THE SPROUTING OF HIGH-THRESHOLD CUTANEOUS NERVES AND
ITS ACCELERATION BY NEURAL ACTIVITY

By

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(ii)
ABSTRACT

Previous studies have shown that in the rat the sprouting of low-threshold ("touch"-sensitive) nerves into adjacent denervated skin can occur only during a brief "critical period" of post-natal life that ends at about 20 d of age. Additionally, for any segmental nerve this sprouting occurred only in the region of that nerve's sensory dermatome. One objective of my thesis was to examine whether the sprouting of high-threshold ("pain"-sensitive) cutaneous nerves is also temporally and spatially constrained.

The presence of high-threshold nerve endings in skin was detected behaviourally by pinching with toothed forceps; normally this elicits a reflex contraction of an underlying skeletal muscle, causing visible skin puckering. Denervated skin failed to respond in this way for the first 2-3 weeks after nerve section, but then a gradual recovery of the response occurred spreading from the adjacent innervated region of skin progressively further into the deprived area. That this recovery was due to sprouting was shown by histological examination of skin, and the findings of nerve fibres within the dermis in sensitive but not insensitive skin. The sprouting occurred in the adult animal, and proceeded beyond the dermatome border of a nerve, showing that intact high-threshold nerves are not under any obvious spatial and temporal constraints.

During the examination for the presence of high-threshold nerve sprouting I noted that repeated skin pinching apparently accelerated the return of high-threshold sensitivity. The second main objective of the thesis was to examine what this phenomenon depended upon. In
animals that were repeatedly stimulated physiologically every 4 d, all of the denervated skin became reinnervated by 20 d, compared to the 40 d or more needed for unstimulated animals. The accelerating affect was mimicked by direct electrical excitation of the remaining nerve. The results revealed that it was the latency for sprouting that was affected; once begun, the rate of sprouting was normal. The effects of electrical stimulation of the intact nerve were prevented if TTX was applied so as to block impulses proceeding centrally from the site of electrical stimulation, but not if they were only prevented from proceeding peripherally. A hypothetical mechanism to explain the effects of impulse activity in causing acceleration of sprouting is provided.
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Fulfillment is a great bondage; it obliges you to achieve an even greater fulfillment.
TABLE OF CONTENTS

SECTION 1

BACKGROUND TO THE INVESTIGATION 2  
A) THE PHENOMENON OF NERVE SPROUTING 2  
   1) Evidence for External Influences Modifying Neuronal Growth During Development 2  
   2) Sprouting After Partial Denervation of the Target 4  
      a) In Partially Denervated Muscle 4  
      b) The Autonomic Nervous System 4  
      c) The Central Nervous System (C.N.S.) 5  
      d) Cutaneous Nerves 6  
   3) Sprouting Into Added or Extra Target Tissue 9  
   4) Sprouting After Colchicine Treatment of Adjacent Nerves 9  
   5) Sprouting in Tissue Culture 10  
B) IDENTIFICATION OF THE SPROUTING STIMULUS 11  
   1) Nerve Growth Factor 11  
   2) Products of Degenerating Nerves 12  
   3) Muscle Activity and the Stimulus to Sprout 13  
C) REGULATION OF COLLATERAL SPROUTING 14  
   1) Blockage of Axonal Transport and its Effect on Sprouting 14  
   2) Role of the Target Tissue in Regulating Axonal Sprouting 15  
   3) Spatial Constraints on Collateral Sprouting 16  
   4) Temporal Constraints on Collateral Sprouting 16  
D) EFFECTS OF IMPULSE ACTIVITY ON THE NEURON 17  
   1) Histological Effects of Impulse Activity 18  
      (vi)
2) Metabolic Effect of Impulse Activity
3) The Effect of Impulse Activity on Axoplasmic Flow
4) The Effect of Impulse Activity on Collateral Sprouting of Intact Nerves

E) PROBLEMS OF BEHAVIOURAL TESTING OF HIGH-THRESHOLD FIELDS
1) Effect of Nerve Sections Distal or Proximal to the D.R.G.

SECTION 2
THE PRINCIPAL AIM AND GENERAL STRATEGY OF THE INVESTIGATION

SECTION 3
METHODS AND MATERIALS
1) Animals
2) Anaesthesia
3) Surgical Procedures
4) Denervation of Skin
5) Electrical Stimulation of Intact Cutaneous Nerves
6) Application of Tetrodotoxin (TTX) to Intact Cutaneous Nerves
7) Mapping of Low and High-Threshold Fields
8) Measurement of Sensitive and Insensitive Skin Areas
9) Measurement of the Field "Axes" of mDCN-T13
10) Histology of Nerves Within Skin
11) Electrophysiological Identification of the Presence of High-Threshold Fibres in Skin
12) Statistical Analysis
SECTION 4

RESULTS

A) THE ANATOMY OF THE CUTANEOUS HIGH AND LOW-THRESHOLD FIELDS OF THE RAT'S TRUNK

1) The Cutaneous Nerves

2) The Low and High-Threshold Mechanosensory Fields Compared

B) SPROUTING OF THE mDCN-T_13 HIGH-THRESHOLD NERVES INTO ADJACENT DENERVATED SKIN

1) Introduction

2) Low-Threshold Nerves: Lack of Collateral Sprouting

3) Are There Silent (Non-Functional) High-Threshold Nerve Endings in Behaviourally Insensitive Skin?

4) Histological Examination for the Presence of Nerves in Skin
   a) Presence or Absence of Axons in Skin

C) QUANTITATIVE CORRELATION OF THE SKIN AREA CONTAINING AXONS AND THAT SENSITIVE TO FORCIP PINCHING: THE BORDER DETERMINATION

D) WHICH FIBRE TYPES SPROUT INTO DENERVATED SKIN IN THE ADULT RAT?

1) Electrical Excitation of mDCN-T_13

2) Electrical Excitation of Skin

E) SPATIAL CONSTRAINTS ON SPROUTING OF THE INTACT HIGH-THRESHOLD NERVES

1) Spatial Constraints on mDCN-T_13 High-Threshold Field Sprouting

2) Spatial Constraints on DCN-T_13 High-Threshold Field Sprouting

3) Sprouting of mDCN-T_13 vs DCN-T_13 High-Threshold Nerve Sprouting
F) EFFECTS OF ACTIVITY ON THE SPROUTING RESPONSE OF INTACT HIGH-THRESHOLD NERVES 53

1) Observations Pending to the Study 63

2) Further Rationale for the Proposed Study of Impulse Effects on Sprouting 64

G) AN INVESTIGATION OF THE BASIS OF THE APPARENT ACCELERATION IN SPROUTING CAUSED BY REPEATED BEHAVIOURAL TESTING 66

1) The Effect of Physiological Stimulation on Sprouting of High-Threshold Nerves 66

2) Examination of the Effects of Tetrodotoxin (TTX) Block of the Physiologically Stimulated mDCN-T13 on the Accelerated Sprouting 67

H) THE EFFECT OF ELECTRICALLY-EVOKED IMPULSE ACTIVITY ON THE SPROUTING OF INTACT HIGH-THRESHOLD NERVES 69

1) The Normal Time Course of Sprouting of the High-Threshold Fibres 69

2) The Time Course of Sprouting of the High-Threshold Nerves of mDCN-T13 After Electrical Stimulation 70

I) DO THE IMPULSES EVOKED BY ELECTRICAL STIMULATION OF THE INTACT HIGH-THRESHOLD NERVES HAVE TO TRAVEL PERIPHERALLY, CENTRALLY, OR BOTH, TO ACCELERATE THEIR SPROUTING? 73

1) Do the Electrically Evoked Impulses Have to Travel Peripherally to Cause the Phenomenon? 73

2) Do the Impulses Have to Travel Centrally? 73

J) PRELIMINARY RESULTS CONCERNING THE CHARACTER OF THE EFFECT OF IMPULSE ACTIVITY ON SPROUTING OF THE HIGH-THRESHOLD CUTANEOUS NERVES 76

1) Concerning the Time Course of the Decay of the Effect of Electrical Stimulation 76

2) Will Physiological Stimulation of One Half of the Receptive Field of the Intact mDCN-T13 Nerve Affect Sprouting of the Non-Stimulated Half? 77

3) Does Electrical Stimulation, in the Adult Rat, of the Low-Threshold ("Touch-sensitive") Nerves in mDCN-T13 Evoke Their Sprouting Into Adjacent Denervated Skin? 79
SECTION 5

DISCUSSION

A) TECHNIQUE

1) The Physiological Excitation of Cutaneous Nerves and the Reflex Response

2) The Resolution of the Mapping Technique

B) RESULTS

1) Sprouting of the High-Threshold Nerves in the Rat

2) Impulse Activity and Its Effect on Collateral Sprouting of Intact High-Threshold Nerves

3) Possible Mechanisms by Which an Increase in Neural Activity Accelerates the Observed Collateral Sprouting

4) Which Types of High-Threshold Nerves Have Sprouted?

APPENDIX I

Mann-Whitney U-Test

APPENDIX II

Comparison of Slopes

APPENDIX III

Raw Data

REFERENCES
LIST OF FIGURES

<table>
<thead>
<tr>
<th>Fig.</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Animal Housing</td>
<td>177</td>
</tr>
<tr>
<td>2</td>
<td>The Dorsal Cutaneous Nerves</td>
<td>179</td>
</tr>
<tr>
<td>3</td>
<td>The Segmental Origin of the Cutaneous Nerves</td>
<td>181</td>
</tr>
<tr>
<td>4</td>
<td>Incision Repair in Experimental Animals</td>
<td>183</td>
</tr>
<tr>
<td>5</td>
<td>The Result of the Surgical Denervation to Produce an mDCN-T_{13} &quot;Island&quot;</td>
<td>185</td>
</tr>
<tr>
<td>6</td>
<td>Electrical Stimulation of mDCN-T_{13}</td>
<td>187</td>
</tr>
<tr>
<td>7</td>
<td>The Application of TTX to mDCN-T_{13}</td>
<td>189</td>
</tr>
<tr>
<td>8</td>
<td>Behavioural Responses to High-Threshold Nerve Stimulation</td>
<td>191</td>
</tr>
<tr>
<td>9</td>
<td>The Behavioural Map of a mDCN-T_{13} Island</td>
<td>193</td>
</tr>
<tr>
<td>10</td>
<td>Low-Threshold Fields of DCNs</td>
<td>195</td>
</tr>
<tr>
<td>11</td>
<td>High and Low-Threshold Field of DCNs</td>
<td>197</td>
</tr>
<tr>
<td>12</td>
<td>Sprouting of High-Threshold Fields: Area of Insensitive Skin Remaining</td>
<td>199</td>
</tr>
<tr>
<td>13</td>
<td>Area of Low-Threshold Field of mDCN-T_{13} at Different Days After Denervation</td>
<td>201</td>
</tr>
<tr>
<td>14</td>
<td>Histological Examination of Sensitive Skin, Insensitive Skin and Skin into which High-Threshold Sensitivity had Returned after Denervation</td>
<td>203</td>
</tr>
<tr>
<td>15</td>
<td>The Correlation Between Behavioural and Histological High-Threshold Field Borders</td>
<td>205</td>
</tr>
<tr>
<td>16</td>
<td>Reflex Activation of the Cutaneous Trunci Muscle by Electrical Stimulation of a Cutaneous Nerve</td>
<td>213</td>
</tr>
<tr>
<td>17</td>
<td>Reflex Activation of the Cutaneous Trunci Muscle by Electrical Stimulation of Behaviourally Sensitive Skin</td>
<td>218</td>
</tr>
<tr>
<td>18</td>
<td>Denervation Pattern for the Experiments Dealing with Spatial Constraints</td>
<td>222</td>
</tr>
<tr>
<td>19</td>
<td>Measurement of High-Threshold Field Axis</td>
<td>224</td>
</tr>
</tbody>
</table>
Fig. 20  The Border Extension Due to Sprouting of mDCN-T_{13} Fields 226
Fig. 21  The Border Extension Due to Sprouting of DCN-T_{13} Fields 229
Fig. 22  The Border Extension Due to Sprouting of mDCN-T_{13} vs DCN-T_{13} Fields 232
Fig. 23  The Increase in Area Due to Sprouting of Intact High-Threshold Nerves of mDCN-T_{13} and DCN-T_{13} Fields 235
Fig. 24  The Effect of Repeated Behavioural Testing on Sprouting of High-Threshold Nerves 238
Fig. 25  The Effect of Physiological Activity on Sprouting of High-Threshold nerves 240
Fig. 26  The Time Course of Sprouting of the High-Threshold Fibres in mDCN-T_{13} 242
Fig. 27  The Effect of Electrical Stimulation (10 minutes) on Sprouting of the High-Threshold Nerves 244
Fig. 28  The Effect of Electrical Stimulation on the Area of High-Threshold Fields Four Days After Denervation 246
Fig. 29  The Effect of Electrical Stimulation (5 minutes) on Sprouting of High-Threshold Nerves 248
Fig. 30  Effect of Electrical Stimulation and TTX on Sprouting of High-Threshold Fibres 250
Fig. 31  The Effect of Electrical Stimulation on the Low-Threshold Fibres in the Adult Rat 253

(xii)
BACKGROUND TO THE INVESTIGATION

A) THE PHENOMENON OF NERVE SPROUTING

When target tissues of nerves are partially, or totally denervated, neighbouring intact nerves usually begin to sprout into the deprived regions (Weddell; Guttman and Gutman, 1941; Livingston, 1947; Speidel, 1941); these sprouts often attain normal function and thus may compensate for the lost innervation. The available evidence largely supports the view that such sprouting is triggered by factors originating in the target tissue, and the nerves themselves have been hypothesized as providing a means whereby the local effectiveness or availability of these proposed sprouting factors is somehow neutralized (Ramon y Cajal, 1919; Aguilar, Bisby, Cooper and Diamond, 1973; Diamond, Cooper, Turner and Macintyre, 1976). This experimentally evoked collateral sprouting is of special interest because of the possibilities that the mechanisms involved both in its initiation and its regulation may relate to those that operate when axonal sprouting occurs during the development of the nervous system, and possibly even in the mature organism (Speidel, 1933; 1935a,b; 1941).

1) Evidence for External Influences Modifying Neuronal Growth During Development.

Ramon y Cajal (1919) noted that during development, nerves grew relatively long distances to reach epithelial target tissues and only then would they sprout collaterals, each growing to a territory devoid of nerves; he suggested that the obvious explanation was the production by the target tissue of nerve sprouting factors. He provided us with a detailed description of the neuronal development in the corneal
epithelium of chick embryos and proposed that powerful alluring substances were produced by the aneuritic epithelium which acted on the growing nerves. Once the epithelium had become innervated he proposed that a state of chemical equilibrium was reached in which the epithelium was no longer capable of attracting new nerve sprouts. Forssman, (1900) coined the term neurotropism to describe the alluring effect of aneurogenic end organs (or targets) on growing nerves. Speidel (1935 b, 1941) observed the growth of nerves in the dermis of the tail fin of the living tadpole. He noted that the ingrowing axons branched profusely before some of the terminals made connections with the skin. Those branches which made contact with the correct target formed presumably permanent connections as a result of a chemoaffinity between the pre and postsynaptic sites. He noted that some of the terminals did not make connections with the skin or muscle and that these "excess" branches were subsequently eliminated by autonomy or withdrawal.

Other evidence in support of the concept of neurotropism was provided by the histological analysis of the innervation of the developing pig's snout (Fitzgerald, 1961). As the pig's snout enlarges during growth new epidermal ridges are added. The new epidermal ridges are subsequently innervated by ingrowing neurites until the density of innervation in the new epidermal ridges reaches that of the older ones. Since the number of nerves entering the snout remained the same, Fitzgerald concluded that the existing neurites in the older epidermal ridges sprouted collaterals in response to a neurotropic substance released from the newer aneuritic epidermal ridges.
The conclusion drawn from the results described above and below (Sec A2. 4, C) is that a chemical substance is released by the aneuritic end organs on target cells which attracts certain nerves towards them; once the target is appropriately innervated the chemotactic substance is neutralized or no longer released (Ramon y Cajal, 1919; Diamond, Cooper, Turner and Macintyre, 1976). This concept has been extended to include several other targets including muscle and even other nerve cells (Diamond 1979).

2) Sprouting After Partial Denervation of the Target

a) In Partially Denervated Muscle

Hoffmann (1950) and Edds (1950) showed histologically that following partial denervation of skeletal muscle in the rat, the surviving intramuscular axons sprouted collateral branches which penetrated into the endoneurial sheaths of degenerating nerve fibres, passed down to the muscle fibres and re-innervated the denervated end plates. The sprouts arose as ultraterminal processes as early as one week after denervation and grew towards the denervated muscle; thus the muscle power was restored to its predenervation level within a short time by the process of extension of the surviving motor units (Weiss and Edds, 1946). After partial denervation the process of re-innervation was apparently completed in about one month, since after this period no uninnervated muscle fibres could be detected.

b) The Autonomic Nervous System

It has been shown that after partial denervation of target organs in the autonomic nervous system the remaining intact nerves will sprout to innervate the denervated targets. Murray and Thompson (1957)
sectioned the preganglionic roots T₁-T₃ in the cat which caused 90% of the fibres to the superior cervical ganglion to degenerate; this included those nerves responsible for pupil dilation.

One month following the operation they stimulated the preganglionic nerve T₄-T₇. These nerves do not normally cause pupil dilation yet one month after section of T₁-T₃ they did evoke dilation; collateral branches of the intact preganglionic roots T₄-T₇ were also seen entering the superior cervical ganglion and functionally innervated the previously denervated muscle.

Sprouting of remaining intact nerves has also been shown histologically and electrophysiologically to occur after partial denervation of parasympathetic ganglion cells in the frog atrial septum (Courtney and Roper, 1976; Roper 1976; Roper and Ko, 1978).

c) The Central Nervous System (C.N.S.)

Although not quite so easy to detect compared with the peripheral nervous system, collateral sprouting has also been described in the C.N.S. Liu and Chambers (1958) chronically isolated one dorsal root on the left flank of the cat by sectioning several roots cranial and caudal to it; these roots were sectioned between their dorsal root ganglion and the spinal cord. Six months after the isolation procedure they noted that the central arborization of the intact root (detected by demonstrating degeneration products after acute section of the root) extended two to three spinal segments further rostrally and caudally than did those of the corresponding contralateral root. Comparable sprouting occurred after partial denervation of the lateral geniculate nucleus and nucleus of the optic tract by sectioning the visual cortical
afferent fibres; the remaining intact retinal projections were shown (by staining for degeneration products) to sprout collaterals into the denervated nuclei (Goodman and Horel 1965). Both of these studies were based on light microscopy and it was not until Raisman (1969) used the electron microscope to show that collateral sprouting in response to partial denervation could indeed form new synaptic connections in the C.N.S. After lesioning the fibres in the medial forebrain bundle of rats, Raisman showed that the fimbrial fibres sprouted to invade the septal nuclei and occupy sites on the cell soma that had been denervated as a result of the lesion (Raisman, 1968; Raisman and Field, 1973). Sprouting of intact hippocampal neurons after lesion of the entorhinal cortex was studied electrophysiologically; the new synapses formed by the remaining intact nerves became functional between 9 to 15 days after the lesion (Cotman and Lynch, 1976).

d) **Cutaneous Nerves.**

Denervation of a region of skin results in collateral sprouting from neighbouring nerves. Undoubtedly the clearest demonstration of this phenomenon was provided by Speidel (1933, 1935a, 1947) using the technique mentioned earlier, of direct microscopic observation of the cutaneous nerves in the transparent, living tadpole tail; he partially denervated a region of the tail and observed a remaining intact nerve sprout two collateral branches 3 and 4 days after denervation. Six days after denervation the two sprouts had given rise to seven endings which supplied part of the denervated zone. The sprouts quickly restored the innervation pattern back to normal (i.e., that seen previous to denervation) in approximately two weeks after denervation.
Zander and Weddell (1951) partially denervated a portion of the corneal epithelium in rabbits and using histological methods observed that the intact axons sprouted collaterals into the denervated regions. Weddell Guttmann and, Guttmann (1941) and Weddell (1942) observed similar evidence of collateral sprouting of sensory nerves in both the adult rabbit hind limb and ear. Immediately after cutting the sural nerve in the leg and the greater auricular nerve in the ear the area of sensory loss could easily be mapped behaviourally; a pin prick in insensitive skin would not elicit a reflex withdrawal. The areas were mapped on consecutive days after denervation and it was found that the area of insensitivity decreased over the next few weeks. Since the originally cut nerves had not been allowed to regenerate Weddell and his colleagues concluded that the surrounding intact nerves had sprouted branches into the denervated skin. These observations were further investigated histologically by infiltrating the skin with the vital dye methylene blue so that the nerves in the skin could be seen with a light microscope. In areas of skin where sensory testing revealed a return of sensitivity, nerve fibres could be detected while nerves were not seen in insensitive skin; there was good agreement (+ 1mm) between where nerve fibres ended and where behavioural cutaneous sensitivity ceased. Jackson (1980) repeated the experiments on the rabbit ear and leg examining specifically whether the low-threshold touch nerves would also sprout into denervated skin. He produced areas of denervated skin in the same manner as Weddell, and then carefully recorded electrophysiologically the impulses from the surrounding intact nerves while lightly brushing the skin. This enabled him to map the area of
loss for the touch sensitive nerves. After as many as sixty-five days after denervation, he was unable to demonstrate any shrinkage of the area of skin insensitive to touch.

Jackson concluded that in the adult rabbit the low-threshold mechanosensory nerves did not sprout into denervated skin. In Weddell's experiments, the nerves that sprouted were shown by behavioural testing to be the high-threshold nociceptive (pin prick sensitive) nerves. The inability of low-threshold nerves to sprout in the adult was also demonstrated by Devor, Schonfield, Seltzer and Wall (1979); they partially denervated the skin of the adult rat's paw and revealed after repeated daily behavioural testing, that the area of insensitivity decreased significantly. They subsequently recorded electrophysiologically impulses from the nerves which had sprouted into the anaesthetic zone and showed that the high-threshold small diameter myelinated nerves (Group III afferents) were the nerves that had sprouted; no low-threshold touch sensitive (Group II afferents) nerves were detected in the previously anaesthetic zone.

Jackson and Diamond (1981) showed that low-threshold nerves could indeed sprout collaterals into denervated skin in the rat, but only during a brief critical period postnatally. It appears that after twenty days of age the low-threshold nerves are no longer capable of sprouting new branches in contrast to high-threshold nerves, which seem to retain this ability throughout life. Diamond and his collaborators also showed electrophysiologically that intact, uninjured cutaneous nerves in adult salamander skin (Ambystoma) will sprout collaterals after a portion of the cutaneous innervation has been eliminated.
surgically; the receptive fields of the remaining intact nerves enlarged (Aguilar, Bisby, Cooper and Diamond, 1973; Diamond, Cooper, Turner and Macintyre, 1976; Cooper, Scott and Diamond, 1977; Diamond, 1979).

3) Sprouting Into Added or Extra Target Tissue.

When supernumary limbs (extra limbs) were grafted onto the thoracic region of the salamander, the fore limb nerves emitted new nerve fibres which grew towards the added limbs, (Detwiler, 1936; Detwiler and van Dyke, 1934). The newly added limb initiated sprouting of the intact nerves and appeared to attract the sprouts towards the denervated limb. Aneurogenic limb buds removed from developing tadpoles, when transplanted to the trunk of the frog tadpole (a region which does not normally supply nerves to the limb) became functionally innervated by the spinal nerves at the level of the grafts (Harrison, 1907). In this case the extra limb appeared to attract "foreign" nerves to it, and these functionally innervated the misplaced limb. The inference is that during development the target tissue (extra or misplaced) will none the less attract nerves to it which subsequently innervate the target.

4) Sprouting After Colchicine Treatment of Adjacent Nerves

Diamond and his collaborators, using the hind limb of the salamander, treated the 16th spinal nerve with a concentration of colchicine that interrupted fast axoplasmic transport without killing the nerve fibres or affecting impulse conduction in them. The cutaneous nerve fields of the neighbouring—untreated 15th and 17th spinal nerves, as well as the treated 16th nerve were reexamined
electrophysiologically one to three weeks later; the 15th and 17th nerves had sprouted and hyperinnervated both skin and muscle supplied by the 16th nerve, just as if the 16th nerve had been cut (Aguilar et al 1973). It has recently been shown that colchicine treatment of the fimbria of the rat hippocampus will induce sprouting of the untreated nerves in the absence of degeneration (Goldowitz and Cotman, 1980). Guth, Smith, Donati and Albuquerque (1980) treated the fourth lumbar spinal nerve in the rat with a concentration of colchicine sufficient to block fast axoplasmic transport, but otherwise left the nerve intact. Two weeks later stimulation of the adjacent fifth lumbar spinal nerve resulted in a larger than normal isometric tension, produced by the muscles in the treated leg; histological examination of the muscles revealed that sprouting of the fifth lumbar nerves had occurred resulting in dually innervated muscle fibres in the treated limb but not in the control limb. (For further discussion see Sec. C1).

5) **Sprouting in Tissue Culture**

To further investigate the environmental influence of selected factors on the growth of nerves many powerful technique were developed; one was tissue culture. Ross G. Harrison (1907, 1910), the inventor of tissue culture, realized the ability of the technique to answer many developmental questions concerning the nervous system:

If it could be shown in tissue culture that there is an attraction between growing nerve fibres taken from a certain part of the nervous system and a particular kind of peripheral cell and between another type of central
neuroblast and a different peripheral cell, then we should have direct evidence for the existence of those more subtle factors which seem to be necessary to account for the definitive establishment of particular nerve connections (Harrison 1910).

Since 1910 many experimentors have used tissue culture for this purpose. When sympathetic ganglia from chick embryos are confronted by many different target tissues in culture, preferential outgrowth is observed. Sympathetic ganglia were found to grow preferentially to the following tissues in diminishing order: heart, kidney, colon, liver, skin, skeletal muscle, spinal cord (Ebendal and Jacobson, 1977). Coughlin (1975) showed that the submandibular ganglia of the fetal mouse shows virtually no axonal growth cultured independently when compared to the vigorous outgrowth that occurs towards a piece of submandibular gland epithelium which is its normal target; this outgrowth seems to show some specificity since a variety of other embryonic mouse tissues do not initiate outgrowth from the ganglia. These results indicate that a substance or substances, produced from the target tissues selectively stimulate growth of the axons towards the targets. The possible specificity of these chemostatic substances seems to be indicated by the results of Coughlin (1976).

B. IDENTIFICATION OF THE SPROUTING STIMULUS

1) Nerve Growth Factor

Bueker (1948) showed that mouse sarcoma implanted into chick embryos would result in a 20-40 percent increase in the size of the spinal sensory ganglia and the sympathetic ganglia that innervated the
tumor. After further rigorous study, it was found that a protein (isolated and named Nerve Growth Factor, N.G.F.) was responsible for the increase in growth; N.G.F. caused an increase in size of the ganglia by increasing the mitotic activity and accelerating the differentiation of the young neurons (Levi-Montalcini and Hamburger, 1951; Levi-Montalcini and Angeletti, 1968). There exist several reports showing that several tissues produce N.G.F., including fast-growing mesodermal tissues (Levi-Montalini 1966) which would be consistent with the evidence that mesenchyme exerts a strong attraction on growing axons (Ramon y Cajal, 1928) To date N.G.F. remains the only clearly identified trophically acting agent affecting the nervous system (Varon and Bunge, 1978). Ebendal, Olson, Seiger and Hedlund (1980) found, using a bioassay, that there was no detectable amount of N.G.F. in rat irides immediately after excision. However, if the irides were cultured for twenty-four hours, N.G.F. appeared; denervation of the irides in situ evoked the appearance of N.G.F. within 10 days which would again fall to undetectable amounts after they were re-innervated. These results indicated that the levels of N.G.F. in a tissue may be regulated by its innervation.

2) **Products of degenerating nerves**

Hoffman (1950, 1951) and Hoffmann and Springell (1951) showed that following partial denervation of the muscles of rats, intact axons sprouted new processes which re-innervated the muscle fibres. They also showed that an extract of either nervous tissue or egg yolk, when injected into normal muscle, evoked the outgrowth of new processes from
motor endplates and nerve fibres. They suggested that the sprouting which occurs in partially denervated muscles is evoked by the diffusion of a lipid substance from degenerating nerve fibres which would stimulate the surviving axons to sprout. Attempts to identify the active substance (neuroctein) were not successful. Further evidence in support of Hoffman's work has been provided by Brown, Holland and Ironton (1978) who deafferented skeletal muscles in the rat by sectioning dorsal roots distal to their dorsal root ganglion. This procedure produced sprouting of the motor nerves in otherwise undenervated muscle. They suggested that the sprouting of the motor nerves was in response to a factor released within the muscle by degenerating nerves.

3) Muscle Activity and the Stimulus to Sprout

When normally innervated muscles in mice are paralysed by injections of botulinum toxin, the intact motor nerves sprout collaterals (Duchen and Strich, 1968). This caused many investigators to wonder whether the inactive muscle fibres could also be a source of a sprouting stimulus (Brown and Ironton, 1977). Further investigation showed that if partially denervated soleus muscles in the mouse were directly electrically stimulated by implanted electrodes, sprouting of the remaining intact motor nerves normally expected, would be prevented (Brown and Holland, 1979). The conclusion drawn from the above experiment was that the denervated muscle fibres liberated a sprouting stimulus and that electrical stimulation of the denervated fibres prevented the release of the stimulus; Van Herrevelde (1947) had earlier suggested that denervated muscle may be the source of a sprouting
stimulus.

C. REGULATION OF COLLATERAL SPROUTING

1) Blockage of Axonal Transport and Its Effect on Sprouting

Ramon y Cajal (1919) was the first to propose a role of the nerve in the regulation of its sprouting and distribution of nerve endings at target tissues (See Sec. A1). Evidence in support of a regulatory role of the nerves was provided by Aguilar et. al. (1973). They blocked fast axoplasmic transport in intact nerves in salamander skin (without killing the nerve or effecting impulse conduction) and found that adjacent untreated nerves would sprout into the treated nerves territory. It was proposed by Diamond et al. that the target tissue manufactures a substance(s) that stimulates intact nerves to sprout, and that the nerves provide a factor(s) which is carried to the nerve endings by fast axonal transport, which in some way neutralize the effects of the growth promoting substance. Sprouting ceases when the effects of the nerve factor(s) balance that of the target tissue stimulus. The above hypothesis could also explain why partial denervation leads to the sprouting of remaining axons; the reduction at the target tissue of an influence exerted normally by intact nerves would no longer neutralize the production of a sprouting stimulus, therefore the sprouting stimulus would initiate growth of the remaining intact nerves. Sprouting of "extra" endings will continue until the imbalance between sprouting stimulus and neutralizing factor is resolved (Diamond et. al., 1976). Additional evidence in support of this hypothesis has been obtained for mammalian motor nerves (Outh et al 1980), and in mammalian brain (Goldowitz and Gotman, 1980).
2. **Role of the Target Tissue in Regulating Axonal Sprouting.**

As mentioned previously the only well characterized sprouting factor is N.G.F., which is produced in the target tissues of post-ganglionic sympathetic nerve cells (Ebbandal et al. 1980). The provision of such sprouting agents by targets would then enable the target tissue to regulate the amount of collateral sprouting; once all the targets have been innervated the target would no longer produce a sprouting stimulus. A recent report indicates that the level of N.G.F. in target tissues is influenced in a manner entirely consistent with the above hypothesis (Ebbandal et al., 1980). Added or extra target tissue or tissue fragments will increase the sprouting of intact nerves in vivo (Bueker, 1948; Levi-Montalcini and Hamburger, 1951; Olson and Malmfors, 1970).

