Neuronal Specification of Cutaneous Nerves through Connections with Skin Grafts in the Frog

Abstract. *Skin grafts were rotated 180 degrees in frog tadpoles. After metamorphosis, cutaneous reflexes were tested, and the receptive fields of cutaneous nerves were mapped electrophysiologically. Accurately localized limb movements were elicited by mechanical stimulation of normal skin, or of dorsal skin reimplanted on the back after 180-degree rotation. Reflexes misdirected to the original site of the stimulated skin were elicited from dorsoventrally inverted grafts, but not from anteroposteriorly inverted grafts. In most cases, the local nerves supplied the grafts, and each nerve entered the skin within its own receptive field. This observation eliminated the possibility that misdirected reflexes were due to selective regrowth of cutaneous nerves. We concluded that cutaneous nerves formed central synaptic associations which were specified by their new terminal connections with grafted skin.*

When the receptive fields of sensory neurons are interchanged, changes in reflexes result which have been thought to indicate that the central connections of sensory neurons may be specified through their peripheral connections (*1*). Miner (*2*) first showed that after 180-degree rotation of a skin graft on one side of a frog, reflex movements were misdirected to the original site of the graft rather than to the point of stimulation. She concluded that the contact of sensory nerves with the grafted skin resulted in a change in their specificity and a readjustment of their central connections. However, there are at least two other possible causes of such misdirected reflexes. First, specific peripheral connections might have reformed as a result of regrowth of cutaneous nerves to their appropriate places in the skin. Second, the local sign of the skin might be due to specific patterns of impulses originating from different places in the skin.

To distinguish between these alternatives, we have repeated Miner's experiments, and have extended them by making two kinds of skin grafts in *Rana pipiens* tadpoles at different developmental stages I through XIV (*3*) and by recording action potentials in nerves from normal and grafted skin. The stages at which the operations were done did not affect the results. In the first type, the skin was cut from the trunk on one side, extending from the middorsal to the midventral line and from behind the eye to the base of the tail. The graft was replaced after 180-degree rotation. These we call DV-inverted grafts. In the second type, the skin on the back was cut from between the eyes to the tail fin and was replaced after 180-degree rotation. These we call AP-inverted grafts. All nervous connections of the grafted skin were broken during these operations. Forty frogs with DV-inverted grafts and 14 with AP-inverted grafts survived through metamorphosis, and their cutaneous reflexes were then tested at intervals over a period of about a year.

