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CHEMOAFFINITY IN THE ORDERLY GROWTH OF NERVE FIBER PATTERNS AND CONNECTIONS*

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In early observations on the outgrowth and termination of nerve fibers, it appeared that different fiber types must be guided to their respective end organs and other connection sites by selective chemical or electrical forces. Explanatory terms like chemotaxis, chemotropism, galvanotaxis, and neurotropism were commonly employed by Cajal[7] and others early in the century. These selectivity concepts later came under attack, especially during the 1930's and 40's when the application of more analytic experimental approaches to the mechanics of nerve growth seemed to rule out the presence of either chemical or electrical selectivity in favor of a predominantly mechanical interpretation.[34, 37]

The numerous examples of apparent selectivity described earlier, as well as the developmental patterning of the central nerve tracts and fiber systems in general, we came to believe, were more properly and correctly explained on a mechanical basis, particularly in terms of the orienting effects of mechanical stresses on tissue ultrastructures and the resultant formation of submicroscopic systems of mechanical guide lines in the colloidal matrix of the growing medium.

At the height of this antiselectivity movement I was led, from evidence indirect and partly behavioral, to postulate again in 1939 a form of chemical selectivity in nerve growth even more extreme in some respects than in the earlier proposals. The hypothesis,[18–24] in brief, suggested that the patterning of synaptic connections in the nerve centers, including those refined details of network organization heretofore ascribed mainly to functional molding in various forms, must be handled instead by the growth mechanism directly, independently of function, and with very strict selectivity governing synaptic formation from the beginning. The establishment and maintenance of synaptic associations were conceived to be regulated by highly specific cytochemical affinities that arise systematically among the different types of neurons involved via self-differentiation, induction through terminal contacts, and embryonic gradient effects.

Coming at a time when "instinctive" was still a disreputable term in most scientific quarters, and when concepts of nerve growth were strongly dominated by the mechanical theory, this seemed a long shot at first and hardly less wild than some of the opposing interpretations of the day like the "resonance principle"[32, 33] that it was proposed to replace. When tested experimentally, however, study after study through the 1940's[18–24, 29] yielded results that fit nicely. In brief, whenever central fiber systems were disconnected and transplanted or just scrambled by rough surgical section, regrowth always led to orderly functional recovery and under conditions that precluded re-educative adjustments. The functional outcome was always *as if* the scrambled fibers somehow unsorted themselves in regeneration and managed to "home in" on their original and proper central nerve terminals.

It seemed a necessary conclusion from these results that the cells and fibers of the brain and cord must carry some kind of individual identification tags, presumably

cytochemical in nature, by which they are distinguished one from another almost in many regions, to the level of the single neuron; and further, that the growing fibers are extremely particular when it comes to establishing synaptic connections, each axon linking only with certain neurons to which it becomes selectively attached by specific chemical affinities.

This chemoaffinity theory included additional features such as the application of morphogenetic gradients in retinal, cutaneous, vestibular, and other systems to explain their orderly topographic projection and central representation, the patterning of central neurotization by peripheral induction, neuronal specification through synaptic contact, and related principles of growth and differentiation as applied to the specialized problems in the functional organization of neuronal connections in neurogenesis.[23–26] It carried previous conceptions of nerve specificity to a new order of refinement and put on a prefunctional chemical basis the ordering of the brain networks for inherited components in behavior.

Taken as a whole, the scheme offered an explanation of the developmental patterning of central nervous organization that seemed to have distinct advantages over alternative concepts applied previously, like "disuse atrophy," "neurobiotaxis," "mechanical contact guidance," "bioelectric fields," "autonomous differentiation of resonance scores," and "stimulogenous fibrillation." The chemoaffinity interpretation also fitted nicely with related developments in animal behavior and ethology on the one hand, and in experimental embryology and genetics on the other to bring together a number of loose concepts into a systematic approach to the inheritance and development of behavior patterns.