The Merkel cell is a specific target cell for the low-threshold mechanosensory endings in salamander skin (Cooper, Scott and Diamond 1977). When the salamander skin is denervated, the Merkel cells survive and the regenerating nerves grow to the denervated marked cells and functionally innervate them (Scott, Cooper and Diamond, 1981). In partially denervated salamander skin the remaining intact nerves sprout new functional endings by an amount that always restores the density of mechanosensory ending to the level that existed before denervation (Cooper, Diamond and Turner, 1977). It seems that sprouting within the salamander skin ceases when all the available Merkel cells have become innervated (those sprouts which have not found a Merkel cell functionally disappear). Merkel cells also act as targets for low-threshold mechanosensory nerves in mammalian skin but their...
effectiveness in initiating sprouting or the ability of the low-threshold nerves to sprout to them seems to be limited to a very short critical period after birth (Jackson and Diamond, 1981).

3) **Spatial Constraints on Collateral Sprouting**

When the hind limb of the salamander is partially denervated, the remaining low-threshold nerves can be shown electrophysiologically to sprout and invade the denervated skin; the sprouting observed in this case seems to be spatially constrained (Diamond et al., 1976, Macintyre and Diamond, 1981). It was found that when a branch of the segmental nerve N17 was cut along with the rest of the segmental nerves in the hind limb, then the remaining intact N17 would readily (within 2-3 weeks) sprout and restore normal innervation within the area originally occupied by its own parent segmental nerve. However, it would not invade (at least for about 8 weeks) the denervated skin originally supplied by the adjacent nerves. Similar findings were obtained for sprouting of mammalian low-threshold nerves. When a cutaneous branch of a segmental nerve was isolated by denervating adjacent nerves in the rat, the intact low-threshold nerves would sprout only into denervated skin formerly innervated by the same segmental nerve, and not into areas formerly supplied by adjacent segmental nerves (Jackson and Diamond 1981). Thus, there appears to be a spatial constraint imposed on sprouting of certain cutaneous nerves (the low-threshold, mechanosensory ones), or rather, the sprouting nerves seem to prefer to sprout into some areas of skin rather than others.

4) **Temporal Constraints on Collateral Sprouting**

The failure of intact motor neurons to sprout after partial
denervation of neonatal skeletal muscle has been well documented by Thompson and Jansen (1977) and Dennis and Harris (1980). These authors partially denervated muscles at birth in the rat and showed both histologically and electrophysiologically that the remaining nerves did not sprout; this differs dramatically to what is seen when adult rat skeletal muscle is partially denervated. However, when skin is partially denervated in the rat, the low-threshold mechanosensory nerves will sprout into denervated areas only during a short critical period ending at approximately 20 days after birth; there is no electrophysiologically detectable sprouting of the low-threshold nerves in groups of animals older than 20 days (Jackson and Diamond, 1981). Not only can sprouting of intact low-threshold mechanosensory nerves no longer be evoked by adjacent denervation after this age, but when sprouting is initiated in the rat pup, it ceases at about 20 days. However, Devor, Schonfeld, Seltzer and Wall (1979) have shown electrophysiologically that after partial denervation of the rat paw, the remaining intact high-threshold (Group III) afferents can sprout into adjacent denervated skin probably throughout the life time of the animal. It appears that along with other constraints on collateral sprouting of certain intact nerves there exists a temporal constraint during which a particular nerve may acquire or lose its ability to sprout.

D. EFFECTS OF IMPULSE ACTIVITY ON THE NEURON

Although never directly demonstrated, the likelihood that impulse activity in neurons might influence normal axonal sprouting during development has long been an attractive one (recent review by Harris,
Indeed in the mammalian visual cortex, normal development of "binocular columns", primarily an organized array of axonal terminal fields, has been shown to depend upon the relative pattern and quantity of inputs onto the retina of both eyes (Hubel, Wiesel and LeVay, 1977). We have now found, serendipitously, that the collateral sprouting of high-threshold (nociceptive) mechanosensory nerves into denervated skin of rats is strikingly accelerated by relatively modest amounts of nerve excitation (Nixon, Jackson, Diamond, Foerster and Diamond, 1980; Diamond, 1982). Aside from its intrinsic interest this result may also explain the extreme variability in the reported values of the apparent onset of such nerve sprouting in mammalian skin (Weddell et al., 1941; Devor et al., 1979). A common feature of these studies is that functional sprouting was examined by periodic physiological stimulation of the skin. As will be seen repeated stimulation itself can be a potent determinant of the onset of sprouting of these nerves.

1) Histological Effects of Impulse Activity

Electrical stimulation of a motor axon is a convenient and effective method of increasing impulse activity in the neuron. Barr and Bertram (1951) used the above method to examine the histological changes due to increased neural activity. They electrically stimulated (20V, 50HZ) the hypoglossal nerve in several cats and sacrificed them at different times (up to 19 days) after stimulation to examine histologically the motoneuron cell bodies. They saw an almost immediate (12 hours after stimulation) depletion of nissil substance followed by an enlargement of the nucleolus; both returned to normal after approximately 17 days after stimulation. Barr and Bertram
concluded that these changes in morphology indicated that the cell was undergoing an increase in protein synthesis as a result of stimulation. Hyden (1943) described similar morphological changes in spinal cord cells of guinea-pigs following exercise to exhaustion. Chromatolysis (the dispersion of nissl substance) of cells of the cochlear ganglion in guinea-pigs was found by Hamburger and Hyden (1945) following exposure of the animals to loud sounds. Increased neural activity, produced electrically or physiologically, evokes a morphological change within the neurons cell body which is an expression of increased metabolic activity. When the peripheral connection of a nerve is severed, the cell body also undergoes chromatolysis in order to increase the production of materials necessary for repair and regeneration of the cut peripheral nerves (Sieberman, 1971). TTX block of a peripheral motor nerve will evoke sprouting of the intact motor nerve (Brown and Ironton, 1977) as well as causing the neuron cell bodies to undergo chromatolysis (Czeh, Gallego, Kudo and Kuno, 1979). During regeneration and sprouting the nerve cell body undergoes chromatolysis and increased metabolic rate; an intact nerve can be forced to undergo chromatolysis and increase its metabolism by prolonged electrical stimulation.

2) **Metabolic Effect of Impulse Activity**

The information above suggests that electrical stimulation of a nerve will increase the metabolic rate in the nerve and this was clearly shown to be the case for motoneurons in the cat (Lux, Schubert, Kreutzberg and Globus, 1970). Lux et al intracellularly injected tritiated glycine into individual motoneurons in the cat, some of the
motor axons were electrically stimulated for twenty minutes (0.1 msec pulse width, just suprathreshold) while other control neurons were unstimulated. They prepared the spinal cords for autoradiography. This histological procedure removed all of the free amino acids so that the radioactivity remaining in the cell soma was taken as a measure of the intensity of the labelled amino acid incorporated predominantly into proteins (Droz and Leblond, 1963). They found that the radioactivity was significantly higher in the stimulated motoneurons when compared with the unstimulated ones and concluded that incorporation of amino acids and subsequent protein metabolism was enhanced in stimulated neurons. Increased protein synthesis has been shown to occur in monkey occipital cortex as a result of physiological stimulation of the eyes (Singh and Talwar, 1969).

3) The effect of impulse activity on axoplasmic flow.

Lux et al. (1970) using the same intracellular injection techniques described above, also measured the amount and rate of movement of radioactivity labelled protein down the axons of stimulated and unstimulated nerves. They found that the amount of radioactivity in stimulated axons was considerably higher than in unstimulated ones. They suggested that the axons of stimulated neurons receive more proteins than do normal unstimulated ones; the difference being a quantitative one. The axons of excited neurons showed two peaks of radioactivity; the slow component was moving down the axon at 12mm/day while the fast component was moving at 40mm/day. In nonstimulated cells the slow and fast components were shown to travel at the same rate as their counterparts in stimulated nerves, but the amount of
radioactivity being transported was significantly less. No difference could be seen in the speed of protein transport in the axons of excited neurons. Fast axoplasmic transport has been shown to continue unchanged when TTX was present to block membrane excitability (Ochs, 1975). Electrical stimulation at high stimulation rates (greater than 350 Hz) may in fact slow the rate of axoplasmic transport (Worth and Och, 1976).

4) The Effect of Impulse Activity on Collateral Sprouting of Intact Nerves

The majority of neurons in the striate cortex (visual cortex) of the normal adult monkey, and about eighty percent of those in the adult cat are, binocularly driven; that is to say, physiological stimulation of either eye will effect the discharge of these cortical neurons. Some of the cells are driven predominantly by one eye while the rest are driven predominantly by the other. Cells with the same ocular dominance are arranged in columns alternating with columns of cortical cells dominated by the other eye (Hubel and Wiesel, 1962; 1968). The ocular dominance columns in the rhesus monkey begin to develop at about 3 weeks before birth and continue to develop postnataally (Hubel et al., 1977). Monocular visual deprivation of monkeys from birth results in an increase in width of the cortical ocular dominance columns of the normal eye and a commensurate decrease in width of the ocular dominance columns of the deprived eye (Hubel, Wiesel and LeVay, 1975). Development of ocular dominance columns occurs normally in animals deprived of visual stimulation in both eyes (Hubel and Wiesel, 1974).
visual deprivation during neonatal life has also been found in cats, (Wiesel and Hubel, 1963b; 1965). Monocular deprivation can cause the visual cortex to become permanently dominated by one eye only if the monocular deprivation occurs during a critical period in early life; deprivation in the adult has no effect (Hubel and Wiesel, 1970). Neurons in the visual cortex appear to require neural activity from both eyes during postnatal life in order for the striate cortex to develop normally. Other non striate visual systems such as the lateral geniculate nucleus and the superior colliculus also require normal visual activity in order to develop normally (Wiesel and Hubel, 1953; Hoffmann and Sherman, 1974; 1975).

As mentioned earlier, a TTX block of action potentials in motor nerves cause these nerves to sprout collaterals in already fully innervated muscle (Brown and Ironton, 1977) and TTX-blocked autonomic fibres can sprout to form synapses on denervated heart ganglion cells (Roper and Ko, 1978); this indicates that impulse activity along the axons is itself not an essential factor needed for the nerves to sprout. However, stimulation of the intact nerves in partially denervated muscles of rat will accelerate sprouting of the motor nerves (Hoffmann, 1952). Hoffmann partially denervated muscles in the hind limb of rats and electrically stimulated the spinal cord rostral to the lumbar plexus or what remained of the sciatic nerve (50-100 Hz 1.5 mA for ten minutes to one hour). He then examined histologically the previously partially denervated soleus and gastrocnemius muscle for evidence of collateral sprouting. He found no difference in the amount of collateral sprouting of stimulated nerves when compared to
unstimulated ones three days after denervation; four days after denervation 23.5 percent of the electrically stimulated nerves had sprouted collaterals as opposed to 8.5 percent for the control unstimulated nerves. He also found that direct stimulation of the sciatic nerve has a greater effect on the rate of collateral sprouting than stimulation of the spinal cord. Hoffmann suggested that the electrical stimulation of the nerve rendered it more responsive to a peripheral sprouting stimulus, possibly by increasing the metabolic activity in the perikaryon. The process was not followed beyond seven days after denervation, but even at seven days the stimulated nerves had significantly more collateral sprouts than the control ones.

Weddell et al. (1941); Weddell (1942); and Devor et al. (1979) analysed the sprouting of high-threshold cutaneous nerves into denervated skin in rabbits and rats. They measured, using behavioural methods, the reduction on anesthetic areas of skin at daily intervals after denervation and found that the adjacent intact fibres sprouted to innervate the denervated skin. In all cases the daily behavioural testing evoked impulses in the adjacent sprouting nerves which would increase the amount of neural activity in the sprouting nerves. Neither investigation controlled for the increase in neural activity. Therefore the possibility existed that the behavioural testing was accelerating the collateral sprouting of the high-threshold cutaneous nerves in a manner similar to that seen after motor nerve stimulation. Recently, Greenfield and Devor (1981) have investigated this possibility in the rat hindlimb; they partially denervated the dorsal and plantar surface of the rat hind limb: in one group of animals the
area of anesthetic skin was mapped behaviourally (using mosquito forceps) every other day; four other groups were mapped only once at 7, 15, 30, and 45 days after denervation. They found no difference in the rate of sprouting of the high-threshold nerves into denervated skin in animals mapped every second day compared to animals mapped only once at a selected time after denervation. However, they do suggest that since the rats were moving around their cages during the post operative period that there was continuous self-generated mechanical stimulation of the partially denervated rat paws and that this amount of stimulation may be sufficient to cause an increase in neural activity and a subsequent acceleration of collateral sprouting of the intact high-threshold nerves.

E. PROBLEMS OF BEHAVIOURAL TESTING OF HIGH-THRESHOLD FIELDS.

The first satisfactory delineation of high-threshold dermatomes (the area of skin functionally innervated by one segmental nerve) was made by Sherrington (1893, 1898) in acute experiments in the frog, cat and macaque monkey. Sherrington used the method of "remaining sensibility" to isolate the areas of innervation of one segmental cutaneous nerve from the overlapping of adjacent cutaneous nerves. He sectioned at least two segmental cutaneous nerves (proximal to their D.R.G.'s) rostral and caudal to the one under study to produce an insensitive area on either side of the isolated dermatome, and tested for sensitivity by observing responses to pinching the skin with toothed forceps. Sherrington found the trunk high-threshold dermatomes to be serial bands extending between the dorsal and ventral midlines with a rostro-caudal overlap of approximately one-half.
Subsequently, behavioural mapping has been used to determine the characteristic pattern of serial overlapping fields in vertebrates. Using the method of "remaining sensibility" the high-threshold receptive fields in man (Foerster, 1933), cat (Kuhn, 1953) ox (Arnold and Kitchell, 1957) and sheep (Kirk, 1968) have been determined.

1) Effect of Nerve Sections Distal or Proximal to the D.R.G.

Although the method of "remaining sensibility" had been used extensively to map high-threshold dermatomes it was not until 1970 that Kirk and Denny-Brown demonstrated the variability of the technique. Using the monkey they showed that the extent of the cutaneous areas from which reflex response could be elicited was dependent in some way upon the integrity of the adjacent cutaneous nerves.

They isolated a dermatome by cutting three adjacent cutaneous nerves cranial and caudal to the one being studied. If the dermatome was isolated by section of the adjacent nerves distal to their D.R.G. its area was approximately twice as large as the dermatome three to four days after surgical isolation by the more usual method of sectioning the dorsal roots of the same nerves proximal to their ganglia within the dura mater. The area of a dermatome isolated by sectioning three adjacent roots proximal to their D.R.G. would be further reduced twenty four hours after a second surgical denervation was done to section three additional roots (proximal to their D.R.G.) caudal and cranial to those already cut (Kirk & Denny-Brown, 1970).

Due to the short period of time (24 hrs) required to produce the change in dermatomal size Kirk, and Denny-Brown concluded that the change was due to a functional alteration within the spinal cord rather
than an anatomical one. They investigated the possibility that a central inhibitory process was responsible for these changes.

The above decreases in dermatomal size were found not to be due to a loss in transmitting peripheral nerve pathways since in all cases a subcutaneous injection of strychnine, a drug which has been found to abolish spinal inhibition (Bradley and Eccles, 1953), would quickly (within 15 minutes) yet temporarily increase the area of the isolated dermatome to one that was larger than the original dermatome isolated by sectioning three adjacent nerves distal to their D.R.G. The effect of dorsal root section on dermatome size is also present in cats and rabbits (Kirk, 1974).

To date the mechanism by which dorsal root section has its effect on the size of an isolated dermatome is still unclear, although Denny-Brown, Kirk and Yanagisawa (1973), have further investigated this problem. They suggested that impulses evoked in nerves located in the skin outside the isolated dermatome are functionally "silent" and unable to cause a behavioural response until a tonic inhibition in the control nervous system is removed. It is clear, however, that if one is attempting to compare isolated dermatomes within a group of animals it is essential that the investigator compares only those animals with identical patterns and methods of denervation.
THE PRINCIPAL AIM AND GENERAL STRATEGY OF THE INVESTIGATION

One principal aim of this study was to define spatial and temporal constraints that operate in the recovery of high-threshold mechanosensory function in denervated mammalian skin. This investigation was prompted in part by results obtained in another project which was ongoing in the laboratory dealing with the recovery of low-threshold mechanosensory function. The latter results suggested that low-threshold nerves do not sprout in the adult mammal but perhaps the high-threshold nerves would. Because several investigators had reported that cutaneous nerves (whether they were high or low-threshold was unknown) readily sprout in the mammal, I decided to investigate, in the adult rat, whether or not the high-threshold nerves would sprout. Serendipidously a collaborative preliminary investigation suggested the possibility that physiological stimulation of the high-threshold nerves could in some way accelerate their sprouting which lead me to the second principal aim of this study; what component of physiological stimulation is responsible for the apparent acceleration of cutaneous nerve sprouting?

The first necessity was to select a mammalian preparation in which the high-threshold receptive field of intact cutaneous nerves could be measured. The cutaneous nerves in the rat's back were easily accessible for experimental manipulation and the distribution of their low-threshold fields was known. I had also been investigating a nociceptive reflex in the rat which was driven specifically by high-threshold afferents in these cutaneous nerves. We hoped that the
high-threshold field of the cutaneous nerves could be mapped behaviourally using the nociceptive reflex as a measure of cutaneous high-threshold sensibility. Behavioural techniques for mapping high-threshold fields had been extensively used in the past but had recently come under criticism regarding their accuracy; this criticism regarding their accuracy would have to be resolved in the rat preparation. The cutaneous nerves of the rat were found to satisfy the experimental requirements, their high-threshold mechanosensory receptive fields could be unambiguously, behaviourally mapped so the initial task was to map the receptive fields of these nerves in the rat back.

The general strategy adopted to investigate the sprouting of high-threshold nerves was to isolate in the skin a receptive field of a selected intact nerve by cutting some or all of the adjacent nerves then to follow this receptive field to examine whether it expanded by sprouting into previously denervated skin. The behavioural technique used measured only the functional sprouting of the high-threshold mechanosensory nerves so a correlative histological analysis of sprouting was also done to provide the morphological basis for sprouting.

To investigate whether physiological stimulation would in fact accelerate the sprouting of these nerves the intact isolated receptive field was physiologically stimulated and compared to the sprouting observed in unstimulated animals. Once it had been confirmed that indeed stimulation of the remaining intact receptive field did accelerate its sprouting, several mechanical and pharmacological
strategies were used to investigate the basis of such an influence of the stimulation. In particular, the possibility that the impulse activity in the nerve was itself responsible.
METHODS AND MATERIALS

1) Animals

All animals used in this investigation were female Wistar albino rats obtained from Woodlyn Farms, Guelph, Ontario. On the day of their initial surgery they weighed 200 ± 20 gm and were approximately 60 days old. After their surgery the animals were housed individually in flat top cages designed to prevent the rats from lacerating their denervated back skin on the cage tops (Fig. 1). Wood chips were placed on the floor of the cage for bedding; food and water were freely available.

2) Anaesthesia

All surgical procedures were performed on animals anaesthetized by an intra-abdominal injection of sodium pentobarbital (Somnotol; MTC standard; 45 mg /kg). Surgical anaesthesia was defined as that level of anaesthesia adequate to eliminate any reflex response to a tail pinch. The animal's core temperature was maintained at 37 ± 1°C; this was achieved by placing a thermistor probe in the animal's rectum which monitored its temperature and, via a feedback control unit, regulated the temperature of the heating pad on which the animal rested during surgery. Normally an animal would recover from anaesthesia within six hours. Those animals in which infection or self mutilation of the denervated back skin was detected were removed from the study. At the end of each "terminal" experiment, (after the necessary skin specimens were collected), the animals were killed by cervical dislocation.

3) Surgical Procedures

When the animal had reached a surgical level of anaesthesia, an
area of hairy skin (2-3 cm wide and 7-8 cm long) was shaved on the right side of the dorsal midline. A surgical incision was made, with a scalpel, through the full thickness of the skin from the ninth rib to the fourth lumbar vertebra; the incision was approximately one cm to the right of the dorsal midline. The skin on the left of the dorsal midline was retracted laterally to expose the Dorsal Cutaneous nerve bundles (DCNs) (Fig. 2); these nerve bundles emerge from the body wall musculature 1-2 cm from the dorsal midline and run down the body wall on top of the latissimus dorsi for approximately 2-3 cm before they enter the skin to supply the afferent innervation of the back. The DCNs each divide into two smaller branches as they emerge from the body wall. The medial branch of DCN-T₁₃ (mDCN-T₁₃) lies above the lateral branch and innervates an area of skin between the dorsal midline and the lateral dorsal cutaneous nerve field supplied by the lDCN-T₁₃ (Fig. 3). The lateral cutaneous nerves (LCNs) emerge from the body wall musculature 3-4 cm lateral to the dorsal midline and run for approximately 1 cm before entering the skin. Each DCN includes afferent nerves from only one segment of the spinal cord, i.e. from one dorsal root ganglion (DRG). The DCNs are easily identified; the DCN from spinal cord segment T₁₁ (DCN-T₁₁) is accompanied by a large vein, and the DCN from spinal segment L₃ (DCN-L₃) lies in the junction between the body wall musculature and the hindlimb musculature. Once the appropriate DCNs were cut to produce the desired pattern of denervation the incision was closed with interrupted gut sutures, in such a way that the sutures were not visible from the exterior (Fig. 4); such internal "blind" sutures were necessary because externally
exposed ones were usually chewed by the animal, causing reopening of the wound. After the incision was closed the wound area was rinsed with physiological saline, then swabbed with 70% ethanol to prevent infection.

4) Denervation of Skin

For most of the experiments in this investigation the DCNs T₁₀-₁₂ and L₁-₃ on the left side of the dorsal midline were cut, with fine scissors below the body wall musculature just medial to where they emerge; the 1DCN-T₁₃ was also cut close to the body wall, and in all cases 1-2 cm of the peripheral stumps was elvulsed to prevent regeneration of the cut nerves. In addition, on the right side the DCNs-T₁₀-L₃ were cut and elvulsed in the same manner. The consequence of these denervations was effectively to produce an isolated "island" of skin, on the left side of the dorsal midline, that was innervated by mDCN-T₁₃ and surrounded by a "sea" of denervated skin formerly supplied by the adjacent DCNs. (Fig. 5).

For the experimental results described in the Results section E, the pattern of denervation was more extensive; the nerves that were cut below the body wall musculature were DCNs T₈-T₁₂, 1DCNT₁₃, and L₁-L₅, and in addition the lateral cutaneous nerves (LCNs) T₈ - L₅ were also cut. These nerve sections resulted in the complete denervation of the left side of the rat's back, except for a small area of skin (1.5 - 2.0 cm in width) on the left side of the ventral midline, and the area of skin supplied by mDCN-T₁₃. Cutting the cutaneous nerves below the body wall musculature, with elvulsion of their peripheral stumps was found to be effective in preventing their subsequent regeneration.
5) **Electrical Stimulation of Intact Cutaneous Nerves**

Once the initial denervation had been performed to leave an isolated island of innervated skin, its nerve supply, the intact mDCN-T₁₃, and its blood vessels were carefully dissected free from the body wall. A small amount of connective tissue was deliberately not freed from the nerve in order to minimize the possibility of damaging it. The dissected nerve was carefully lifted up to a point approximately one half centimeter from the body wall, and a piece of plastic was placed between the nerve bundle and the wall. Two platinum wire electrodes were embedded in the plastic about one quarter of a centimeter apart, and perpendicular to the intact nerve; the nerve was placed on top of electrodes (Fig. 6). Special care was taken to minimize the tension on the nerve during electrical stimulation; this was assisted by having the electrode assembly connected to the isolated stimulator by two very flexible thin insulated copper wires which allowed the electrodes to "float" independent of the stimulator, and to move freely with the respiratory movements of the rat. The nerve bundle and electrodes were then covered with petroleum jelly to prevent them from drying out. After the nerve had been electrically stimulated the petroleum jelly was removed and the nerve was carefully lifted from the electrodes and replaced to its in situ location.

6) **Application of Tetrodotoxin (TTX) to Intact Cutaneous Nerves.**

The concentration of TTX used throughout these studies was 10μg/ml (31μl) (TTX Sigma Chemical Comp. St. Louis, Mo. U.S.A.) in physiological saline. This concentration has been shown to block impulse propagation in a wide variety of mammalian nerves, including the cat sciatic nerve,
without affecting axonal transport (Ochs and Hollingsworth, 1971; McClure, 1972). TTX was applied to one of two locations on the intact nerve, depending on the experiment. For experiments in which TTX was to be used to block impulses conducting to the skin from the region of the nerve across the stimulating electrodes, a small portion (2-3 mm) of the nerve was carefully dissected peripheral to the stimulating electrodes and a bath was formed around the cleaned nerve with petroleum jelly, into which was placed 0.1 ml of the TTX solution. When the TTX was to be applied central to the stimulating electrodes in order to block impulses conducting towards the dorsal root ganglion, and when it was to be used to block impulses generated by physiological stimulation of the skin, an incision through the first layer of muscle was made about 0.5 cm medial to the point where the cutaneous nerve emerged from the body wall musculature. The incision was extended approximately 0.5 cm caudally and rostrally, and a flap of muscle retracted from the body wall, exposing the intact nerve as it emerged from an inner layer of body wall musculature (Fig. 7). This procedure created a "muscle trough" at the bottom of which was the intact cutaneous nerve. Approximately 2-3 mm of the nerve was carefully dissected clean, then 0.1 ml of the TTX solution was placed in the muscle trough.

To determine whether the TTX had blocked impulse propagation the peripheral field of the treated intact nerve was physiologically stimulated. Pinching the skin with toothed forceps results in a reflex response only if the impulses are allowed to conduct to the central nervous system (C.N.S.). When the TTX is effectively blocking these
impulses the skin pinch fails to evoke a reflex response. Twenty minutes after TTX was applied to a cutaneous nerve its skin field was pinched. If the pinch evoked a reflex response then the animal was retested at 5 minute intervals; normally the TTX blocked the reflex response to a skin pinch within 20–30 min after its application to the nerve. Once the TTX had blocked impulse conduction in the nerve (as determined above), the intact nerve was electrically stimulated or the skin was pinched (depending on the experiment), then the TTX was immediately removed from the bath, and before closing the incision the nerve was bathed in several changes of physiological saline. Three to four hours after the removal of the TTX solution skin pinches evoked mild reflex response, and by 24 hours these responses did not look detectably different from normal.

7) **Mapping of Low and High-Threshold Fields**

To map the high-threshold fields of cutaneous nerves in the rat a behavioural method, similar to that first used extensively by Sherrington, was employed. The usual objective was to map the area of skin supplied by the high-threshold fibres of a remaining mDCN-T₁₃.

Previous studies from this lab (Nixon et al., 1981) have shown that in anaesthetized rats the high-threshold cutaneous nerves of mDCN-T₁₃ will, when stimulated, evoke a reflex contraction of the **cutaneous trunci** muscle (CTM), a thin sheet of skeletal muscle that lies just below, and inserts into, the back and flank skin of the rat. Thus a pinch with toothed forceps of the dorsal skin evokes a contraction of the underlying CTM. The afferent inputs which drive this reflex are the nociceptive high-threshold Group III (A β) and
C-fibres which run in the DCNS. Low-threshold touch-sensitive afferents (Group II or Aβ fibres) do not evoke a reflex contraction of CTM. The motor output to CTM emerges from the brachial plexus and was essentially unaffected by the surgical manipulation used in these experiments except when it was necessary to record directly from the motor nerves to CTM.

Rats which had previously been surgically denervated to produce a mDCN-T₁₃ "island" (Methods Sec. 4) were anaesthetized at selected times after denervation, and the skin on the back was shaved. The rats were now ready to be behaviourally mapped. A skin fold (2-3 mm long) was grasped between the points of a pair of fine toothed forceps and compressed firmly; (Fig. 8a) while the presence or absence of a visible reflex contraction of CTM was noted (Fig. 8). A skin location some 2-3 mm away from the first was similarly examined and in this manner the back of the rat was systematically pinched and the response to each pinch recorded. A point was reached where a skin pinch would elicit a response at one spot but not two to three mm further along the skin; this was taken as the border between sensitive skin and an adjacent area of insensitive skin.

A fine felt tipped pen was used to mark the site on the rat's back between the spot where a pinch elicited a reflex response and the adjacent spot where a pinch failed. When the back skin had been completely mapped the marks, indicating the border between insensitive and sensitive skin, were joined together with a continuous line (Fig. 9). To map the area of sensitive skin supplied only by mDCN-T₁₃, its "autonomous zone", the initial incision made to produce the mDCN-T₁₃
island (Methods Sec. 3) was reopened and the intact mDCN-T_{13} nerve was cut. The back skin of the rat was again mapped with fine toothed forceps and the new area of insensitive skin that was formerly supplied entirely by mDCN-T_{13} was found.

Finally, the peripheral stump of the cut mDCN-T_{13} was cleaned and raised onto platinum electrodes; signals from the recording electrodes were amplified and displayed on an oscilloscope as well as relayed through a loud speaker. Impulses were recorded extracellularly from the nerve while lightly brushing the skin with a fine bristle which stimulated the low-threshold touch nerves. In this way the low-threshold field of the previously intact mDCN-T_{13} was mapped (Jackson, 1980); the borders of this field were also marked on the skin with a fine tipped felt pen. By recording several low-threshold fields of DCNs on both sides of the dorsal midline the overlap between adjacent DCN fields was determined (Fig. 10). The medial borders of the medial branches of the DCNs overlap very little at the dorsal midline, and therefore the medial border of a medial DCN can be taken to represent the dorsal midline of the rat's back (Jackson, 1980); a line drawn along the medial border of the low-threshold receptive field of mDCN-T_{13} was routinely used to indicate the dorsal midline for further measurements.