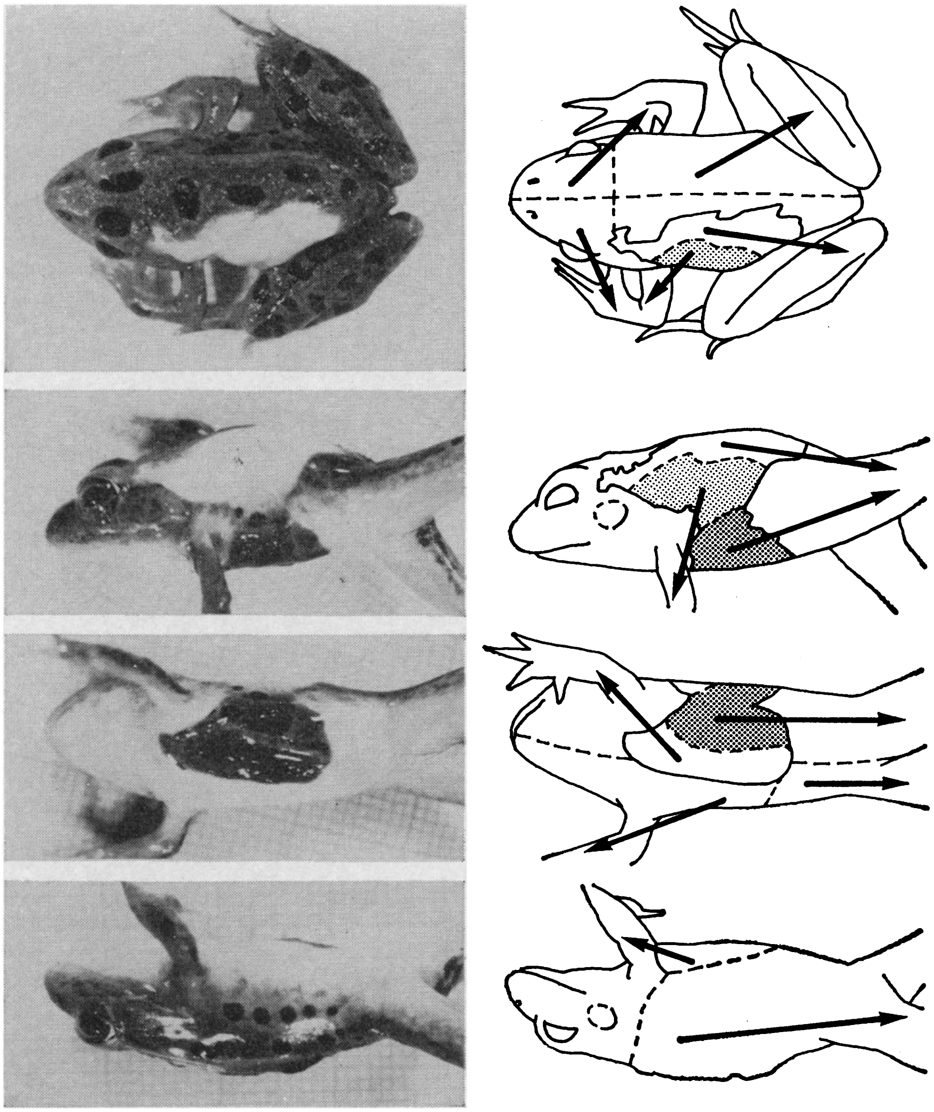

Fig. 1 (right). Different views of the same frog with a DV-inverted skin graft, prepared 280 days previously at larval stage XIV. Each arrow points from the reflexogenic zone of skin to the limb which responded when the skin-zone was stimulated. Misdirected limb movements resulted from stimulating the stippled zones: when the lightly stippled zone was stimulated, the forelimb wiped the darkly stippled zone; when the darkly stippled zone was stimulated, the hindlimb wiped the lightly stippled zone.

Stimulation of normal skin with a nylon bristle (0.4 mm diameter, exerting a pressure of 200 mg) resulted in limb movements directed at the point of stimulation. However, stimulation of DV-inverted grafts frequently resulted in reflex movements which were misdirected to the original site of the grafted skin. When back skin grafted to the belly was stimulated, the ipsilateral hindlimb wiped at the back. Stimulation of belly skin grafted to the back caused a forelimb wipe directed at the belly (Fig. 1). These observations agree with those of Miner (*2*). However, misdi-

