Temporal and Spatial Constraints on the Recovery of Mechanosensory Function in Denervated Skin

by

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ABSTRACT

One approach to the study of how nerves can establish appropriate connections, and of possible "plasticity" intrinsic to the connections themselves, is to partially denervate a target tissue and examine the reestablishment of functional connections by nerves. Cells or tissues whose normal nerve supply has been lesioned can become reinnervated by two means: the sprouting of new collaterals from nearby intact nerves, or the regeneration of the damaged fibres. Both mechanisms have been reported as involved in the recovery of function after nerve lesions in mammals.

In the course of experiments on the reinnervation of skin in rabbits, however, evidence was obtained suggesting that intact low-threshold mechanosensory nerves in adult mammals may not sprout into denervated skin. The investigations described in this thesis were undertaken to examine this possibility further, and in particular to use the rat to study the ability of such nerves to establish functional connections in denervated skin.

Direct electrophysiological recording from cutaneous nerve bundles was used to detect impulses generated by tactile stimulation of the skin. The area of skin supplied by a nerve (its low-threshold mechanosensory receptive field) and in particular the number of touch domes (specific sensory structures in the skin) innervated by that nerve were determined after various manipulations;

both the area of the field and the population of touch domes were used as measures of the distribution in the skin of functional endings. Intact low-threshold mechanosensory nerves in the rat were found to sprout into adjacent denervated skin only during a remarkably brief "critical period" that begins at about 15 days of age and ends at about 20; such sprouting could neither be evoked nor did it continue after this age. Moreover, during the critical period, the intact low-threshold mechanosensory nerves often failed to sprout into denervated skin except that available within the same ("parent") dermatome. These intact nerves therefore are under constraints both in time and space, which operate to limit the extent of functional sprouting and also the location of such newly established endings. Regenerating nerves however were found to be subject to neither of these constraints, and even in the adult they would freely grow across dermatomal borders to establish functional endings in denervated skin. Of special significance, when the regenerating nerves arrived at skin that during the critical period had been reinnervated by sprouts of intact nerves, the regenerating fibres replaced functionally the earlier sprouted endings; those endings within the usual territory of the intact nerve that had sprouted were not displaced however.

It was concluded that intact and regenerating nerves are differentially regulated; intact nerves, but not regenerating ones, are subject to temporal and spatial constraints in their ability to establish functional endings in denervated skin. The endings

which are established in response to denervation of skin during the critical period however incompletely suppress some quality of the target which allows regenerating nerves to recognize it, and to engage succesafully in competition for its innervation.

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SECTION 1

Rationale and Overall Objectives

In the course of embryogenesis neural connections and the circuits thus formed eventually emerge in a well ordered fashion. The exact manner in which the specific circuitry is determined is as yet incompletely understood. For any given neuron the input it receives and the distribution of its own connections with its target cells can dictate the role it plays in the behaviour of the animal. Much of the primary development of the nervous system in mammalia occurs *in utero* and is therefore relatively inaccessible to experimental manipulation. However, one potentially productive approach to an understanding of the development of neuronal connections is to examine post-natally the formation of new connections made with targets after these have been deprived of some or all of their normal innervation. This approach has produced much information about processes which may be similar to those operating in primary development. There is also the possibility that such investigations may yield information of clinical usefulness, especially about the ability or inability of neurons to compensate after lesions by making new connections.

In general, when peripheral nerves are damaged, either experimentally or through trauma or disease, their target regions may become partially or totally denervated. In most nervous systems of vertebrates this deficit in innervation cannot be made up by

further division and differentiation of new nerve cells. A functional innervation can be restored in the deprived region by two means only: - regeneration of the damaged nerves or invasion of the affected area by new branches arising from nearby intact nerves. Several aspects of these processes are examined in the present study, which concentrates in particular on the peripheral connections of primary mechanosensory afferent axons.

In man, the reporting of sensation is most often used to assess the return of a sensory innervation to denervated skin following peripheral nerve lesion. In other mammals the recovery of sensory function in denervated skin has usually been assessed by the presence or absence of behavioural responses to irritating or noxious stimulation. It was however quite clearly demonstrated by Kirk and Denny-Brown (1970) that such responses may depend upon the "state" of the central nervous system (CNS) at the moment of testing, and therefore can be misleading as to the actual distribution of the peripheral connections of primary afferents. The direct electrophysiological recording of impulse activity in the primary afferent nerve fibres, when it can be performed, is therefore the most accurate method of determining the distribution of functional sensory endings in the skin.

The principal objective of this study was to investigate, in a mammalian preparation, the ability of intact cutaneous mechanosensory nerves to functionally compensate for denervation of adjacent skin by making new endings (that in certain instances are known to

contact specific mechanosensory structures) in the denervated skin. The dorsal cutaneous nerves (DCNs) of rats were selected for this investigation because (a) it is possible to make high resolution direct electrophysiological recordings of impulse activity from them, at least of the touch-sensitive receptors innervated by the larger fibres, and so measure the distribution of their mechanosensory endings in the skin; (b) the receptive fields of these nerves are so organized as to permit an accurate measure of the areas of skin that particular DCNs supply, both normally and after they have functionally sprouted into denervated skin; and (c), specific target structures, the "touch-domes", can be seen on the surface of the skin and stimulated individually, permitting an analysis of results to be made at the level of individual identified mechanosensory structures.

The outcome of these investigations has provided a principally descriptive account of temporal and spatial constraints on the ability of low-threshold mechanosensory cutaneous nerves to establish or reestablish sensory function in experimentally denervated skin. Although the mechanisms underlying the phenomena presented here remain unknown, the results point to future experiments that can explore possible mechanisms which might underlie the observations.

SECTION 2

Background to the Investigation

1. Evidence for a Role of the Target on the Branching of Axons During Development

On the basis of histological observations, Santiago Ramon y Cajal (1852-1934) and Wilhelm His (1831-1904) arrived at a dynamic description of the outgrowth of axons from the nerve cell bodies. From their pioneering work came the recognition that during development neurons tend to extend axons over relatively long distances, and sprout collateral branches, largely and often only, in the vicinity of their end organs (or targets). In developing his concept of "neurotropism", Cajal (1919) went on to say of the developing innervation of the corneal epithelium of chick embryos:

> "Each fibre is destined for an epithelial territory devoid of nerves, and there are no vast aneuritic spaces in some regions nor excessive collection of fibrils in others. One could say that after invasion of the epithelium a state of chemical equilibrium is created, by which the innervated territories are incapable of attracting new sprouts".
>
> Guth translation, 1960; page 162.

Harrison (1910) first described the outgrowth of nerve fibres from young neurons *in vivo*. The primary sensory Rohon-Beard cells are visible in the dorsal part of the neural tube of frog embryos. Harrison observed that the axons of these cells grow out into the sub-epidermal tissue where they give rise to many branches. In a series of studies on living nerves in the transparent tail of the tadpole, Speidel (1932, 1935, 1941, 1942, 1964) made obser-

vations which were entirely consistent with Cajal's suggestion quoted above. In the course of directly observing the growing nerves, Speidel saw that the majority of cutaneous fibres grew directly towards the skin, sprouting collaterals there. Sprouting continued as the tail grew larger. A few aberrant branches sprouted into the deep tissues away from the skin, but these were apparently subjected to continual remodelling; some of these branches would suddenly change their direction and turn superficially; others retracted variable distances, changed their direction, and continued to grow towards the skin. A few of these aberrant collaterals retracted completely or perhaps degenerated. Although strongly suggestive, these observations were not proof of Cajal's original hypothesis that sprouting of axons in the vicinity of the end organ is evoked by a stimulus from the target tissue.

Other evidence in favour of a cutaneous sprouting stimulus was provided by Fitzgerald (1961) who examined histologically the density of free nerve endings in the skin of the pre- and post-natal pig's snout. As a pig grows the number of epidermal ridges on the snout increases. He found that, although no new axons arrived at the skin during this time of increasing numbers of epidermal ridges, the density of innervation remained constant; Fitzgerald therefore concluded that the maintenance of this constant density was accounted for by the sprouting of new collateral branches by the existing nerves. Significantly, he found that the number of endings increased in direct proportion to the increasing number of epidermal ridges,

suggesting that some component of the epidermis was the source of a local sprouting stimulus.

Equally intriguing as the sprouting of axons in the vicinity of their targets is the observation that sprouting eventually ceases. This observation was incorporated into Cajal's proposal of a "chemical equilibrium" that exists between some component of the available target (in this case epithelia) and another component that was related to the nerves themselves; subsequently this concept was extended to include all targets, such as muscle or even other nerve cells (Aguilar, Bisby, Cooper and Diamond, 1973; Diamond, Cooper, Turner and Macintyre, 1976).

2. Collateral Sprouting of Intact Axons After Partial Denervation

Collateral sprouting of nerves occurs not only during early development; it can be evoked during adult life as well. The clearest examples of collateral sprouting by intact nerves in adult animals have been obtained by partially denervating a target tissue. This possibility was first made explicit by Exner (1884, 1885) who reported that muscle atrophy seen after complete denervation was prevented if the muscle was only partially denervated; he suggested that either (1) remaining undamaged motor nerve axons sprout and takeover the denervated muscle fibres, or (2) muscle fibres are innervated by more than one neuron. The first possibility, "denervation sprouting", has been shown to be a widespread phenomenon occurring in virtually every system examined (see below).

(a) Cutaneous nerves

For the clearest demonstration of denervation sprouting we return to the observations of Speidel (1935) on the innervation of the transparent skin of the tadpole tail. Three days after sectioning one part of the nerve supply, he observed that the remaining intact axons near the denervated skin began sending out new collateral branches which successfully formed new endings in the denervated region within the following 3-10 days. Using histological methods Zander and Weddell (1951a) investigated the behaviour of intact axons in the corneal epithelium of rabbits following lesions to a portion of its innervation. They found that denervated regions of the corneal epithelium were invaded by collateral branches newly sprouted from intact axons in adjacent regions. The earlier work of Weddell et al. (Weddell, Guttmann and Gutmann, 1941; Weddell, 1942) on the cutaneous innervation of rabbit skin however yielded the classic statement of functional sprouting in mammalian skin. Using both the adult rabbit ear and leg, Weddell and his colleagues found that after cutting selected cutaneous nerves, sensory function in skin returned over the next few weeks. This recovery was assessed behaviourally by marking, in repeated tests, the area within which pin-prick was not effective in eliciting a withdrawal reflex; this area grew smaller at each successive test. Since regeneration of the originally cut nerve was prevented, Weddell and his collaborators interpreted these results as being due to collateral sprouting from nerves surrounding the denervated zone. This interpretation was confirmed by histological

observations carried out in the skin using primarily the technique of infiltrating the skin with dilute solutions of methylene blue (Weddell, 1941), a vital stain taken up by nerves. In regions where repeated behavioural tests revealed a return of sensibility, the presence of nerve fibres was detected. There was good agreement (usually within 1 mm) between the border of areas that were behaviourally sensitive and the location of demonstrable nerve fibres; nerves were not seen in insensible areas.

The clinical observations by Pollock (1920) that areas of recorded sensory loss following peripheral nerve injuries shrank circumferentially before regeneration could have taken place were interpreted by Weddell et al. to be a result of a similar new growth of fibres (collaterals) from surrounding intact nerves. Livingston (1947) too, concluded that following complete interruption of the median nerve in patients, the denervated thenar muscles and denervated skin of the affected hand became invaded by collaterals from the intact ulnar nerve. The possibility was not excluded however that the recovery was due to regeneration (Weddell, 1942) of median nerve axons through a communicating anastomosis between the medium and the ulnar nerves (Guttmann, 1977). More recently, Devor et al. (Devor, Schonfeld, Seltzer and Wall, 1979) examined the return of sensory function to the skin of the rat's foot following section of selected nerves. Repeated behavioural testing using "pinch" stimuli revealed that after the sciatic nerve was sectioned (and prevented from regenerating) the remaining sensitive area expanded into the anaesthetic zone. In

animals which showed evidence of behavioural recovery the electrophysiologically mapped receptive fields of single high-threshold mechanosensory afferents (Group III) were found to be larger than those of similar afferents in normal controls. They concluded that the recovery of sensory function in experimentally denervated skin was due to the collateral sprouting of remaining intact axons. The use of behavioural responses for infering the location of the terminal arborizations of sensory nerves in the skin has previously been called into question by Kirk and Denny-Brown (1970). Amongst other observations, they found that systemic injection of sub-convulsive doses of strychnine (which interferes with inhibitory activity in the spinal cord) could dramatically, and almost immediately (within 15 minutes), enlarge the behaviourally determined sizes of dermatomes that were "isolated" by cutting either neighbouring dorsal roots or whole spinal nerves. They suggested that impulses evoked in nerves located in the skin outside the first-mapped dermatome were unable to cause a behavioural response until a tonic inhibition in the central nervous system was removed. Stirling (1973) and Diamond and his collaborators (Aguilar, Bisby, Cooper and Diamond, 1973; Diamond, Cooper, Turner and Macintyre, 1976; Cooper, Scott and Diamond, 1977; Diamond, 1979) recorded the afferent impulses evoked in nerves by mechanical stimulation of the skin in salamanders (Ambystoma) and demonstrated that collateral sprouting of intact nerves occurred after a portion of the cutaneous innervation had been eliminated surgically; the receptive fields of the remaining intact nerves enlarged.

(b) Somatic motor nerves

That intact motor nerves were capable of sprouting collaterals was demonstrated by partially denervating skeletal muscle. The first evidence was indirect. Weiss and Edds (1946) demonstrated that as early as 1 week after unilaterally sectioning the ventral spinal root of L5, which provides some of the innervation to the hind limb muscles in rats, supramaximal stimulation of the adjacent ventral roots (L4 and L6), which also contribute to the sciatic plexus, caused a larger tension to be developed in hind limb muscles than that obtained from corresponding stimulation on the contralateral side. By 6-8 weeks after cutting L5 the muscle tension generated by stimulation of L4 and L6 on the ipsilateral side increased until eventually it equalled that produced by stimulation of roots L4, L5 and L6 on the contralateral unoperated side. They interpreted these results as indicating that the intact L4 and L6 motor nerves on the operated side had sprouted collaterals and reinnervated muscle fibres denervated by the original L5 nerve section. However, the increase in the muscle tension might have been attributable to hypertrophy of the muscle fibres remaining innervated on the operated side. This was shown not to be the explanation by Edds (1950) and Hoffmann (1950), who both demonstrated histologically that one week after partial denervation of muscle remaining intact axons sprouted collateral branches (that originated within 1 mm of their own terminations on muscle fibres) and formed connections with nearby denervated muscle fibres.

(c) Autonomic nerves

Sprouting of intact fibres has also been found to occur after partial denervation of targets in the autonomic nervous system (see Langley, 1900). After unilateral section of preganglionic roots T1-T3, 90% of the fibres to the superior cervical ganglion degenerated, including the nerves responsible for pupil dilation (Murray and Thompson, 1957). One month post-operatively electrical stimulation of the preganglionic nerves of T4-T7 (which normally are not involved in pupil dilation), evoked dilation (Murray and Thompson, 1957; Guth and Bernstein, 1961). Collateral branches of intact preganglionic fibres within the ganglion were detected at the light microscopic level. More recently, Nja and Purves (1977) showed physiologically that post-ganglionic neurones in the superior cervical ganglion may receive "weak" connections from the "non-dominant" segments (T4-T7), and Purves and Lichtman (1978) raised the possibility that the effects observed by Murray and Thompson might represent the sprouting (strengthening) of connections that were already present. Sprouting of intact remaining nerves has also been demonstrated by histological and by intracellular recording techniques following partial denervation of parasympathetic ganglion cells in the frog atrial septum (Courtney and Roper, 1976; Roper, 1976; Roper and Ko, 1978).

(d) Axons in the central nervous system (CNS)

In the CNS too, sprouting of collaterals from intact nerves has been described. This was first demonstrated in cat spinal cord (Liu and Chambers, 1958); six months after isolation of one dorsal root by section of several adjacent roots cranially and caudally on the left side, the arborization of the central process of that root (detected by demonstrating degeneration products after acute section of the root) extended further rostrally and caudally than did those of the corresponding contralateral root. This result was confirmed by Murray and Goldberger (1974) who found that after spinal cord hemisection the central processes of ipsilateral dorsal roots arborized more extensively than usual within the spinal cord as demonstrated autoradiographically and by the distribution of products of degeneration following acute section of dorsal roots. Goodman and Horel (1966) showed by staining for degeneration products that retinal projections to the ventral part of the lateral geniculate nucleus, and to the nucleus of the optic tract, sprouted after these regions were partially denervated by chronic section of the visual cortical efferent fibres. That collateral sprouting in the CNS could lead to formation of synapses was shown electronmicroscopically (Raisman, 1969; Raisman and Field, 1973); after lesions of the medial forebrain bundle (MFB), fimbrial fibres (which normally occupy the dendrites of neurons in the septal nuclei) sprouted to occupy synaptic sites on the cell soma that were vacated as a result of the lesion. Similar sprouting and formation

of new synapses by remaining intact fibres has been demonstrated electronmicroscopically after partial denervation in the ventral cochlear nucleus of the adult rat (Gentschev and Sotelo, 1973) and in the nucleus gracilis of the cat (Rustioni and Sotelo, 1974). Intact adrenergic fibres in the central nervous system also undergo extensive collateral sprouting after neighbouring adrenergic axons are cut (Stenevi, Bjorklund, and Moore, 1973; Moore, Bjorklund and Stenevi, 1973). Sprouting by intact neurons in the hippocampus after lesion of the entorhinal cortex has been studied in some detail (Cotman and Lynch, 1976); electrophysiological investigations have shown that new synapses formed by remaining intact nerves become functional between 9 and 15 days after the lesion (Steward, Cotman and Lynch, 1973, 1976; reviewed by Cotman and Nadler, 1978).

3. Evidence for Sprouting in Mature Animals in the Absence of Degenerating Nerves

The nature of the stimulus (or stimuli) which leads to sprouting of intact nerves has not yet been clearly defined. The observations that regenerating fibres appear to seek out and enter the degenerating distal portion of a nerve led Cajal (1919, 1928) to propose that an attractant substance was released from the degenerating distal stump of the nerve. It has also been thought that after section of nerves a stimulus for nearby intact axons to sprout was provided by "products of nerve degeneration" (see Edds, 1953; Brown, Holland and Ironton, 1978). Weiss and Taylor (1944) however obtained evidence that did not support the hypothesis

that regenerating nerves responded to some factor released from nerve debris. They directed a cut nerve into the long end of a "Y" shaped arterial sleeve, one branch of which contained portions of degenerating nerve. After the nerves had regenerated through the arterial sleeve, there was no difference in the number of regenerating fibres growing into the debris filled branch as compared to the other.

Conclusive evidence that axon growth can be stimulated or evoked simply by products of nerve degeneration has proved elusive; other factors have been sought that might account for the sprouting observed after partial denervation of a target tissue. Of particular relevance is the evidence obtained in mature animals that sprouting of intact axons can be evoked in the absence of such "products of degeneration".

(a) Motor nerves to skeletal muscle

Since lesioning peripheral motor nerves leads to paralysis of muscles as well as nerve degeneration, Duchen and Strich (1968) investigated the effects of muscle paralysis produced without nerve degeneration; they injected small quantities of botulinum toxin into gastrocnemius muscles of mice. It had been shown earlier by electronmicroscopy that in toxin treated (paralysed) muscles the motor nerve terminals showed no evidence of degeneration (Thesleff, 1960). With silver staining Duchen and Strich found that sprouting of intact intramuscular axons had occurred. These sprouts did not initially make functional connections with muscle fibres since the

onset of recovery of function was delayed for several weeks. Muscles recovered from such paralysis showed an abnormal branching pattern of axons within the muscle (Duchen, 1970); coincident with the recovery of function, there was an apparent regression of many sprouted terminals. Similar results were obtained by injecting botulinum toxin into the sternomastoid muscles of rats (Watson, 1969). Sprouting of motor nerves also occurs in "motor end-plate disease" of mice (Duchen and Stefani, 1971) and myasthenia gravis in man (Brownell, Oppenheimer and Spalding, 1972). Treatment of motor nerve axons with tetrodotoxin (TTX) which produces paralysis by blocking impulse conduction (Hille, 1970) was also found to induce sprouting of the intact motor axons in the absence of degenerating nerve (Brown and Ironton, 1977). Muscle inactivity is common to all these otherwise different conditions, and Brown and Ironton concluded that inactivity of muscles gives rise to a sprouting stimulus that is effective on nearby motor nerves. The work of Lømo et al. (Lømo and Rosenthal, 1972; Lømo and Westgaard, 1975) has shown that inactivity induced by a local anaesthetic nerve block causes changes (e.g. development of sensitivity to applied acetylcholine in non-synaptic muscle membrane) similar to those after denervation, and that these changes can be prevented by direct stimulation of the muscle (see also Lavoie, Collier and Tenenhouse, 1977; Pestronk, Drachman and Griffin, 1976). When impulse activity was blocked in only a portion of the motor nerve supply to a muscle (Betz, Caldwell and Ribchester, 1980b) there was sprouting of the untreated nerves.

However, this sprouting, demonstrated histologically, was not as extensive as that following partial denervation of the muscle.

(b) Autonomic nerves to uninnervated target tissues

Olsen and Malmfors (1970) obtained excellent evidence suggesting that target tissues can cause nerves to sprout. They completely denervated irides of rats for three months *in situ*; presumably the products of degenerating nerves within the denervated tissue would be removed within that time. They then transplanted a piece of the now nerve-free iris to the anterior chamber of the eye of a host rat. Using the Falk-Hillarp method for histochemically visualizing catecholamines in adrenergic nerves, they demonstrated that the undamaged sympathetic axons of the host iris sprouted to innervate the transplant; after sprouting had occurred the density of innervation in the transplant was similar to that of a normally innervated iris. In other experiments they further noted that implants such as intestinal smooth muscle or arterial smooth muscle were also able to evoke sprouting of the host sympathetic nerves. The density of innervation in these implants however resembled that of the normal donor tissue rather than that of the host iris. Similar experiments involving fetal hippocampus implants into the anterior chamber of rabbit eyes yielded comparable results; the hippocampal pyramidal cells survived and became innervated by sprouts of intact autonomic fibres (Olson, Friedman, Sieger and Hoffer, 1977). The results of these studies strongly suggest that a diffusible factor was produced by these

particular uninnervated tissues, which evoked sprouting of the autonomic nerves, and further that the amount of sprouting is regulated by the target tissue. The sprouting agent suggested by these experiments could well be Nerve Growth Factor (NGF, see 4 below).

(c) Sprouting after colchicine application

Stirling (1973) showed that partial denervation of the skin of the salamander hind leg leads to collateral sprouting of the remaining intact low-threshold mechanosensory nerves, and subsequently Diamond and his colleagues used the hind limb nerves in this animal to investigate the hypothesis that factors transported down the axons may regulate nerve sprouting at the level of the target tissue (see below). Aguilar, Bisby, Cooper and Diamond (1973) confirmed that in the salamander, sectioning the 16th segmental nerve on one side causes the two adjacent nerves (15 and 17) to sprout and completely takeover the denervated muscles and skin of the hind limb. These workers then treated 16th nerves topically with a concentration of colchicine that they showed interrupted fast axoplasmic transport apparently without killing nerve fibres or interfering with impulse conduction. The cutaneous fields and limb muscles supplied by spinal nerves 15, 16 and 17 were investigated 1-3 weeks after colchicine treatment; the motor innervation of muscle and the sensory cutaneous fields of the 16th nerve appeared completely normal and indistinguishable from the control side. However, the 15th and 17th nerves had sprouted into both skin and

muscle supplied by the 16th nerve, just as if the latter had been cut. The possibility that the colchicine treatment caused sprouting by having a direct action on the target tissue (Cangiano and Fried, 1976), or that the colchicine caused a scattered degeneration of only some of the axons or terminals of the treated nerves, leading to sprouting by the remainder (see Edds, 1953), was investigated in a subsequent quantitative study which showed that the distribution and sensitivity of the mechanosensory endings of treated nerves remained normal in limbs in which the adjacent nerves had sprouted (Cooper, Diamond and Turner, 1977). Similarly, application of colchicine to the fimbria of the rat hippocampus induced sprouting and increased synaptic density in the molecular layer of the dentate gyrus in the absence of observable degenerative changes (Goldowitz and Cotman, 1980). Guth, Smith, Donati and Albuquerque (1980), treated spinal nerve L4 in the rat with a concentration of colchicine sufficient to block fast axonal transport of acetylcholinesterase without producing degeneration of the treated nerve (cf. Jackson and Diamond, 1977). Two weeks later, stimulation of the treated L4 resulted in normal isometric tensions in the plantaris muscle; stimulation of L5 however resulted in a larger than normal isometric tension, similar to that obtained by electrical stimulation of L5 after transection of L4. Histological examination of muscles, using combined silver and cholinesterase staining, revealed abnormalities of innervation characteristic of preterminal sprouting, and numerous examples of dually-innervated muscle fibres were seen in every experimental specimen examined (but not in normal control muscles).

4. Identity of Sprout Promoting Agents

Nerve growth factor (NGF, Levi-Montalcini and Angelleti, 1968) remains the only clearly identified tissue agent affecting sympathetic and sensory neurons "trophically" (Varon and Bunge, 1978). NGF can also cause nerves to grow towards sources of this substance (Campenot, 1977; Letourneau, 1978). The synthesis, storage, and biological effects of NGF produced by target tissues of sympathetic neurons has recently been reviewed (Harper and Thoenen, 1980; Greene and Shooter, 1980). It has recently been reported that NGF activity was not detectable by bioassay in irides immediately after their excision; within 24 hours however detectable levels of NGF were found in irides explanted to culture on grafted into a host eye. Furthermore, denervation of this tissue in situ evoked the appearance of NGF activity within 10 days (Ebendal, Olson, Sieger and Hedlund, 1980). These authors also reported that the NGF activity of denervated irides falls to undetectable levels concomitant with reinnervation of the iris grafts. These results provided the first demonstration that the level of NGF in a tissue in situ may be regulated by its innervation.

Van Harreveld (1947) claimed that sprouting of remaining motor nerves in partially denervated sartorius muscle of the rabbit was enhanced when the nearby quadriceps muscles were totally denervated. He further demonstrated that partially denervated muscles, injected with ether extracts of denervated muscles, eventually developed a significant extra increase in muscle tension as compared

to those injected with extracts of normally-innervated muscle. Hoffman (1950) similarly claimed that ether extracts of denervated muscle, spinal cord, CNS white matter, and egg yolk, all could provoke nerve sprouting when injected into partially denervated or normal muscles, as evaluated by gold chloride nerve staining. Attempts to identify the active substance (neurocletin) were not successful (Hoffman and Springell, 1951). More recently Tweedle and Kabara (1977, 1978) corroborated the previous work of Van Harreveld and found that lipophilic material found in denervated muscles (but not in normal muscles nor in degenerating nerves) can stimulate sprouting of intact nerves. Brown, Holland and Ironton (1978) reported that sectioning of dorsal roots produced sprouting of motor nerves in otherwise undenervated skeletal muscles in the rat; since nerve sections were carried out distal to the dorsal root ganglia they suggested that the observed sprouting of the motor nerves was in response to a factor released within the muscle by degenerating sensory nerves (see also Section 2.6).

Other than that for NGF, evidence for the specific identity and mechanism of action of possible sprouting factors has not yet been obtained.

5. Regulation of Collateral Sprouting at the Target: An Hypothesis

An hypothesis has been proposed by Aguilar, Bisby, Cooper and Diamond (1973) to explain how nerve sprouting is regulated; this hypothesis is a more general form of one suggested by Ramon y Cajal (1919) to explain sprouting during primary development. It was proposed by Diamond et al. that the target tissue manufactures a

substance that stimulates intact nerves to sprout and that the nerves provide factor(s) at their endings, carried there in the fast transport system, which in some way neutralizes or offsets the effects of a growth promoting substance or causes it to cease being produced; sprouting ceases when the effects of the nerve factor(s) balance that of the target stimulus. Evidence in support of this hypothesis has been obtained from experiments on amphibian peripheral sensory nerves (Aguilar, Bisby, Cooper and Diamond, 1973), mammalian skeletal motor nerves (Guth, Smith, Donati and Albuquerque, 1980), and mammalian CNS (Goldowitz and Cotman, 1980). In each case disruption of axonal transport in one set of nerves by topical application of colchicine resulted in sprouting of other nerves which shared the target region (see Section 2.3c). Also consistent with this hypothesis are the results of experiments in which target organs or tissues are partially denervated (see Section 2.2); the axonal lesions would be expected to reduce the neural factors previously supplied by axoplasmic transport in the lesioned nerves. The observation that nerves sprout after exposure to botulinum toxin (Duchen and Strich, 1968; Watson, 1969) would be consistent with the hypothesis if the toxin prevents the release of the hypothetical neutralizing factor(s) as well as the release of acetylcholine at the nerve terminal. According to the hypothesis, in all the above cases the sprouting and formation of "extra" endings will continue until the imbalance existing between the target produced stimulus to sprout and the neutralizing factor produced by nerve endings is resolved (Diamond, Cooper, Turner and Macintyre, 1976).

6. Initiation of Sprouting: Other Observations

Morphological studies on the endings of nerves in skeletal muscle (Barker and Ip, 1966; Tuffery, 1971), cornea (Zander and Weddell, 1951b), skin (Burgess, English, Horch and Stensaas, 1974), and brain (Sotello and Palay, 1971) have led to the suggestion that new endings normally are continually being formed perhaps as others degenerate.

Recently Rotschenker (1979) showed by intracellular recording from muscle fibres that denervation of the cutaneous pectoris muscle on one side of the frog induces the formation of new "extra" synapses on the fibres of the normally innervated homologous muscle on the opposite side. The delay with which new synapses were formed on the unoperated side was dependent upon the distance from the spinal cord of the contralateral axotomy, suggesting that the signal for sprouting may arise in the damaged nerves at the site of injury and is then communicated transneuronally within the spinal cord to the intact motor nerves on the opposite side. Erulkar (1980), by recording intracellularly from the motor nerves on one side of the frog's spinal cord and antidromically stimulating the motor nerves on the opposite side, obtained preliminary evidence that motor nerves may be bilaterally electrically coupled; this then could indicate a means of transfer of small molecules between these nerve cells.

As previously noted sensory fibre degeneration in muscle with a normal motor innervation has been shown to affect muscle membrane sensitivity to acetylcholine and also to lead to terminal sprouting of the intact motor nerves (Brown, Holland and Ironton, 1978).

It seems likely that in the skeletal neuromuscular system, and possibly other systems also, there may be more than one condition sufficient to initiate sprouting of nerves; specific signals, or a common mechanism of action for the initiation or regulation of sprouting have not yet been conclusively demonstrated.

7. Evidence for a Failure of Sprouting to Occur

Although there is clear evidence that sprouting of intact nerves in the central nervous system can occur (Section 2.2c; 2.3c), there is also evidence that such sprouting fails to occur following partial deafferentation of certain central nuclei. In kittens and adult cats no sprouting of cervical primary afferents onto the spinal nucleus of the trigeminal nerve could be detected, either histologically or physiologically, even a year or more after trigeminal denervation (Kerr, 1972, 1975a, b); sprouting of cervical primary afferent axons was expected since trigeminal denervation results in partial deafferentation of the dorsal horn of the spinal cord at the level C1. Absence of sprouting in the dorsal column nuclei has also been reported after chronic partial deafferentation (Rustioni and Molenaar, 1975). Sprouting of auditory nerve fibres into the medial superior olive was not detected after removal of the antero-ventral cochlear nucleus (Liu and Liu, 1971; White and Nolan, 1974). There are also several reports of failure of central aminergic neurons to sprout in deafferented regions such as the superior colliculus, lumbar spinal cord, hypothalamus, and other regions

of the central nervous system where there is normally a demonstrably low density of existing adrenergic innervation (Stenevi, Bjorklund and Moore, 1973). A lack of intralaminar sprouting of retinal axons in partially denervated lateral geniculate nuclei of monkeys was reported by Stelzner and Keating (1977), and similarly, after removal of the optic input, in the adult cat the lateral geniculate nucleus does not become reinnervated by sprouts (Guillery, 1972). More recently, Field (1980) has shown a failure of fimbrial axons to sprout and reinnervate the bed nuclei of the stria terminalis.

Edds and Small (1951) partially denervated leg muscles in adult monkeys and found functional and histological evidence that intact motor nerves in these muscles sprouted very little if 25-50% of the motor nerves had been cut and not at all if 80-90% of the normal innervation had been removed. In partially denervated neonatal rat muscles remaining intact motor axons continue to retract synapses (a normal developmental phenomenon), albeit with a slower time course than normal (Brown, Jansen and Van Essen, 1976; Thompson and Jansen, 1977). One suggestion raised by these studies was that in the young rat motor nerves are dominated by an intrinsic requirement to reduce the number of synaptic connections (but see Section 2.9b). Recently, Dennis and Harris (1980) demonstrated that although cut nerves could regenerate readily, intact thoracic motor nerves did not sprout following partial denervation of the intercostal muscles in neonatal rats.

8. Evidence that the Ability of Nerves to Sprout May Depend on the Age of the Animal

Clinical case studies (Head and Sherren, 1905; Onne, 1962; Peacock, 1963) indicate that complete recovery of sensory function after peripheral nerve lesions is rare in adults but may be more common in children under 11 years of age (see also Leonard, 1973). As has been previously noted, however, subjective reporting on the return of sensation as evidence for sprouting of intact nerves must be viewed with caution (Kirk and Denny-Brown, 1970). In an earlier cited study (Weddell, Guttmann and Gutmann, 1941) the authors stated simply that recovery after denervations carried out on the skin of four week old rabbits was "more rapid" than that seen in older animals.

Several investigations of plasticity in the central nervous system (see below) have provided clear evidence that the age of the animal in which targets of intact nerves are partially denervated influences the rate and/or extent of sprouting.

In kittens, after removal of the optic input to the lateral geniculate nucleus, there is a slight amount of translaminar sprouting (Guillery, 1972; Hickey, 1975) which is absent under similar denervation conditions in the adult cat (Guillery, 1972). In the neonatal rat hippocampus, ablation of the entorhinal cortex evokes sprouting of the commissural fibres (which share the innervation of the molecular layer of the dentate gyrus); this sprouting is both more rapid and more extensive than that seen after similar lesions performed in the

adult (Lynch, Stanfield and Cotman, 1973; Zimmer, 1973; Gall and Lynch, 1978; Gall, McWilliams and Lynch, 1979). Partial transection of the lateral olfactory tract in the neonatal hamster has been reported to cause extra (abnormal) connections to be formed by sprouts of the surviving lateral olfactory tract fibres only if the lesions were performed before 14 days of age (Devor, 1976). Similarly, if one eye is removed not later than 10-12 days of age, retinofugal projections from the opposite eye have been found to develop aberrant branches that penetrate regions normally innervated by the enucleated eye (Schneider, 1973; Frost and Schneider, 1979). Enucleation of one eye in rats younger than 8 days of age has also been shown to result in extra branching of retinofugal fibres (Lund, Cunningham and Lund, 1973; Lund and Lund, 1976); in neither rats nor hamsters does enucleation of one eye in the adult produce these extra branches. It has been shown that if the superficial layers of the right superior colliculus of the newborn hamster are removed, fibres from the left eye terminate not only in the surviving deep layers of the right superior colliculus, but also cross the tectal midline and terminate in the medial third of the left superior colliculus; if the right eye is also removed at birth, the abnormally recrossing fibres from the left eye will spread over the entire surface of the left superior colliculus (Schneider, 1973). More recently, So and Schneider (1978) performed similar experiments including eye removal at later postnatal ages and found that after a critical age (14 days) is reached, even when

terminal space is available, the axons and axon terminals will not "move" (sic) at least over any appreciable distance.

In other instances the distribution of axonal branches or their connections has been shown to be subject to external influences which may be effective only relatively early in the animal's life. Normal formation of visual circuitry appears to be in some way dependent upon congruent incoming sensory activity; monocular deprivation, performed variously by lid suture or eye enucleation, has been shown to reduce greatly the number of binocularly driven cortical neurons (e.g. Wiesel and Hubel, 1963; Pettigrew and Konishi, 1976). The loss of binocularity occurs if monocular deprivation is imposed during a critical period, which extends into the third postnatal month (Hubel and Wiesel, 1970). Much of the evidence relating to the critical period for the striate cortex has been recently reviewed (Pettigrew, 1978). It is of interest that two independent studies in man have demonstrated the existence of a critical period for human binocular vision development (Banks, Asland and Letson, 1976; Hohmann and Creutzfeld, 1976). Critical, or sensitive, periods, within which changes can be evoked by appropriate experimental manipulations, have been identified for: the role of testosterone in initiating the differentiation of hypothalamic neural circuitry (Gorski, 1971), the dependence of muscle spindles on sensory innervation for normal development (Zelena, 1957, 1964; Werner, 1973), for retinal respecification of Xenopus following 180° rotation of the eye (Jacobson, 1967, 1968), and for the sensitivity of spinal sensory ganglion cells of chick embryo to nerve growth

factor (Weis, 1970, 1971). The times when various critical conditions or stimuli are effective are concentrated in the same relatively short period early in life for birds and mammals, the animals in which such critical periods have been most extensively studied (reviewed by Thorpe, 1964; Scott, Stewart and DeGhett, 1974; Gottlieb, 1976).

9. Evidence for Competition Between Nerves During Primary Development

(a) Cell death

The naturally occurring cell death during embryogenesis, which has been reported in most regions of developing nervous systems (reviewed by Cowan, 1973), can become more pronounced if the peripheral target is removed prior to its innervation (Shorey, 1909; Hamburger, 1934, 1958; Prestige, 1967; Chu-Wang and Oppenheim, 1978). This reduction in neuron number, usually assessed histologically, occurs about the time neurons make peripheral connections (Landmesser and Pilar, 1974; Landmesser and Morris, 1975; Hamburger, 1975). Histological and ultrastructural investigations have indicated that degenerating neurons had in fact sent axons to the target (Landmesser and Pilar, 1974; Landmesser and Pilar, 1976; Clark and Cowan, 1976; Oppenheim and Chu-Wang, 1977). In converse situations the addition of supernumerary limbs (extra target) to developing amphibian (Detwiler, 1936) and chick (Hamburger and Keefe, 1944) embryos produced an increase in the number of dorsal root ganglion cells of those segments contributing to innervation of the extra limb. It would seem possible then that growing peripheral structures attract nerves towards them (Detwiler, 1936; Speidel, 1941; Levi-Montalcini, 1966) and

these nerves may compete for some aspect of the target necessary for their survival (Hamburger, 1958).

In another approach, instead of increasing the size of the target, Pilar et al. surgically reduced the number of axons available to compete for the ciliary muscles of the chick eye (Pilar, Landmesser and Burstein, 1980); cutting selected nerve bundles to the ciliary muscles in ovo led to a reduction (by approximately 40%) of cell death amongst the remaining intact neurons. Retrograde labelling of these nerve cell bodies with horseradish peroxidase identified their locations and physiological examination of the innervation of the ciliary muscle indicated that the "rescued" neurons were able to form functional connections with the target. In this situation, as with the supernumerary limbs, survival of developing neurons seemed to be dependent upon successful competition for some factor associated with the peripheral target; experimental removal of a portion of the competing nerve supply apparently allowed remaining nerves to be successful in establishing and maintaining connections in the end organ. Evidence has been obtained that a similar matching of neuronal number with target size during development occurs in the sympathetic nervous system (reviewed by Purves and Lichtman, 1978; Purves and Lichtman, 1980).

Of particular relevance to this thesis are observations that neurons may continue to compete for successful innervation of end-organs or targets following the primary development of the nervous system (and the matching of pre- and post-synaptic populations). Evidence for a continuing competition between nerves is presented below.