8) Measurement of Sensitive and Insensitive Skin Areas

After the rat had been behaviourally mapped, and the low and high-threshold fields of mDCN-T_{13} had been marked on the rat's back as described above, the back skin was returned to its normal position so that the medial border of the low-threshold receptive field of mDCN-T_{13}
was lying along the vertebral column of the rat; this minimized
distortion of the receptive fields. A thin acetate sheet was layer
over the rat's back and the outlines of the areas of sensitive and
insensitive skin, and the low and high-threshold fields of mDCN-T_{13}
were drawn on the acetate sheet. The outlines on the sheet were then
traced onto paper as a permanent record for further measurements. A
straight line was drawn on the paper along the medial border of the
mDCN-T_{13} low-threshold field to indicate the dorsal midline of the
rat's back. The receptive field areas of mDCN-T_{13} and the areas of
insensitivity lateral to the dorsal midline were measured by
superimposing the acetate sheet on graph paper \((\text{mm}^2)\), and by counting
the number of squares contained within each area; partially enclosed
squares were counted as half squares.

9) Measurement of the Field "Axes" of mDCN-T_{13}

To determine the linear extent to which a high-threshold field
border shifted due to sprouting, the changes in the length of two
selected axes of the field were measured, the rostro-caudal and the
dorso-ventral axes (See Fig. 19). It was assumed that sprouting in the
rostro-caudal axis of the high-threshold mDCN-T_{13} field was uniform;
to determine the extension of just one border, rostral or caudal, the
maximum linear distance of the rostro-caudal axis was divided by two.
The maximum distance by which the ventral border of the high threshold
mDCN-T_{13} field had moved was measured directly in the direction
perpendicular to the dorsal midline. This indicated the distance that
the ventral border had extended into denervated skin.

10) Histology of Nerves Within Skin
Nerves in the skin were stained using a silver impregnation method (Loots et al., 1979), modified for frozen sections as follows:

The piece of skin to be examined was removed from the rat and frozen in isopentane (industrial grade) cooled with liquid nitrogen. Thirty micron frozen serial sections were cut on a cryostat and air dried onto clean microscope slides, then fixed in 10% buffered Formalin for 3 hr. The slides were then transferred to 80% ethanol for 1 hr for partial dehydration. The slides were then immersed at room temperature for 25 min in an impregnation solution; this was made by dissolving 0.5 g Protarol-S in 100 ml distilled water, following which were added (in order) 0.005 - 0.010 g of allantoin, 1.0 ml of 1% Cu(NO₃)₂ solution, 1.0 ml of 1% AgNO₃ solution and 1-2 drops of 30% H₂O₂. The slides were then rinsed in deionized water and placed in reducer number 1 at room temperature for five minutes. Reducer number 1 consisted of 1 g hydroquinone and 1 ml (35%) formalin in 100 ml distilled water. The slides were again rinsed in deionized water and then transferred to silver-allantoin intensifier solution for five minutes. The latter solution was made by adding 1 g of AgNO₃ and 0.01 g of allantoin to 100 ml distilled water. The slides were again placed in reducer number 1 for 10 seconds, and then in the unused reducer number 1 for a further 5 minutes. The slides were washed in deionized water and fixed for 2 min in a solution of 2% sodium thiosulphate. The slides were dehydrated in 80% ethanol and two changes of absolute ethanol, then cleared in xylene and mounted.

To determine the extent of the correlation between a behaviourally determined border of a high-threshold field of mDCN-T₁₃ and its
histologically determined border, the following protocol was followed. The behavioural borders were mapped and drawn on the rat's back (Method Sec. 7). A sharp razor blade was drawn along the behavioural border so as to cut through the epidermis and just into the dermis; this incision was later used as a visible indicator of the behaviourally determined border in cross sections of the skin. A piece of skin 1.5 cm long and 1.0 cm wide was removed from the animal in such a way that the blade incision ran perpendicular to the long axis of the piece of skin, with approximately one half of the piece of skin, the behaviourally sensitive, to one side of the incision, the other half in the behaviourally insensitive portion. The objective was to determine histologically where the nerves were in relation to the behavioural border (the incision). The skin was mounted, frozen and 30 μm serial sections were cut on a cryostat parallel to its long axis; thus approximately one half of the section was behaviourally sensitive and the other half behaviourally insensitive. The sections were processed and mounted on slides as described above, and then viewed with a light microscope (objective 25X, NA 0.60 overall magnification 250X). When a nerve was found in the dermis (epidermal ones were not resolved by this technique) the position of the nerve relative to the behavioural border (the nick in the section) was measured and recorded, and also recorded whether the nerve was on the sensitive or insensitive side of the border. The data were plotted as indicated in Fig. 15.

11) **Electrophysiological Identification of the Presence of High-Threshold Fibres in Skin.**

This method for identifying the presence of high-threshold Group
III (Aδ) fibres in skin arose out of the observations that electrical stimulation of the high-threshold group III afferents (pulse width 0.05 msec) evokes a motor output to the CTM with a reflex latency of 10-20 msec while a much larger voltage, (e.g. 2-3x amplitude) and a pulse width of about 0.5 msec causes excitation of the C-fibre afferents, which evokes a second independent reflex volley to CTM with a latency of 40-60 msec (see Results, Section D1 and Nixon, Jackson, Theriault and Diamond, 1981). Since the Group III afferents evoke a reflex volley with a different latency than do the C-fibre afferents, an attempt was made to differentiate between the high-threshold afferents in the sprouted high-threshold receptive field of mDN-T13. The results were used to examine, electrophysiologically, the presence or absence of high-threshold nerves in the skin. The rats were anaesthetized, shaved and the motor nerves to CTM dissected free from the brachial plexus; platinum wire electrodes were used to record the reflex volley evoked in these nerves by stimulation either of DCNs or of skin directly. For the latter experiments the dorsal skin was cleaned with alcohol and conductive cream was rubbed in. Bipolar platinum electrodes (0.5 cm apart) connected to an isolated stimulator were used to stimulate the skin directly. After positioning the stimulating electrodes, the voltage of the stimulating pulse was slowly increased to a maximum (100V, 1.0 msec pulse width) while recording the latency of the evoked reflex response in the CTM motor nerves. A latency of 10-20 msec would indicate that excitation of Group III afferents had occurred, while one of 40-60 msec would indicate the presence of C-fibre afferents in the skin. Finally, the selected DCN was cut, and the skin excitation
repeated in the middle of the previously mapped high-threshold field. The absence of a reflex volley in the motor output to CTM indicated that the cut nerve had indeed conveyed the sensory input evoked from the skin by the electrical stimulus.

12) **Statistical Analysis**

All statistical tests comparing significance between groups were carried out using the Mann-Whitney U-test. An example of the use of this test is given in Appendix I.

To compare the rate of expansion of high-threshold receptive fields after different treatments, a linear regression line was fitted to each group of data according to the least squares analysis; the slopes of various lines were compared using an analysis of variance. An example of this analysis is given in Appendix II.
RESULTS

A. THE ANATOMY OF THE CUTANEOUS HIGH AND LOW-THRESHOLD FIELDS OF THE RAT'S TRUNK

1) The Cutaneous Nerves

The sequential origin of the sensory ganglia giving rise to the pattern of afferent innervation of the rat's trunk (unlike that of the limbs or head) (see Fig. 10) is clearly preserved anatomically. Each segmental afferent output is organized into three primary rami, the dorsal, lateral and ventral rami (see Fig. 3). As the rami emerge from the body wall musculature, each divides into two smaller rami; the dorsal ramus becomes the dorsal cutaneous nerve (DCN), which divides to form the medial branch of the dorsal nerve (mDCN) and the lateral branch (lDCN). Each DCN innervates skin on the back from the dorsal midline to a region of the flank approximately one quarter of the total distance to the ventral midline. The lateral cutaneous nerves (lCNs) innervate the lateral flank skin, and the ventral cutaneous nerves the remaining, largely ventral, skin. The sensory dermatomes on each side thus run from the dorsal to ventral midline, and each one is composed of three receptive fields of the three rami of the same segmental nerve. When the DCNs are exposed for experimental manipulation their segments of origin are easily identified (Hebel and Stromberg, 1976): the DCN of the eleventh thoracic segment (DCN-T₁₁) as it emerges from the body wall has been found invariably to be accompanied by a conspicuously large vein which makes it easily distinguishable from other DCNs. Also it has been noted that the DCN of the third lumbar segment (DCN-L₃) runs in the readily-observed groove between the trunk
and the pelvis. Using these landmarks the rest of the DCNs can be reliably identified as they emerge from the body wall. The division of the DCNs into their medial and lateral branches occurs soon after the DCNs emerge, and each of these branches runs independently along the body wall for approximately two to three cm before penetrating into the overlying skin. The medial branch always runs superficially to the lateral branch, and with practice the medial branch can be carefully pushed aside with a glass rod to expose the lateral.

2) The Low and High-Threshold Mechanosensory Fields Compared

Low-threshold fields of DCNs were routinely mapped electrophysiologically (by recording the skin area from which afferent impulses were evoked by stroking with a light bristle – see methods) in these studies as an important "reference area" for the high-threshold mapping; after the low-threshold DCN field borders were drawn on the back of the rat the medial and lateral branches (the DCN sub-fields) were mapped independently. In animals of 180 to 220g, the low-threshold fields of the medial and lateral branches of one DCN overlap one another by 1-4 mm, a value which varies among segmental levels and among animals. Adjacent DCN low-threshold fields also overlap one another by 1-5 mm, an amount that also varies among segmental levels and among animals. More caudally the DCNs-L3 and L4 overlap one another by 4-5 mm, while the smaller more rostral DCN field overlap one another to a lesser extent, 1-3 mm. The low-threshold fields of mDCNs begin at the dorsal midline and do not cross it; consequently the medial borders of the right and left mDCNs do not significantly overlap with each other, but rather the two fields abut.
at the dorsal midline (Fig. 10).

Unlike the low-threshold fields, the mapping of high-threshold fields was done behaviourally, and to do this the DCN field was "isolated"; this was done by cutting DCNs adjacent to that selected for study, i.e., by denervating the skin surrounding the selected high-threshold DCN field. The field was then mapped behaviourally by noting the area of skin that, when pinched, evoked a reflex contraction of underlying cutaneous muscle (Fig. 8); the field borders were drawn on the animal's back. After the behavioural map was completed the DCN was sectioned and the low-threshold field of the same nerve was mapped electrophysiologically (Fig. 9). The high-threshold field was always larger than the low-threshold field of the same nerve, extending some two to three mm beyond the latter in all directions (Fig. 5). In contrast to the low-threshold receptive field of mDCNs the high-threshold ones cross the dorsal midline, by two or three mm, sharing this skin with the corresponding mDCN field on the contralateral side of the dorsal midline (Fig. 5). Since the behavioural technique used to map the high-threshold receptive fields required the section of adjacent cutaneous nerves, it was impossible to determine the amount of overlap between adjacent high-threshold fields within one animal. To gain some idea concerning the amount of this overlap, several different DCNs and their branches were mapped both behaviourally and, for the low-threshold fields, electrophysiologically in different animals, and the results combined to give a picture of the probable pattern of the innervation in the individual rat (Fig. 11). The consistent finding was that for any DCN the high-threshold
receptive field was larger than the low-threshold one, extending two to three mm beyond it in all directions (Fig. 11). Since adjacent low-threshold fields of DCNs could be accurately determined in any one animal, and since the high-threshold field borders extend by a known amount beyond those of the low-threshold ones, a predictable map of the high-threshold field pattern of the DCNs innervating the rat back can be constructed (Fig. 11). On the average the region of overlap between adjacent high-threshold fields was some 4 to 6 mm, indicating that if only one DCN was removed there would be virtually no detectable area of high-threshold insensitivity (although there would be a somewhat less sensitive region in the area of skin corresponding to the overlap).

B. SPROUTING OF THE mDCN-T13HIGH-THRESHOLD NERVES INTO ADJACENT DENERVATED SKIN

1) The first experiments were done to determine if intact high-threshold nerves sprout functional collaterals into adjacent denervated skin. The technique used in these initial experiments measured changes in the total area of insensitive skin left after the standard denervation procedure, i.e., shrinkage of this area (see Methods), and not the expansion of a remaining area of sensitive skin supplied only by the "isolated" nerve (the latter, more rigorous method, was however adopted for the later experiments).

Five groups of animals were surgically denervated to produce an "island" of innervated skin supplied only by the medial branch of DCN-T13 (mDCN-T13) (Fig. 5). One group of animals was behaviourally mapped immediately after the surgical denervation (day zero); this group (C) provided the control values against which all other groups
were compared. One group of experimental animals (E1) was mapped eight days after denervation, a second group (E2) at sixteen days, a third (E3) at twenty-four and a fourth (E4) at forty days after denervation. The areas of insensitive skin (measured lateral to the dorsal midline), i.e., skin that when pinched failed to evoke a reflex response, was measured in each animal. A mean reduction in area of insensitive skin was considered to be the result of intact nerve sprouting into adjacent denervated skin, since none of the previously cut nerves were able to regenerate back to the skin (at least during the periods used in these studies) after they had been cut, ligated and buried below the body wall musculature. The possibility that such a shrinkage could be due to central changes, e.g., excitability of neurons in the reflex pathway for example, is dealt with below. The area of insensitive skin produced by the surgical denervation on day zero (Groups C) was $968 \pm 72 \text{ mm}^2 \text{ S.E.M.}$; eight days later (Group E1) the insensitive area was not significantly different from this control value ($987 \pm 188 \text{ mm}^2 \text{ S.E.M.} \ p > 0.005$) nor was it significantly different at sixteen days (Group E2, $838 \pm 79 \text{ mm}^2 \text{ S.E.M.} \ p > 0.005$). However, by twenty-four days (Group E3) the insensitive skin area was significantly reduced, to $626 \pm 59 \text{ mm}^2 \text{ S.E.M.} \ (p < 0.01)$. Finally, forty days after denervation (Group E4) the remaining insensitive skin was only $67 \pm 42 \text{ mm}^2 \ (\text{S.E.M.})$, significantly less ($p < 0.001$) than the sensitive skin remaining at twenty-four days after denervation (Group E3). The insensitive area is plotted against the number of days after surgical denervation in Fig. 12. It can be seen that it began to decrease only sixteen or more days after the denervation. These results suggest that it took this long
for the intact high-threshold nerves to sprout functional collaterals into the insensitive skin (see later). There thus appeared to be a latent period for such functional sprouting of at least sixteen days. Once such sprouting began (sometime after sixteen days and before twenty-four days) the rate of reinnervation of the deprived skin was approximately thirty-five mm$^2$ of insensitive skin per day. By forty days there was almost no insensitive skin remaining.

That the eventual decrease in the area of insensitive skin was not due to reinnervation by regeneration of the previously cut nerves was shown by recutting the same nerves; this procedure caused no changes in the areas of sensitive or insensitive skin in any of the experimental animals.

2) **Low-Threshold Nerves: Lack of Collateral Sprouting**

It has been shown in this laboratory that the low-threshold nerve fields (the touch sensitive receptive fields) of the intact DN do not sprout functional collaterals into denervated skin in the adult rat (Jackson and Diamond, 1981); this was confirmed in the present experiment. Using electrophysiological procedures (see Methods) the area of the low-threshold mechanosensory receptive field of the intact mDCH-T$\text{,}^1$ was measured, i.e., the area of skin from which action potentials were initiated in the nerve by stroking with a fine bristle. On the initial day of surgery (Group C, day zero) this area was $127 \pm 19$ mm$^2$ S.D. and 40 days after denervation it had not changed significantly $121 \pm 16$ mm$^2$ S.D. (Group E) (Fig. 13).

3) **Are There Silent (Non-Functional) High-Threshold Nerve Endings in Behaviourally Insensitive Skin?**
The reason for examining this question comes principally from a paper by Kirk and Denny Brown (1970) in which they studied the high-threshold receptive fields of intact cutaneous nerves in the monkey (Macaca mulatta). They isolated the intact eighth thoracic nerve by cutting three segmental cutaneous nerves rostral, and three caudal, to it, and then behaviourally mapped (using a reflex response to a pin prick) its high-threshold receptive field. Next they injected the monkey with a strychnine sulphate solution (0.25 mg/kg), and fifteen minutes later remapped the high-threshold field. They noted a remarkable change; immediately after the injection the area of high-threshold sensitivity had increased to a value some two to three times its original size. Their explanation for this was that there were cutaneous axons (in the eighth thoracic nerve) that innervated the insensitive skin surrounding the first-measured high-threshold receptive field, but in normal (experimental) conditioning stimulation of these "surround" nerves failed to evoke a reflex response because the effects of their impulses were inhibited in the cord. However strychnine, which can abolish spinal inhibition (Bradley and Eccles, 1953) caused an "unmasking" of the input in these "silent" nerves, which were now capable of evoking a reflex response. The possibility that a similar phenomenon might occur in the rat was therefore examined.

Four rats were anaesthetized, and in each a mDOM-T13 island was produced by appropriate denervations, and mapped behaviourally; the map outlines were drawn on the back of each rat. A solution of strychnine sulphate (1.5 mg/kg) was then injected subcutaneously, and the
high-threshold fields were remapped. In all four animals there was no
difference in the area of these fields after the injection, and the
positions of the border between the sensitive and insensitive skin was
also unchanged. By this criterion therefore, there were no "silent"
high-threshold afferents in insensitive skin surrounding the normal
high-threshold receptive field of mDCN-T\textsubscript{13} analogous to those in the
monkey. However, better evidence in this regard came from an
appropriate morphological examination of the skin (see below).

4) Histological Examination for the Presence of Nerves in Skin
   a) PRESENCE OR ABSENCE OF AXONS IN SKIN

The behavioural mapping technique used in this study relies both
on the high-threshold nerves possessing normal function (their endings
in the skin being excited by the pinch stimulus), and on their evoked
impulses being capable of activating the appropriate reflex motor
output from the CNS. One of the problems for the histological studies
now to be described is that normal intact high-threshold nerves might
conceivably extend some of their branches beyond a behaviourally
determined high-threshold field border; these "extended" high-threshold
endings might be genuinely non-functional, or "silent", (Mark, 1974).
If such endings existed, it is possible that they could become
functional for some reason after other nerves supplying the same skin
region are cut (Kirk and Denny-Brown, 1970), thus giving the appearance
of having sprouted de novo into denervated skin, rather than already
being present and silent. As discussed in the preceding section, there
could be a fringe of endings around a measured field whose impulses
failed to evoke a reflex response. Moreover, a fringe of
high-threshold cutaneous axons (very small myelinated and unmyelinated nerves) may extend into insensitive skin in such small numbers that they could be missed during a histological examination of insensitive skin. Despite these hypothetical difficulties the histological examination of skin seemed a worthwhile study to make. Such an examination would be unable to differentiate between high and low-threshold nerves. The proposed histological study, however, was simplified by the assumption that intact low-threshold nerves do not sprout functionally "silent" collaterals into denervated skin in the adult; this is unlikely (Jackson, 1980).

The histological examination of skin that was especially interesting was that of behaviourally insensitive skin, and skin in which sensitivity was believed to have been restored by sprouting of neighbouring intact nerves. In addition to "control" skin taken from normally-innervated regions, pieces of insensitive skin were removed from eight rats that had been surgically denervated to produce an mDCN-T13 island and behaviourally mapped either sixteen or twenty-four days later. In such animals, the cut-off peripheral axons would have had ample time to degenerate (Ramon y Cajal, 1928; Weddell, Guttmann and Gutmann, 1941). The pieces of skin averaged one cm square and were taken from an area at least two to three mm outside the border of the behaviourally sensitive skin. The skin samples were frozen, silver stained and viewed in the light microscope as described in the Methods Sec 10. Four pieces of behaviourally sensitive skin were also processed for histological examination, taken from the same animals; these pieces of skin were removed from regions that had been
denervated, and which would have been insensitive on day zero (this is known from results described in Sec. A2), but by 24 days had become behaviourally sensitive, consistent with their having been invaded by sprouts from neighbouring intact nerves.

A normal ("control") skin is shown in Fig. 14A. Clearly visible in it are the silver-stained axons, running in small fascicles in the dermis. However, the histological examination of the behaviourally insensitive skin revealed no such cutaneous axons in either the dermis or the epidermis; nevertheless the motor nerves to CTM, which were not touched during the initial denervation, showed up well with the silver stain. Clearly visible in the dermis of such behaviourally insensitive skin however were many axon-free "Schwann tubes" (Fig. 14B). These tubes are formed by the Schwann cells which remain and multiply after the axons and myelin have degenerated and been phagocytized by macrophages (Ramón y Cajal, 1928). The Schwann tubes appeared to run throughout the dermis in a kind of network; this would seem to correspond to the normal "network" of cutaneous nerves (Weddell et al 1941). Figure 14B shows a portion of the normal network. In the skin which had become sensitive to forcep pinching, however, there were clearly identifiable axons in the dermis, most of which appeared to lie within Schwann tubes (Fig. 14C). The sprouted axons did not fill the Schwann tubes to the extent seen in normal skin, but what seemed to be products of degenerating nerves were frequently seen within them (Fig. 14C). In the low-threshold field of the intact mDOM-T, in which the Schwann tubes were filled with axons (Fig. 14A) no appearances suggestive of degenerating nerves or their products were seen. The
histological results thus revealed that while behaviourally insensitive skin was devoid of visible axons, skin in which behavioural sensitivity had been restored contained numerous axons which were almost all within what seemed to correspond to the Schwann tubes seen in the denervated skin at earlier times. However, the sprouted nerves obviously did not completely restore the normal axonal population within the Schwann tubes although this conclusion is essentially qualitative since axon counts in skin were not made in these studies.

The results thus directly support the inference from the behavioural evidence that cutaneous nerve sprouting occurred into denervated skin, and that this was responsible for the return of high threshold mechanosensory function. A stronger support was provided by the measurements now to be described.

C QUANTITATIVE CORRELATION OF THE SKIN AREA CONTAINING AXONS AND THAT SENSITIVE TO FORCEP PINCHING: THE BORDER DETERMINATION

The accuracy with which the behavioural mapping technique defines the border of high-threshold field is determined primarily by the amount of skin which is stimulated by the toothed forceps and was estimated to be ± 1.0 - 1.5 mm (see DISCUSSION).

The next experiments attempted to assess the correlation between the behaviourally and histologically determined high-threshold field borders. Three experimental animals were used; one had been behaviourally mapped sixteen days after its initial surgery, a second had been physiologically stimulated with toothed forceps at the time of the denervations and behaviourally mapped sixteen days later, and the third animal was one whose intact mDCN-T₁₃ had been electrically
stimulated on the day of its initial surgery and mapped sixteen days later.

The behaviourally determined high-threshold field borders of the intact mDCN-T13 were drawn on the back of each (anaesthetized) rat, then a sharp razor blade was drawn along the behavioural border with sufficient pressure to cut through the epidermis and extend slightly into the dermis. This incision was later used as a reference point to indicate the location of the behavioural border in cross sections of skin. A piece of skin (approximately 1.5cm long and 1.0cm wide) was removed from each animal, each piece consisting of approximately half behaviourally sensitive and half insensitive skin; with the razor blade incision running along the border between them. The skin was frozen, serially sectioned and stained with silver.

Each section was viewed under the light microscope (objective 25x, NA 0.60 overall magnification 250x). The behaviourally insensitive skin was carefully examined for the presence of stained axons; in every third section the behaviourally sensitive skin was similarly examined. Each time an axon or bundle of axons was observed, its location was noted (as being in behaviourally sensitive or insensitive skin) and its distance measured in tens of microns from the behaviourally determined border (the razor blade incision). After all the sections had been viewed, the data were plotted as in Fig. 15B, C and D on an enlarged representation of the piece of skin examined. The presence of a nerve or group of nerves in each viewed section was indicated by a dot situated on the appropriate side and distance from the incision. (See Fig. 15A legend for a further description of the method of
presentation). In all three skin samples examined no axons were seen in the behaviourally insensitive skin (i.e., beyond the limits of the behaviourally determined field border (see Fig. 15B, C and D); Schwann tubes were present, however, as described above (see Fig. 14B). Axons were seen within the Schwann tubes in the behaviourally determined border region, and in the behaviourally sensitive skin, which contained prominent bundles of axons within Schwann tubes (see Fig. 14A). From these results the histologically defined border in each of the three samples was approximately coincident with the behaviourally defined border, within the ± 1.0mm limitation on this; these results are consistent with the conclusion that the behavioural techniques used in this study indicate the histological border of the intact high-threshold nerves with an accuracy of ± 1 mm.

D. WHICH FIBRE TYPES SPROUT INTO DENERVATED SKIN IN THE ADULT RAT?

There are three classes of sensory nerves to the skin; these are the large diameter myelinated fibres (Group II) associated with low-threshold mechanoreceptors, the small-diameter myelinated (Group III) and the very fine unmyelinated C-fibres associated with nociception. The histological examination was "inadequate to differentiate between the two classes of fibres of interest, the Group III and the C-fibres' associated with high-threshold nociception." We do, however, have a method of distinguishing between the two classes in the present experiments involving electrical stimulation of either the axons in the nerve, or in the skin.

1. **Electrical excitation of mDCN**

There are two classes of high-threshold afferents which, when
stimulated, will evoke a reflex contraction of CTM (Methods Sec. 11); these are the small-diameter myelinated group III (Aβ) afferents (2 to 6 μ), and the much smaller unmyelinated C-fibre afferents (<2μ). Either or both of these groups of nerves may sprout into adjacent denervated skin in the adult rat to give rise to enlarged high-threshold receptive field of nerves. The latencies of the reflex response to excitation of these two classes of nerves were determined in the following way: the intermDGN-T13 was electrically stimulated and both the compound action potential proceeding centrally in the mDGN-T13 and the evoked reflex output, the efferent volley in the motor nerves to CTM on the ipsilateral side, were recorded (Fig. 16). Stimulation of mDGN-T13 with small voltages (e.g., 0.5 volt square wave of 0.05msec width) excited the Group II low-threshold afferent fibres (conduction velocity of approximately 50m/sec) (Fig. 16A); however, there was no reflex volley in the motor nerves to CTM (Fig. 16A). A small increase in the voltage, up to two to five volts, excited the Group III (Aδ) afferent fibres (conduction velocity of approximately 20m/sec) (see Fig. 16B); this now evoked a reflex excitation of the motor nerves to CTM, with a latency of 10-15msec. (Fig. 16B). A much larger voltage and pulse width (15 volts, 0.5msec pulse width) excited a C-fibre afferent volley (conduction velocity of 1.9m/sec) and was associated with a second output in the motor nerves to CTM with a latency of 40 to 60msec (Fig. 16C). This was a repeatable finding. It was hoped therefore that direct electrical stimulation of the skin would also allow the differentiation between the reflex output evoked by activation of the Group III and the C-fibres. If so, it would then
be possible to determine, by electrical stimulation of the formerly
denervated skin into which the high-threshold field had expanded, which
group or groups of high-threshold nerves had sprouted.

2. Electrical Excitation of the Skin

Three animals with "sprouted" high-threshold receptive fields of
mDCN-T\textsubscript{13} were examined. The animals were behaviourally mapped and the
high-threshold receptive field of mDCN-T\textsubscript{13} was drawn on the back of
each. The low-threshold field of mDCN-T\textsubscript{13} was determined
electrophysiologically, but for these studies the mDCN-T\textsubscript{13} was kept
intact, i.e., it was not cut to improve the signal-to-noise ratio. In
this way impulses in the cutaneous nerves due to electrical stimulation
of the skin could propagate to the spinal cord and evoke reflex
responses. The low-threshold receptive field (which does not enlarge
into adjacent denervated skin in the adult rat, see Fig. 17a) was used
as a reference to allow the determination of the region of skin
containing the enlarged portion of the high-threshold field; in normal
animals the high-threshold field does not extend beyond the
low-threshold one by more than two to three mm (see Sec. A2) so that an
area of only high-threshold sensitivity well beyond this, caused by
sprouting of mDCN-T\textsubscript{13} fibres, should be innervated only by the sprouted
high-threshold nerves (see Fig. 15c and D). In order to measure the
latency of the reflex output from the cord, the evoked impulse activity
in the motor nerves to CTM was recorded directly. The skin region that
included the receptive field of the sprouted high-threshold nerves in
mDCN-T\textsubscript{13} was shaved, cleaned with 70% ethanol, and conductive cream was
rubbed into the skin. Electrical stimulation of the skin in the middle
of the low-threshold field of mDCN-T13 was done first, i.e., the region supplied by both high and low-threshold fibres (Fig. 17A and B).

The largest stimulus voltage used (100V, pulses of 1.0msec pulse width) evoked only a single reflex output in the motor nerves to CTM; its latency was 10 - 15 msec; no second reflex response was ever seen characteristic of C-fibre excitation. This result thus indicated that in these experiments the electrical skin stimulus was able to excite the group III afferents but not the C-fibre ones.

Electrical stimulation (90V, pulses of 1.0msec pulse width) of the skin in the sprouted region of the intact high-threshold field of mDCN-T13 (Fig. 17C and D) did evoke a reflex response in the motor nerves to CTM; its latency was 10 to 15 msec, indicating that the Group III afferents fibres had clearly been activated. Again, the stimulus failed to evoke a long latency reflex response characteristic of C-fibre excitation. When the mDCN-T13 was cut the electrical stimulation of skin failed to evoke any reflex response.

Since we could not excite C-fibre endings in normal skin by direct stimulation, the finding that electrical stimulation of the skin in the sprouted region of the intact high-threshold field of mDCN-T13 (Fig. 17C and D) also failed to evoke a long latency reflex response did not allow the conclusion that C-fibres had not sprouted. It was certain, however, that at least some of the group III afferents had sprouted into the adjacent denervated skin.

E. SPATIAL CONSTRAINTS ON SPROUTING OF THE INTACT HIGH-THRESHOLD NERVES

Although low-threshold mechanosensory nerves do not sprout in
adult rats (Results Sec. B2), there does exist a short critical period postnatally during which these nerves will sprout into denervated skin (Jackson and Diamond, 1981). This sprouting in neonatal rats is spatially confined; the low-threshold nerves seem to sprout only into denervated skin that was formerly part of their "parent" dermatome. It is evident from the results already described in this thesis that the high-threshold mechanosensory nerves are not similarly constrained (e.g., Fig. 12). These nerves can sprout into denervated skin of adjacent dermatomes, apparently as readily as within their own. In this then they differ not only from the low-threshold fibres sprouting within the critical period mentioned above but also from low-threshold mechanosensory fibres in the salamander whose sprouting is similarly confined to the parent dermatome (Macintyre and Diamond, 1981).

1. Spatial Constraints on mDGN-T₁₃ High-Threshold Field Sprouting

The following experiments were designed to determine whether the high-threshold nerves have any directional preference in their sprouting. It was conceivable for example, that these nerves might initially sprout ventrally to innervate the denervated skin within their dermatome, and only subsequently sprout into an available adjacent dermatome. If there was no such preference the sprouting in the rostro-caudal direction would be no different in initial rate from that in the dorso-ventral axis.

