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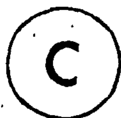
# NORMAL AND DISORDERED FUNCTION
# IN
# A MAMMALIAN TOUCH RECEPTOR


By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# NORMAL AND DISORDERED FUNCTION IN A MAMMALIAN TOUCH RECEPTOR

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ABSTRACT

Mechanosensory transduction changes both during development and after the application of vincristine, a neurotoxic agent. Specifically, cutaneous sensitivity mediated by Type I slowly adapting cutaneous mechanoreceptors (touch domes) changes over the course of development in two ways. The first is a reduction in the density of touch domes in the skin from birth to adulthood. The second mechanism involves an increase in threshold to mechanical stimulation from birth to old age.

In control adult animals, administration of the neurotoxic drug vincristine increased mechanosensory threshold within 24 hours after treatment. This raised threshold was maintained for two weeks but there was a return to control values by three weeks post-treatment. Measurements of response latency indicated that the rise in receptor threshold could occur without impulse propagation being impaired in the axon. Both the morphological and physiological state of the axon twenty-four hours after vincristine treatment indicated that vincristine modified sensory transduction at the level of the touch domes or of the fine nerve terminals abutting it. Vincristine may have produced the temporary high threshold state of the receptor by direct action on the receptor or indirectly by interfering with axoplasmic flow. If the latter is the case, this observation provides indirect evidence for the existence of a functional connection between the flow of trophic factors in the nerve fibres supplying touch domes and the mechanosensory transduction process in the receptor.

Possible mechanisms for the changes observed in receptor properties during ageing and drug treatment are presented. It is proposed that during ageing a functional deafferentation occurs; the implications of this proposed process on the ageing nervous system are discussed. It is suggested that similar mechanisms may underlie threshold changes seen during ageing and after the administration of vincristine.

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# I. INTRODUCTION

## A. Rationale for Research

### 1. Development and Ageing of a Sensory Receptor

> "Uniformity and orderliness in the sequence of exposure to the environment may be governed and programmed by the developmental timetables of different parts of the nervous system"
>
> Goldman (1976) p. 71

Our link to the outside world is the sensory nervous system. Exteroceptive information is transmitted by sensory nerve fibers to the central nervous system after external energy has been transduced into nerve impulses by receptors. The manner in which neural transduction takes place will determine the temporal and spatial pattern of impulses that is transmitted to the central nervous system. In man and in other animals, the interpretation of the incoming impulse pattern by central neurons may be regarded as a perception of the environment, irrespective of its consequences at conscious or subconscious levels.

In the adult, the threshold and density of individual receptors gives one perceptual window on the outside world, a window that may be extended or reduced in younger or older animals. Organisms may, therefore, experience different perceptions of the same physical stimuli in their environment over the course of their lives. In the following series of experiments, I will explore the changing response of one type of somatosensory receptor during the life of a rat. I will then examine the alteration of the response of that receptor after pharmacological treatment.

a. The Effects of Sensory Activity on Development

1. Somatosensory System

If, during development, attachment is fostered by skin contact (Blass, Hall and Teicher, 1978), then the neonate's nervous system must be able to receive and process tactile information. Indeed, touch stimuli can even elicit crude reflexes in several species of fetal mammals (Bradley and Mistretta, 1975).

The role of peripheral input in the development of central connections of one somatosensory system has been studied in detail for the rodent facial vibrissae. Each vibrissa projects to a discrete location in the contralateral somatosensory cortex, termed a barrel. If vibrissae are destroyed at birth, there is loss of the characteristic barrel structure in both the somatosensory cortex (Van der Loos and Woolsey, 1973) and thalamic projections (Woolsey et al., 1979; Killackey, 1979). There is a critical period after birth when receptor destruction will have characteristic central effects (Weller and Johnson, 1975).

In addition to the vibrissal system, the development of the somatotopic representation of the hindlimb in the gracile nucleus of the dorsal columns has been studied. After amputation of the hindlimb in neonatal opossums, the neurons of the gracile nucleus that would have represented the hindlimb disintegrated (Johnson et al., 1972). The activity from the peripheral inputs, therefore, may also be necessary for normal central development in this system.

