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Reprinted from *GROWTH, Symposium Vol. X*, pp. 63-87, 1951  
PRINTED IN THE U.S.A.

# REGULATIVE FACTORS IN THE ORDERLY GROWTH OF NEURAL CIRCUITS\*

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Nearly all of us have been exposed at one time or another to the general features of neural development. It will be recalled that the nervous system starts as a thickened plate of cells the edges of which curl together to form a tube, and that this simple tube is then gradually transformed through a series of stages into the complicated structure of the adult brain and cord. The internal microscopic changes that take place during this transformation also have been studied and described in some detail. It has been shown that the nerve cells multiply mainly in the germinal layer lining the lumen of the neural tube. After division the free neuroblasts migrate radially outward into the walls of the tube where they collect in columns, layers, and nuclei to form the future grey matter of the nervous system. At first the nerve cells are roughly globular in shape but as they undergo differentiation, they begin to spin out those long fiber-like processes, the axons and dendrites, that characterize the mature neuron. Some of these fiber processes extend peripherally to connect with various types of end organs. The majority grow for varying distances upward, downward, and across the midline within the neuraxis to form synaptic connections with other nerve cells.

This discussion will be concerned only with these latter phases of neurogenesis, i.e., with the outgrowth of the nerve fibers and particularly with the patterning of their terminal connections. The problem is largely that of the developmental organization of the synaptic associations in the centers and their orderly arrangement into adaptive reflex pathways and integrative circuits. Putting the problem in its simplest terms from the developmental standpoint, it may be expressed as follows: How do the outgrowing fibers of the developing nerve cells

\*Part of the work reported herein was aided by grants from the National Institutes of Health, Public Health Service, and the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

succeed in acquiring their proper end-organ connections in the periphery and their proper synaptic linkages within the nerve centers?

It is necessary to emphasize the word "proper" here, for it is evident that the nerve fiber connections must be laid down in a selective and precise design in order to mediate the complex functions of the developed system. This particular phase of neural growth represents one of the most complicated and refined steps in neurogenesis and is certainly one of the most interesting and important from the functional standpoint.

For a long time this problem was thought to lie more properly within the province of psychology and learning theory. The brain circuits seemed to be too complicated and too precisely adapted for their functions to conceive of their being installed by the forces of growth alone without aid of functional adjustment. Accordingly hypotheses were advanced suggesting that function somehow channels adaptive pathways out of initially equipotential nerve networks, or that synaptic hook-ups which happen to prove adaptive in function are reinforced and maintained while the others are resorbed or undergo atrophy and degeneration. In the widely accepted theory of "neurobiotaxis", electrical potentials generated in the nerve centers in the course of activity were presumed to influence the course and termination of the growing fibers. Those nuclei and tracts that happened to be activated simultaneously or in close succession were supposed to develop interconnections.

This tendency to favor the functional and to minimize the innate and genetic factors in the developmental patterning of brain circuits was carried to extremes during the late 20's and 30's. Witness the infamy into which the term "instinct" had fallen in this period. And remember the extreme efforts which were made, in some instances, to account for all of the organized behavior of the new-born mammal and the newly-hatched bird or reptile on the basis of practice effects obtained from the constrained movements of the fetus in utero or of the developing chick within the egg shell. Reference was made to the "conditioning" of the motor patterns under these circumstances and to the "education" of the sensory surfaces.

Of late the pendulum of opinion has been swinging rapidly in the other direction. Today most of us are convinced we have ample proof that highly refined integrative circuits and coordination patterns can be, and are, built into the brain quite independently of functional

organization. Furthermore, after ten years of investigating the patterning effects of synaptic formation in the development and in the regeneration of the central nervous system, we feel that we are beginning to perceive the first faint outlines of at least a plausible picture of the general nature and the overall scheme of the developmental processes responsible.

It will be seen that our current notions involve a return in part to something like the old chemotactic theories that held favor at the turn and early part of the century. But the up-to-date picture is a compound one including phenomena like cell differentiation, determination, induction, etc., such as are recognized routinely to be operative in the ontogenetic patterning of other organ systems. In fact, if our present hypothesis is correct, the developmental organization of the brain circuits is effected in large measure through mechanisms with which the experimental embryologist already is quite familiar.

Before turning to the evidence, I should warn in advance that our data thus far have been obtained almost exclusively from the amphibians and fishes. There are certain advantages to working with the regeneration of synaptic linkages in the developed organism, and the central nervous system of these animals has been found to possess a remarkable capacity for functional regeneration that is almost entirely lacking in the higher forms. Also it should be pointed out, perhaps, that the morphology of synaptic patterning in the nerve centers, i.e., the end product of growth in this case, is not something that is visible under the microscope. Hence we have been obliged to rely heavily on behavioral criteria.

