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FURTHER EXPERIMENTAL INVESTIGATIONS ON
THE PHENOMENON OF HOMOLOGOUS RESPONSE
IN TRANSPLANTED AMPHIBIAN LIMBS

III. HOMOLOGOUS RESPONSE IN THE ABSENCE OF SENSORY
INNERVATION

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ONE FIGURE

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In the preceding article (Weiss, '37 b) new evidence was put forward proving that there is no selectivity in the re-establishment of nervous connections between the central nervous system and transplanted limbs that could account for the phenomenon of homologous response (Weiss, '36 b). The reinnervation of the transplants is achieved by their being replenished indiscriminately with branches regenerating from whatever nerve fibers are available. The specificity of the response of the muscles in the transplants (Weiss, '37 a), lacking morphological counterpart, must therefore be explained on a physiological basis. The assumption was made that this physiological principle consists of some selective functional relationship between the centers on the one hand and the muscles, or the nerves specified by their muscles, on the other. One might make the reservation, however, that before accepting the cogency of such far-reaching modifications of our notions concerning the activities of the nervous system, one other alternative should first be tested which might

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possibly reconcile the new facts with the old ideas of a purely geometrical linkage between the centers and the periphery. This alternative was thought by some to lie in the principle of sensory control: the contention was that homologous response may be due to influences reaching the centers from the transplanted muscles by way of sensory pathways.

That the phenomenon of homologous response is certainly not the result of secondary adjustments in the sense of learning or conditioning, has been established beyond doubt by the experiments described in the first article of this series (Weiss, '37 a) as well as by those in which a reversal of locomotion was obtained after the bilateral exchange of limbs (Weiss, '35). The absence of functional and adaptive value for the body of the movements of transplanted limbs is too obvious to deserve further comment; but this cannot obviate the objection that sensory effects might be responsible in some other, non-adaptive, sense for informing the centers of the presence and the names of the newly inserted muscles. As a matter of fact, the specificity of proprioceptive excitations has been demonstrated by Verzár and Weiss ('30; see also the first article of this series, Weiss, '37 a), so that it was not unfounded speculation to assume that the centers may receive sensory clues as to what particular muscles had come to lie at the ends of the various lines. And if this specific afferent excitation could, owing to its specificity, affect the central connections in such a way as to produce the observed association between synonymous muscles, a plausible explanation of the phenomenon of homologous response along conventional lines may seem feasible.

It has been shown, however, that this would seem so only to a rather superficial observer, and that if one actually tries to elaborate the scheme in concrete terms, one soon gets into conflict with the facts (Weiss, '28, 29). However, instead of relying on such indirect arguments, it seemed preferable to conduct a critical experiment and to examine directly what the function of transplanted legs would be in the absence of sensory innervation. Inasmuch as the phenomenon of homolo-

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gous response is a spinal phenomenon persisting after decerebration (Weiss, '37 a), and since the limbs are outside of the visual field of the animal, not to mention the improbability that visual control can interfere with muscular coordination in Amphibia, the proprioceptive control from the limbs themselves was the only type of sensory influx that had to be considered. Accordingly, the experiments consisted of complete deafferentation of the left fore limb without damage to the motor innervation, and of transplanting then into the vicinity of this limb a supernumerary fore limb, to be supplied by one of the purely motor nerves of the plexus.

MATERIALS AND METHODS

As in most experiments of this series, *Amblystoma mexicanum*, the axolotl, was used, the specimens measuring between 4 and 6 cm. of length. In chloreton anaesthesia a flap of skin covering the back over the shoulder level was lifted. Then by a longitudinal incision, the axial musculature was teased away from the spine, and the ribs were transected until the brachial nerves became visible in the depth. By tracing these nerves proximally under the dissecting microscope, the spinal ganglia were located. The ganglia were severed from their distal nerves and then pulled out, whereby the dorsal roots remained attached to them. Since the main innervation of the fore limb comes from the third, fourth and fifth segments, with the second segment adding a minor contribution, the second to the fifth spinal ganglion inclusive were removed. In some cases the sixth ganglion was also included. After the resection the wound was closed and the skin readjusted. Mortality after the operation was about 50%. Thirteen animals survived.