In spite of the attractions and the considerable supporting data, there have always been a number of persisting objections and gaps in the evidence to prevent our accepting the hypothesis completely. In the first place, we had never actually seen growing nerve fibers bypass a series of empty neuron slots to settle on their own proper terminals. This always had to be inferred indirectly, mainly from behavioral evidence. Moreover, this same behavioral evidence could be accounted for in other terms without recourse to all the postulated chemical affinities and without the assumption of selective reconnection—by schemes involving certain physiological coding and resonance phenomena that could operate in randomized nerve nets. The "resonance principle" of Weiss,[32, 33] which had remained for nearly 20 years the favored explanation of related phenomena produced by peripheral nerve derangements, was just such a scheme in which the growth of synaptic connections was conceived to be completely nonselective, diffuse, and universal in downstream contacts. Nothing in the evidence, including the postregenerative mapping data from electrical[9, 15] and lesion[21] studies on the optic tectum, could be considered critical in deciding between these alternatives; direct histological evidence was needed to settle the questions involved.

The chemoaffinity interpretation also met objections on the grounds that there are not enough distinct chemical labels available in the embryo. The scheme requires literally millions, and possibly billions, of chemically differentiated neuron types, each distinguishable from all others on the same side of the midsagittal plane. Each half of the nervous system is presumed to be a chemical mirror map of the other. This labeling problem, plus the further task of interconnecting in precise detail all the postulated millions of chemically specific neuron units into functionally

adaptive brain circuits, also seemed prohibitive from the standpoint of information theory because of a supposed lack of enough "bits of information" within the zygote to handle all the developmental decisions involved in building a brain on this plan.

Evidence obtained recently seems to provide a direct experimental answer to such objections. This has come in the past few years from histological studies started in 1958 on the optic system of fishes, in which I was joined in 1959–1960 by Dr. Attardi,[4, 5] and in the past year and a half by Dr. Arora.[1, 2] In brief, we think we have finally man-i aged to demonstrate quite directly by hstological methods the postulated selectivity in nerve growth and synaptic formation. The new evidence shows that fibers arising from different parts of the retina preferentially select separate central pathways as they grow into the brain, and that they eventually find, and connect with, specific predesignated target zones in the midbrain tectum.

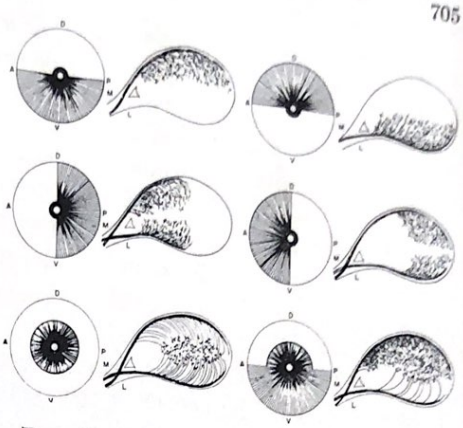

FIG. 1.—Diagrammatic reconstructions of regeneration patterns formed in optic tracts and tectum by fibers originating in different retinal halves, as indicated (after Attardi and Sperry[4, 5]).

In these experiments, the main optic trunk was severed in a rough manner to enhance the inevitable scrambling among the hundreds of thousands of constituent fibers. The corresponding eye was then opened, and half of the retina was removed (as indicated in Figs. 1, 2, and 3) in order that the course and termination of the remaining fibers from the intact half-retina might be differentiated histologically. A summary of the results from the different types of cases is presented in Figure 1. As shown in the diagrams, removal in separate cases of the *top* half of the retina, the *bottom* half, the *front*, the *back*, or the *outer* peripheral hemiretina resulted, respectively, in quite different and consistently distinctive regeneration patterns. At each of the successive forks in the system of trails leading back to their tectal destinations, the various fiber groups made different and correct choices.