The nature of the evidence and the kind of conclusions to which it has led may be illustrated with a brief survey of some of the early experiments dealing with the patterning of synaptic connections in the visual pathways. The studies of Matthey (4-7) in France, later confirmed by Stone and Ussher (28) in this country, had demonstrated that the optic nerve of the adult urodele amphibian is capable of functional regeneration with good visual recovery following its complete transection or even after transplantation of the entire eyeball. The optic fibers have their cell bodies located in the ganglion cell layer of the retina. In development the optic fibers grow toward the brain in the optic stalk, and, in the submammalian vertebrates, cross completely in the chiasma to terminate principally within the optic lobe of the midbrain (Fig.1). According to Matthey's description of the recovered

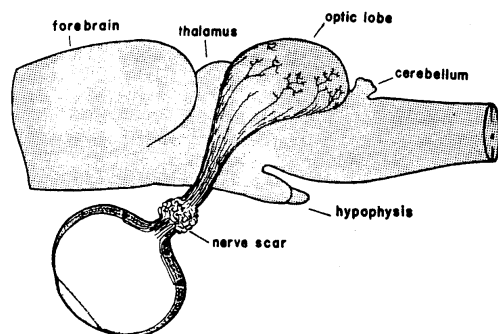


FIGURE 1.

The regenerating optic fibers grow toward the brain, cross completely in the chiasma, and terminate mainly—in the amphibians—in the contralateral optic lobe of the midbrain.

vision, his newts would, among other things, swim toward a small moving lure presented outside the glass walls of the aquarium and attempt to snap at it through the glass. This ability to perceive a small object discretely rather than as a diffuse blur, and to perceive correctly its location in space and the direction of its movement would imply an orderly restoration of the original functional relations of the optic fibers in the brain centers.

It seemed curious that one should get this orderly visual recovery in view of the predominant opinion that nerve fibers regenerate and terminate at random. If the fibers connected at random, one would expect instead a blurred confusion; unless, of course, some kind of functional factor were influential in governing the formation of synaptic relations or in adjusting the new central hook-ups once they had been formed. The possibility that some such functional adjustment was involved seemed to be favored further by the inclusion among the earlier reports of cases in which histological checks showed that the optic nerve had connected with the wrong side of the brain and of others in which the eyes in the course of transplantation had become rotated out of normal position from 90 to 180 degrees. Presumably it could only be through some kind of functional adjustment that normal

vision could be recovered in the face of anatomical rearrangements of this kind.

We became interested in these reports back in 1941 and ran a repeat test in the common newt *Triturus viridescens*, in which optic nerve transection was combined with deliberate surgical rotation of the eye through 180 degrees on the optic axis. With more careful and detailed tests than had been applied in the previous studies, it was possible to show that the recovered vision under these conditions was neither normal, as in the earlier reports, nor a confused blur, as might be expected with the fibers terminating at random. Instead the visual perception was systematically and clearly inverted 180 degrees correlated directly with the rotated position of the retinal field (10, 13). That is to say, the animals responded throughout as if they saw everything up-side-down and reversed front-to-back. Furthermore, the resultant maladaptive visuomotor coordinations persisted indefinitely with no evidence of correction by reeducation.

The reversed reactions were found to be exactly similar in character to those in animals in which the eye was rotated with the normal nerve connections left intact (12). In other words the normal spatial local sign properties of the retinal field had been restored in their original form. This was further substantiated by the fact that when the eye was re-rotated into the normal position after the animals had been responding in reverse for several months, they directly began again to respond in the normal manner.

Under the conditions of the experiments, the orderly patterning of the central hook-ups could not be ascribed to any kind of functional adaptation. Animals do not learn to see things up-side-down and backwards. In fact it was possible to show that these animals were at a greater disadvantage with inverted vision than with no vision at all. Mechanical guidance and orderly timing of fiber ingrowth also could be ruled out as possible factors responsible for the orderly adjustment of the central relations. Chaotic interspersions of the optic fibers was found to prevail generally upon histological examination of the nerve scars (Fig. 2A).

With these possibilities eliminated it became necessary to infer that optic fibers arising from different retinal loci must differ from one another in quality. If the regenerating fibers were all alike and indistinguishable from one another, there would be no way in which they could form differential reflex relations in the centers on an orderly

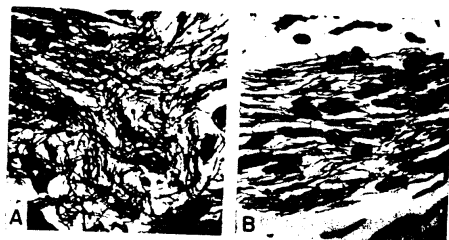


FIGURE 2.

Photomicrographs of silver impregnated sections of regenerated optic nerves in *T. viridescens*. A: Regeneration scar at region of transection. B: newly formed optic nerve at entrance of optic foramen following degeneration and regeneration of the neural retina (from Sperry, 1943c).

plan following their random dispersion within the nerve scar. We suggested that this optic fiber specificity might be brought about through a polarized field-like differentiation of the retina in development with the specificity of the ganglion cells being extended into the optic fibers throughout their length. It was also suggested that normal vision might prevail if the retina or its anlage were rotated prior to the stage at which its polarity had become irreversibly fixed.

It was necessary to conclude further that the retinal differentiation must occur on at least 2 axes. A dorsoventral differentiation alone, for example, would not be sufficient because the anterior and posterior retinal quadrants would be indistinguishable. An anteroposterior differentiation superimposed upon the dorsoventral, however, would stamp the fibers from each retinal locus with a unique combination of qualities (Fig. 3). One is reminded of Harrison's (2), studies showing the establishment of an anteroposterior and a dorsoventral axis in the differentiation of the early limb bud. It was suggested that contralateral transplantation of the eye or its anlage during the critical developmental stages might similarly reveal a separate determination of two such axes in retinal differentiation.