After the operation the animals were kept under observation and their reactions were studied. The results of these studies were reported in a previous paper to which we refer for further information (Weiss, '36 a). It was outlined there that, in agreement with what had been found in the toad, deafferentation fails to entail an essential impairment of the

locomotor function of the limbs. The deafferented limbs played their normal and coordinated part in the locomotor patterns. The only difference from normal was a certain hypertonicity of the adductor and extensor muscles in the resting posture of some of the animals. During locomotor activities this hypertonicity was acutely superseded by seemingly normal movements of the limb, and after a few weeks it had subsided altogether. The results were very uniform and applied to all animals except one with motor paralysis in which apparently the motor nerves had been damaged accidentally in the operation. It was concluded from these observations that the central locomotor patterns survive the removal of the sensory innervation of the limbs; that limbs with purely motor innervation exhibit normal activities; and that, in general, the role of sensory control for establishment and maintenance of motor coordination patterns is overrated in lower vertebrates.

In eleven cases in which the persistence of motility in the deafferented limb had been ascertained, a supernumerary fore limb was grafted near the deafferented limb. The interval between the deafferentation and the transplantation varied from 6 to 19 days. Table 1 summarizes the results. The method of transplantation was described in the first paper of this series (Weiss, '37 a). The transplants received for their innervation either the fifth nerve alone or the fifth and part of the fourth nerve.

Before the transplantation was performed, the deafferented region was tested for the presence or absence of sensibility. A few remarks must be added concerning these tests: Animals of the sizes used are extremely sensitive to mechanical stimulation applied to the skin. Three types of stimuli were applied which, in the order of their effectiveness, are: pinch with the forceps, passive movements of the limb, and touch with a glass rod with blunt end. The threshold of sensitivity seemed to vary greatly with the general state of activity of the animal. Under normal conditions the lightest touch possible in using a rigid instrument causes an instantaneous depression

of the gills; by stimulating jerk of the and never fail in

The same stimulated limbs, remaining pinching and squ on the part of that the operation

PROTOCOL NO.	MOTILITY OF O AFTER DEAFFERENTATION	D T F T
T28	Normal	
T29	Normal	
T30	Normal	
T31	Normal	
T32	Normal	
T33	Normal	
T36	Paralyzed	
T37	Normal	
T38	Normal	
T39	Normal	
T40	Normal	

sensory connection and that the motor.

The first movement of the limb, namely the time of the innervated by motor individual case 28 days, not of time must be motor roots, damage in the exper

of the gills; by stronger stimulation one obtains a quick swimming jerk of the whole animal. These reactions are prompt and never fail if elicited from normal limbs.

The same stimulations, however, if applied to the deafferented limbs, remained without any effect. Even the strongest pinching and squeezing of the limbs failed to elicit any response on the part of the animal. From this it could be concluded that the operation had been successful in interrupting all the

TABLE 1

PROTO- COL NO.	MOTILITY OF O AFTER DEAFFER- ENTATION	INTERVAL BE- TWEEN DEAF- FERENTATION OF O AND TRANSPLANTA- TION OF T, IN DAYS	INTERVAL BE- TWEEN TRANS- PLANTATION OF T AND AP- PEARANCE OF FIRST MOVE- MENTS, IN DAYS	SENSIBILITY AT TIME OF APPEARANCE OF HOMOLOGOUS RESPONSE OF T, IN		LATER REAPPEAR- ANCE OF SENSI- BILITY, IN DAYS AFTER DEAFFER- ENTATION
				O	T	
T28	Normal	15	29	Absent	Absent	—
T29	Normal	15	26	Absent	Absent	65
T30	Normal	15	25	Absent	Absent	71 (trace)
T31	Normal	15	27	Present	Absent	64 (in T, trace)
T32	Normal	19	22	Present	Present	+
T33	Normal	14	29	Present	Present	+
T36	Paralyzed	7	37	Absent	Absent	77
T37	Normal	7	34	Absent	Absent	38 (only in T)
T38	Normal	6	Regenerated limb	Present	Present	+
T39	Normal	10	Preserved			
T40	Normal	11	33	Dubious	Dubious	+

sensory connections contained in the respective limb plexus, and that the nerves assigned to the transplants were purely motor.

