Reprinted from THE ANATOMICAL RECORD
Vol. 102, No. 1, September, 1948

ORDERLY PATTERNING OF SYNAPTIC ASSOCIATIONS IN REGENERATION OF INTRACENTRAL FIBER TRACTS MEDIATING VISUOMOTOR COORDINATION[1]

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TWO FIGURES

The manner in which the integrative circuits of the nervous system became organized in development is still conjectural. For many years Kappers' ('32) electrical theory of "neurobiotaxis" was favored as the most likely explanation of how the synaptic associations become arranged in appropriate patterns. The timing of nerve discharges he supposed to be the crucial factor in determining the adaptive selectivity of neuronal interconnections. This interpretation was in accord with the widespread conviction that the refined precision, complex organization and functional adaptiveness of the underlying neural structures required for mediation of central nervous integration could hardly be attained independently of function.

A recent series of experiments on the inherent patterning of synaptic connections in the amphibian central nervous system (Sperry, '43–'48) prompted by an entirely different working hypothesis which puts no dependence upon functional factors has repeatedly yielded results consistent with the alternative view and incompatible with the neurobiotatic interpretation. In brief, without reference to the supporting evidence which has been reviewed at length elsewhere (Sperry,

[1] Aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

-native hypothesis assumes that the developing
ntinue to undergo a refined biochemical differ-
ng themselves far beyond that which becomes
ly and which closely approaches in degree the
rentiation attained in their inherent structural
It is postulated further that selective affinities
ibilities of a physico-chemical nature develop
ious neuron types as a result of their differenti-
t synaptic linkages are formed in a discrimina-
nly between cells which have special affinity for
he advancing tips of the developing nerve fibers
to by-pass many of the nerve cells which they
their outgrowth forming synaptic endings only
with particular neurons the chemical properties
pecially matched to those of the growing fibers.
nents thus far have been concerned with the
ich appropriate connections become established
r and periphery and accordingly have dealt only
formation in the primary sensory and motor
uestion has therefore arisen as to whether the
ynapses deeper in the nervous system, involving
d higher order association neurons, might not
a quite different and more functional type of
t was to investigate this possibility that the
eriments were undertaken.

ations are concerned with the reestablishment of
tic relations in regeneration of central associa-
lowing brain transection near the caudal end of
halon. Although regeneration of many different
oth descending and ascending, was included, at-
ncentrated primarily upon the descending tracts
uomotor coordination. These tracts were singled
he differential adjustment and organization of
ctions are comparatively easy to measure, and
the factor of functional adaptation in recovery
y tested in the case of vision by surgically ro-

tating or inverting the eyes or by cross-uniting the optic nerves.

EXPERIMENTAL PLAN AND METHODS

All the descending intracentral fiber tracts which link the primary visual centers with the medulla and spinal cord were severed by transecting the brain posterior to the optic lobes. The object was to find out if the divided fibers would regenerate and restore functional associations with the lower centers of the spinal cord and medulla, and, if so, whether the central synaptic associations would be restored in a haphazard, indiscriminate fashion or in an orderly, selective manner. Finally, the experiments were designed, in the latter case, to determine whether the adjustment of the central connections might be dependent upon functional types of adaptation like learning or whether it was regulated by factors intrinsic to the regeneration process itself.

If the central course and termination of the regenerating fibers were determined merely by mechanical guidance, one would anticipate only confusion and disorder in the recovered function because of the inevitable admixture of regenerating fibers of different functional types. With division of all the tracts which link cord and medulla to the higher brain centers, ascending as well as descending, there would be opportunity for intermixing of fibers not only within those tracts subserving vision but also between these and many nearby tracts of various other functions. Furthermore, even if a few fibers should chance to be directed across the cut into their original channels, they would not necessarily be guided to their former terminals because the final portion of their course within the neuropil, where the synaptic connections are made, remains unchanneled. This latter factor alone would cause much confusion in fiber termination were it dependent entirely upon mechanical guidance. Any orderliness in the recovery of function under the experimental conditions would thus necessarily indicate the action of discriminative organizing agencies of some sort.

ncy might conceivably be the "conditioning"
cess by which adaptive synaptic connections
rced and maladaptive ones eliminated on the
nctional effects. Surgical rotation of the eyes
grees was carried out in some of the animals
od in which the divided central tracts were
r the purpose of testing this possibility. If
ecovered normal visuomotor responses despite
ture of the eyes, it would certainly imply a
of adjustment. On the other hand if the
s of synaptic associations were restored in the
ne result would be a recovery of systematically
motor coordinations, highly maladaptive in
, '43–'45). In view of the detrimental func-
n this latter case the orderliness of recovery
e ascribed to any functional process of adap-

echanical guidance and functional adjustment
regenerating fibers would have no means of
natically arranged efferent relations in the
the fibers were all alike in character. Accord-
of reversed vision would constitute evidence of
pecificity among the regenerating fibers and
The idea that the formation of synaptic con-
ılated by selective affinities between the regen-
and lower level neurons.