(b) Synapse elimination

Redfern first showed that skeletal muscle fibres in neonatal mammals are innervated differently than they are in the adult (Redfern, 1970). The intracellular post-synaptic potential recorded from the majority of muscle fibres in mature animals is all or none, indicating that a single axon contacts each muscle fibre. In the neonatal muscles studied by Redfern, multiple end-plate potentials were elicited by increasing the strength of nerve stimulation, indicating that the terminals of more than one axon connect with each muscle fibre. This work was confirmed and extended by Bagust, Lewis and Westerman (1973), Brown, Jansen and Van Essen (1976) and Thompson and Jansen (1977). By applying similar electrophysiological techniques, this polyneuronal innervation was shown to decrease gradually with increasing age; the 1:1 ratio of the mature muscle being established by a few weeks after birth. The number of motor units does not change during the period of synapse elimination (Brown, Jansen and Van Essen, 1976) showing that the number of motor nerve fibres was not being reduced by cell death; that the synapse elimination on the individual muscle fibres involved physical retraction of nerve endings was proposed on morphological grounds (Korneliussen and Jansen, 1976).

Electrophysiological investigation of neonatal autonomic ganglia has produced evidence that during development there is a hyperinnervation of target cells with subsequent elimination of connections; intracellular recordings from neurons in the parasympathetic submandibular ganglion of the rat have shown that at

birth each neonatal ganglion cell is innervated by an average of five different axons. Synapse elimination occurs during the first month of life until the mature pattern of one cell receiving innervation from one axon is established (Lichtman, 1977). Similar synapse elimination during early neonatal life, suggested to occur in the sympathetic superior cervical ganglia (Landmesser and Pilar, 1978), has been shown (Lichtman and Purves, 1980).

There is evidence of synapse elimination in the central nervous system too. In the cerebellum of the mature rat each Purkinje cell is contacted by a single climbing fibre; in newborn rats however, electrophysiological evidence has been obtained that Purkinje cells are innervated by more than one climbing fibre, of which all but one relinquish their connections over the first few weeks of life (Crepel, Mariani and Delhaye-Bouchaud, 1976).

The results of all the above investigations suggest that there is competition between neurons for innervation of targets, and that this competition is resolved in many instances fairly late in development, even after birth of the animal. In rat soleus muscles, partially denervated at 1-5 days of age, the motor unit size of intact remaining axons decreases almost to the normal adult value even when there are nearby denervated muscle fibres present, suggesting that in addition to a competitive mechanism of elimination, many synapses are withdrawn by a mechanism intrinsic to the individual motor neurons (Brown, Jansen and Van Essen, 1976; Thompson and Jansen, 1977). More recently however Betz, Caldwell and Ribchester (1980a) found that isolated motor units in partially

denervated rat lumbrical muscles maintained their expanded neonatal size and did not decrease to the normal adult level; this result was obtained from partially denervated muscles in which only a single motor unit remained intact. Their conclusion was that, at least in the rat lumbrical muscle, the normal post-natal reduction in motor unit size depends entirely on the competition between motor units.

10. Experimental Evidence that Competition Between Nerves Can Occur in Mature Systems

One approach to the investigation of competitive interactions between nerves has been to deprive a target of its innervation, allow (or induce) it to become innervated from another (foreign) source and then to allow the original (native) nerve to grow back to the former target. This has been most extensively studied in the skeletal neuromuscular system (see below).

(a) Regenerating nerves: foreign and appropriate

The most extensive investigations of regenerating nerves in potentially competitive conditions have been carried out in the skeletal neuromuscular system. Regeneration of motor nerves to specific targets has been recently reviewed (Landmesser, 1980).

In teleost fish, Mark and his collaborators (Marrotte and Mark, 1970a, b; Mark and Marrotte, 1972; Mark, Marrotte and Mart, 1972) cross-reinnervated extraocular muscles and allowed the original nerves to regenerate. Using principally behavioural criteria they concluded that in cross-reinnervated extraocular muscles the function of foreign synapses was suppressed upon re-

innervation by the correct nerve and that the absence of degenerating nerve terminals was evidence that the suppressed foreign synapses were morphologically normal, but non-functional (silent). Scott (1975, 1977) repeated and extended these experiments using intracellular recording from such muscles and found that in animals exhibiting apparent behavioural suppression of a foreign innervation, stimulation of the foreign nerve produced end-plate potentials in dually innervated muscle fibres; innervation persisted even after regeneration of the correct nerve. Similarly, intracellular recordings from cross-reinnervated gill muscles in perch produced evidence that foreign synapses survived when the original nerve was allowed to regenerate and reinnervate its former target (Frank and Jansen, 1976).

In amphibia, Cass, Sutton and Mark (1973) cut and rerouted the two major motor nerve branches to axolotl hind limbs and observed the subsequent behavioural recovery and innervation of antagonist muscle groups by the two regenerating nerves. Stimulation of the original extensor or flexor nerves during the onset of behavioural recovery failed to demonstrate that muscles had become functionally cross-reinnervated; when examined between 1½-9 months later, the appropriate muscles contracted to stimulation only of their original nerve trunk. In salamander forelimbs, flexor motor nerves were induced (by surgical transposition) to form connections with denervated extensor muscles; the flexor muscles were removed. Intracellular recordings from the cross-reinnervated extensor muscle

fibres showed that upon the return of the correct nerve, synaptic transmission from the foreign nerve was suppressed; the suppression involved a reduction in quantal content of transmitter release (Dennis and Yip, 1978). From the latter experiments two lines of evidence indicated that suppressed foreign nerve endings are retracted from the muscle: (1) a second lesion of the correct nerve after the observed suppression of foreign transmission produced no significant increase in the proportion of fibres exhibiting foreign transmission and (2) muscles which showed complete suppression of foreign transmission were removed, bathed in medium containing horseradish peroxidase (HRP) and the correct nerve was repetitively stimulated; HRP is taken up by active nerve terminals (Heuser and Rees, 1973). Histochemical staining and examination of synapses by electronmicroscopy demonstrated that ≈ 94% of the axon terminals had HRP incorporated into vesicles; at least that percentage of all identifiable synapses were from the correct nerve. A variety of studies in amphibia investigating the ability of regenerating original and foreign nerves to re-innervate denervated muscle fibres has indicated that although the initial reinnervation may be non-selective (either nerve being successful) some form of competition or selection ensues which results in correct reinnervation. In toads, Hoh (1971) cut the common nerve trunk which supplies motor innervation to both a fast and a slow muscle and studied the subsequent reinnervation. After 5-7

months he measured the contraction characteristics of each muscle and found that each had acquired and retained only correct nerve innervation. Schmidt and Stefani (1976) found that after cutting the nerve to a muscle, which contained both fast and slow fibres, fast axons regenerated more quickly and were able to form functional connections on both fast and slow fibres; the functional connections with slow fibres were suppressed upon regeneration by the native slow axons. Surgical transposition of frog skeletal muscle to the dorsal lymph sac and subsequent reinnervation of them by two regenerating foreign nerves (Grinnell, Rheuben and Letinsky, 1977; Grinnell, Letinsky and Rheuben, 1979) showed that each of the foreign nerves was able to takeover portions of the denervated muscle when allowed to regenerate either simultaneously or in a staggered fashion. The regions of muscle responding to single shocks of either nerve did not overlap; however, tetanic stimulation of each motor input generated 80-90% of the tension developed by direct muscle stimulation. Thus some mutual suppression of synaptic efficiency by both foreign inputs had occurred. Intracellular recordings showed that in dually innervated muscle fibres one of the inputs exhibited a quantal content sufficiently small to render that input ineffective for eliciting muscle contractions after single stimuli.

If, in mammalian muscle, the original nerve supply is cut (Elsberg, 1917), poisoned with botulinum toxin (Fex, Soneson, Thesleff and Zelena, 1966), or reversibly blocked with local anaesthetics (Jansen, Lømo, Nicolayson and Westgaard, 1973) foreign

axons that had been surgically transposed will regenerate and innervate the muscle. Gutmann and Hanzlikova (1967) observed that foreign innervation of a mammalian muscle did not prevent subsequent reinnervation by the original nerve. In a series of experiments utilizing electrophysiological as well as histological techniques, Frank, Jansen, Lømo and Westgaard (1975) transplanted the fibular nerve onto the soleus muscle, and then they interrupted the original soleus muscle nerve to allow the foreign nerve to form connections; regeneration of the original nerve was not prevented. The extent of reinnervation by the original nerve depended upon the method of denervation. Virtually complete reinnervation of muscle fibres including those already innervated by the foreign nerve, was achieved by the original nerve if it had sustained only a single crush. When reinnervation by the original nerve was delayed by resecting a segment of it, only muscle fibres without foreign nerve innervation were reinnervated. Denervation by simply cutting the original nerve gave an intermediate result. Twitch tensions of soleus muscles produced by stimulation of the foreign nerve were not reduced following reinnervation by the correct nerve indicating no suppression of foreign synapses after reinnervation of the soleus by its original nerve supply. In other experiments on mammalian skeletal muscle, foreign transmission was also found to persist upon reinnervation by the correct nerve (Bernstein and Guth, 1961; Tonge, 1974; Brown, Jansen and Van Essen, 1976).

Potentially competitive conditions have been produced in the mammalian autonomic nervous system where denervated superior cervical ganglia were simultaneously reinnervated by regenerating

original and foreign nerves (Purves, 1976a). The cervical sympathetic trunk and the nearby vagus nerve were cut and positioned such that the foreign (vagal) axons grew into the ganglion along with the regenerating original fibres. Intracellular recording from ganglion cells revealed that initially about 25% were reinnervated by the vagus nerve alone, and 25% by the original nerve alone; the remaining 50% of ganglion cells received contacts from both original and foreign nerves. Approximately the same fraction of ganglion cells remained dually innervated a year or more after the initial operation and therefore under these circumstances competitive suppression of foreign terminals by original axons did not take place.

In experiments on the mechanosensory innervation of salamander skin, Scott, Macintyre and Diamond (1981) showed that the first regenerating axon to contact the target Merkel cell (Parducz, Leslie, Cooper, Turner and Diamond, 1977) effectively captures it whether or not the axon belonged to the nerve bundle originally supplying that area; no evidence was obtained indicating that a more appropriate nerve, for that location, could displace or suppress other innervation.

(b) Intact versus regenerated nerves

Competitive conditions have also been produced following partial denervation of target tissues; where this leads to sprouting of the remaining intact innervation (see 2.2) the ability of the lesioned nerves to reestablish functional connections with their original targets have been examined.

Using histological and behavioural techniques Zander and Weddell (1951a) found that denervated regions of the rabbit cornea became reinnervated, first by branches of surviving intact nerves and later by regenerating lesioned nerves; histological examination indicated that the sprouted intact nerves withdrew from the originally denervated territory as the regenerating nerves arrived. The absence of demonstrable degenerating debris from these sprouts led to the authors to suggest that the evoked collateral sprouts retracted rather than died. It has recently been shown that lesions of the sciatic nerve in the hind leg of the rat produced a subsequent expansion (sprouting) of the residual innervation in the rat foot supplied by the intact saphenous nerve (Devor, Schonfeld, Seltzer and Wall, 1979). Following regeneration of the lesioned sciatic nerve, behavioural and electrophysiological examination of the innervation of the rat foot showed that the previously expanded receptive fields of high-threshold mechanosensory afferents in the saphenous nerve returned to their normal control sizes.

In contrast, cutaneous mechanosensory nerves in the salamander that had sprouted into denervated skin did not retract when regeneration of the lesioned nerves was allowed (Scott, Macintyre and Diamond, 1981). Competition between these nerves appears to be simply a matter of timing; the first axon that reached the denervated Merkel cells established a permanent connection regardless of whether that axon was regenerating or sprouting.

In the skeletal neuromuscular system evidence that regenerating nerves are able to displace or suppress endings formed as a result of

sprouting by intact nerves has been obtained chiefly in amphibia. Cass, Sutton and Mark (1973) cut segmental nerve 16 in the hind limb of salamanders; electrophysiological evidence was obtained indicating that motor nerves in segmental nerve 17 sprouted to innervate denervated muscle fibres. Upon regeneration by nerve 16 however, nerve 17 was found to functionally innervate only those muscles that it had supplied prior to the original lesion. Since a second lesion of nerve 16, after its successful regeneration, produced a functional expansion of nerve 17 within 3 days (as opposed to 3 weeks as seen after the original lesion) they concluded that the regenerating nerve had successfully suppressed the sprouts of nerve 17 without causing their physical retraction. Similarly, Bennett and Raftos (1977) cut nerve 16 to the hind leg of salamanders; sprouting and regression of synapses on denervated muscles formed by sprouts of nerve 17 was assessed using intracellular recording techniques. As the cut nerve 16 reestablished connections with its former muscle fibres, the sprouted endings of nerve 17 were found to undergo a reduction in quantal content to the point where they were undetectable. Similar results have also been obtained in investigations of sprouting and regression of synapses in axolotl musculature (Genat and Mark, 1977). In experiments on axolotl hind limb musculature it was found that if regenerating cut nerves were delayed, for approximately 50 days, they were unable to functionally displace sprouted intact ones (Slack, 1978). It would seem therefore that if sufficient time is allowed for the maturation of newly formed synapses by sprouts (see Bennett and Raftos, 1977; Thompson, 1978) they

are able to successfully compete with regenerating nerves that originally supplied that muscle. Evidence for sprouting and regression of sprouts in the superior oblique muscles of Xenopus has been obtained using both histological and electrophysiological techniques (Fangboner and Vanable, 1974; Fangboner, 1979). Oculomotor nerves sprouted into superior oblique muscles denervated by cutting the trochlear nerve; following regeneration, stimulation of the oculomotor nerve ceased to elicit contraction of the superior oblique muscle. Histological evidence was obtained that the sprouts of the intact oculomotor nerve degenerated after the regenerating trochlear nerve arrived.

Brown and Ironton (1978) examined both the capacity of motor nerves to sprout after partial denervation of muscle in the mouse and the ability of the regenerating axons subsequently to suppress endings of sprouted intact ones. Motor units remaining intact after partial denervation of muscle were found to expand, completely innervating the muscle within 12 days; reinnervation of the muscle by the severed motor axons occurred from 14-19 days after the injury. Following reinnervation by the original nerve, the size of sprouted motor units decreased. However the regenerating axons innervated fewer fibres than normal and the sprouted ones continued to innervate more fibres than normal; approximately 10% of the muscle fibres remained functionally innervated by both the intact, sprouted nerve and the regenerated, original nerve.

After crushing the upper thoracic ventral roots in the cat, removing about 90% of the innervation to the superior cervical ganglion (SCG), the residual nerves supplying the SCG from lower thoracic segments were shown to sprout collaterals (Murray and Thompson, 1957). This work was confirmed and significantly extended in experiments where the crushed nerves were allowed to regenerate (Guth and Bernstein, 1961); six weeks after crushing of the preganglionic roots T1-T3, electrical stimulation of the preganglionic nerves of T4-T7 (which normally are not involved in pupil dilation), evoked dilation. However, after a further six months, regeneration of the originally crushed axons had occurred, and the pupil again dilated in response to stimulation of roots T1-T3 but stimulation of T4-T7 no longer produced pupil dilation. The interpretation offered was that most of the synapses formed by the earlier sprouting within the ganglion were functionally replaced (or suppressed) by the regenerating appropriate axons (but see Purves, 1976a; Nja and Purves, 1977). Sprouting of residual intact vagal axons occurs after partial denervation of the cardiac ganglion in the frog (Courtney and Roper, 1976); 1-6 days following crush of one vagus nerve, the proportion of cells receiving dominant functional contact from remaining contralateral intact vagus nerves was found to increase; following regeneration of the crushed vagus, intracellular recordings demonstrated that the proportion of cells receiving dominant functional contact from the sprouted nerve declined as connections were reestablished by the original nerve (Roper, 1976; Roper and Ko, 1978).

11. Spatial Constraints on Intact Sprouting Nerves: The Domain Hypothesis

There is evidence that collateral sprouting of intact cutaneous mechanosensory nerves is, in the salamander, under a form of territorial, or spatial, regulation (Diamond, Cooper, Turner and Macintyre, 1976; Macintyre and Diamond, 1981). The experiments involved partial denervation of the salamander hind limb and direct electrophysiological measurement of sprouting by the remaining intact cutaneous innervation. It was found that when skin within the segmental field was partially denervated, the sprouting of the remaining intact mechanosensory nerves of that segment was able to reconstitute the density and distribution of the original field within 2-3 weeks (Cooper, Diamond and Turner, 1977). However, for a period of 2 months or longer, segmental cutaneous nerves failed to enlarge significantly into adjacent denervated skin formerly occupied by a neighbouring spinal nerve (Macintyre and Diamond, 1981). The region of skin within which intact axons would readily sprout was defined as the "domain" of the segmental nerve. The borders of these domains do not always coincide exactly with those of the segmental mechanosensory dermatomes. In some experiments the fields of nerves 15 and 17 were smaller than usual and clearly not abutting (Aguilar, Bisby, Cooper and Diamond, 1973); following section of nerve 16, nerves 15 and 17 sprouted beyond their dermatomes (defined as that region originally innervated). These nerves expanded into territories which, in the majority of animals, are the usual ones for these nerves. In the latter

set of experiments therefore the segmental nerve domains were somewhat larger than the originally observed segmental dermatomes; in general though, the borders of the domain and those of the corresponding dermatome have been found to be not greatly different.

Devor, Schonfeld, Seltzer and Wall (1979) observed that the behaviourally assessed recovery of sensory function in denervated skin of the rat foot due to sprouting of the intact saphenous nerve never extended into the lateral two toes. They concluded that, since the same phenomenon was observed in similarly denervated skin of neonatal rats (which are much smaller in total skin area), the cessation of behavioural spread was not due to an upper limit on sprouting being reached. Although the authors did not comment upon the possible existence of a spatial mechanism acting to restrict the growth of these intact nerves, the results suggest that high threshold mechanosensory nerves in mammals may also respect "domain" boundaries. Regenerating cutaneous nerves in both the rat, and salamander were able to grow freely into denervated skin from which intact nerves appeared to be excluded.

SECTION 3

The Principal Aim and General Strategy of the Investigations

The principal aim of this study was to define spatial and temporal constraints that operate in the recovery of low-threshold mechanosensory function in denervated mammalian skin. The investigation was prompted in part by results obtained in studies of cutaneous nerve sprouting in the salamander (see Background), and in particular by the unexpected results of a Pilot Study carried out on the cutaneous innervation of adult rabbits. The latter results suggested that intact low-threshold mechanosensory nerves in mammalian skin may not sprout into denervated skin. Because the extent of the investigations with rabbits were limited, in comparison to the studies on the rat that constitute the body of this thesis, the results of the rabbit work have been sequestered at the end (Appendix I).

The first necessity was to select a mammalian preparation in which a moderately large region of skin is innervated by known and accessible cutaneous nerves, whose fields are so distributed that isolation of one could be achieved by section of neighbouring nerves; in addition, a "whole nerve" extracellular recording should reveal easily-detectable impulse activity in single axons. It was hoped too, that such nerves would be of sufficient accessable length to allow for whole nerve recordings to be made from them "in continuity", i.e. without cutting them.

The known segmental "banding" of mammalian sensory dermatomes, particularly in the trunk region, together with the observation that trunk skin is often loosely bound to the underlying body wall (suggesting the likelihood of long lengths of sub-cutaneous nerve bundles), led to an examination of the dorsal skin of the rat.

The Dorsal Cutaneous Nerves (DCNs) of the rat, and their receptive fields in the skin, were found to satisfy the experimental requirements. The general strategy adopted was to "isolate", in skin, the low-threshold mechanosensory receptive field of a selected intact nerve by cutting some or all of the neighbouring cutaneous nerves supplying adjacent skin, and then to examine whether or not the selected nerve established low-threshold mechanosensory endings in the denervated skin. Functional sprouting of the intact, but isolated, nerve would be measured as an increase in the area of the low-threshold mechanosensory receptive field of the selected nerve. Throughout this work the evidence for the presence of low-threshold mechanosensory nerve endings in the skin is physiological; that is, when appropriate tactile (mechanical) stimuli evoked detectable impulses in a nerve, that nerve was accepted as innervating that skin. Since mechanoreceptive field areas can be influenced by stimulus spread (stronger stimuli can result in larger measured areas) a standardized stimulus was used, principally involving displacement of hairs and excitation of the associated tactile mechanoreceptors. A particular experimental advantage is the presence of "touch domes" in the hairy skin; these highly mechanosensitive punctate

sensory structures, readily visible after depilation of the skin, respond characteristically only to vertical and not lateral displacement.

The quantitative analysis of the areas of low-threshold mechanosensory receptive fields, and the number of touch domes supplied by identified cutaneous nerves, thus refer always to functional innervation. In this study any nerve endings that did not acquire mechanosensory function were "invisible" to the technique, and in this sense were regarded as non-existent. Recent results from this laboratory concur with the available literature and support the working assumption that classically "silent" nerve endings probably do not occur in the skin of the rat, and that the physiological analysis of low-threshold mechanosensory nerve distribution in the skin accurately defines the presence or absence of nerve endings (see Discussion).

SECTION 4

Methods

1. Animals

All animals used in this investigation were male and female Wistar albino rats obtained from Woodlyn Farms, Guelph, Ontario. Those older than 20 days of age were separated by sex and housed in groups of 2-3 per cage on wood chip bedding with food and water freely available.

Multiparous pregnant females were purchased, caged individually, and checked each morning for the birth of pups. At 10 days of age (except where noted) the litters were culled and typically, the eight pups closest to the mean litter weight were returned to the lactating female; the remainder were killed by ether overdose. Surviving pups were weaned at 20 days of age.

2. Anaesthesia

All surgical procedures were performed on anaesthetized animals using "clean" (but not aseptic) technique. Anaesthesia was induced and maintained in animals 5 or 10 days of age by ether inhalation. In animals 15 days of age and older anaesthesia was induced by intra-abdominal injection of sodium pentobarbitol (Somnotol; M.T.C. Standard) (45 mg/kg). When necessary, anaesthesia was prolonged by supplementary injections (5 mg/kg). Adequate anaesthesia for surgical manipulations was determined by a lack of response by the animal to a tail pinch. Core temperataure was monitored with a rectal thermister probe and maintained at 37 ± 1°C

by a feedback-controlled circulating water pad placed beneath the animal. Anaesthesia and surgery were well tolerated by animals of all ages, and recovery was generally uneventful; in those rare instances where post-operative infections were detected (usually small incision line abscesses) the animal was removed from the study. At the conclusion of each "terminal" experiment, animals were administered a lethal dose of the anaesthetizing agent.

3. Exposure of the Dorsal Cutaneous Nerves

The Dorsal Cutaneous Nerves (DCNs) were exposed through an incision in the skin to the right of, and parallel to, the dorsal midline (see Fig. 1). The incision was made through the full thickness of the skin and usually extended from the tenth rib to the level of the third lumbar vertebra. Gentle traction applied to the wound margin exposed, on both sides, the DCNs of spinal segments T9-L4 at the points where they emerged from the musculature of the body wall (Fig. 2).

4. Denervation of Skin

Depending upon the pattern of peripheral denervation desired, various DCNs or their individual branches were crushed or cut; in the latter case the selected nerves were grasped with watchmaker's forceps and a length of about 10 mm (in older animals) was removed. When regeneration of these nerves was not desired, the central stump was ligated and, with the aid of forceps, thrust into the body wall musculature. Nerve crushes were done with specially flat-ground watchmaker's forceps. The nerve bundle was compressed

FIGURE 1. Right Paramidline Incision

This figure depicts diagramatically the incisions made to the right of, and parallel to, the dorsal midline for the purpose of exposing the dorsal cutaneous nerves (DCNs). The dorsal midline was estimated by placing the animal in a prone position and manually locating the tips of the spinous processes of the vertebrae in the midtrunk region.

A. Incisions were made 2-5 mm to the right of the dorsal midline and through the full thickness of the skin; this exposed the underlying DCNs of the right side.

B. The DCNs on the left side were exposed by applying traction (arrows) to the left margin of the incision. This traction displaced the skin, and the incision, making the left DCNs available for surgical manipulation or electrophysiological recordings; the low-threshold mechanosensory fields of the left DCNs were not, therefore, surgically interfered with by this method of exposure.

FIGURE 1.

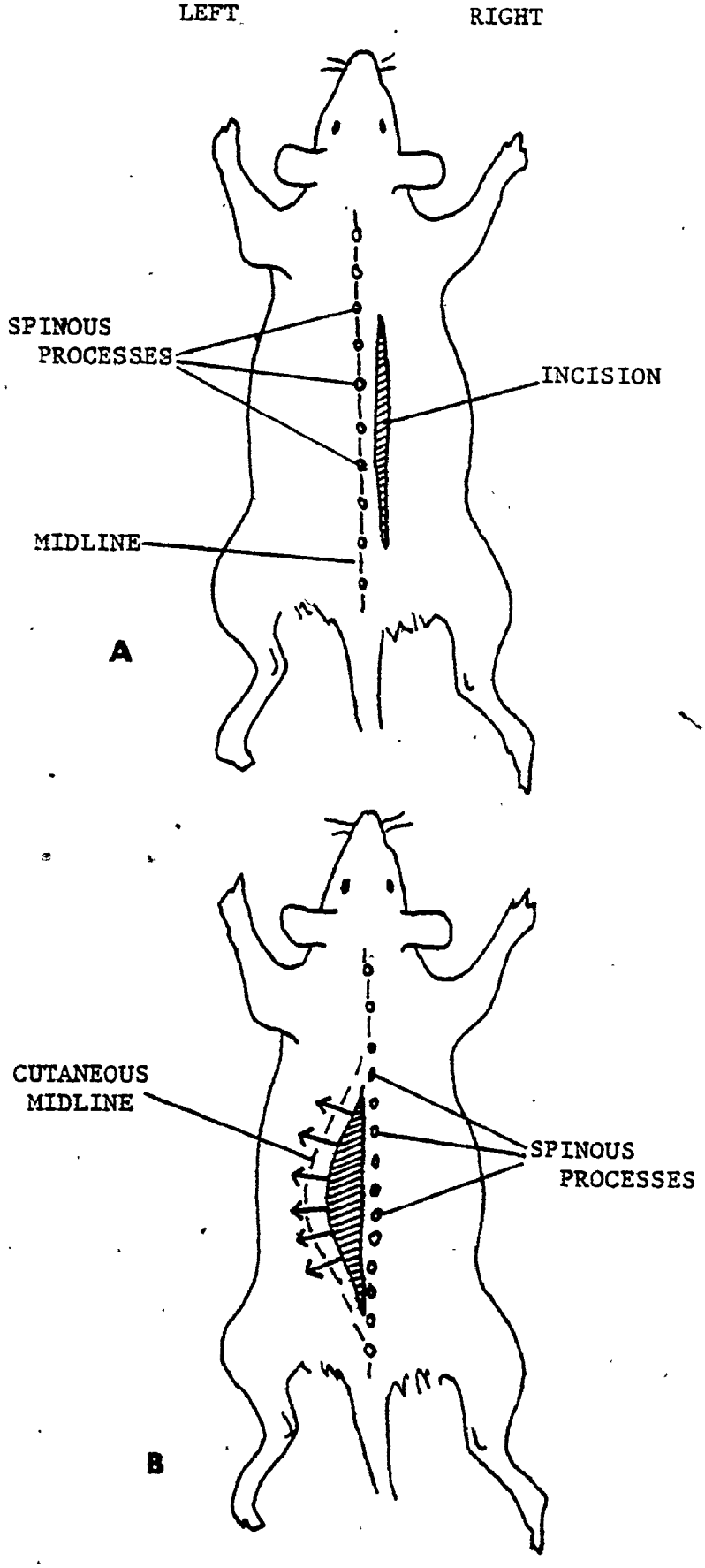

LEFT
RIGHT
SPINOUS PROCESSES
INCISION
MIDLINE
A
CUTANEOUS MIDLINE
SPINOUS PROCESSES
B

FIGURE 2. The Dorsal Cutaneous Nerves

In this photograph the back skin has been reflected to expose four adjacent Dorsal Cutaneous Nerves (DCNs) of T11-L1 at the point where they emerge from the body wall musculature (arrows) on the left side. The exit of DCN-T13 is indicated by the large arrow. The head of the animal is to the left of this photograph; the midline (spinous processes of the vertebrae) is indicated by the interrupted white line. The DCN of T11 (far left) is typically accompanied by a larger blood vessel than that which accompanies the rest. This characteristic, together with the distinctive location of the points of DCN exits, was routinely used to identify DCNs as the segment-of-origin.

several times, each compression lasting 2-3 seconds within a 1-2 mm segment. Immediate and persistent (several days) failure of impulse conduction through the crushed region (assessed by whole nerve recording) testified to the effectiveness of this procedure.

5. Skin Incisions and Repair

Surgical incisions of skin were made using single edged disposable razor blades. With sufficient care the underlying body wall musculature remained undamaged. Following the surgical procedure, in animals scheduled for recovery, incisions were closed in a single layer with interrupted gut sutures inserted through the full width of the skin. Closed incisions were liberally rinsed with sterile saline and then lightly swabbed with a 70% alcohol solution and rinsed again before recovery.

6. Preparation of Nerves for Recording Afferent Impulses

The DCNs of segments T10-L3 were exposed and the branches (medial and lateral, see Fig. 8) of selected DCNs were dissected free of the loosely investing connective tissue over a 5-10 mm length, ligated separately, and cut at the point where they branched. In those experiments in which functional regeneration of previously cut or crushed nerves was to be investiagated, the DCNs were exposed central to the site of the previous nerve lesion through a small incision made in the musculature of the body wall. In these cases the whole DCN was cleared of connective tissue, ligated, and cut. The peripheral stump (with ligature) was then brought through the

incision to rest on the surface of the body wall. During surgical procedures the nerves were kept moist with a Kreb's physiological saline of the following composition: NaCl 118.0 mM; KCl 4.7 mM; KH_2PO_4 1.8 mM; $MgSO_4$ 1.6 mM; $CaCl_2$ 2.5 mM; $NaHCO_3$ 24.9 mM; glucose 10.0 mM.

In several experiments the recordings were made from nerves that were not cut. These nerves, to be recorded from in continuity, were very gently dissected clear of fat and loose connective tissue for a length of 10-15 mm. Special care was taken to avoid damage to any accompanying blood vessels.

7. Mapping of Low-Threshold Mechanoreceptive Fields

(a) Nerve recording

Nerve impulses were recorded extracellularly from multi-axon nerve bundles. Under the usual conditions, in which the nerve was cut just prior to recording, the peripheral stump was raised into air on bipolar platinum electrodes; to hinder drying of the nerve the exposed portion was moistened and then it, and the ends of the electrodes over which the nerve lay, were coated with petroleum jelly. Stable recordings have been made under these conditions for at least six hours without obvious deterioration of the nerve.

In continuity recording from intact axon bundles required that the exposed portion of the nerve be gently raised with fire-polished glass probes onto the electrodes. Special care was taken to avoid placing tension on the nerve. While raised in air, the exposed portion of the nerve was prevented from drying by placing

along it several cotton threads moistened in saline. Recordings under these conditions were generally completed within 1-2 minutes after raising the nerve into air; the nerve was then returned to its original position.

Signals from the recording electrodes were amplified using a Grass P511 AC differential pre-amplifier in line with a Tektronix 5A22N amplifier. Signals were filtered (LF 100 Hz, HF 10 KHz) to obtain optimum signal-to-noise ratios and displayed on a Tektronics D13 dual beam storage oscilloscope, from which photographs were taken with a Tektronics C5 oscilloscope camera; impulse activity was also detected as audible signals by relaying the oscilloscope output through a loudspeaker which was equipped with earphones. The animal and the equipment were connected to a common ground. Figure 3 is a schematic diagram of the recording set-up. Recordings of impulse activity in nerves under these conditions are seen in Figures 4; 6, and 10.

(b) Stimulation of the skin

The low-threshold mechanosensory receptive field of a nerve is defined throughout these studies as that area of skin within which displacement of hairs or light contact of the skin with a fine bristle evokes detectable impulse activity in that nerve. Prior to dissection of nerves for recording, the fur on the back of the animal from the nape of the neck to the base of the tail was clipped to approximately 0.5 mm in length. While recording from the nerve the skin was stimulated mechanically by a hand-held nylon water-color

FIGURE 3. Schematic Diagram for the Recording of Nerve Impulses

Low-threshold mechanosensory nerve activity was recorded from the whole nerve which was lifted into air and laid across bipolar platinum hook electrodes. Impulse activity was differentially amplified, displayed on a cathode ray oscilloscope (CRO) in a free run mode, and from there relayed through an audiomonitor. Photographic records were taken from the oscilloscope screen, in the "storage" mode, using polaroid film in a mounted camera.

FIGURE 3.

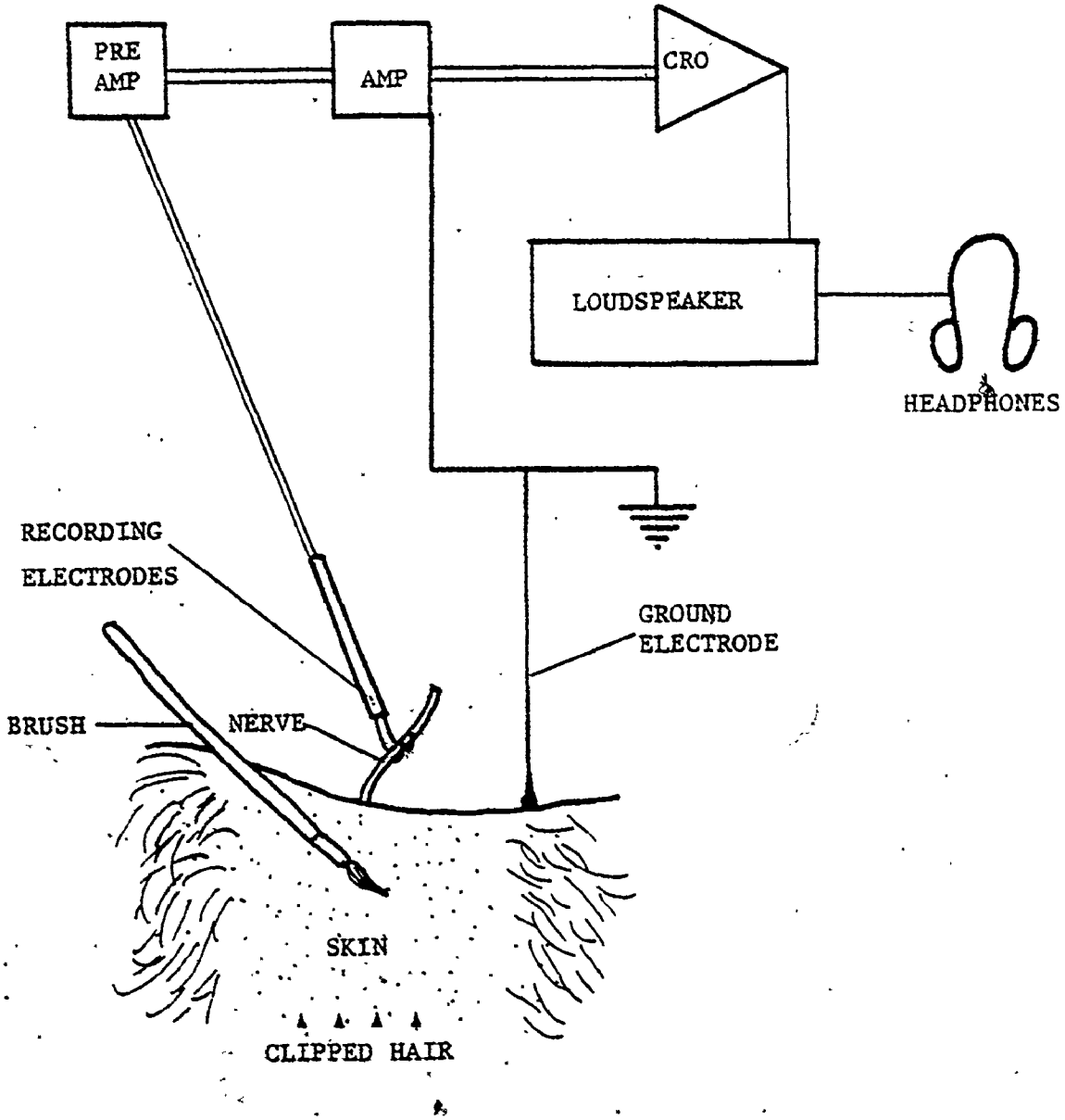
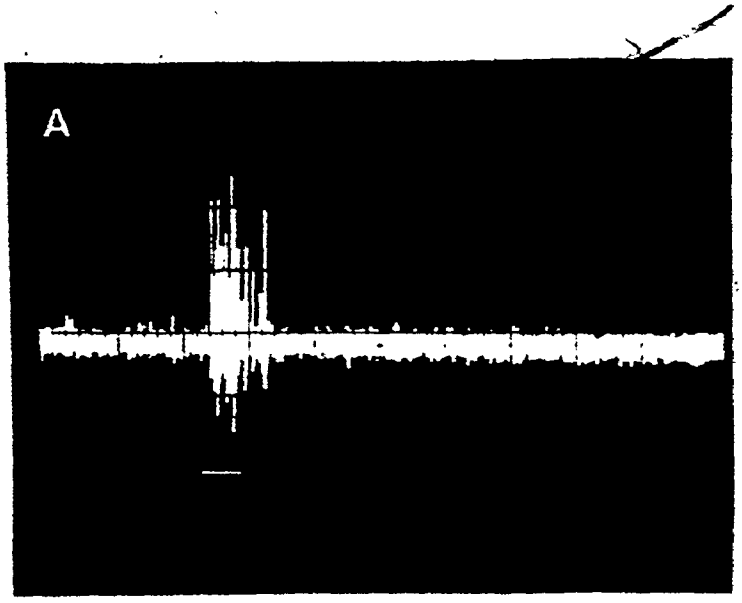

PRE AMP
AMP
CRO
LOUDSPEAKER
HEADPHONES
RECORDING ELECTRODES
GROUND ELECTRODE
BRUSH
NERVE
SKIN
CLIPPED HAIR

FIGURE 4. Evoked Impulse Activity

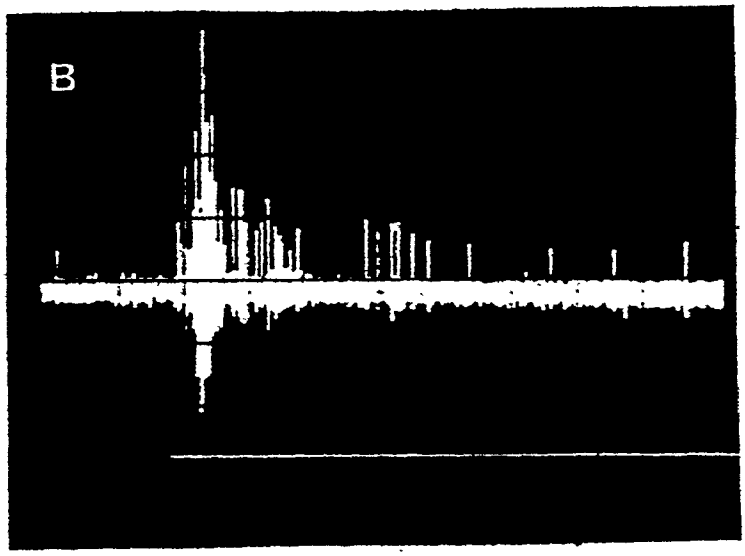
A. This photographic record shows a typical burst of impulses recorded from the medial branch of DCN-T13 following a brief displacement of clipped hairs by a hand-held brush applied within that nerve's receptive field. The solid line below the oscilloscope traces indicates the approximate time of stimulus application.

B. Recording as in A., the mechanical stimulus was applied within the field approximately 1.5 mm from the nearest mechanosensory touch dome and maintained for the duration indicated by the solid white line below the oscilloscope trace. Amplitude and time calibration is the same for both records.