RESULTS

The first movements in the transplants appeared at approximately the time when they were known to be due in limbs innervated by mixed nerves. The exact time needed for each individual case can be seen from table 1. The average was 28 days, not counting case T36 in which a certain amount of time must be allowed for the repair of the damage to the motor roots, done in the process of deafferentation. The average in the experiments with mixed nerves was 23 days (Weiss,

'37 a). The difference of 5 days seems to be significant, although it cannot be decided whether it is due to the general weakening of the animals during the first weeks after an operation as serious as the removal of the spinal ganglia, or to the difference in the seasons when the two sets of experiments were done, the one with mixed nerves having started in September, the one with purely motor nerves in November. One animal, T39, was sacrificed before function in the transplant was recovered; the rest were kept for several months.

The movements in the transplants reappeared in the same manner as was described in the first article of this series (Weiss, '37 a). From the very beginning there could be no doubt but that the contractions of the muscles in T were again strictly homologous with the contractions in O.² Homologous response appeared and persisted in exactly the same manner as in experiments with mixed nerves.

The persistent lack of sensory control is of crucial importance for the interpretation of these results. Regeneration of sensory connections from remnants of the spinal ganglia that might have been left behind had to be excluded. Therefore sensibility tests were resumed at the critical stage when movements in the transplants first appeared. At this time, sensitivity was found to be entirely absent in five of the cases (table 1). In one case, T31, the normal limb showed a certain amount of sensibility while the transplant was entirely anaesthetic. In another case, T40, sensitivity to stimuli of ordinary strength was absent but strong pinching could evoke a reaction. Finally, in three cases, T32, T33 and T38, a certain amount of sensibility was present in both limbs. Although it required considerably stronger stimuli than normal to evoke a response in these three cases, the fact that some sensory connections had been reestablished since the date of deafferentation seems beyond question. That the sensory recovery was due to regeneration rather than to an imperfection of the operation, is demonstrated by the fact that immediately after the operation sensitivity had been absent in

²T = transplant, O = the nearby original limb.

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all cases. There are strong indications that this regeneration came from collateral fibers of neighboring segments and not from possible remnants left in the operated segments. At least in cases T33 and T40, which were examined histologically in detail (see below), the absence of sensory connections within the operated segments could be ascertained beyond doubt.

Ruling out as inconclusive the cases with sensory recovery, our evidence rests upon six absolutely clear cases in which no trace of sensibility could be discovered in the transplants at the time when they resumed function. In five of these cases the normal limbs were also devoid of sensibility. This is all that it was necessary to prove with regard to our problem: that homologous response appears in the same perfection with or without sensory control.

Continued observation of the animals revealed that eventually, if sufficient time is allowed, a certain amount of sensibility reappears in practically every limb. Only in one animal, T28, did sensibility remain permanently wanting. In one animal, T37, it reappeared in the transplant but not in the normal limb, and in four more cases it became noticeable between 9 and 11 weeks after the deafferentation, which is about 4 weeks after function in the transplants was restored. The sensory responses of the animals were clear and well marked in these cases, although sometimes very strong stimuli were required to evoke them. The reason why they must be considered as due to collateral regeneration rather than to segmental regeneration will be discussed in the histological part of this paper.