In six groups of animals DGNs T₈₋₁₂, L₁₋₁₅ and mDGN-T₁₃ were cut (a more extensive denervation than used hitherto) to produce an intact mDGN-T₁₃ island (Fig. 5) and in addition the lateral cutaneous nerves
(LCNs) innervating the flank skin just lateral and adjacent to the DCNs were also cut [LCN-T3 to L5 (Fig. 18)]. The purpose of the extensive denervation was to provide the intact mDCN-T13 with as much denervated skin as was experimentally practicable, into which it could sprout before reaching already innervated skin. During the time of these experiments (40 days) the mDCN-T13 was in fact unable fully to innervate all the denervated skin available to it, i.e., its field never extended enough to abut with already innervated surrounding skin. Groups of animals were behaviourally mapped at 0, 16, 20, 25, 30 and 40 days after denervation, and the maximum dorso-ventral and rostro-caudal "axes" of the isolated field were measured (as in Fig. 19). A comparison was thus possible between the extension of the intact nerve field into denervated skin within its own "parent" dermatome, and that into either of the adjacent denervated dermatomes. The data are plotted in Fig. 20A and the lines of linear regression computed from the data are plotted in Fig. 20B. A comparison (Appendix II) of the rate of movement of the rostro-caudal border and the dorso-ventral one indicates that there is no preferred direction of sprouting (P > 0.05); all the borders of the intact high-threshold field advanced into denervated skin at the same rate. Thus there was no apparent preference of the intact high-threshold nerves to sprout into their parent dermatomes, and it is concluded that there are no spatial constraints on sprouting of the intact high-threshold nerves analogous to those of the low-threshold ones within the neonatal rats (Jackson and Diamond, 1981), or to those of the low-threshold nerves in the skin of the salamander (Macintyre and Diamond, 1981).
2. Spatial Constraints on DCN-T₁₃ High-Threshold Field Sprouting

The next experiments examined for the presence or absence of spatial constraints on the sprouting of an intact entire DCN. It is conceivable that the above results for the medial branch depended upon sprouting being initiated into the parent dermatomal territory and then spilling over into the adjacent foreign dermatomal skin. In the new experiments only the latter (denervated) skin would be available for invasion by axons of the medial branch.

Six groups of animals were denervated as in E1 above, but in these animals the lateral branch of DCN-T₁₃ was left intact along with the medial branch. Groups of animals were behaviourally mapped on days 0, 16, 20, 25, 30 and 40 after denervation and the maximum dorso-ventral and rostro-caudal axes of the field were measured. The border extensions were again compared as in E1 above. The data is plotted in Fig. 21A and the lines of linear regression computed from the data are plotted in Fig. 21B. A comparison (see Appendix II) of the rate of extension of the rostro-caudal border, measured from the medial portion of the DCN field, and the dorso-ventral border (i.e., the lateral extension within dermatomal skin) indicated that the high-threshold axons of the whole DCN-T₁₃ did indeed readily sprout within its own denervated dermatome as well as adjacent denervated dermatomes and that there was no preferred direction of sprouting (p > 0.05); all the borders of the intact high-threshold field expanded into denervated skin at approximately the same rate. It is therefore not essential for the medial branch of mDCN-T₁₃ to be presented with denervated skin within its own dermatome in order to initiate sprouting.
of high-threshold nerves into adjacent denervated dermatomes.

3. Sprouting of mDCN-T₁₃ vs DCN-T₁₃ High-Threshold Nerve Sprouting

This experiment examined the possibility that a field supplied by a given number of axons would expand more rapidly than a field with a smaller number, even though the density of the skin innervation (in the case examined) probably would be the same for the larger and the smaller fields. If so, an isolated DCN-T₁₃ field (supplied by the medial and lateral divisions of DCN-T₁₃) should expand into the surrounding denervated skin at a higher rate than would the medial subfield. The rate of increase in area, and of extension of the rostro-caudal and dorso-ventral axes of isolated DCN-T₁₃ fields, were compared with those of isolated medial subfields of the same segment T₁₃. As shown in Fig. 22A and Appendix II the rostro-caudal borders of both the sprouting DCN-T₁₃ and mDCN-T₁₃ high-threshold fields extended into denervated skin at the same rate (p > 0.05). Similarly, the dorso-ventral borders of both fields expanded outwards at the same rate (p > 0.05) (Fig. 22B Appendix II). These results indicate then that, as expected, the extension by sprouts into denervated skin was unaffected by the size of the remaining innervated field.

The increases in areas of both the sprouting DCN-T₁₃ and mDCN-T₁₃ are plotted in Fig. 23A and the lines of linear regression computed from the data are plotted in Fig. 23B. It is clear that DCN-T₁₃ and mDCN-T₁₃ when surrounded by denervated skin will sprout and increase the area of their receptive fields at significantly different rates (p < 0.05); this would be expected since the rate of change in area is directly proportional to the square of the radius of the quasi-circular
receptive fields. Since the whole DCN field has a larger initial radius than its smaller subfield then it should, as it does (Fig. 23B), increase its receptive field area faster than its smaller subfield even though their axes are increasing at the same rate. There is thus no detectable difference in either the rate or the direction of sprouting of the large (whole DCN) or small (medial DCN branch) nerves into denervated skin.

F. EFFECTS OF ACTIVITY ON THE SPROUTING RESPONSE OF INTACT HIGH-THRESHOLD NERVES

1) Observations Pending to the Study

In initial experiments designed to examine the sprouting of intact high-threshold nerves the strategy was to follow the time course of sprouting within each animal. Four animals were denervated to produce an mDCN-T13 island in each, and each animal was subsequently behaviourally mapped every four days. In these early experiments the area of remaining insensitive skin was measured every time the animals were mapped, rather than an area of "isolated" sensitive skin as in the experiments described so far (Fig. 5). The area of insensitive skin was 968 ± 72 mm² S.E.M. on the initial day immediately following surgery. After a "latent period" of a few days the insensitive area decreased at a roughly uniform rate (Fig. 24). Twenty-four days after surgery there was no insensitive skin remaining (Fig. 24); by this time each animal had been mapped on at least five different occasions. Surprisingly, in another group of animals (used for a different experimental objective), which had received a similar denervation, but
had not been further examined until sixteen days later, there was very little shrinkage of the area of skin insensitive to a pinch. The speculation arose to whether the repeated behavioural mapping could itself affect the rate of sprouting of intact high-threshold nerves. For this reason, the strategy was altered; groups of animals were mapped only once, each group at a selected number of days after denervation. When the amount of remaining insensitive skin in such a group mapped at twenty-four days is compared to that of the repeatedly examined animals (Fig. 24), it is clear that the areas of insensitive skin are significantly different ($p < 0.001$); the animals that had been mapped only once had $626 \pm 59 \text{mm}^2$ S.E.M. of remaining insensitive skin, while those mapped five times had almost none. It seemed then, that the testing procedure was somehow responsible for apparently accelerating the rate of sprouting of the high-threshold nerves. In the absence of any intermediate behavioural testing, the high-threshold nerves succeeded in innervating all the available denervated skin only by approximately forty days, while with behavioural testing every four days the same result was achieved in approximately twenty days (Fig. 24). At first sight it appeared that the sprouting was accelerated to roughly twice the normal value by the repeated behavioural testing procedure.

2) **Further Rationale for the Proposed Study of Impulse Effects on Sprouting**

In response to partial denervation of the back skin of the adult rat, remaining intact high-threshold nerves clearly sprout to innervate the deprived skin. This was suggested by the expansion of the
behavioural fields, and confirmed by the finding that nerve fibres reappear in denervated skin pari passu with the redevelopment of sensitivity in it. The behavioural and the morphological data agree well. Low-threshold nerves do not sprout (at least functionally) into denervated skin in the adult rat (Jackson and Diamond, 1981; Devor et al., 1979). Evidence that the sprouted nerves certainly include high-threshold ones has been given (Sec. D2). The present results are in accord with those of Devor et al. (1979) who demonstrated physiologically that high-threshold cutaneous nerves sprout into denervated glaborous and hairy skin in the adult rat hind paw. They utilized the behavioural withdrawal of the hind limb to a skin pinch to determine the areas of innervation and denervation; as well, they recorded impulses in the intact nerve and demonstrated electrophysiologically that some intact fiber had unusually large receptive fields, extending into the previously denervated skin.

Devor et al. (1979) and Weddell et al. (1941) analysed sprouting of intact nerves by behaviourally mapping each animal, sometimes every day, to follow the often daily expansion of a remaining intact nerve field. That repeated examination of an intact nerve field may affect such results has been indicated by the present findings, which suggest that repeated behavioural mapping of the intact high-threshold mechanosensory nerves will apparently accelerate their sprouting. The repeated behavioural mapping used in the experiments of Devor et al. (1979) might account for the rapid rate of sprouting of the intact nerves they reported. This will be further discussed later.

Several possible mechanisms could be responsible for an increased
rate of sprouting caused by repeated behavioural testing. Pinching may release growth factors within the skin which act on the intact nerves. The pinching may damage the intact neurites within the skin, and cause them to regenerate rather than sprout; the rate of such regeneration may be faster than the rate of sprouting. Finally, pinching within the intact high-threshold nerve field will increase the impulse activity in the axons, and this may somehow accelerate their rate of sprouting into adjacent denervated skin. Increased impulse activity in intact motor nerves has been claimed to accelerate the rate of sprouting of these nerves in partially denervated muscle (Hoffman, 1952) although this has never been confirmed.

It seemed clear that the possibility should be investigated that impulse activity per se can cause an acceleration of cutaneous nerve sprouting.

G. AN INVESTIGATION OF THE BASIS OF THE APPARENT ACCELERATION IN SPROUTING CAUSED BY REPEATED BEHAVIOURAL TESTING

1. The Effect of Physiological Stimulation on Sprouting of High-Threshold Nerves

During the behavioural testing procedure several skin pinches were made actually within the receptive field of the isolated intact high-threshold nerve (the mDCN-T₁₃); the rest of the pinches were in insensitive skin surrounding the island field, or in the outermost, surrounding, skin (sensitive skin innervated by adjacent uncut cutaneous nerves such as D CN-T₉, the lateral cutaneous nerves (L CNs-T₉-L₄) and D CN-L₄). During the repeated behavioural mapping, over a four day period, the intact mDCN-T₁₃ field was estimated as
receiving approximately 135 pinches. These pinches were spread out over five behavioural testing procedures. The question arose - what would be the effect of a similar number of pinches of the field done according to a different regime, in particular all on the initial day of surgery?

In these experiments the effects of physiological stimulation (skin pinching) were examined on the isolated intact high-threshold fields of mDCN-T\textsubscript{13} in a group of six surgically denervated animals. After the animals were sutured, the skin that included the mDCN-T\textsubscript{13} field was pinched 135 times; each pinch had a duration of approximately one second, and the pinches were scattered throughout the entire area of the field. Sixteen days later the animals were behaviourally mapped. In these and subsequent experiments areas of the high-threshold field mDCN-T\textsubscript{13} were measured, i.e., the sensitive skin supplied by this nerve, rather than the areas of insensitive skin as in the earlier experiments. The value obtained (619 ± 25;S.E.M.) is significantly larger (p <0.001), indeed more than double the area of the intact high-threshold field of mDCN-T\textsubscript{13} in a control group of animals mapped sixteen days after denervation, which had not been pinched (250 ± 19mm\textsuperscript{2};S.E.M.) (Fig. 25). There was no doubt that the skin pinches delivered in one stimulation regime were little different in their effectiveness from these spread out over many days. This was good, since it permitted a better and more practicable approach to subsequent analysis of the phenomenon.

2) Examination of the Effects of Tetrodotoxin (TTX) Block of the Physiologically Stimulated mDCN-T\textsubscript{13} on the Accelerated
Sprouting.

If the physiological stimulation of the intact high-threshold field of mDCN-T\textsubscript{13} was causing an accelerated sprouting of the intact nerves by increasing the amount of neural activity in them, then the block of such activity should prevent the effect. The following experiments were undertaken to examine this point.

One group of six rats were denervated to produce an mDCN-T\textsubscript{13} island. The intact mDCN-T\textsubscript{13} trunk was exposed and carefully cleaned in the region beneath the lattisimus dorsi, and about 0.1 ml of TTX solution (10µg/ml in 0.9% saline) was placed on the nerve for 25 min (Methods Sec. 6), at which time a forceps pinch within the field no longer elicited a reflex response of any kind. The skin of the intact nerve field was then pinched 135 times. The pinches were distributed randomly throughout the whole field. The TTX was then washed away with 0.9% saline and the animals were sutured. The reflex response to a skin pinch within the mDCN-T\textsubscript{13} field was not detectably different from normal 24 hr later indicating that impulses were again travelling along the intact nerve to the C.N.S., and that the effects of TTX had therefore worn off.

These animals were behaviourally mapped sixteen days after their initial surgery and the area of the intact high-threshold receptive field of mDCN-T\textsubscript{13} was measured. This area was significantly (p<0.001) smaller (311 ± 25mm\textsuperscript{2}.S.E.M.), in the animals that had received the experimental physiological stimulation during the time that TTX was on the intact nerve than in animals without TTX (619 ± 22mm\textsuperscript{2}.S.E.M.). Moreover the area of the fields of the animals that had TTX on the
nerve was not significantly different (p>0.05) from the area of the mDCN-T13 field in animals that had not been physiologically stimulated, but were simply mapped sixteen days after denervation (250 ± 19mm² S.E.M.) (Fig. 25).

These results show that impulse propagation in the physiologically stimulated nerves was essential for the stimulation to have its effect, that is to cause an apparent acceleration in their sprouting and thus it was the increased neural activity caused by the pinching that must have been responsible for the phenomenon.

H. THE EFFECT OF ELECTRICALLY-EVOKED IMPULSE ACTIVITY ON THE SPROUTING OF INTACT HIGH-THRESHOLD NERVES.

A more direct and quantitative method of increasing neural activity in the intact high-threshold nerves of mDCN-T13 would be to electrically stimulate them directly. The following experiments were designed to answer the questions: (1) will direct electrical excitation of these nerves mimic the effects of physiological activation on their sprouting into denervated skin? (2) if it does, what is the time course of the effect? (3) is there a "dose response" relationship?

1) The Normal Time Course of Sprouting of the High-Threshold Fibres.

To determine the time course of sprouting of the high-threshold cutaneous axons in the absence of experimentally-applied physiological stimulation, it was necessary to measure the fields in separate groups of animals at different times after denervation, since sequential behavioural mapping in the same rat caused the apparent acceleration described above. Eight groups of animals were denervated to isolate their mDCN-T13 fields and each group was mapped once only, at a
selected period after denervation (day zero, 8, 12, 24, 28, 32, 36 and 40 days). The area of the high-threshold receptive field of mDCl-T13 was $239 \pm 23 \text{mm}^2 \text{S.E.M.}$ on the initial day of surgery (day zero), which was not significantly different ($p > .05$) from the area of the field sixteen days after denervation ($260 \pm 19 \text{mm}^2 \text{S.E.M.}$). By twenty-four days however the area was significantly larger ($424 \pm 37 \text{mm}^2 \text{S.E.M.}$) ($p < .001$).

After twenty-four days following denervation the area of the intact high-threshold field increased approximately linearly (Fig. 26) until, at forty days all the available denervated skin was sensitive; the area of the high-threshold receptive field of mDCl-T13 at that time was $814 \pm 41 \text{mm}^2 \text{S.E.M.}$ From the graph it can be seen that normally there is a latent period of about sixteen days after denervation during which the high-threshold nerves of mDCl-T13 do not sprout detectably into adjacent denervated skin.

2) The Time Course of Sprouting of the High-Threshold Nerves of mDCl-T13 After Electrical Stimulation.

Although an increase in neural activity induced by physiological stimulation (skin pinches) clearly seemed to accelerate the sprouting of the intact high-threshold nerves into denervated skin, there was no practicable means of measuring the actual amount of this activity. However, direct electrical stimulation of the intact nerves will similarly increase neural activity, and thus should also be able to accelerate the sprouting of remaining high-threshold nerves into denervated skin. Electrical stimulation of the intact nerve, however, should allow a more quantitative approach to the relationship between
impulse activity and sprouting.

An attempt was made to mimic electrically the effects of 135 pinches. If each pinch evoked approximately 50 to 100 impulses in all the high-threshold mechanosensory axons (Burgess and Perl, 1967), there would be approximately 12,000 (or fewer) impulses in each axon as a consequence of the pinches. It was decided, therefore, to stimulate the intact nerve electrically 12,000 times (20 Hz for 10 min, with a pulse width of 0.5 msec) on day zero, with a voltage sufficient to excite all the high-threshold nerves. This then was the "standard" electrical conditioning stimulus regime.

Four groups of animals were surgically denervated to produce an mDN-T13 island and the remaining intact nerve was electrically stimulated for 10 min at 20 Hz, i.e., 12,000 times; the animal was then sutured. One group was behaviourally mapped once on each of days 4, 8, 12 and 16 days after denervation. The area of the receptive field of mDN-T13 began to increase (Fig. 27) after a "latent period" of about four days, and by sixteen days the area was $597 \pm 55 \text{mm}^2$ S.E.M., which is significantly larger ($p<0.001$), in fact almost three times larger, than the area of the receptive field at sixteen days without stimulation ($250 \pm 19 \text{mm}^2$). The latent period before which sprouting began was reduced from the normal of about sixteen days for unstimulated nerves, to four days for the stimulated nerves; however, once the sprouting began the area of the receptive field increased linearly, at the same rate ($p>0.05$) as did the expanding receptive field of unstimulated nerves (Appendix II).

The results indicated that the electrical stimulation will shorten
the latent period before sprouting will begin, but once the nerves have
begun to sprout they do so at a rate independent of whether they have
been stimulated or not.

To determine if there was a "dose-response" relationship between
electrical stimulation and sprouting, three additional groups of
experimental animals were similarly denervated and electrically
stimulated at 20 Hz; one group was stimulated for 5 min (total 6,000
stimuli), a second for 40 min (total of 48,000 stimuli) and a third for
80 min (total of 96,000 stimuli). The area of the receptive field four
days after the electrical stimulation were not significantly different
(p>0.05) for the two groups stimulated for 40 minutes (244 ± 5 mm²
S.E.M.) or 80 minutes (232 ± 15 mm² S.E.M.); nor were these values
significantly different than that of the field sizes on the initial day
of surgery (239 ± 23 mm² S.E.M.) (Fig. 28). Evidently, the additional
electrical stimulation was unable to produce a further detectable
decrease in the latent period before sprouting would commence.

For those animals of the first group, i.e., those stimulated 6,000
times at 20 Hz, one group was behaviourally mapped on each of days 8,
12 and 16 after denervation. The area of the remaining high-threshold
field eight days after denervation (225 ± 22 mm² S.E.M.) was not
significantly different (p>0.05) from the area of the receptive field
on the initial day of surgery (239 ± 23 mm² S.E.M.), indicating that the
intact nerves had not yet begun to sprout. However, sixteen days after
the denervation and stimulation the area was 507 ± 20 mm² S.E.M., which
was significantly larger (p<0.01) than the area of the receptive field
in a similar group that had not had stimulation (260 ± 19 mm² S.E.M.)
Thus, if the remaining intact nerve is stimulated 6,000 times, then the latent period before sprouting begins is reduced to eight days from the control value of sixteen days in unstimulated animals; always, once the nerves had begun to sprout, the area of the receptive field enlarged linearly at a rate independent of whether the nerve had been stimulated or not \((p>0.05)\) (Appendix II). The results indicate that there exists a "dose-response" relationship between electrical stimulation and the onset of sprouting: 10 min of stimulation at 20 Hz reduced the latent period to four days, but any additional amount of stimulation apparently fails to produce a detectable decrease in the latent period. In all cases, once the sprouting had begun the rate of increase in the size of the receptive field was the same in stimulated and unstimulated nerves.

I. DO THE IMPULSES EVOKED BY ELECTRICAL STIMULATION OF THE INTACT HIGH-THRESHOLD NERVES HAVE TO TRAVEL PERIPHERALLY, CENTRALLY, OR BOTH, TO ACCELERATE THEIR SPROUTING?

Direct electrical stimulation of the cutaneous nerves evokes impulses which travel peripherally to the neurites in the skin and centrally to the cell bodies in the dorsal root ganglion and to their central neurites in the spinal cord. The mechanism responsible for the accelerated sprouting could be activated as a consequence of impulses arriving at any or all of these locations, or by simply being conducted along axons. The following experiments were designed to examine these possibilities.

1) Do the Electrically Evoked Impulses Have to Travel Peripherally to Cause the Phenomenon?
One group of animals was surgically denervated to produce a mDCN-T\textsubscript{13} island, and the intact mDCN-T\textsubscript{13} was placed on platinum wire electrodes for electrical stimulation. Before the stimuli were delivered, TTX (10\textmu g/ml) was placed on the nerve at a point peripheral to the stimulating electrodes (Method Sec. 6) to block impulse propagation to the skin. Once impulse propagation had been blocked (Methods Sec. 6) the intact mDCN-T\textsubscript{13} nerve was electrically stimulated at 20Hz over a period of 10 min (approximately 12,000 stimuli), then the animals were sutured closed. This group of animals was behaviourally mapped sixteen days later; the area of the intact high-threshold field of mDCN-T\textsubscript{13} was found to be $777 \pm 32\text{mm}^2$ S.E.M., which was not significantly different ($p>.05$) than the area of the same field in animals that had been similarly denervated and had received a similar episode of electrical stimulation, but which did not have TTX applied to the nerve ($697 \pm 55\text{mm}^2$ S.E.M.) (Fig. 30). Thus electrical stimulation of the intact high-threshold nerves in mDCN-T\textsubscript{13} will accelerate their sprouting even when the evoked impulses are prevented from reaching the periphery. This result also indicates that TTX does not apparently affect the axonal transport of materials needed for the stimulated nerves to sprout.

2) Do the Impulses Have to Travel Centrally?

Another group of animals was surgically denervated to produce a mDCN-T\textsubscript{13} island and the intact nerve placed on the stimulating electrodes. In this group, TTX was placed on the nerve central to the stimulating electrodes to block impulse propagation to the dorsal root ganglion and spinal cord; once impulse propagation had been blocked
(Methods Sec 6) the intact nerve was electrically stimulated at 20Hz for 10 min and then the animals were sutured closed. Sixteen days later the animals were behaviourally mapped and the area of the high-threshold field of mDCN-T13 determined. In this group the area was \(334 \pm 10\text{mm}^2 \text{ S.E.M.}\), a value significantly smaller (\(p<0.001\)) than the area of the same field in animals sixteen days after denervation combined with a similar episode of electrical stimulation, but without TTX \((697 \pm 55\text{mm}^2 \text{ S.E.M.})\) (Fig. 30). Thus when the TTX was placed central to the stimulating electrodes, i.e., when impulse propagation was blocked centrally, the effect of electrical stimulation was significantly reduced, to approximately 15% of that expected for electrical stimulation without TTX.

The possibility that simply placing TTX on the nerve might accelerate sprouting was examined. A group of animals were surgically denervated to produce an mDCN-T13 island and TTX was placed on the intact nerve (as above) to block impulse propagation, then the animals were sutured closed.

Sixteen days later the animals were behaviourally mapped and the area of the intact high-threshold mDCN-T13 field was determined. The area \((331 \pm 29\text{mm}^2 \text{ S.E.M.})\) was not significantly different (\(p>0.1\)) than the area of the same field measured in animals sixteen days after electrical stimulation with TTX placed central to the stimulating electrodes \((334 \pm 10\text{mm}^2 \text{ S.E.M.})\) (Fig. 30). It appears that in order for electrical stimulation to accelerate sprouting of high-threshold nerves, the impulses must at least propagate centrally towards the dorsal root ganglion. The mechanical manipulations used to place TTX...
on the intact nerve may accelerate the sprouting of the high-threshold nerves; this may be due to the manipulations evoking some impulse activity in the intact nerves. If the mechanical manipulation did initiate an increased impulse activity in the mDCN-T₁₃ during the experiment then this increased activity could itself cause a slight acceleration of sprouting of the high-threshold nerves.

J. PRELIMINARY RESULTS CONCERNING THE CHARACTER OF THE EFFECT OF IMPULSE ACTIVITY ON SPROUTING OF THE HIGH-THRESHOLD CUTANEOUS NERVES

1) Concerning the Time Course of the Decay of the Effect of Electrical Stimulation

The results show that electrical stimulation of the intact high-threshold nerves in mDCN-T₁₃ on day zero (the initial day of surgery) would accelerate their sprouting into denervated skin, and that the effect was dose dependent; 12,000 impulses delivered at 20 Hz accelerated sprouting approximately twice as much as 5,000 impulses at the same frequency. It was decided therefore to examine how long the effects of electrical stimulation lasted, i.e., what is the time course of the "expected" decay of the effect.

The area of the high-threshold receptive field of mDCN-T₁₃ sixteen days after denervation combined with electrical stimulation of the spared nerve for 10 min at 20Hz on day zero (the initial day of surgery) was 697 ± 55 mm² S.E.M. In five other animals the intact mDCN-T₁₃ received the same standard period of stimulation either 6, 7 or 8 days prior to the surgical denervation required to produce a mDCN-T₁₃ island. Sixteen days later the animals were behaviourally
mapped and the area of the high-threshold receptive field of mDCN-T13 was determined. The mean value of the area in the two animals that were stimulated six days prior to denervation was 540 mm²; of the one animal stimulated seven days prior to denervation 670 mm²; and of the two animals stimulated eight days earlier 671 mm². All these values were within one standard deviation of the area of the high-threshold receptive field of mDCN-T13 in the animals that were examined sixteen days after combined denervation and electrical stimulation on day zero (697 ± 121 mm² S.D.). It appears then, from these preliminary observations, that the effects of electrical stimulation do not begin to decay significantly for at least eight days. These experiments are continuing.

2) Will Physiological Stimulation of One Half of the Receptive Field of the Intact mDCN-T13 Nerve Affect Sprouting of the Non-stimulated Half?

Previous published reports by Devor et al (1979) and Burgess and Perl (1967) showed electrophysiologically that in both the rat and cat, the receptive fields of single high-threshold small diameter myelinated axons usually covered a large portion, if not all, of the receptive field of their parent segmental nerve. If this were true for the high-threshold nerves in mDCN-T13 then physiological stimulation of a portion of its receptive field should increase the impulse activity in, and therefore accelerate the sprouting of, nerves that innervate both the stimulated and the unstimulated portion of the receptive field of the parent DCN. On the other hand, if the axons in the stimulated portion of skin did not have endings in the unstimulated (but still
innervated) portion of the skin field, they would not be expected to sprout through the innervated skin into the denervated skin adjacent to (i.e., beyond) the innervated region. We wondered therefore, whether physiological stimulation of the high-threshold endings within one region of the receptive field of mDCN-T\textsubscript{13} would or would not cause them to display an accelerated sprouting into the skin not directly affected by the physiological stimulus.

Two animals were surgically denervated to produce a mDCN-T\textsubscript{13} island and only the caudal half of this isolated high-threshold field was physiologically stimulated (100 forceps pinches). Sixteen days later the animals were behaviourally mapped and the high-threshold receptive field of mDCN-T\textsubscript{13} was determined. The rostral border of the field had sprouted into adjacent denervated skin by approximately 7.0 mm, which is significantly different from the sprouting of the rostral border in animals sixteen days after simple "surround denervation" without stimulation (0.0 mm). The caudal border of the high-threshold receptive field of mDCN-T\textsubscript{13} (i.e., the border of the stimulated region of skin) had also extended into denervated skin, by approximately 7.0 mm. It seems then that physiological stimulation of only the high-threshold endings within the caudal half of the receptive field of mDCN-T\textsubscript{13} had led to the apparent acceleration of sprouting not only of the stimulated endings in the caudal half but also the unstimulated endings (presumably of the same axons) in the rostral half of the receptive field.

The probable explanation for the above result is that in fact all, or at least many, of the high-threshold nerve fibres supplying the
receptive field of mDCN-T₁₃ were activated; the physiologically stimulated axons supplying the caudal half of the receptive field were the same ones that innervated the rostral half. Physiological stimulation of the caudal half increased the impulse activity in all the high-threshold nerves in mDCN-T₁₃ and therefore accelerated the sprouting of all the high-threshold nerves equally in all directions.

3) Does Electrical Stimulation, in the Adult Rat, of the Low-Threshold ("Touch-sensitive") Nerves in mDCN-T₁₃ Evoke Their Sprouting Into Adjacent Denervated Skin?

The present results have confirmed the findings of Jackson and Diamond (1981) which showed that in adult rats, the intact low-threshold cutaneous touch nerves do not sprout into adjacent denervated skin (Fig. 13). It seemed worthwhile, however, to examine the possibility that electrical stimulation of intact low-threshold nerves, coupled with denervation of adjacent skin, would evoke their sprouting. The experiment was simple, since the identical procedure used for the high-threshold nerves provided the required condition to make the test. All that remained was to examine the reaction of the low-threshold nerves in the same animals in which acceleration of high-threshold nerves had occurred. In all animals in which the isolated intact nerve had been stimulated to evoke the accelerated sprouting of the high-threshold receptive field, the intact low-threshold field did not sprout significantly (Fig. 31). Electrical stimulation plus denervation thus failed to evoke collateral sprouting of the low-threshold nerves, even at a time when the high-threshold nerves had sprouted and enlarged their receptive field by as much as
DISCUSSION

A. TECHNIQUE

Since in this investigation the presence in skin of high-threshold cutaneous nerve endings was primarily determined behaviourally, the resolution of the mapping technique will be considered first.

1) The physiological excitation of high-threshold cutaneous nerves and the reflex response

The electrophysiological findings (Results Sec. D1) show that the reflex response of the C.T.M. in the anaesthetized rat was driven by only small myelinated and unmyelinated cutaneous high-threshold nerve stimulation (Group III or C-fibre); electrical or physiological stimulation of the large diameter myelinated (Group II) cutaneous low-threshold fibres stimulation was ineffective (Nixon et al., 1966). The physiological stimulus used to map the high-threshold receptive fields in this study was a skin pinch by toothed forceps; the response to a pinch of the trunk skin was a visible contraction of the C.T.M. which could be clearly seen on both the side on which the stimulus was applied as well as the contralateral side of the trunk. In partially denervated trunk skin, the border between behaviourally sensitive and behaviourally insensitive skin could be easily and clearly determined. The reflex contraction of C.T.M. was maintained throughout the period during which the stimulus was applied, usually between two and three seconds. A pinch in a region of totally denervated skin would on occasion elicit a twitch of the underlying CTM, however, this twitch was not bilateral, nor did it maintain if the stimulus was maintained. This response in totally denervated skin was considered to
be the result of direct mechanical excitation of the underlying muscle fibres and not a reflexly evoked response.