In 18 or almost 1/3 of the original experimental group, interference with the blood supply to the eye during the operations resulted in degeneration of the retina itself along with the entire optic nerve. This was followed by regeneration of a new retina and a new optic

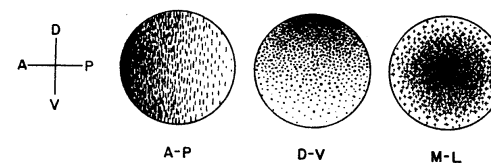


FIGURE 3.

Diagrams illustrating how each retinal locus might acquire its unique properties through polarized differentiation of the retinal field in development. Differentiation on the anteroposterior (A-P), or nasotemporal, axis alone would not be sufficient as all points along lines perpendicular to the A-P axis would remain equivalent. The addition of a dorsoventral (D-V) gradient, qualitatively distinct from the former, however, would endow each retinal point with its distinct local properties. A separate mediolateral (M-L) or radial differentiation, by itself, or in combination with either the A-P or the D-V gradient would not be adequate, but superimposed upon the combination of the first two, might increase the degree of differentiation.

nerve (Fig. 2B). The remarkable regenerative capacity of the urodele retina has been known for a long time. As early as 1920, Wachs (30) had demonstrated that the neural retina regenerates from surviving cells in the pigmented layer. The functional results in our 18 cases with regenerated retinæ were similar to those in which the original retina survived. Accordingly the inferences regarding biaxial polarization were extended to include regenerating as well as developing retinæ.

A further deduction was drawn from the results, namely, that the central neurons on which the optic fibers terminate are also subject to qualitative differentiation. If the central cells were all alike, there would again be no way in which differential reflex relations could be laid down in a systematic pattern. Presumably the optic lobe, like the retina, is subject to a polarized differentiation across at least two separate axes.

Our general conclusion, then, was that the optic fibers do not obey the rule of random termination but instead form their terminals both in regeneration and in development in a highly selective and discriminative manner in accordance with specific affinities and incompatibilities between the ingrowing fibers and the central neurons. A fiber from any given retinal point apparently is predisposed to terminate on the cells of a particular local area of the optic lobe.

As the ingrowing fibers enter the optic centers they encounter many types of elements including glia cells, capillaries, and axons from other nuclei, as well as the various types of neurons and their dendrites

belonging to the optic tectum. We picture the advancing fiber tips as growing over and beyond most of the elements they encounter until eventually neurons are contacted the particular chemical nature of which causes a special reaction in the fiber tip resulting in the cessation of further elongation and the formation of an intimate and lasting synaptic connection. In a sense, the central synaptic patterning is governed from the periphery through the differentiation of the retina and we accordingly have referred to the "peripheral regulation of central synapsis."

When Stone (26), in checking our report shortly afterward, employed the same functional tests we had used, i.e., the optokinetic responses to a moving visual field (Fig. 4) plus careful observation of

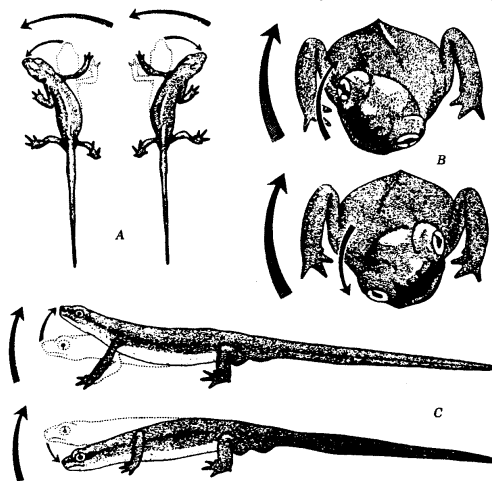


FIGURE 4. Reversal of optokinetic responses caused by inverted vision. Large arrows indicate the direction of movement of the striped walls of a drum when rotated about the three primary axes of the body in A, B, and C respectively. The normal pursuit phase of the response (same direction as drum rotation) is shown for comparison along with the reversed pursuit reactions (from Sperry, 1951).

the direction of response in localizing small objects in space, he was able to confirm the visual inversion. We have rechecked the results many times ourselves in the course of student demonstrations and have yet to find any exceptions.

Also it has been possible to extend the experiments, along with various modifications (Figs. 5 and 6) but with essentially the same results, to the anuran amphibians, the frogs and toads, in both larval and adult stages (14, 15); and also to adult and young teleost fishes of several different families including both marine and fresh water forms (21, 22); as well as to prefunctional embryonic stages in the urodeles (27).