## 2. Other Sensory Systems

Central representation of visual input has been shown to be altered by eyelid suture, dark rearing, eye enucleation and exposure to various patterns of light and dark (Daniels and Pettigrew, 1976). The orientation specificity of cells of the kitten visual cortex is shifted towards a preference for the orientation pattern that the animal has been exposed to during development (Blakemore and Cooper, 1970). If a kitten has experienced monocular deprivation during development the deprived eye is ineffective in driving cortical cells that are easily driven by the non-deprived eye. During the development of the visual system of the cat there are critical periods when the amount and character of sensory input plays an important role in the formation of functional central circuitry (Hubel and Wiesel, 1970).

Studies in the gustatory and olfactory system indicate that early sensory experiences can influence adult preferences (Mistretta and Bradley, 1978). If the nares of the rat are blocked during early development, the olfactory bulbs are smaller (Meisami, 1976). In another study olfactory bulb neurons were shown to degenerate if the animals had been exposed to specific odors during development (Doving and Pinching, 1973; Pinching and Doving, 1974). This indicated a functional localization in the olfactory bulb. Later studies using the 2-deoxyglucose method have indicated that this is indeed the case (Sharp et al., 1975). Behavioral studies have shown that exposure to an odor early in development produces a preference for that odor in older pups (Mistretta and Bradley, 1978). In the gustatory system

behavioral studies in rats have shown that exposure to a taste during nursing and weaning results in a preference for that taste as an adult (Capretta and Rawls, 1974).

In several avian species behavioral studies have shown that auditory deprivation produces abnormal responses to auditory stimuli (Gottlieb, 1976). The responsiveness of rats to auditory patterns is greatly reduced if the animals have been deprived of auditory input during devlopment (Tees, 1967). Conversely, exposing rats to a particular sound pattern during development increases the responsiveness of cells of the inferior colliculus to that pattern (Clopton and Winfield, 1976).

### 3. Present Research

It is clear from the evidence in several sensory systems that the activity level of different inputs is a critical factor for the determination of central circuitry during development. Therefore, the ability of individual receptors to respond to environmental stimuli may have an effect on the level of afferent input and the final pattern of central circuitry during normal development. The first question to be answered in this thesis is whether the sensitivity of the peripheral touch input changes during normal development.

The development of touch sensitivity was studied using a single identified receptor in the skin, the touch dome (Iggo, 1963a). Touch sensitivity of an area of skin that is mediated by touch dome structures

would depend on the density and threshold of receptors, and these two variables were measured during development, maturity and ageing.

B. Background

1. Neural Receptors

Muller (1840) postulated 'specific nerve energies' existed for vision and audition, suggesting that the optic nerve could convey only messages regarding sight and the auditory nerve could carry only messages regarding hearing. Blix (1884; 1885) subsequently extended this concept of specificity to skin receptors for warmth to cold, using psychophysical techniques. Von Frey (1894) then suggested that there were only four kinds of nerve endings in the skin that specifically transduced pressure, or warmth, or cold, or pain. Morphologists, however, subsequently described the structure of many more than four types of cutaneous sensory nerve endings, and specific non-neural receptor structures associated with sensory nerve endings were seldom identified (Munger, 1971, review; Hagen et al., 1953).

By the early 1950's, Weddell and others (Sinclair et al., 1952) questioned the relevance of a correlation between nerve ending structure and specific modality, as they found that structurally similar free nerve endings subserved different sensory modalities in both human ear skin and in the rabbit cornea (Zander and Weddell, 1951). It was suggested that the central perception of the modality of a particular stimulus might be signaled by the pattern of firing of the group of non-specific free nerve endings (Sinclair, 1955; Weddell and Miller, 1962). Electrophysiological recordings from peripheral

nerve fibers, though, demonstrated a correlation between sensory nerve fiber diameter and the stimuli that preferentially excite the fiber. Generally, large diameter, Aα nerve fibers subserve touch while smaller diameter, AΔ myelinated and unmyelinated "C" fibers subserve tactile or thermal stimuli, including those of noxious intensity (Table I summarizes this data). More recent recordings from single sensory nerve fibers have clearly shown that, apart from some of the C and smaller AΔ fibers, each fiber is typically selectively activated by a specific stimulus modality (Brown and Iggo, 1967; Burgess, Petit and Warren, 1968; Burgess and Perl, 1973; Perl, 1968).