the experiments were carried out on adult
riturus viridescens, during the winter months
r to March. After the animals had been deeply
ith ether, the ventral wall of the cranium was
arp-pointed watchmakers' forceps and broken
the junction of midbrain and medulla. After
s had been reflected rostrally the brain was
approximately the level indicated in figure 1,
time, by repeated crushing with an extremely
rceps, the points of which had been especially

sharpened and ground to blade-like thinness. The division of the brain substance by this method was clearly visible. It was thought that separate section of each side of the brain in bilateral cases might help reduce the mixup of fibers across the median raphe. This is important because other experiments (Sperry, '45a, '48) have indicated that some neuron types, if given a chance, readily form their characteristic associations on the opposite as well as on the normal side of the brain or cord.

Transection was performed unilaterally in 10 cases and bilaterally in 19 cases, only 11 of the latter of which survived. It was found that the mortality of the bilateral cases could be greatly reduced by keeping the animals first in cold (approxi-

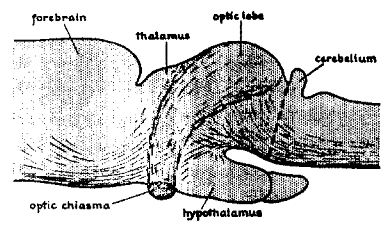

Fig. 1 The level of transection, diagrammatic.

mately 8°C.), moist containers and later in cool, shallow, well-aerated water from 4 to 8 weeks after operation or until they had reached an advanced stage of recovery. In 7 of the bilateral cases both eyes were rotated 180 degrees on the optic axis approximately 26 days after brain transection and at least 15 days before any signs of visual recovery. Records of the individual cases were kept separately, the animals being easily distinguished by their pigmentation patterns.

Optokinetic responses elicited by rotating a striped drum around the animals on the three primary axes of the body and localization and pursuit responses elicited by moving a small lure in the field of vision served as the principal criteria of visual performance. The lure consisted of a piece of dark brown gum rubber 3 mm in diameter and was kept within a roximately 10 cm. After the tests of functional been completed in the animals with rotated were surgically reset in their normal orienta- ests repeated. The results of the latter are in- along with those of the cases which retained the osture throughout. After the functional obser- been completed, the brains were prepared for examination by the Bodian silver proteinate

RESULTS

effects of transection. In the unilateral cases the sponses toward the side opposite the hemisection normal or exaggerated whereas the responses ther side were extremely weak or lacking. Re- tation of the visual field on the longitudinal and dy axes were distorted by the persistent tend- and turn to the side opposite the lesion. The alize small lure objects was markedly impaired. ere usually affected to some extent with the im- e severe on the side of the lesion in the majority ause all the optic fibers cross in the chiasma in nd because the optokinetic responses to the left only through the right eye and vice versa, the suggest that the fibers subserving these opto- nses are largely uncrossed at the level of section se involved in the localization responses are ed. In the bilateral cases all visual responses ed except in one animal which retained weak eactions toward one side only, indicating that on had not been complete on one side. Various mptoms such as opisthotonus, rolling and veer- le, swimming upside down, weakness, ataxia, etc. d in varying degree and form but no attempt the present experiment to keep any detailed non-visual defects.

regeneration with eyes normally oriented. In the ses optokinetic responses toward the side of the

hemisection began to reappear at the end of the third post-operative week. These reactions rapidly became stronger and were accompanied by other signs of functional improvement during the following several weeks. Recovery was delayed approximately three to 6 weeks in the bilateral cases which had been kept at lower temperatures. The final tests of recovery were made in all cases between 95 and 110 days after operation.

In general, after regeneration, the lost functions were found to have been restored in an orderly systematic manner. In the unilateral cases the optokinetic responses in all three primary planes were restored to approximately their pre-operative status. The ability to localize small objects in space also underwent great improvement until no conspicuous defects remained on either side. The best cases were indistinguishable from normal with the tests employed. The bilateral cases recovered optokinetic responses correctly directed in all three planes. Responses in the frontal plane were roughly normal in character. Those in the transverse and sagittal planes, however, which normally are less pronounced, were noticeably weak in most cases. In one case the responses seemed to be lacking entirely in the transverse plane and in another no reliable optokinetic reactions could be elicited in either vertical plane.