Of special interest are those cases in which the regenerating fibers, in order to

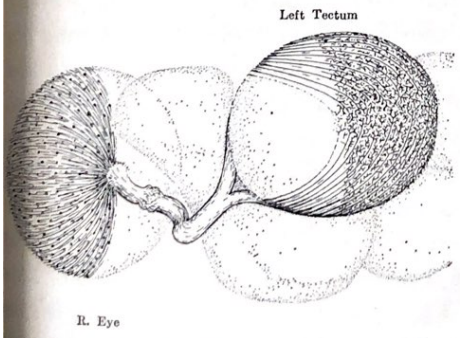

FIG. 2.—Following ablation of temporal retina and optic nerve section, regenerating optic fibers grow through extensive stretches of the semi-denervated anterior tectum, but form synaptic layer and connections only in posterior tectum.

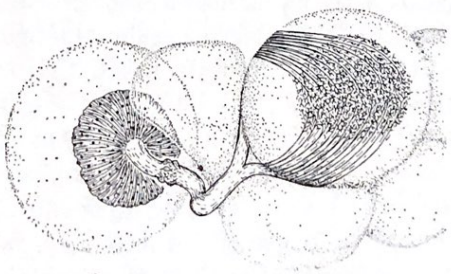

FIG. 3.—Fibers from center of retina grow through denervated peripheral zone of tectum without establishing plexiform layer and synaptic connections until they reach central zone.

reach their own synaptic zones, were obliged to first grow through and across non-terminal sectors of optic tectum. This was true when the posterior half-retina was removed, and the fibers from the anterior hemiretina, particularly those of the medial tract, had to cross large stretches of the partially denervated anterior half of the tectum without forming synaptic linkages (Fig. 2). In this situation the regenerated fibers remained in the superficial parallel layer and coursed straight through without branching or synapsis to bypass the whole population of potential terminal neurons that lay empty and readily accessible all through the front half of the tectum.

Something similar occurred when the peripheral part of the retina was removed (Fig. 3). In these cases, all the regenerating fibers, in order to reach their proper synaptic zones in the center of the tectum, were obliged to grow from the outer tectal border across the denervated peripheral zone. Here again they remained confined to the superficial parallel layer passing straight through without synapsis. Only within the central zone of the tectum did these fibers from the central retina ramify and form the deeper synaptic layer.

It is evident that optic fibers advancing along the same central channels grew very differently depending on their retinal origins. On reaching a given point in the peripheral tectum, for example, one fiber type dipped centrally and ramified in the deeper plexiform layer to form connections among the neuronal elements of the immediate vicinity, whereas other fibers in the same position continued to grow right on through and beyond these same free, denervated neurons, bypassing them and many others for varying distances, until the fiber tips reached the appropriate tectal zone that matched their retinal origin. With the mechanical conditions of the growing medium identical for these different fiber types, and with other factors like timing, rate of growth, and functional feedback seemingly eliminated,[21, 23, 24] the systematic variance in the course and termination seems most reasonably explained on the basis of specific chemoaffinities between the different optic fibers and the elements encountered in growth. Studies still in progress show that, when the main medial and lateral tracts in these fish are freed and surgically interchanged in the brain where they approach the tectum, the displaced fiber bundles promptly recross in growth to regain their proper channels.[2] Fiber bundles, deflected still farther centrally at the edge or within the tectum, tend to form new short-cut pathways in the parallel layer oriented in a direction in which no optic fibers would ordinarily grow in the given region.[1] We also find that fibers from neighboring points in the retina tend to segregate at the first opportunity within the nerve scar and may remain thus segregated through the chiasma and all the way to the tectum.