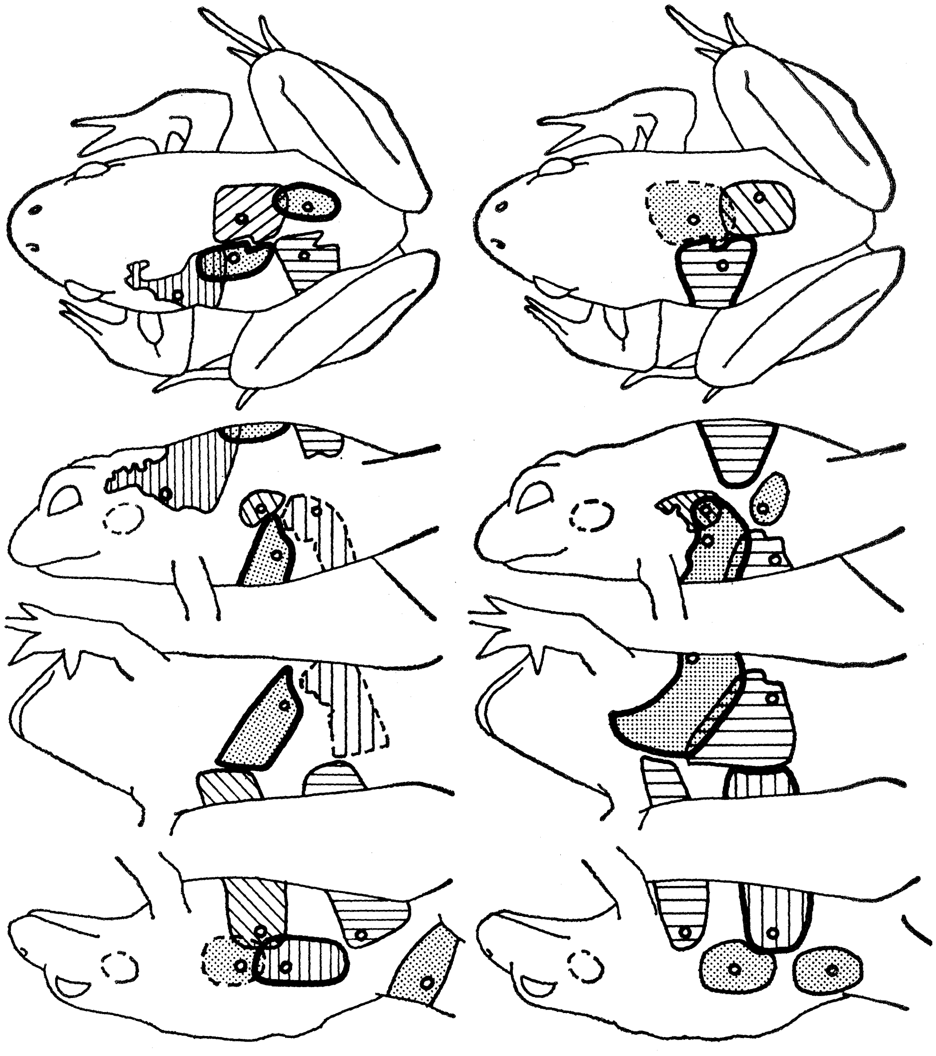

Fig. 2. Different views of the same frog as in Fig. 1 with DV-inverted graft. Receptive fields of cutaneous nerves were mapped by recording action potentials in the nerve which entered the skin at the position shown by a circle within each receptive field.

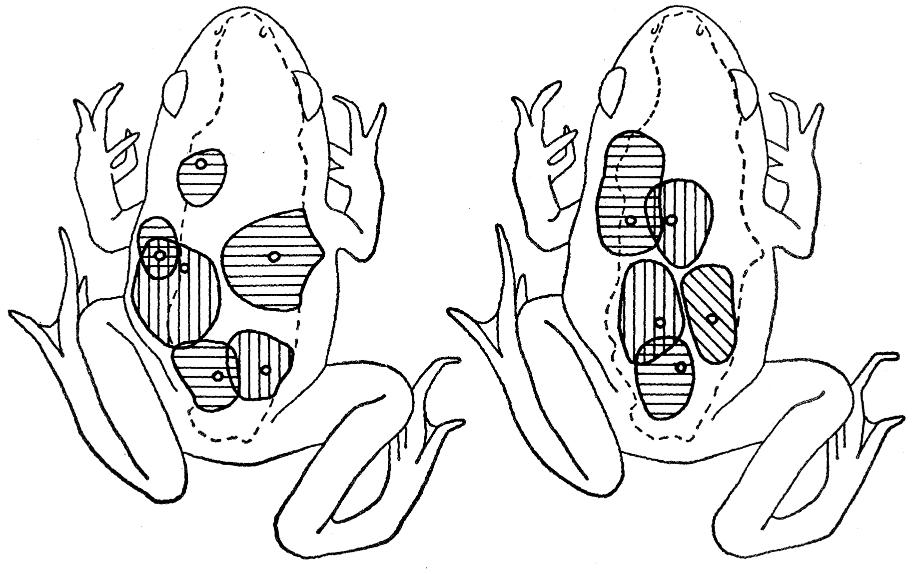

Fig. 3. Frog with AP-inverted graft prepared 326 days previously at larval stage I. Graft is outlined with dashed line. Each circle shows the point of entry of the nerve into its receptive field.

rected responses were never elicited by stimulating AP-inverted grafts; only normal limb movements occurred.