With the advent of electron microscopy, specialized non-neural receptor structures could, at last, be visualized (Andres and von During, 1973), and Table II summarizes some mammalian receptor structures and their identified function. The coordination of neurophysiological with anatomical studies by Iggo (1963a; Iggo and Muir, 1969) on touch domes and by Hensel (1973) on cold receptors have demonstrated the existence of modality specific receptor structures.

In addition to having specific varied structures in the skin that are selectively activated by specific modalities, different receptors have a different pattern of central connections in the spinal cord. Both polymodal nociceptors and high threshold mechanoreceptors project to neurons in lamina I of the dorsal horn of the spinal cord (Christensen and Perl, 1970). Some lamina I cells form part of the lateral spinal thalamic tract in the monkey, a tract important for pain in man (Kumazawa et al., 1971). Low threshold mechanoreceptors, in

TABLE 1

Cutaneous Receptor Types Classified on the Basis of Nerve Fiber Diameter (Erlanger and Gasser, 1937; Gasser and Erlanger, 1955; 1960)

| Fiber Diameter | Classification and Conduction Velocity | Receptor type/ Adequate stimulus | Adaptation rate | Reference/Animal |
|---|---|---|---|---|
| | | Guard hair, G1 and G2 mechanical (touch) | rapid | Brown and Iggo, 1967; Burgess, Petit and Warren, 1968; Brown and Hayden, 1971/cat and rabbit; cat; rabbit |
| | | Tylotrich hair, mechanical (touch) | rapid | Brown and Iggo, 1967; Brown and Hayden, 1971/cat and rabbit; rabbit |
| 6μm-17μm | Aα 30-100 m/sec | Touch dome, type I, slowly adapting, mechanical (touch) | slow | Iggo, 1963a; Burgess, Petit and Warren, 1968; Tapper, 1965; Iggo and Muir, 1969; Hunt and McIntyre, 1960a; Brown and Hayden, 1971/ cat; cat and rabbit |
| | | Type II, slowly adapting (Ruffini ending), mechanical | slow | Witt and Hensel, 1959; Burgess, Petit and Warren, 1968; Chambers et al., 1972; Hunt and McIntyre, 1960a/cat |
| | | Pacinian corpuscle, mechanical (touch/ vibration | rapid | Hunt and McIntyre, 1960c; Burgess, Petit and Warren, 1968; Talbot et al., 1968/cat; cat; monkey |

| | | | | |
|---|---|---|---|---|
| 1μm-5μm | AΔ<br>4-30 m/sec | Down hair<br>D hair<br>(touch) mechanical | rapid | Hunt and McIntyre, 1960b; Brown and Iggo, 1967; Burgess, Petit and Warren, 1968; Perl, 1968/cat; cat and rabbit; cat; monkey |
| | | Mechanical nociceptors noxious, punctate, high threshold mechanical | slow | Burgess and Perl, 1967; Perl, 1968/cat; monkey |
| | | Mechanical thermal nociceptors, noxious heat, noxious mechanical | slow | Iggo and Ogawa, 1971; monkey |
| | | Thermoreceptors (cooling) | slow | Iggo, 1963b; Perl, 1968; Iggo, 1969; Hensel and Iggo, 1971/ monkey; monkey; dog and monkey; monkey |
| 0.3μm-1.5μm | C<br>0.4-2.0 m/sec | C mechanoreceptors (touch) | slow | Bessou, Burgess, Perl and Taylor, 1971; Iggo, 1963b; Zotterman, 1939/ cat; monkey; monkey |
| | | Thermal and mechanical nociceptors noxious heat, high threshold mechanical | slow | Iggo and Kornhuber, 1968; Douglas and Ritchie, 1957; Iggo, 1959; Iriuchijima and Zotterman, 1960/ cat; cat; cat; rat and dog |
| | | Polymodal nociceptors noxious heat, chemicals and high threshold mechanical | slow | Bessou and Perl, 1969; Bessou, Burgess, Perl and Taylor, 1971/ cat; cat |
| | | Mechanical nociceptors noxious mechanical | slow | Iggo, 1960; Bessou and Perl, 1969/ cat; (some in subcutaneous fatty tissue) cat |
| | | Warm receptors | | Hensel et al., 1960; Bessou and Perl, 1969; Hensel and Iggo, 1971; Iruichijima and Zotterman, 1960; Iggo, 1969/cat; cat; monkey; dog and rat; dog |

contrast, have spinal cord connections in laminae III, IV and V of the dorsal horn.