In the cat this similar type of pinch stimulus has been shown by electrophysiological technique to be ideally suited for the study of high-threshold receptive fields of both Group III afferents (Burgess and Perl, 1967) and C-fibre afferents (Iggo, 1960; Iggo, 1977). Initially, a maintained skin pinch would evoke a high frequency discharge in both Group III and C-fibre afferents which would rapidly decrease in frequency, but would normally stop only when the pinch was terminated (Burgess and Perl, 1967; Iggo, 1960). Burgess and Perl demonstrated that repeated stimulation by toothed forceps at a randomly selected point within the high threshold receptive field of a cutaneous nerve would result in a progressive decrease in the number of impulses in the stimulated nerve, so that eventually a skin pinch would not elicit any impulses in the nerve; sometimes the high-threshold fibres would be rendered excitable by a single stimulus. During the present studies, after the skin had been pinched, subsequent pinches in the same place evoked a behavioural response that was visibly reduced or there might be no behavioural response at all; this seems likely to reflect the same adaptive type of phenomenon as that seen by Burgess and Perl in the cat. If the pinches were separated by at least a five minute interval, however, a second pinch in the same place would again produce a behavioural response not visibly different from the initial normal response. In order to attain an accurate indication of the innervation status of the skin, the behavioural response to the initial skin pinch was always taken as significant.
2) The Resolution of the Mapping Technique

The accuracy of the behavioural mapping technique in revealing the presence or absence of high-threshold mechanosensory cutaneous nerve endings was studied by correlative behavioural and histological methods. The behavioural borders of the high-threshold fields were approximately coincident with the histological ones and both had a variability of about ±1 mm and thus the overall correlation was taken to be not better than ±1 mm. This resolution is similar to that of the physiologically determined receptive field of Group III afferents (+1 mm, Burgess and Perl, 1967) and C-fibre afferents (+2 mm, Iggo, 1960) in the cat. In the rabbit, behaviourally determined borders of high-threshold cutaneous nerve fields have also been found to be approximately coincident with the histologically determined borders within ±1 mm (Weddell, Guttman and Guttman, 1941).

Since, within the experimental error defined above, the behavioural response to a skin pinch was always well-correlated with the presence of histologically observable nerve fibres, any physiologically "silent" endings in denervated, and thus behaviourally insensitive, skin (i.e., endings that did not evoke a reflex response when the skin was pinched), could not have extended much beyond 1 mm beyond the border of a remaining nerve field. When the fields enlarged, they could extend considerably further than this (e.g., 10-12 mm); the correspondence between the boundary observed physiologically and that found histologically is therefore consistent with the intact nerves having sprouted into formerly behaviourally insensitive skin, and thus rendering it sensitive. It is true, nevertheless, that the
histological analysis did not allow the visualization of the fine unmyelinated terminals (< 0.3μ diameter); this introduces a possible error since it is unknown how far such terminals might extend beyond the visible large parent axons that were observed. However, as discussed below, the burden of the evidence, coupled with the knowledge that collateral sprouting of intact nerves in other systems certainly occurs after partial denervation, gives confidence in the conclusion just stated.

B. RESULTS

The principal findings in this study are that intact, high-threshold, mechanosensory nerves will sprout into adjacent denervated skin in the adult rat; there appears to be a latent period of at least sixteen days after denervation before this sprouting is detectable (functionally or histologically). Increased neural activity in the intact nerve, however, decreases this latent period substantially, but has little, or no, effect on the rate of sprouting once it has begun.

1) Sprouting of High-Threshold Nerves in the Rat

Unlike low-threshold mechanosensory nerves in the rat (Jackson & Diamond, 1981) which will sprout into denervated skin only during a short critical period in early life, the high-threshold mechanosensory nerves will sprout into denervated skin in rats of any age (although very old rats (>2 years) were not studied). Intact high-threshold nerves in the rabbit leg (Weddell, Guttmann and Guttmann, 1941) have also been shown behaviourally and neuro-histologically to sprout collaterals into denervated skin. More recently, Devor, Schonfield,
Seltzer and Wall (1979) have also shown behaviourally, electrophysiologically and neurohistologically that high-threshold nerves in the rat paw will sprout collaterals into denervated skin.

When sprouting is determined behaviourally, an apparent functional enlargement of a high-threshold receptive field after adjacent denervation may be due to the elimination of a tonic central suppression of neurons involved in the behavioural response rather than collateral sprouting of the remaining intact nerves (Kirk and Denny-Brown, 1970). This possibility was tested; both the pharmacological and the neurohistological results obtained, provided evidence that this was not the case. The expansion of the high-threshold receptive fields expanded into adjacent denervated skin therefore can be associated to collateral sprouting of the remaining intact nerves.

I determined electrophysiologically that Group III small diameter myelinated fibres were certainly among those that had sprouted into the denervated skin. This was also the case in the experiments on the rat paw done by Devor et al. (1979). It was not possible to determine whether C-fibre afferents had also sprouted. Weddell et al. (1941) using a technique described by Weddell (1941) showed histologically using a light microscope that in the rabbit ear, the majority of the cutaneous nerves which sprout into denervated skin were unmyelinated; an unambiguous identification of unmyelinated C-fibres is difficult at the light microscope level, and to be absolutely sure that the C-fibres are sprouting one would have to undertake a difficult electron microscopic examination.
In the present experiments the high-threshold nerves sprouted into the denervated skin mainly by advancing along the Schwann tubes previously occupied by adjacent nerves. This finding suggests that either new sprouts were being attracted towards the denervated skin by the empty Schwann tubes (or by the products of degeneration with them), or perhaps that they simply used the relatively unimpeded pathways offered by the now empty Schwann tubes. Either of these suggestions is consistent with the findings, and it certainly seems as though these Schwann tubes were now serving to guide the neurites to the denervated skin.

The stimulus to sprout may derive from denervated targets, or target tissue, in the surrounding skin. It has been suggested (Jackson, 1980; Jackson and Diamond, 1981) that in the salamander and rat denervated Merkel cells, the targets of certain of the low-threshold mechanosensory nerves, release a trophic substance which causes these nerves to sprout. Once an appropriate nerve has innervated the target tissue it seems to neutralize the trophic substance and sprouting stops (Aquilar, Bisby, Cooper and Diamond, 1973; Cooper, Diamond and Turner, 1977). Finally, collateral sprouting into denervated tissue has been well documented with functional, histological and electrophysiological methods in partially denervated muscle (Edds, 1953) sympathetic ganglia (Langley, 1897; Guth and Bernstein, 1961) and more recently in the central nervous system (Bernstein and Goodman, 1973).
2) Impulse Activity and Its Effect on Collateral Sprouting of Intact High-Threshold Nerves

The results of this thesis provide evidence that an increase in the amount of neural activity (produced either physiologically or electrically), within the high-threshold Group III afferent cutaneous nerves in the rat, will accelerate their collateral sprouting into adjacent denervated skin. This is not the first time that neural activity (evoked electrically) has been reported to affect collateral sprouting in mammals; Hoffman (1952) provided histological evidence that electrical stimulation of remaining intact motor nerves in partially denervated muscles of rats accelerated their sprouting. Recently, Maehlen and Nja (1982) showed electrophysiologically and morphologically that electrical stimulation of the remaining intact preganglionic sympathetic nerves accelerated their sprouting in partially denervated post ganglionic neurons in guinea pigs; it was essential that transmission through the ganglion remained intact since ganglionic blocking agents prevented the effect (Maehlen and Nja, 1982). Electrical stimulation of the post-ganglionic neurons accelerated the sprouting of the remaining intact preganglionic neurons. They suggested that the increased activation of the post ganglionic neurons (the targets of the sprouting nerves) enhance their ability to receive the sprouts. However, in partially denervated mammalian muscle it seems unlikely that an increase in activity of the denervated target will increase its ability to receive sprouting intact motor nerves since increased activity (produced by direct electrical stimulation) in partially denervated muscle has been shown to inhibit
the sprouting of intact motor nerves (Hoffman, 1952; Brown, Holland and Ironton, 1980). Furthermore, chronic inactivity (produced by placing TTX on the motor nerve) of muscle fibres in fully innervated muscles will evoke sprouting of the intact nerves (Brown and Ironton, 1977). In the present study physiological or electrical activation of the denervated target tissue was not tested to see what effect it may have on the sprouting of adjacent intact high-threshold nerves. However, it was shown that increased neural activity (produced electrically or physiologically) in the intact sprouting nerve would accelerate its sprouting. It was not essential for the electrically evoked impulses to travel to the cutaneous targets in order for the electrical stimulation to accelerate the sprouting of the intact nerves, rather, it was essential for the impulses to travel to the spinal cord; TTX placed on the nerve between the stimulating electrodes and skin would not prevent the acceleration of sprouting due to increased impulse activity.

In both cases mentioned above (mammalian muscle and sympathetic ganglion) neither provided evidence that physiological stimulation could accelerate nerve sprouting. This study provides the first evidence that physiological stimulation of the intact nerves will accelerate their sprouting; currently within the laboratory, other sensory modalities in rat skin are being examined (thermosensitive and touch sensitive nerves) to see if physiological stimulation of their endings will selectively accelerate their sprouting.

Normally without stimulation the high-threshold nerves would begin to sprout into denervated skin after a latent period of about sixteen
days after denervation; electrical and/or physiological stimulation of the nerves reduced this latent period. Once the nerves had begun to sprout, however, they did so at the usual rate, i.e., it seemed not to matter whether they had been stimulated or not.

Previous studies of sprouting of cutaneous nerves in mammals (Weddell et al., 1941; Devor et al., 1979) have been carried out by examining the enlargement of cutaneous receptive fields in an animal on several occasions after denervation thus providing continuous physiological stimulation to the sprouting intact nerves (this is similar to the experimental results in Results Sec. B1); this could explain the rapid onset of sprouting of the cutaneous nerves in their studies since in both studies the intact nerves were physiologically stimulated every two to three days after adjacent denervation. Had they not repeatedly stimulated the cutaneous nerves then they may have delayed the onset of their sprouting. Even though Devor et al. (1979) did frequently stimulate the sprouting nerves they did not see any significant sprouting of them until four days after denervation; this is consistent with the present study showing that there is no significant sprouting before four days after denervation no matter how much stimulation has occurred.

After a preliminary abstract of the results presented in this thesis had been published, Devor and his colleagues examined the possibility that physiological stimulation may indeed accelerate the cutaneous high-threshold nerve sprouting in their preparation, the rat paw (Greenfield and Devor, 1981). Their conclusion was that in their preparation added physiological stimulation, above that normally
received by a rat paw, did not significantly accelerate sprouting. They realized of course, that the amount of stimulation received by the rat while walking could in itself be enough to maximally accelerate the sprouting of the high-threshold nerves. Perhaps had they partially denervated the rat paw and then placed TTX on the remaining intact nerve they may have been able to show that by blocking the impulses evoked in the intact nerve while the rat was walking in its cage they would have been able to increase the latent period before the nerves began to sprout into denervated skin; this would also show that the amount of stimulation received by the sprouting nerves while the rat walked around its cage was sufficient to maximally accelerate their sprouting. The evidence provided in this thesis shows that only a minimal amount of neural activity (10 min at 20 Hz) is required to maximally accelerate the sprouting of high-threshold nerves; this amount could certainly be achieved by the nerves innervating the rat's paw as the animal moves around in its cage.

Finally, this study provides evidence in support of a "dose-dependent" relationship between electrical stimulation and collateral sprouting (see discussion below).

3) Possible Mechanisms by Which an Increase in Neural Activity Accelerates the Observed Collateral Sprouting

The quantitative findings from this study indicate that the main effect of an increase in neural activity in the remaining high-threshold nerves is to reduce the latent period before sprouting of these nerves will begin. The TTX results indicate that the electrically evoked impulses need to be conducted centrally, but not
peripherally to the skin in order to accelerate nerve sprouting. These results indicate that electrical stimulation has its effect either in the neurons in the dorsal root ganglion, or by a mechanism involving the central projections to the spinal cord; in this study it was not possible to differentiate between the two. A study of the literature suggests at least one mechanism by which an increase in neural activity could accelerate collateral sprouting; this is presented below.

In order for intact nerves to sprout in response to a sprouting stimulus they need necessary membrane lipids and proteins; these must be produced in the cell body (Verra, Grafstein and Ross, 1979) and then transported down the axon by axoplasmic flow to the region of the growing nerve endings. The bulk of the cytoskeletal proteins which have been implicated in the process of axonal growth (Yamada, Spooner and Wessels, 1971; Bray, 1973; Bunge, 1973; Heacock and Agranoff, 1976; Lasek and Hoffman, 1976) are major identified constituents of slow axonal transport (Grafstein, McEwen and Shelesnik, 1970; Hoffman and Lasek, 1975). After electrical stimulation (12,000 times on day zero) the peripheral nerves in the present study began to sprout after four days. Since the average distance from the cell bodies of the intact nerves to the peripheral neurites is six cm, membrane constituents produced in the cell body would have to travel at approximately 150 mm/day to reach the neurites and provide the materials for growth; since slow axoplasmic flow in rat peripheral nerves is approximately 1 mm/day (Hoffman and Lasek, 1975) the membrane components in slow flow would be unable to contribute to the axonal growth noted after.
electrical stimulation. Therefore, the effect of electrical stimulation would be either to increase the rate of axoplasmic flow of the membrane components produced in the neuron or somehow increase the production of membrane components which normally are transported by fast axoplasmic transport; either of these effects of electrical stimulation of the intact autonomic nerve would possibly render it more responsive to the sprouting stimulus.

On the other hand, we know that electrical stimulation does not increase the rate of axoplasmic transport (Lux et al., 1970; Worth and Ochs, 1970). We are therefore left with the possibility that electrical stimulation evokes an increase in the production of membrane constituents which are produced in the cell body (Lux et al., 1970; Singh and Talwar, 1969) and travel by fast axoplasmic transport to the peripheral neurites.

The transport of membrane components in fast transport have been suggested by Bisco (1973) in regenerating rat sensory nerves to account for the early onset of growth after lesions. Pate Skene and Willard (1981) have identified at least two essential membrane components which travel by fast axoplasmic transport in rabbit regenerating nerves and, finally, Goldowitz and Cotman (1980b) have shown that the membrane components necessary for the initial axonal sprouting of intact fibres in the rat brain are provided not by slow axoplasmic flow, but probably by fast flow.

As mentioned earlier, (Introduction Sec. D2) an increase in neural activity (either physiologically or electrically produced) will increase the metabolic rate in the cell bodies of the stimulated nerves.
(Barr and Bertram, 1951; Singh and Talwar, 1964; Lux et al., 1970). Not all proteins manufactured by the neurons show accelerated synthesis during axonal growth; it is generally considered that priority is given to the production of structural proteins which are incorporated into the cell membrane and that a reduction occurs in the synthesis of proteins associated with synaptic transmitter metabolism (Reis and Ross, 1973; Cheah and Geffen, 1973). It is most likely, therefore, that the increased neural activity should accelerate both the switch to the production of membrane components, in response to the sprouting stimulus, as well as increase the rate of their synthesis; this could result in more membrane components being synthesized and transported to the peripheral neurites sooner than would occur if the neurites had not been stimulated.

Finally, as mentioned before, it appears that the effect of electrical stimulation on the acceleration of sprouting of the intact nerve is dose dependent. Electrical stimulation only shortens the latent period before which sprouting will begin; once the sprouting has begun the nerves sprout at the same rate whether they are stimulated or not. This result indicates that the major effect of electrical stimulation of the intact nerve is to switch the metabolic machinery from producing maintenance proteins to producing membrane constituents; once the switch has occurred then the rate of production of membrane constituents is the same in both stimulated and unstimulated nerves. Electrical stimulation provides the signal to switch.

There may be a threshold level of neural activity needed before the switch can occur. Once the amount of neural activity has surpassed
the threshold level required the metabolic production of membrane constituents will begin. If this were true, then one may expect that electrical stimulation for 80 min (96,000 stimuli) would shorten the latent period even further than that seen at 10 min (12,000 stimuli) of electrical stimulation. Two possible reasons why 80 min does not shorten the latent period further are discussed below. The first reason could be that there exists another physical limitation which does not permit the latent period to be less than four days. Even at the fastest switching time it may be physically impossible to mobilize the ribosomes, mRNA and amino acids necessary to produce the membrane constituents sooner than four days after electrical stimulation. Cat moto-neurons stimulated electrically for eight hours first begin to show signs of increased metabolism 48 hrs after stimulation with a peak RNA and protein synthesis occurring at approximately 4 days after electrical stimulation (Barr and Bertram, 1951).

The second possible reason is that indeed 80 min electrical stimulation does cause a switch in metabolism sooner than does 10 min, but both amounts of stimulation are above the threshold amount needed to produce the effects on metabolism. The reason that we see no shortening of the latent period may be that we are unable to resolve such a small difference between the latent period after 80 min as opposed to that after 10 min of electrical stimulation.

Since no amount of electrical stimulation will increase the rate of axoplasmic flow then in all cases the metabolites produced in the cell body will take the same amount of time to reach the peripheral neurites. This may account for the short latency before onset of
sprouting. Even if the metabolites needed for axonal growth are produced immediately after electrical stimulation they will have to travel by axoplasmic flow to the peripheral endings before sprouting of the intact axons can begin. The travel time needed (perhaps four days) for the metabolic products to reach the peripheral neurites may account for the apparent absolute shortest latency (4 days) before which sprouting cannot begin.

(4) Which Types of High-Threshold Nerves Have Sprouted?

The results of this thesis have indicated that at least the Group III high-threshold afferent nerves have sprouted into denervated skin. This result is consistent with other reports in the literature concerning high-threshold nerve sprouting in the rat (Devor et al., 1979), rabbit (Weddell et al., 1941) and man (Head; 1920). Devor et al (1979) were unable to determine electrophysiologically whether the C-fibres had sprouted into denervated skin in the rat paw; this may be due to their sampling bias and therefore they were not able to rule out the possibility that the high-threshold C-fibres sprouted into denervated adult skin. Low-threshold nerves certainly regenerate after nerve section in adult rats (Diamond & Jackson, 1981) and cat (Burgess and Horch, 1973), yet, are unable to sprout into adjacent denervated skin of adult rats.
Appendix I

Mann-Whitney U-Test

All statistical tests were carried out using the Mann-Whitney U-test. This is a simple, non-parametric test designed for use with small samples which can be placed in order with respect to one variable. The two sets of data are plotted on a graph, one above the other. Then a value is calculated which is call the U statistic (U₃); this value is found on a table which indicates the appropriate p level.

A sample calculation is illustrated below.

Area of the high-threshold receptive field of mDN-T₁₃ sixteen days after denervation and

B. Ten minutes electrical stimulation

| 4 | 6 | 5 | 5 | 7 |

A. Five minutes electrical stimulation

| 400 | 500 | 600 | 700 | 800 | 900 |

area in mm²

To perform this test, a count is made of the number of values in sample A which are smaller (i.e. to the left) of each value in Sample B. These values are added together to yield a value C. The Mann-Whitney statistic (U₃) is the larger of the two quantities, C, or (n₁n₂ - C). In this case, C = 28, n₁n₂ - C = 30 - 28 = 2, therefore in this example, U₃ = 23. On the table for n₁ = 5, and n₂ = 5a value of 23 indicates a 0.005 level of significance (see Sokal and Rohlf, 1973).
Appendix II

The rate of sprouting of the intact high-threshold pDCN-T13 and DCN-T13 fields after different amounts of electrical stimulation was analysed by comparing the slopes of the lines of linear regression which were fitted to the data according to a linear least squares analysis. The lines of linear regression were calculated for data points taken from the curves during the actual growth phases.

The actual strategy for the comparison of slopes is as follows:

Comparison of Slopes

Let's consider the general equation of a regression line, which is fitted to a certain set of experimental data. This equation would be:

\[ y = C_0 + C_1 x \]  \hspace{1cm} (1)

where \( y \) is the dependent and \( x \) the independent variable. \( C_0 \) is the intercept and \( C_1 \) gives the slope of the regression line.

Let's hypothesize now that another regression line derived from a second set of experimental data is described by:

\[ y = C_0' + C_1' x \]  \hspace{1cm} (2)

and that one would be interested in comparing the slopes \( C_1 \) and \( C_1' \) of the two lines, so as to check if they are significantly different or not.

Inserting a dummy variable \( z \), which would be zero for the first set and one for the second, a general equation could be obtained, which would describe both regression lines. This general equation would be of the form:

\[ y = C_0 + C_1 x + C_2 z + C_3 (x \times z) \]  \hspace{1cm} (3)
For the first line, $z$ is zero and (3) is reduced to (1). For the second line, $z$ equals one and (3) gives:

$$y = C_o + C_1 x + C_2 + C_3 x$$  \hspace{1cm} (4)

In the latter case (4), the equation can be written as

$$y = (C_o + C_2) + (C_1 + C_3) x$$  \hspace{1cm} (5)

and compared with (2) it gives the following:

$$C'_o = C_o + C_2$$  \hspace{1cm} (6)

and

$$C'_1 = C_1 + C_3$$  \hspace{1cm} (7)

From (7) it can be easily deduced that, if one wanted to compare the slopes $C_1$ and $C'_1$, it would be the same to check whether $C_3$ is significantly different than zero or not. If $C_3$ is zero, then $C_1$ is exactly the same as $C'_1$, i.e. the two lines have the same slope.

This was applied to compare the slopes of the following lines of linear regression: See Appendix II A-G.
Appendix II

A - The increase in the area of the high-threshold field due to sprouting of mDCN-T_{13} compared to DCN-T_{13}.
### DATA LIST
- **INPUT MEDIUM**: CARDS
- **N OF CASES**: 77
- **VAR LABELS**: *X, Y, AREA OF FIELD, TYPE OF FIELD*
- **VALUE LABELS**:  
  - *X*: 1, 2, 3, 4, 5
  - *Y*: 6, 7, 8, 9
- **COMPUTE**: *1*
- **VAR LABELS**:  
  - *INTERACTION*
  - *REGRESSION VARIABLES*: *Y, X, Z, I*
  - *REGRESSION WITH*: *X, Z, I*
- **STATISTICS**: ALL
- **READ INPUT DATA**

---

**OPTION**: 1

**IGNORE MISSING VALUE INDICATORS**

**NO MISSING VALUES DEFINED... OPTION 1 WAS FORCED**

---

### AREAS OF SUPER ISLANDS

**FILE NAME (CREATION DATE = 02/02/15):**

---

#### MULTIPLE REGRESSION

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<th>CASES</th>
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#### CORRELATION COEFFICIENTS
A value of 0.00000 is printed if a coefficient cannot be computed.

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## Areas of Super Islands

### Multiple Regression

#### Dependent Variable: Area of Field
- **Mean Response:** 4659.70130
- **Std. Dev.:** 2946.97880

#### Variable(s) Entered on Step Number 1:
- **Day**
- **Type of Field**

### Multiple R
- **R Square:** 0.41887
- **Adjusted R Square:** 0.17029

### Analysis of Variance
- **Sum of Squares:**
  - Regression: 73.11561561
  - Residual: 73.782698
  - Total: 146.898314
- **Mean Square:**
  - Regression: 3974.895
  - Residual: 73.782698
- **F Significance:** 1.20764

### Coefficient of Variability:
- 5.6 PCT

### Variables in the Equation

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<th>Std Error</th>
<th>F</th>
<th>Beta</th>
<th>Significance</th>
<th>Elasticity</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>96.514870</td>
<td>37.022468</td>
<td>6.796063</td>
<td>.396083</td>
<td>.013</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>194.3664</td>
<td>1329.4954</td>
<td>2.138869</td>
<td>.331802</td>
<td>.068</td>
<td></td>
</tr>
<tr>
<td>(Constant)</td>
<td>3154.1020</td>
<td>967.8346</td>
<td>10.528523</td>
<td>.34491</td>
<td>.002</td>
<td></td>
</tr>
</tbody>
</table>

### Variables Not in the Equation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Partial</th>
<th>Tolerance</th>
<th>F Significance</th>
</tr>
</thead>
</table>

### Coefficients and Confidence Intervals

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>Std Error</th>
<th>T</th>
<th>95.0 PCT Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>96.514870</td>
<td>37.022468</td>
<td>2.685879</td>
<td>22.729198 - 170.300546</td>
</tr>
<tr>
<td>T</td>
<td>194.3664</td>
<td>1329.4954</td>
<td>1.442845</td>
<td>25.3143 - 459.0469</td>
</tr>
<tr>
<td>(Constant)</td>
<td>3154.1020</td>
<td>967.8346</td>
<td>1.987928</td>
<td>1225.2029 - 5083.0812</td>
</tr>
</tbody>
</table>

## Areas of Super Islands

### Multiple Regression

#### Dependent Variable: Area of Field

### Variance/Covariance Matrix of the Unnormalized Regression Coefficients
**AREAS OF SUPER ISLANDS**

FILE NAME (CREATION DATE = 02/02/15)

---

MULTIPLE REGRESSION

DEPENDENT VARIABLE: Y

AREA OF FIELD

### SUMMARY TABLE

<table>
<thead>
<tr>
<th>STEP</th>
<th>VARIABLE ENTERED</th>
<th>VARIABLE REMOVED</th>
<th>F TO ENTER OR REMOVE</th>
<th>SIGNIFICANCE</th>
<th>MULTIPLE R SQUARE CHANGE</th>
<th>SIMPLE R SQUARE CHANGE</th>
<th>OVERALL F</th>
<th>SIGNIFICANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>X</td>
<td></td>
<td>5.79607</td>
<td>0.011</td>
<td>0.9448</td>
<td>0.00893</td>
<td>0.0093</td>
<td>0.09448</td>
</tr>
</tbody>
</table>
Appendix II

B - The increase in the dorso-ventral axis of the high-threshold field due to sprouting of mDCN-T_{13} compared to DCN-T_{13}.
ACADEMIC COMPUTING SERVICES
McMASTER UNIVERSITY
S P S S -- STATISTICAL PACKAGE FOR THE SOCIAL SCIENCES
VERSION 4.0 -- FEBRUARY 16, 1981

RUN NAME: D-V'S OF SUPER ISLANDS
DATA LIST: FIXED(11/1 X 1-2 2'4 Y 6-9)
THE DATA LIST PROVIDES FOR 7 VARIABLES AND 1 RECORDS PER CASE.
A MAXIMUM OF 9 COLUMNS ARE USED ON A RECORD.

INPUT MEDIUM CARD
N OF CASES 77
VAR LABELS:
VALUE LABELS:
COMPUTE:
VAR LABELS:
REGRESSION:
STATISTICS:
READ INPUT DATA
00090400 CM NEEDED FOR REGRESSION:

OPTION: 1
IGNORE MISSING VALUE INDICATORS
NO MISSING VALUES DEFINED...OPTION I WAS FORCED

D-V'S OF SUPER ISLANDS

FILE: NOMAME (CREATION DATE = 02/02/15)

MULTIPLE REGRESSION

VARIABLE MEAN STANDARD DEV CASES
X 22.5714 12.9574 77
Y 2.7729 2.1783 77
Z -5.192 5.925 77
I 12.1598 14.4965 77

CORRELATION COEFFICIENTS.
A VALUE OF 99.999999 IS PRINTED
IF A COEFFICIENT CANNOT BE COMPUTED.

X Y Z

4.7596 .87182 - .69755
.27182 -.69755 - .01210
.46761 - .39737 .81210
X Y Z
FILE: WONAME (CREATION DATE = 02/02/15)

MULTIPLE REGRESSION

DEPENDENT VARIABLE: Y
LENGTH OF AXIS

MEAN RESPONSE: 2.77732
STD. DEV.: 0.72830

VARIABLE(S) ENTERED ON STEP NUMBER: 1...

VARIABLES IN THE EQUATION:

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>B</th>
<th>STD ERROR</th>
<th>T</th>
<th>SIG.</th>
<th>BETA</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>0.13452458E-01</td>
<td>0.0077237E-2</td>
<td>4.422093</td>
<td>0.053911</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>-1.0073901</td>
<td>0.00737568</td>
<td>-1.35581</td>
<td>-0.053911</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>2.5852721E-02</td>
<td>0.00035318</td>
<td>1.140377</td>
<td>0.011432</td>
<td></td>
</tr>
</tbody>
</table>

CONSTANT: 2.6775079

ALL VARIABLES ARE IN THE EQUATION.

COEFFICIENTS AND CONFIDENCE INTERVALS:

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>B</th>
<th>STD ERROR</th>
<th>T</th>
<th>95.0% CONFIDENCE INTERVAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>0.33652468E-01</td>
<td>0.0087237E-2</td>
<td>3.811348</td>
<td>0.2330540E-01 - 0.4399940E-01</td>
</tr>
<tr>
<td>I</td>
<td>-2.0057901</td>
<td>0.00375688</td>
<td>-6.135581</td>
<td>-1.163899E-01 - 0.643812E-01</td>
</tr>
<tr>
<td>CONSTANT</td>
<td>2.5775079</td>
<td>0.11321654</td>
<td>21.820780</td>
<td>2.2339956 - 2.8152001</td>
</tr>
</tbody>
</table>

DAYS OF SUPER ISLANDS

FILE: WONAME (CREATION DATE: 02/02/15)

MULTIPLE REGRESSION

DEPENDENT VARIABLE: Y
LENGTH OF AXIS

VARIANCE/COVARIANCE MATRIX OF THE UNNORMALIZED REGRESSION COEFFICIENTS:

<table>
<thead>
<tr>
<th></th>
<th>X</th>
<th>Z</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>0.00822</td>
<td>0.00822</td>
<td>0.03026</td>
</tr>
<tr>
<td>Z</td>
<td>0.00822</td>
<td>0.00105</td>
<td>0.0005</td>
</tr>
<tr>
<td>I</td>
<td>0.03026</td>
<td>0.00105</td>
<td>0.0005</td>
</tr>
</tbody>
</table>
### Multiple Regression

**Dependent Variable:** \( Y \)  
**Length of Axis**

<table>
<thead>
<tr>
<th>Step</th>
<th>Entered</th>
<th>Removed</th>
<th>( F ) to Enter or Remove</th>
<th>Significance ( F )</th>
<th>Multiple ( R )</th>
<th>( R^2 ) Square Change</th>
<th>Simple ( R )</th>
<th>Overall ( F )</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( Y )</td>
<td></td>
<td>48.82005</td>
<td>0.0</td>
<td>0.7794</td>
<td>0.23015</td>
<td>0.3748</td>
<td>0.60792</td>
<td>0.00046</td>
</tr>
<tr>
<td>2</td>
<td>( T )</td>
<td></td>
<td>33.56257</td>
<td>0.0</td>
<td>0.8768</td>
<td>0.23015</td>
<td>0.5078</td>
<td>0.60792</td>
<td>0.00046</td>
</tr>
</tbody>
</table>
Appendix II

C - The increase in the rostro-caudal axis of the high-threshold field due to sprouting of mDGN-T13 compared to DGN-T13.
**SPSS -- STATISTICAL PACKAGE FOR THE SOCIAL SCIENCES**  
**VERSION 8.0 -- FEBRUARY 16, 1981**

**RUN NAME** 8-CMS OF SUPER ISLANDS  
**DATA LIST** FILE11/1 2 3 4 5 6 7 8 9

The data list provides for 3 variables and 1 record per case.  
A maximum of 3 columns are used on a record.