FIGURE 4.



20 μv
0.2 sec.



paint brush that had been trimmed down to a few filaments. When wetted, these filaments constituted a fine, flexible tip capable of displacing the clipped, protruding hairs. Working from near the centre of the receptive field outward, stimuli were delivered at close intervals radially across the skin. Under these conditions the borders of the nerve field were characterized by an abrupt loss of stimulus-evoked impulses from one stimulus to the next (e.g. Fig. 10). A fine-tipped felt pen was used to mark the boundaries of the receptive field directly on the skin. The reliability of the method was established in representative experiments by making repeated measurements during which independent observers scored the presence or absence of an evoked response either on the oscilloscope trace or through the audio monitor; in such tests the placement of border points by as many as three persons rarely differed by more than one stimulus "step" (< 0.5 mm).

(c) Measurement of field areas

After the receptive fields had been mapped and the borders marked, the skin was returned to its normal resting position. In order to measure the areas of the marked receptive fields, a thin acetate sheet was lightly molded to the back of the animal and the outlines of the fields were traced; these outlines were then traced onto paper for permanent record. The acetate tracings were superimposed on graph paper (mm^2) and the number of squares contained within the field being measured were counted; partially enclosed squares were counted as half squares.

(d) Measurement of field "axes"

In addition to obtaining the areas of low-threshold mechanosensory receptive fields, other measurements were made on which to base subsequent estimates of the linear distance that a boundary might become displaced, either by expansion or by shrinkage of a field. For these measurements a straight edge rule marked at 0.5 mm intervals was used to obtain the maximum linear extent (MLE) of the field in the long (approximately dorso-ventral) and short (approximately rostro-caudal) axes of the dermatome (see Fig. 23).

8. Touch-Domes Innervated by mDCN-T13

In several experiments, after the low-threshold receptive field of a nerve was mapped, the number of touch-domes innervated were counted. Tattoo dots were placed in the skin (see below) at the edges of the field; a commercially obtained "facial quality" depilatory cream (Neet, Whitehall Labs) was applied to the clipped hairs of that region and removed 2-5 minutes later. Under incident and tangential illumination rounded epidermal elevations were visible in the depilated regions (Fig. 5). These elevations have a characteristic appearance and distribution and are highly sensitive punctate mechanosensory structures; very slight vertical displacements of these elevations evokes a characteristic (Iggo and Muir, 1969), somewhat irregular, slowly-adapting discharge in the afferent nerve (Fig. 6). The number of these domes innervated by a given nerve was obtained

FIGURE 5. Touch Domes in the Skin of the Rat

After depilation of the skin of the back, the touch domes are visible to the naked eye as rounded pale elevations reminiscent of "goose flesh". This photograph shows the partly depilated back skin of a rat tangentially illuminated. Each dome before depilation is normally associated with a large hair protruding from near the centre of the elevation.

FIGURE 6. The Discharge of a Touch Dome

Slight vertical displacement of a touch dome evokes a characteristic high frequency, slowly-adapting, and somewhat irregular train of impulses in the afferent nerve(s) innervating it.

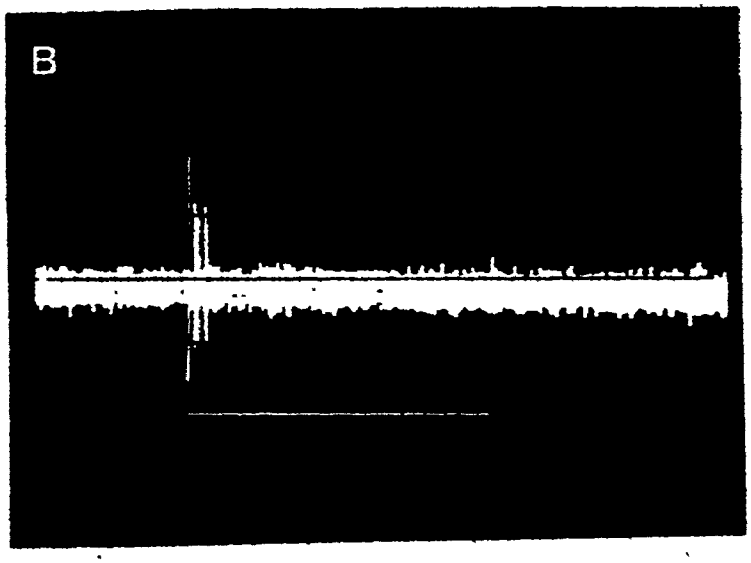
A. This record shows the discharge recorded from a mDCN-T13 during mechanical stimulation by a bristle applied directly to the surface of a dome. The stimulus was maintained during the time indicated by the solid white line below.

B. A similar mechanical stimulus (solid line) was applied to the skin immediately adjacent to the dome stimulated in record A. The characteristic "dome-discharge" is absent; there is no stimulus spread.

FIGURE 6.

20 uv

0.2 sec.



by applying a hand-held bristle to the surface of these elevations while recording from the nerve and observing the response.

9. Tattooing

In some cases permanent reference dots were placed in the skin by dipping a 27 gauge hypodermic needle in China Black ink and inserting the needle through the epidermis into the dermis. To ensure a uniform penetration by the needle, a custom made motorized hand-held needle driver (Fig. 7) with adjustable excursion distances was used. Over 90% of the tattooed dots so placed could be routinely identified up to 2 years later. There were no instances of infection as a result of this procedure.

10. Statistical Methods

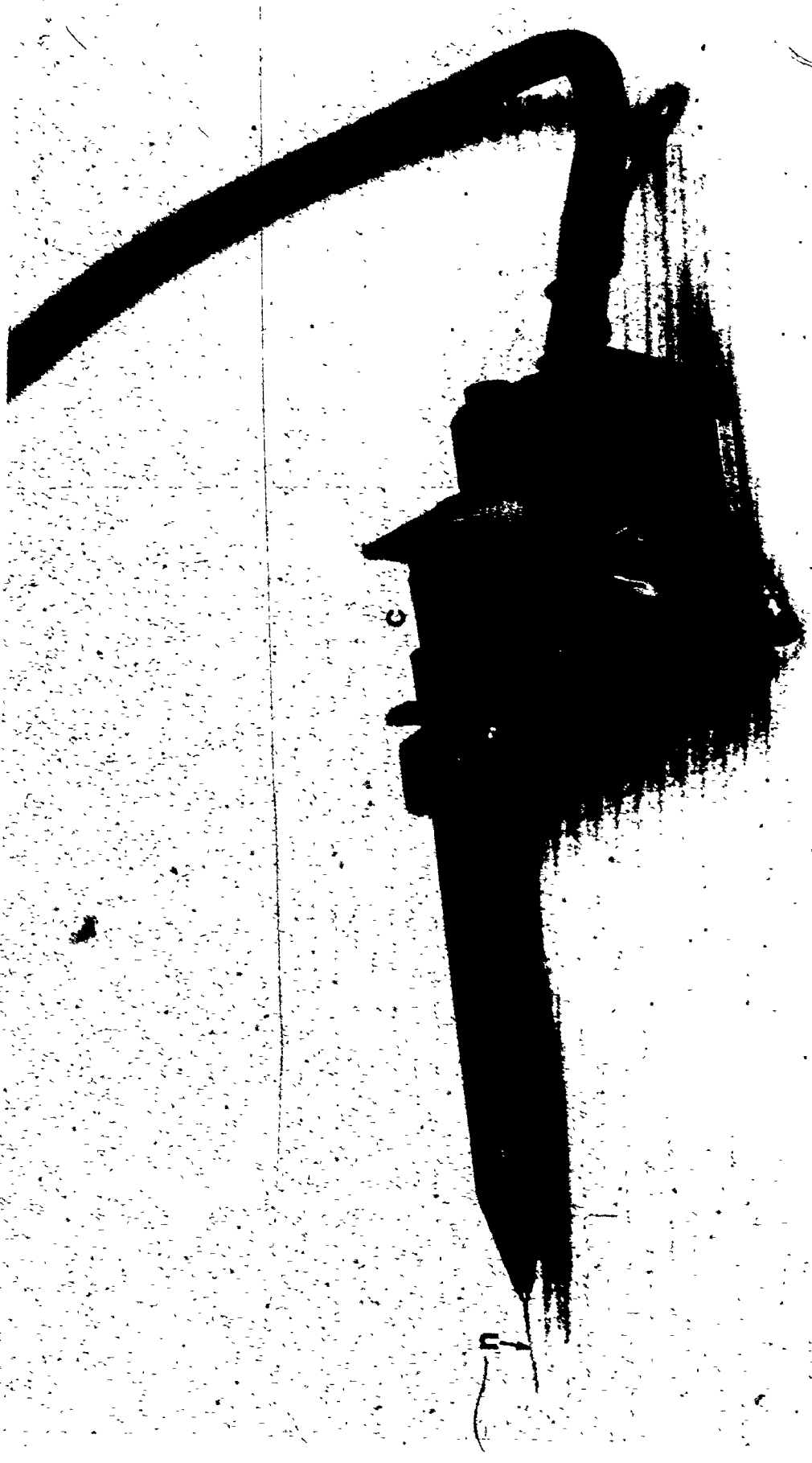
Two statistical tests were applied to all data collected from groups of animals. Student's t-test for unpaired groups was used to test for significant differences between the means of an experimental group and its control. Since most groups contained less than 30 animals, N-1 weighting of the formula was used.

The same data were also compared using the Mann-Whitney U-test, a simple distribution free test designed for use with small samples which can be placed in order (ranked) with respect to one variable.

In tables, graphs, and text, data from groups are presented as the mean ± one standard deviation.

FIGURE 7. Tattooing Hand Piece

This device was designed and built by Mr. C.W. Ikeson for the purpose of placing permanent reference tattoo dots a controllable depth into the skin. A disposable ½ inch 27 gauge hypodermic needle (n) is inserted into the needle carrier. The excursion distance of the needle tip was adjustable by varying the tension on a retaining spring clamped to the drive shaft. Ink was drawn into the needle by capillary action; the tip of the needle was then plaçed lightly on the surface of the skin and the operating foot-switch (not shown) closed, powering the electromagnetic coil (c). The needle tip was driven the selected distance into the skin where it oscillated (60 Hz), ejecting a small amount of ink.



SECTION 5

Results

1. The Cutaneous Innervation of the Rat Back

In the trunk region of the rat each segmental spinal nerve gives rise to a dorsal ramus from which is derived the Dorsal Cutaneous Nerve (DCN) of that segment. This is shown diagrammatically in Figure 8. Each DCN emerges from the body wall musculature in a location typical for its segment of origin; the origin of particular DCNs was determined in this study by dissecting the nerve centrally to its point of departure from the spinal nerve. The DCN of spinal nerve T11, in particular, is reliably identified both by its gross anatomical location and by a large blood vessel which characteristically accompanies it in its course to the skin (see Fig. 2). Other DCNs were most easily identified as to segment-of-origin by their position relative to the DCN of segment T11.

Upon emerging from the body wall musculature each DCN divides into two branches, one of which runs slightly more superficially in the loose subcutaneous connective tissue. The low-threshold mechanosensory fields of the DCNs, or their branches separately, were mapped using the techniques described in the Methods section. These nerves were found to innervate characteristic regions of back skin, extending laterally from the dorsal midline. The receptive fields of adjacent DCNs overlap along their borders, but each DCN has an "autonomous zone" - the central portion of its field of innervation.

When mapped separately, the more superficial branch of each DCN was found to innervate only the medial portion of the DCN field, while the lateral portion was supplied by the second branch. The pattern of innervation of skin by these nerves is shown in Figure 9. The remainder of each segmental dermatome (i.e. the skin extending further laterally around the flank to the ventral midline) is innervated by other segmentally arising cutaneous nerves (first the lateral cutaneous nerve, and then the ventral cutaneous nerve, see Fig. 8) which pursue separate anatomical routes; the low-threshold mechanosensory fields of the lateral cutaneous nerves were not studied in detail, but were found to have very little overlap with the DCN fields (Fig. 9). Throughout this thesis, "medial" and "lateral" fields will respectively always refer to the two sub-fields of the dorsal cutaneous nerves.

Displacement of the hairs located in skin innervated by a given nerve evokes a short burst of impulses in that nerve (Fig. 4). The resolution of the mapping technique was such that two adjacent mechanical stimuli separated by less than 0.5 mm, could be reliably identified as being "in" and "out" of the receptive field respectively (see Methods). An example of this is shown in Figure 10. The majority of the low-threshold mechanosensitive afferents encountered were rapidly adapting, although a maintained stimulus could clearly evoke trains of impulses in some axons. Frequently the stimulus evoked a very characteristic high frequency slowly-adapting and irregular burst of impulses (Fig. 6). Such responses, usually in one or a very few axons, were evoked specifically by mechanical stimulation of

FIGURE 8. The Segmental Origin of the DCNs (Diagramatic)

Each spinal nerve in the trunk region gives rise to a DCN by way of the dorsal ramus. Each DCN divides into two branches (medial and lateral) which have separate points of entry into the overlying skin. The Lateral Cutaneous Nerve and the Ventral Cutaneous Nerve of each segmental spinal nerve pursue a separate anatomical route (via the ventral ramus of the spinal nerve) and emerge from the body wall musculature at a location distant from that of the DCNs. Although more difficult than the DCNs to expose through the standard right paramidline incision, the left lateral cutaneous nerves are accessible for surgical manipulation.

FIGURE 8.

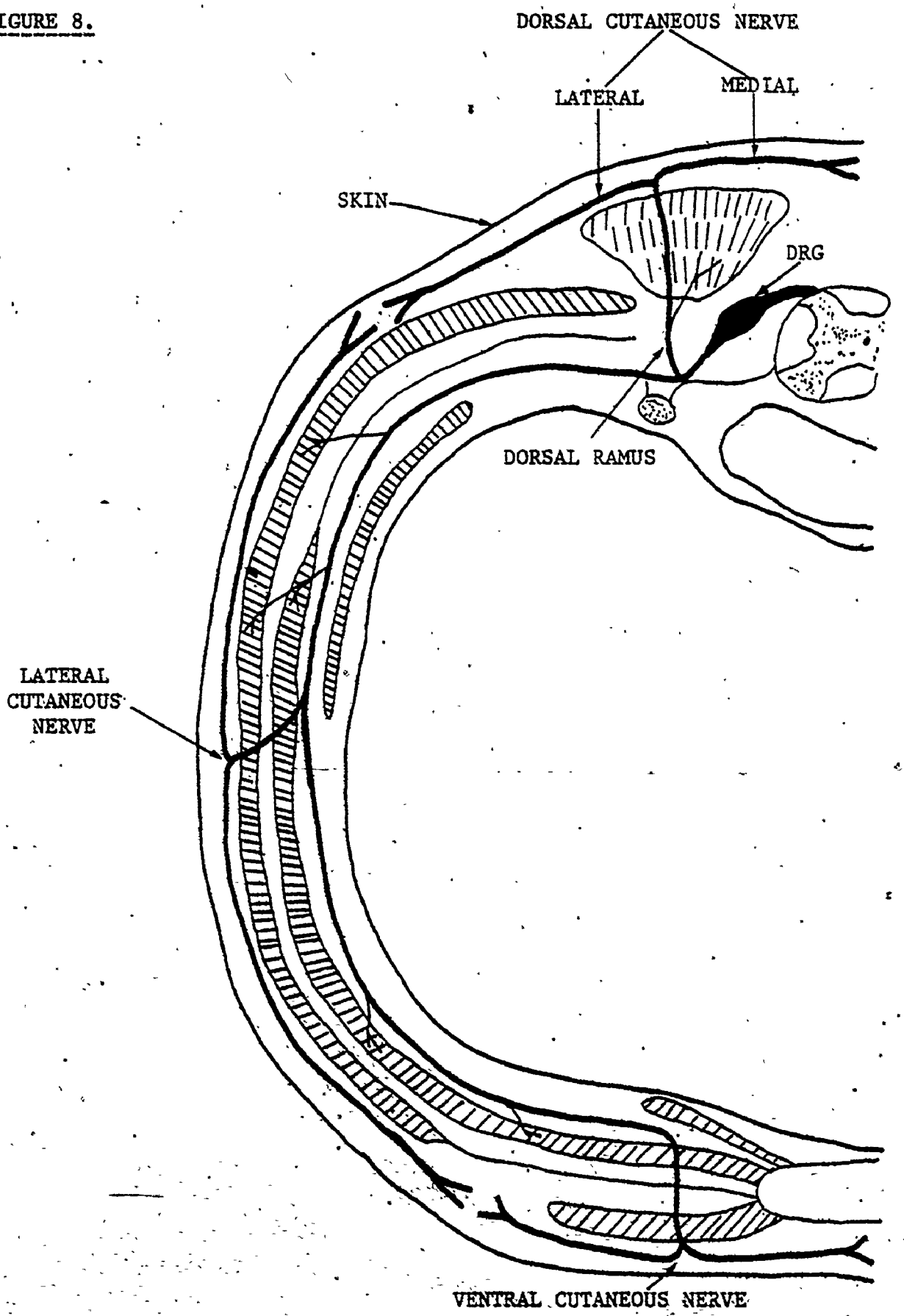

DORSAL CUTANEOUS NERVE
LATERAL
MEDIAL
SKIN
DRG
DORSAL RAMUS
LATERAL CUTANEOUS NERVE
VENTRAL CUTANEOUS NERVE

FIGURE 9. Low-Threshold Mechanosensory Fields of DCNs

This figure illustrates the pattern of innervation of dorsal skin by the DCNs. The low-threshold mechanosensory fields shown here were obtained by separately recording from both branches of several adjacent DCNs; the pattern of innervation obtained for this normal adult rat (260 gm) was a consistent finding in animals of all ages; the absolute sizes of the fields of course increased as the animals grow. The low-threshold mechanosensory fields of the Lateral Cutaneous Nerves, although unable to be mapped in their entirety owing to the positioning of the animal, are indicated on the left side as the lightly hatched area; the small amount of overlap between the fields of the DCNs and the lateral cutaneous nerves was also a consistent finding. The "serial" banding pattern exhibited by the dorsal cutaneous nerves of successive segments shown is also characteristic of those more rostral and more caudal. Isolated "island" fields were produced by leaving only the medial branch of DCN-T13 on the left side (⊕) intact; both branches of DCNs supplying the surrounding skin were interrupted.

FIGURE 9.

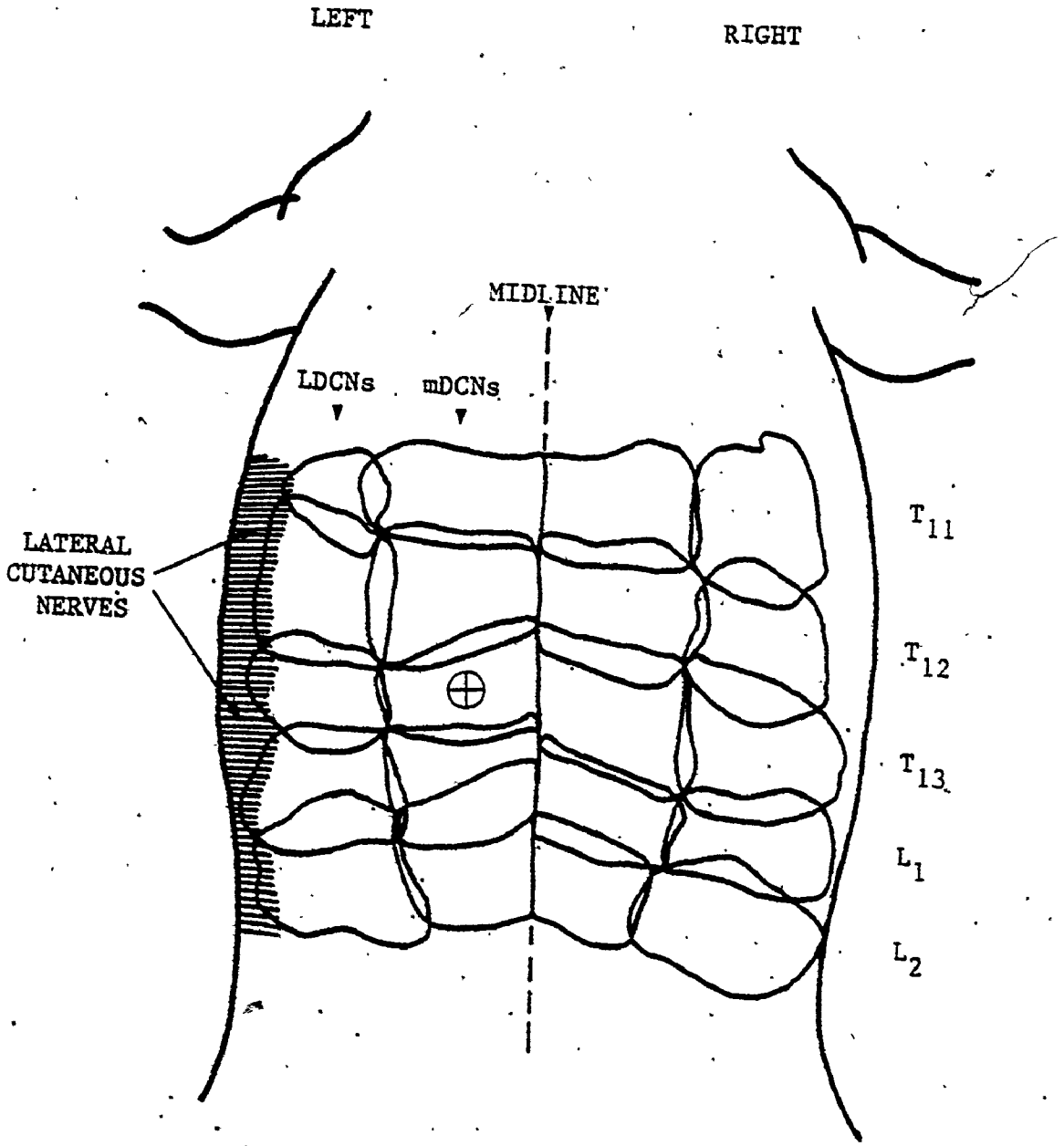
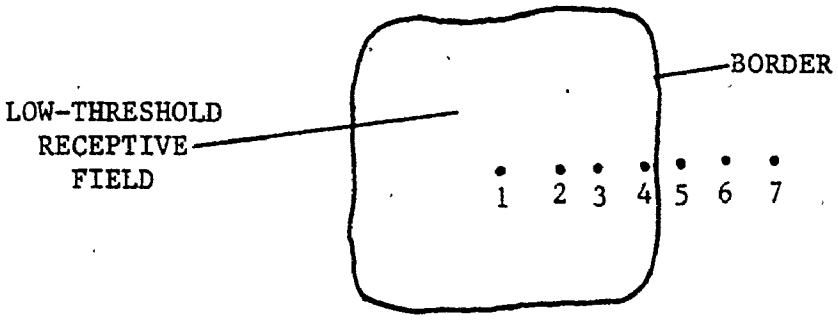

LEFT
RIGHT
MIDLINE
LDCNs
mDCNs
LATERAL CUTANEOUS NERVES
T11
T12
T13
L1
L2

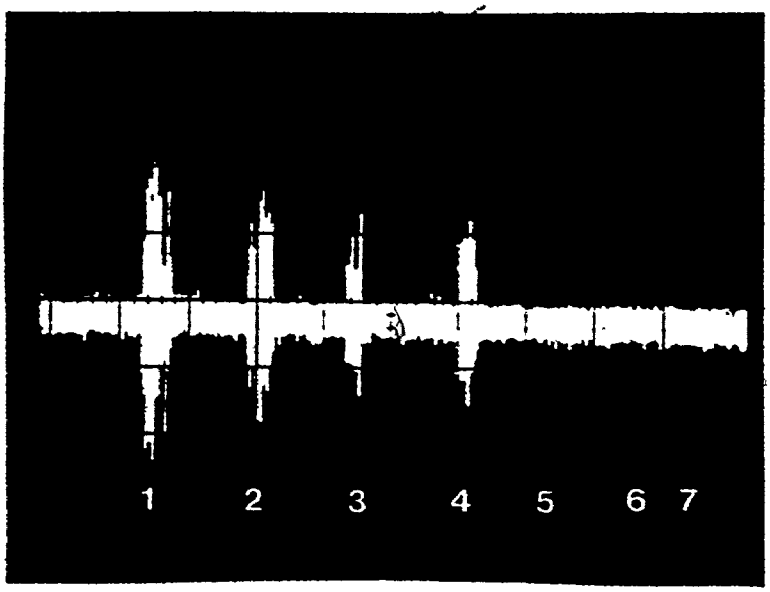
FIGURE 10. Mapping the Border of a Low-Threshold Mechanosensory Field

While recording from a given nerve, mechanical stimuli were delivered to the skin in a straight line outwards from the centre of the low-threshold mechanosensory field, with the oscilloscope in the storage mode and with a slow sweep speed. The border of the low-threshold mechanosensory receptive field is characterized by an abrupt loss of evoked impulse activity from one stimulus presentation to the next; stimuli 1-4 are clearly inside the receptive field whereas stimuli 5-7 are clearly outside it. A diagram of approximate stimulus locations is shown in the upper portion of the figure; the activity evoked by each is shown in the photographic record below. The borders of receptive fields were marked directly on the animal during the mapping procedure.

FIGURE 10.



LOCATION OF STIMULUS



20 uv

0.5 sec.

rounded epidermal elevations in the skin (Fig. 5), the touch domes.

No distinction was made between afferents responding to hair movements and those responding only to direct light contact of the brush tip with the skin between the hair shafts; the borders obtained for receptive fields after depilation of the skin were found to coincide with those obtained immediately before depilation.

2. The Effects of Adjacent Skin Denervation on the Receptive Field of an Intact Cutaneous Nerve in Adult Rats

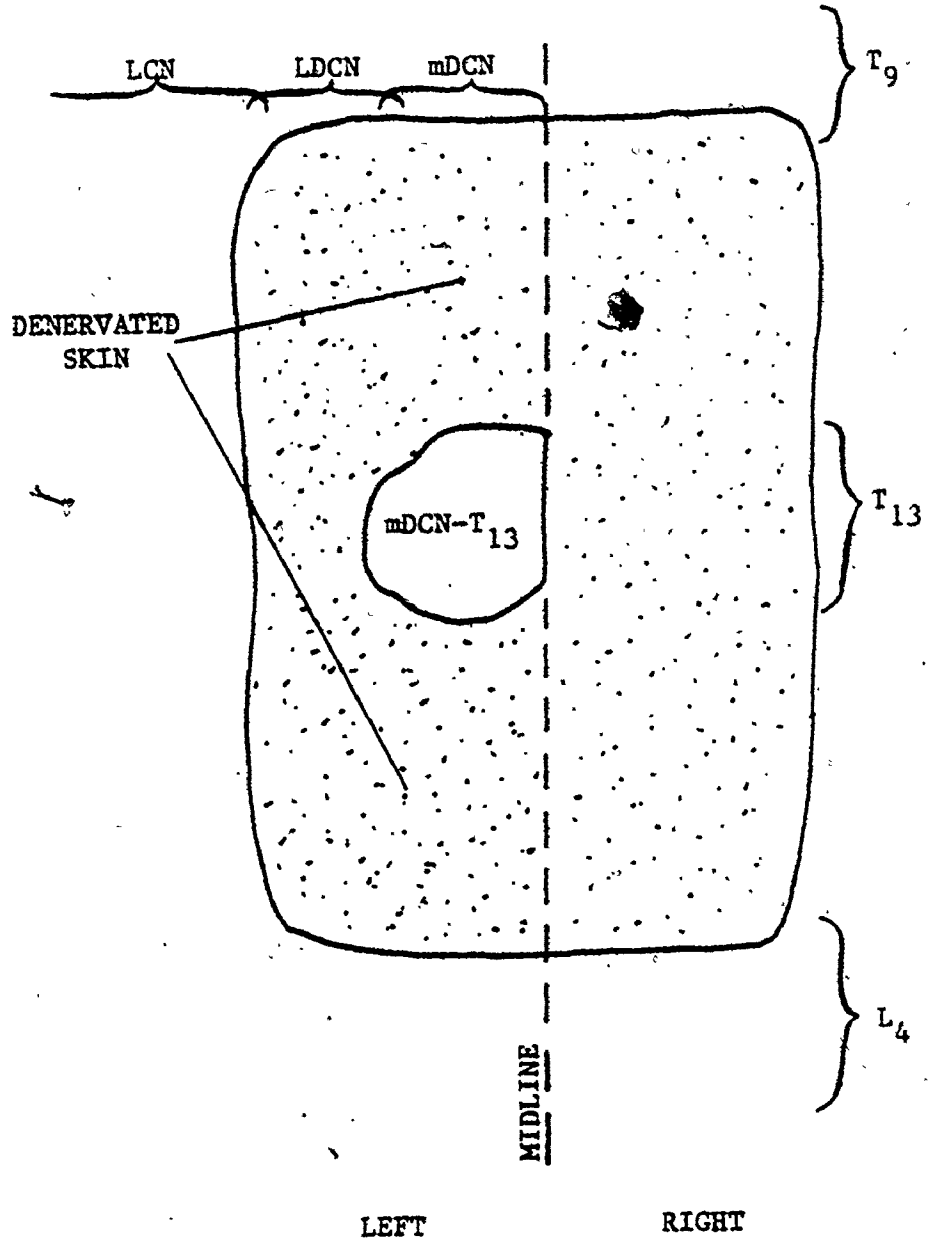
The first experiments were carried out to determine if intact low-threshold mechanosensory nerves sprout functional collaterals into adjacent denervated skin.

In 42 adult (male and female, 150-200 g) rats the medial field of the DCN of 13 (mDCN-T13) on the left side was mapped "in continuity", i.e. while recording from the intact medial branch of the nerve (see Methods). After mapping, tattooed dots were placed in the skin at the edges of the receptive field. In 30 animals the skin surrounding this field was then denervated by ligating and cutting all branches of DCNs T10-L3 on both sides excepting only the mDCN-T13 on the left side. This procedure effectively isolates the mDCN-T13 producing, as it were, an "island" of innervated skin in a "sea" of denervated skin (see Fig. 11). In the remaining 12 animals the mDCN-T13 fields were similarly mapped and tattooed, but the neighbouring DCNs were allowed to remain intact.

FIGURE 11. The Receptive Field of mDCN-T13 as an "Island" of Innervation

This figure shows diagramatically the pattern of denervation produced in order to "isolate" the receptive field of the medial branch of DCN-T13 (mDCN-T13). The stipled areas indicate the regions of skin from which low-threshold mechanosensory function is lost by surgically interrupting both branches of DCNs of segments T10-L3 except, on the left side, the medial branch of DCN-T13.

FIGURE 11.


LCN
LDCN
mDCN
T_9
DENERVATED SKIN
mDCN-T_{13}
T_{13}
L_4
MIDLINE
LEFT
RIGHT

A second mapping of these mDCN-T13 fields was done at various times ranging from 10-65 days later. In all animals, of both the experimental and the control series, the borders of the mDCN-T13 fields at this second mapping were found to coincide with the tattooed dots placed earlier at the edges of the first mapped (original) field (Fig. 12). In the experimental series adjacent regions of skin formerly innervated by cut nerves remained denervated; functional regeneration of these nerves had not occurred. It was concluded therefore that in adult rats intact low-threshold mechanosensory afferents did not sprout functional collaterals outside their normal fields of innervation in response to denervation of adjacent skin.

Because of the generally held tenet that younger animals, and systems, are more "plastic" in terms of their ability to recover function after various perturbations, similar experiments were done in younger animals - juveniles (20-30 days of age) and pups (10 days of age). To do these experiments however it was first necessary to investigate the disposition of the cutaneous nerve fields during the normal increase in surface area that occurs during the growth of the animal at the ages of interest.

3. <u>The mDCN-T13 Fields During Growth from Pup to Adult</u>

As a newborn rat grows, the total surface area of its skin is vastly increased. This increase in size must be reflected by an increase in the absolute <u>area</u> of the low-threshold mechanosensory receptive fields of the cutaneous nerves. Three lines

FIGURE 12. Lack of Sprouting in the Adult Rat

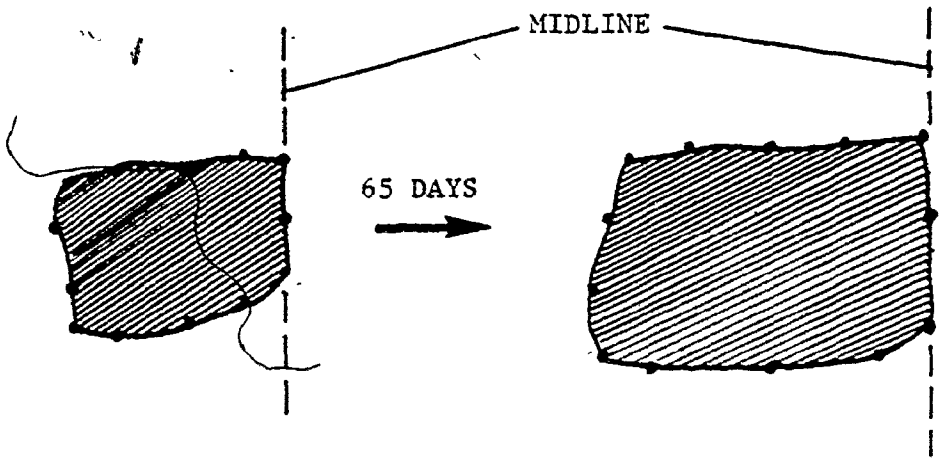
Shown in this figure are the low-threshold mechanosensory fields of the left mDCN-T13 of two animals mapped in continuity at the beginning of the experiment (day 0) and again 65 days later. Tattoo dots were placed at the border of the fields mapped at day 0.

A. In this animal the skin surrounding the receptive field of the intact mDCN-T13 was denervated for 65 days; there was no functional regeneration of the cut nerves.

B. In this animal the low-threshold mechanosensory field of mDCN-T13 was mapped and tattooed, as in A., but no denervation of adjacent skin was carried out.

The borders of all receptive fields at the second mapping were found to coincide with the tattoo dots placed in the skin at the earlier mapping, whether or not the immediately adjacent skin was denervated. The same result was obtained from animals in both experimental and control groups mapped at shorter intervals.

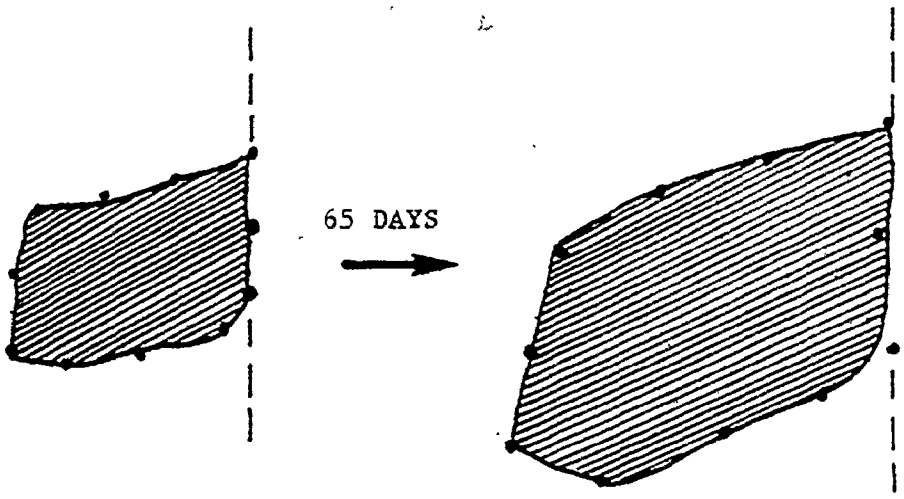
FIGURE 12.



A

SURROUNDING SKIN DENERVATED

1 CM.



B

SURROUNDING SKIN INNERVATED

of evidence were obtained describing the mDCN-T13 receptive fields in growing animals.

(a) The low-threshold mechanosensory fields of mDCNs-T13 were mapped acutely in groups of animals ranging in age from 10-60 days of age. The areas thus obtained (Fig. 13) show that between 10 and 60 days of age the low-threshold receptive field of mDCN-T13 increases in absolute area by approximately seven-fold.

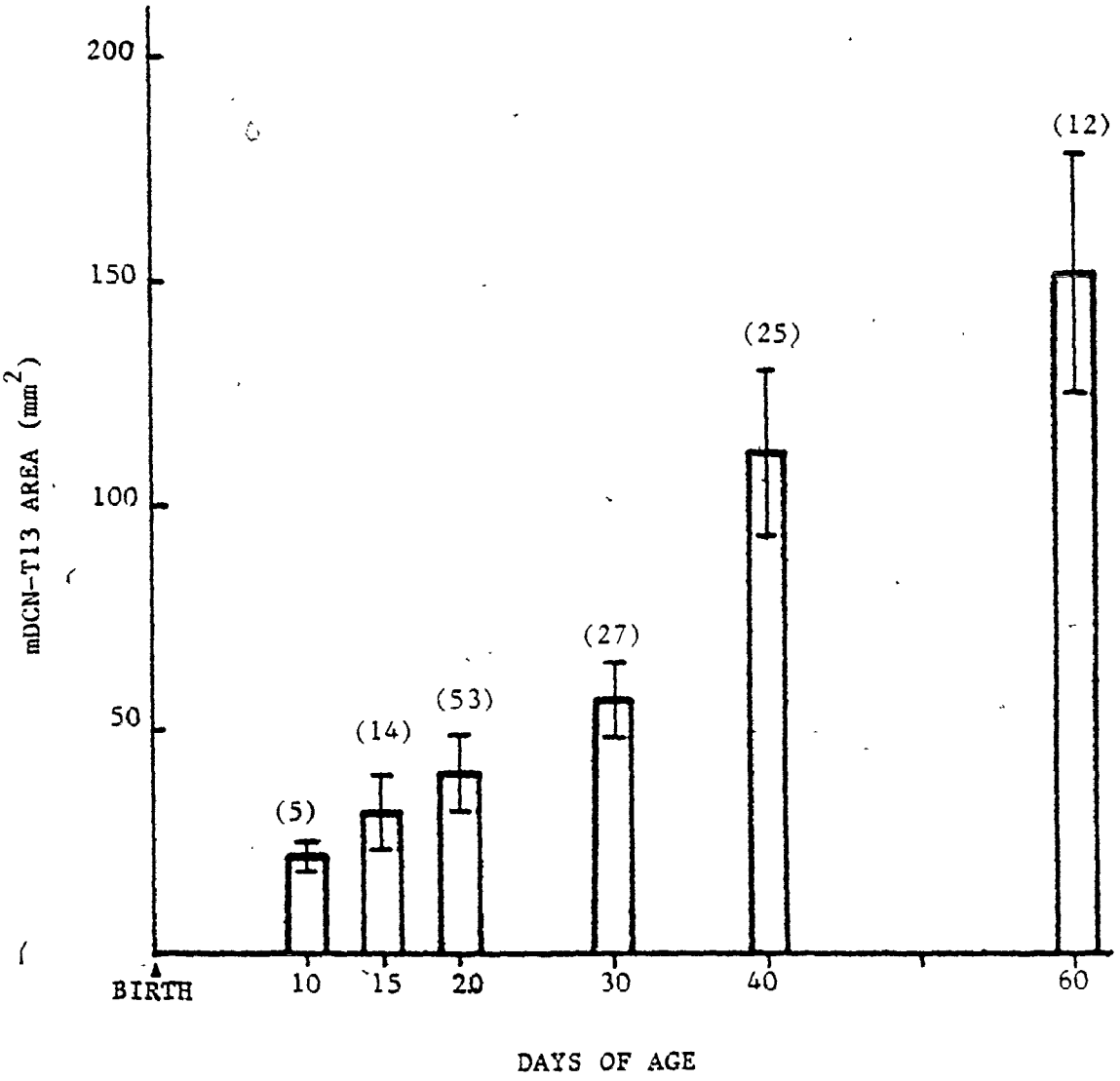
(b) In order to determine whether the borders of a low-threshold mechanosensory receptive field retain their topographic relationship to one another as the skin (and the receptive field) increases in area, the receptive fields of mDCNs-T13 in nine young animals (15 or 20 days of age) were mapped while recording from the nerve in continuity; tattooed reference dots were placed in the skin at the borders thus obtained and a second mapping was done from 5-7 weeks later. At this second mapping the borders of these fields were found to coincide with the reference dots (as in Fig. 12B). This result shows that the borders of the low-threshold mechanosensitive receptive fields established in early life are stable, "passively" becoming further apart as the skin increases in area.