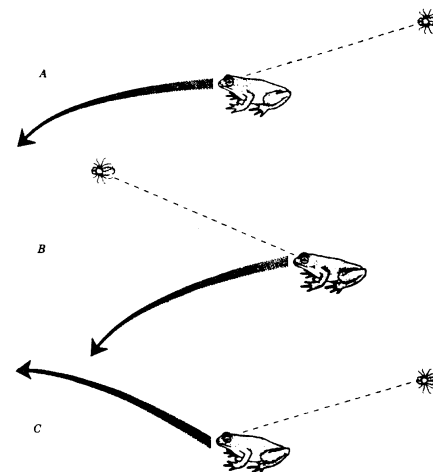


FIGURE 5.

Sample types of error in spatial localization as produced by: A: 180 degree rotation of the eye with optic nerve transection. B: contralateral transplantation of the eye with inversion of the dorsoventral axis only. C: contralateral transplantation with inversion of the nasotemporal axis only. More complicated illusory displacements are obtained by intermediate degrees of retinal rotation and by combinations of eye rotation, contralateral transplantation, and optic nerve cross union (from Sperry, 1951).

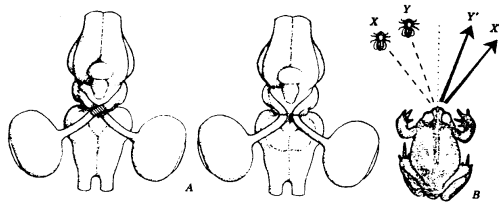


FIGURE 6.

Contralateral transfer of retinal projection on the brain. A: By excising the optic chiasma and cross-uniting the two sets of optic nerve stumps as diagrammed, the central projection of the two retinae is interchanged. B: After recovery the animals respond as if everything viewed through either eye were being seen through the opposite eye. For example, when a lure is presented at X or Y, the animal strikes at X' or at Y' respectively (from Sperry, 1951).

The interpretation of selective central termination has been confirmed to the following extent: localized lesions in the optic lobe of the normal frog were found to produce a distinct blind area in the corresponding quadrant of the visual field as indicated by the frog's lack of response to a lure presented in this area compared with immediate approach responses upon presentation in any other area. If regenerating optic fibers terminate at random in the optic lobe, local lesions made subsequently would be expected to produce a generalized lowering of visual acuity, but not a discrete blind area. The same type of scotomata are found after regeneration, however, as in the normal animal indicating that fibers from the various retinal areas reestablish their functional associations in the same locality of the optic lobe in which they had formerly terminated (14). In animals in which the eye has been rotated, the scotomata are found to be displaced accordingly.

Experiments involving transection and centripetal regeneration of the vestibular nerve root (16) have led to similar conclusions regarding the patterning of synaptic connections in the vestibular nuclei of the hindbrain. The results indicate that the sensory fibers innervating the various endorgans of the labyrinth differ from one another in quality. For example, those fibers connected with the crista of the horizontal semi-circular canal differ from those connected with the crista of the anterior or the posterior vertical canals and these differ in turn from those supplying the macula of the utricle, etc.

The endorgans themselves must undergo a correlated differentiation. In the case of the macula of the utricle the functional relationships suggest a biaxial polarized specification similar to that of the retina such that fibers to any given point in the macula may be stamped with unique properties distinguishing them from all other macular fibers. The central neurons of the vestibular nuclei must also be qualitatively specified, for if they were all alike and indistinguishable, there would be no basis on which the ingrowing vestibular fibers (Fig. 7) could form their proper differential reflex relations.

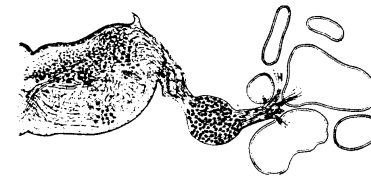


FIGURE 7.

Diagrammatic section through regenerated vestibular nerve root at its entrance into the medulla. Fibers innervating the various sensory endings in the labyrinth become intertangled in the nerve scar but nevertheless reestablish central reflex relations in a systematic pattern (from Sperry, 1951).

Considering the manner in which the labyrinth differentiates (3) and the way in which the innervation of its sense organs is developed (1), it seems probable that here, as in the optic system, central synapsis is governed from the periphery with the endorgans leading the way in differentiation and inducing specificity in their nerve fibers. In the labyrinth, however, the bodies of the nerve cells are not embedded within the endorgan tissue, as they are in the retina, but lie in the ganglion at some distance with only their fiber tips in contact. We are therefore obliged to assume in this case that terminal fiber connections are sufficient for the induction of specificity in the peripheral nerve cells.

This idea that an endorgan may impose specific chemical changes in its nerve fibers, and that this in some way determines its functional relations with the centers—something which we have now come to regard as a general principle in neurogenesis—was suggested by Weiss (31) in 1936 under the term "modulation" to account for the "homologous" or "myotopic" function of supernumerary limbs. The

phenomenon of homologous response is based essentially upon excellent, normal muscle coordination in the presence of highly abnormal nerve-muscle connections (31, 32, 33), and as such, has remained a challenge to neurological theory for more than 20 years. The phenomenon becomes explainable now in terms consistent with orthodox reflex physiology when one views the motor nerve "modulation" in the light of our present chemoaffinity concepts of synaptic growth. One must postulate that the formation of synaptic endings upon the motoneurons is regulated initially in ontogeny by selective affinities between the soma and dendrites of the motoneurons and the fibers which fire them. When the nerve-muscle connections are rearranged, as by limb transplantation or by more direct crossing of nerves into foreign muscles, it may be assumed that the resultant alteration of chemical properties in the motoneurons leads to some kind of breakdown in the original synaptic linkages and that this is followed by the establishment of new synaptic relations in accordance with the new pattern of interneuronal affinities. In other words when one alters the peripheral connections, there follows a compensatory switch of synaptic relations in the centers, the end result of which is to restore the original, or typical over-all pattern of neuronal linkages.