**INPUT MEDIUM** CARD  
**N OF CASES** 69  
**N OF VARIABLES** 9  
**LABELS**  
**VAR LABELS**  
**VALUE LABELS**  
**COMPUTE**  
**REGRESSION**  
**STATISTICS**  
**READ INPUT DATA**

00000400 CM NEEDED FOR REGRESSION

**OPTION** 1  
**IGNORE**  
**MISSING VALUE INDICATORS**

No missing values defined...option 1 was forced.

---

**R-CMS OF SUPER ISLANDS**

**FILE** NAME1 (creation date = 02/02/15)

***MULTIPLE REGRESSION***

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>MEAN</th>
<th>STANDARD DEV</th>
<th>CASES</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>22.5714</td>
<td>12.0574</td>
<td>77</td>
</tr>
<tr>
<td>Y</td>
<td>1.5577</td>
<td>1.4418</td>
<td>77</td>
</tr>
<tr>
<td>Z</td>
<td>10.4156</td>
<td>13.8548</td>
<td>77</td>
</tr>
</tbody>
</table>

**CORRELATION COEFFICIENTS.**

A value of .9999999 is printed if a coefficient cannot be computed.

<table>
<thead>
<tr>
<th></th>
<th>Y</th>
<th>Z</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>.87057</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z</td>
<td>.57129</td>
<td>.72799</td>
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</tr>
</tbody>
</table>

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**Multiple Regression**

**Dependent Variable:** Y  
**Length of Axis**

<table>
<thead>
<tr>
<th>Variable (s) Entered on Step</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <strong>DAY</strong></td>
<td></td>
</tr>
<tr>
<td>2. <strong>AXIS</strong></td>
<td></td>
</tr>
<tr>
<td>3. <strong>INTERACTION</strong></td>
<td></td>
</tr>
</tbody>
</table>

**Multiple R**  
8.7710

**Adjusted R Square**  
7.0900

**Analysis of Variance**

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>13.57990</td>
<td>4.32366</td>
<td>81.1421</td>
<td>*0.00001</td>
</tr>
<tr>
<td>Residual</td>
<td>4.08379</td>
<td>4.05775</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>17.66379</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Variables in the Equation**

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>Std Error B</th>
<th>t</th>
<th>Significance</th>
<th>Elasticity</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>320562206E-01</td>
<td>32031742E-02</td>
<td>103.33050</td>
<td>0.8163174</td>
<td>0.00001</td>
</tr>
<tr>
<td>Z</td>
<td>-2623956E-01</td>
<td>11502750</td>
<td>-22.32021</td>
<td>0.00001</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>4956817E-02</td>
<td>5045318E-02</td>
<td>9.34018</td>
<td>0.00001</td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>81429439</td>
<td>8377124E-01</td>
<td>94.58421</td>
<td>0.00001</td>
<td></td>
</tr>
</tbody>
</table>

**Variables Not in the Equation**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Partial Tolerance</th>
<th>Significance</th>
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</table>

**Coefficients and Confidence Intervals**

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>Std Error B</th>
<th>t</th>
<th>95.0% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>320562206E-01</td>
<td>32031742E-02</td>
<td>103.33050</td>
<td>2.51722E-01 to 6.48641E-01</td>
</tr>
<tr>
<td>Z</td>
<td>-2623956E-01</td>
<td>11502750</td>
<td>-22.32021</td>
<td>-3.28123E-01 to -2.81043E-01</td>
</tr>
<tr>
<td>T</td>
<td>4956817E-02</td>
<td>5045318E-02</td>
<td>9.34018</td>
<td>-2.03879E-02 to 1.18741E-02</td>
</tr>
<tr>
<td>Constant</td>
<td>81429439</td>
<td>8377124E-01</td>
<td>94.58421</td>
<td>-1.98118E-02 to 1.98118E-02</td>
</tr>
</tbody>
</table>

---

**R-C's of Super Islands**

**Dependent Variable:** Y  
**Length of Axis**

**Variance/Covariance Matrix of the Unnormalized Regression Coefficients**

<table>
<thead>
<tr>
<th></th>
<th>X</th>
<th>Z</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>0.00001</td>
<td>-0.123</td>
<td>-0.00001</td>
</tr>
<tr>
<td>Z</td>
<td>0.00001</td>
<td>-0.00004</td>
<td>-0.00002</td>
</tr>
<tr>
<td>T</td>
<td>0.00001</td>
<td>-0.00004</td>
<td>-0.00002</td>
</tr>
</tbody>
</table>
### Multiple Regression Summary Table

<table>
<thead>
<tr>
<th>Step</th>
<th>Enter/Remove</th>
<th>F to Enter</th>
<th>Significance</th>
<th>Multiple R</th>
<th>R Square</th>
<th>R Square Change</th>
<th>Simple R</th>
<th>Overall F</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E</td>
<td>103.3286</td>
<td>.000</td>
<td>.7067</td>
<td>.75784</td>
<td></td>
<td></td>
<td>81.14123</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>6.85203</td>
<td>.016</td>
<td>.87496</td>
<td>.76958</td>
<td>.00270</td>
<td>.92497</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix II

D. The increase, due to sprouting, in the rostro-caudal compared to the dorso-ventral axis of the high-threshold field of DCN-T13.
RUN NAME : DCMTL3 AXTS
DATA LIST : FIXED(I)1/5 X 1-2 Z 4 Y 5-9

THE DATA LIST PROVIDES FOR 3 VARIABLES AND 1 RECORDS PER CASE.

INPUT MEDIUM : CARD
M OF CASES : 7
VAR LABELS : 7.8DAYS
VALUE LABELS : 2.XAXIS 3.YAXIS
COMPUTE : 4.X4X4 REGRESSION : 1.INTRA
VAR LABELS : 1.INTERACTION
STATISTICS : 5.REG
PEAD INPUT DATA

OPTION = 1
IGNORE MISSING VALUE INDICATORS
(NO MISSING VALUES DEFINED...OPTION 1 WAS FORCED)

FILE .HOMAME (CREATION DATE = 82/02/15.)

**MULTIPLE REGRESSION**

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>MEAN</th>
<th>STANDARD DEV</th>
<th>CASES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>21.6757</td>
<td>12.3401</td>
<td>74</td>
</tr>
<tr>
<td>Z</td>
<td>2.4494</td>
<td>1.9174</td>
<td>74</td>
</tr>
<tr>
<td>T</td>
<td>19.4370</td>
<td>12.9174</td>
<td>74</td>
</tr>
</tbody>
</table>

CORRELATION COEFFICIENTS.
A VALUE OF 99.9999% IS PRINTED IF A COEFFICIENT CANNOT BE COMPUTED.

Y T
Z .4290 .8473
T .4414 .8370 .76100
Y .4414 .8370 .76100
Z .8370 .76100
**MULTIPLE REGRESSION**

**DEPENDENT VARIABLE:** \( Y \)  
**LENGTH OF AXIS**

**MEAN RESPONSE:** 2.49135  
**ST. DEV.:** 1.01745

**VARIABLES ENTERED ON STEP NUMBER 1.**  
**X**  
**Z**  
**INTERACTION**

**MULTIPLE R** 0.96108  
**ADJUSTED R SQUARE** 0.96069  
**STDEV:** 0.93604  
**COEFF. OF VARIABILITY:** 13.7 PCT

**ANALYSIS OF VARIANCE**
- **SUM OF SQUARES:** 67.85798  
- **MEAN SQUARE:** 22.95119  
- **F:** 199.00429  
- **SIGNIFICANCE:** .000

**VARIABLES IN THE EQUATION**

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>( b )</th>
<th>STD ERR ( b )</th>
<th>F</th>
<th>BETA</th>
<th>SIGNIFICANCE</th>
<th>ELASTICITY</th>
<th>TOLERANCE</th>
<th>SIGNIFICANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>( x )</td>
<td>.17458719E-01</td>
<td>.45149059E-02</td>
<td>68.655016</td>
<td>.4647028</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>( z )</td>
<td>1.799524</td>
<td>1.5899329</td>
<td>126.64589</td>
<td>.4584729</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>( t )</td>
<td>-4.0543214E-02</td>
<td>.63850458E-02</td>
<td>32.234929</td>
<td>0.0598544</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>(CONSTANT)</td>
<td>1.78805543</td>
<td>1.1242523</td>
<td>49.134111</td>
<td>-0.01771</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

**ALL VARIABLES ARE IN THE EQUATION.**

**VARIANCE/COVARIANCE MATRIX OF THE UNNORMALIZED REGRESSION COEFFICIENTS**

\[
\begin{pmatrix}
0.0002 & -0.0004 & -0.0028 \\
-0.0002 & 0.0004 & -0.0004 \\
-0.0008 & -0.0004 & 0.0004
\end{pmatrix}
\]

**X**  
**Z**  
**I**
### Summary Table

<table>
<thead>
<tr>
<th>Step</th>
<th>Entered</th>
<th>Removed</th>
<th>F to Enter</th>
<th>F to Remove</th>
<th>Significance</th>
<th>Multiple R</th>
<th>R Square</th>
<th>R Square Change</th>
<th>Simple R</th>
<th>Overall F</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Y</td>
<td></td>
<td>6.83502</td>
<td>.000</td>
<td>.42999</td>
<td>.18488</td>
<td>.18488</td>
<td>.47999</td>
<td>.000</td>
<td>197.04299</td>
<td>.000</td>
</tr>
</tbody>
</table>
Appendix II

E - The increase, due to sprouting, in the rostro-caudal compared to the dorso-ventral axis of the high-threshold field of mDCN-T13.
DATA LIST FIXED 1/1 X 1-2 Z 4 Y 6-9.

THE DATA LIST PROVIDES FOR 3 VARIABLES AND 3 RECORDS PER CASE.
A MAXIMUM OF 120 CHARACTERS ARE USED ON A RECORD.

INPUT MEDIUM
N OF CASES 80
N OF VARS 3
VALUES LABELS 1:0, 2:1, 3:R-C (110-V)
COMPUTE 1:5, 2:2
VAR LABELS 1:INTERACTION
REGRESSION VARIABLES X, Y, Z, / REPRESSION WITH X, Z, T

STATISTICS:
READ INPUT DATA

00500400 CM NEEDED FOR REGRESSION.

OPTION = 1
IGNORE MISSING VALUE INDICATORS
(NO MISSING VALUES DEFINED...OPTION 1 WAS FORCED)

---

MULTIPLE REGRESSION

VARIABLE MEAN STANDARD DEV CASES
X 27.4000 11.7250 80
Y 1.5000 2.9037 80
Z 11.7000 14.4015 80

CORRELATION COEFFICIENTS.
A VALUE OF .9999999 IS PRINTED IF A COEFFICIENT CANNOT BE COMPUTED.

Y .64057     .62842     .61754
Z .64057     .76126     .81754
X

2
MULTIPLE REGRESSION

DEPENDENT VARIABLE: Y LENGTH OF AXIS
MEAN RESPONSE 1.93437 STD. DEV. 58068
VARIABLE(S) ENTERED ON STEP NUMBER 1: X DAY AXIS INTERACTION

MULTIPLE R
R SQUARE .89788
ADJUSTED R SQUARE .78774
STD DEVIATION 26753
COEF OF VARIABILITY 13.9 PCT

--------------------------------- VARIABLES IN THE EQUATION -----------------------------

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>B</th>
<th>ST. ERROR B</th>
<th>F</th>
<th>SIGNIFICANCE</th>
<th>BETTA</th>
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<tbody>
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<td>X</td>
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<td>.3623281E-02</td>
<td>89.499613</td>
<td>.0576911</td>
<td></td>
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<tr>
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<td>.13147609</td>
<td>93.178642</td>
<td>.0349216</td>
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<td></td>
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<tr>
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<td>.34877398E-01</td>
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ALL VARIABLES ARE IN THE EQUATION.

COEFFICIENTS AND CONFIDENCE INTERVALS.

<table>
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<tr>
<th>VARIABLE</th>
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<th>T</th>
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</table>

MULTIPLE R GOODNESS OF FIT.
### Multiple Regression Table

<table>
<thead>
<tr>
<th>Step</th>
<th>Variable</th>
<th>Entered</th>
<th>Removed</th>
<th>F to Enter</th>
<th>Significance</th>
<th>Multiple R</th>
<th>R Square</th>
<th>Simple R Change</th>
<th>Overall F</th>
<th>Significance</th>
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</thead>
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<td>.81951</td>
<td>.70511</td>
<td>.81951</td>
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<td></td>
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</tbody>
</table>

Legend:
- **X**: Independent variable
- **F to Enter**: F value for entering the variable
- **Significance**: Significance level for entering the variable
- **Multiple R**: Multiple correlation coefficient
- **R Square**: Coefficient of determination
- **Simple R Change**: Change in simple correlation coefficient
- **Overall F**: Overall F statistic
- **Significance**: Significance level for the overall model
Appendix II

\[ F = \text{The increase in area of the high-threshold field due to sprouting of mDCN-T_{13} after electrical stimulation 6,000 times (5 min stimulation) compared to no stimulation.} \]
RUN NAME: TIME COURSE OF 5 MIN. AND NO STIM
DATA LIST FIXED (1/1 X 1-2 Z 4 Y 6-9)
The DATA LIST provides for 3 VARIABLES and 1 RECORDS PER CASE.
A maximum of 9 columns are used on a RECORD.

INPUT MEDIUM CARD
N OF CASES 3
VAR LABELS X: DAY/S
VALUE LABELS X: 1/0 IS 5 MIN. STIM. 11 NO STIM.
COMPUTE X*Z
VAR LABELS Z: INTERACTION
REGRESSION Y WITH X, Z, T
STATISTICS ALL
READ INPUT DATA

00000000 CM NEEDED FOR REGRESSION

FILE NONAME (CREATION DATE = 02/02/15)

************** MULTIPLE REGRESSION **************

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>MEAN</th>
<th>STANDARD DEV</th>
<th>CASES</th>
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<td>X</td>
<td>27.0000</td>
<td>10.7081</td>
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<tr>
<td>Z</td>
<td>502.788</td>
<td>199.659</td>
<td>34</td>
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<tr>
<td>T</td>
<td>16.0000</td>
<td>16.4108</td>
<td>34</td>
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</table>

CORRELATION COEFFICIENTS:
A value of 0.000000 is printed if a coefficient cannot be computed.

<table>
<thead>
<tr>
<th></th>
<th>Y</th>
<th>Z</th>
<th>T</th>
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</thead>
<tbody>
<tr>
<td>Y</td>
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<td>.70594</td>
<td>.98315</td>
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<td>Z</td>
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<td>T</td>
<td>.98315</td>
<td>.98315</td>
<td>.98315</td>
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TIME COURSE OF 5 MIN. AND NO STIM

FILE NONAME (CREATION DATE = 82/02/15.)

** DEPENDENT VARIABLE ** Y AREA OF FIELD

MSE 5021.47099 STD. DEV. 1995.65913

VARIABLE(S) ENTERED ON STEP NUMBER 1. X DAY
INTERACTION

MULTIPLE R 0.94688
ADJUSTED R 0.94624
R S Q U A R E 0.94688
ADJUSTED R S Q U A R E 0.94624
R S Q U A R E 0.94688
STDEV 673.84117
COEFL OF VARIABILITY 1.4 PCI

--- VARIABLES IN THE EQUATION ---

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<th>F</th>
<th>SIG</th>
<th>ELASTICITY</th>
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<tr>
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<td>-5336.6176</td>
<td>1675.9916</td>
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<tr>
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<td>36.258989</td>
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<td>29823.78</td>
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<tr>
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<td>89917.8989</td>
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--- VARIABLES NOT IN THE EQUATION ---

COEFFICIENTS AND CONFIDENCE INTERVALS.

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TIME COURSE OF 5 MIN. AND NO STIM

FILE NONAME (CREATION DATE = 82/02/15.)

** DEPENDENT VARIABLE ** Y AREA OF FIELD

VARIANCE/CovARIANCE MATRIX OF THE UNNORMALIZED REGRESSION COEFFICIENTS.

<table>
<thead>
<tr>
<th>x</th>
<th>z</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>2150.29181</td>
<td>2515.29181</td>
<td>1.29E+07</td>
</tr>
<tr>
<td>2.29E-05</td>
<td>2.88E-07</td>
<td>1.29E+07</td>
</tr>
<tr>
<td>1.23E+04</td>
<td>1.23E+04</td>
<td>1.29E+07</td>
</tr>
</tbody>
</table>
TIME COURSE OF 5 MTH AND NO STIM

FILE NONAME (CREATION DATE = 02/02/15.)

MULTIPLE REGRESSION

DEPENDENT VARIABLE: Y AREA OF FIELD

<table>
<thead>
<tr>
<th>STEP</th>
<th>VARIABLE ENTERED</th>
<th>VARIABLE REMOVED</th>
<th>F TO ENTER OR REMOVE</th>
<th>SIGNIFICANCE</th>
<th>MULTIPLE R</th>
<th>R'SQUARE</th>
<th>R SQUARE CHANGE</th>
<th>SIMPLE R</th>
<th>OVERALL F</th>
<th>SIGNIFICANCE</th>
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<tbody>
<tr>
<td>1</td>
<td>Y</td>
<td></td>
<td>52.10797</td>
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<td>.75682</td>
<td>.69395</td>
<td>85.71240</td>
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<td></td>
<td>T</td>
<td></td>
<td>16.29864</td>
<td>.003</td>
<td>.66995</td>
<td>.75682</td>
<td>.75682</td>
<td>.69395</td>
<td>70.3744</td>
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</table>
Appendix II

G - The increase in area of the high-threshold field due to sprouting of mDCN-T13 after electrical stimulation 12,000 times (10 min stimulation) compared to no stimulation.
ACADEMIC COMPUTING SERVICES
RAISE (Y) UNIVERSITY
S P S S -- STATISTICAL PACKAGE FOR THE SOCIAL SCIENCES
VERSION 8.0 -- FEBRUARY 16, 1981

RUN NAME: TIME COURSE OF 10 MIN. AND NO STIM.
THE DATA LIST PROVIDES FOR 3 VARIABLES AND 1 REPEATED PER CASE.
A MAXIMUM OF 9 COLUMNS ARE USED ON A RECORD.
INPUT MEDIUM: CARD
N OF CASES: 33
VAR LABELS: 2
VALUE LABELS: 7 AREA OF FIELD:
COMPUTE: 10010 MIN. STIM. (1) NO STIM.
VAR LABELS: 2 INTERACTION
REGRESSION: VARIABLES=X,Y,Z,I/
STATISTICS = ALL
READ INPUT DATA
005040 CM NEEDED FOR REGRESSION

OPTION = 1
IGNORE MISSING VALUE INDICATORS
NO MISSING VALUES DEFINED...OPTION 1 WAS FORCED

TIME COURSE OF 10 MIN. AND NO STIM.
FILE: MDNAM0 (CREATION DATE = 07/02/15.)

* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
MULTIPLE REGRESSION
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

VARIABLE  MEAN  STANDARD  DEV  CASES
X  22.1014  12.8290  33
Y  59.04393  17.192417  33
I  16.4840  16.3943  33

CORRELATION COEFFICIENTS.
A VALUE OF .99,.99999 IS PRINTED.
IF A COEFFICIENT CANNOT BE COMPUTED.

Y  .48740  .27514  .8887
Z  .49976  .35282  .9888
X  .37290  .58952  .7888
I  .37290  .58952  .7888
**TIME COURSE OF 10 MIN. AND NO STIM.**

FILE: **HNAME** (CREATION DATE = 02/02/15.)

**MULTIPLE REGRESSION**

DEPENDENT VARIABLE: \( y \)  
AREA OF FIELD

MEAN RESPONSE: 3949.39394  
STD. DEV.: 1750.40167

VARIABLES ENTERED ON STEP NUMBER 1:  
- DAY DIGITATION OF STIM.
- INTERACTION

<table>
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<th>VARIABLE</th>
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<th>STD. ERROR</th>
<th>A. SIG.</th>
<th>BETA</th>
<th>SIGNIFICANCE</th>
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<tbody>
<tr>
<td>X</td>
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<td>-208.33333</td>
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<td>1.2177789</td>
<td>0.96203705</td>
<td>0.0000000</td>
<td>0.96203705</td>
</tr>
<tr>
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<td>1249.2571</td>
<td>1875.1751</td>
<td>1.35039589</td>
<td>0.255</td>
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ALL VARIABLES ARE IN THE EQUATION.

**COEFFICIENTS AND CONFIDENCE INTERVALS.**

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<th>VARIABLE</th>
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<th>STD. ERROR</th>
<th>A. SIG.</th>
<th>95.0% CONFIDENCE INTERVAL</th>
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<tr>
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<td>16.557644</td>
<td>170.26683 4</td>
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**TIME COURSE OF 10 MIN. AND NO STIM.**

FILE: **HNAME** (CREATION DATE = 02/02/15.)

**MULTIPLE REGRESSION**

DEPENDENT VARIABLE: \( y \)  
AREA OF FIELD

VARIANCE/COVARIANCE MATRIX OF THE UNNORMALIZED REGRESSION COEFFICIENTS:

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<tr>
<td>( t )</td>
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</table>

\( x \) \( z \) \( t \)
### Multiple Regression Summary Table

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<tr>
<th>Step</th>
<th>Entered Variable</th>
<th>Removed Variable</th>
<th>P to Enter</th>
<th>P to Remove</th>
<th>Significance</th>
<th>Multiple R</th>
<th>R Square</th>
<th>R Square Change</th>
<th>Simple R</th>
<th>Overall F</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
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<td>Z</td>
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<td>0.001</td>
<td>0.000</td>
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<td>0.23756</td>
<td>0.4740</td>
<td>14.39508</td>
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Appendix III

Raw Data
Appendix III (a).

The area of remaining insensitivity in animals mapped only once on the specified times after their initial surgery, which produced an mDCN-T₁₃ Island.

<table>
<thead>
<tr>
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<th>B</th>
<th>C</th>
<th>D</th>
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</thead>
<tbody>
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<td>27A</td>
<td>250</td>
<td>1066</td>
<td>141</td>
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</tr>
<tr>
<td>85A</td>
<td>233</td>
<td>773</td>
<td>140</td>
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<tr>
<td>86A</td>
<td>238</td>
<td>969</td>
<td>105</td>
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<td>102A</td>
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<tr>
<td>103A</td>
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<td></td>
<td></td>
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</tbody>
</table>

\[ C - \overline{x} = 968.4 \pm 72.4 \text{ S.E.} \]

\[ D - \overline{x} = 129 \pm 6.6 \text{ S.E.} \]

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
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<td>1325</td>
<td>142</td>
<td></td>
</tr>
<tr>
<td>2A</td>
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<td>970</td>
<td>130</td>
<td></td>
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<td>3A</td>
<td>219</td>
<td>875</td>
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<tr>
<td>4A</td>
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<td>780</td>
<td>128</td>
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</tr>
</tbody>
</table>

\[ C - \overline{x} = 987.5 \pm 188.9 \text{ S.E.} \]

\[ D - \overline{x} = 132.7 \pm 3.1 \text{ S.E.} \]

A - Identification 
B - Weight of Animal on the day of Operation in Grams 
C - Area of Insensitivity Remaining in mm² 
D - Area of the low-threshold field of mDCN-T₁₃ in mm²
### Animals Mapped Sixteen Days After Denervation

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
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<tr>
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<td>144</td>
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<td>210</td>
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<td>220</td>
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<tr>
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<td>145</td>
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<td>6B</td>
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<td>653</td>
<td>155</td>
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<tr>
<td>7B</td>
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<tr>
<td>8B</td>
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<td>999</td>
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<tr>
<td>9B</td>
<td>203</td>
<td>690</td>
<td>187</td>
</tr>
<tr>
<td>11B</td>
<td>210</td>
<td>630</td>
<td>178</td>
</tr>
</tbody>
</table>

C – \( \bar{x} = 389.4 \pm 79.8 \text{ S.E.} \)

D – \( \bar{x} = 182 \pm 10 \text{ S.E.} \)

### Animals Mapped Twenty Four Days After Denervation

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<th>C</th>
<th>D</th>
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<td>222</td>
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<td>388A</td>
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<td>871</td>
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<td>392A</td>
<td>178</td>
<td>664</td>
<td>220</td>
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<td>393A</td>
<td>174</td>
<td>545</td>
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<tr>
<td>394A</td>
<td>180</td>
<td>714</td>
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<td>395A</td>
<td>178</td>
<td>586</td>
<td>185</td>
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</table>

C – \( \bar{x} = 626.5 \pm 59.6 \text{ S.E.} \)

D – \( \bar{x} = 202.1 \pm 7.0 \text{ S.E.} \)

A – Identification 
B – Weight of Animal on the Day of Operation in Grams
C – Area of Insensitivity Remaining in mm²
D – Area of the low-threshold Field of mDON-T₁₃ in mm²
Appendix III (b)

The Area of Remaining Insensitivity Sixteen Days After Denervation In Animals Mapped on Days 4, 8, 12 and 16 After Their Initial Surgery

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
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<tr>
<td>L-249</td>
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</tr>
<tr>
<td>L-250</td>
<td>205</td>
<td>295</td>
</tr>
<tr>
<td>L-252</td>
<td>203</td>
<td>13</td>
</tr>
<tr>
<td>L-253</td>
<td>219</td>
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</tbody>
</table>

$\bar{X} = 95.7 \pm 67.3$ S.E.

A = Identification 
B = Weight of animal on the day of operation in grams
C = Area of insensitivity remaining in mm$^2$
Appendix III (c)

The area of the high and low-threshold receptive fields of mDCN-T\textsubscript{13} mapped only once on the specified number of days after denervation which produced an mDCN-T\textsubscript{13} island.

<table>
<thead>
<tr>
<th>Animals Mapped Zero Days After Denervation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
</tr>
<tr>
<td>-----</td>
</tr>
<tr>
<td>119</td>
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<td>117</td>
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<td>115</td>
</tr>
<tr>
<td>181</td>
</tr>
<tr>
<td>180</td>
</tr>
</tbody>
</table>

C - \( \bar{x} = 239 \pm 23 \) S.E.

D - \( \bar{x} = 146.8 \pm 9.8 \) S.E.

E - \( \bar{x} = 1.3 \pm 0.12 \)

F - \( \bar{x} = 0.9 \pm 0.06 \) S.E.

A - Identification
B - Weight of Animal on the Day of Operation in grams
C - Area of the high-threshold field of mDCN-T\textsubscript{13} in \( \text{mm}^2 \)
D - Area of the low-threshold field of mDCN-T\textsubscript{13} in \( \text{mm}^2 \)
E - Maximum Length of the rostro-caudal Axis of the high-threshold field
F - One half the maximum rostro-caudal Axis
Animals Mapped Eight Days After Denervation

<table>
<thead>
<tr>
<th>A</th>
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<th>D</th>
<th>E</th>
<th>F</th>
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<tbody>
<tr>
<td>4</td>
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<tr>
<td>3</td>
<td>219</td>
<td>264</td>
<td>131</td>
<td>1.7</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

C - $\bar{x} = 251 \pm 25$ S.E.

D - $\bar{x} = 132.7 \pm 3.1$ S.E.

E - $\bar{x} = 1.5 \pm 0.1$ S.E.

F - $\bar{x} = 0.75 \pm 0.05$ S.E.

A - Identification 
B - Weight of Animal on the Day of Operation in grams
C - Area of the high-threshold field of mDCN-T, in mm$^2$
D - Area of the low-threshold field of mDCN-T, in mm$^2$
E - Maximum Length of the rostro-caudal Axis of the high threshold field
F - One half the maximum rostro-caudal Axis
### Animals Mapped Sixteen Days After Denervation

<table>
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<td>187</td>
<td>207</td>
<td>116</td>
<td>1.5</td>
<td></td>
</tr>
</tbody>
</table>

\[
C - \bar{x} = 260.5 \pm 19.1 \text{ S.E.}
\]

\[
D - \bar{x} = 130 \pm 6.2 \text{ S.E.}
\]

\[
E - \bar{x} = 1.8 \pm 0.07 \text{ S.E.}
\]

\[
F - \bar{x} = 0.9 \pm 0.03 \text{ S.E.}
\]

- **A**: Identification
- **B**: Weight of Animal on the day of operation in grams
- **C**: Area of the high-threshold field of mDCI-T13 in mm²
- **D**: Area of the low-threshold field of mDCI-T13 in mm²
- **E**: Maximum Length of the rostro-caudal Axis of the high threshold field
- **F**: One half the Maximum rostro-caudal Axis
### Animals Mapped Twenty Four Days After Denervation

<table>
<thead>
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<td>139</td>
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<td>346</td>
<td>131</td>
<td>2.5</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

\[ C - \bar{x} = 424.5 \pm 37 \text{ S.E.} \]

\[ D - \bar{x} = 136 \pm 4.1 \text{ S.E.} \]

\[ E - \bar{x} = 2.5 \pm 0.06 \text{ S.E.} \]

\[ F - \bar{x} = 1.25 \pm 0.03 \text{ S.E.} \]

- **A**: Identification \#  
- **B**: Weight of Animal on the day of operation in grams  
- **C**: Area of the high-threshold field of mDCN-T₁ in mm²  
- **D**: Area of the low-threshold field of mDCN-T₁ in mm²  
- **E**: Maximum Length of the rostro-caudal Axis of the high threshold field  
- **F**: One half the Maximum rostro-caudal Axis
### Animals Mapped Twenty Eight Days After Denervation

<table>
<thead>
<tr>
<th>A</th>
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<th>E</th>
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<td>168</td>
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<td>152</td>
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<td>8</td>
<td>180</td>
<td>451</td>
<td>165</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>9</td>
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<td>339</td>
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<tr>
<td>11</td>
<td>200</td>
<td>502</td>
<td>128</td>
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</tr>
</tbody>
</table>

C - $\bar{x} = 485 \pm 42$ S.E.

D - $\bar{x} = 150 \pm 8$ S.E.

E - $\bar{x} = 2.3 \pm 0.12$ S.E.

F - $\bar{x} = 1.4 \pm 0.06$

**Legend**

A - Identification
B - Weight of animal on the day of operation in grams
C - Area of the high-threshold field of mDCN-T, in mm$^2$
D - Area of the low-threshold field of mDCN-T, in mm$^2$
E - Maximum length of the dorso-ventral axis of the high-threshold field
F - Maximum length of the rostro-caudal axis of the high-threshold field
G - One half the maximum rostro-caudal axis
### Animals Mapped Sixteen Days After Denervation

<table>
<thead>
<tr>
<th>A</th>
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<th>C</th>
<th>D</th>
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<th>G</th>
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<td>21⑦</td>
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<td>2.7</td>
<td>1.35</td>
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<tr>
<td>*HT35</td>
<td>200</td>
<td>461</td>
<td>207</td>
<td>2.2</td>
<td>2.3</td>
<td>1.25</td>
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<td>540</td>
<td>263</td>
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<td>1.4</td>
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<td>373</td>
<td>195⑧</td>
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<td>1.1</td>
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<td>132</td>
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<td>155</td>
<td>1.5</td>
<td>2.0</td>
<td>1.0</td>
</tr>
<tr>
<td>125</td>
<td>182</td>
<td>348</td>
<td>226</td>
<td>2.2</td>
<td>1.8</td>
<td>0.95</td>
</tr>
</tbody>
</table>

C - $\bar{x} = 403 \pm 42$ S.E.