(c) The number of touch domes supplied by the left mDCN-T13 as the animal grows was determined. In groups of animals ranging in age from 15-60 days of age the touch domes within the low-threshold mechanosensory field of mDCN-T13 were identified visually

FIGURE 13. Normal Increase in Receptive Field Area During Growth of the Rat

These histograms show the area of low-threshold mechanosensory innervation provided by mDCN-T13 (mean ± S.D.) mapped acutely in groups of animals ranging from 10 to 60 days of age. As the animal grows and the total surface area increases so does the absolute area of the receptive field. These histograms were compiled from data obtained from normal control animals. The number of animals in each group appears in paranthesis above the results for that group.

FIGURE 13.


200
150
100
50
mDCN-T13 AREA (mm^2)
(5)
(14)
(53)
(27)
(25)
(12)
BIRTH
10
15
20
30
40
60
DAYS OF AGE

under tangential illumination (Fig. 5). The number of domes supplied by mDCN-T13 in each animal was counted by stimulating each dome successively with a fine bristle while recording from the nerve (see Fig. 6). It was found that the number of domes supplied by mDCN-T13 at 15 days of age (18.6 ± 1.5) is stable at least up to 60 days of age (Fig. 14B); during this period however the field increases dramatically in area (Fig. 14A). The domes simply become further apart as the surface enlarges.

4. The Effects of Adjacent Skin Denervation on the Receptive Field of an Intact Cutaneous Nerve in Juvenile Rats

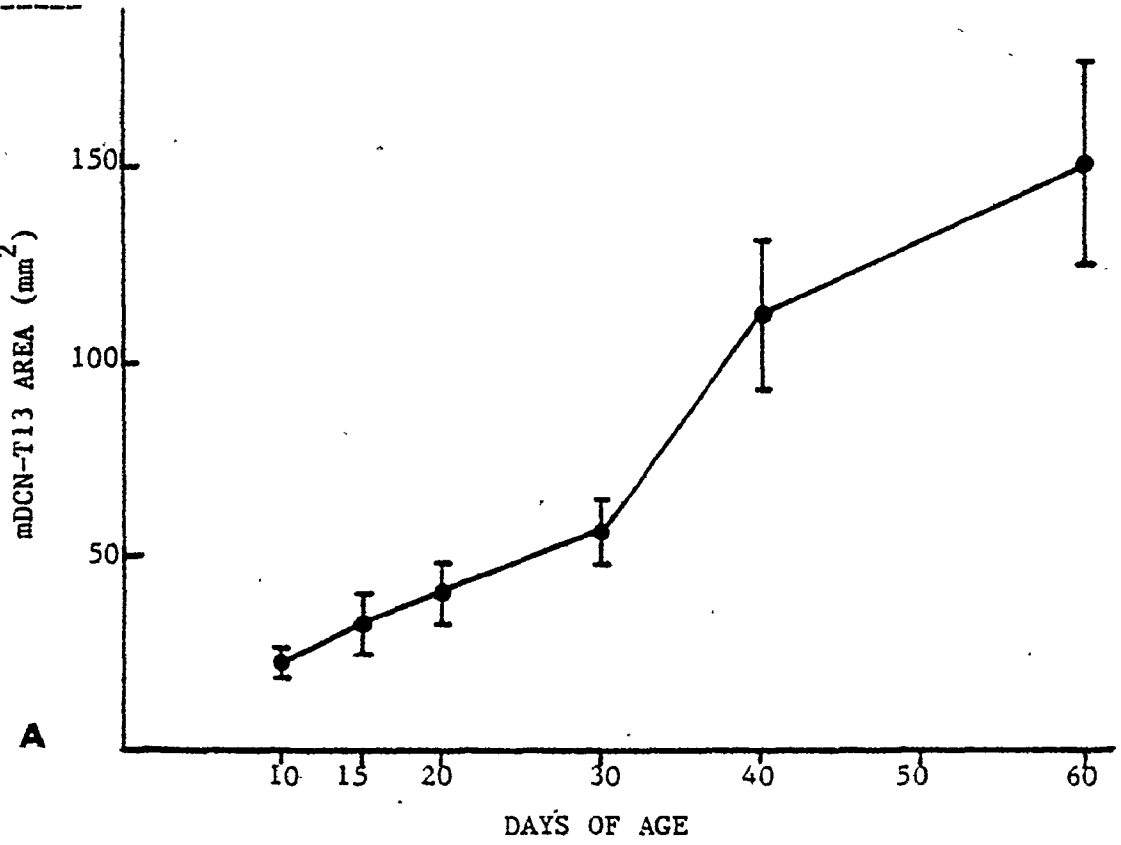
In 57 animals ranging in age from 20-30 days of age, "islands" (Fig. 11) of intact innervation were produced; the mDCN of T13 on the left was mapped in continuity and its peripheral field borders tattooed as described above. In a control group of 16 animals of the same age, the mDCN of T13 on the left was mapped in continuity but adjacent DCNs were allowed to remain intact. Animals of both groups were investigated at various times during the subsequent 85 days. The results were the same as those obtained from the previous experiments on adult animals (see Fig. 12); in both series at the second mapping of the mDCN-T13 fields there was no discernable difference between the obtained borders and the original ones defined by the tattooed dots. There was no detectable functional regeneration of the nerves originally cut to produce the "islands". The last five animals of the experimental ("island") and control (map and tattoo only) groups were mapped for

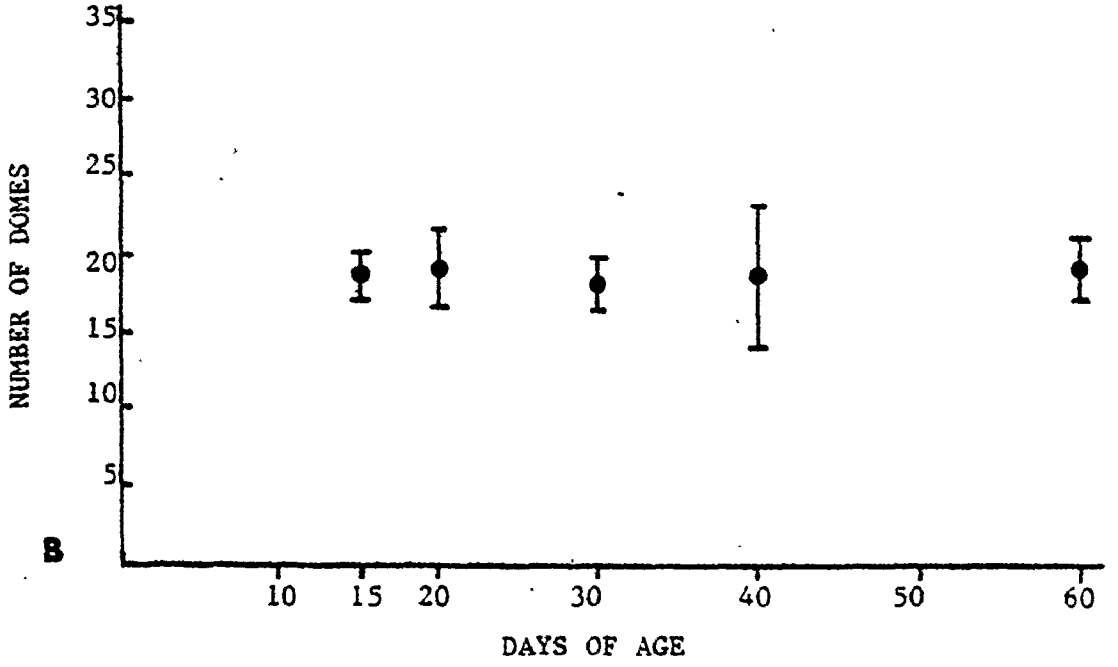
FIGURE 14. The Number of Domes Innervated by the Normal mDCN-T13

A. Reproduced here, for comparison purposes, are the results of receptive field area measurements shown in the previous figure.

B. Domes in the region of the low-threshold mechano-sensory field of mDCNs-T13 were mechanically stimulated and the number supplied by the mDCN-T13 determined in groups of animals ranging in age from 15 to 60 days of age. As shown, the number (mean ± S.D.) of touch domes supplied by mDCN-T13 remains stable over the 15 to 60 day period. Domes in the skin of 10 day old rats were indistinct and could not be reliably counted. The normal increase in absolute area as the animal grows is not accompanied by an increase in the number of touch domes within the field.

FIGURE 14.


150
100
50
mDCN-T13 AREA (mm^2)
A
10
15
20
30
40
50
60
DAYS OF AGE


35
30
25
20
15
10
5
NUMBER OF DOMES
B
10
15
20
30
40
50
60
DAYS OF AGE

the second time 84-85 days after the original surgery, along with a control group of five previously unoperated animals of the same age. The results from these animals are shown in Figure 15. There was no significant difference in the area of the receptive field of mDCN-T13 between the experimental "island" group and either of the two control groups; neither were the control groups significantly different from each other. Clearly there was no evidence of low-threshold mechanosensory nerve sprouting outside their normal territory into denervated skin even in animals as young as 20-30 days of age. A different result was obtained in the next series of experiments, in which still younger animals were investigated.

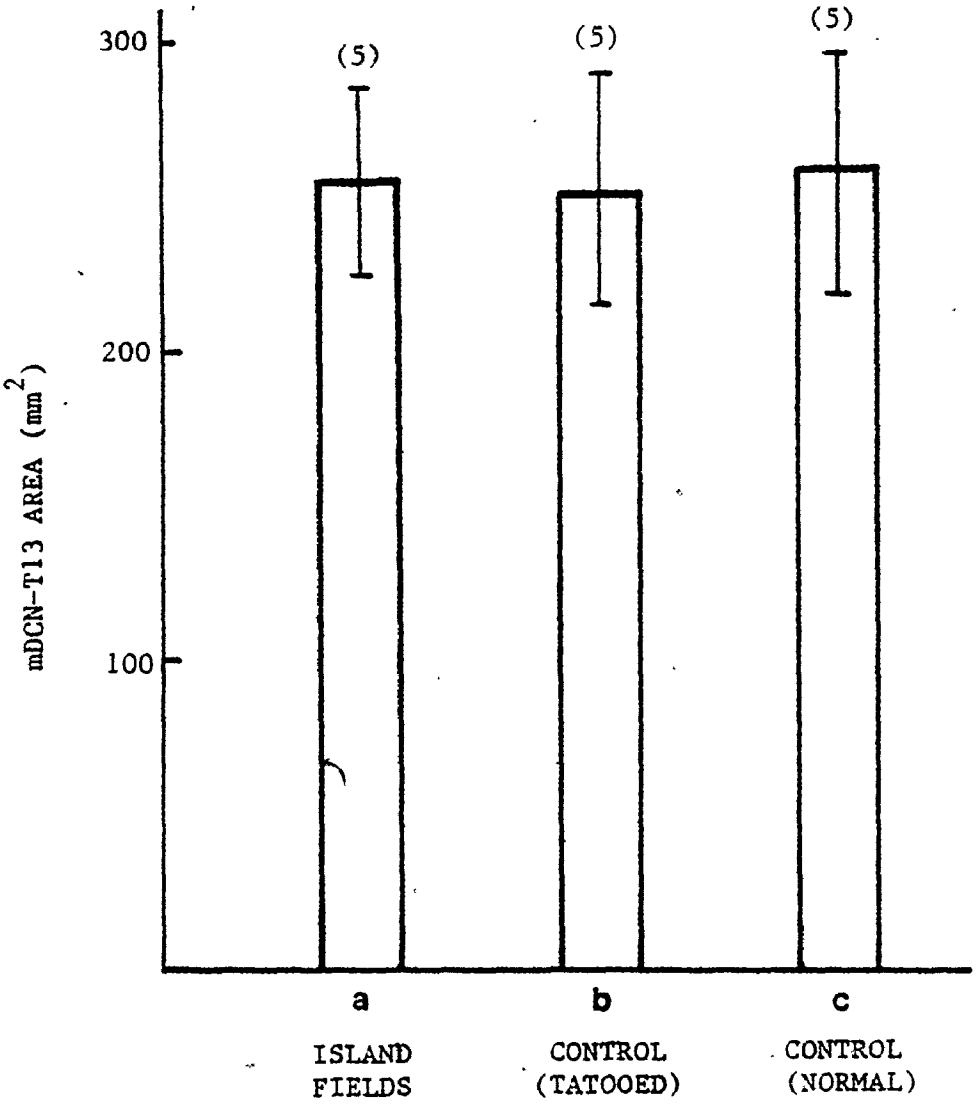
5. Sprouting by Intact Nerves in Rat Pups

Next investigated was the effect on intact nerves of denervation of skin at 10 days of age. In such young animals the DCNs were found to be too delicate to tolerate in continuity recordings. Therefore instead of having each experimental animal serve as its own control (by tattooing borders) for a later mapping, islands of intact innervation (mDCN-T13) were produced but not mapped in a total of twenty ten day old rat pups, as in older animals, by cutting the branches of adjacent DCNsT10-L3 bilaterally (see Fig. 11). Control 10 day old pups were anaesthetized but not operated on. Both groups were of normal weight at 20 days of age. The low-threshold mechanosensory fields of mDCNs-T13, island and control, were mapped at 20 days of age;

FIGURE 15. Lack of Sprouting in Juvenile Rats

Shown in this figure are the low-threshold mechanosensory fields of mDCN-T13 (mean ± S.D.) mapped (a) in 5 animals 85 days after the field had been mapped in continuity, its borders tattooed, and the surrounding skin denervated; (b) in 5 animals 85 days after the field had been mapped, and tattooed, but with no denervation of adjacent skin, and (c) in 5 normal control animals of the same age. There was no functional regeneration of experimentally cut nerves. There was no significant difference between the areas of any of these groups ($p>0.1$). The fields of nerves which had been previously mapped and tattooed (a and b) were coincident with the tattooed dots at the time of the second mapping.

FIGURE 15.


300
200
100
mDCN-T13 AREA (mm^2)
(5)
(5)
(5)
a
b
c
ISLAND FIELDS
CONTROL (TATOOED)
CONTROL (NORMAL)

the results (Fig. 16) clearly showed that the island fields had become larger in area than the fields of control mDCNs-T13. Although not counted in all of these animals, the number of touch domes supplied by mDCNs-T13 whose receptive fields had been isolated as islands of innervation was found to be increased (28.5 ± 2.0, n=8) as compared to the number innervated by mDCN-T13 in the control animals (19.0 ± 2.4, n=27).

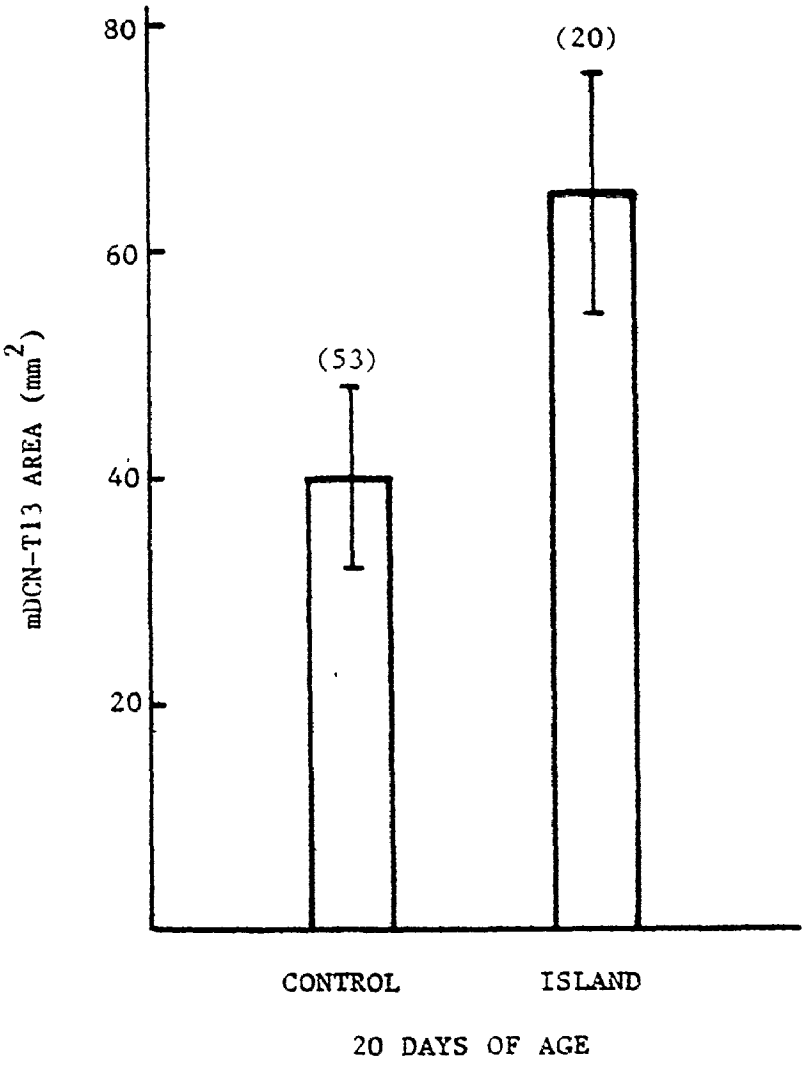
The possibility was examined that this increase in area of the island field was due simply to a reduced growth of denervated skin with an attendant compensatory stretching of the innervated skin. To test this, two groups of 10 day old animals were prepared (six per group). In both groups identical rectangular outlines were tattooed into the skin of the back just to the left of the dorsal midline. In one group this region of skin and its surroundings were denervated by cutting the appropriate DCNs; the second group was anaesthetized but not otherwise operated on. At 20 days of age the areas enclosed by the tattooed dots were measured and compared. The results obtained showed no difference ($p>0.2$) between the area enclosed by tattoo dots in innervated skin (45.0 ± 2.4 mm^2) and the area enclosed by tattoos in denervated skin (44.6 ± 1.6 mm^2).

From this, and the calculation that the number of domes/cm^2 in island fields (44.5 ± 4.5) was not different ($p>0.2$) than that in control fields (46.7 ± 6.4), it was concluded that the "extra"

FIGURE 16. Expansion of Fields into Denervated Skin: Rat Pups

The histograms show the area (± S.D.) of the low-threshold mechanosensory fields of mDCNs-T13 in two groups of animals, both mapped at 20 days of age. On the right is a group of animals in which the receptive field of mDCN-T13 was isolated as an island of innervation at 10 days of age. On the left is a group of normal control animals. The number of animals in each group appears in paranthesis above each histogram. The island fields were significantly larger ($p<0.001$), indicating an expansion of the "island" field (sprouting) into experimentally denervated skin had occurred between 10 and 20 days of age.

FIGURE 16.


80
60
40
20
mDCN-T13 AREA (mm²)
(53)
(20)
CONTROL
ISLAND
20 DAYS OF AGE

area occupied by mDCN-T13 in the 10 day period after isolation of its receptive field in the 10 day old rat pup is not attributable to a passive stretching of skin with its contained nerves. These results clearly indicated that in the rat, expansion of an intact nerve's low-threshold mechanosensory field into adjacent denervated skin is confined to an early period of life; that is, it has a "critical period".

The likeliest explanation of this result is that the island fields expanded by functional sprouting of the intact nerves into denervated skin; to simplify the writing, this interpretation has been adopted. The alternatives and the relevant arguments are presented in the Discussion (7.1).

6. The Critical Period for Sprouting

The apparent critical effect of age on the ability of intact nerves to establish functional endings in denervated skin (compare Figs. 12, 15 and 16) was examined in further experiments. Defining the end of the critical period was of particular interest; a more detailed description of sprouting by these isolated intact nerves during the critical period was also desired. The former was the easier to deal with and therefore was examined first.

(a) The end of the critical period

(i) The first question concerned the end of the critical period: "at what age is denervation of the skin no longer able to evoke functional sprouting into it of neighbouring intact

low-threshold mechanosensory nerves?" To answer this question island fields innervated by the intact medial branch of DCN-T13 were produced in animals at 15, 20, and 30 days of age; at selected times thereafter (5-20 days) the areas, and number of domes, innervated by these isolated intact mDCNs-T13 were obtained compared to those of mDCN-T13 in unoperated controls of the same ages. Island fields isolated at 15 days of age showed an extra enlargement when mapped at either 20 or 30 days of age (Fig. 17A). The number of domes innervated by these isolated mDCNs-T13 was also increased (Table I). There was however no detectable evoked enlargement of island fields isolated at 20 days of age and mapped either at 30 or 40 days of age (Fig. 17B). Similarly, receptive fields of mDCNs-T13 isolated at 30 days of age and mapped at 50 days of age showed no extra enlargement (Fig. 17C). In the latter experimental groups (fields isolated at 20 or 30 days of age) there was also no increase in the number of domes innervated by the intact mDCNs-T13 at the later mappings (Table I).

From these results it was concluded that the critical period for sprouting of functional endings by intact low-threshold mechanosensory cutaneous nerves into denervated skin ends at about 20 days of age; denervation of skin in animals older than this is ineffective in evoking such sprouting.

(ii) A second, related, question was: "does sprouting that is evoked before the end of the critical period, <u>continue</u>

FIGURE 17. The Effect of Isolating the Island Field at 15, 20, or 30 Days of Age

These histograms show the area (± S.D.) of mDCN-T13 receptive fields in groups of control animals and groups in which the receptive fields of mDCNs-T13 had been isolated as islands of intact innervation at 15, 20 and 30 days of age. The number of animals in each group is given in paranthesis above the appropriate column. Dome counts obtained from these groups of animals are presented overleaf in Table I.

A. The island fields isolated at 15 days of age had become significantly larger than control values mapped at 20 ($p<0.025$) or 30 ($p<0.001$) days of age.

B. These histograms show the area (± S.D.) of mDCN-T13 receptive fields isolated as islands of intact innervation in two groups of animals at 20 days of age. Neither group of island fields was significantly different from its control ($p>0.2$); denervation of the surrounding skin produced no subsequent extra enlargement of the isolated field into the denervated skin.

C. The histograms show the area (± S.D.) of isolated island and control, mechanosensory fields in two groups of animals mapped at 50 days of age; the island fields were isolated at 30 days of age. As in B., there was no extra enlargement ($p>0.2$) of the island fields evoked by the denervation.

FIGURE 17.

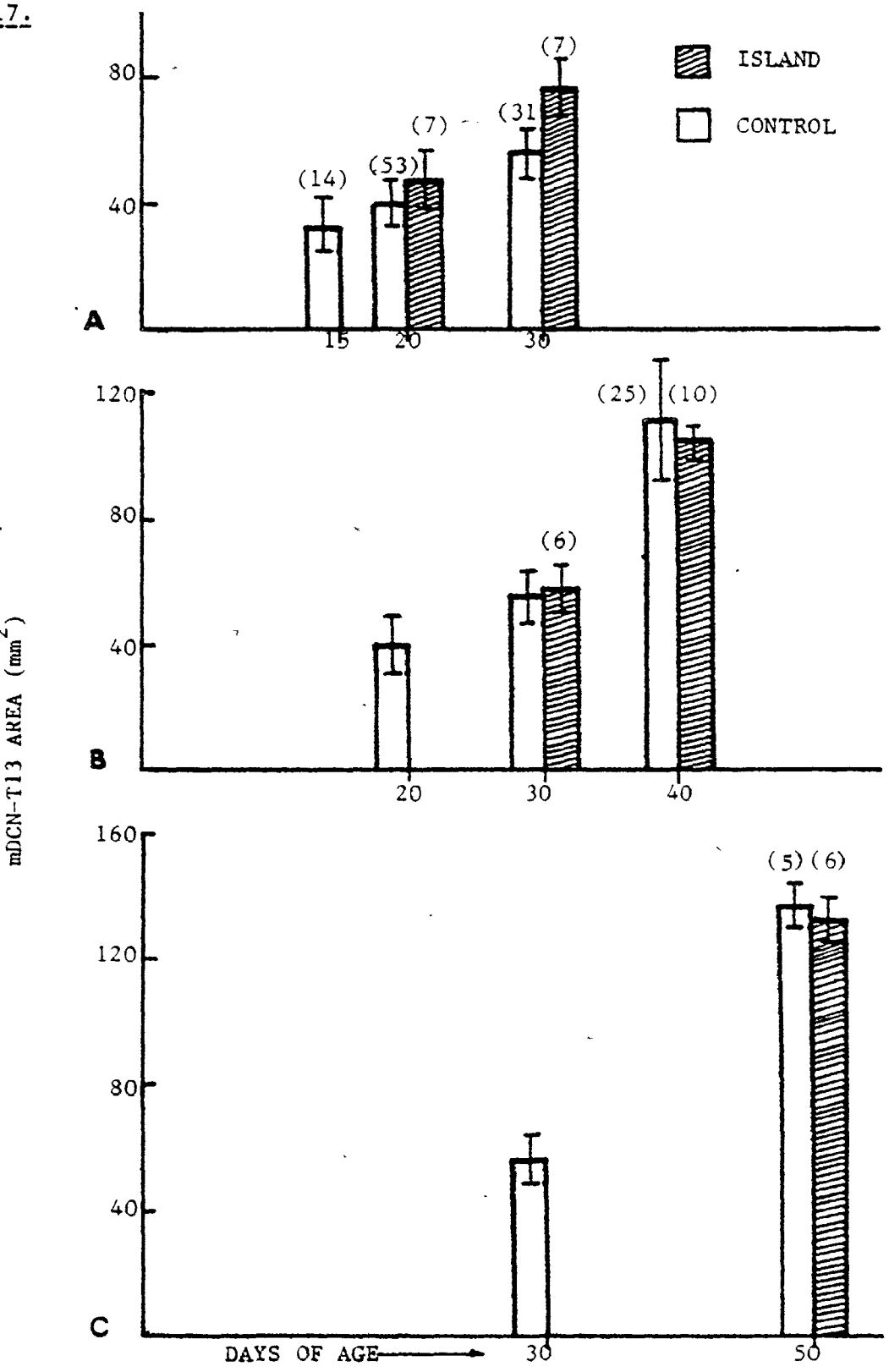

ISLAND
CONTROL
mDCN-T13 AREA (mm²)
A
B
C
80
40
120
160
(14)
(53)
(7)
(31)
(7)
(6)
(25)
(10)
(5)
(6)
15
20
30
40
50
DAYS OF AGE

TABLE I Dome Counts in Fields Isolated at 15, 20 and 30 Days of Age

Shown here are the numbers of touch domes (± S.D.) supplied by mDCN-T13 with peripheral fields isolated at 15, 20 or 30 days of age; the counts were obtained from the groups of animals presented in the immediately preceding Figure (Fig. 17). Dome counts were significantly increased over controls only in island fields isolated at 15 days of age (Groups A); in groups B and C there was no difference between the numbers of innervated domes in isolated or control fields (N.S. $p>0.2$).

TABLE I.

DOME COUNTS

	DAYS	CONTROL				ISLAND
GROUPS A.	15	18.6 + 1.5 n=8				-------
	20	19.0 + 2.4 n=27	-	p<0.01	-	22.0 + 2.4 n=7
	30	18.1 + 1.8 n=18	-	p<0.001	-	24.3 + 1.5 n=7
GROUPS B.	20	19.0 + 2.4 n=27				----------
	30	18.1 + 1.8 n=18	-	N.S.	-	19.3 + 2.2 n=6
	40	18.6 + 4.7 n=7	-	N.S.	-	19.0 + 3.0 n=4
GROUPS C.	30	18.1 + 1.8 n=18				----------
	50	18.7 + 2.1 n=5	-	N.S.	-	18.4 + 2.3 n=6

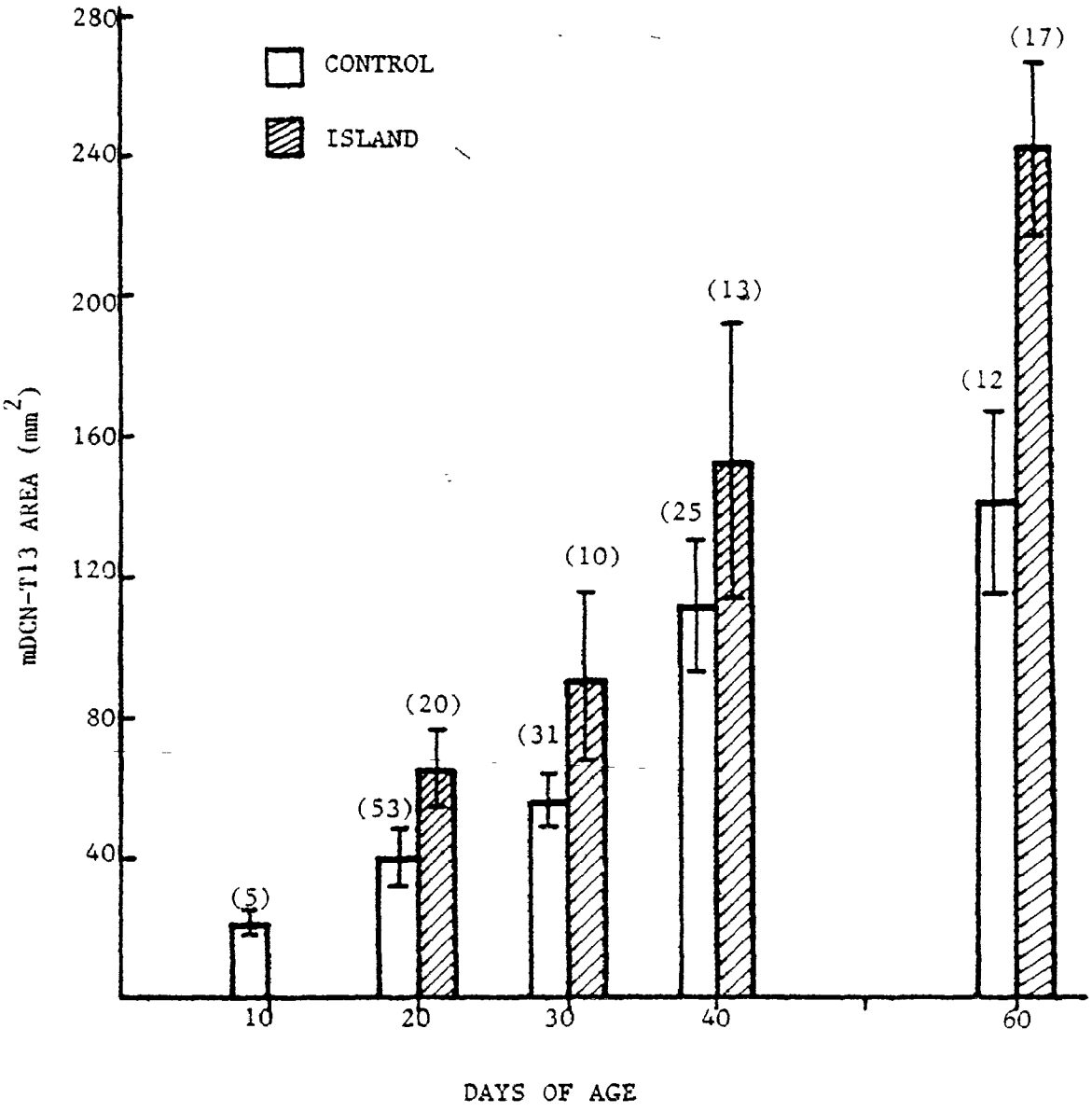
beyond it?" To address this question, the receptive fields of mDCNs-T13 were isolated as islands of intact innervation in 10 day old rat pups (and mapped in groups at 30, 40, or 60 days of age). Regeneration of nerves cut at 10 days of age was prevented by ligating the central stumps; the success of this was confirmed by recordings made from these nerves central to the ligatures at the subsequent mapping of mDCNs-T13. The extra enlargement of isolated mDCN-T13 fields, detected at 20 days of age (see Fig. 16) was also readily detectable at 30, 40, and 60 days of age (Fig. 18). It was observed in these animals that, provided there was no regeneration of the originally cut nerves, the previously isolated island field remained completely surrounded by denervated skin even at the longest time interval investigated. The functional sprouting by the intact axons of isolated mDCNs-T13 had, therefore, ceased prior to complete occupation of experimentally denervated skin (see also Fig. 26). That sprouting ceased at 20 days of age was suggested by the results presented in Figure 23 where the "maximum linear extension" (see following section) of these isolated mDCN-T13 fields was examined. The measurements describe the increase of isolated and control fields in both of the major axes as the animal grows; after 20 days of age the increase of experimental and control fields parallel each other in both axes, indicating that no extra extension was occurring.

The most convincing evidence for the cessation of the

FIGURE 18. Maintained Enlargement of Island Fields Isolated at 10 Days of Age

This figure shows the area (± S.D.) of mDCN-T13 receptive fields isolated in four groups of 10 day old animals and mapped at successively longer intervals. Control groups are also shown. The number of animals in each group is given in paranthesis above the appropriate column. At each age examined (20, 30, 40, and 60 days of age) the isolated fields were significantly ($p<0.001$) larger than the fields in the control group of the same age. In no group was there detectable regeneration of the nerves originally cut and ligated at 10 days of age.

FIGURE 18.


280
240
200
160
120
80
40
mDCN-T13 AREA (mm²)
CONTROL
ISLAND
(5)
(53)
(20)
(31)
(10)
(25)
(13)
(12)
(17)
10
20
30
40
60
DAYS OF AGE

"extra" growth (sprouting) at 20 days of age comes from a consideration of the number of touch domes in the field of mDCN-T13. As shown earlier (Fig. 14), in fields of control animals this number (approximately 18) normally remains stable, between 15 and 60 days of age. In Table II is shown the number of touch domes supplied by intact mDCNs-T13 counted at 20 days of age and later following denervation of the adjacent skin at 10 days of age. By 20 days of age, concomitant with the extra enlargement of field areas, there is an increase (to approximately 28) in the number of touch domes supplied; after 20 days of age there is, however, no <u>further</u> increase in this number (Table II). Consistent with a cessation of evoked sprouting at about 20 days of age is the observation that the increase in area and number of domes supplied by intact mDCNs-T13 isolated at 15 days of age is less than for nerves isolated at 10 days of age (Fig. 19).

(iii) An experiment was performed to determine if isolation of the receptive field of mDCN-T13 earlier than 10 days of age would result in an even greater expansion of the intact nerve into the denervated skin. Islands of intact innervation were produced by isolating the receptive field of mDCNs-T13 in 5 day old animals. When mapped at 20 days of age (the end of the critical period) these isolated intact mDCNs-T13 were found to have expanded into adjacent denervated skin, innervating an increased number of domes. This increase in touch

TABLE II. The Evoked Increase in the Number of Domes Supplied by Isolated mDCNs-T13

Shown here are the dome counts (± S.D.) obtained for mDCNs-T13 isolated at 10 days of age, and those of normal age matched controls. Although the skin and domes surrounding the isolated island fields remained denervated, the number of domes supplied by the intact mDCN-T13 did not increase after 20 days of age.

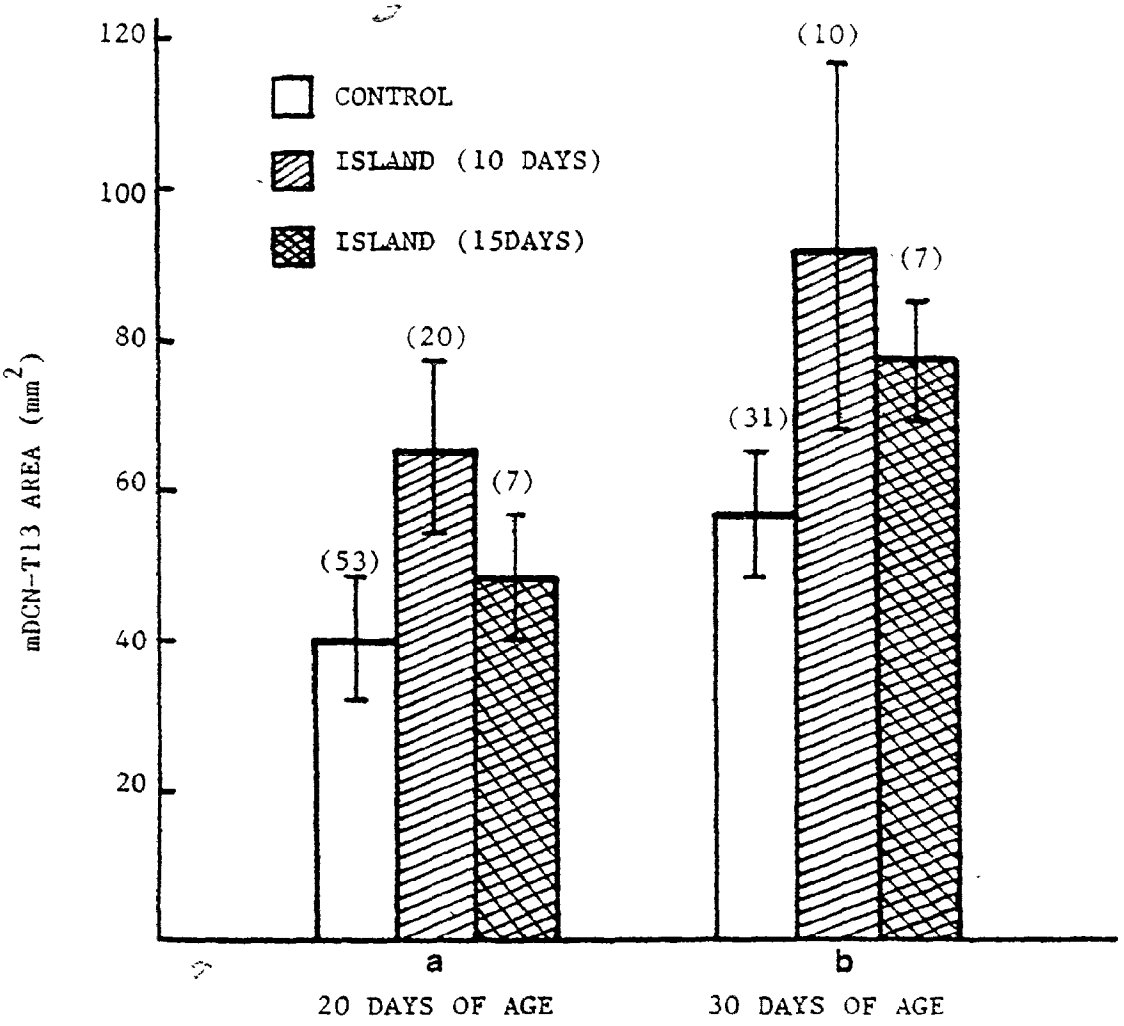
TABLE II.

AGE (DAYS)	CONTROL				ISLAND
15	18.6 ± 1.5 n=8	-	N.S. p>0.2	-	19.0 ± 3.4 n=4
20	19.0 ± 2.4 n=27	-	p<0.001	-	28.5 ± 2.0 n=8
30	18.1 ± 1.8 n=18	-	p<0.001	-	28.0 ± 0.8 n=4
40	18.6 ± 4.7 n=7	-	p<0.001	-	27.7 ± 1.5 n=3
60	18.8 ± 1.0 n=11	-	p<0.001	-	28.4 ± 3.2 n=11

FIGURE 19. Comparison of Island Fields Isolated at 10 or 15 Days of Age

These histograms show the areas (± S.D.) of mDCN-T13 receptive fields mapped at 20 (a) or 30 (b) days of age; island fields were isolated in two groups at either 10 or 15 days of age. The field isolated at 10 days of age became significantly larger ($p<0.01$) than those isolated at 15 days of age; the same was true for the number of domes supplied by these nerves (mean ± S.D., as shown below the histogram).