We mapped the receptive fields in four frogs with AP-inverted grafts and in 12 frogs with DV-inverted grafts by recording with a bipolar platinum electrode, occasionally from a single fiber but usually from several fibers firing simultaneously, when the skin was stimulated with the same nylon bristle used for testing reflexes. In most cases, regardless of the original position of the grafted skin, the cutaneous nerve entered the skin within its own receptive field (Figs. 2 and 3). Of 218 nerves tested, only six entered the skin a short distance outside their receptive fields. There was overlap of receptive fields, in agreement with previous observations (*4*). However, the receptive fields of normal skin and DV-inverted grafts did not overlap but always ended precisely at the edge of the graft (Fig. 2). It is unlikely that mechanical factors prevented nerves from growing across the edge of the graft, because the receptive fields of normal skin and AP-inverted grafts did overlap (Fig. 3). This receptive field overlap may be one reason why the reflexes elicited from AP-inverted grafts were normal.

Receptive fields of dorsal cutaneous nerves were roughly circular or oval with the anteroposterior axis longer than the mediolateral. Receptive fields of ventral cutaneous nerves were always much longer in the mediolateral than in the anteroposterior axis (Fig. 2). These distinctive shapes of dorsal and ventral receptive fields were not altered by the grafts. Therefore, the shape of the receptive field is mainly determined by the nerve rather than by the skin into which the nerve grows.

Our observations eliminate the possibility that misdirected reflexes were due to selective regrowth of sensory nerves to their original places in the skin grafts. The second alternative, that specific patterns of impulses originate from different places in the skin, though difficult to exclude, is unlikely. The pattern of impulses in any single cutaneous nerve fiber varied according to the mode of application of the stimulus, in agreement with more detailed studies of action potentials originating from cutaneous mechanoreceptors in the frog (*5*). Therefore, of three possible causes of misdirected reflexes elicited from 180-degree rotated skin grafts we have excluded specific peripheral reconnection and have found no evidence of specific patterns of impulses in different sensory nerves. It is most likely that each region of the skin has a local property which determines the specific central connection of its nerves.

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Fatty Acids in Blue-Green Algae: Possible Relation to Phylogenetic Position

Abstract. *Analyses of the lipids in five species of blue-green algae show that the fatty acids are largely the C_{16} and C_{18} acids. The only alga that could be grown heterotrophically,* Chlorogloea, *formed the triply unsaturated C_{18} acid in the light but only the doubly unsaturated C_{18} acid in the dark. Examination of these results and the results of others suggest that, except for one species, the more highly unsaturated acids are found in the morphologically more complex algae. The fatty acid compositions of blue-green algae are different from the fatty acid composition of the other prokaryotic organisms, the bacteria. It is speculated that the diversity of the patterns of fatty acid composition among the blue-green algae could be of phylogenetic significance.*