D - $\bar{x} = 212 \pm 13$ S.E.

E - $\bar{x} = 2.0 \pm 0.11$ S.E.

F - $\bar{x} = 2.3 \pm 0.15$ S.E.

G - $\bar{x} = 1.15 \pm 0.07$ S.E.

A - Identification

B - Weight of Animal on the day of operation in grams

C - Area of the high-threshold field of mDCN-T13 in mm$^2$

D - Area of the low-threshold field of mDCN-T13 in mm$^2$

E - Maximum length of the dorso-ventral axis of the high-threshold field

F - Maximum length of the rostro-caudal axis of the high-threshold field

G - One half the maximum rostro-caudal axis
### Animals Mapped Twenty Days After Denervation

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
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<th>G</th>
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<td>546</td>
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<td>3.1</td>
<td>1.55</td>
</tr>
<tr>
<td>128</td>
<td>188</td>
<td>624</td>
<td>193</td>
<td>2.2</td>
<td>3.7</td>
<td>1.75</td>
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<tr>
<td>HT3</td>
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<td>482</td>
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<td>1.35</td>
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<td>124</td>
<td>199</td>
<td>356</td>
<td>127</td>
<td>2.1</td>
<td>2.1</td>
<td>1.05</td>
</tr>
</tbody>
</table>

C - \[ \bar{x} = 516.3 \pm 48.7 \text{ S.E.} \]

D - \[ \bar{x} = 151.1 \pm 11 \text{ S.E.} \]

E - \[ \bar{x} = 2.26 \pm 0.07 \text{ S.E.} \]

F - \[ \bar{x} = 2.8 \pm 0.25 \text{ S.E.} \]

G - \[ \bar{x} = 1.4 \pm 0.12 \text{ S.E.} \]

A - Identification
B - Weight of Animal on the day of operation in grams
C - Area of the high-threshold field of mDCN-T13, in \( \text{mm}^2 \)
D - Area of the low-threshold field of mDCN-T13, in \( \text{mm}^2 \)
E - Maximum length of the dorso-ventral axis of the high-threshold field
F - Maximum length of the rostro-caudal axis of the high-threshold field
G - One half the maximum rostro-caudal axis
### Animals Mapped Twenty Five Days After Denervation

<table>
<thead>
<tr>
<th>A</th>
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<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
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<tbody>
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<td>HT26</td>
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<td>2.8</td>
<td>3.4</td>
<td>1.7</td>
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<tr>
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</table>

**C** = \( \bar{x} = 688.5 \pm 50 \text{ S.E.} \)

**D** = \( \bar{x} = 194.8 \pm 7 \text{ S.E.} \)

**E** = \( \bar{x} = 2.33 \pm 0.15 \text{ S.E.} \)

**F** = \( \bar{x} = 3.37 \pm 0.1 \text{ S.E.} \)

**G** = \( \bar{x} = 1.68 \pm 0.05 \text{ S.E.} \)

A - Identification
B - Weight of Animal on the day of operation in grams
C - Area of the high-threshold field of mDCN-T,3 in mm$^2$
D - Area of the low-threshold field of mDCN-T,3 in mm$^2$
E - Maximum length of the dorso-ventral axis of the high-threshold field
F - Maximum length of the rostro-caudal axis of the high-threshold field
G - One half the maximum rostro-caudal axis
Animals Mapped Thirty Days After Denervation

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
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<td>192</td>
<td>808</td>
<td>220</td>
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<td>3.9</td>
<td>1.95</td>
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<td>217</td>
<td>2.6</td>
<td>3.8</td>
<td>1.9</td>
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</tbody>
</table>

C - $\bar{x} = 835.6 \pm 61$ S.E.

D - $\bar{x} = 205.6 \pm 5$ S.E.

E - $\bar{x} = 2.47 \pm 0.12$ S.E.

F - $\bar{x} = 3.42 \pm 0.04$ S.E.

G - $\bar{x} = 1.71 \pm 0.02$ S.E.

A - Identification
B - Weight of Animal on the day of operation in grams
C - Area of the high-threshold field of mDCN-T13 in mm²
D - Area of the low-threshold field of mDCN-T13 in mm
E - Maximum length of the dorso-ventral axis of the high-threshold field
F - Maximum length of the rostro-caudal axis of the high-threshold field
G - One half the maximum rostro-caudal axis
### Animals Mapped Forty Days After Denervation

<table>
<thead>
<tr>
<th>Identification</th>
<th>Weight of Animal on the day of operation in grams</th>
<th>Area of the high-threshold field of MDCH-T₁₃ in mm²</th>
<th>Area of the low-threshold field of MDCH-T₁₃ in mm²</th>
<th>Maximum length of the dorso-ventral axis of the high-threshold field</th>
<th>Maximum length of the rostro-caudal axis of the high-threshold field</th>
<th>One half the maximum rostro-caudal axis</th>
</tr>
</thead>
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<td>3.9</td>
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C - $\bar{x} = 1017 \pm 88$ S.E.

D - $\bar{x} = 196.1 \pm 15$ S.E.

E - $\bar{x} = 2.81 \pm 0.13$ S.E.

F - $\bar{x} = 3.95 \pm 0.24$ S.E.

G - $\bar{x} = 1.97 \pm 0.11$ S.E.

A - Identification
B - Weight of Animal on the day of operation in grams
C - Area of the high-threshold field of MDCH-T₁₃ in mm²
D - Area of the low-threshold field of MDCH-T₁₃ in mm²
E - Maximum length of the dorso-ventral axis of the high-threshold field
F - Maximum length of the rostro-caudal axis of the high-threshold field
G - One half the maximum rostro-caudal axis
Appendix III (f)

The area of the high and low-threshold fields of DCN-T_{13} mapped only once on the specified number of days after denervation which produced an intact DCN-T_{13} receptive field on the flank; the rest of the flank was totally denervated.

**Animals Mapped Zero Days After Denervation**

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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C = $\bar{x} = 340.3 \pm 24.5$ S.E.

D = $\bar{x} = 283.3 \pm 15$ S.E.

E = $\bar{x} = 2.46 \pm 0.15$ S.E.

F = $\bar{x} = 1.61 \pm 0.08$ S.E.

G = $\bar{x} = 0.80 \pm 0.04$ S.E.

A = Identification

B = Weight of Animal on the day of operation in grams

C = Area of the high-threshold field of DCN-T_{13} in $\text{mm}^2$

D = Area of the low-threshold field of DCN-T_{13} in $\text{mm}^2$

E = Maximum length of the dorso-ventral axis of the high-threshold field

F = Maximum length of the rostro-caudal axis of the high-threshold field

G = One half the maximum rostro-caudal axis
### Animals Mapped Sixteen Days After Denervation

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C - $\bar{x} = 570.4 \pm 78.3$ S.E.

D - $\bar{x} = 379.6 \pm 24$ S.E.

E - $\bar{x} = 2.95 \pm 0.16$ S.E.

F - $\bar{x} = 2.47 \pm 0.14$ S.E.

G - $\bar{x} = 1.23 \pm 0.07$ S.E.

**A** - Identification

**B** - Weight of Animal on the day of operation in grams

**C** - Area of the high-threshold field of DCN-T13 in mm²

**D** - Area of the low-threshold field of DCN-T13 in mm²

**E** - Maximum length of the dorso-ventral axis of the high-threshold field

**F** - Maximum length of the rostro-caudal axis of the high-threshold field

**G** - One half the maximum rostro-caudal axis
### Animals Mapped Twenty Days After Denervation

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C - $\bar{x} = 943 \pm 35$ S.E.

D - $\bar{x} = 340 \pm 19$ S.E.

E - $\bar{x} = 3.4 \pm 0.12$ S.E.

F - $\bar{x} = 3.2 \pm 0.2$ S.E.

G - $\bar{x} = 1.6 \pm 0.1$ S.E.

A - Identification
B - Weight of Animal on the day of operation in grams
C - Area of the high-threshold field of DCM-T₁₀ in $\mu^2$
D - Area of the low-threshold field of DON-T₁₀ in $\mu^2$
E - Maximum length of the dorso-ventral axis of the high-threshold field
F - Maximum length of the rostro-caudal axis of the high-threshold field
G - One half the maximum rostro-caudal axis
Animals Mapped Twenty Five Days After Denervation

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C - $\bar{x} = 1127 \pm 74$ S.E.

D - $\bar{x} = 415 \pm 18$ S.E.

E - $\bar{x} = 3.6 \pm 0.08$ S.E.

F - $\bar{x} = 3.5 \pm 0.25$ S.E.

G - $\bar{x} = 1.75 \pm 0.12$ S.E.

A - Identification
B - Weight of Animal on the day of operation in grams
C - Area of the high-threshold field of DCN-T12 in mm²
D - Area of the low-threshold field of DCN-T12 in mm²
E - Maximum length of the dorso-ventral axis of the high-threshold field
F - Maximum length of the rostro-caudal axis of the high-threshold field
G - One half the maximum rostro-caudal axis
## Animals Mapped Thirty Days After Denervation

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C - \( \bar{x} = 1236 \pm 118 \) S.E.

D - \( \bar{x} = 379 \pm 40 \) S.E.

E - \( \bar{x} = 3.7 \pm 0.12 \) S.E.

F - \( \bar{x} = 4.0 \pm 0.29 \) S.E.

G - \( \bar{x} = 2.0 \pm 0.14 \) S.E.

**A** - Identification

**B** - Weight of Animal on the day of operation in grams

**C** - Area of the high-threshold field of DCN-T13 in \( \text{mm}^2 \)

**D** - Area of the low-threshold field of DCN-T13 in \( \text{mm}^2 \)

**E** - Maximum length of the dorso-ventral axis of the high-threshold field

**F** - Maximum length of the rostro-caudal axis of the high-threshold field

**G** - One half the maximum rostro-caudal axis
### Animals Kept Forty Days After Denervation

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C - $\bar{x} = 1370 \pm 92.4$ S.E.

D - $\bar{x} = 391.5 \pm 28.6$ S.E.

E - $\bar{x} = 3.65 \pm 0.19$ S.E.

F - $\bar{x} = 4.4 \pm 0.16$ S.E.

G - $\bar{x} = 2.2 \pm 0.08$ S.E.

A - Identification #
B - Weight of Animal on the day of operation in grams
C - Area of the high-threshold field of DGN-T.12 in $\text{mm}^2$
D - Area of the low-threshold field of DGN-T.13 in $\text{mm}^2$
E - Maximum length of the dorso-ventral axis of the high-threshold field
F - Maximum length of the rostro-caudal axis of the high-threshold field
G - One half the maximum rostro-caudal axis
Appendix III (g)

The area of the high and low-threshold receptive fields of DCN-T_{13} mapped only forty days after the animals would have normally been denervated but in these animals all the cutaneous nerves were left intact and therefore they are the control animals for series III(f).

**Animals Mapped Forty Days After They Would Have Normally Been Denervated**

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C - $\overline{x} = 380 \pm 11.7$ S.E.

D - $\overline{x} = 279 \pm 13$ S.E.

E - $\overline{x} = 2.56 \pm 0.05$ S.E.

F - $\overline{x} = 1.58 \pm 0.06$ S.E.

G - $\overline{x} = 0.79 \pm 0.01$ S.E.

A - Identification #

B - Weight of Animal on the day of operation in grams

C - Area of the high-threshold field of DCN-T_{13} in mm^2

D - Area of the low-threshold field of DCN-T_{13} in mm^2

E - Maximum length of the dorso-ventral axis of the high-threshold field

F - Maximum length of the rostro-caudal axis of the high-threshold field

G - One half the maximum rostro-caudal axis
Appendix III (h)

The area of the high and low-threshold receptive fields of mDCN-T13 mapped only once on the specified number of days after denervation and five minutes electrical stimulation (6,000 impulses) of the intact mDCN-T13 island.

<table>
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<td>180</td>
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C = \bar{x} = 225 \pm 11.7 \text{ S.E.}
D = \bar{x} = 123 \pm 3.3 \text{ S.E.}
E = \bar{x} = 1.78 \pm 0.04 \text{ S.E.}
F = \bar{x} = 0.89 \pm 0.02 \text{ S.E.}

A - Identification #
B - Weight of Animal on the day of operation in grams
C - Area of the high-threshold field of mDCN-T13 in mm²
D - Area of the low-threshold field of mDCN-T13 in mm²
E - Maximum length of the rostro-caudal axis of the high-threshold field
F - One half the maximum rostro-caudal axis
### Animals Mapped Twelve Days After Denervation

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C - $\bar{x} = 354.8 \pm 30.7$ S.E.

D - $\bar{x} = 131.8 \pm 7.4$ S.E.

E - $\bar{x} = 2.1 \pm 0.20$ S.E.

F - $\bar{x} = 1.05 \pm 0.1$ S.E.

A - Identification number
B - Weight of animal on the day of operation in grams
C - Area of the high-threshold field of mDCN-T13 in $mm^2$
D - Area of the low-threshold field of mDCN-T13 in $mm^2$
E - Maximum length of the rostro-caudal axis of the high-threshold field
F - One half the maximum rostro-caudal axis
### Animals Mapped Sixteen Days After Denervation

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<td>193</td>
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C - \( \bar{x} = 507 \pm 20 \) S.E.

D - \( \bar{x} = 153 \pm 9.5 \) S.E.

E - \( \bar{x} = 2.6 \pm 0.06 \) S.E.

F - \( \bar{x} = 1.3 \pm 0.03 \) S.E.

A - Identification 
B - Weight of Animal on the day of operation in grams
C - Area of the high-threshold field of mDCN-T₁ in mm²
D - Area of the low-threshold field of mDCN-T₁ in mm²
E - Maximum length of the rostro-caudal axis of the high-threshold field
F - One half the maximum rostro-caudal axis
Appendix III (i)

The area of the high and low-threshold receptive fields of mDCN-T₁₃ mapped only once on the specified number of days after denervation and ten minutes electrical stimulation (12,000 impulses) of the intact mDCN-T₁₃ island.

**Animals Mapped Four Days After Denervation**

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<td>180</td>
<td>119</td>
<td>1.6</td>
<td>&quot;</td>
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</table>

C - $\bar{x} = 222 \pm 22$ S.E.

D - $\bar{x} = 145 \pm 14$ S.E.

E - $\bar{x} = 1.7 \pm 0.08$ S.E.

F - $\bar{x} = 0.85 \pm 0.04$ S.E.

A - Identification
B - Weight of Animal on the day of operation in grams
C - Area of the high-threshold field of mDCN-T₁₃ in mm²
D - Area of the low-threshold field of mDCN-T₁₃ in mm²
E - Maximum length of the rostro-caudal axis of the high-threshold field
F - One half the maximum rostro-caudal axis
### Animals Mapped Eight Days After Denervation

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<td>228</td>
<td>139</td>
<td>1.6</td>
<td></td>
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</table>

- **C**: \( \bar{x} = 410 \pm 79 \text{ S.E.} \)
- **D**: \( \bar{x} = 161 \pm 22 \text{ S.E.} \)
- **E**: \( \bar{x} = 2.4 \pm 0.33 \text{ S.E.} \)
- **F**: \( \bar{x} = 1.2 \pm 0.16 \text{ S.E.} \)

**A**: Identification 
**B**: Weight of Animal on the day of operation in grams
**C**: Area of the high-threshold field of mDCN-T13 in \( \text{mm}^2 \)
**D**: Area of the low-threshold field of mDCN-T13 in \( \text{mm}^2 \)
**E**: Maximum length of the rostro-caudal axis of the high-threshold field
**F**: One half the maximum rostro-caudal axis
### Animals Mapped Twelve Days After Denervation

<table>
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<td>735</td>
<td>153</td>
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</table>

\[ C - \bar{x} = 557 \pm 51 \text{ S.E.} \]

\[ D - \bar{x} = 146 \pm 3 \text{ S.E.} \]

\[ E - \bar{x} = 2.9 \pm 0.27 \text{ S.E.} \]

\[ F - \bar{x} = 1.45 \pm 0.13 \text{ S.E.} \]

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<td>Weight of Animal on the day of operation in grams</td>
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<tr>
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<td>Area of the high-threshold field of mDCN-T13 in ( \text{mm}^2 )</td>
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<tr>
<td>D</td>
<td>Area of the low-threshold field of mDCN-T13 in ( \text{mm}^2 )</td>
</tr>
<tr>
<td>E</td>
<td>Maximum length of the rostro-caudal axis of the high-threshold field</td>
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<td>F</td>
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### Animals Mapped Sixteen Days After Denervation

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<td>3.4</td>
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<td>843</td>
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<td>4.2</td>
<td>&quot;</td>
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<td>222</td>
<td>186</td>
<td>781</td>
<td>543</td>
<td>3.9</td>
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<tr>
<td>223</td>
<td>186</td>
<td>543</td>
<td>127</td>
<td>3.2</td>
<td>&quot;</td>
<td></td>
</tr>
</tbody>
</table>

\[
\bar{C} = \frac{x}{n} = 597 \pm 55 \text{ S.E.}
\]

\[
\bar{D} = \frac{x}{n} = 5 \pm 13 \text{ S.E.}
\]

\[
\bar{E} = \frac{x}{n} = 3.7 \pm 0.18 \text{ S.E.}
\]

\[
\bar{F} = \frac{x}{n} = 1.9 \pm 0.09 \text{ S.E.}
\]

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<td>Weight of Animal on the day of operation in grams</td>
</tr>
<tr>
<td>C</td>
<td>Area of the high-threshold field of mDCN-T in mm²</td>
</tr>
<tr>
<td>D</td>
<td>Area of the low-threshold field of mDCN-T₁₂ in mm²</td>
</tr>
<tr>
<td>E</td>
<td>Maximum length of the rostro-caudal axis of the high-threshold field</td>
</tr>
<tr>
<td>F</td>
<td>One half the maximum rostro-caudal axis</td>
</tr>
</tbody>
</table>

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\[\text{S.E.}\] = Standard Error of the Mean
Appendix III (j)

The area of the high and low-threshold receptive fields of mDCN-T, mapped only once four days after denervation and the specified number of minutes of electrical stimulation of the intact mDCN-T, island.

### Animals Mapped Four Days After Forty Minutes of Electrical Stimulation (49,000 impulses)

<table>
<thead>
<tr>
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<td>253</td>
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<td>2</td>
<td>224</td>
<td>189</td>
<td>253</td>
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<tr>
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<tr>
<td>4</td>
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<td>185</td>
<td>252</td>
<td>136</td>
<td>1.7</td>
<td>&quot;</td>
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</table>

C - \( \bar{x} = 244.5 \pm 8.1\) S.E.

D - \( \bar{x} = 135.2 \pm 3.2\) S.E.

E - \( \bar{x} = 1.7 \pm 0.07\) S.E.

F - \( \bar{x} = 0.35 \pm 0.03\) S.E.

A - Identification of
B - Weight of Animal on the day of operation in grams
C - Area of the high-threshold field of mDCN-T, in \(\text{mm}^2\)
D - Area of the low-threshold field of mDCN-T, in \(\text{mm}^2\)
E - Maximum length of the rostro-caudal axis of the high-threshold field
F - One half the maximum rostro-caudal axis
Animals Mapped Four Days After Eighty Minutes of Electrical Stimulation (26,000 impulses)

<table>
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<th>D</th>
<th>E</th>
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</thead>
<tbody>
<tr>
<td>267</td>
<td>193</td>
<td>223</td>
<td>133</td>
<td>1.5</td>
<td>1/2 RC</td>
<td></td>
</tr>
<tr>
<td>268</td>
<td>188</td>
<td>201</td>
<td>129</td>
<td>1.3</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>269</td>
<td>194</td>
<td>222</td>
<td>130</td>
<td>1.6</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>270</td>
<td>203</td>
<td>273</td>
<td>181</td>
<td>1.7</td>
<td>&quot;</td>
<td></td>
</tr>
</tbody>
</table>

C = \bar{x} = 232 \pm 15.1 \text{ S.E.}

D = \bar{x} = 144 \pm 12 \text{ S.E.}

E = \bar{x} = 1.5 \pm 0.3 \text{ S.E.}

F = \bar{x} = 0.7 \pm 0.4 \text{ S.E.}

A = \text{Identification of animal}

B = \text{Weight of animal on the day of operation in grams}

C = \text{Area of the high-threshold field of mDCN-T, in mm}^2

D = \text{Area of the low-threshold field of mDCN-T, in mm}^2

E = \text{Maximum length of the rostro-caudal axis of the high-threshold field}

F = \text{One half the maximum rostro-caudal axis}
Appendix III (k)

The area of the high and low-threshold receptive fields of mDCN-T\textsubscript{13} mapped only once sixteen days after denervation which produce a mDCN-T\textsubscript{13} island. In these animals TTX and/or vehicle was used in the specified way in order to block impulses in the intact nerve.

Animals in which TTX was placed on the nerve to block impulses then the receptive field of mDCN-T\textsubscript{13} was pinched one hundred and thirty-five times

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
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<td>187</td>
<td>336</td>
<td>129</td>
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<td>195</td>
<td>218</td>
<td>124</td>
<td>2.0</td>
</tr>
<tr>
<td>254</td>
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</tr>
<tr>
<td>255</td>
<td>167</td>
<td>258</td>
<td>137</td>
<td>2.0</td>
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<td>257</td>
<td>183</td>
<td>344</td>
<td>130</td>
<td>2.2</td>
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<td>260</td>
<td>186</td>
<td>315</td>
<td>153</td>
<td>1.9</td>
</tr>
</tbody>
</table>

C - \( \bar{x} = 311 \pm 25 \) S.E.

D - \( \bar{x} = 142 \pm 9 \) S.E.

E - \( \bar{x} = 2.1 \pm 0.75 \) S.E.

A - Identification of
B - Weight of Animal on the day of operation in grams
C - Area of the high-threshold field of mDCN-T\textsubscript{13} in mm\textsuperscript{2}
D - Area of the low-threshold field of mDCN-T\textsubscript{13} in mm\textsuperscript{2}
E - Maximum length of the rostro-caudal axis of the high-threshold field
Animals in which vehicle was placed on the nerve than the receptive field of mDCN-T was pinched one hundred and thirty-five times.

<table>
<thead>
<tr>
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<td>236</td>
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<td>590</td>
<td>160</td>
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<td>183</td>
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<td>164</td>
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<td>259</td>
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<td>189</td>
<td>622</td>
<td>173</td>
<td>3.3</td>
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</table>

C - $\bar{x} = 619 \pm 25$ S.E.

D - $\bar{x} = 164 \pm 3$ S.E.

E - $\bar{x} = 3.3 \pm 0.1$ S.E.

A - Identification
B - Weight of Animal on the day of operation in grams
C - Area of the high-threshold field of mDCN-T in mm²
D - Area of the low-threshold field of mDCN-T in mm²
E - Maximum length of the rostro-caudal axis of the high-threshold field
Animals in which TTX was placed on the intact nerve to block impulses travelling centrally from the point of electrical stimulation (12,000x) of the intact nerves.

<table>
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<tr>
<th>A</th>
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<th>D</th>
<th>E</th>
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<td>200</td>
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<td>184</td>
<td>347</td>
<td>107</td>
<td>2.0</td>
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<td>181</td>
<td>320</td>
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<td>265</td>
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<td>296</td>
<td>167</td>
<td>2.1</td>
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</table>

C - $\bar{x} = 334 \pm 10$ S.E.

D - $\bar{x} = 150 \pm 14$ S.E.

E - $\bar{x} = 2.0 \pm 0.05$ S.E.

A - Identification
B - Weight of Animal on the day of operation in grams
C - Area of the high-threshold field of mDCN-T in mm$^2$
D - Area of the low-threshold field of mDCN-T in mm$^2$
E - Maximum length of the rostro-caudal axis of the high-threshold field.
Animals in which TTX was placed on the intact nerve to block impulses travelling peripherally from the point of electrical stimulation (12,000x) of the intact nerve.

<table>
<thead>
<tr>
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<th>E</th>
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<td>186</td>
<td>747</td>
<td>167</td>
<td>4.0</td>
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C - $\bar{x} = 777 \pm 32$ S.E.

D - $\bar{x} = 165 \pm 5$ S.E.

E - $\bar{x} = 3.9 \pm 0.16$ S.E.

A - Identification $	heta$
B - Weight of Animal on the day of operation in grams
C - Area of the high-threshold field of mDCN-13 in mm$^2$
D - Area of the low-threshold field of mDCN-13 in mm
E - Maximum length of the rostro-caudal axis of the high-threshold field
Animals in which IX was placed on the intact nerve but the nerve was not stimulated

<table>
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<th>C</th>
<th>D</th>
<th>E</th>
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<td>190</td>
<td>295</td>
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<td>174</td>
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</tr>
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</table>

\[ \bar{x} = 331 \pm 28.7 \text{ S.E.} \]

\[ \bar{x} = 155 \pm 13.9 \text{ S.E.} \]

\[ \bar{x} = 1.96 \pm 0.07 \text{ S.E.} \]

A - Identification of animal
B - Weight of animal on the day of operation in grams
C - Area of the high-threshold field of mDCN-T13 in mm²
D - Area of the low-threshold field of mDCN-T13 in mm²
E - Maximum length of the rostro-caudal axis of the high-threshold field
Animals in which vehicle was placed on the intact nerve but the nerve was not stimulated.

<p>| | | | | |</p>
<table>
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<tr>
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<td>293</td>
<td>159</td>
<td>1.9</td>
</tr>
<tr>
<td>289</td>
<td>192</td>
<td>316</td>
<td>155</td>
<td>1.8</td>
</tr>
</tbody>
</table>

C - $\bar{x} = 296 \pm 1.2$ S.E.

D - $\bar{x} = 139 \pm 6.7$ S.E.

E - $\bar{x} = 1.85 \pm 0.05$ S.E.

A - Identification #
B - Weight of Animal on the day of operation in grams
C - Area of the high-threshold field of mDCN-T13 in mm²
D - Area of the low-threshold field of mDCN-T13 in mm²
E - Maximum length of the rostro-caudal axis of the high-threshold field
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(Quoted text)


Animal Housing

The cage tops were made by Mr. C.W. Ikerson and were used to cover the rat cages in place of regular cage tops. When the regular cage tops were used, the rats were continually irritating their denervated back skin by rubbing against the food trough; this would cause the incisions to open. The flat tops prevented the rats from rubbing against them since they were elevated more than 15 cm above the floor of the cage. The animals were housed individually, wood chips were placed on the floor; food and water were freely available.
The Dorsal Cutaneous Nerves

In this photo of the rat's back, the skin has been shaved and an incision has been made along the dorsal midline through the full thickness of the skin. The dorsal skin on the left side has been retracted ventrally to expose the dorsal cutaneous nerves and accompanying blood vessels as they exit the body wall musculature and extend to the skin. The dorsal cutaneous nerve from segment T_{13} is marked with an arrow as it exits the body wall. Just above the 11.6 cm mark is a large vein which always accompanies the dorsal cutaneous nerve from segment T_{11}; this vein was one of the anatomical landmarks used to identify specific dorsal cutaneous nerves in this study.
The Segmental Origin of the Cutaneous Nerves

This figure is a diagramatic representation of the typical anatomical arrangement of the branches of a single cutaneous nerve (i.e., the cutaneous nerve from spinal cord segment T₁₂). The spinal nerve divides soon after the dorsal root ganglion (DRG) to give rise to a dorsal ramus which exits the body wall musculature (hatched area) and divides again into the medial and lateral dorsal cutaneous nerves. The ventral ramus of the spinal nerve divides laterally and ventrally to give rise to the lateral and ventral cutaneous nerves respectively.
Incision Repair in Experimental Animals

These two photos indicate the typical time course of incision repair. The top photo is of a rat on the initial day of surgery; this animal has just been sutured closed with cat gut suture (A), the sutures were placed internally and are therefore invisible from the exterior. Internal suturing was necessary to prevent the animals from removing the sutures prematurely. The suture line is indicated (B). The bottom photo is of a typical suture line along the rat's back (C) sixteen days after the initial day of surgery.
Figure 5

The Result of the Surgical Denervation to Produce an mDCN-T₁₃ "Island"

This figure is a diagramatic representation of an mDCN-T₁₃ "Island" of innervation (solid line) surrounded by denervated skin. The stippled area represents the area of skin formerly innervated by dorsal cutaneous nerves T₁₀-L₃ which have now all been surgically cut bilaterally, except for mDCN-T₁₃ on the left side. In experiments described in Results Sec E two additional DCN's rostral to T₉ and caudal to L₃ and all the LCN's on the left side were cut to produce a larger area of denervated skin. Typically the high-threshold field border (dotted line) of mDCN-T₁₃ would extend beyond that of the low-threshold field by 2-3 mm in all directions.
Electrical Stimulation of mDCN-T₁₂.