The localizing reactions of all but three of the bilateral cases were made consistently toward the proper quadrant of the visual field for all quadrants on both sides after the eyes had been returned to normal position. There was a range in the degree of recovery, however, from the best case which responded with roughly the normal speed and accuracy to the worst cases which were obviously slower to react and less certain of the precise direction and distance of the lure. In one of the three exceptional cases no localizing responses could be elicited on one side. In the second exception, distinct erroneous reactions toward the corresponding quadrant of the visual field on the contralateral side were made consistently when the lure was presented in the dorsal quadrants on either

is the animal in which the vertical optokinetic
e lacking. Apparently regenerating fibers which
rossed the midline had failed to do so, or vice
hird exception frequently displayed crossed re-
r to those of the preceding case when the lure
l in the dorsal quadrant of the left visual field.
gave no information on visual acuity and it is
acuity was quite low after regeneration. Nor
furnish an exact measure of directional precision
of gross quadrant discrimination. One got the
at the responses of most of the animals, particu-
teral cases, were not aimed as precisely after
as before. Nevertheless, correct optokinetic re-
vement of the visual field around the three main
ody and accurate localization even though it was
extent of turning to the proper quadrant of the
equire together a high degree of selectivity and
underlying neural mechanisms.
sual functions likewise appeared to have under-
lerly recovery. The various abnormalities of
movement had almost completely disappeared in
l cases and had very largely disappeared in the
up at the time of the final tests.
with eyes rotated. When visual responses reap-
e 7 animals in which the eyes had been rotated,
l the same systematic reversal observed after
tion of the eyes in animals otherwise normal
a). Reversal of the optokinetic responses in the
nd transverse planes, spontaneous optokinetic
swaying movements of the head, most pronounced
ontal plane, and erroneous localization of small
appeared in characteristic fashion to a degree
the subsequent display of normal reactions al-
ibed above. These maladaptive coordinations per-
out correction longer than a month in all cases
es were reset in the normal orientation.

Effect of optic lobe lesions. It was possible that regeneration had taken place at random and that by "induction" effects via the efferent connections a reorganization of synaptic relations had occurred in the higher centers to suit the new patterns of connections at lower levels. There was no indication of this in the functional results of destruction of the median part of the optic lobes in 5 of the bilateral cases after the eyes had been reset. The effects of these lesions were found to be similar to those obtained in normal cases, namely, an elimination of all responses to the lure when it was presented

Fig. 2 Photomicrograph of a parasagittal section through the scar region showing the deflection and intermixing of the regenerated fibers despite which there occurred an orderly recovery of function. (475 ×.)

anywhere in the dorsal part of the visual field plus retention of normal reactions to the lure when it was presented in the ventral portions of the visual field. Had there been a functional readjustment in the optic lobe to suit random connections in the lower centers, the above operation should have impaired vision in the ventral as well as in the dorsal quadrants and also might possibly have left some residual vision in the dorsal quadrants. Apparently, however, the afferent system had retained its original organization and the efferent relations had been adjusted accordingly.

checks. Microscopic examination of the sec-
vealed a copious supply of regenerated fibers
p where the transection had been made. The
tortion visible in the fascicular and intra-
patterns varied in degree from two cases in
fficult to locate the scar line to others in which
tion was evident. The photomicrograph (fig.
a case in which the deflection of fibers was
ich nevertheless had exhibited an orderly re-
tion. The erratic intertangled course taken by
ugh the scar in the majority of cases amply
nference that the methodical character of func-
could not possibly be ascribed to mechanical
bers across the lesion into their former path-

DISCUSSION

egree of orderliness with which functional re-
ween the primary visual and lower-level centers
by regeneration following brain transection in
experiments cannot be accounted for by mere
idance of the regenerating fibers. The results
icate the action of systematic discriminatory
me kind in the recovery process. That learning
ation through functional adjustment might be
as been ruled out by the fact that the central
were restored in the same systematic manner
he eyes had been previously rotated 180 degrees
nctional results of recovery highly maladaptive.
s are explicable on the assumption that the re-
bers differ in character in accordance with their
erent associations and that this constitutional
s able to influence in a selective manner the type
associations which were formed in the efferent
must be concluded that the lower-level neurons
er qualitatively among themselves, for, if they
e, there would have been no basis on which the
fibers could have established associations selec-

tively. Presumably the formation of synaptic connections in the spinal and medullary centers is regulated by differential affinities between the various types of regenerating fibers and the different kinds of lower-level neurons. The regenerating fibers in their descent into the cord may be pictured as giving off numerous collaterals which arborize profusely in the central grey and superficial neuropil zones encountering many types of nerve cell bodies, dendrites, axons, glia cells, and capillaries with only a few of these contacts, limited to specific neuron types, resulting in the formation of synaptic endings.