Several species of blue-green algae appear to be the only organisms now known that do not contain polyunsaturated fatty acids (*1–3*) but do carry out green-plant photosynthesis in which oxygen is evolved. Prior to our analyses of *Anacystis nidulans* (*1*), the correlation between the ubiquity of polyunsaturated acids and oxygen evolution in green plants suggested a relation of these acids to oxygen production (*4*), because in photosynthetic bacteria the polyunsaturated acids are absent and no oxygen is evolved during photosynthesis. Recent studies (*5*), in which the concentration of the polyunsaturated acid α-linolenate has been compared to photosynthetic evolution of oxygen by two species of the green alga *Chlorella,* have resulted in disagreement in the interpretation of results as to whether the content of α-linolenate is related to photosynthetic production of oxygen. It is clear that the functions of polyunsaturated acids in green plants are not yet understood, and further knowledge is desirable. Parker, Van Baalen, and Maurer (*3*) concluded that the blue-green algae were probably not the source of fatty acids found in the organic matter of sediments and noted that the 11 species that they studied could be divided into three categories on the basis of the content of oleic, linoleic, and linolenic acids. Our data confirm and extend these data and suggest a possible correlation of the classical phylogenetic position based on morphology with that suggested by the qualitative content of fatty acids.

Growth conditions used for the algae we studied are given in Table 1. Although the temperature was not rigidly controlled, we reported earlier that temperature affects quantitatively but not qualitatively the composition of fatty acids of *Anacystis* (*1*). Algae were harvested by centrifugation, washed in distilled water, lyophilized, and stored at −18°C until they were analyzed.

Lipids were extracted from 1 g of lyophilized algae and were saponified and esterified according to the procedures of Nichols, Harris, and James (*6*). A second saponification of the lipid fraction with methanolic KOH containing 5 percent water was done to insure complete saponification (*7*). Nonsaponifiable material was removed with peroxide-free ether and gas chromatographic analyses were made on the reesterified acid fraction. To aid in the interpretation of the results, a portion of each ester mixture was reduced with hydrogen and platinum dioxide and analyzed in the same manner as the unreduced mixture.

As Parker *et al.* (*3*) noted in their survey of 11 species of blue-green algae, the fatty acid compositions of the algae we studied appear to be quite simple, but there are significant qualitative variations between one species and another (Table 2). The unicellular form *Synechococcus cedrorum* contains only the mono-unsaturated 18 : 1 (*8*) acid which has also been found in the other unicellular forms investigated, *Anacystis nidulans* (*1*) and *A. marina* (*3*). The simple filamentous species *Oscillatoria* contained both the polyunsaturated 18 : 2 and 18 : 3 acids, as had been shown by Schmitz (*9*) for *O. chalybea,* although only the 18 : 2 acid was present in marine filamentous species *O. williamsii* (*3*). We found polyunsaturated acids in *Nostoc muscorum,* as Parker *et al.* did (*3*).

The unexpected results involve two species. *Hapalosiphon laminosus* is a thermophilic blue-green alga found in many hot springs (*10*). Morphologically, it is more complex than any of the previously mentioned algae because it has true branches and specialized cells akinetes and heterocysts. Yet only mono-unsaturated acids were found in this organism.

Table 1. Growth conditions of algae. For medium D, see Kratz and Myers (*18*); medium A and A as given by Allen and Arnon (*19*) was supplemented with $10^{-2}M$ sucrose and 2 g of KNO_3 per liter; medium No. 11, see Hughes *et al.* (*20*). Cultures of the first four organisms were gassed with 0.5 percent CO_2 in air, *Oscillatoria* was grown in stagnant culture, and the *Hapalosiphon* was grown in shake culture. Au, autotrophic growth in the light; He, heterotrophic growth in the dark.

Organism*	Growth conditions		Gas phase
	Temp. (°C)	Medium	
Synechococcus cedrorum	26–30	D	0.5% CO_2
Nostoc muscorum A	26–30	D	.5% CO_2
Chlorogloea fritschii (Au)	26–35	A and A	.5% CO_2
Chlorogloea fritschii (He)	35	A and A	.5% CO_2
Oscillatoria sp.	25	No. 11	Air
Hapalosiphon laminosus	40	No. 11	Air

* Sources of the organisms were as follows: *Synechococcus* and *Nostoc,* Indiana University Culture Collection of Algae; *Chlorogloea,* Dr. G. E. Fogg, Westfield College, University of London; *Oscillatoria,* isolated from a soil sample from Knoxville; and *Hapalosiphon,* isolated by R. W. Holton (*10*).