HISTOLOGICAL EXAMINATION

Longitudinal serial sections in frontal orientation were made of the preserved animals. The sections were stained with iron-hematoxylin. The results of six cases, treated in this manner and examined thoroughly, were as follows:

The dorsal roots 2, 3, 4, 5 and in one case also 6, were completely absent on the operated side (fig. 1). Throughout the extent of the operated area the meninges were uninterrupted and no fiber was seen to enter the vertebral canal in

the region of the former dorsal roots. The ventral roots were intact and symmetrical on both sides. The operated side was carefully searched for remnants of ganglionic cells. These cells can be recognized so easily by their size, staining properties, and the large nucleus with the two darkly stained nucleoli, that even single cells scattered in the mesenchyme can be detected. In four cases not a single cell of this type was found within the segments from which the spinal ganglia had been removed (fig. 1).

In two cases, T29 and T36, the operation had not been quite radical; some spinal ganglion cells had been left behind, although all their connections with the spinal cord were interrupted and destroyed. In case 29, the left ganglia 2, 3 and 5 were completely absent. But on the fourth segmental nerve a remnant of the fourth ganglion, containing thirty-one cells, was discovered. The number of cells contained in the normal fourth ganglion of the opposite side was counted to 1969 cells. The thirty-one cells mentioned were located in a cluster at considerable distance from their original site, embedded in connective tissue which had formed a postoperative scar. They were attached to the motor root, but there was positively no trace of a dorsal root connecting these cells with the cord. In the second case of this sort, T36, we find a similar situation. Here the ganglia 2, 3 and 4 on the operated side were completely absent but a fragment of the fifth ganglion containing seventy cells had been left behind. Again the peripheral fibers of this remnant were found to join the motor root peripherally, but not a single fiber was found to have entered the spinal cord.

Therefore, all five studied cases were consistent in revealing the complete interruption of sensory communication between the transplanted limbs and the spinal cord from the second to the fifth segments inclusive.

It is interesting to compare this absence of sensory connections within the limb segments with the observation that eventually some sort of sensibility had reappeared in the limbs. The obvious objection that the sensory reactions may have been due simply to a mechanical transmission from the point



Fig. 1 Frontal section of the spinal cord showing the absence of dorsal roots and the presence of spinal ganglia of the fifth segment. Note the absence of dorsal roots thereof in the second, third, fourth, and fifth segments.

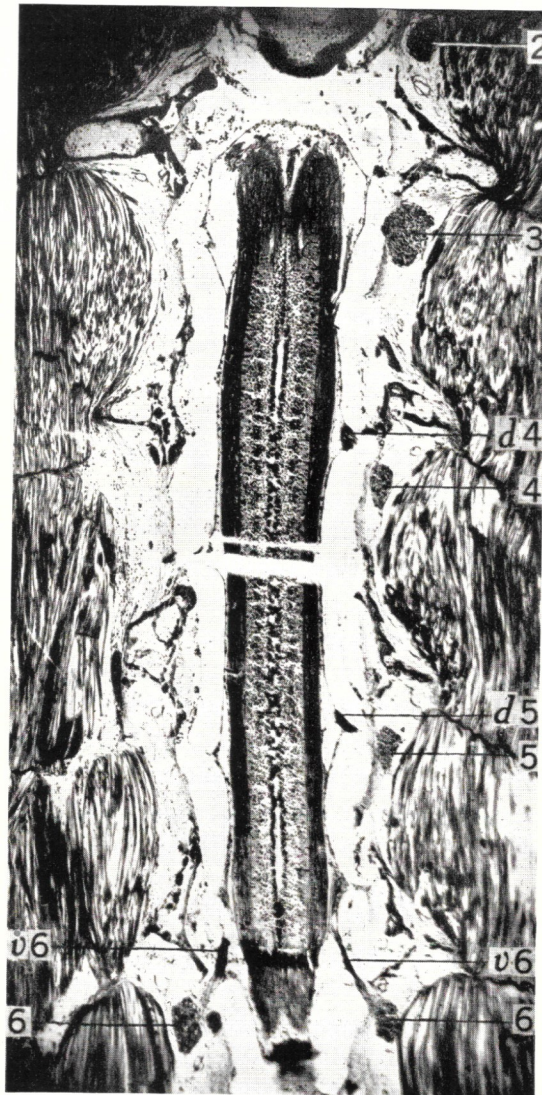
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Fig. 1 Frontal section through the spine of case T40 (table 1). Slight curvature with dorsal concavity has developed as a result of fixation. 2, 3, 4, 5, 6, spinal ganglia of the second, third, fourth, fifth and sixth segments; d4, d5, dorsal roots of the fourth and the fifth ganglia; v6 ventral roots of the sixth segment. Note the absence on the left side of ganglia and dorsal roots or traces thereof in the second, third, fourth and fifth segments.

