and several species of mammals. The morphology and positional specificity of both developing and regenerating retinal ganglion cell axons terminal arbors have been extensively studied and reviewed (Cline, 1998; King, 1999; O'Leary et al., 1999; Loschinger et al., 2000; Udin, 2000; Thanos and Mey, 2001). Even in this well-studied system, there is little agreement about whether there is a common sequence of events that characterize formation of the map. The answer seems to depend on the methodology used for labeling axons, the methodology used for analysis of the tissue, the class of vertebrate studied, and even the individual species. Some studies report that the precision (grain) of the topographic map is initially as precise as it will ever be, whereas others assert that the precision improves markedly during the early stages of functional development. Even within mammals, a unified description of the events does not seem to be possible. For example, Simon and O'Leary (1992) indicate that neonatal rat retinal ganglion cell axon terminal arbors initially branch widely over the tectum, forming an "initially diffuse projection". It then becomes much more precisely topographically aligned due to elimination of errors, pruning of axon branches in "topographically inap-

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propriate positions along with expansion of the terminals in appropriate positions." On the other hand, Chalupa and Snider (1998), investigating development of retinocollicular projections in embryonic and postnatal ferrets, report that the specificity of projections is nearly perfect from the time retinal fibers reach the tectum. Similar differences in results or interpretation can be found in the literature on other vertebrate classes. It appears that the developmental studies of retinotectal and retinogeniculate topographical map precision can be summarized as follows. From the time axons first grow toward their target, there is at least a rough topographic mapping, in that there is a significant correlation between the location of the ganglion cell body and the position along the target in which axons first begin to form terminal arbors. Early maps of terminal arborizations demonstrate somewhat more overlap in their positions than mature maps, but this is always due, in part, to the small size of the embryonic or neonatal target, compared with the adult. During maturation, axon arbors expand to form a dense array of endings and usually grow in absolute size. During this period, axon arbors may also be spatially refined, pruned, so that they occupy only the appropriate territory within the target.

It is generally agreed that activity (as defined by action potential generation) is not involved in the initial topography (ordering) of projections from the retina to the tectum or in the initial formation of other sensory maps. That is where agreement ends. Several studies have provided convincing demonstrations that refinement of the topographic projection from the retina to the tectum or, more commonly, maintenance of the specificity of terminal arbors, requires neuronal activity. Such conclusions are usually based on the finding that chronic activity blockade increases the number of "mistakes" made by retinal axons, or the overlap of the projections from adjacent receptor sites, or the absolute size of terminal arbors. In the examples where activity is thought to be essential for the initial establishment of mature specificity in the retinotectal map or other retinotopic maps in the visual system, the timing appears to be before maturation of the photoreceptors and light-evoked responses. Hence, it is usually thought that the relevant activity is independent of information from the external environment. As an aside, it is worth pointing out that hypotheses about the role of afferent input (experience) in map formation did not begin in the past decade or two. For example, Paul Weiss called such a role "restrictive individuation," and the late Marcus Jacobson dubbed it "functional respecification."

The auditory system provides another opportunity to address these questions because of its precise topographic organization. Each auditory nerve fiber is thought to form terminal arbors in each subdivision of the CN, thus establishing three separate maps of the receptor surface: in the anteroventral, posteroventral, and dorsal cochlear nucleus. Comparison of the relative precision and timing of map formation in these three areas may shed light on generalizable events. In addition, because spiral ganglion cells are of placodal origin and directly contact the receptor cells, i.e., hair cells, this system may provide less ambiguity than that seen in the visual system, with relative heterogeneity found in retinal ganglion cells. The present study by Leake, Snyder, and Hradek, along with their previous studies (Snyder and Leake, 1997), represent the first thorough examination of the ontogeny of topographic precision of connections between the cochlea and the cochlear nucleus known to us. In these two outstanding sets of experiments, the authors made small injections of Neurobiotin into the basal region of the spiral ganglion in perinatal and neonatal kittens. They then evaluated both the size of the cochlear region contacted by the labeled axons and the position of terminals in each subdivision of the cochlear nucleus. Careful, objective quantitative evaluations of the projections and of the postnatal growth of the cochlear nucleus allowed the authors to relate both the *absolute* size and the *relative* size of projections into the cochlear nucleus of neonatal and adult cats before, during, and after the development of auditory function. Accepting a variety of reasonable assumptions about the injection, the analysis methods and the growth and orientation of the cochlear nucleus, the results can be summarized as follows. The earlier study (Snyder and Leake, 1997) showed that the mature precision of tonotopic projection is observed as early as postnatal day 6 (P6), when hearing is still very immature; thresholds are at or above 100 dB SPL, and there are few if any spontaneous action potentials seen when microelectrode recordings are made from the eighth nerve. In the experiments presented in this issue, they have examined the projection at earlier times (2 days prenatal to 3–4 days postnatal). The major results are as follows.