We have since found a comparable restoration of muscle coordination following disarrangement of nerve-muscle connections in the oculomotor system of larval amphibians (19), and in the innervation of the pectoral fin musculature of adult teleost fishes (23). The same interpretation would account for the results in these cases. The orderly proprioceptive function in supernumerary limbs as indicated in their myotatic reflexes (29, 32) can also be explained in similar terms. An orderly restoration of proprioception has been found recently to follow the contralateral cross-connection of the spinal sensory roots of the hind limb in the frog (24). The central processes of the proprioceptive neurons under these conditions regenerate into the contralateral limb centers (Fig. 8) and establish their typical reflex relations despite non-adaptive functional effects and a chaotic intermixing of the fibers as they enter the cord. The suggested interpretation in terms of selective synaptic linkages is perhaps easier to accept under these circumstances than under those in which only the peripheral relations are disarranged as above.

Though definitely speculative, this interpretation of the pioneer experiments of Weiss as well as of the more recent findings would

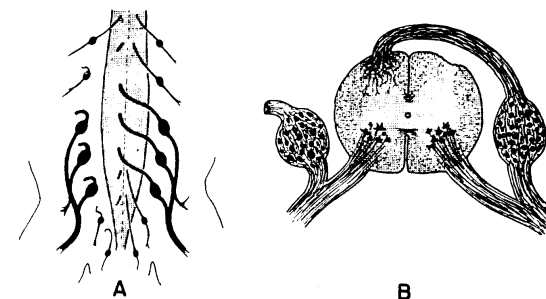


FIGURE 8. Contralateral cross connection of the sensory roots of the hind limb nerves in frog tadpoles (A: dorsal view, B: transverse section). The various types of proprioceptive and cutaneous fibers establish contralateral reflex relations in an orderly, selective manner despite intertangling of fibers and maladaptiveness of functional results.

bring the development of the motor and of the proprioceptive circuits into line with that suggested for the visual and vestibular systems. Further support for these concepts has been found also in recent studies dealing with the development of cutaneous local sign.

A patient on the examining table with eyes closed is capable of localizing with considerable accuracy a pin prick applied anywhere on the body surface provided his nervous system is intact and normal. Similarly the dog or cat turn directly to the point where a flea is biting and even the frog displays pretty fair localization of cutaneous stimuli. There is something about impulses entering the brain from different points on the body surface that registers the cutaneous locus from which they arise and we refer to this as the "local sign" quality of cutaneous sensibility. When cut nerve fibers misregenerate in man into foreign areas of skin, sensations are thereafter referred erroneously to the former site of termination (17). Thus, for accurate localization, it is necessary that the central synaptic associations of the cutaneous fibers be neatly adjusted during development to suit the pattern of peripheral innervation. In a sense the topography of the entire body surface must be reflected in an orderly way in the central circuits.

The circuits involved are not simple. Consider that a stimulated spot on the end of the left thumb, for example, is readily pointed

out by the right index finger in a blindfolded subject even though the spot may be at any one of a hundred different points in space depending on the posture of the wrist and arm at the moment. Perhaps more than in the case of any other basic sensory function, it has been thought that we learn to distinguish and to locate different points on the skin. In an earlier investigation of this question in the rat (11), the cutaneous nerves of the left hind foot were crossed into the right hind foot. The operations were performed as early as 14-26 days of age before the animals could have had much experience in localizing cutaneous stimuli. Following recovery of function, painful stimulation of the right foot caused the rats to withdraw and to lick the left foot. At the same time they extended the right foot more strongly against the offending stimulus. In several cases trophic sores developed on the sole of the right foot. This caused the animals to walk about on three legs holding the uninjured left foot off the floor and thus placing extra weight upon the open lesions of the inflamed right foot.

The incorrigible persistence of these maladaptive reactions, throughout extensive training procedures suggested that cutaneous local sign, contrary to general opinion, is something that is built into the nervous system and not a product of experience and training. It has since been possible to confirm this conclusion in amphibians and to analyze in part the developmental forces involved (8, 9, 24, 25).

We have been able to eliminate functional adaptation as the responsible factor by experiments like the following: when skin flaps, with original innervation retained, are transposed in frog tadpoles across the midline of the back, from the left to the right side, the frogs, after metamorphosis, wipe erroneously with the left leg at the former site of the skin flap when it is stimulated on the right side. After contralateral cross connection of the sensory nerve roots of the hind limb in frog tadpoles, the metamorphosed frogs make characteristic, but useless and maladaptive responses of the left limb when the right foot is stimulated (Fig. 8). After contralateral cross-union of the ophthalmic nerves in frog tadpoles, the metamorphosed frogs wipe off the right side of the snout when the left side is stimulated. After cross-union of the ophthalmic and mandibular nerves in efts, the recovered withdrawal responses to needle pricks under the mandible are reversed such that the head is depressed against the needle point instead of being lifted away (Fig. 9).