FIGURE 19.



AGE (DAYS)		20	30
DOMES:	CONTROL	19.0 ± 2.4 n=27	18.1 ± 1.8 n=18
	ISOLATED (15)	22.0 ± 2.4 n=7	24.3 ± 1.5 n=7
	ISOLATED (10)	28.5 ± 2.0 n=8	28.0 ± 0.8 n=4

domes innervated was, however, no greater than that seen after isolation of the island at 10 days of age (Fig. 20).

(iv) A fourth experiment addressed the possibility that the extra enlargement of an island field might cease because sprouting nerves were inhibited in some was as the endings grew nearer innervated skin even though there might still be a significant extent of denervated skin. Consequently experiments were done to determine whether increasing the area of the skin that was denervated would influence the amount of enlargement of the island field. The receptive fields of mDCNs-T13 were isolated as islands in animals at 5 days of age, however, in addition to cutting the adjacent branches of DCNsT10-L3 to produce the island, the lateral cutaneous nerves of segments T12-L1 were also cut on the left side. This produces a very much larger area of denervation surrounding the island field (see Fig. 9). The island fields in these animals were subsequently mapped at 20 days of age. As expected, the intact low-threshold mechano-sensory nerves supplying the isolated fields invaded the adjacent denervated skin; the expansion of the fields and the number of domes acquired, was no greater than that found in animals with the lesser areas of denervation (Fig. 21).

The conclusion drawn from these experiments is that at or about 20 days of age a critical period ends for evoking, and also for the continuation of, functional sprouting by intact low-threshold mechanosensory nerves into denervated skin.

FIGURE 20. The Number of Domes Innervated by mDCNs-T13 Isolated at 5 Days of Age

The histograms show the number of domes (± S.D.) innervated by mDCNs-T13 at 20 days of age, (a) in control animals; (b) in animals with fields isolated at 10 days of age; and (c), in animals with the fields isolated at 5 days of age. The number of animals in each group is given in paranethesis above the appropriate column. Even though denervated at different times before the end of the critical period, there was no significant difference in the number of domes innervated ($p>0.1$) between groups isolated at 5 days of age and those isolated at 10 days of age.

(Note: the animals denervated at 5 days of age (c) failed to thrive following the first surgery and at 20 days of age were found to have not achieved a normal weight gain. The mean body weight of this group was 30.3 ± 2.6 g, compared to normal littermate control weights of 40.3 ± 4.5 g. Since the surface area of these animals would be correspondingly smaller, the receptive field areas of intact mDCNs-T13 in these animals (48.4 ± 6.9 mm^2) are beyond interpretation, and as such are not included in this figure.

No other group was subject to this failure to thrive; no experimental group was significantly different in weight than its control ($p>0.2$).

FIGURE 20.

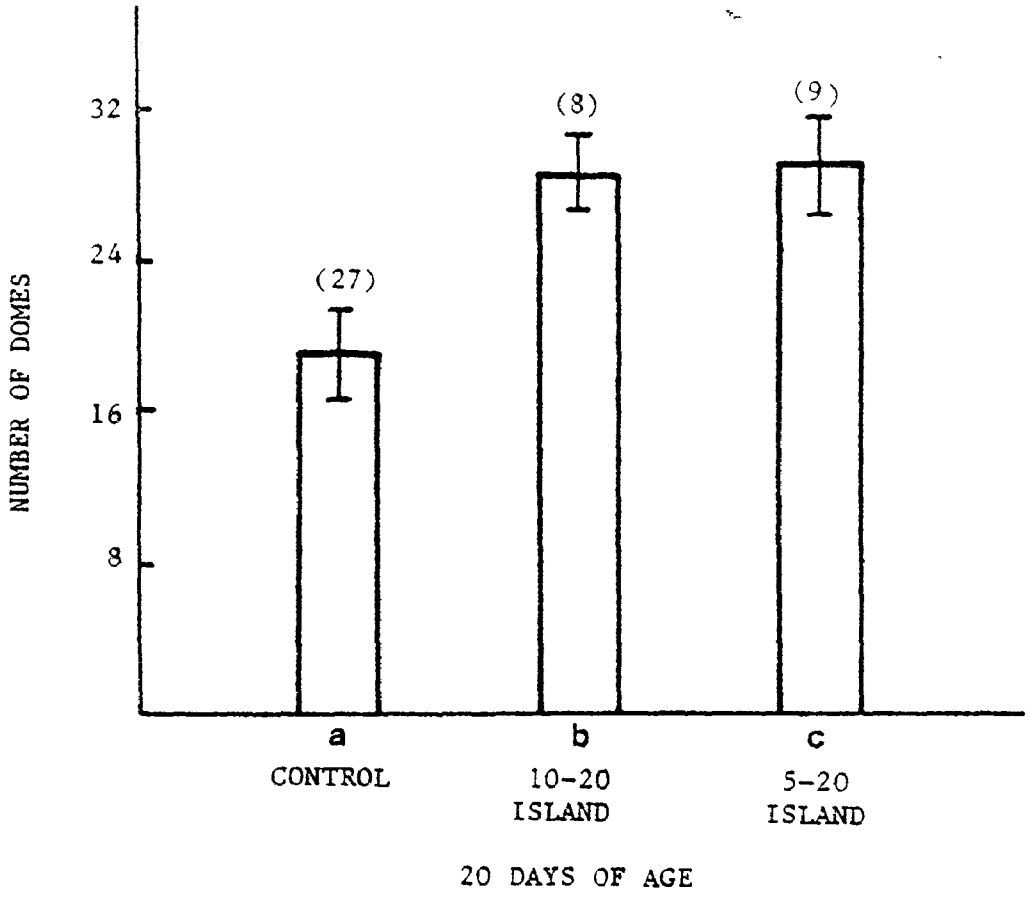
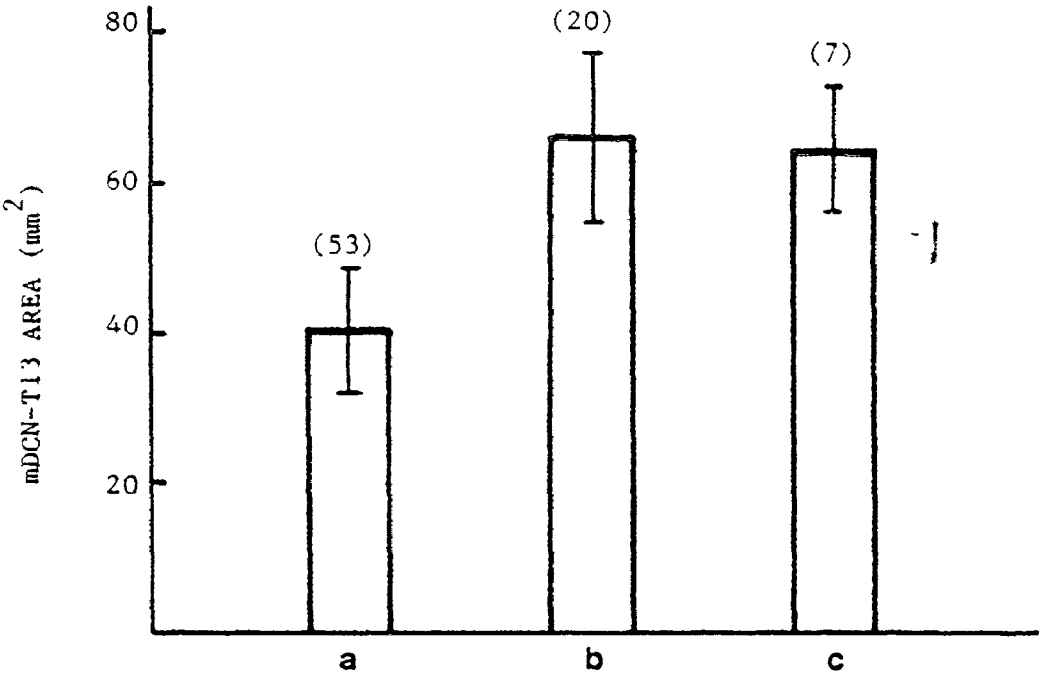

NUMBER OF DOMES
32
24
16
8
(27)
(8)
(9)
a
CONTROL
b
10-20
ISLAND
c
5-20
ISLAND
20 DAYS OF AGE

FIGURE 21. Failure of More Extensive Denervation to Evoke More Extensive Expansion of an Isolated Field

The histograms show the area (± S.D.) of mDCN-T13 fields mapped at 20 days of age in control animals (a), and in two groups of animals (b and c) isolated at 10 or 5 days of age respectively. The number of animals in each group is given in paranthesis above the appropriate column. In the group denervated at 10 days of age the island field was produced by cutting, as usual, the adjacent branches of the DCNs of segments T10-L3; island fields at 5 days of age were similarly produced but, in addition, a more extensive denervation of skin was carried out by cutting also the lateral cutaneous nerves of segments T12-L1 on the left side. The island fields of both experimental groups were significantly larger ($p<0.001$) than the age matched controls. However there was no significant difference ($p>0.1$) between the areas of the experimental groups. The number of domes (± S.D.) innervated by mDCNs-T13 in these groups are given below in the histograms. The mDCNs-T13 isolated earlier and more extensively, at 5 days of age, came to innervate larger than normal number of domes ($p<0.001$) but no more so than those isolated at 10 days of age by the lesser denervation ($p>0.1$).

FIGURE 21.


80
60
40
20
mDCN-T13 AREA (mm²)
(53)
(20)
(7)
a
b
c
CONTROL
10-20
ISLAND
5-20
SUPER-ISLAND
DOMES
19.0 ± 2.4
n=27
28.5 ± 2.0
n=8
29.6 ± 2.6
n=7

(b) The beginning of the critical period

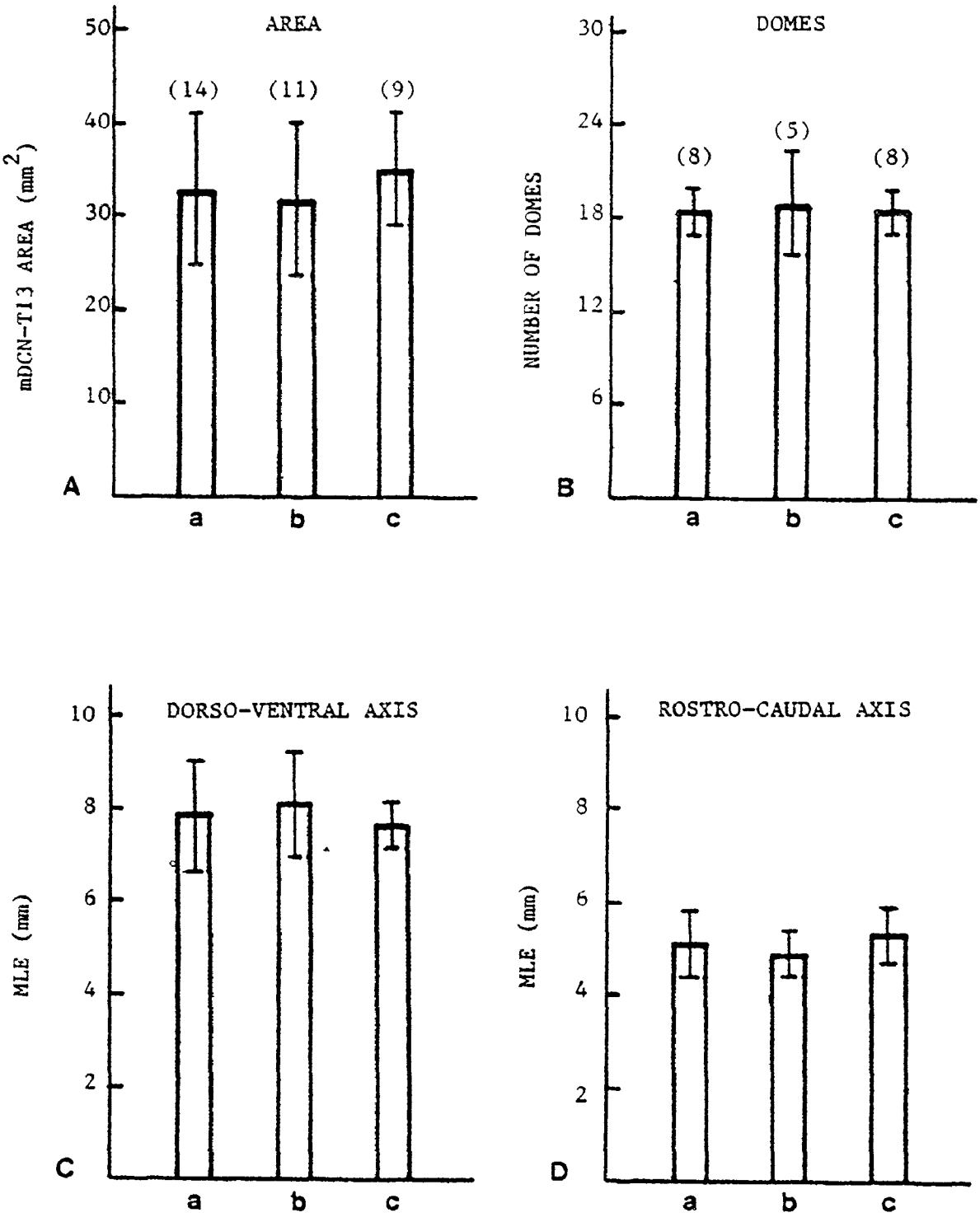
The observation that island fields isolated at 5 days of age expanded to the same extent at 20 days of age, as those isolated at 10 days of age (Figs. 20 and 21) suggested the possible existence of a postnatal beginning to the critical period for sprouting of intact low-threshold mechanosensory nerves into denervated skin. This possibility was tested. The receptive fields of mDCNs-T13 were isolated in 5 and 10 day old rats and mapped at 15 days of age. The results showed clearly that the isolated fields in the animals of both groups, when mapped at 15 days of age, were neither significantly different from each other, nor from normal 15 day old control fields (Fig. 22). That is, no detectable sprouting of the intact isolated nerves occurred before 15 days of age in either experimental group. It follows from these findings that the critical period for establishing functional endings, or for sprouting itself, must begin after 15 days of age; if sprouting was initiated earlier, the endings were not excitable.

It can be concluded from these investigations that, in the rat, there is a remarkably brief critical period during which intact low-threshold mechanosensory nerves will sprout (as indicated by establishment of sensory function) into adjacent denervated skin. This period begins at, or a little after 15 days of age and ends at about 20 days of age.

FIGURE 22. Lack of Field Expansion into Denervated Skin Between 5 and 15 Days of Age

Shown in this figure are the results obtained by mapping mDCNs-T13 in three groups of animals at 15 days of age: (a) normal unoperated controls (n=14), (b) island fields isolated at 10 days of age (n=11), (c) island fields isolated at 5 days of age (n=9). The number of animals in each group is given above the appropriate column. The top two histograms give the area (A) and number of domes (B) supplied by the mDCNs-T13 of these three groups. Neither experimental group was significantly different from controls ($p>0.1$) in area or number of domes innervated, nor were they different ($p>0.1$) from each other. The lower pair of histograms (C and D) in this figure gives the measurements made of the Maximum Linear Extent (see text) of these fields in the two major axes of the parent dermatome. Neither group of isolated fields was significantly extended, in either axis, nor were they different from each other ($p>0.1$).

FIGURE 22.


AREA
mDCN-T13 AREA (mm²)
50
40
30
20
10
(14)
(11)
(9)
A
a
b
c
DOMES
NUMBER OF DOMES
30
24
18
12
6
(8)
(5)
(8)
B
a
b
c
DORSO-VENTRAL AXIS
MLE (mm)
10
8
6
4
2
C
a
b
c
ROSTRO-CAUDAL AXIS
MLE (mm)
10
8
6
4
2
D
a
b
c

7. Preferential Sprouting of Intact Nerves Within their "Parent" Dermatome

In the course of measuring the areas of the island and control fields of mDCNs-T13, the enlargement of fields isolated before the end of the critical period appeared to be accentuated in the lateral (dorso-ventral) direction. This was examined in order to determine, quantitatively whether the sprouting of the intact mDCNs-T13 had occurred uniformly into the surrounding denervated regions or if it had in fact occurred preferentially into the denervated skin of the "parent" (T13) dermatome.

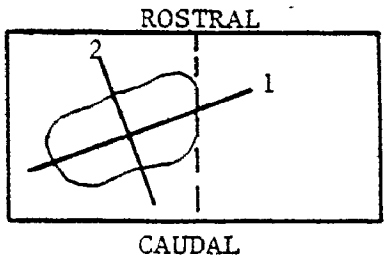
A straight edge rule was used to measure the "Maximum Linear Extent" (MLE) of mDCN-T13 fields both in the long axis of the dermatome and at right angles to it. An increase over controls of the MLE in the long axis (approximately dorso-ventral) would reflect sprouting of mDCN-T13 into denervated skin of the parent dermatome; an increase in the short axis (approximately rostro-caudal) would reflect sprouting of mDCN-T13 into denervated skin of the adjacent dermatomes. These measurements were carried out on control fields and on island fields isolated at 10 days of age and mapped subsequently. The results of these measurements are shown in Figure 23.

The island fields isolated at 10 days of age had enlarged in both major axes; sprouting had thus occurred into skin both within and adjacent to the dermatome of the original field. The

FIGURE 23. Maximum Linear Extension of Intact mDCNs-T13 into Denervated Skin

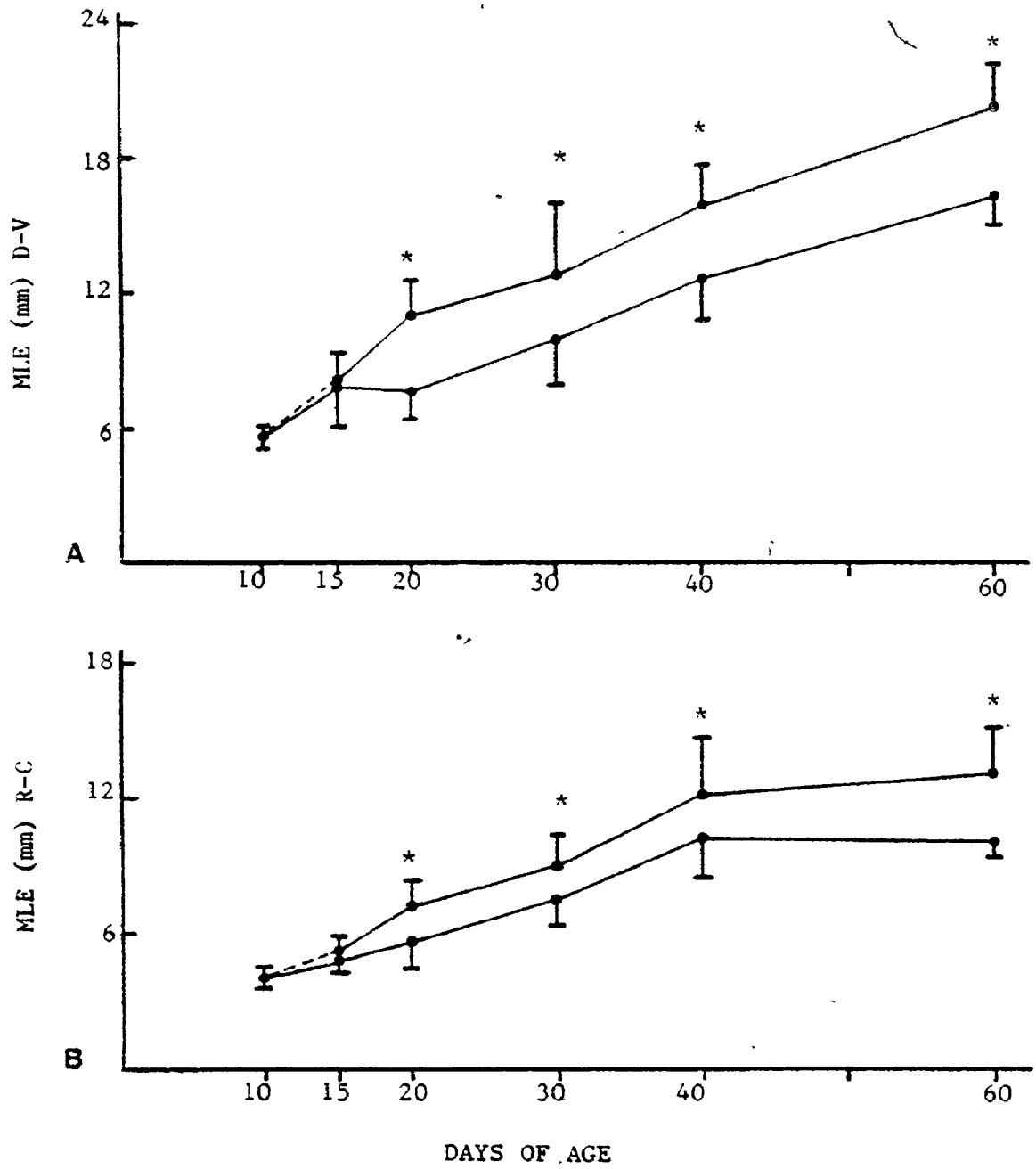
This figure shows the "Maximum Linear Extent" (MLE) of mDCN-T13 fields in the long axis of the dermatome, and in the axis at the right angles to this. The insert (see box above line graphs) demonstrates diagramatically both of these measurements. The orientation of the long axis of the dermatome of T13 was estimated based on experience with many of these preparations. A straight edge ruler marked at 0.5 mm intervals was then placed parallel to this axis and drawn across the map of the field. The Maximum Linear Extent (distance between two points on the border) in this axis was measured (A). The procedure was repeated with the straight edge rule at right angles to the long axis (B). Measurements of this sort were made on the fields isolated as islands of intact innervation at 10 days of age and on the fields of normal control animals. A significant difference ($p<0.001$) between measurements made on isolated fields and those of age matched controls is indicated by an asterisk (*) above that point of the line graph.

FIGURE 23.



1-LONG AXIS(D-V)

2-SHORT AXIS (R-C)



nerves supplying isolated island fields had however extended further into denervated skin which lay within the parent dermatome. This asymmetrical expansion of isolated island fields was experimentally examined in more detail.

(a) Sprouting in the dorso-ventral axis

The first question was: "how much, if any, of the observed increase in the length of the MLE in the dorso-ventral axis was due to sprouting of the island nerves across the dorsal midline into the denervated skin of the opposite side?"

In 10 day old rats the dorsal midline of the trunk region is visible to the naked eye as a faint dark line running the length of the animal. The low-threshold mechanosensory fields of the DCNs abut bilaterally along this line with little if any detectable overlap. In two groups of six animals tattoo dots were placed at roughly 0.5 mm intervals along this line in the region of the T13 dermatomes. In one group the receptive field of the left mDCN-T13 was isolated as previously described; the other group was anaesthetized but not surgically manipulated. When mapped at 20 days of age the medial border of the low-threshold mechanosensory field of the left mDCN-T13 in both groups were found to coincide with the previously tattooed line - there was clearly no functional sprouting of the intact isolated nerves across the midline, even though denervated skin was ostensibly available there. The evoked increase observed in the dorso-ventral axis of the expanded fields (Fig. 23A) was therefore entirely attributable to the intact mDCNs-T13

establishing functional endings across their lateral border in denervated skin of the T13 dermatome.

(b) Sprouting in the rostro-caudal axis

In the skin there are no visible surface landmarks indicating the normal rostral or caudal boundaries of the sensory dermatomes. Although not specifically investigated in these experiments, there were no observations to suggest that the expansion of the island field in the short axis of the dermatome was preferentially either rostral or caudal. Since it was not possible to distinguish between expansion rostrally and expansion caudally the simplest interpretation has been adopted, namely that the measured "extra" increase in the rostro-caudal axis of isolated fields (Fig. 23B) was due to a small but significant sprouting of the intact nerves equally into both of the adjacent dermatomes. Even if one was to make the assumption that the evoked increase of the MLE in the rostro-caudal axis (1.5 mm) of island fields was entirely into one or the other of the adjacent denervated dermatomes, it is still clear that there was a greater evoked expansion (3.5 mm) into the denervated skin lying within the parent dermatome.

(c) Lack of sprouting outside a fully innervated dermatome

Because of the above findings on the asymmetrical expansion of the isolated field, an attempt was made to "improve" the expansion in the apparently less favoured direction (rostro-caudal) by providing denervated skin, during the critical period,

only at the rostral and caudal borders of the field. To achieve this, at ten days of age DCNs of T10-T12 and L1-L3 were cut on the left side of six animals; the left DCN-T13 (both medial and lateral branches) remained intact. On the right side the DCNs of T10-L3 were cut (Fig. 24). Since functional sprouting was known not to occur across the dorsal midline, if evoked enlargement of the left DCN-T13 were to occur it would be observed only as a consequence of sprouting into the denervated skin of the T12 and L1 dermatomes on the left side. These partially isolated fields of the intact DCN-T13 were mapped at 20 days of age. Unexpectedly there was no detectable evoked expansion of the intact DCN-T13 into the adjacent denervated dermatomes (Fig. 25); neither areas nor MLE axis measurements were larger than normal control fields for either the medial or lateral branches.

It appeared then that intact low-threshold mechanosensory fields were able to expand functionally into adjacent denervated dermatomes only when they were also able to expand into denervated skin within the same dermatome.

8. Sprouting of Dorsal Cutaneous Nerves into Lateral Cutaneous Nerve Territories

The medial branch of DCNs-T13 with peripheral receptive fields isolated at 10 days of age ceased sprouting in the lateral direction (within the dermatome) before they reached the boundary between the whole DCN field and that of the lateral cutaneous nerves

FIGURE 24. The "Isolated Dermatome"

Shown diagramatically is the pattern of denervation produced as follows:

The DCNs of segments T10-T12 and L1-L3 were cut on both sides; DCN-T13 was cut on the right side only. The stipled area indicates the denervated region of skin which partly surrounds the receptive field of the intact, whole DCN-T13 of the left side.

FIGURE 24.

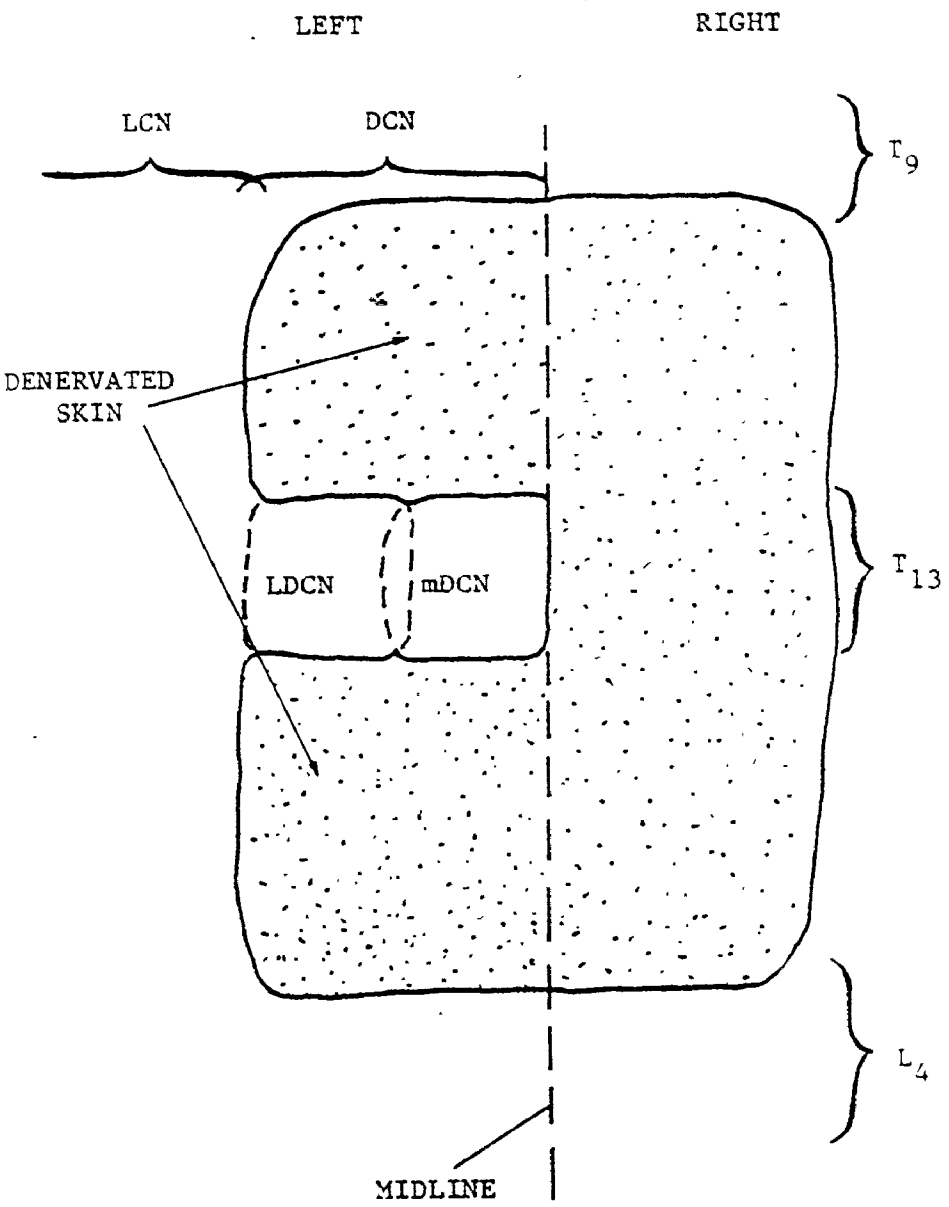
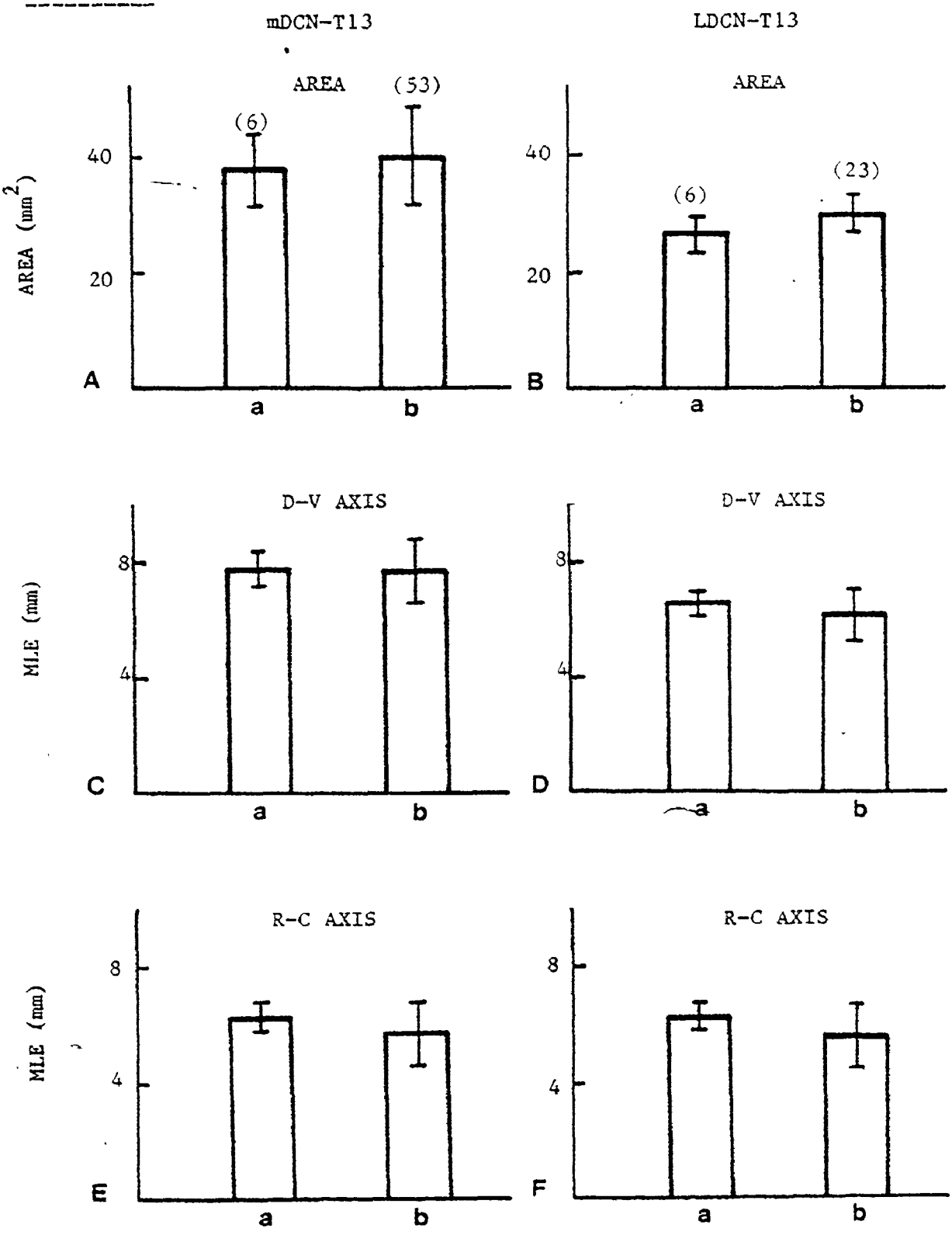

LEFT
RIGHT
LCN
DCN
T_9
DENERVATED SKIN
LDCN
mDCN
T_{13}
L_4
MIDLINE

FIGURE 25. Failure of DCN Fields to Enlarge into Denervated Skin of Adjacent Dermatomes

Nerve sections were done in six pups at 10 days of age to produce the pattern of denervation shown in Figure 24. At 20 days of age the medial and lateral branches of DCN-T13 were mapped separately. In each histogram (A-F) the experimental group is on the left (a) and the control groups on the right (b). The receptive field areas of neither medial (A) nor lateral (b) branches of intact DCNs-T13 were found to be larger than control fields in 20 day old normal unoperated animals, nor were there significant differences in the dorso-ventral axis (C and D) or in the rostro-caudal axis (E and F). For all measurements $p > 0.1$.

FIGURE 25.


mDCN-T13
LDCN-T13
AREA
AREA
(53)
(6)
(6)
(23)
AREA (mm²)
40
20
A
B
a
b
D-V AXIS
D-V AXIS
MLE (mm)
8
4
C
D
R-C AXIS
R-C AXIS
MLE (mm)
E
F

(Fig. 26; see also Fig. 9). It was possible therefore that the intact axons supplying an isolated sub-field could establish functional endings readily only in denervated skin within the whole DCN field. Experiments were designed therefore to determine if intact DCN axons would also functionally invade the territory of the lateral cutaneous nerve of the same segment if the skin were made available by denervation. These experiments involved, of necessity, the lateral branch of the DCN of T13 (LDCN-T13).

It was first necessary to establish that this branch resembled the medial one in being competent to expand its low-threshold mechanosensory sprout functional field into denervated skin during the critical period. In 10 day old animals the DCNs of T10-L3 were cut bilaterally except for the LDCN-T13 on the left side (see Fig. 9). The low-threshold mechanosensory field of this intact LDCN-T13 was subsequently mapped at 20 days of age and compared to those of normal controls. The results resembled those already described for the isolated medial branch of the same nerve. The LDCN-T13 field had expanded into denervated skin and was clearly larger in area than fields of LDCNs-T13 in normal controls (Fig. 27). Measurement of MLE of these fields in the two major axes of the dermatome showed that for this nerve too, the sprouting intact axons extended preferentially into the denervated skin that lay within the same dermatome (Fig. 27).

FIGURE 26. The Maximum Linear Extent of Expanded mDCN Fields Compared to the DCN Field

In this figure the maximum linear extent of island fields (isolated at 10 days of age) in the long axis of the dermatome is shown at 20, 30 and 40 days of age; shown too is the maximum linear extent of normal whole DCNs (medial plus lateral branches) in the same axis at the same ages. The isolated mDCN-T13 fields extended in the long axis of the dermatome but stopped short of complete occupation of the denervated skin within the parent dermatome.

FIGURE 26.

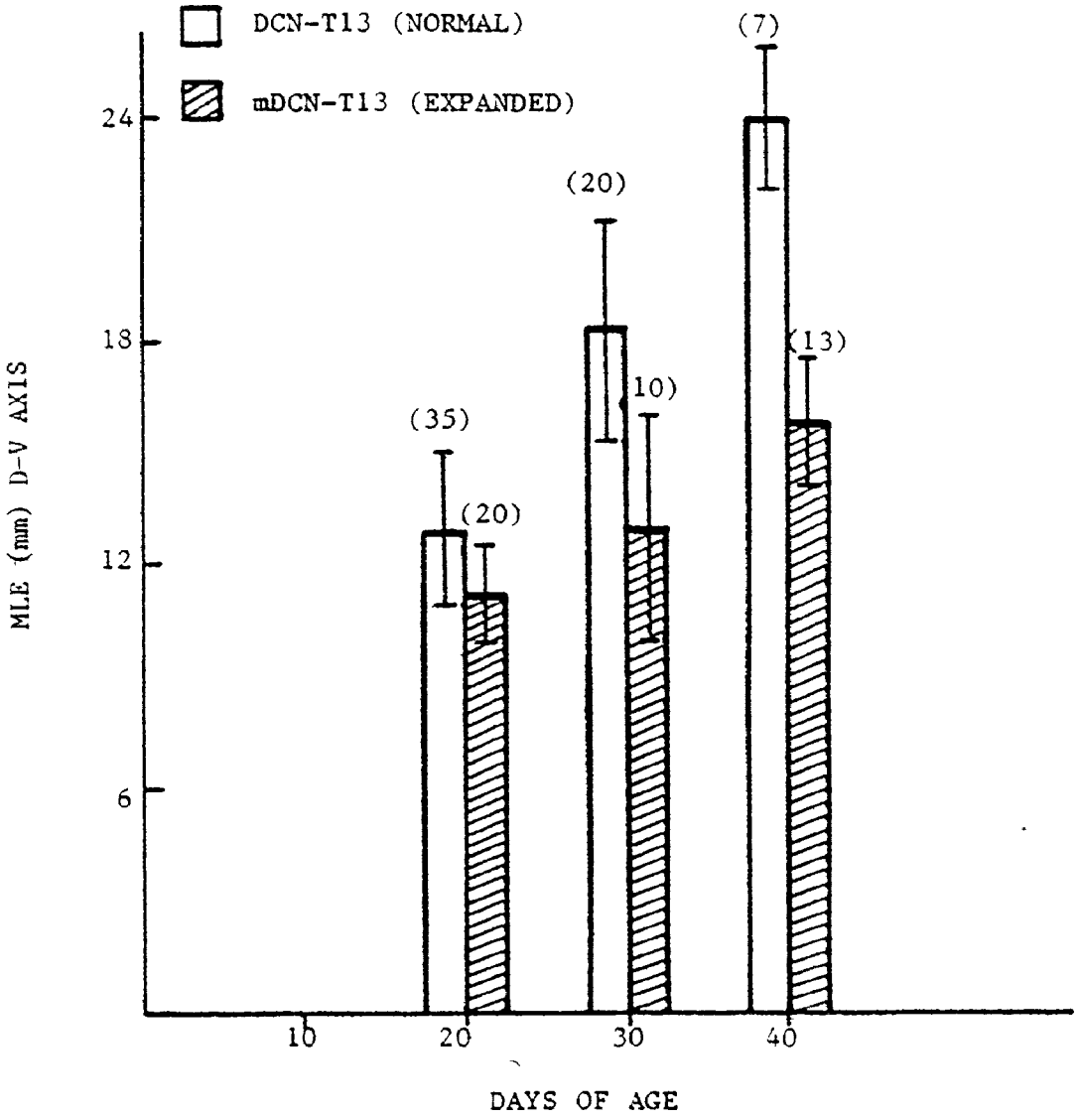
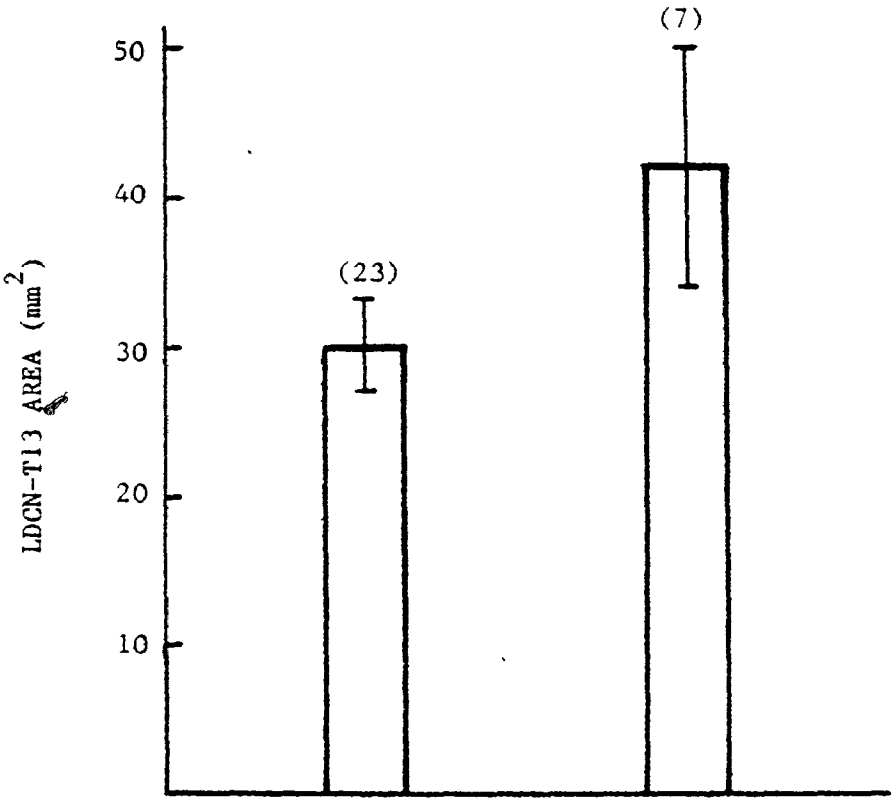

DCN-T13 (NORMAL)
mDCN-T13 (EXPANDED)
MLE (mm) D-V AXIS
24
18
12
6
(35)
(20)
(20)
10)
(7)
(13)
10
20
30
40
DAYS OF AGE

FIGURE 27. Expansion of the Lateral Branch of DCN-T13 into Denervated Skin

The histograms show the area (± S.D.) of receptive fields mapped for the lateral branch of DCNs-T13 at 20 days of age in control animals and those in which the mDCN-T13 and adjacent DCNs T10-L3 were cut at 10 days of age. These partly isolated LDCN-T13 fields were larger ($p<0.01$) than control fields mapped at the same age. The maximum linear extent (MLE) of these fields in the dorso-ventral and rostro-caudal axes of the dermatome are shown below the histogram. The number of animals in each group is given in paranthesis above the appropriate column.