1. At all ages, the projections are highly precise and topographically organized;
2. From birth to adult, the *absolute* size of the projections from a given area of the cochlea actually grow in extension along the tonotopic axis by approximately 25%, whereas comparable measurements of the size of the target regions of the cochlear nucleus grew by approximately 45%;
3. The *relative* size of cochlear nucleus projections from a given area of the cochlea are 32–50% larger in the perinatal kitten than expected on the basis of relative size of the CN (i.e., the projection is less precise earlier in development than in the adult).

These results were supported by double injections of the tracer in some perinatal animals, and there was an indication that the three divisions of the cochlear nucleus may vary in their rate of maturation.

Hence, the results presented here by Leake and colleagues suggest that axonal projections from the cochlea to the brain undergo refinement of the topographic precision, pruning, early in development, similar to findings reported in the visual system of several species. Two questions naturally arise. Are the underlying principles and mechanisms similar in the two sensory systems? What might be the functional significance of the developmental changes described? Although neither of these questions can be answered, speculation is certainly warranted.

POTENTIAL PRINCIPLES

To understand whether the perinatal enhancement of topographic specificity described by Leake and colleagues is mechanistically similar to developmental changes seen in retinal projections to the brain, comparisons at several other levels should be addressed.

The dynamics of synapse formation present an interesting problem in the formation of topographic maps. Leake et al. showed that axonal projections from a given region of the spiral ganglion grow in absolute size over time, but

at the same time, they constitute a proportionally smaller region within the overall projection to each division within the cochlear nucleus. From these data on the projection patterns of small numbers of ganglion cells it may be reasonable to extrapolate to single axon arbors. The data would thus suggest that individual arbors grow during development, but do not expand as much as the total extent of each cochlear nucleus division. These unequal rates of expansion alone could account for the improvement in topographic precision. In other words, the data suggest, but do not prove, that the axons make synaptic contact with a relatively broader area of the cochlear nucleus subdivisions at early postnatal ages than at later ages. At this time, we have little information on the extent of rearrangements of synapses during this period of development in the kitten, nor do we know whether this rearrangement involves synapse elimination (Jackson and Parks, 1982; Ryugo and Fekete, 1982). As the authors suggest, cell death in the spiral ganglion might also account for the changes observed.

Although these dynamics are not fully understood in either retinotopic or tonotopic maps, some interesting insights can be learned from neuromuscular projections. The projections of some motor pools to individual muscles are topographically organized. For example, the projection to the diaphragm and the serratus anterior muscles have an orderly innervation such that neighboring regions within the motor pool in the spinal cord innervate neighboring groups of muscle fibers within the muscle. In mammalian skeletal muscle, fibers are polyinnervated at single endplates, and excess terminals are removed in an activity-dependent process until exactly one input remains at each endplate. In topographically innervated muscles, the initial innervation has significant topography and refinement of topography to the mature state is the result of this synapse elimination (Laskowski and High, 1989; cf Cramer and Van Essen, 1995). Therefore, in this system, unlike most sensory systems, we know that synapse elimination accounts for refinement of the map after the initial topographic innervation.

Another level of analysis not experimentally addressed in this study, but clearly of interest, involves the potential role of activity (action potentials) in the immature spiral ganglion cells in the final stages of topographic precision. What little evidence exists suggests that the first auditory nerve synapses in the kitten cochlear nucleus form before the time of birth, before the functional maturation of auditory hair cells (D. Ryugo, personal communication; Rubel and Fritzsch, 2002). Furthermore, before the onset of acoustically evoked responses in the cochlear nucleus, spiral ganglion cells may exhibit low levels of spontaneous activity. Therefore, it is feasible that this level of activity could influence the cellular events necessary for refinement of the tonotopic map. On the other hand, it remains to be shown that this activity is required or even plays a "permissive" role in formation or maintenance of the map. Studies of spontaneous activity in the eighth nerve and cochlear nucleus of perinatal kittens find few axons with any spontaneous activity and in this minority of cells it is at a very low rate—under 10 Hz. From this information, one might conclude that it is unlikely that spiral ganglion cell activity is playing a major role in the formation of topographic specificity. However, it is unknown in any sensory system exactly how much activity is required and the biophysical/biochemical nature of the activity remains undefined. Although it has been postulated that the coincidence of propagated action potentials provides important information, one cannot rule out the possibility that very low levels of activity may be sufficient and/or that action potentials, per se, are not necessary or sufficient to provide the essential signals.