It is apparent from the results that not only the synaptic terminals, but also in these fishes the route by which the growing optic fibers reach those terminals, is selectively determined, presumably on the basis of similar or identical chemoaffinity factors. From direct observation and photography of living nerve fibers,[17] it is known that, as the growing tip advances, it continuously sends out a spray or flare of rapidly elongating and retracting microfilaments that extend outward into the surrounding front in all possible directions (Fig. 4). The above evidence suggests that factors more chemical than mechanical determine which of the various microfilament probes will preponderate at each point to set the course of growth. The results lead us to what is essentially a chemotactic view of nerve outgrowth, though

without the "distance action" imputed in some definitions of chemotaxis. The general principle of contact guidance is assumed to apply here as it always has in any chemical, electrical, or mechanical theory of nerve growth since about 1913 when Harrison made it clear that nerve fibers are able to grow only in contact with surfaces, never freely into or across fluid spaces.[14]

There remains the problem of explaining the topographic plan inherent in the "homing behavior" of the optic fibers that is responsible for the neat maplike projection that is laid down among retino-tectal connections. For this, I still go back

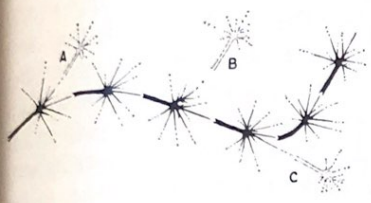

FIG. 4.—Schematic representation of sequential steps in chemotactic guidance of a growing nerve fiber. A spreading flare of microfilaments constantly reaches out in front of the advancing fiber tip testing the surroundings in all directions. The critical factors determining which microfilaments will prevail to set the course of growth would appear from present evidence to be mainly chemical. Numerous alternative possible paths, as represented at A, B, and C, are open and mechanically feasible at each point but fail to develop because of differential chemical attraction.

to my initial interpretation[19, 23] proposing an orderly cytochemical mapping in terms of two or more gradients of embryonic differentiation that spread across and through each other with their axes roughly perpendicular. These separate gradients successively superimposed on the retinal and tectal fields and surroundings would stamp each cell with its appropriate latitude and longitude expressed in a kind of chemical code with matching values between the retinal and tectal maps. The inversion of the retinal map on the tectum suggests complementary relations in the affinity forces involved in linking corresponding points in the two fields. Similar inversions in other systems point to general use of complemental gradient values in synapsis. The same set of cytochemical factors extended from the ganglion cells of the retina into the microfilament flare at the tip of the growing optic axons and also stamped on the optic pathways could be utilized for guiding the respective fiber types into their separate proper channels at each of the numerous forks or decision points which they encounter as they make their way back through what essentially amounts to a multiple Y-maze of possible pathways. The final course laid down by any given fiber reflects the history of a continuous series of decisions based on differential affinities between the various advance filaments that probe the surroundings ahead and the diverse elements that each encounters.

Prediction that the nasotemporal (anteroposterior) gradient might be shown to be fixed separately and prior to the dorsoventral gradient,[21] has since been confirmed in the experiments of Székely[31] and Stone.[30] These and other embryological studies with correlated electrical analyses by Gaze[10] and his associates give further credence to the gradient interpretation. Apparent discrepancies in the recent report of Burgen and Grafstein[6] are instructive regarding the dynamics of gradient organization, but require no change in the basic hypothesis. We have been able to show in behavioral studies that the discrimination of color and brightness, like the perception of directionality and spatial factors in vision, also undergoes an orderly restoration in optic nerve regeneration.[3] This means that additional specification of the optic fibers is required to assure the appropriate tectal linkages for the differ-

ent types of color and luminosity fibers. The same would seem to be true for the "on," "off," and "on-off" classes of optic fibers in order to explain the observed orderly recovery of optokinetic responses and of learned pattern discriminations.