These two photographs are of the same rat. The rat has been surgically denervated to produce an mDCN-T₁₂ island, then the remaining intact mDCN-T₁₂ nerve has been dissected, along with some connective tissue, away from the body wall and placed on the stimulating electrodes. A is the thermostatically controlled heating pad used to keep the rat at 37°C throughout the experiment. B is the dissecting scope used during the dissection and stimulation. C is the stimulating electrode holder. Fine flexible insulated copper wires lead from the electrode holder to the rat back. D is an incision in the body wall musculature. E is a piece of clear plastic into which are embedded two platinum wires connected to the insulated copper wires.
Figure 7

The Application of TTX to mDCN-T

This photograph is a close up of the incision in the body wall musculature seen in Figure 6D. A - is the cutaneous nerve (mDCN-T13) which runs along the bottom of the trough made by the retraction of the body wall muscular. B - is a thin piece of cotton pulled beneath the cutaneous nerve to make it more visible for photographic purposes. The trough is filled with a TTX solution until the impulses in the nerve have been blocked. After the experiment is completed the trough is washed several times with a normal saline solution.
Figure 8

Behavioural Responses to High-threshold Nerve Stimulation

These two photographs are of the same rat. The bottom photograph is of a shaved anesthetized rat on whose back skin has been drawn five lines perpendicular to the spinal column. When the toothed forceps are used to stimulate an area of skin innervated by high-threshold cutaneous nerves (top photograph), the cutaneous muscle beneath the skin (the cutaneous trunci muscle) is reflexly activated and the parallel lines are drawn closer together to produce an observable response. If the forceps pinch is placed in denervated skin there will be no response seen and the parallel lines will remain as they are in the bottom photograph.
The Behavioural Map of a mDCN-T₁₃ Island

This is a photograph of an animal which has been denervated to produce an mDCN-T₁₃ "Island" by cutting DCNs T₁₀-L₃ on the right side, T₁₀-T₁₂, L₁-L₃, and lDCN-T₁₃ on the left side, the animal was behaviourally mapped using toothed forceps. The hatched areas are those areas that are sensitive to a pinch. The island of sensitivity - C - surrounded by insensitive skin is innervated by cutaneous nerves running in mDCN-T₁₃. Once mDCN-T₁₃ is cut this island becomes insensitive to a skin pinch. A - is the para-midline incision through which the initial denervations are made. B - indicates the low-threshold touch field of mDCN-T₁₃ which begins at the dorsal midline (white dashed line) and runs laterally. C - is the border of the high-threshold field of mDCN-T₁₃.
Low-Threshold Fields of DCNs

This figure illustrates the typical banding pattern of the low-threshold fields of segmental dorsal cutaneous nerves innervating the rat's back. This map was made by recording from individual branches of each segmental nerve while brushing the skin with a fine bristle. The diagram illustrates the typical amount of overlap between branches of the same segmental nerve as well as the amount between adjacent segmental nerves. A portion of the area of skin innervated by the lateral cutaneous nerves is illustrated on the left side by the hatched region and shows the typical pattern of overlap between them and the dorsal cutaneous nerves. The cross within a circle indicates the low-threshold field of mDCN-T13.
High and Low-threshold Field of DCNs

In this diagram the high-threshold field borders of DCN-T₁₃, mDCN-T₁₃, mDCN-T₁₂, and mDCN-L₁ (dotted lines) are superimposed on the low-threshold fields (solid lines) of the rat's back. The high-threshold fields of mDCN-T₁₂ and L₁ have been lightly stippled in. The whole high-threshold field of DCN-T₁₃ was mapped initially in one animal, then the lateral branch of the DCN-T₁₃ was cut and the medial field of the same DCN-T₁₃ was remapped (diagonally hatched). As is evident from the diagram, the areas of overlap among the high-threshold fields of mDCN-T₁₃ and its adjacent fields are so great that it would be very difficult to find insensitive skin on the left side of this animal if only the medial branch of DCN-T₁₃ were cut. A forceps pinch throughout most of the denervated mDCN-T₁₃ field would elicit impulses in cutaneous nerves in the overlapping adjacent DCN fields; for this reason it was impossible to behaviourally map all the adjacent high-threshold field within one animal. The high-threshold fields were mapped in different animals and then combined, using their low-threshold fields as a reference, to give a picture of the probable pattern of innervation in an individual rat.
Sprouting of High-Threshold Fields: Area of InSensitive Skin Remaining

Each dot represents the area (mean ± S.E.M.) of insensitive skin remaining in different groups of animals mapped at the specified number of days after denervation. For each group the denervation performed on day zero was done to produce an "island" of sensitive skin innervated by αDCH-T13 surrounded by insensitive skin. The area of insensitive skin 16 days after denervation was not significantly different (p > 0.05) from that on day zero. After day sixteen the area of insensitive skin decreased so that forty days after denervation there was almost no insensitive skin remaining.
Figure 13

Area of the Low-Threshold Field of mDCN-T<sub>13</sub> at Different Days After Denervation

This bar graph represents the area (mean ± S.D.) of the low-threshold field of mDCN-T<sub>13</sub> in two groups of animals mapped either at zero or forty days after denervation. For both groups the denervation performed on day zero produced an "Island" of innervation supplied by mDCN-T<sub>13</sub> surrounded by denervated skin. The area of the low-threshold field forty days after denervation was not significantly different (p > 0.05) than the area on day zero; this result confirmed the earlier results of Jackson & Diamond (1981) that low-threshold nerves do not sprout into denervated skin in the adult rat.
Histological Examination of Sensitive Skin, Insensitive Skin and Skin into which High-Threshold Sensitivity had Returned after Denervation

These three photographs are of three pieces of skin from the rat's back which have been impregnated with silver in order to stain the cutaneous nerves (see methods). A is a piece of skin from a normal control (undenervated) rat whose skin is behaviourally sensitive to a forceps pinch; it shows a typical bundle of cutaneous nerve fibres running in the dermis. B is a piece of insensitive skin from a rat that has been denervated sixteen days earlier to produce an "Island" of sensitive skin innervated by 
\[ \text{MCN-T}_{13} \] surrounded by an area of insensitive skin; this photograph of insensitive skin shows a typical Schwann tube devoid of any axons. The empty Schwann tubes were seen to run throughout the insensitive skin and formed a kind of network. C is a photograph of a piece of skin which was previously insensitive on the day of denervation but twenty-four days after denervation had become behaviourally sensitive to a forceps pinch. In the photograph one can see a typical Schwann tube as in photograph B but in this case there are a few axons running within the Schwann tube; this was typically found to be the case for axons in the dermis of skin which had previously been insensitive but was now sensitive to a forceps pinch.
A
NORMAL CONTROL SKIN

B
BEHAVIOURALLY INSENSITIVE SKIN

C
SKIN IN WHICH BEHAVIOURAL SENSITIVITY HAD RETURNED
The Correlation Between Behavioural and Histological High-Threshold Field Borders

A - The Techniques

A - represents a diagramatic version of how the pieces of skin were obtained in order to carry out the histological analysis and subsequent correlation with the behaviourally high-threshold field border. Once the high-threshold field border of mDCI-T₁₂ was marked on the rat's back a razor blade was drawn along the behavioural field border leaving a small incision through the epidermis and into the dermis. A piece of skin was then excised in such a way that approximately half of the skin was sensitive and the other half insensitive while the razor blade incision ran through the middle. The rat skin was frozen, mounted in a cryostat and 30μ sections were cut perpendicular to the razor blade incision and finally mounted on the microscope slide. After the slides had been processed they were examined using a light microscope (objective 25x, NA 0.60, overall magnification 250x). When a nerve was found in the dermis the position of the nerve relative to the razor blade incision (the behavioural border) was measured and recorded, and also whether the nerve was on the sensitive or insensitive side of the border. In every section the area of insensitive skin and the behavioural border region was investigated for the presence of axons; the sensitive area of skin was examined in every third section.
Figure 15

B - Denervation and No Stimulation

B - is a diagramatic representation of the histological analysis of a piece of skin obtained from a rat (0132) sixteen days after a denervation procedure which produced an "Island" of sensitive skin innervated by mDCN-T13 surrounded by a large area of insensitive skin. A 0.0um represents the razor blade incision which divided sensitive from insensitive skin. The error in the technique used to determine the behavioural field border was judged to be ± 1mm. Each dot represents
a) the presence of an axon or group of axons in each serial section and
b) their position relative to the razor blade incision. The low-threshold field border of mDCN-T13 is indicated. It can be seen that sixteen days after denervation, the behaviourally determined high-threshold field border is coincident (within experimental error) with the histologically determined border and that no axons were seen in behaviourally insensitive skin.
Figure 15

C - Denervation with Physiological Stimulation

C - is a diagramatic representation of the histological analysis of a piece of skin obtained from a rat (#296) sixteen days after a denervation procedure which produced an "Island" of sensitive skin innervated by mDCN-T_{13} surrounded by an area of insensitive skin; following the denervation on day zero, the island of sensitive skin was physiologically stimulated with toothed forceps 100 times. Again "0"um represents the razor blade incision which marked the behaviourally determined border; the low-threshold border is indicated. In this animal (#296) the high-threshold cutaneous sensitivity had extended into previously insensitive skin by approximately 4.5mm and, as can be seen in the diagram, so had the axons extended into previously denervated skin. Again one can see that the behaviourally determined border is coincident with the histologically determined border.
D - Denervation with Electrical Stimulation

D - is a diagramatic representation of the histological analysis of a piece of skin obtained from a rat (#159) sixteen days after a denervation procedure which produced an Island of sensitivity as in "C" above; following the denervation the intact mDCN-T13 nerve was electrically stimulated for 5 min (6000 impulses, 15 Volts, with a pulse width of 0.5msec) on day zero. The area of sensitive skin was mapped on day sixteen and a razor blade was drawn along the behaviourl border as in the cases above. Again in this animal, as in the animal "C", the area of high-threshold sensitivity had extended into previously insensitive skin and, as can be seen from the diagram, so too had the axons extended into previously denervated skin: the behaviourally defined border and the histologically determined border are coincident. The above three diagrams provide results which are consistent with the conclusion that the behavioural techniques used in this study indicate the histological border of the intact high-threshold nerves with an accuracy of ± 1mm.
Reflex Activation of the Cutaneous Trunci Muscle by Electrical Stimulation of a Cutaneous Nerve

These photographs are of oscilloscope traces. The top trace in each pair shows the impulses recorded in the motor nerves innervating the cutaneous trunci muscle (CTM) reflexly evoked by electrical stimulation of mDCN-T_{13} of an anaesthetized rat. The bottom trace is the compound action potential recorded in mDCN-T_{13} after electrical stimulation of the nerve. Photograph "A" shows what happens when a small voltage (0.5 volts, pulse width of 0.05msec) is applied to mDCN-T_{13}. The change seen in the bottom trace represents the depolarization of a group of fibres whose conduction velocity is approximately 50m/sec (Group II fibres); the depolarization of this group of fibres never evoked a reflex volley in the motor nerves to CTM as indicated by the flat base line in the upper trace of photograph "A".

Photograph "B" (i) and (ii) indicates what happens when the stimulus voltage applied to mDCN-T_{13} is raised to five volts; a second peak appears in the bottom trace which represents the depolarization of a group of fibres with a smaller diameter whose conduction velocity is approximately 20 m/sec (Group III or Aδ fibres). When the second peak appears in the compound action potential (lower trace) the first reflex volley is recorded from the motor nerves to CTM. The reflex volley evoked by the excitation of Group III fibres in mDCN-T_{13} has a latency of 10-15 msec. If one varies the stimulus voltage so that the second
peak disappears or reappears so the reflex volley will disappear and reappear. The only difference between the two photographs is that in the second photograph the sweep speeds have decreased.
A

VOLLEY RECORDED IN THE MOTOR NERVES

COMPOUND ACTION POTENTIAL RECORDED IN mDCN-T13.

B

(I)

STIMULUS: 5 VOLTS
0.05 mSEC PULSE WIDTH

(II)

STIMULUS: 5 VOLTS
0.05 mSEC PULSE WIDTH
Photograph "C" - shows what happens when the stimulus voltage is raised much further (15 volts, pulse width of 0.5 msec); a third peak is seen on the lower trace which represents the depolarization of a group of fibres with a conduction velocity of approximately 1.9 m/sec (C-fibres). When the C-fibre peak appears on the compound action potential (lower trace) a second reflex volley with a latency of 40 to 60 msec appears in the motor nerves to CTM.
Reflex Activation of the Cutaneous Trunci Muscle by Electrical Stimulation ofBehaviourally Sensitive Skin

These photographs are oscilloscope traces showing impulses recorded in the motor nerves innervating the cutaneous trunci muscle (CTM) evoked by electrical stimulation of skin innervated by mDCN-T_{13} of an anaesthetized rat (9276). On day zero the rat was denervated to produce an "Island of sensitive skin innervated by mDCN-T_{13}" surrounded by an area of insensitive skin, then, the intact nerve (mDCN-T_{13}) was electrically stimulated for 5 min (6000 impulses, 15 volts, 0.5 msec pulse width). Sixteen days later the rat was behaviourally mapped, the intact nerve was carefully placed on recording electrodes and the low-threshold field of mDCN-T_{13} was mapped with a fine bristle. The motor nerves to CTM on the ipsilateral side were then dissected free and placed on recording electrodes.

Figure 17A is the actual map of the low and high-threshold fields of mDCN-T_{13}. Normally the high-threshold field does not extend more than 2-3 mm beyond the low-threshold one but in this case the intact nerve had been stimulated which caused the nerve to sprout into adjacent denervated skin. Points "A" and "C" within the high-threshold field are therefore in areas into which cutaneous high-threshold nerves had sprouted; electrical stimulation of these areas of skin would therefore be exciting impulses in only sprouted nerve endings.

The two photographs in Figure 17B are of the impulses recorded in the motor nerves to CTM after electrical stimulation of the skin in the
A

INCISION

LOW-THRESHOLD FIELD OF mDCN-T_{13}

AUTONOMOUS HIGH-THRESHOLD FIELD mDCN-T_{13}

INSENSITIVE SKIN

B

ELECTRICAL STIMULATION OF SKIN AT POINT "B"

STIMULUS: 50 VOLTS
0.5 mSEC PULSE WIDTH

STIMULUS: 100 VOLTS
0.5 mSEC PULSE WIDTH
middle of the low-threshold field (point "B"). (i) shows the impulse evoked by a 50 volt stimulus; this reflexly evoked impulses in the motor nerves to CTM with a latency of approximately 15 msec and correspond to those evoked by electrical stimulation of Group III cutaneous afferent fibres. (ii) shows the impulses evoked by stimulation in the same place as (i) above but with a higher voltage (100 volts) and the sweep speed of the trace has been reduced to 20 msec/division; this was done in order to optimize the possibility of seeing a second reflex volley due to excitation of C-fibre afferents. In none of the animals studied was I able to evoke a second reflex volley by electrically stimulating the skin.

Figures 17 C and D show the reflexly evoked impulses in the motor nerves to CTM evoked by electrical stimulation of skin into which high-threshold cutaneous nerves had sprouted. Figure C are those impulses evoked by a 90 volt stimulus of the skin at position "A" on Figure 17A while those in "D" are the result of electrical stimulation (90 volts) at position "C" in Figure 17A. No impulses could be evoked from any of these positions once mDCN-T13 had been cut.
ELECTRICAL STIMULATION OF SKIN AT POINT "A"

STIMULUS
90 VOLTS
0.5 mSEC PULSE WIDTH

20μV
-10mSEC

ELECTRICAL STIMULATION OF SKIN AT POINT "C"

STIMULUS
90 VOLTS
0.5 mSEC. PULSE WIDTH

20μV
20mSEC
Denervation Pattern for the Experiments Dealing with Spatial Constraints

This is a diagrammatic representation of the area of denervated and innervated skin on the initial day of surgery for animals in Results Sec. E. Normally, the area of high-threshold sensitivity innervated by mDCN-T<sub>13</sub> (cross hatched area) is surrounded by denervated skin formerly innervated by DCNs T<sub>10</sub> - L<sub>3</sub> bilaterally leaving only mDCN-T<sub>13</sub> intact on the left side (area within the dotted line). However, for these experiments DCNs T<sub>8</sub>, T<sub>9</sub>, L<sub>4</sub>, and L<sub>5</sub> were cut bilaterally as well LCNs T<sub>8</sub> - L<sub>5</sub> on the left side (diagonally hatched area). The purpose of this more extensive denervation was to provide the intact mDCN-T<sub>13</sub> with as much denervated skin as was experimentally practicable, into which it could sprout before reaching already innervated skin.
**Figure 19**

**Measurement of High-Threshold Field Axis**

This diagram demonstrates diagramatically the method used to measure the rostro-caudal and dorso-ventral areas of the high-threshold field of an intact nerve. The dorso-ventral axis is measured along a line perpendicular to the midline and extending to the most ventral part of the high-threshold field border. The rostro-caudal axis is measured along a line perpendicular to the dorso-ventral axis and extending to the two points which are furthest apart on the high-threshold border; this value is then divided by one half in order to obtain the extension, due to sprouting, of one border which then allowed us to compare the extension of one rostro-caudal border to that of the ventral border of the high-threshold field.
The Border Extension Due to Sprouting of mDCN-T_13 Fields

Figure "A" is a graph of the data (mean ± S.E.M.) obtained from measuring the maximum rostro-caudal axis, then dividing it by one half, (see Figure 19) "squares" and the maximum dorso-ventral axis "circles" of the high-threshold field of mDCN-T_13 in different animals at selected times after denervation. The denervation performed on day zero was the same for each animal and is described in figure 18. The graph indicates that following denervation both intact field borders begin to extend into denervated skin which would be expected if the high-threshold nerves were sprouting within both their parent dermatome as well as the adjacent denervated skin. A comparison was made between these two groups to determine whether there was a preferential extension of either border, i.e., do the sprouting high-threshold nerves prefer to sprout within their parent dermatome or does it not matter? The star represents the rostro-caudal border and the triangle the dorso-ventral border forty days later after no denervation. Figure 20B are the two lines of linear regression computed from the data in Figure 20A. The dorso-ventral border data significantly (p < 0.05) fit line "A" (N = 40, correlation coefficient = 0.76, slope = 0.030). The rostro-caudal data significantly (p < 0.05) fit line "B" (N = 40, correlation coefficient = 0.88, slope = 0.030). The slopes of these lines are not significantly different (p > 0.05 Appendix II).
BORDER EXTENSION OF mDCN-T₁₃ HIGH-THRESHOLD FIELDS

DAYS AFTER DENERVATION

DORSO-VENTRAL BORDERS

ROSTO-CAUDAL BORDER
BORDER EXTENSION OF mDCN-T13 HIGH-THRESHOLD FIELDS

DORSO-VENTRAL BORDER (A)

ROSTRO-CAUDAL BORDER (B)

DAYS AFTER DENERVATION

CM
The Border Extension Due to Sprouting of DCN-T₁₃ Fields

Figure A is a plot of the data (mean ± S.E.M.) obtained from measuring the maximum extension of the rostro-caudal (squares) and dorso-ventral (circles) borders of DCN-T₁₃ high-threshold fields into adjacent denervated skin (see Figure 19). The animals used for the data in this Figure differ from those in Figure 20; these animals have a larger intact high-threshold field (the whole DCN-T₁₃, mDCN-T₁₃ plus lDCN-T₁₃) on day zero than those used in Figure 20. Again, the denervation performed on day zero was the same for each animal and is described in Figure 18 with one exception, in these animals, lDCN-T₁₃ was left intact. The graph indicates that following denervation of the surrounding skin on day zero, both of the high-threshold field borders begin to extend (due to sprouting of intact high-threshold nerves) into the denervated skin. Again a comparison was made between these two groups to see whether there was a preferential extension of either border (Appendix II). Figure 2B are the two lines of linear regression computed for the data in Figure 21A. The dorso-ventral data significantly (p < 0.05) fit line A (N = 37, correlation coefficient = 0.73 slope = 0.033). The rostral-caudal data significantly (p < 0.05) fit line "B" (N = 37, correlation coefficient = 0.87, slope = 0.037). The slopes of these lines are not significantly different (p > 0.05 Appendix II).
BORDER EXTENSION OF DCN-T13 HIGH-THRESHOLD FIELDS

DORSO-VENTRAL BORDER (A)

ROSTRO-CAUDAL BORDER (B)

CM

DAYS AFTER DENERVATION
The Border Extension Due to Sprouting of mDCN-T₁₃ vs DCN-T₁₃ Fields

A - is a graph of the lines of linear regression representing the rostro-caudal border extension due to sprouting of high-threshold nerves into denervated skin of the mDCN-T₁₃ fields (dotted line) and the DCN-T₁₃ fields (solid line). The slopes of these lines are not significantly different (p > 0.05 Appendix II).

B - is a graph of the lines of linear regression representing the dorso-ventral border extension due to sprouting of the intact high-threshold nerves into denervated skin of the mDCN-T₁₃ fields (dotted line) and the DCN-T₁₃ fields (solid line). Again, as above, the slopes of these lines are not significantly different (p > 0.05 Appendix II).
ROSTRO-CAUDAL BORDER EXTENSION OF THE mDCN-T₁₃ AND DCN-T₁₃ FIELDS

CM

DCN-T₁₃
mDCN-T₁₃

DAYS AFTER DENERVATION
DORSO-VENTRAL BORDER EXTENSION OF THE mDCN-T₁₃ AND DCN-T₁₃ FIELDS

CM

DAYS AFTER DENERVATION

DCN-T₁₃
mDCN-T₁₃
The Increase in Area Due to Sprouting of Intact High-threshold Nerves of mDCN-T_{13} and DCN-T_{13} Fields

A - is a graph of the area (mean ± S.E.M.) of the high-threshold fields in different groups of animals mapped at the specified times after denervations. The denervation procedure on day zero was more extensive than normal and is described in Figure 18. The only difference between the two groups is that on the initial day of surgery the DCN-T_{13} "Island" of innervation (that area supplied by the mDCN-T_{13} plus the lDCN-T_{13}) was larger than that of the subfield mDCN-T_{13}. As is evident from the graph, the area of the high-threshold fields increased as the intact nerves sprouted into adjacent denervated skin.

The triangle represents the area (mean ± S.E.M.) of the high-threshold fields of DCN-T_{13} after forty days without denervation; the star represents the area (mean ± S.E.M.) of mDCN-T_{13} after forty days without denervation.

B - represents the lines of linear regression computed from the data in Figure 22A. Each line significantly fits the data (p < 0.05). Line "A" has a slope of 27.816 and a correlation coefficient of 0.876 (N = 37), Line "B" has a slope of 21.508 and a correlation coefficient of 0.844 (N = 38); the slopes of these lines are significantly different (p < 0.05), indicating that the area of DCN-T_{13} increases at a faster rate than that of mDCN-T_{13}.
THE INCREASE OF AREA OF DCN-T₁₃ AND mDCN-T₁₃ FIELDS

HIGH-THRESHOLD FIELD AREA IN mm²

DAYS AFTER DENERVATION

DCN-T₁₃

mDCN-T₁₃
THE INCREASE IN AREA OF DCN-T_{13} AND mDCN-T_{13} FIELDS

HIGH-THRESHOLD AREA IN mm^2

DAYS AFTER DENERVATION

DCN-T_{13} (A)
mDCN-T_{13} (B)
The Effect of Repeated Behavioural Testing on Sprouting of High-Threshold Nerves

This is a graph of the area (mean ± S.E.M.) of insensitive skin remaining at a selected number of days after a denervation procedure was performed to produce an "Island" of sensitive skin innervated by mDCN-T13 surrounded by insensitive (denervated) skin (Figure 5). The solid circles represent 5 groups of animals which were behaviourally mapped only once on either of zero 3, 16, 24 or 40 days after denervation. The open squares represent one group of four animals mapped every four days after denervation. As can be seen from the graph the area of remaining insensitive skin decreases more rapidly (due to the sprouting of the intact high-threshold nerves into adjacent denervated insensitive skin) when the animals were repeatedly mapped every four days than when they were only mapped once. Twenty-four days after denervation when the group mapped repeatedly had no insensitive skin left (open box) the group that was only mapped once (on day 24, solid circle) had a significantly larger area of insensitive skin remaining (626 ± 59 mm² S.E.M. p < 0.001). Apparently the behavioural testing had accelerated the rate of sprouting of the high-threshold nerves.
AREA OF INSENSITIVE SKIN REMAINING

(DAYS AFTER DENERVATION)

(1000 800 600 400 200)

(40 32 24 16 8 0)

(1 mm²)
The Effect of Physiological Activity on Sprouting of High-Threshold Nerves

These bar graphs represent the area (mean ± S.E.M.) of the high-threshold field of mDCN-T13 behaviourally mapped sixteen days after a denervation procedure that produced an "Island" of sensitive skin innervated by mDCN-T13 surrounded by insensitive denervated skin (Figure 5). The control animals (A) were sutured closed and then behaviourally mapped sixteen days later. The animals in group B were sutured closed and then the high-threshold field innervated by mDCN-T13 was physiologically stimulated (forcep pinch) 135 times. The intact mDCN-T13 was carefully exposed in the animals in group C and a TTX solution (10µg/ml in 0.9% saline) was placed on the nerve for 25 min at which time a forcep pinch with its high-threshold receptive field no longer elicited a reflex response of any kind; the receptive field was then pinched 135 times. The animals in group C were then sutured closed and behaviourally mapped sixteen days later. The graph indicates that physiological stimulation of the intact high-threshold field of mDCN-T13 will accelerate the sprouting of the intact high-threshold nerves; this acceleration of sprouting due to physiological stimulation can be stopped by blocking impulse conduction in the intact nerve using TTX.
The Time Course of Sprout of the High-Threshold Fibres in mDCN-T<sub>13</sub>

This is a graph of the areas (mean ± S.E.M.) of high-threshold fields (mDCN-T<sub>13</sub>) mapped only once in eight different groups of animals at the specified number of days after denervation (Fig. 5). The graph shows that there is a latent period of about sixteen days before the area of the high-threshold field begins to increase; once the high-threshold nerves have begun to sprout (after day sixteen) the area of the high-threshold field increases linearly until the previously denervated skin is fully innervated at approximately forty days after denervation.
Figure 27

The Effect of Electrical Stimulation (10 minutes) on Sprouting of the High-Threshold Nerves

This is a graph of the areas (mean ± S.E.M.) of high-threshold fields of mDCN-T13 which were behaviourally mapped at the specified number of days after surgical denervation (Fig. 5). The intact mDCN-T13 was electrically stimulated 12,000 times (10 min, 20 Hz) on day zero in all the animals in groups A–D then one of the groups of animals was mapped once on either 4, 8, 12 or 16 days after denervation. The animals in group E were surgically denervated on day zero but not electrically stimulated; these animals served as the control and were mapped sixteen days later. The graph shows that the area of the receptive field in the stimulated animals began to increase linearly after a latent period of about four days and by sixteen days (Group D) the area was significantly larger (p < 0.001) than the area of the receptive field at sixteen days without stimulation (Group E).
THE EFFECT OF ELECTRICAL STIMULATION (10 minutes) ON HIGH-THRESHOLD FIELDS

HIGH-THRESHOLD FIELD AREA IN mm²

DAYS AFTER DENERVATION

A
B
C
D
E
The Effect of Electrical Stimulation on the Area of High-Threshold Fields Four Days After Denervation

These four bar graphs represent the areas (mean ± S.E.M.) of high-threshold receptive fields (mDCN-T₁₃) in four different groups of animals. Groups B, C and D were animals denervated on day zero to produce an isolated mDCN-T₁₃ "Island" of innervation (Figure 5) then the intact high-threshold nerve was electrically stimulated (20 Hz, 0.5 msec pulse width) for either 10 minutes (Group B), 40 minutes (Group C) or 80 minutes (Group D). The animals were sutured closed and behaviourally mapped four days later. The animals in group A were denervated as above and then behaviourally mapped the same day. The graph shows that there is no difference (p >0.05) between the areas of the high-threshold fields four days after denervation and electrical stimulation either for 10, 40 or 80 minutes; these areas are not different (p >0.05) than the area of the receptive field on day zero (Group A).
THE EFFECT OF ELECTRICAL STIMULATION ON THE AREA OF HIGH-THRESHOLD FIELDS FOUR DAYS AFTER DENERVATION

A- No stimulation mapped on day zero
B- 10 minutes stimulation mapped on day four
C- 40 minutes stimulation mapped on day four
D- 80 minutes stimulation mapped on day four

AREA OF HIGH-THRESHOLD FIELD IN mm²
The Effect of Electrical Stimulation (5 minutes) on Sprouting of

High-Threshold Nerves

This is a graph of the areas (mean ± S.E.M.) of high-threshold fields of mDCN-T13 which were behaviourally mapped at the specified number of days after surgical denervation (figure 5). The intact mDCN-T13 was electrically stimulated for 5 minutes (20 Hz approximately 6000 times) on day zero in all the animals in groups A, B and C then one of these groups of animals was mapped once on either 8, 12 or 16 days after denervation. The animals in groups "D" and "E" were surgically denervated on day zero but not electrically stimulated; these animals served as the control groups and were mapped at either eight or sixteen days after denervation. The graph shows that the area of the high-threshold receptive fields eight days after denervation and electrical stimulation for 5 minutes (Group A) is not significantly different (p >0.05) than the animals eight days after denervation without stimulation. However, sixteen days after denervation and electrical stimulation, the area of the receptive field of mDCN-T13 is significantly larger (p <0.05) than the area of the receptive field in animals mapped sixteen days after denervation but without electrical stimulation. Apparently 5 minutes of electrical stimulation will reduce the latent period for sprouting from sixteen days to eight days.
THE EFFECT OF
ELECTRICAL STIMULATION (5 minutes)
ON HIGH-THRESHOLD FIELDS

HIGH-THRESHOLD FIELD AREA IN mm²

DAYS AFTER DENERVATION

0  10  20  30  40
Figure 30

Effect of Electrical Stimulation and TTX on Sprouting of High-Threshold Fibres

The five bars in this graph represent the area (mean ± S.E.M.) of the high-threshold receptive field of mDCN-T₁₃ sixteen days after denervation (Figure 5) and electrical stimulation for ten minutes. Group A - represents the control group that was electrically stimulated but no TTX was placed on the intact nerve. The animals in group B were also electrically stimulated but before they were stimulated TTX was placed on the intact nerve between the stimulating electrodes and the skin to block the electrically evoked impulses from travelling peripherally. The animals in Group C were similarly electrically stimulated but TTX was first placed on the intact nerve between the stimulating electrodes and the dorsal root ganglia to block the electrically evoked impulses from travelling to the spinal cord. Finally the animals in Group D were not electrically stimulated, rather TTX was placed on the intact nerve central to the stimulating electrodes until impulse propagation in the nerve was blocked, then the animals were sutured and behaviourally mapped sixteen days later; this group served as a TTX control. The diagram shows that electrical stimulation of the intact nerve will accelerate the sprouting of that nerve (Group B, if the evoked impulses are prevented from travelling peripherally), the effect of electrical stimulation is unchanged (Group B). However, if the evoked impulses are prevented from travelling centrally the acceleration of sprouting due to electrical stimulation...
is significantly decreased ($p < 0.05$) and the area of these receptive fields (Group C) is not significantly different ($p > 0.05$) from those whose nerve was not stimulated but rather the impulse propagation in them was temporarily stopped (Group D).

Group E represents the area of the high-threshold receptive field of animals denervated on day zero then mapped sixteen days later; the intact nerve in these animals was not electrically stimulated, nor was the intact nerve manipulated in any way to place TTX on it. The area of the receptive field in this group of animals is significantly smaller ($p < 0.05$) than the areas of either group C or D. Possibly the mechanical manipulations necessary to place TTX on the nerve may be significantly increasing the impulse activity in the nerve and thereby slightly accelerating the sprouting of the stimulated intact nerves.
EFFECT OF ELECTRICAL STIMULATION AND TTX ON SPROUTING OF HIGH-THRESHOLD FIBRES

AREA OF HIGH-THRESHOLD FIELD IN mm²

SIXTEEN DAYS AFTER DENERVATION
The Effect of Electrical Stimulation on the Low-Threshold Fibres in the Adult Rat

The two bars in this graph represent the areas (mean ± S.E.M.) of low-threshold receptive fields in two groups of animals; "B" sixteen days after denervation plus electrical stimulation and "A" on the initial day of surgery. The animals in group "A" were denervated on day zero and mapped on the same day (146 ± 22 S.D.). The animals in group B were denervated as in group A but the intact mDCN-T13 was electrically stimulated for 10 minutes (20 Hz) then the animals were sutured and mapped sixteen days later (166 ± 29 S.D.). The areas of the low-threshold fields in Group A are not significantly different (p >0.05) than those in Group B. Electrical stimulation apparently does not cause low-threshold nerves to sprout into adjacent denervated skin in the adult rat.
THE EFFECT OF ELECTRICAL STIMULATION ON THE LOW-THRESHOLD FIBRES IN THE ADULT RAT

AREA OF LOW-THRESHOLD FIELD IN mm²

SIXTEEN DAYS AFTER DENERVATION

A

B

5

5