FIGURE 27.



MLE:	D-V	6.2 ± 1.0 n=23	8.0 ± 0.7 n=7
	R-C	5.7 ± 0.7 n=23	6.4 ± 0.6 n=7

Having established the ability of the intact LDCN to establish functional endings in denervated skin, a second group of animals was selectively denervated in order to answer the specific question - "will the LDCN-T13 sprout into denervated skin within the parent dermatome, but beyond the normal boundaries of the whole DCN field"? In this group of animals the DCNs of T10-L3 were cut bilaterally at 10 days of age except that both branches of the left DCN-T13 remained intact. In addition to these denervations the Lateral Cutaneous Nerves (LCNs) of segments T12-L1 were cut on the left side. (Note - these are not the lateral branches of the DCNs, but the yet more lateral cutaneous divisions of the parent segmental nerves.) The entire DCN-T13 field (medial plus lateral sub-fields) was thus effectively isolated as an island of intact innervation in a sea of denervated skin. The only skin available to these DCNs was that of the adjacent T12 and L1 dermatomes already shown not to be preferred territory, and the more lateral skin formerly supplied by the LCNs. At 20 days of age the isolated DCNs-T13 were mapped; the medial and lateral branches were also mapped separately. As expected the medial branch of DCN-T13 had not expanded to takeover any of the denervated skin or touch domes of adjacent dermatomes (Table III). As also shown in the Table the lateral branch of DCN-T13 had, however, clearly enlarged its field, principally into the denervated skin of the parent dermatome formerly supplied by the LCN-T13; the number of domes supplied by the expanded LDCNs-T13 was also increased.

TABLE III The DCN-T13 as an Isolated Island of Innervation

Shown here are the results obtained from a group of animals (n=11) in which the entire receptive field (both branches) of DCN-T13 was isolated at 10 days of age by cutting adjacent DCNs of the segments T10-L3, plus the Lateral Cutaneous Nerves of segments T12-L1 on the left side of the animal. At 20 days of age the medial and lateral branches of the remaining intact DCN-T13 were mapped. The results are presented for whole DCN fields, and for the medial and lateral branches considered separately; significant differences between island and control measurements are enclosed by heavy black lines and the p value given.

TABLE III.

	DCN-T13			mDCN-T13		LDCN-T13		
	ISLAND		CONTROL	ISLAND	CONTROL	ISLAND		CONTROL
AREA mm^2	81.1 + 11.4 n=11	p<0.001	66.4 + 10.0 n=35	39.9 + 7.5 n=11	40.0 + 8.3 n=53	45.8 + 7.9 n=11	p<0.001	30.0 + 3.1 n=23
R-C AXIS mm	6.0 + 0.5 n=11		6.0 + 0.9 n=35	6.0 + 0.5 n=11	5.7 + 1.1 n=53	6.0 + 0.5 n=11		5.8 + 0.7 n=28
D-V AXIS mm	15.8 + 1.6 n=11	p<0.001	12.3 + 2.1 n=35	8.5 + 1.6 n=11	7.7 + 1.1 n=53	8.8 + 1.6 n=11	p<0.001	6.3 + 1.0 n=23
DOMES	35.4 + 4.1 n=11	p<0.001	29.6 + 2.7 n=27	19.9 + 2.0 n=11	19.0 + 2.4 n=27	15.5 + 0.4 n=11	p<0.001	10.7 + 1.6 n=20

In addition to showing that low-threshold mechanosensory DCN axons could establish functional endings across their normal boundaries in denervated LCN skin, the results of these last two experiments also demonstrated that intact DCN axons are able to extend their mechanosensory fields into denervated skin earlier towards or away from the dorsal midline; expansion in either direction was preferred to that rostrally or caudally.

9. Do Similar Temporal and Spatial Constraints Operate on Regenerating Nerves?

It seemed possible that the preferred extension of intact nerves into denervated skin within the parent dermatome might result from the guidance of the growing endings by some structural or mechanical feature of the skin. If so, these might be expected to act also on growing endings of nerves that were regenerating after section. Low-threshold mechanosensory fields established by regenerating nerves in denervated skin were, therefore, examined.

The mechanosensory field of the medial branch of DCN-T13 was mapped, in continuity, in seven 40 day old animals, and the borders obtained were tattooed into the skin. The field was then isolated by cutting the lateral branch of DCN-T13 and both branches of the surrounding DCNs of segments T10-L3 on both sides. The central stump of the cut nerves were ligated to prevent their regeneration. The intact mDCN-T13 was then crushed at the point where it enters the underside of the skin. Crushing, rather than cutting,

was done in order to promote successful regeneration. Three weeks later the previously crushed mDCNs-T13 of three animals were mapped, again in continuity, to determine if low-threshold mechanosensory function had been restored. In each animal the mDCN-T13 had regenerated and reestablished a small field of low-threshold mechanosensory innervation located within the original borders of the field (Fig. 28). These animals were again allowed to recover, and after a further 2 week period (total - 5 weeks after crush) the mDCNs-T13 of all seven animals were remapped. In each animal the regenerating mDCN-T13 was found to have established a low-threshold mechanosensory field which not only occupied its original territory, but also extended beyond the tattooed boundaries (Fig. 29). The fields were found to have expanded equally in the rostro-caudal axis (22.5 ± 1.4 mm) and in the dorso-ventral axis (22.1 ± 1.2 mm; Fig. 29). In four of the seven animals at this mapping (5 weeks after crush) the low-threshold mechanosensory field of the regenerating mDCN-T13 extended 2-3 mm across the dorsal midline into denervated skin of the opposite side as indicated by the tattooed boundary.

These findings show that there were no obvious mechanical constraints on the regenerating low-threshold mechanosensory axons and indicate that such are unlikely to operate on intact ones when sprouting. The regenerating fibres appeared freely able to cross dermatomal boundaries into "foreign" skin. Of interest was the finding that regenerating nerves were

FIGURE 28. Recovery of Low-Threshold Mechanosensory Function by a Regenerating Nerve

Shown in this figure is the result obtained from the three animals in which the mDCN-T13 on the left side had been crushed immediately under the skin three weeks earlier; at that time the LDCN-T13 and adjacent DCNs of segments T10-L3 had been cut and ligated to prevent their regeneration. By three weeks the regenerating mDCN-T13 was found to have established the peripheral field indicated by the hatched area; the tattoo dots indicated marked the original boundaries of the mDCN-T13 mapped (in continuity) at the time of the initial surgery. The star (★) indicates the point of entry into the skin of the regenerating mDCN-T13.

FIGURE 28.

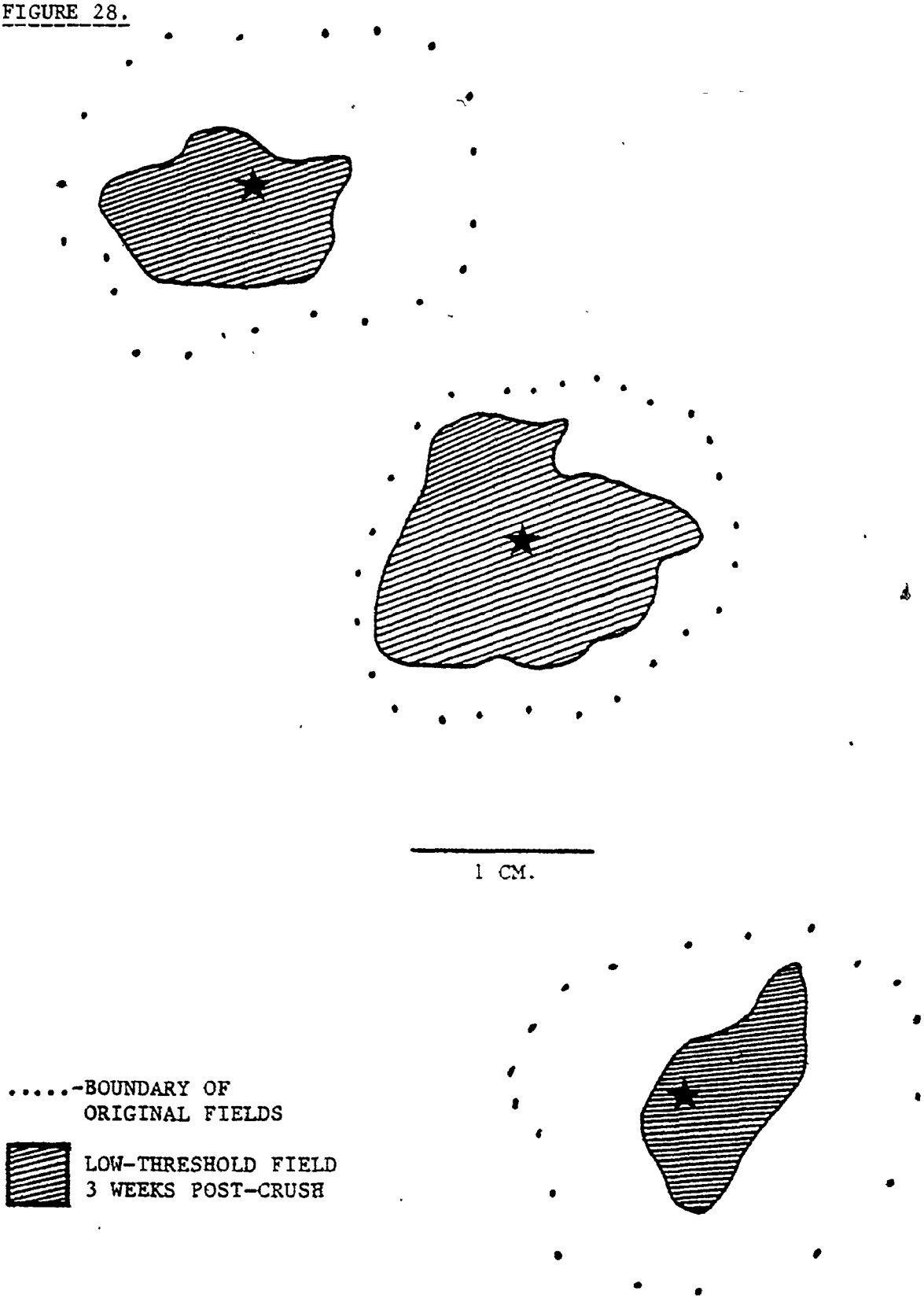
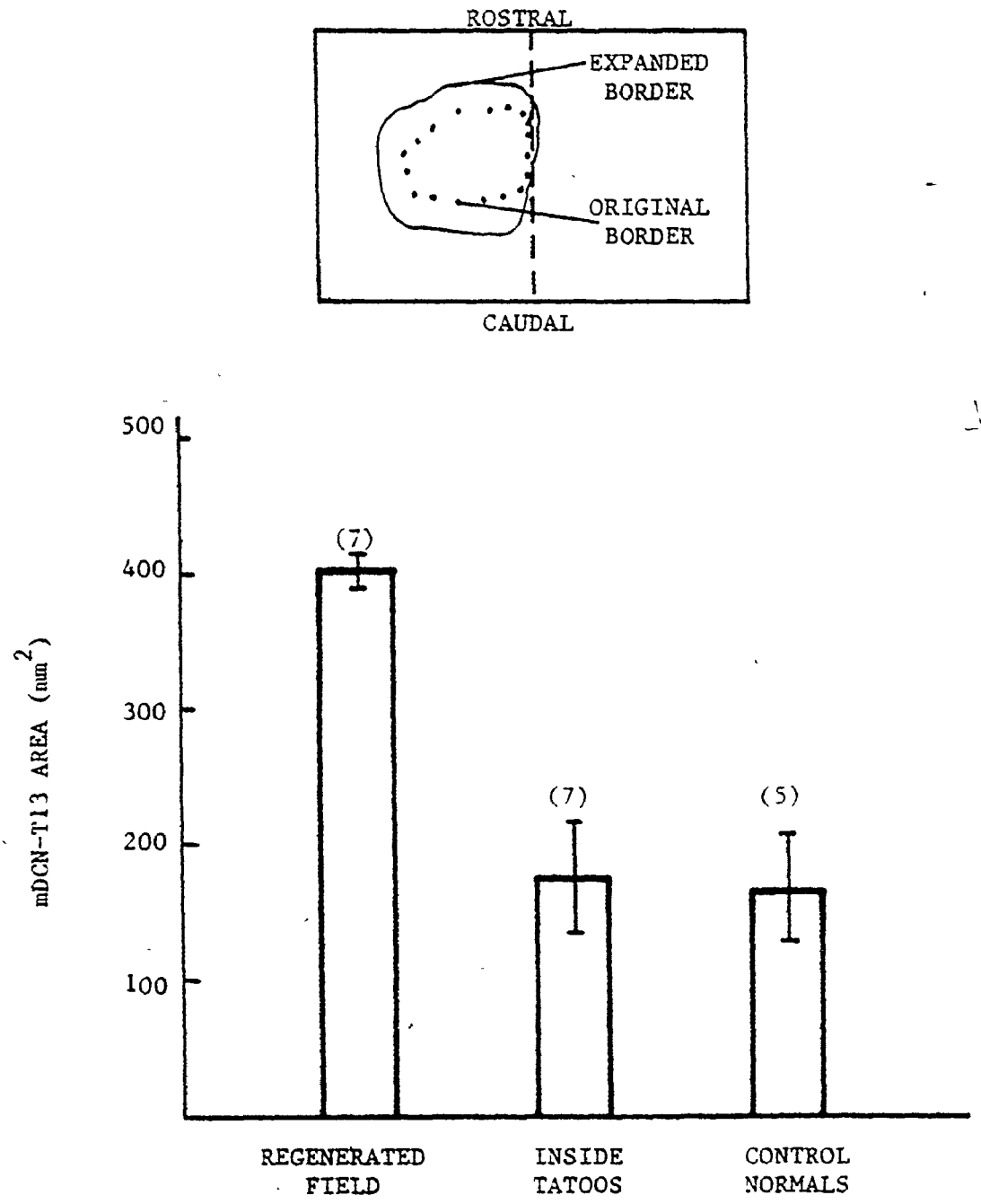

1 CM.
.....-BOUNDARY OF
ORIGINAL FIELDS
LOW-THRESHOLD FIELD
3 WEEKS POST-CRUSH

FIGURE 29. Extension of a Regenerating Nerve's Peripheral Field Beyond its Former Boundaries

The upper part of this figure depicts the low-threshold mechanosensory field established by a regenerating mDCN-T13 5 weeks after it had been crushed immediately under the skin; the adjacent branches of DCNs T10-L3 had been cut and ligated, to prevent regeneration, at the time mDCN-T13 was crushed. The low-threshold mechanosensory receptive fields established by this regenerating nerve, as in the six other animals, had clearly extended beyond the normal boundaries as indicated by the tattoo reference dots. In the lower part of the figure the histograms show the area (± S.D.) of the receptive feilds established by regenerating mDCNs-T13 in this group of animals; for comparison the areas enclosed by the tattoo reference dots were also measured in these animals. The receptive fields of mDCNs-T13 in a group of unoperated control animals of the same age are also shown.

FIGURE 29.


ROSTRAL
EXPANDED BORDER
ORIGINAL BORDER
CAUDAL
500
400
300
200
100
mDCN-T13 AREA (mm²)
(7)
(7)
(5)
REGENERATED FIELD
INSIDE TATOOS
CONTROL NORMALS

able to cross the midline and establish mechanosensory function in the denervated skin of the opposite side; the midline boundary was the most powerful spatial constraint operating on intact nerves within the critical period.

10. Competition Between Intact and Regenerating Nerves

(a) Regenerating nerves in their former territories: "original" nerves

The low-threshold mechanosensory receptive fields of intact mDCNs-T13 that had expanded into denervated skin during the critical period are maintained up to at least 60 days of age (Fig. 18). In the absence of regeneration by the initially cut nerves these expanded fields seem stable, even though surrounded by denervated skin. Since regeneration of the DCNs is not confined to early life, the question was asked - "what is the consequence to the expanded island field of allowing the initially cut nerves to regenerate back to their former territories?" Of special interest was the possibility that a competition may ensue between the sets of nerves, the sprouted and the regenerated, for both the territory and specific targets in it.

As before, the mechanosensory fields of mDCNs-T13 were isolated in a group of 10 day old rat pups. To ensure complete denervation of the surrounding skin, but at the same time to encourage regeneration of nerves, the branches of DCNs of segments T10-L3 (except for the left mDCN-T13) were simply cut near the

body wall; the cut nerves were neither resected nor ligated and, since there is virtually no tension on these nerves, the central and peripheral stumps of these cut nerves remained in close apposition, a condition conducive to successful regeneration of peripheral nerves.

At weekly intervals one or two animals were taken from this group, anaesthetized, and the appropriate nerves exposed. By recording from the originally cut nerves central to the site of the initial axotomy, functional regeneration of low-threshold mechanosensory nerves was examined. Recovery of mechano-sensitivity in the skin by the regenerating nerves was first detected only after approximately 35 days had elapsed (45 days of age) before which the fields of the intact mDCN-T13 had expanded into denervated skin. At ~~60~~ days of age in the remaining animals the mechanosensory fields of the intact mDCNs-T13 were mapped as were those of the now-regenerated adjacent branches of DCNs-T12 and L1. The regenerating nerves were found to have established their fields in roughly their normal pattern (Fig. 30). The formerly isolated island field of the intact mDCN-T13, was not obviously expanded. The areas of these mDCN-T13 fields, now surrounded by regenerated innervation, were found to be not different than control values (Fig. 31); these once sprouted mDCNs-T13 had apparently relinguished the extra territory acquired in adjacent denervated skin (Fig. 18) to the regenerating original nerves. Of special interest was the finding that the number of touch domes now

FIGURE 30. Fields Established by Regenerating Nerves Around an Island of Intact Innervation

In this figure is shown the general pattern of low-threshold mechanosensory fields established in the skin by 60 days of age; the branches of DCNs T10-L3 cut at 10 days of age were, in this group, neither resected nor ligated, thus allowing their regeneration. The mDCN-T13 remained intact.

FIGURE 30.

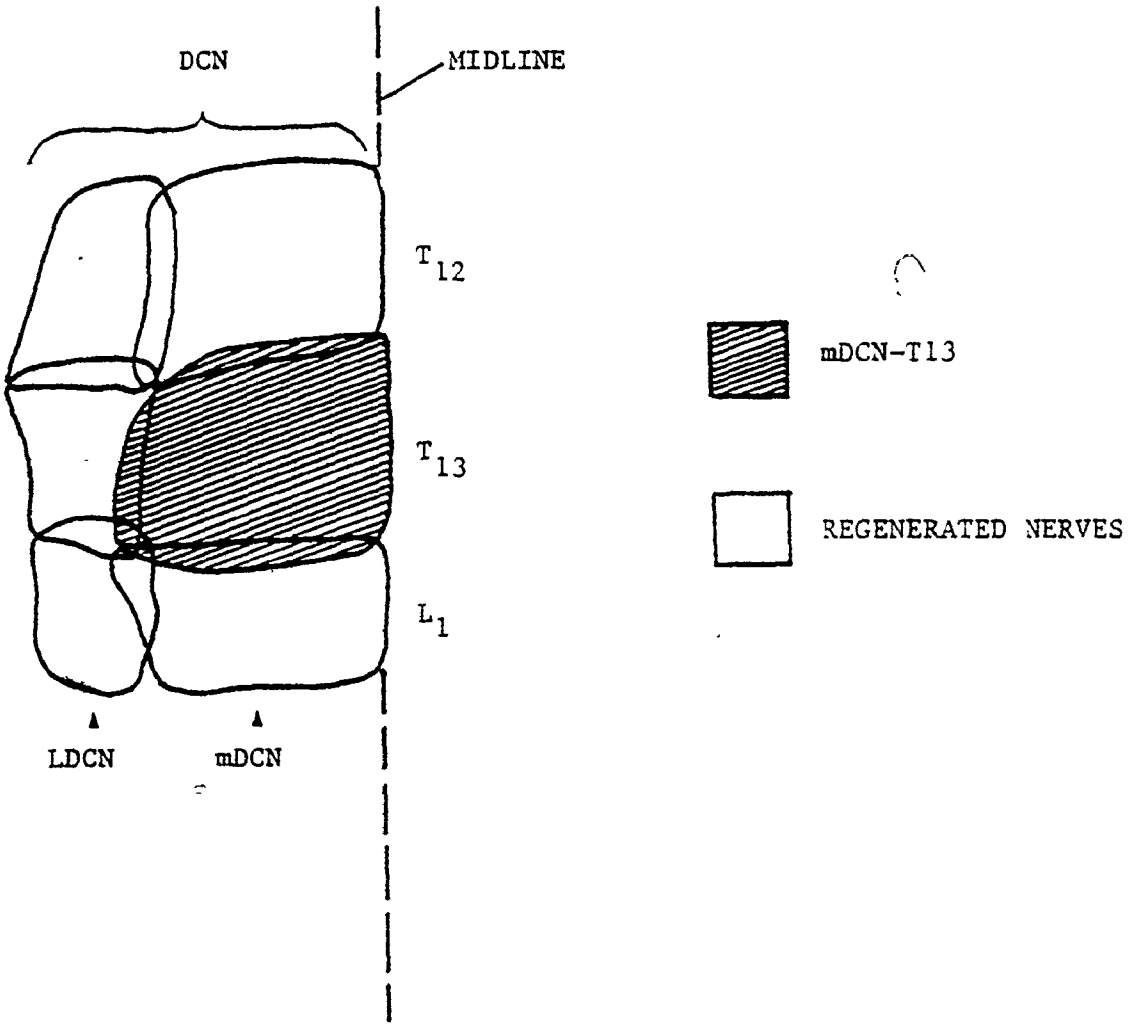
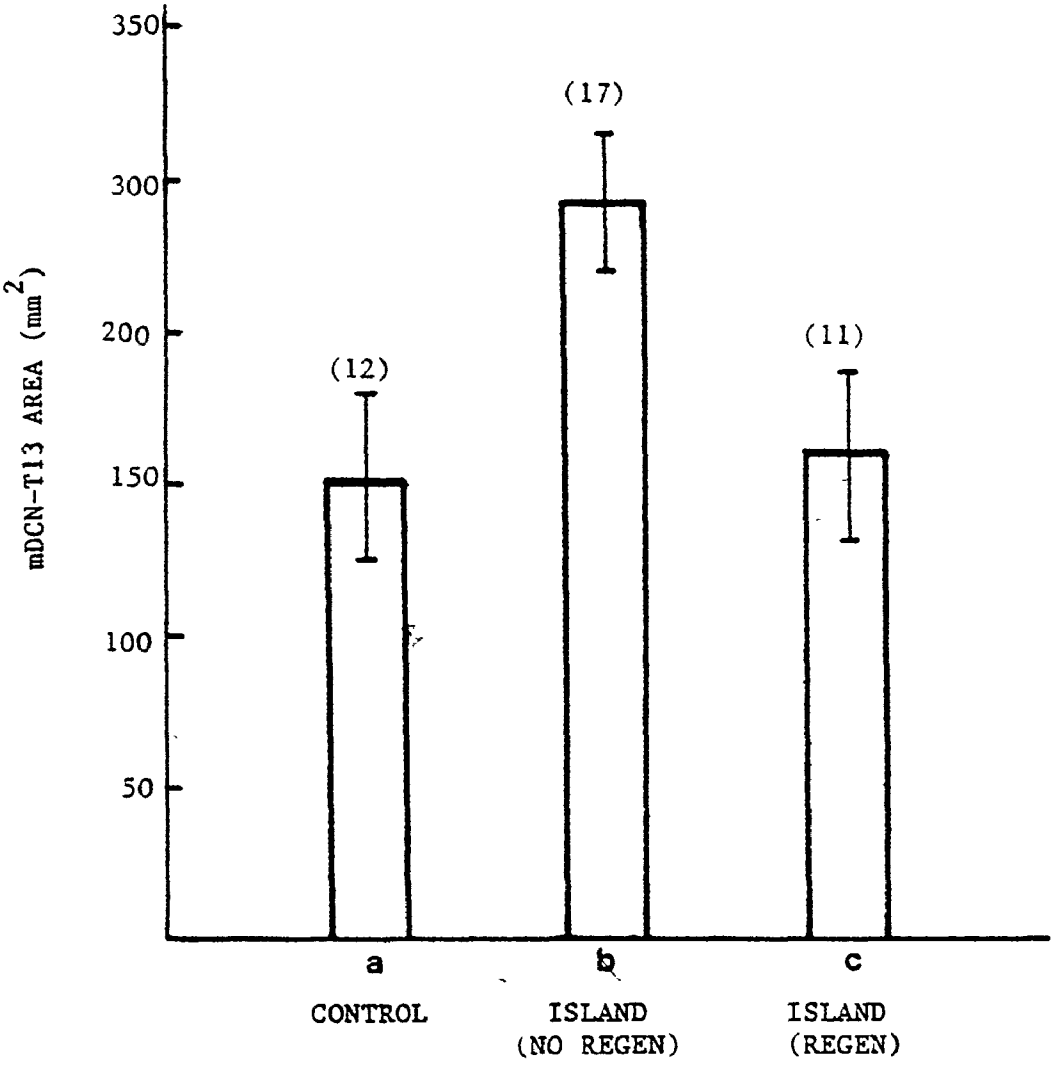

DCN
MIDLINE
T12
T13
L1
LDCN
mDCN
mDCN-T13
REGENERATED NERVES

FIGURE 31. Retracting of Enlarged Isolated Fields Upon Regeneration of the Original Nerves

These histograms show the area (± S.D.) of mDCNs-T13 in three groups of animals mapped at 60 days of age: (a) normal unoperated controls, (b) isolated as an island of intact innervation at 10 days of age with regeneration of the cut nerves prevented, (c) isolated as an island of intact innervation at 10 days of age but allowing subsequent regeneration of the cut nerves. The number of animals in each group is given in paranthesis above the appropriate column. The fields of intact mDCNs-T13 now surrounded by successfully regenerated original nerves are reduced in area ($p<0.001$) compared to island fields enlarged by sprouting where regeneration was prevented, and are not different ($p>0.1$) than the same field in normal unoperated control animals of the same age.

FIGURE 31.


350
300
200
150
100
50
mDCN-T13 AREA (mm^2)
(12)
(17)
(11)
a
b
c
CONTROL
ISLAND
(NO REGEN)
ISLAND
(REGEN)

supplied by these mDCNs-T13 (19.6 ± 2.2) was no different than the number of touch domes supplied by mDCNs-T13 in normal unoperated control animals of the same age (18.8 ± 2.0). In the absence of regeneration of the original nerves these mDCNs-T13 would have been expected to supply 28.4 ± 3.2 domes (see Fig. 32). Domes outside the now retracted mechanosensory fields of these mDCNs-T13 were functionally innervated only by regenerated original nerves.

These results show that, if allowed to regenerate, a nerve originally supplying a region of skin was able to replace (at least functionally) an innervation previously established in that area by neighbouring intact nerves.

(b) Regenerating nerves outside their former territories: "Foreign" nerves

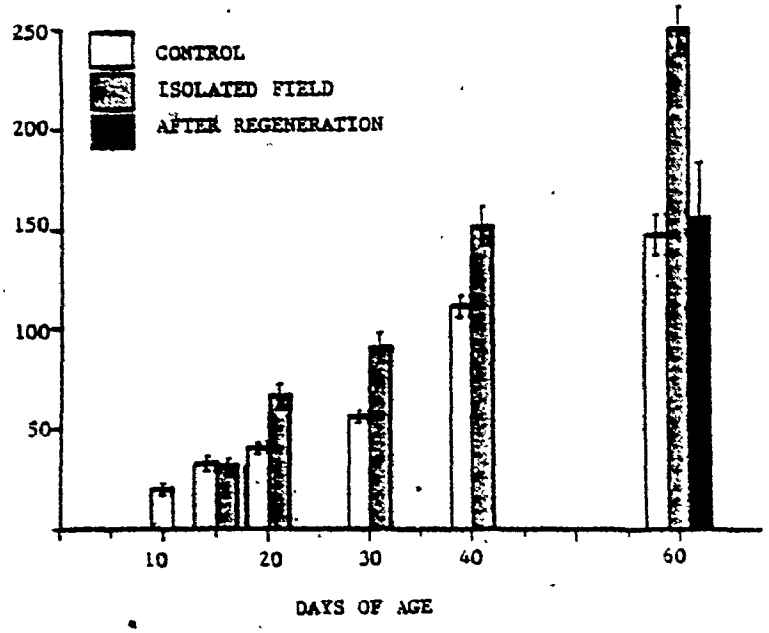
In the above experiments the regenerating nerves returned to their former territories. The possibility was also examined that a "foreign" regenerating nerve, that is, a nerve that formerly supplied another area of skin, would be able to replace denervation-evoked expanded innervation of intact mDCNs-T13. An experiment similar to that described immediately above was designed to investigate this possibility.

As in the previous experiment, mechanosensory fields of mDCNs-T13 were isolated as islands of innervation in a group of 10 day old rats; all cut nerves were allowed to regenerate except for lateral branch of the left DCN-T13. It's central stump was

FIGURE 32. Domes in mDCN-T13 Fields after Competition with Regenerated Nerves

The earlier results showing expansion (Fig. 18) and retraction (Fig. 31) of isolated island fields are presented in the upper portion of this figure. The Table below presents the number of domes (± S.D.) in the receptive fields of mDCNs-T13 in animals between the ages of 15 and 60 days of age; island fields were isolated at 10 days of age. The increased number of domes supplied by these mDCNs-T13, acquired between 15 and 20 days of age is maintained up to at least 60 days in the absence of regeneration by the originally cut nerves. Upon regeneration of the original nerves the number of domes innervated by these mDCNs-T13 was no different than the control values in unoperated animals ($p>0.1$).

FIGURE 32.



AGE (DAYS)	CONTROL		ISLAND
15	18.6 ± 1.5 n=8	- N.S. p>0.2 -	19.0 ± 3.4 n=4
20	19.0 ± 2.4 n=27	- p<0.001 -	28.5 ± 2.0 n=8
30	18.1 ± 1.8 n=18	- p<0.001 -	28.0 ± 0.8 n=4
40	18.6 ± 4.7 n=7	- p<0.001 -	27.7 ± 1.5 n=3
60	18.8 ± 1.0 n=11	- p<0.001 -	28.4 ± 3.2 n=11
60 after regeneration			19.6 ± 2.2 n=8

ligated and thrust into the body wall musculature, effectively preventing its successful regeneration. At 60 days of age the previously isolated fields of mDCNs-T13 and those of the regenerated DCNs of T12 and L1 were mapped. The regenerated DCNs-T12 and L1 were found to have established low-threshold mechanosensory innervation as before, but now supplied not only their original territories, but also the former territory of the LDCN-T13 (Fig. 33). The receptive field areas of the intact mDCNs-T13, formerly isolated and now surrounded by regenerated innervation, were of normal control values (Fig. 34), significantly reduced in area from that obtained for similarly isolated mDCNs-T13 where regeneration of the cut nerves was prevented.

It was concluded therefore that regenerating low-threshold mechanosensory nerves per se have a competitive advantage over intact ones that have endings established in denervated skin; whether or not a regenerating nerve originally supplied that territory it can, at least functionally, replace or suppress endings of intact nerves.

11. Nerves Regenerating to Undenervated Skin

In light of the above observations on the ability of regenerating nerves to functionally replace endings produced by intact ones, an experiment was performed to determine if a regenerating nerve was able to establish function in skin already supplied by its appropriate intact nerve. Basically the experiment consisted of allowing a nerve whose own territory had been

FIGURE 33. Regeneration of DCNs T12 and L1 into the Former Field of LDCN-T13

Depicted in this figure are typical low-threshold mechanosensory fields mapped for DCNs T12-L1 in a group of 60 day old animals (n=11); the mDCN-T13 had been isolated as an island of intact innervation at 10 days of age by cutting all adjacent branches of DCNs T10-L3 except that the LDCN-T13 was cut and ligated to prevent its regeneration. In the absence of functional regeneration by the LDCN-T13, the regenerating DCNs of T12 and L1 established atypical "dog-leg" shaped low-threshold mechanosensory fields which completely surrounded the field of the intact mDCN-T13.

FIGURE 33.

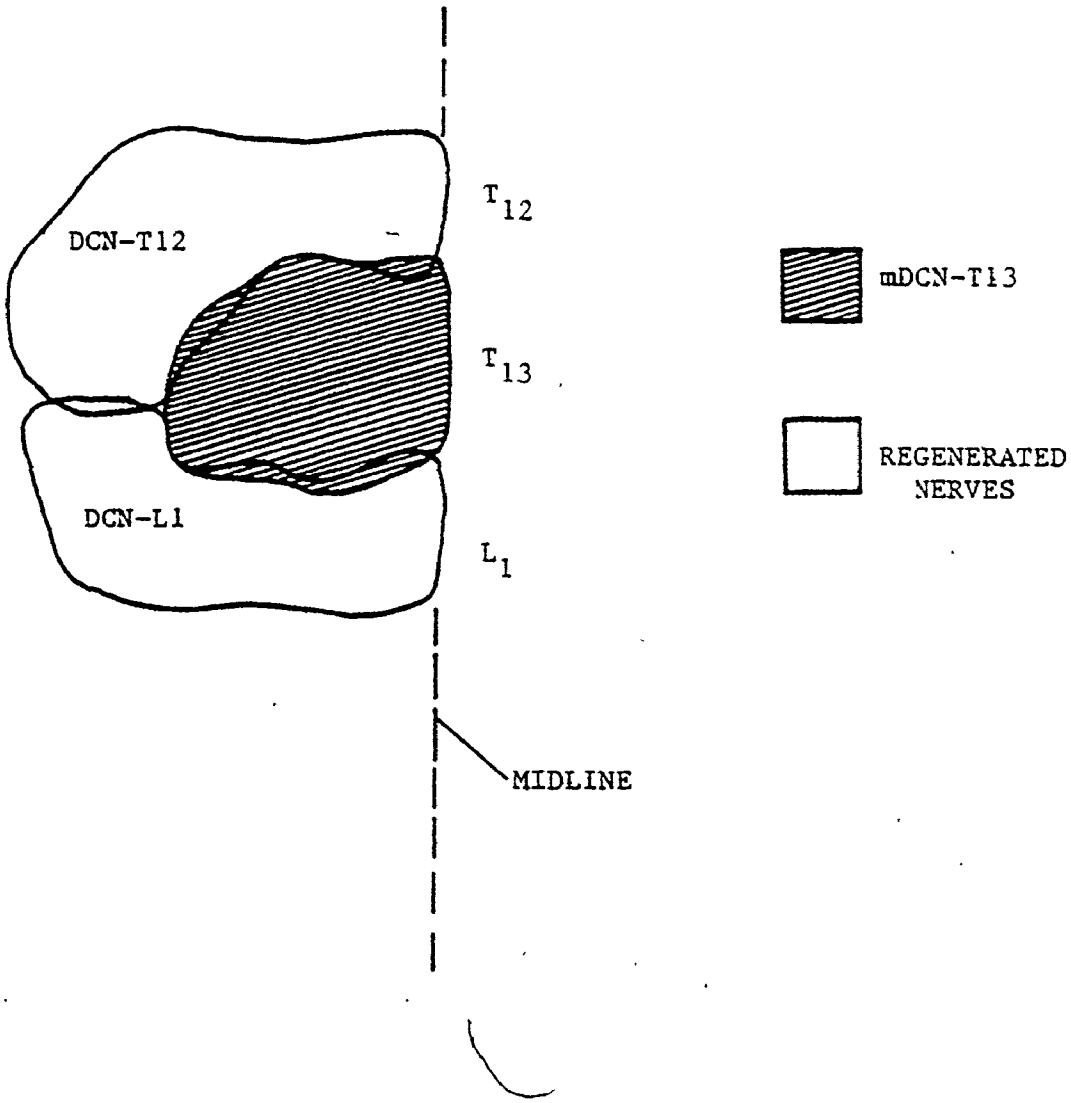
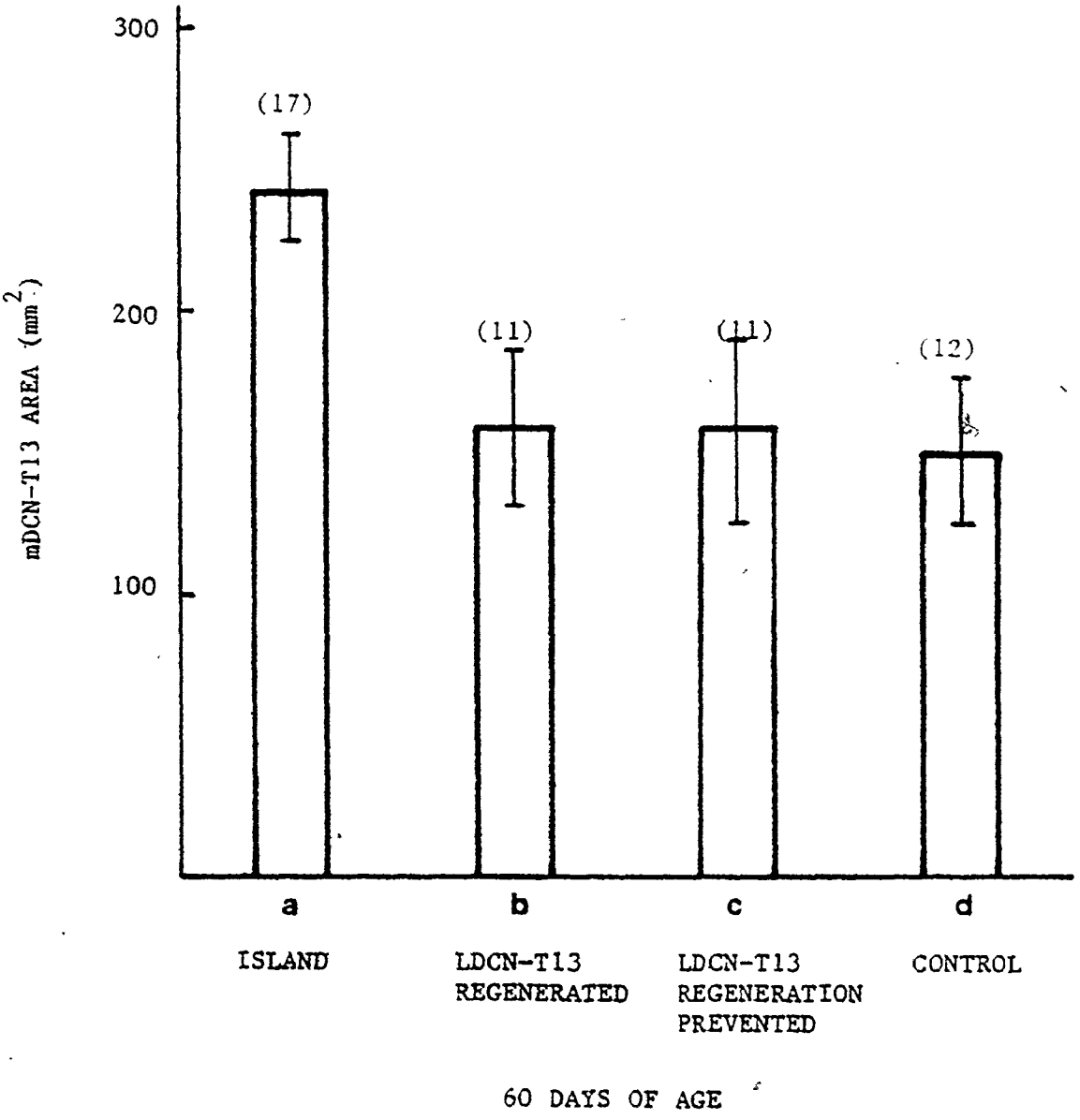

DCN-T12
T12
T13
DCN-L1
L1
MIDLINE
mDCN-T13
REGENERATED NERVES

FIGURE 34. Displacement of Intact Nerves by Regenerating "Foreign" Nerves

These histograms give the areas (± S.D.) of the mDCN-T13 receptive fields in four groups of animals at 60 days of age: (a) the mDCNs-T13 had been isolated at 10 days of age, and regeneration of the cut nerves was prevented; (b) the mDCNs-T13 had been similarly isolated but the cut nerves were successful in regaining their former territories (see Fig. 30); the animals of group (c) were isolated as in group (b), but the lateral branch of DCN-T13 had been prevented from regenerating and its former territory was subsequently invaded by regenerating axons of DCN-T12 and DCN-L1 (Fig. 33). The last column to the right (d) shows the field areas of mDCNs-T13 in normal unoperated control animals at 60 days of age. Groups b, c, and d are all smaller ($p<0.001$) than (a). Neither (b) nor (c) are different from control values, or each other ($p>0.2$).