In addition to the plethora of studies addressing the role of activity in the refinement of retinotopic maps, there has been an explosion of new information and insights on molecular signaling mechanisms that might provide essential signals for the topography that is established as the retinal axons initially form a map in the tectum or lateral geniculate body. In the past few years, several molecules have been found that are expressed in gradients along topographic axes, and it is thought that combinations of these molecules are both necessary and sufficient to accurately specify targets (e.g., Yates et al., 2001). Whether or not similar gradients of these molecules are expressed in the developing spiral ganglia and cochlear nuclei remains to be determined and is the subject of ongoing studies.

As we consider the relative roles of chemospecific molecules and activity-dependent processes in the development and refinement of projections, it is important to note that in most systems examined thus far, the topography of projections is quite well established in the initial projection of axons onto the target, even in examples where the actual target cells continually shift during development. Subsequent events may fine-tune this topography. This has led to the common simplification that an initial "rough" topography is established by endogenous chemospecific molecular mechanisms and activity-dependent mechanisms then take over to form the finishing touches. It may be tempting to interpret the data presented by Leake and colleagues by this intuitive and easily digested two-stage model. Another view is that such a model is a gross oversimplification along several lines. First, as we will stress below, the initial topography of projections is by no means "rough". Second, there are clearly bidirectional interactions between neuronal activity and the molecules thought to influence axonal growth and positional specificity. For example, signaling through neurotrophins and other axon guidance molecules such as netrin-1 and myelin-associated glycoprotein (e.g., Ming et al., 2001) can be altered by activity. In addition, eph/ephrin signaling and neurotrophin signaling are involved in long-term potentiation (e.g., Gao et al., 1998; Xu et al., 2000; Murai and Pasquale, 2002), in which patterns of neuronal activity alter the strength of existing synapses. Moreover, nervous system injury, including CNS injury as well as peripheral axotomy, results in up-regulation of molecules involved in development of axonal projections. Thus, it may appear that activity, as defined by action potentials, has relatively little influence on the development of topography. However, it is entirely possible, even likely, that there is an ongoing interplay between the biophysical properties of axons and their target cells that influences the expression of growth and position signaling molecules; it is this interaction that drives the maturation of topographic maps throughout development. Conversely, many recent experiments have pointed out that it is also an oversimplification to consider patterns of action potential generation to be the necessary and sufficient events to drive refinement of topography that takes place during the final stages of topographic map formation. In fact, most of our experi-