As in the visual, vestibular, and proprioceptive systems it has also

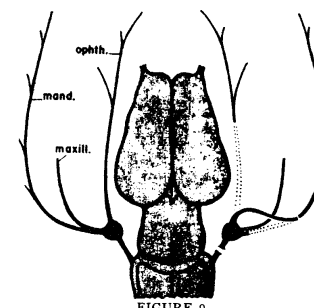


FIGURE 9.

Cross union of ophthalmic and mandibular nerves with section of root V. Regardless of the maladaptive response effects produced by the peripheral cross, the central processes of the ophthalmic fibers reestablish their typical pattern of synaptic relations (from Sperry and Miner, 1949).

been possible to show that the patterning of the central hook-ups is not dependent upon any orderly developmental plan based on an exact timing and mechanical guidance of sensory fiber ingrowth. Normal reflex patterns are systematically established in centripetal regeneration of the transected spinal sensory roots and of the cranial trigeminal root. In the case of root V, normal central relations are restored even when the regenerating fibers are forced to enter the brain over the foreign pathways of nerve VII.

Implicit in these results is the conclusion that sensory fibers distributed peripherally to different cutaneous loci differ from one another in character and are thereby enabled to form specific reflex relations in the centers. The central neurons among which the sensory fibers terminate must also be qualitatively specified in accordance with their various efferent linkages. Again we may picture the synaptic connections as being laid down selectively in accordance with specific chemo-affinities and incompatibilities between the sensory and the central neurons.

Also implicit in the results is the further conclusion that the integument itself undergoes in the course of development a highly refined differentiation that correlates closely with the limen of 2-point discrimination. As in the retina and utricular macula the local sign specificity



of the integument and its nerve supply must be field-like in nature and at least biaxial in dimension. A given spot on the forearm, for example, must be localized with reference to the dorsoventral as well as the anteroposterior limb axis. In all these biaxial or multiaxial fields, the differentiation along any one axis may be viewed as a quantitative gradient, but the gradients on separate axes must be qualitatively distinct.

The foregoing experiments leave unanswered the problem of how the peripheral connections of the cutaneous fibers become adjusted. Do the outgrowing sensory fibers, during development, for example, seek out selectively each its proper cutaneous terminal? This possibility has been negated in favor of another by the experiments of Miner (8, 9). Dr. Miner excised in frog tadpoles the sensory nerves that normally supply the hind limb. As a result the deafferented skin of the growing limb bud was invaded by cutaneous fibers from neighboring segments that otherwise would have connected with trunk skin. After metamorphosis normal cutaneous reflexes were elicitable from the limb indicating that the sensory fibers had formed functional connections in atypical areas of skin and central relations appropriate for those atypical terminals. The same occurred when an extra hind limb bud was transplanted to the back in the mid-trunk segments (Fig. 10). After metamorphosis stimulation of the transplanted limb

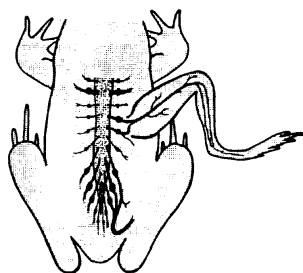


FIGURE 10.

By the transplantation of an extra limb bud to the back, cutaneous fibers, destined normally to innervate trunk skin, can be made to connect with limb skin. The central processes thereupon form synaptic relations appropriate for the limb instead of the trunk (see Miner, 1951b).

elicited reflex responses of the ipsilateral hind limb similar to those evoked from corresponding points on the normal limb. In other words, cutaneous fibers normally destined to form central hook-ups appropriate for the belly, flank, and back skin of the trunk, formed instead connections appropriate for the digits, heel and knee of the limb.

From these results, it appears that the local sign specificity is stamped secondarily upon the cutaneous fibers after, and in consequence of, the formation of their integumental connections. This would obviate any need for individual fibers to seek out their proper cutaneous terminals. Nonselective outgrowth with endorgan specification thus seems to be the rule here as in the innervation of the musculature already extensively analyzed in the earlier experiments of Weiss (31-33). According to our present interpretation, this peripheral determination of central associations may be regarded as a method that is widely employed throughout the organism for insuring the development of the appropriate patterns of fiber connections between the nerve centers on the one hand and the various endorgans of the periphery on the other.

In brief, the genesis of cutaneous local sign appears to depend upon (a) random outgrowth and termination of the cutaneous fibers in the various regions of the body (b) refined local differentiation of the entire cutaneous covering (c) integumental induction of a correlated local specificity in the cutaneous neurons via their peripheral contacts (d) qualitative differentiation of the central neurons among which the sensory fibers make synapsis (e) selective termination of the central processes of the cutaneous fibers according to specific affinities and incompatibilities between the sensory and central neurons. No exact chronological order is implied; presumably many of the steps are concurrent, with extensive overlap throughout.