FIGURE 34.


300
200
100
mDCN-T13 AREA (mm²)
(17)
(11)
(11)
(12)
a
b
c
d
ISLAND
LDCN-T13 REGENERATED
LDCN-T13 REGENERATION PREVENTED
CONTROL
60 DAYS OF AGE

surgically excised to regenerate into skin which had an essentially normal innervation.

The low-threshold mechanosensory fields of the left mDCNs-T13 of six animals were mapped, recording in continuity, and the borders marked on the skin. Immediately after these maps were completed both branches of DCN-T13 were cut (not ligated) at the point where they enter the underside of the skin; the skin comprising the low-threshold mechanosensory field of this DCN was then excised and the edges of the excision wound brought together and sutured (Fig. 35). In three of these animals the DCNs of segments T10-T12 and L1-L3 were ligated and cut at the body wall, denervating the skin in the region of the excision wound. No DCNs in the other three animals were cut (with the exception of the DCN-T13); in these animals the skin on either side of the excision wound remained innervated (by DCNs-T12 and L1). Recovery from the surgery was uneventful and the animals did not appear unduly distressed by the skin removal.

Viewed under the dissecting microscope (x25) seven weeks later, the cut DCNs-T13 in all six animals were seen to have apparently grown towards and penetrated the dermal side of the skin within 1-2 mm of the scar resulting from the previous excision. When mapped for mechanosensory function however, only in the three animals in which skin adjacant to the excision wound was denervated

FIGURE 35. Removal of a Regenerating Nerve's Target

Represented is the pattern of innervation seen after the area of skin comprising the low-threshold mechanosensory field of DCN-T13 had been excised after mapping (see text). The severed central ends of both branches of DCN-T13 remained in roughly their normal positions under the skin near the excision wound.

FIGURE 35.

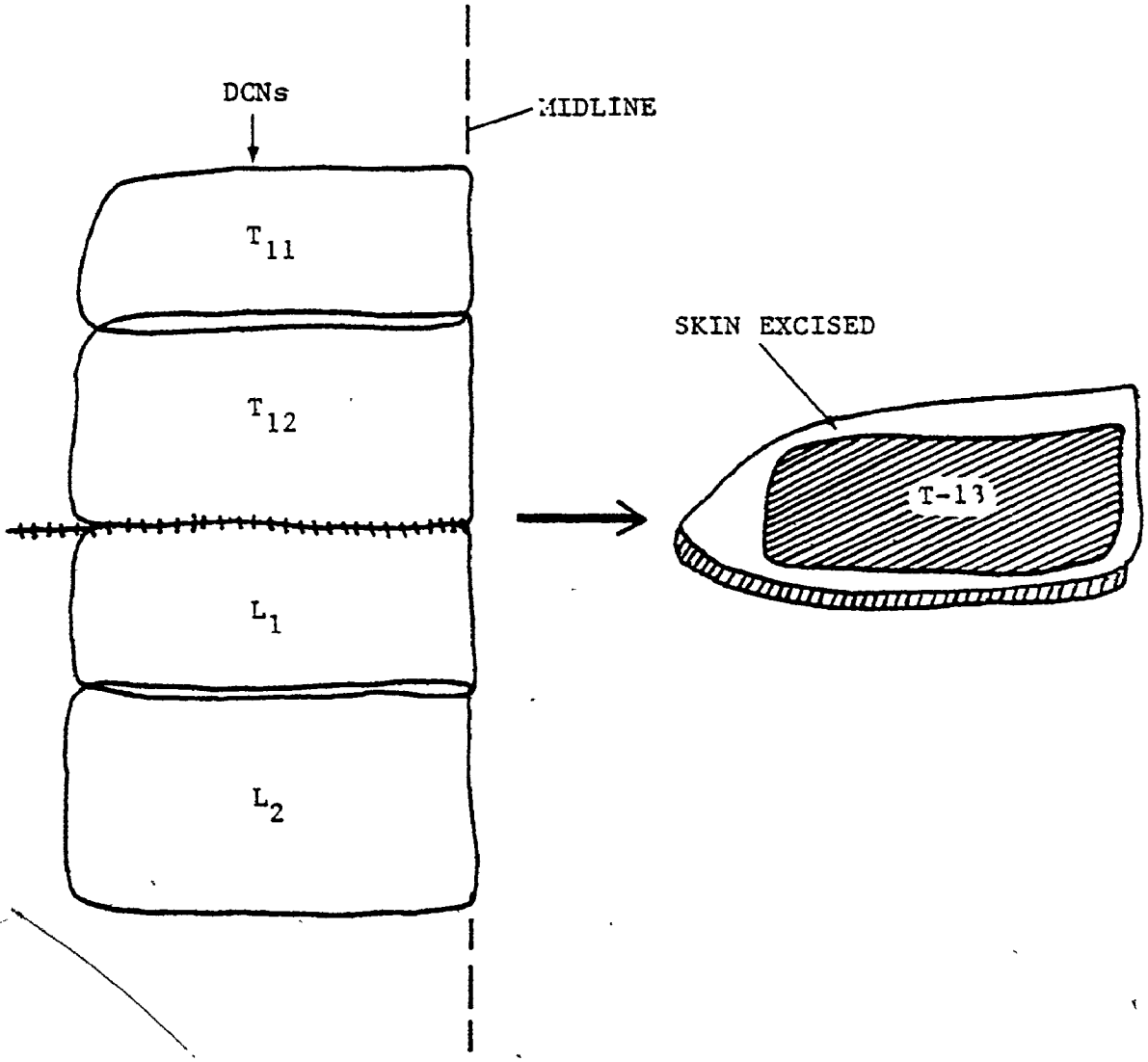
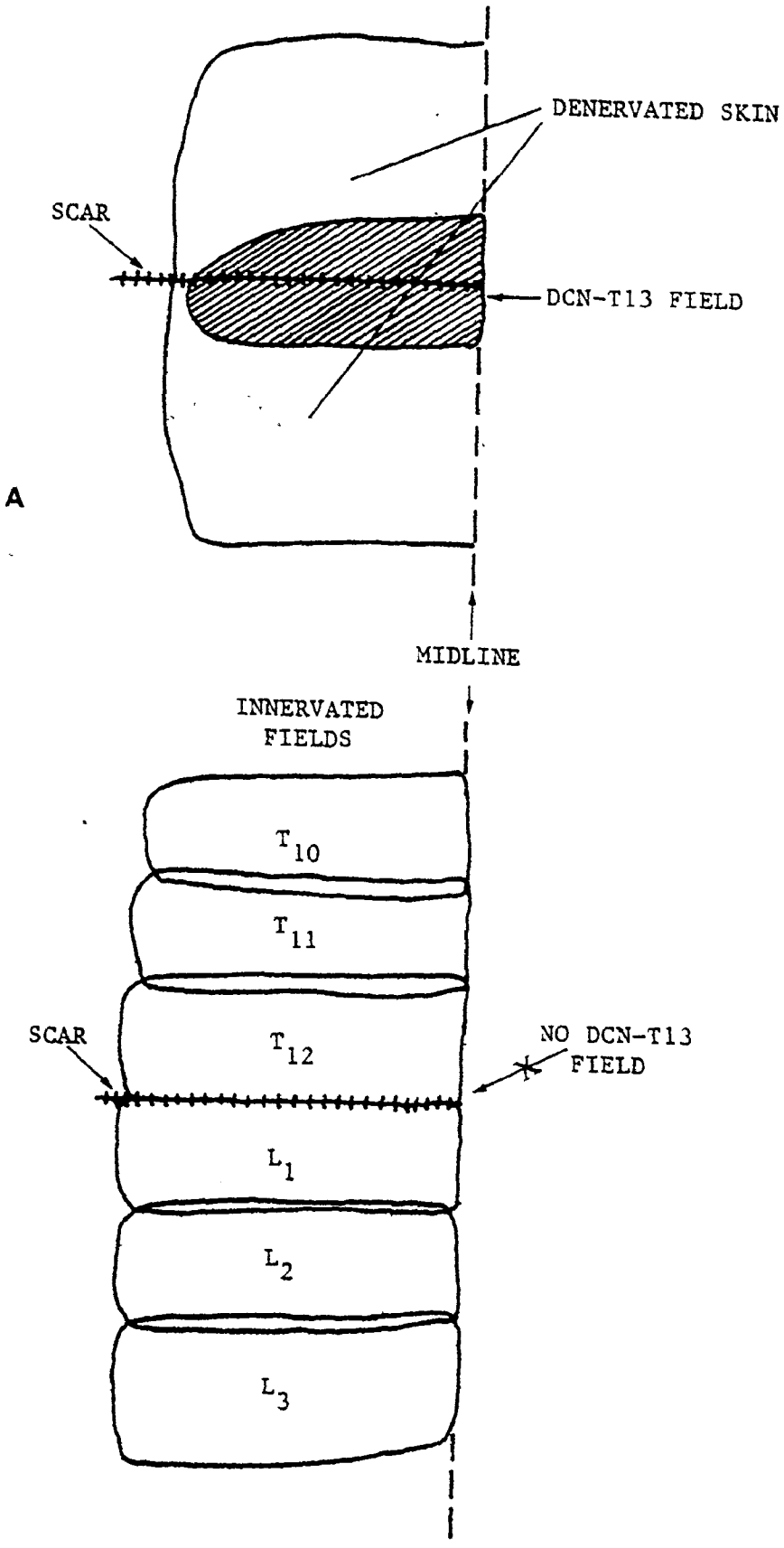


FIGURE 36. Regenerating Nerves in the Absence of their Original Target

A. Low-threshold mechanosensory field (diagrammatic) established by a regenerating DCN-T13 following excision of its normal target area, and denervation of the skin on both sides of the resultant scar at the time of the original operation seven weeks earlier. No detectable regeneration of the DCNs-T10-T12 and DCNs-L1-L3 was present at the time of the second mapping.

B. The innervation of the skin in the region of the scar where the original field of DCN-T13 had been excised and the wound edges drawn together; there were no denervations of the skin by cutting adjacent DCNs in these animals. Seven weeks after the original manipulation, the low-threshold mechanosensory receptive fields of DCN-T12 and DCN-L1 were found to still abut along the scar line and the cut DCN-T13 had not successfully established any detectable low-threshold mechanosensory function in the skin.

FIGURE 36.


DENERVATED SKIN
SCAR
DCN-T13 FIELD
A
MIDLINE
INNERVATED FIELDS
T_{10}
T_{11}
T_{12}
SCAR
NO DCN-T13 FIELD
L_1
L_2
L_3
B

had the regenerated DCNs-T13 established a successful innervation (Fig. 36). In the three animals where the skin adjacent to the excised field remained normally innervated, the (presumably) regenerating fibres of DCN-T13 had been totally unsuccessful in acquiring low-threshold mechanosensory function.

It was concluded from these experiments in which nerves were allowed to compete for a functional innervation of skin that regenerating nerves have a competitive advantage over endings established in denervated skin by intact ones; they are, however, unable to displace mechanosensory endings originally established by an intact nerve within its normal territory. The endings evoked by denervation of skin within the critical period are, therefore, likely to be the ones replaced by those of regenerating nerves.

SECTION 6

Discussion: Technique

Since in this investigation the presence of low-threshold mechanosensory innervation was determined functionally rather than morphologically, the technique used to obtain the data will be considered first, prior to a treatment of the results.

1. The Receptors in Hairy Skin Excited by the Stimulus

In the present study displacement of hairs constituted an adequate stimulus for the excitation of several receptor types. Very similar mechanical manipulations have been shown to constitute adequate stimulation for excitation of "low-threshold mechanosensory" nerves in many investigations using single unit analysis of sensory afferent function (see below). The cutaneous mechanoreceptors involved, all showing a very high sensitivity to mechanical deformation of the skin, can be divided into two major categories: rapidly adapting and slowly adapting. In the hairy skin of most mammals, including the rat, the hair follicle receptors are rapidly adapting mechanoreceptors, discharging impulses only during displacement of the hair (Brown and Iggo, 1967). These follicles are innervated by myelinated afferent nerve fibres and usually there is extensive branching so that one myelinated axon may supply many hair follicles (Whitehorn, Howe, Lessler and Burgess, 1974). There are several different kinds of hair follicle afferent units (Brown and Iggo, 1967; Burgess, Petit and Warren, 1968) which respond to movement

of the fine down hairs (that form the majority of the body cover), the larger guard hairs, and the still larger tylotrich (Straile, 1960) hairs. In general, the larger the hair, the larger the sensory afferent fibre supplying its follicle, and the fewer the number of follicles innervated by a single afferent unit (Brown and Iggo, 1967). Hairy skin also contains "field" receptors (freely terminating myelinated nerve fibres) which are also rapidly adapting low-threshold mechanosensory units (Burgess, Petit and Warren, 1968). The slowly adapting low-threshold mechanosensory units of hairy skin are categorized as Type I and Type II, both of which respond continuously to a maintained stimulus (Hunt and McIntyre, 1960a, Iggo and Muir, 1969). The Type I receptor discharge is characteristically irregular while the Type II is more constant in frequency. The Type I receptors are present in non-primate hairy skin as raised epidermal elevations ("touch domes") and respond to very slight vertical deformation of the dome. The Type II receptor responds to slight indentation of the skin, but is not associated with a visible surface feature; further, Type II receptors often carry a resting discharge, and are easily excited by stretching of the skin (Werner and Mountcastle, 1965; Chambers, Andress, Von Duering and Iggo, 1972). Since Type I (dome) receptors are sensitive only to vertical displacement, the two kinds of receptors can be distinguished on both morphological and physiological grounds.

Aside from the specific identification and excitation of the Type I receptors, in the present study the stimulus used was taken as adequate for the described receptors, which have been categorized simply as constituting the "low-threshold mechanosensory" innervation.

2. Detection of the Low-Threshold Mechanosensory Innervation

(a) Activity in single axons

As referred to in the beginning, low-threshold mechanosensory innervation of skin was chosen for investigation because the axons whose endings supply or constitute the receptors are, as a general rule, large myelinated fibres (Hunt and McIntyre, 1960b; Brown and Iggo, 1967). It has been shown that with multi-axon whole nerve recording (as was used in this investigation), the larger the diameter of the axon, the larger the recorded spike height (Blair and Erlanger, 1933; Zotterman, 1939). In the cited studies the recorded amplitudes of impulses in axons of various diameters varied not with the diameter itself but with the square of the diameter. For example, in the present study, the usual maximum spike amplitude recorded was approximately 100 μV; accordingly, an impulse recorded from an axon half the size of the one generating such a spike would give rise to a 25 μV signal. The largest cutaneous mechanosensory afferents have axons which are between 10 and 14 μ in diameter (Hunt and McIntyre, 1960b); on this basis an impulse in an axon 5 μ in diameter would be just detectable above the background noise level of 10 μV. In the present study noise levels were routinely 10 μV or less.

Nerves have been categorized according to their axon diameters (and conduction velocities) in two generally accepted ways. In order of decreasing diameters, myelinated axons have been divided into Aα, β, γ, δ and B groups; unmyelinated axons have been labelled C fibres. Alternatively, and also in order of decreasing size, myelinated axons have been classified into groups I, II, and III; unmyelinated axons constitute group IV (see Boyd and Davey, 1968). Conduction velocity measurements of down hair receptors (Burgess, Petit and Warren, 1968; Burgess, Howe, Lessler and Whitehorn, 1974) have established that their parent axons fall into the upper range of group III (Aδ) fibres (axon diameter 2-6 μ). With an exception, the remainder of the cutaneous low-threshold mechanosensory receptors are supplied by groups I or II fibres (Burgess, Petit and Warren, 1968; Iggo, 1977). The exception is low-threshold mechanosensory cutaneous innervation which can be supplied by group IV (C) fibres (Iggo, 1959; Bessou, Burgess, Perl and Taylor, 1971); activity in such axons (<1 μ in diameter) would not be resolved with the present technique. From these considerations, in the present experiments impulses in myelinated low-threshold mechanosensory axons, including the group III ones, would be resolved and only those in group IV (C) fibres would be "invisible". Since the stimulus used in the present investigation generated several impulses in very likely more than one axon (Brown and Iggo, 1967; Burgess, Howe, Lessler and Whitehorn, 1974), individual spikes generated by the rapidly

adapting mechanoreceptors were not attributable to specific receptor types. The potential for summation of impulses in two or more axons also served to increase their detectability from the recordings.

(b) The borders of the mechanosensory nerve fields

The edges of the receptive fields mapped in the present study were characterized by an abrupt loss of the evoked impulses that were detected visually from the oscilloscope screen or audibly through the headphone monitor. In the region of overlap between adjacent fields, the density of innervation is decreased (Kuhn, 1953), as judged by the magnitude of the response evoked by the standard stimulus. The resolution of the recordings was, however, sufficient to identify unambiguously the border of functional low-threshold mechanosensory innervation. Behaviourally determined nociceptive fields of the cutaneous nerves investigated in this study are somewhat larger than the low-threshold mechanosensory fields (Jackson and Diamond, 1979); consequently, a direct morphological demonstration of the accuracy of the physiological technique is complicated but is the subject of a separate investigation currently being pursued in this laboratory. The preliminary results of combined histological and physiological investigations in this laboratory indicate that the low-threshold receptive field, mapped as in the present investigation, accurately describes the anatomical distribution of the endings of low-threshold afferents.

Another possible source of error in the determining of borders for receptive fields is stimulus spread. Since mechanoreceptors are by definition sensitive to deformation of skin, supra-threshold stimuli applied at sites remote from the actual endings of nerves may be transmitted through the skin and cause excitation of the endings, thus producing a "receptive field" larger than the skin area containing the actual peripheral arborization of endings. This potential source of error was minimized in the present investigations; in order to increase the apparent area of a given field in this way it was found necessary to apply the stimulus with sufficient force to cause visible dimpling of the skin by the bristle. In no case in the present investigation was the stimulus allowed even to approach this magnitude. In determining the presence or absence of functional innervation for individual touch domes, stimulus spread was not a factor. As has been shown previously (Iggo and Muir, 1969; Burgess, English, Horch and Stensaas, 1974), Type I afferents respond only to direct stimulation of the raised portion of the epithelium itself (the dome); stimuli delivered to the skin immediately adjacent to a dome, or stronger stimuli at a distance, fail to evoke characteristic discharge.

The discussion to follow thus deals with the distribution in the skin of functional low-threshold mechanosensory innervation that is supplied by myelinated afferents of groups I, II and the largest group III fibres.

SECTION 7

Discussion: Results

The results of this thesis provide evidence that intact low-threshold mechanosensory nerves are under constraints both in time and space which operate to restrict their ability to restore sensory function to denervated skin; regenerating nerves are not subject to the same constraints. Further, regenerating nerves are able to compete with, and functionally replace, the extra endings which are established in denervated skin by intact nerves.

1. Functional Reinnervation of Denervated Targets: Sprouting?

During the development of cutaneous innervation, sensory targets are formed and acquire function; functional innervation is established by nerves which sprout in the vicinity of such targets both pre- and post-natally (see 2.5). In the present experiments, the innervation of skin was removed (or prevented from being established) by cutting selected cutaneous nerves, and the ability of other low-threshold mechanosensory cutaneous nerves to establish new functional connections with the extra target tissue thus made available was examined. In one sense then, the development of the cutaneous innervation is being experimentally recapitulated by confronting cutaneous nerves with essentially available target tissue. One important question is: are new axons still arriving at the skin in the neonate? If they are not, then this possible explanation for the expansion of receptive fields

into denervated skin is excluded. This would leave, to account for the expanded field, only the development of new sprouts from axons already present at the skin or alternatively, a failure of such axons to retract an excess of sprouts that were already present in the skin but more widely distributed i.e.) outside what would normally become the maintained receptive field.

The last birth day of neurons in the thoraco-lumbar dorsal root ganglia (DRGs) of rats and mice occurs at approximately 15 days of gestation with peak production occurring between 11-14 days (Lawson, Caddy and Biscoe, 1974; Lawson and Biscoe, 1979; Lawson, 1979). Between 17-20 days of gestation, Narayanan, Fox and Hamburger (1971) found increasing numbers of axons in the skin which was correlated with a marked increase in foetal responses to tactile and nociceptive stimuli. Since, in the rat DRGs, there is no naturally occurring cell death post-natally (Hatai, 1902; Lawson, Caddy and Biscoe, 1974) it is likely that by birth the peripheral axon of each surviving DRG neuron has reached the vicinity of peripheral targets and established functional endings; generally, neurons which do not maintain connections with targets during development die (Hamburger, 1975; Hollyday and Hamburger, 1976; Landmesser, Pilar and Burstein, 1980).

At birth in rats, Merkel cells are contacted by unmyelinated neurites which become myelinated by 13 days of age. If, in normal development, a late population of axons were to arrive, establish connections and survive, then at least some Merkel cells should be

seen to exhibit connections from immature unmyelinated neurites at this later time; such is not the case (English, 1977).

An unlikely possibility which must, however, be considered is that in the early development of cutaneous innervation the endings of a given nerve are initially more widely distributed in the skin and subsequently withdraw their borders of functional innervation through suppression, retraction, or death of endings outside this region (see 2.6). There is however no evidence in the literature for either a radial retraction of cutaneous nerve fields in normal development which might be reversed by (for example) surgically denervating the surrounding skin, or for the presence in skin of morphologically sound endings which do not transduce impulses. Indeed, in the present investigation, it is difficult to understand how such hypothetical endings of mDCNs-T13 would be rescued in the T12 and L1 dermatomes only if the region supplied by the lateral branch of DCN-T13 was also denervated; functional expansion of the mDCN-T13 field of intact nerves into adjacent dermatomes did not occur unless denervated skin was also present in the ipsilateral (T13 dermatome).

Also addressing this point, in the present study it was found that the normal number of touch domes supplied by mDCNs-T13 is unambiguously established and visible in the skin of rat pups between 10 and 15 days of age; there was no reduction in this number at any later time. Similarly, receptive field borders of intact nerves established in the skin at 15 days of age are the same borders detected 5-7 weeks later. Radial retraction, or

suppression, of functional endings does not therefore occur after 15 days of age and is unlikely to occur earlier. This is not to say that retraction of endings does not occur in the development of cutaneous innervation (see Fitzgerald, 1966; English, 1977) - only that, if present in the normal establishment of low-threshold mechanosensory innervation of skin, it does not lead to either a reduction in area or number of targets functionally innervated by the whole nerve and is an unlikely candidate to be invoked to explain the obtained expansion of receptive fields into denervated skin during the critical period.

The remaining possibility, that experimentally denervated targets become reinnervated by newly formed endings of intact nerves, is supported by evidence that in normal development, cutaneous nerves continue to sprout new endings post-natally as available targets appear (see below). During growth the increased size of the animal is of course associated with an increase in the total surface area of the skin; as expected, the present results show that this increase in surface area is accompanied by a normal increase in the absolute area of skin functionally innervated by a cutaneous nerve. Moreover, the borders of the fields established by these nerves retain their topological positions, i.e. the normal increase in receptive fields area is due to a radial expansion of skin which "tows" the endings along with it. The full complement of cutaneous sensory nerves is likely

present at birth in the hairy skin of rats; morphological studies of the snout pads of pre- and post-natal pigs (Fitzgerald, 1961) and rats (MacIntosh and Sinclair, 1978) have shown that as more target tissue (epithelial ridges) appears through growth of the animal sprouting of new nerve endings by axons already present in the skin continues post-natally and maintains or establishes the mature density of endings in the skin. In the hairy skin of rats (Butcher, 1934) and mice (Gibbs, 1935) all the primary (tylotrich) follicles are present at birth, but guard and down hair follicles continue to appear until 7-8 days post-natally. Merkel cells are present and innervated at birth in their characteristic (in rats) location, surrounding the primary hair follicles (Smith, 1968; English, 1977); these locations later become visible as epidermal elevations - the touch domes. In rats (English, 1977) and cats (Kasprzac, Tupper and Craig, 1970) Merkel cells continue to differentiate post-natally within the already established touch dome sites. Since hair follicles acquire low-threshold mechanosensory innervation and each Merkel cell in the mature touch dome is typically contacted by a single ending, functional sprouting of low-threshold mechanosensory nerves in hairy skin post-natally is certainly a feature of normal development. This sprouting presumably occurs within the confines of the field established early on since it does not normally lead to an expansion of field area beyond that accomplished by radial growth of the skin.

2. The Critical Period

In the present investigation, evidence was obtained that the receptive field of intact low-threshold mechanosensory nerves are able to expand in response to denervation of adjacent skin only during a brief period between 15 and 20 days post-natally. The possibility that the observed "extra" increase in receptive field area was an artefact due to a difference in growth between innervated and denervated skin was excluded; an alternative possibility that need not be considered at length is that the denervation in some way substantially lowers the threshold of excitation for the remaining intact nerves, leading to excitation of endings by remote stimuli. If this had been the case, the standard stimulus would have been expected to excite more endings (Burgess, Howe, Lessler, Whitehorn, 1974) when applied within an island field, leading to a larger evoked response; no such gross difference in evoked responses to the standard stimulus were noted in the present study. Directly to the point, the normally stable number of touch domes supplied by intact mDCNs-T13 increased in parallel with the increase in area of an isolated island field; these receptor structures are insensitive to stimulus spread in that they are not excited by lateral displacement of skin (Iggo and Muir, 1969; Burgess, English, Horch and Stensaas, 1974). The expansion of the intact nerve's receptive field into denervated skin and acquisition of extra touch domes is therefore due to the establishment of functional endings in such skin by the intact nerve.

In the absence of evidence that the enlarged receptive fields produced subsequent to denervation of adjacent skin was due to increased sensitivity of receptors, rescue or activation of functionally silent endings, or an artefact due to altered skin growth, the remaining possibility - sprouting of new endings - was accepted as being the most likely explanation of the results; histological confirmation is pending. Since in the present investigation an evoked expansion of isolated low-threshold mechanosensory receptive fields was found to occur only between 15 and 20 days of age, there is therefore a critical period for denervated targets to become functionally reinnervated by sprouting of the remaining undamaged nerves. The origin or mechanism of this temporal constraint is as yet unknown; it could conceivably lie within the nerve, the target (cell or tissue), or be external to both.

(a) Lack of functional sprouting after 20 days of age

The inability of low-threshold mechanosensory nerves in the rat to sprout functionally beyond their established receptive field borders into denervated skin after 20 days of age stands in marked contrast to low-threshold mechanosensory cutaneous nerves in the salamander (Aguilar, Bisby, Cooper and Diamond, 1973) and also high-threshold mechanosensory cutaneous nerves in the rabbit (Weddell, Guttmann and Gutmann, 1941; Weddell, 1942) and rat (Devor, Schonfeld, Seltzer and Wall, 1979; Jackson and Diamond, 1979) all of which retain into adulthood the ability to sprout

and enlarge their receptive fields into such denervated skin. Diamond (e.g. 1979) would explain such sprouting to be the result of a stimulating factor released by denervated targets (normally neutralized by innervation), acting on intact nerves in the vicinity, causing them to sprout new endings. In the present case, the denervated targets (e.g. hair follicles and Merkel cells of touch domes) of low-threshold mechanosensory nerves might cease producing such a stimulus at or about 20 days of age, or the nerves might become unable to respond to it. In this regard it would be premature to speculate on the observation that between 17 and 22 days of age the hair follicles in rat skin become "quiescent" in terms of cellular proliferation and follicular growth (Butcher, 1934).

On the present evidence it is not possible to determine if the cessation of sprouting after 20 days of age is due to some factor inherent in the maturation of the neurons. It is known however, that the morphological (Lawson, Caddy and Biscoe, 1974) and histochemical (Kalina and Wolman, 1970) appearance of DRG neurons approaches that of adult rats during the third and fourth post-natal weeks. This maturation may render an undamaged low-threshold nerve unable to command further axonal growth after a certain point in time, or to recognize an externally originating signal to grow; damage to a mature neuron may overcome or reverse this process, accounting for their observed ability to regenerate even in the adult. Maturation of the low-threshold mechanosensory

neurons may not completely account for the end of the critical period since there is the suggestion that low-threshold mechanosensory endings may "turnover" (i.e. some being lost, others being formed) even in the adult (Burgess, English, Horch and Stensaas, 1974). Such turnover might require that a residual sprouting capacity be retained, however the question has not been examined in detailed, controlled experiments. If such a residual sprouting capacity is retained in the adult rat, sprouting of intact low-threshold nerves in adult skin is confined to that territory already innervated by it since no topographical change in field borders with age was detected. The intermediate evoked increase in area of fields isolated at 15 days of age compared with those isolated at 10 days of age is entirely consistent with there being less time remaining for sprouting of the former group before the end of the critical period is reached.

(b) Lack of functional sprouting before 15 days of age

Low-threshold mechanosensory fields isolated in rats at either 5 or 10 days of age did not show any expansion evoked by denervation of surrounding skin until after 15 days of age. The mDCNs-T13 of both groups, by 20 days of age, had expanded equally into denervated skin. Functional sprouting in both groups was thus delayed until after a critical age. In the absence of histological evidence, it is not possible to distinguish between a failure of these nerves to sprout or a failure of sprouts to establish functional endings. Although not previously reported

for cutaneous nerves, a failure of intact motor nerves to sprout and expand their field of innervation after partial denervation of muscles in neonates has been described (Thompson and Jansen, 1977; Brown, Jansen and Van Essen, 1976; cf. Betz, Caldwell and Ribchester, 1980b); Gilad and Reis (1980) have recently reported a failure of mesolimbic axons to sprout in early post-natal development. The failure of intact motor nerves to sprout in neonates however may not be directly comparable to the lack of functional sprouting before 15 days found in the present investigation; neonatal motor nerves are initially "overexpanded" in that the size of the motor unit in the neonatal muscles is larger than that in the adult (Redfern, 1970). In contrast, the available evidence on the development of sensory innervation of the skin shows that postnatally, cutaneous nerves normally continue to form new endings (e.g. Fitzgerald, 1961; MacIntosh and Sinclair, 1978).

There is no reason to assume that the factors responsible for a lack of functional sprouting before 15 days of age are the same as those responsible for the lack of sprouting seen after 20 days of age. Indeed, if one were to propose that a sprouting stimulus was not produced by sensory targets before 15 days of age one would then be left with the paradoxical task of accounting for the sprouting which does occur in normal postnatal development. Interestingly, neonatal rat motor neurons damaged at birth are able to regenerate quite quickly (3-5 days) back to the denervated muscle but, regardless of the age at denervation, these regenerating

days of age; sprouting of the remaining intact motor nerves in the partially denervated muscle did not occur (Dennis and Harris, 1980). In the hamster the mature response of facial motor neurons to axotomy (e.g. mitochondrial swelling) is not seen in animals less than 3 weeks old (LaVelle and LaVelle, 1970; LaVelle, 1964). Watson (1974, 1976) proposed that the response of the cell body to axotomy is associated with a change from production of proteins supporting synaptic functions to production of proteins supporting axonal growth. Perhaps both motor nerves and sensory nerves in the neonate are unable to undergo this conversion until a particular stage of development. This suggestion may be premature since in normal development sprouting of low-threshold mechanosensory cutaneous nerves is almost certainly continuing between 5 and 15 days in the hairy skin of rats; during this time intact nerves may be unable to recognize or respond to a signal from experimentally denervated targets outside their normal territory; alternatively, denervated targets may not be able to re-express a factor signalling a need for innervation until a particular stage of development. In rats, neonatal cutaneous nerves may already be sprouting maximally to innervate newly appearing targets and be able to detect, and sprout to, denervated ones only after the normal appearance of new targets ceases or is reduced. As already stated, histological investigations can distinguish a lack of sprouting from a failure of sprouts to form functional connections; crucial testing of the remaining possibilities awaits the specific identification of the sprout-promoting signal.

3. Spatial Restrictions

A striking feature of the expansion of the isolated low-threshold mechanosensory fields into denervated skin during the critical period is that it is not uniform in all directions. Moreover in certain circumstances there was virtually no expansion, even within the critical period. The "island" fields expanded into denervated skin more so in the dorso-ventral axis than the rostro-caudal axis. Evidence that this apparent preferred expansion is not simply an artefact due to structural features of the skin was obtained in this investigation from observations that as crushed nerves regenerated they produced mechanosensory fields which expanded into the adjacent denervated skin equally in both axes. Macintyre and Diamond (1981), in analogous experiments on the cutaneous innervation in salamanders, obtained evidence that intact low-threshold mechanosensory nerves sprout collaterals which establish functional connections with denervated targets located largely in the "parent" dermatome, but not in adjacent ones. They defined the territory into which intact nerves will sprout freely as the "domain" of that nerve. In their experiments too they showed that the apparent hindrance to intact nerves at the domain borders was not due to mechanical barriers there; in the salamander the spatial (domain) restriction is overcome after approximately 2 months. Since the removal of a dorsal root ganglion in amphibian embryos, before innervation of the skin is established, results in adjacent DRGs supplying the innervation to the region which would have been deprived (Miner, 1956; Bibb, 1978;

see Castro, 1963) domains probably arise secondarily to the establishment of sensory dermatomes in the skin. It would be of interest to know if intact nerves supplying such expanded dermatomes exhibit the predicted domain behaviour when skin is later denervated.

The results of the present investigations support the domain hypothesis in that isolated intact low-threshold mechanosensory nerves expanded into skin within the same "parent" dermatome to a greater extent than into denervated skin of adjacent dermatomes. The constraint on sprouting into adjacent denervated dermatomes is not likely to be due to a simple guidance (Weiss, 1941) of the growing endings along lines of stress in skin which run generally parallel to the long axis of dermatomes (i.e. Langer's "cleavage lines" in Man); regenerating nerves are able to grow and establish function equally well at right angles to these gross stress lines. It would appear then that in the rat (cf. Devor, Schonfeld, Seltzer and Wall, 1979) and in the salamander the domain constraint is expressed on the sprouting of intact nerves but not on the sprouting of regenerating ones.

In developing systems the spatial organization of tissues probably depends upon the presence of "positional information" within the organism (Wolpert, 1971; French, Bryant and Bryant, 1976). Insect compartments (Garcia-Bellido, 1975; Morata and Lawrence, 1977) are determined too early in development to be a

secondary phenomenon, however the borders may well be respected by nerves in a manner analogous to that of domains in vertebrates (Lawrence, 1975). Quasi-compartmentation during vertebrate embryogenesis has recently been proposed (Jacobson, 1978).

The present results do not indicate the site of action of the domain constraint. Macintyre and Diamond (1981) have suggested, from results of skin rotation experiments in salamander, that denervated skin provides a non-specific sprouting stimulus to intact nerves and that the spatial constraint (the domain) is defined in terms of its body position. Such results do not however rule out the possibility of a regionally specific stimulus produced by skin, or its targets, to which nerves occupying other parts of the same dermatome are particularly sensitive.

A generalized stimulus to sprout emanating from denervated targets, whatever their position, coupled with a recognition of appropriate dermatomal markers, perhaps mediated by cell surfaces (see Pfenninger and Rees, 1976; Mirsky, 1980), might account for the observed non-uniform field enlargement into denervated skin. In this instance, intact nerves would initially sprout during the critical period into adjacent dermatomes but be retarded by a "difficulty" in growing into foreign skin to foreign targets. This suggestion is difficult however to reconcile with the ease with which regenerating nerves are able to sprout to, and establish function in, supposedly foreign skin. It should be

pointed out again that sprouts of regenerating nerves should not be considered necessarily the equivalent of sprouts of intact nerves. Sperry (1965) has proposed that in ontogeny, a complex system of chemical gradients is responsible for determining the rostro-caudal, dorso-ventral, and proximo-distal axes of the body. Cells could then obtain specific information as to their location by their position in this set of coordinates. Strong support for this hypothesis has been obtained (Hunt and Jacobson, 1973; French, Bryant and Bryant, 1976; Wolpert, Lewis and Summerbell, 1975; see also Keating, 1976). Connections between cells might be influenced by recognition of cell surface labels. One potential distinguishing surface label is the sequence of glycoproteins on the cell surface. Marchase, Barbera and Roth (1975) were able to identify different cell populations by the sequence of glycoproteins attached to their surfaces. In light of this it is interesting that suspended cells from half retinas have been found to adhere preferentially to the region of the optic tectum to which their processes would normally project (Barbera, 1975). Intact nerves, once stimulated to sprout, might preferentially adhere to and grow along cellular and extracellular surfaces which exhibit the appropriate positional labels (cf. Singer, Nordlander and Egar, 1979).