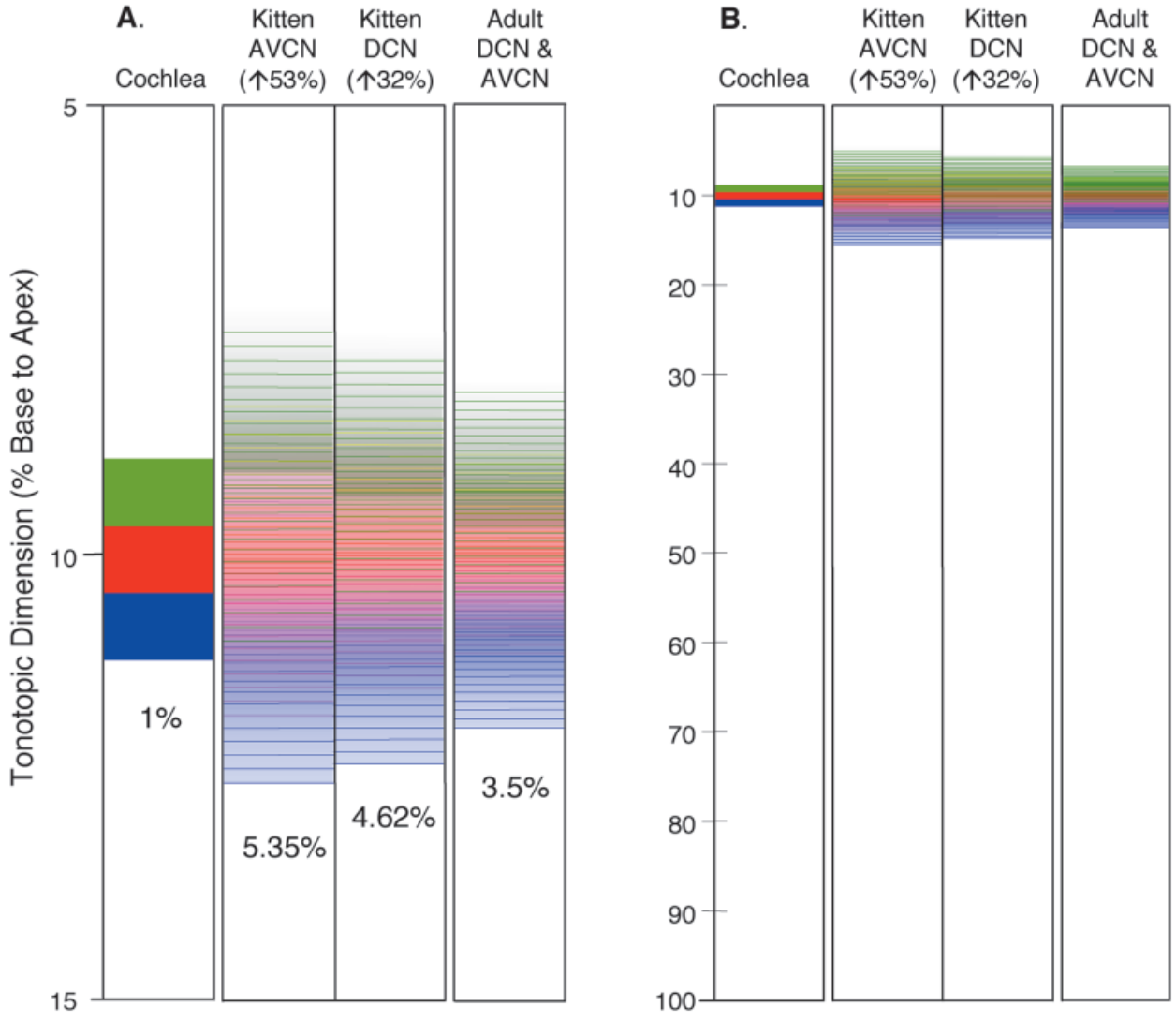

Fig. 1. Graphic depiction of the projection territory of small cochlear regions to the anteroventral cochlear nucleus (AVCN) and the dorsal cochlear nucleus (DCN) of the kitten and mature cat. **A:** Representation of the projection of a small area of the cochlea. **B:** Representation of the whole cochlea. Details are provided in the text.

ments do not allow such a conclusion. Although the existence and/or pattern of action potential generation may be shown important by a manipulation that disrupts activity, this is seldom done in a situation where the molecules known to be important for earlier stages of map formation are disrupted. An exception, although dealing with a somewhat different process (binocular segregation/integration), is recent elegant work on the interaction of form deprivation with mutations of molecules thought to be important for growth and topography (e.g., Pham et al., 2001).

SIGNIFICANCE OF TOPOGRAPHIC "PRUNING"

An outstanding, and relatively rare, feature of the contributions by Leake, Snyder, and Hradek is the careful and thorough quantitative presentation of results, allowing the authors, readers, and commentators to estimate the absolute and relative sizes of the developmental changes examined. As pointed out by the authors, the expectation that initial projections from the cochlea into the three divisions of the cochlear nucleus would be diffuse and nonspecific was not born out. In fact, as has been found in the vast majority of developmental studies of topographic specificity in sensory systems, the earliest projections are actually remarkably accurate. This appears to be the case in secondary and tertiary auditory projections as well (e.g., Young and Rubel, 1986; Sanes and Rubel, 1988). As found in most studies that have quantified terminal arbor size, Leake et al. stress that the absolute size of terminal zones actually increases quite dramatically during the period from birth to maturity. But, when the size of terminal regions labeled from a

discrete injection of tracer into the ear is normalized across age—when the projection size is "corrected" for the growth of the target region—it is *relatively* larger in newborn kittens than in the adult cats. These relationships are found to a differing extent in the three divisions of the cochlear nucleus. The *relative exuberance* reported in the immature projections varies from 32% in the dorsal cochlear nucleus (DCN) to 53% in the anteroventral cochlear nucleus (AVCN).

To exemplify the change in relative size and to provide perspective on its relevance to the overall topographic organization, we have used the data provided by Leake et al. to generate the simple models presented in Figure 1. The tracer injections made into the spiral ganglion resulted in labeling of approximately 1% of the length of the cochlea, which we show in the form of three adjoining regions near the basal end of the cochlea in Figure 1A,B (green, red, and blue bands centered at 10% of the distance from the basal end of the cochlea; each band represents 1% of the length). We then modeled the densities of terminals that might be seen in DCN and AVCN as Gaussian distributions of lines along the tonotopic dimensions of the nuclei in perinatal kittens (middle two columns in 1A,B), and in the adult cats (right columns). In both cases, the length of the tonotopic dimension of the nucleus has been normalized between the kittens and the adults. The only difference between Figure 1A and 1B is that only 10% of the total tonotopic dimension of the nucleus is shown in Figure 1A, whereas 100% is shown in Figure 1B. Figure 1A emphasizes the refinement, or "pruning"; Figure 1B emphasizes the remarkable degree of specificity as early as the projections are examined. Given the degree of specificity seen in the original axonal projections in this and other sensory projections, an important question for future investigations will be, what is the functional significance of the modest refinement observed? In other words, if the location is great, the house is about the right size, and the neighbors are friendly, why do **any** remodeling?

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