This general picture finds further support in a final experiment in which the possible inductive influence of the deeper tissues was eliminated. In frog tadpoles a strip of trunk skin extending transversely from near the mid-dorsal to near the mid-ventral line and of the width of approximately  $3\frac{1}{2}$  dermatomes was cut free, rotated 180 degrees, and replaced so that the dorsal skin was located ventrally and vice versa. As expected, the skin grafts underwent self differentiation with development of typical pigmentation in the reversed positions as shown in figure 11. Localizing responses elicited from these rotated grafts after metamorphosis are aimed erroneously in correlation with



FIGURE 11. Skin grafts, cut free and rotated 180 degrees in early tadpole stages (A: dorsal and ventral views, B: transverse section). The central reflex connections of the sensory fibers that terminate in such grafts are laid down to suit the local specificity of the ingrafted rather than the topography of the body surface (see Miner, 1951a; 1951b).

the intrinsic specificity of the graft rather than the topography of the body surface.

When the white ventral skin on the back is touched, the frog wipes its belly with the forelimb, and when the black dorsal skin on the belly is stimulated, the frog wipes its back with the hind limb. Fibers of the dorsal rami therefore form central reflex connections appropriate for the ventral rami of the spinal nerves and vice versa as a result of the imposed switch in their peripheral relations. The kind of reflex relations which a given cutaneous nerve fiber will form in the centers is thus shown to be determined by the local character of the skin with which it happens to connect in the periphery.

It will be noted that these latter results are contradictory to those mentioned for man, the rat, and for later developmental stages in amphibians. The peripherally determined adjustment in the central relations seems to take place only during the early plastic stages of growth, the duration of which varies for different regions of the body and for different species. The specificity, or the synaptic linkages of the cutaneous fibers, or both, seem to become irreversibly fixed in the course of time and, once fixed, the nerves thereafter retain their original local sign properties. The additional problems involved in the modal differentiation of the cutaneous neurons for pain, tactile, and thermal sensibility have not been explored. One would suppose the local sign specificity to be superimposed upon the more basic modal differentiation.

Thus far we have dealt with the patterning of synaptic formation only in the primary sensory and motor nuclei. Perhaps deeper within the association centers of the neuraxis the synaptic relations are laid down on a different basis with function playing a more important role? What little evidence we have on this point indicates that the same principles apply in the deeper nuclei. Regeneration of the tectobulbar and tectospinal tracts have been found to result in an orderly re-establishment of the typical functional relations despite disorderly intermixing of fibers in the nerve scar and despite maladaptive functional results (20). We are inclined to believe at present that all of the complicated interconnections of the nervous system which form the stock-in-trade of the neuroanatomist, and the headache of the medical student, are built into the system with the refined aspects of synaptic patterning dependent upon factors like those we have been discussing. Nearly all the organization of the cord and brain stem

would be included plus the selective interconnections of cerebellum and cerebrum insofar as they are constant within a species. This leaves plenty of room for learning in the higher centers and more remote by-ways of the neural matrix. The structural relations we are referring to constitute the basic plan of the machine that remains constant and has to be used generally for all activities. To modify it for a particular performance by learning would only exclude or impair its use in other activities.

It may help to tie this material together, if we consider the foregoing data with reference to the developmental patterning of some complete reflex circuits. Collecting our information on the motor, central, and various sensory systems, one can begin to assemble a tentative working picture of the ontogenetic organization of a number of sensori-neuro-motor arcs, such as those mediating the proprioceptive, cutaneous, visuomotor, oculogyric, vestibulo-ocular, and other related reflexes.

As an example, consider the vestibulo-ocular reflex arc which in function helps to maintain a steady view of the visual field during disturbing movements of the head and body. This is commonly referred to as a 3-neuron arc, i.e., only three orders of neurons are involved in the main reflex pathway, the sensory, the motor, and one set of associational neurons between them. The central neurons in this case lie in the vestibular nuclei and their fibers run in the medial longitudinal fasciculus of the same and opposite sides to terminate among the motor ocular nuclei III, IV and VI (Fig. 12). Factors governing the adjustment of connections at both the peripheral and central ends of the motor and also of the sensory neurons have been discussed along with inferences concerning the differentiation and associations of the central neurons (16, 24). This covers all sets of neuronal linkages in the main reflex pathway. By putting these data together in proper chronological order along with other available information on the development of the structures involved, it becomes possible to outline a general picture of the growth and differentiation of this reflex system.

Many details are still missing. For instance, there exist alternate and accessory circuits superimposed upon the main route about which we can say almost nothing at present. Direct inhibitory as well as excitatory synaptic relations also appear to be involved and we have no information about the factors operative in the development of

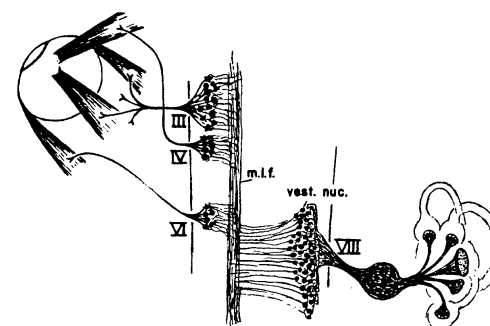


FIGURE 12.  
Simplified diagram of the vestibulo-ocular reflex arc.

these two opposing elements. We can conclude only that various types of neurons form differential synaptic relations, but we know little about the exact patterns of these connections. Many of the sensory and central neurons form linkages with a wide variety of cells. For example, a given axon from the vestibular nuclei may bifurcate to form connections at various levels of the spinal system as well as among the motor ocular nuclei. The underlying chemical relationships become correspondingly complex and one can only speculate about their nature. In short we have hardly begun to scratch the surface of the problems involved and our present diagrams and descriptions remain highly simplified and incomplete. When it comes to these finer details of synaptic patterning, however, we are as ignorant of the finished adult structure and its physiology as we are of its development.