A curious result obtained in the present work is that extension of the isolated intact nerve into denervated adjacent

dermatomes is only observed when extension into the same dermatome is also allowed. There is no easy explanation for this, but it would appear to implicate another factor controlling the ability of intact nerves to sprout. From these results one might speculate that for these intact nerves to respond to a signal initiating sprouting to denervated targets, their cell bodies must lie within the same dorsal root ganglion as nerves which have been damaged. Such would imply that in the present experiments the signal to sprout might originate at the site of injury to axons and be communicated centrally to intact nerves (cf. Rotschenker, 1979). Once stimulated to sprout the direction and formation of endings might fall under a local control at the periphery. There is evidence that the cell bodies in dorsal root ganglia are roughly somatotopically organized with respect to the region of the dermatome innervated (Burton and McFarlane, 1973; Baker, Corner and Veltman, 1978). If the stimulus to sprout is received by intact nerves from damaged ones, those closest to cell bodies of injured neurons might respond more strongly. The evidence of the present study does not allow for a decision to be made on the origin of the sprouting signal. Although there are similarities (Graftstein and McQuarrie, 1978), the cell body ultrastructure and cytochemistry of regenerating neurons has been reported to differ from that of intact neurons undergoing sprouting (Gilad, 1977). Perhaps the cellular machinery of a regenerating neuron is under a stronger central drive from the cell body to grow and is able to overcome spatial restrictions imposed in the

4. Competition

In this thesis, evidence has been presented that endings established by intact low-threshold mechanosensory nerves outside their "normal" boundaries can be, at least functionally, replaced by endings of regenerating nerves. This functional replacement can be accomplished by regenerating nerves regardless of whether they originally innervated the particular territory (or targets) concerned. The latter observation excludes the possibility of specific "conduit" guidance by empty endoneurial tubes (Horch, 1979) being solely responsible for the arrival of regenerating sprouts at targets. It appears more likely that new endings established by the intact nerve during the critical period did not completely suppress a "target quality" (Diamond and Jackson, 1980), allowing regenerating nerves to locate them. In this regard it would be interesting to determine if the entire Merkel cell population in denervated touch domes becomes contacted by sprouted endings of the intact nerve; if not, Merkel cells which remain uninnervated may continue to produce a target factor (Scott, Macintyre and Diamond, 1980) that, although ineffective on intact nerves, might attract regenerating nerves to their vicinity. The proximity between one set of potentially competing endings and another has been suggested to be a factor in the synaptic competition seen during establishment of normal innervation (Bennett and Pettigrew, 1975) and reinnervation (Kuffler, Thompson and Jansen, 1977) of skeletal muscle.

Bennett and Raftos (1977) showed that sprouts of intact motor nerves incompletely occupy vacated end plates initially and require a longer than normal time to mature in size and quantal content; a regenerating motor nerve usually contacts the unoccupied portion of such an endplate and rapidly competes locally to replace the sprouted endings (Thompson, 1978). In the present case perhaps the incomplete suppression of the target quality results from a similar incomplete occupation of individual target sites, i.e. the junctional membrane of individual Merkel cells.

Delaying the arrival at the target of regenerating nerves can reduce their ability to compete successfully with others already present (Frank, Jansen, Lømo and Westgaard, 1975; Slack, 1978; Thompson, 1978). This could conceivably result from a more complete (or mature) occupation of available targets by the first arriving nerves. In the present investigation some endings of intact nerves, after functioning for approximately 25 days or more, were able to be replaced by regenerated ones; it is not yet known whether a longer delay in the arrival at the skin of regenerating nerves would similarly reduce their competitive advantage over endings established in denervated skin by intact ones.

From the results of this thesis, it would appear that it is only the extra endings of intact nerves established outside their normal boundaries during the critical period which are replaced by the regenerated nerves. Faced with regenerating nerves,

the number of touch domes as well as the area supplied by the formerly isolated intact mDCNs-T13 returned to control values and regenerating nerves did not establish a functional receptive field in skin which was, to all intent and purposes, normally innervated. This is consistent with the observations that in the neuromuscular system transposed regenerating nerves are able to grow into a normally innervated muscle but do not establish functional connections unless the normal nerve supply has been interfered with (Fex and Jirmanova, 1969).

In other work, functional or indeed morphological withdrawal of experimentally induced connections in the face of regeneration by the original nerve has been suggested to be possibly due to an over-taxing of the sprouted nerves metabolic machinery (Jansen, Thompson and Kuffler, 1978; Purves and Lichtman, 1978). The longer time required by foreign synapses to mature on salamander limb muscles (Bennett and Raftos, 1977) was however attributed to mismatching of the pre- and post-synaptic elements (see also Purves, 1976; Changeux and Danchin, 1976; Jacobson, 1970). Resolution of competition on the basis of a mechanism which can distinguish between foreign and appropriate inputs (Dennis and Yip, 1978; Schmidt and Stefani, 1976) rather than simple relative numbers of endings being supported by each nerve is appealing and is indeed the most consistent with the evidence obtained in this thesis. That regenerating T12 and L1 axons were able to functionally replace sprouted endings of T13

axons within its own dermatome, might reflect that, as seen by the target, any regenerating nerve is potentially more appropriate than the definitely misplaced endings formed by sprouting of intact nerves outside their normal boundaries.

That regenerating sensory nerves were able to cause the withdrawal of sprouts arising from intact ones was first shown in the rabbit cornea (Zander and Weddell, 1951). Electrophysiological and behavioural evidence for a similar phenomenon occurring for high-threshold nociceptive nerves in glaborous skin of the rat foot has been reported (Devor, Schonfeld, Seltzer and Wall, 1979). Although the sprouting evoked by denervation in the present study differs dramatically from that seen in both of these instances, competition appears to be resolved in a similar fashion - the extra endings withdraw. Under analogous competitive conditions in salamander skin, competition between regenerating nerves and sprouts of intact ones is resolved differently. Denervated Merkel cells appear to be permanently "captured" by the first low-threshold mechanosensory nerve to reach them whether regenerating or intact, foreign or appropriate, and apparently independent of the time allowed for new endings to mature (Scott, Macintyre and Diamond, 1981).

It is not yet known if the functional replacement of endings of intact (but sprouted) nerves by those regenerating ones, found in the present study, is accompanied by an actual physical withdrawal of the endings; the available evidence (e.g. Zander and Weddell, 1951;

Korneliussen and Jansen, 1977; Dennis and Yip, 1978) suggest it to be likely however this can only be determined conclusively by combined physiological and histological investigations.

5. Overall Considerations

From the results of this thesis it is apparent that the ability of cutaneous nerves to establish functional connections in denervated mammalian skin is dictated according to (i) the type of nerve - high- or low-threshold mechanosensory, (ii) the status of the nerve - intact or regenerating, (iii) the spatial location of the available targets, and (iv) the age of the animal. The lack of functional recovery of low-threshold mechanosensory innervation in partially denervated adult rabbit skin (Appendix I) is likely explicable on the basis of the present results that after a certain age, intact low-threshold mechanosensory nerves in mammals cannot sprout outside their borders. It is of interest that in the same preparation sprouting of high-threshold mechanosensory nerves does occur (Weddell, Guttmann and Gutmann, 1941; Weddell, 1942). Similarly, partial denervation of skin of the adult rat's hind foot leads to sprouting by remaining high-threshold nerves; apocryphally, sprouting of intact low-threshold mechanosensory nerves was reported to be "not unequivocal" (Devor, Schonfeld, Seltzer and Wall, 1979). In the hairy skin of the rat back too, high-threshold nerves sprout into denervated skin while low-threshold ones do not (Jackson and Diamond, 1979). The distinctive difference between the ability of high- and low-threshold mechanosensory

nerves to sprout in adult mammals therefore seems likely to be quite general. For denervated sensory targets to reaquire low-threshold mechanosensory innervation in the adult, regeneration of damaged nerves is required.

The present results indicate that in clinical conditions where injury to cutaneous nerves occurs and tactile (epicritic) sensation fails to be reestablished through sprouting of intact nerves or by regeneration of the damaged ones, it might become feasible to damage intentionally the remaining intact nerves in the region of denervated skin; these now regenerating nerves may in fact be able to invade the insensitive region and restore some semblance of normal sensitivity. It is not known whether such "foreign" innervation would be adaptive or even desirable at the perceptual level (see Sunderland, 1980).

The evidence that intact nerves are confined within domain borders could reflect a necessity of the nervous system to maintain, wherever possible, the topographical relationships established in development; such spatial restrictions on the peripheral endings might permit (or instruct) appropriate central connections of the sensory nerves to be made during development. In the developing cutaneous innervation of frogs, the skin has been shown to influence the central connections which allow the animal to correctly localize the site of stimulation (Miner, 1956; Jacobson and Baker, 1969); misdirected cutaneous reflexes following skin grafts within a critical period have been shown to occur.

Perhaps the failure of intact mammalian low-threshold mechanosensory nerves to sprout, outside their established borders, beyond a critical period results in an advantage to the animal in precisely localizing the perceived site of tactile stimulation. Equally, the persistent ability of high-threshold nerves to sprout into denervated skin could reflect the relative importance to the animal of being able to detect potentially injurious events without the necessity of completely accurate localization.

It is tempting to speculate that the temporal and spatial constraints similar to those found to operate on low-threshold mechanosensory nerves post-natally might be general phenomena of development in the nervous system. When discovered, the mechanisms responsible may be manipulable in such a way as to yield a more complete understanding of the development, maintenance, and possible repair of the nervous system.

6. Questions Outstanding

The results of this thesis have laid the ground work from which hypotheses concerning the mechanisms responsible for the phenomena may be tested. One major question remaining is: what are the morphological correlates of the physiological evidence? Investigations are currently underway at both the light- and electron-microscopic level, particularly of the neurite-Merkel cell complexes in touch domes at the various stages of sprouting and competition discovered in the present work. Combined morphological and physiological studies will also establish whether the lack of

functional sprouting is due to a failure of the nerves to sprout or to a failure in the formation of functional endings.

The innervation of the skin by the dorsal cutaneous nerves in the rat is a preparation within which neuron-target phenomena can be dissociated, and which exhibits temporal, spatial, and competitive interactions among nerves for their targets which may directly relate to those operating during the formation of the nervous system and about which information may be obtained through experimental manipulation.

The results of this thesis provide evidence for a remarkably brief critical period during which undamaged low-threshold mechanosensory afferents are able to establish, and maintain for long periods of time, functional innervation outside their normal boundaries. The evidence is consistent with the hypothesis that there are, in the skin, domains within which intact nerves are competent to establish endings and outside of which they are constrained from doing so; regenerating nerves are constrained neither by a critical period nor by a domain boundary. Further, endings established by intact nerves within their domains but outside their normal boundaries incompletely suppress a target quality of the receptor cells; this enables a regenerating nerve, if present, to locate them and allows for competition to occur.

It is concluded that there are temporal and spatial constraints on the recovery of tactile function in denervated mammalian skin which operate differently on intact and regenerating nerves and which at least potentially ensure re-establishment of function by the most appropriate nerve. Similar permissive or restrictive factors may operate during the course of development and could account in part for several aspects of the ontogeny of neural circuits and adjustments of nerve endings in later life.

APPENDIX I

Initial Investigation of Cutaneous Nerve Sprouting in the Rabbit

Sprouting of collaterals by intact cutaneous axons in the vicinity of experimentally denervated skin, associated with recovery of behavioural sensibility to stimuli (pin-prick or electric shock), was described in the skin of the leg and ear of adult rabbits (Weddell, Guttmann and Gutmann, 1941; Weddell, 1942). These studies did not address the question of whether or not low-threshold (touch) mechanosensory innervation was reestablished in their denervated skin along with high-threshold (nociceptive) sensibility. In this appendix are presented the results of a preliminary series of experiments undertaken to answer this question. Cutaneous nerves innervating known regions of skin in the rabbit were cut, and at various times thereafter, adjacent low-threshold mechanoreceptive fields of intact cutaneous nerves were mapped electrophysiologically to determine whether they had sprouted functional collaterals into the denervated skin.

Methods

1. Animals

Adult male and female New Zealand white rabbits (2-2.5 kg) were obtained from Woodlyn Farms, Guelph, Ontario. They were housed both before and after surgery in individual cages with

raised wire-mesh flooring, with food and water freely available.

2. Anaesthesia

All surgical procedures were carried out using clean (not sterile) technique on deeply anaesthetized animals. Anaesthesia was induced and maintained through an open inhalation system by 3% halothane (BDH) in nitrous oxide and oxygen (3:1). After induction of anaesthesia, a loose fitting mask was placed over the animal's muzzle and the total flow rate adjusted to approximately 1.5 litres/minute. Core temperature was monitored with a rectal thermister probe and maintained at 37 ± 1.5 C with a radiant heat lamp.

3. Denervations

(a) Leg - Anaesthetized animals were placed in a prone position with the hind legs fully extended. The fur of the dorsal aspect of the left thigh was clipped short. An incision was made exposing the Sural and Lesser Sural nerves in their course through the popliteal fossa. The nerve to be cut was grasped with watchmaker's forceps, cut, and a 5-10 mm length of the nerve removed. The incision was then closed in one layer with stainless steel surgical clips.

(b) Ear - In anaesthetized animals the Greater Auricular nerve of the left ear was exposed through an incision just proximal to the base auricular cartilage. The nerve was cut at the point where it enters the cartilage and a 5-10 mm length removed. The incision was closed in a single layer with stainless steel surgical clips.

4. Post-operative

All surgical wounds after closure were liberally swabbed with cotton wool soaked in 70% alcohol. Recovery from anaesthesia was usually without incident. Upon recovery the animals were returned to their cages and checked daily for post-operative infections and general health. Those few animals (less than 5%) which developed infections were removed from the study and administered a lethal dose of sodium pentobarbital intravenously.

5. Preparation of Nerves for Recording

(a) Leg - Anaesthetized animals were placed in a prone position with both hind legs fully extended and slightly elevated. The fur of the leg from the popliteal fossa to the toes was clipped to approximately 0.5 mm in length. An incision was made in the skin exposing the sural and lesser sural nerves in the proximal portion of the popliteal fossa. Exposure of the nerves was continued centrally by blunt dissection and retraction of the knee flexors. Both nerves were gently dissected free of surrounding connective tissue and cut as far centrally as practicable, usually 2-3 cm above the popliteal fossa. This procedure allowed nerves which had been cut as part of the experiment in earlier surgery to be recorded from central to the site of the lesion; functional regeneration of these nerves to the skin, if present, would therefore be detected.

(b) Ear - In anaesthetized animals the Greater Auricular nerve was exposed central to the base of the ear through incisions

made in the skin medially from the base auricular cartilage; nerves previously cut were exposed central to the position of the nerve lesion. A 10-15 mm length of the nerve was dissected free of loosely investing connective tissue and cut further centrally. The Occipital nerves (which also supply the dorsal surface of the ear were exposed through a transverse incision near the base of the skull, approximately 5 mm caudal to the occipital protuberance. The nerves were cleared of connective tissue over a length of 10-15 mm and cut centrally. The fur on the dorsal surface of the ear was clipped to a length of approximately 0.5 mm. During dissection all nerves and exposed tissues were kept moist with a Krebs physiological saline, composition: NaCl 118.0 mM; KCl 4.7 mM; KH_2PO_4 1.8 mM; $MgSO_4$ 1.6 mM; $CaCl_2$ 2.5 mM; $NaHCO_3$ 24.9 mM; glucose 10.0 mM.

6. Recording Impulses in Low-Threshold Mechanosensory Axons

The nerve to be mapped was raised from the underlying tissue onto bipolar platinum wire recording electrodes and coated with petroleum jelly to prevent drying of the exposed portion. With a nerve on the recording electrodes the skin was lightly stimulated mechanically with a 200 μ diameter blunt-tipped stainless steel wire. Nerve impulses were recorded extracellularly from the whole nerve. The signals were amplified using a Grass P511 AC differential pre-amplifier in series with a Tektronix 5A22N amplifier, filtered (LF 100 Hz, HF 10 KHz) and displayed on a Tektronix D13 dual beam storage oscilloscope. The

signal was also monitored by relaying the oscilloscope output through a loudspeaker equipped with headphones. The animal and the equipment were connected to a common ground. Short trains of impulses evoked in the nerve were, in these experiments, readily detectable audibly above the baseline noise level. However, the poor signal-to-noise ratios obtained from these whole nerves precluded visual resolution of spikes in the oscilloscope trace.

7. Mapping of Receptive Field Areas

While recording afferent impulses in the selected nerve, mechanical stimuli were applied beginning near the centre of the detectable receptive field and working systematically outwards. The edge of the receptive field of a given nerve was considered to be reached at the position on the skin where the mechanical stimulation ceased to evoke detectable impulse activity in the nerve. The border of the receptive field thus determined was marked directly on the skin with a fine-tipped pen.

The receptive fields of the Sural and Lesser Sural nerves, marked on the skin as described above, were drawn onto a standardized outline of the hind leg of the rabbit, using the head of the tibia, the calcaneus, and the fifth metatarsal as landmarks. The areas of the receptive fields were then measured from these drawings using a fixed arm planimeter. After mapping the receptive fields of the Greater Auricular or Occipital nerves, the ear was removed by cutting central to the base auricular

cartilage. This cartilage was then split in the long axis of the ear and the ear itself flattened onto a sheet of paper and its outline traced. The actual border of a mapped field was transferred to paper by inserting a pin through the ear and into the paper at approximately 1 mm intervals along the border. The perforations in the paper were then connected by lines and the area of the receptive field measured using a fixed arm planimeter.

Results

1. Rabbit Leg

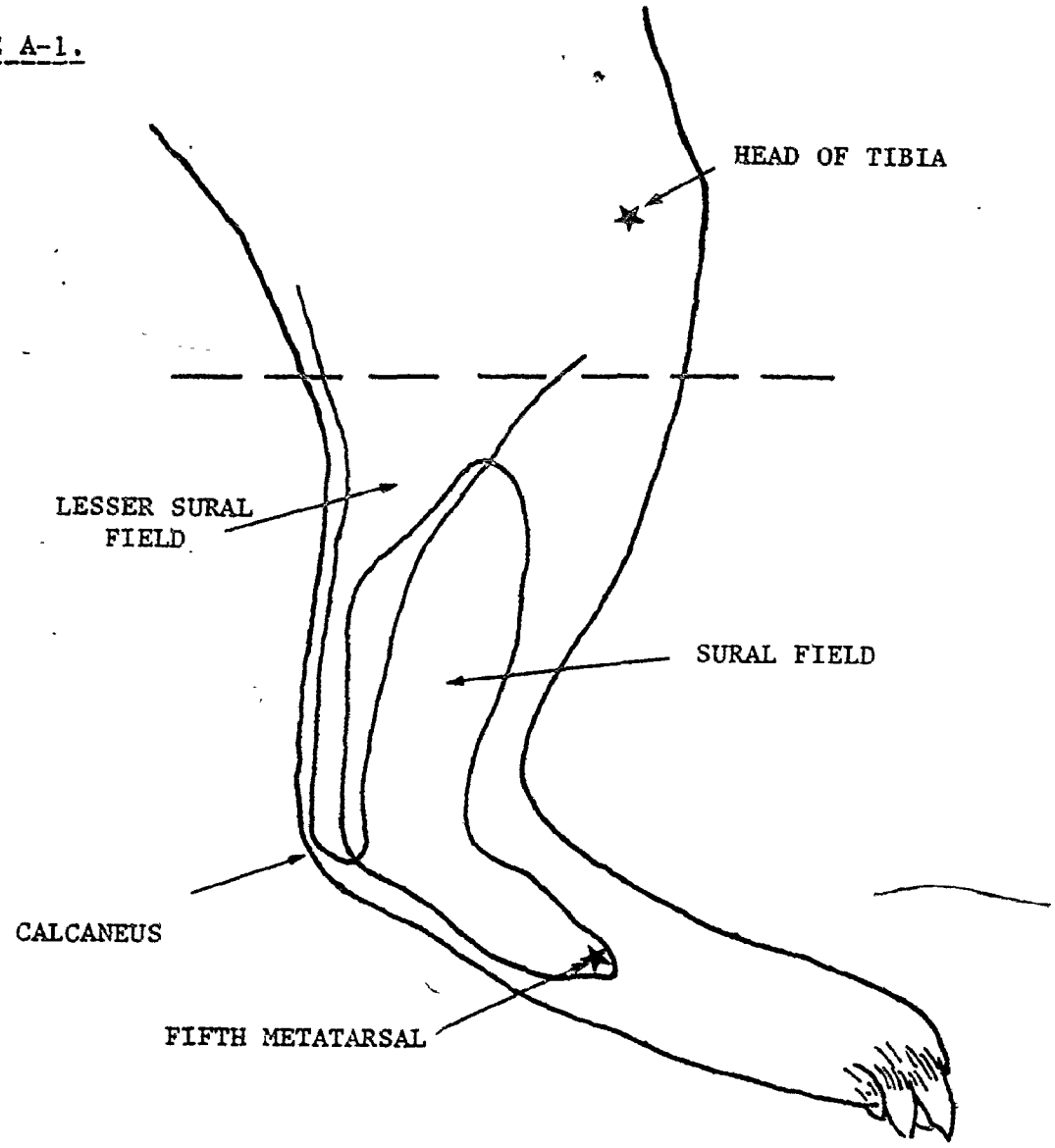
In the rabbit leg the receptive fields of the Sural and Lesser Sural nerves, mapped as described above, occupy adjacent regions of skin, with some overlap (Fig. 1). In 22 animals the Lesser Sural nerve was cut in the left leg; at approximately weekly intervals thereafter one or two animals were taken from the original group, anaesthetized, and the low-threshold mechanoreceptive fields of the Sural nerves in both legs mapped. The Sural nerve receptive fields of the last five surviving animals were mapped 60 days after cutting the Lesser Sural nerve in the left leg; the results (Fig. 2) were representive of the maps obtained from the animals examined at earlier times, and show that the receptive fields of the intact sural nerves had not expanded in response to denervation of adjacent skin as would be expected if these intact nerves had sprouted functional collaterals into the denervated region. In a different

LEGENDS TO APPENDIX I

FIGURE A-1 The Low-Threshold Mechanosensory Fields of the Sural and Lesser Sural Nerves

The low-threshold mechanosensory fields shown here were mapped by direct electrophysiological recording of evoked activity in each of the nerves. Taken together these fields extend from the lateral aspect of the ankle and foot into skin overlying the calcaneal tendon on the dorsal aspect; here the fields are shown as if the skin had been removed from the leg and placed on a flat surface. The field of the Lesser Sural nerve is shown as a discontinuous line since the incision made to expose the nerves for cutting or recording was located in the proximal portion of the field; the fields of the Lesser Sural nerve shown in Figure 3 refer to that area of skin distal to a horizontal line drawn across the field 2.5 cm below the head of the tibia.

FIGURE A-1.



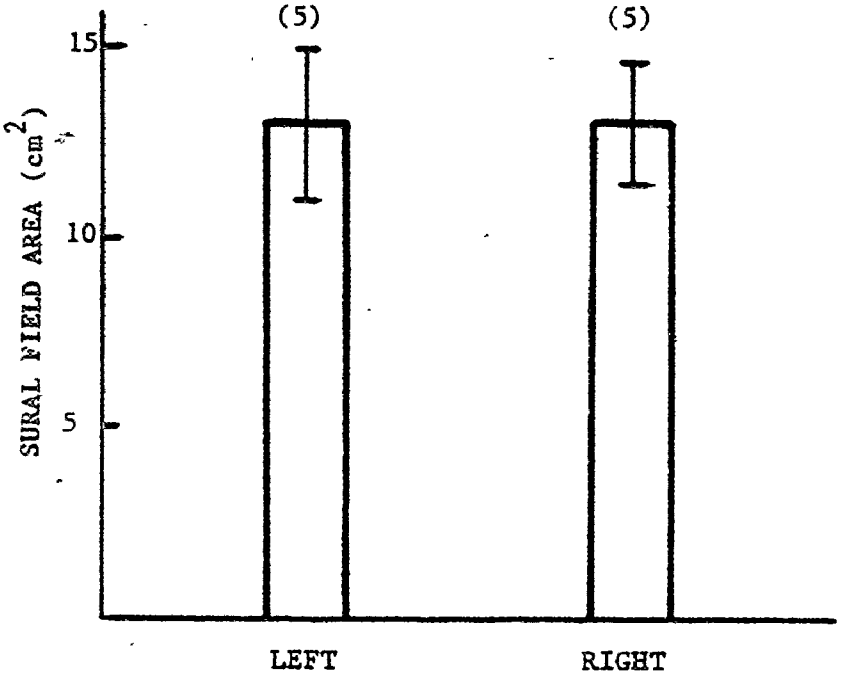
RIGHT HIND LEG

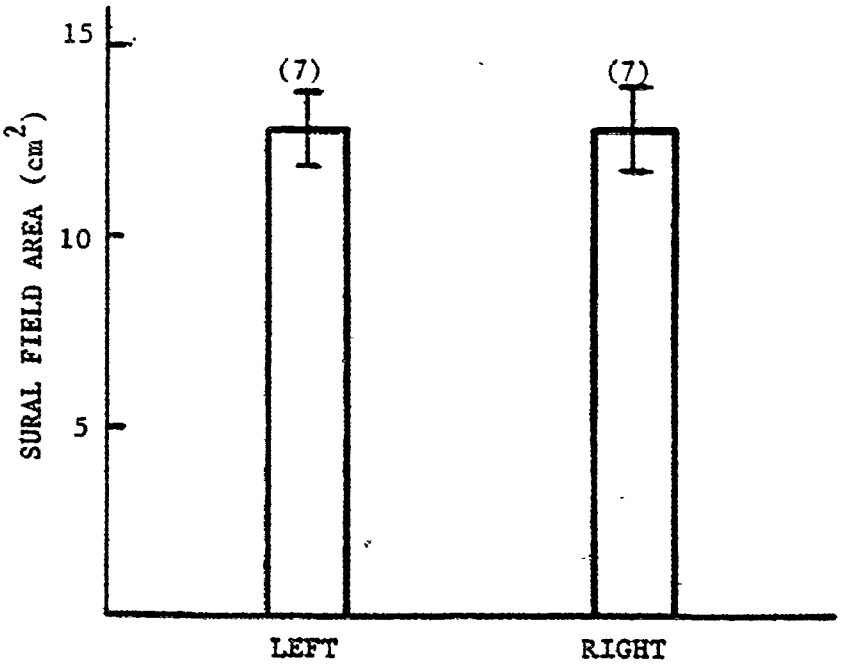
FIGURE A-2. The Effect of Cutting the Lesser Sural Nerve on the Field of the Sural Nerve

A. This histogram shows the area (± S.D.) of the left and right sural nerve fields in 5 acutely mapped normal control animals. There is no significant difference ($p>0.2$) in these areas.

B. This histogram shows the area (± S.D.) of Sural nerve fields in 7 animals in which the left Lesser Sural nerve was cut 60 days earlier. There is no difference between the area of sural nerve fields adjacent to denervated skin (left leg) and those in normally innervated skin (right leg) ($p>0.2$).

FIGURE A-2.


(5)
(5)
15
10
5
SURAL FIELD AREA (cm^2)
LEFT
RIGHT


15
(7)
(7)
10
5
SURAL FIELD AREA (cm^2)
LEFT
RIGHT

group of animals (N=9) the Sural nerve was cut in the left leg leaving the Lesser Sural nerve intact. The mechanosensory receptive fields of both nerves in both legs were mapped 60 days after the unilateral axotomy. As in the previous experiment, there was no detectable enlargement of the receptive field adjacent to denervated skin (Fig. 3). Neither Sural nor Lesser Sural nerves, axotomized at the original surgery, had functionally regenerated to the skin; successful regeneration of similarly axotomized sural nerves was detectable 80-90 days later in a third group of animals.

2. Rabbit Ear

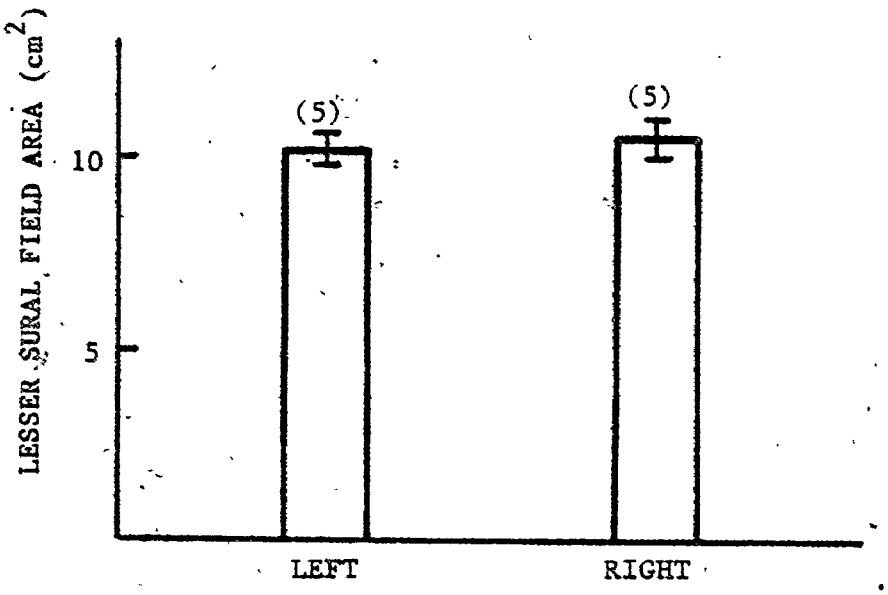
Innervation of the dorsum of the rabbit ear is provided by the Greater Auricular nerve and the Occipital nerve (Fig. 4). The low-threshold mechanosensory receptive fields, in the ear, of left and right Occipital nerves were mapped acutely in four normal animals; there was no significant difference between the fields of the left ear and the fields of the right ear (Table I). In a total of 17 rabbits the Greater Auricular nerve of the left ear was cut, denervating the region of the ear not supplied by the Occipital nerve (see Fig. 4). Every 5-7 days later an animal was taken from this group and the mechanosensory fields of both left and right Occipital nerves mapped in a terminal experiment. There was no clearly apparent change in the receptive fields of Occipital nerves adjacent to denervated skin. After 65 days had elapsed since the initial denervation, the mechanosensory

FIGURE A-3. The Effect of Cutting the Sural Nerve on the Field of the Lesser Sural Nerve

A. The histogram shows the area (± S.D.) of the receptive fields of Lesser Sural nerves in the left and right legs acutely mapped in 5 normal control animals. The field area in the left legs was not different ($p>0.2$) than those in right legs.

B. This histogram shows the area (± S.D.) of Lesser Sural nerve fields in a group of nine animals 60 days after unilateral Sural nerve axotomy in the left leg. There was no difference ($p>0.2$) between fields adjacent to denervated skin (left leg) and those in completely innervated skin (right leg).

FIGURE A-3.


LESSER SURAL FIELD AREA (cm^2)
10
5
(5)
(5)
LEFT
RIGHT

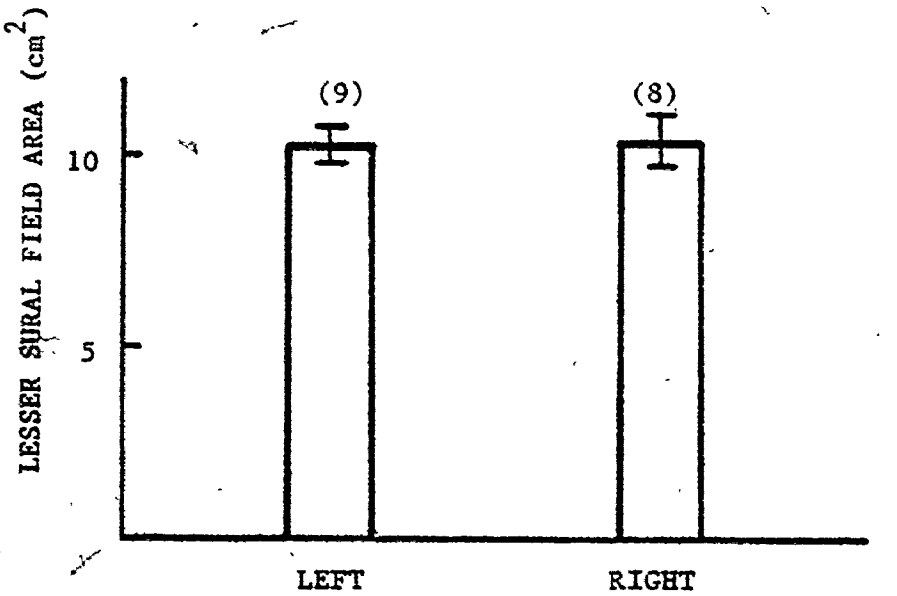

LESSER SURAL FIELD AREA (cm^2)
10
5
(9)
(8)
LEFT
RIGHT

FIGURE A-4. The Receptive Fields of the Greater Auricular Nerve and the Occipital Nerve on the Dorsum of the Rabbit Ear

This figure shows the low-threshold mechanosensory fields of the two nerves which supply innervation to the dorsal surface of the rabbit ear. The fields occupy characteristic positions with a small degree of overlap along their common border. Cutting the Greater Auricular nerve completely denervates the lateral two thirds of the ear.

FIGURE A-4.

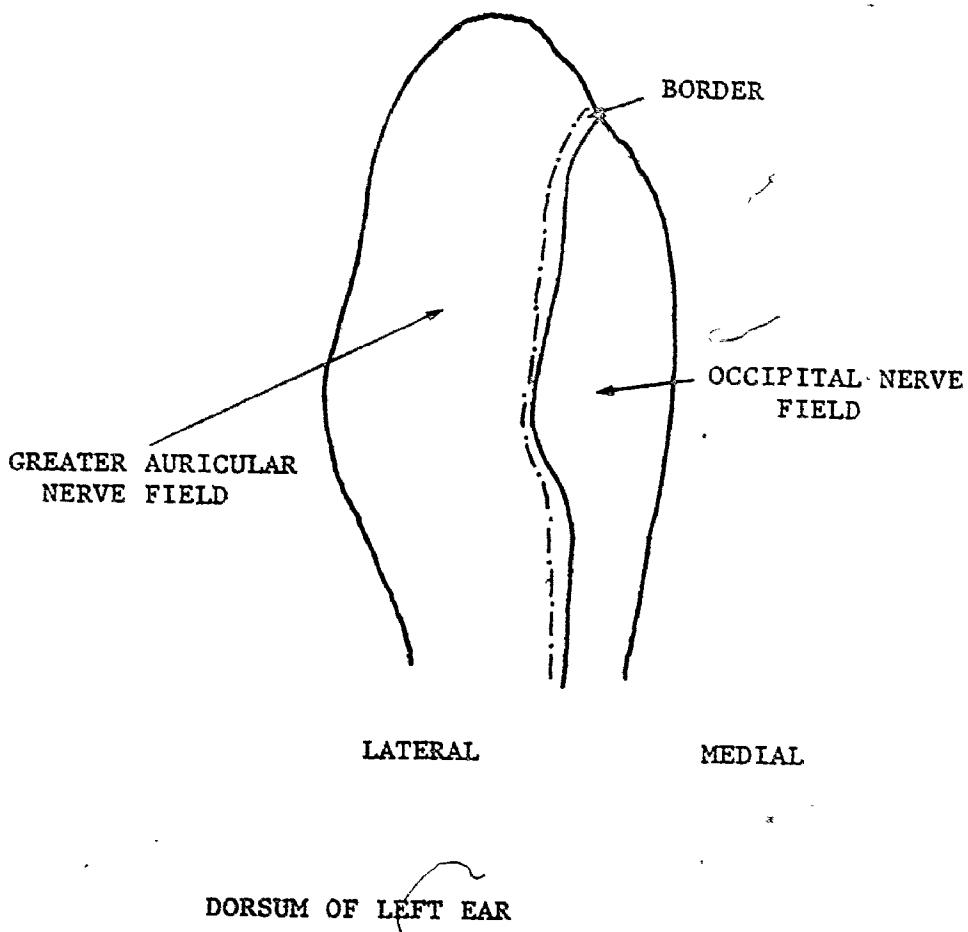

BORDER
OCCIPITAL NERVE FIELD
GREATER AURICULAR NERVE FIELD
LATERAL
MEDIAL
DORSUM OF LEFT EAR

TABLE A-I. The Occipital Nerve Fields in Normal and Partly Denervated Rabbit Ear

A. Shown here are the areas of Occipital nerve fields mapped acutely in four normal control animals.

B. This shows the Occipital nerve fields acutely mapped in a group of five animals in which the left Greater Auricular nerve had been cut 65 days earlier. Denervation of adjacent skin had no effect ($p>0.2$) on the field of the intact occipital nerve supplying the remainder of the ear.

TABLE A-I.

AREAS OF OCCIPITAL NERVE FIELD (cm^2)

Left Ear		Right Ear
22.0		21.3
23.1		24.5
23.6		21.5
24.0		22.6
23.2 ± 0.9	$\bar{x}$ ± S.D. N.S. $p>0.2$	22.4 ± 1.5

Left Ear (Greater Auricular Cut)		Right Ear
23.0		24.2
22.8		24.0
25.0		26.6
20.1		20.6
20.8		22.0
22.9 ± 1.5	$\bar{x}$ ± S.D. N.S. $p>0.2$	22.3 ± 1.9

fields of the Occipital nerves in the remaining 5 animals were mapped. Not only were the occipital nerve fields in the left ears not different than those in the contralateral undenervated ear, they were also not different from those mapped in completely normal controls (Table I). In none of the experimental animals was the originally cut greater auricular nerve found to have regenerated and successfully established mechanosensory function in the skin.

Discussion

The results seemed to indicate that, although regenerating low-threshold mechanosensory nerves were able to grow into denervated skin and resume function, the neighbouring intact ones were unable to sprout new functional collaterals outside their normal fields into experimentally denervated skin, at least for a period up to 65 days after denervation. Weddell (Weddell, Guttmann and Gutmann, 1941; Weddell, 1942) working earlier with the same preparation, had shown that, in the absence of regeneration, histologically verified invasion of denervated skin by collaterals from intact nerves was responsible for the recovery of sensibility to noxious stimuli. This recovery was detectable as early as three days following denervation and was, in most cases, completed by 28 days. It seemed most likely that low-threshold nerves would behave similarly. At the time these experiments were conceived and carried out, recent work had clearly demonstrated

in salamanders, the ability of intact low-threshold mechanosensory nerves to sprout functional collaterals into denervated skin (Aguilar, Bisby, Cooper and Diamond, 1973).

Further investigation of this possible difference in compensatory sprouting between classes of neurons (high- and low-threshold), and between species, was warranted; there were technical considerations, however, which rendered both the leg and ear preparation in the rabbit problematical for more detailed investigations. The major concern derived from the technique of recording afferent impulse activity from whole nerves, and was common to both the ear and leg preparations. Since the whole nerve bundles being recorded from were of relatively large cross-sectional diameter, the recorded signal-to-noise ratios were correspondingly poor. Although short bursts of evoked impulses were detectable audibly it was difficult to define crisply and objectively the border of a given field owing to the decreased density of innervation, and therefore the reduced number of impulses evoked by a standard stimulus as the edges of the receptive field are approached (see Kuhn, 1953). For this reason further experiments using the rabbit leg and ear preparation were abandoned. Exploratory maps of the cutaneous innervation in different regions of several mammalian species were carried out and the cutaneous innervation supplied by dorsal cutaneous nerves in the rat was selected for investigation. The results of these investigations form the substance of this thesis.

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