Those familiar primarily with the visual system of man or other mammals may see objections in the foregoing interpretation. The partial crossing of fibers in the mammalian chiasma gives difficulty; and worse, the fact that the *nasal* half-retina of one eye terminates in close register with the *temporal* half-retina of the other eye in primates may look at first like a direct contradiction. Actually, with the incorporation of certain minor developments that we assume must have taken place in the course of evolution, the same gradient hypothesis works nicely also for the mammals, including man. First, we must postulate that fibers from the temporal pole of the retina and gradually from the whole temporal half-field have evolved a lateral growth affinity that prevents their crossing in the chiasma region. Secondly, keeping the dorsoventral axis as before to mark latitude in the retinal field, we may postulate that in mammalian evolution the *nasotemporal* gradient came to be replaced by the *radial* gradient for topographic labeling. This would give the required cytochemical correspondence for functionally identical points in nasal and temporal half-fields (see Fig. 5); it would also leave the differential nasal-temporal and analogous matching properties in the centers for determining decussation, laterality, layered projection, and related features of the mammalian system associated with stereoscopic vision and retinal rivalry, that in themselves have always been puzzling from the standpoint of neurogenesis. At present the possibility cannot be excluded that the decussation patterns in the optic chiasma are determined mechanically in development in combination with rate and timing of fiber ingrowth. However, previous attempts to explain chiasma formation on a mechanical basis[8] have run into difficulties that appear to be more easily resolved on the chemoaffinity plan.

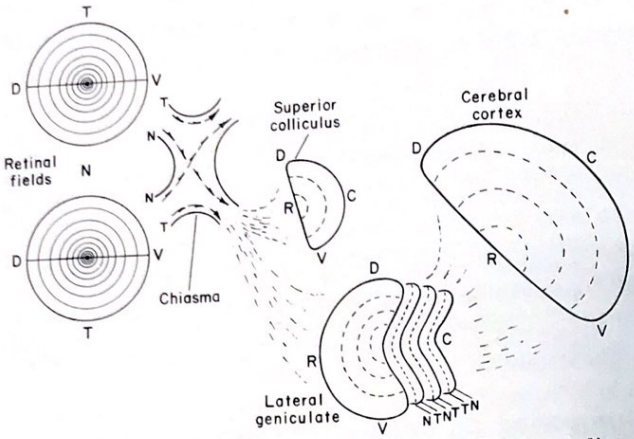

FIG. 5.—Schematic diagram indicating possible application of chemoaffinity interpretation to genesis of mammalian visual system (see text). Axial labeling of gradients for brain centers is highly tentative as the effective embryonic gradients underlying their topographic differentiation remain uncertain. D·V: dorsoventral gradient; N·T: nasotemporal; R·C: rostrocaudal.

There is reason to think, from the evidence available,[16, 23–25, 29] that the above principles, as outlined here for the visual system, apply to the patterning of pathways and connections in the vertebrate nervous system in general, though it is to be expected that their manifestation will be found to vary in form and degree in different species and in different parts of the system. A considerable backlog of supporting observations suggestive of chemotaxis has accumulated during the last two decades, but has not been emphasized owing to the prevailing unpopularity of anything suggestive of neurotropism and chemical guidance. With the demonstr

tion of chemotactic regrowth in the optic system leaving little room for further doubt of the selectivity effects,[4] the way seems cleared for retrospective reassessment of some of the earlier observations with an increased recognition of the importance of selective chemoaffinity in nerve growth and connection in general.[13]

Extension of chemoaffinity concepts to the growth of fiber patterns and connections in the peripheral nervous system may require brief comment at this point, since it is in reference to peripheral nerve growth and regeneration that the strongest evidence has been gathered in support of earlier impressions that nerve outgrowth and termination are basically nonselective. Furthermore, many of the observations indicating absence of selectivity in peripheral nerve regeneration remain in good standing.[11, 35–37] On the other hand, there are exceptions where peripheral innervation is clearly selective, and other cases where it appears to be more selective than not.[11, 12, 22, 23] Though it must thus remain for the present partly a matter of emphasis, it may nevertheless be worthwhile to caution here that, in the changing total picture as we now see it, the established examples of indiscriminate nerve outgrowth and termination begin to look more and more as if they might represent the exceptions rather than the general rule.

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