of stimulation to some intact areas of the skin can be discounted in view of the fact that in the tests effects on distant regions were rigorously excluded. The explanation seems to be that a certain amount of collateral fibers from the neighboring segments had found its way into the denervated parts. The penetration of stray fibers into denervated regions is a well-known phenomenon. Not very much is known, however, about the conditions under which it occurs, nor can our experiments throw much light on the question. Moreover, the matter has no immediate bearing on the problem here under discussion.

CONCLUSIONS

The experiments reported in this article supplement earlier indirect evidence by a direct and crucial proof showing that sensory control is not among the functional mechanisms to be taken into consideration as possible explanations of the phenomenon of homologous response. Not only does sensory control in general seem to be immaterial for the establishment and performance of the normal basic locomotor patterns in Amphibia, as has been shown in a former paper, but it is equally insignificant in the appearance of homologous response in transplanted legs. In the transplanted limb every muscle, though innervated exclusively by motor fibers, contracted synchronously and syndynamically with the synonymous muscle in its nearby fellow limb.

It seems that no further doubt is possible as to the true nature of the phenomenon of homologous response after the results of this article and the two preceding ones (Weiss, '37 a, b). It is utterly erroneous to conceive of homologous response as being due to one of those processes of central adjustment which are known to occur in the higher vertebrates in the role of adapting, modifying or refining patterns of coordination in accordance with the needs of the body as a whole. Homologous response is nothing of the sort: it is merely a primary manifestation of the fundamental mechanism by means of which the centers and the periphery es-

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establish their specific relationships and entertain mutual correspondence. This mechanism employs specific and selective relationships between centers and periphery of such a nature that each individual muscle can be called into activity separately and with proper intensity whenever it is its turn in a given reflex pattern, even though connected with the centers in a new and haphazard way, and even though reports back to the centers of the peripheral effects by way of sensory messages have been excluded. Some assumptions concerning a mechanism that could satisfy these conditions have been made in the form of the so-called resonance principle; it was discussed under new angles in a recent paper, to which the reader may be referred for further information (Weiss, '36 b).

SUMMARY

1. In free-swimming larvae of the axolotl, *Amblystoma mexicanum*, the left arm region was deafferented by removing the spinal ganglia and dorsal roots of the second, third, fourth and fifth segments.
2. As described in a previous paper, the motility and coordinated function of limbs is not affected by deafferentation while sensibility is completely absent.
3. A supernumerary fore limb was then grafted to the deafferented area, and innervation for it was secured by inserting into it one of the deafferented, purely motor, nerves of the fore limb plexus.
4. About 4 weeks after the transplantation the first movements appeared in the transplants. These were typically homologous responses, each muscle of the transplant contracting at the same time and with the same strength as the synonymous muscle in the normal limb. The lack of sensibility in the limbs at this time was ascertained by mechanical stimulation. The function of a transplant could be resumed to its full extent and specificity in the complete absence of sensory innervation.
5. Histological examination of the animals proved the complete absence of sensory connections between the limbs and the spinal cord within the operated area.

6. The experiments furnish definite proof that sensory messages are not instrumental in the establishment of homologous response.

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