It had already been inferred indirectly from the results of regeneration of spinal limb nerves and of the IInd, IIIrd, Vth, VIIth, and VIIIth cranial nerves (Sperry, '48) that the secondary central neurons connected with these various nerves must be qualitatively specified. The present results are entirely in accord with those obtained on synaptic formation within the primary nuclei and lend additional support to the "biochemical affinity" hypothesis of synaptic organization outlined above.

The only other possible basis of interpretation recognizable at present lies in the "resonance" and "specific nerve energy" concepts formulated by Hering ('13) and elaborated more recently by Weiss ('36, '41). It could be supposed that the visuomotor coordination of these animals is independent of selectivity among neuronal interconnections and is regulated instead through the emission of specific frequencies or modes of excitatory energies plus selective sensitization to these on the part of the different neuron types. Orderly recovery of function might be possible on this basis despite a diffuse and indiscriminate reestablishment of synaptic connections. Reasons discussed elsewhere (Sperry, '48) for continuing at this time to favor the connectionist interpretation are applicable to the present observations.

Good discussion material to contrast w/ the chemoaffinity theory

The misdirection of localizing responses toward the corresponding quadrant of the contralateral visual field clearly detectable in two of the bilateral cases indicated that the fibers of these intracentral neurons, like those of the optic inal sensory roots (Sperry, '45a; '48) are able r characteristic pattern of synaptic connections y on either side of the neuraxis. Since such con- the wrong side of the brain or cord are highly maladaptive from the functional aspect, this is cation that the patterning of these synaptic rela- egulated on a functional basis. The fact that fibers outed sufficiently to terminate in the wrong side also provides further evidence that there was tunity mechanically for extensive intermixing of ber types.

SUMMARY

intracentral fiber tracts subserving visuomotor n were transected in adult newts slightly posterior al border of the mid-brain, the divided fibers re- and reestablished functional relations with the ar and spinal centers in an orderly systematic echanical guidance of the regenerating fibers and by function were both eliminated as factors re- or the orderliness of recovery. It is suggested that biochemical affinities exist between the higher and neurons and that the selective patterning of syn- ections is regulated by these affinities in accordance lernized chemotactic interpretation of the develop- anization of neuronal interconnections.

LITERATURE CITED

1913 Memory: Lectures on the specific energies of the nervous tem. Open Court Publishing Co., Chicago, 72 pp.
V. A. 1932 Principles of development of the nervous system eurobiotaxis) in Cytology and Cellular Pathology of the Nervous stem. (ed. W. Penfield), P. Hoeber, N. Y., pp. 45–89.
. 1943a Effect of 180 degree rotation of the retinal field on visuo- otor coordination. J. Exp. Zool., *92:* 263–279.
1943b Visuomotor coordination in the newt (Triturus viridescens) ter regeneration of the optic nerve. J. Comp. Neur., *79:* 33–55.
1944 Optic nerve regeneration with return of vision in anurans. J. europhysiol., *7:* 57–69.

SPERRY, R. W. 1945a Restoration of vision after crossing of optic nerves and after contralateral transplantation of eye. J. Neurophysiol., *8:* 15–28.
——— 1945b Centripetal regeneration of the 8th cranial nerve root with systematic restoration of vestibular reflexes. Am. J. Physiol., *144:* 735–741.
——— 1945c The problem of central nervous reorganization after nerve regeneration and muscle transposition. Quart. Rev. Biol., *20:* 311–369.
——— 1946 Ontogenetic development and maintenance of compensatory eye movements in complete absence of the optic nerve. J. Comp. Psychol., *39:* 321–330.
——— 1947 Nature of functional recovery following regeneration of the oculomotor nerve in amphibians. Anat. Rec., *97:* 293–316.
——— 1948 Mechanisms of neural maturation. In Handbook of Experimental Psychology, (ed. Stevens), John Wiley and Sons, N. Y. (in press).
WEISS, P. 1936 Selectivity controlling the central-peripheral relations in the nervous system. Biol. Rev., *11:* 494–531.
——— 1941 Self-differentiation of the basic patterns of coordination. Comp. Psych. Monog., *17:* 1–96.