Such evidence as we now have, however, seems sufficient to warrant the conclusion that almost the entire nervous system is subject to a refined differentiation of the above sort which in many regions approaches the level of the individual nerve cell. The bulk of the somatic and visceral periphery likewise must undergo a similar subtle specification insofar as differential motor or sensory functions prevail. This would include, in addition to parts already mentioned and other

specialized sensory and motor organs, such structures as the fascias and other subcutaneous connective tissues, the ligaments, tendons, joint surfaces, the periosteum of the bones, and the parts of the various viscera. In the case of the periosteum, for example, pain is fairly well localized particularly in bones close to the surface. Therefore the periosteum at one end of a bone, let us say the tibia of the shank, must differ qualitatively from that in the middle and at the other end. Again, since the local sign quality appears to require it, we would postulate a field-like, biaxial specification of the tibial periosteum.

Thus, in a sense the differentiation of almost the entire extra-neural organism is stamped upon the nervous tissue and is represented in a condensed and abbreviated form within the sensory and motor nuclei. To this we must add the even more varied specification of the intracerebral neurons with all the manifold subtleties required by their complicated interrelations. One gains the impression that in the nervous system the mechanisms of growth and differentiation reach their peak of refinement and complexity.

It remains possible, although the old idea has been under fire in recent times, that the chemical effects of neuronal differentiation may be influential in determining not only terminal linkages but also the course of the growing nerve fibers and thereby the configuration of connecting pathways. We would visualize the growing fiber tips as responding, not so much to chemicals diffusing in the tissue fluids, as to the local, stabilized chemical properties of the differentiating cells and intercellular ground substance which the tips encounter in their advancement. With these chemical qualities disposed in constant focal areas, in gradients, in contrasting borders, and interfaces, and along preexisting fiber pathways, it would be surprising indeed to find that the outgrowing fibers completely ignore all these possibilities for chemical guidance. It is not difficult to conceive of chemical guidance based on the selective reinforcement and resorption of the exploratory filaments and incipient branches of the advancing fibers, and our minds should remain open to the possibility of other mechanisms yet unguessed. There is also suggestion that the neuronal differentiation results in variant fiber growth potentials such that the elongating processes of different types of neurons may respond diversely, each in a characteristic manner, to the same chemical substrate. No renunciation of the importance of mechanical guidance in nerve fiber growth is implied in this recognition of chemical influences. The chemical factors merely supplement, not replace, the mechanical factors.

Neuronal specification, as noted, may arise through induction effects imposed from without via fiber contacts as well as by intrinsic differentiation, and both processes are probably involved in many instances. The special conditions under which induction and differentiation occur in the nervous system may be found to have special implications for the underlying chemistry of these phenomena. I have in mind particularly the induction effects that take place merely through fine fiber connections at a long distance from the nerve cell body and nucleus and which then spread along the fibers back to the cell body and beyond throughout the ramifications of the entire neuron unit.

Consider also the numerous dimensions of chemical differentiation that must be present within the limited confines of the finest collaterals of the nerve fiber. In the dorsal horn of the cord, for example, a given collateral may be specified first as a pain fiber distinguishing it from fibers of other functional modalities in the neighborhood and further as a cutaneous pain fiber setting it off from fibers mediating other types of pain. In addition it must be stamped for the locus of its peripheral termination with a particular dose of one quality to mark its anteroposterior alignment and another of another quality for its dorsoventral alignment. Most fibers must be stamped with at least three such specifying properties.

Consider further that these specifying qualities must be extended into the remotest advancing processes during growth and that the combination of qualities must be maintained without dilution or alteration throughout all the dozens or perhaps even hundreds of terminal ramifications. Under such conditions one can hardly ascribe a gradient of differentiation to an underlying gradient in the density of some kind of molecule. One is prompted to look rather for a gradient of properties within some large molecule or other reproducible element the properties of which as a unit can be modified through many degrees and reduplicated, or extended in some form of chain reaction, throughout the entire neuron without alteration of its specificity.

It may be worth pointing out, in conclusion, that any advances one might be able to achieve in these phases of neural development take on added importance for their implications concerning many yet obscure elements in the structural and functional organization of the adult nervous system.

Finally, to return to our original theme, it would seem that with the foregoing picture of the developmental processes, almost no behavior pattern need be considered too refined or too complicated for its detailed organization to be significantly influenced by genetic factors. The extent to which our individual motor skills, sensory capacities, talents, temperaments, mannerisms, intelligence, and other behavioral traits may be products of inheritance would seem to be much greater on these terms than many of us had formerly believed possible.

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