TABLE II
Identified Receptor Structures

| Morphological description | Adequate Stimulus | Fiber Type | Reference |
|---|---|---|---|
| 1. Touch domes associated with tylotrich hairs | light touch slowly adapting Type I | Aα | Iggo, 1963a; Iggo and Muir, 1969 |
| 2. Ruffini ending | light touch slowly adapting Type II | Aα | Chambers et al., 1972 |
| 3. Cold receptor | cold | AΔ | Hensel, 1973 |
| 4. Pacinian corpuscle | rapidly adapting pressure vibration | Aα | Talbot et al., 1968; Andres and von During, 1973 |
| 5. Meissner corpuscle | slowly adapting light touch | AΔ | Iggo, 1963b; Andres and von During, 1973 |

Within the grouping of light touch receptors, central connections of structurally different receptors are also distinct. Slowly adapting touch domes have elliptical areas of arborization of axon collaterals in laminae III, IV and dorsal V (Brown, Rose and Snow, 1978), whereas the central branches of rapidly adapting hair follicle afferents end mainly in lamina III of the spinal cord forming flame-shaped arborizations (Brown, Rose and Snow, 1977).

The segregation of cutaneous and deep modalities within nuclei is maintained in the brainstem dorsal column nuclei (Dykes et al., 1982), thalamus (Dykes et al., 1981), and somatosensory cortex (Dykes

et al., 1980). In the somatosensory cortex there is a segregation of cutaneous rapidly adapting and cutaneous slowly adapting submodalities within a cytoarchitectonic area (Dykes et al., 1980).

Although some anatomists currently do not accept the concept of distinct receptor structures in the skin that are modality specific (cf. Montagna and MacPherson, 1973; Montagna, 1977), all morphologically-distinct receptor structures that have been physiologically identified thus far are preferentially excited by specific modality stimuli (Iggo, 1963a; Iggo and Muir, 1969; Hensel, 1973). Furthermore, the branches of axons identified physiologically have been found to supply only one type of receptor (Iggo and Muir, 1969; Brown and Iggo, 1967). The small diameter fibers responsive to noxious and touch stimuli are an exception to this. However, it is not known which morphologically-distinct structures, if any, are associated with these fibers (Bessou and Perl, 1969; Bessou et al., 1971).

To summarize, the classification of receptors in the skin is not as simple as von Frey's (1894) analysis suggested, as there may be several types of receptor structures and free nerve endings that transduce a stimulus in a given modality. The evidence suggests that the selective activation of receptors--the receptor specificity--initiates the sorting out of information from environmental stimuli. To a large extent, this discrimination is maintained in modality-specific spinal connections, though some dorsal horn neurons have been shown to receive polymodal inputs (Wall, 1967).

a. Neural Transduction

The cell body of the primary afferent neuron is located in the dorsal root ganglia adjacent to the spinal cord. The axon of the dorsal root ganglion cell bifurcates, with one branch traveling centrally and the other peripherally. In the skin, the primary afferent axon terminates either by branching and producing fine free nerve endings or by having its nerve endings associated with a morphologically-distinct structure. Together, the neural and morphologically-distinct elements are referred to as a primary afferent unit, and it is within the primary afferent unit that sensory transduction takes place (Gray, 1959).

1. Generator Potentials

Stimulus energy produces a conductance change in the membrane of the sensory neurite that results in movement of ions along ionic gradients and a depolarizing current. This current produces a generator potential (Granit, 1955; Bernhard, Granit and Skoglund, 1942) or receptor potential (Gray, 1959). Increasing the stimulus intensity increases both the size and the velocity of the generator potential (Gray, 1959). The graded generator potential is produced by conductance changes that are independent of the conductance changes that produce the regenerative action potential in sensory nerves, since blocking action potentials with tetrodotoxin (Lowenstein, Terzuolo and Washizu, 1963) or procaine (Katz, 1950a) does not block the generator potential. It is commonly accepted that the receptor potential of a mechanoreceptor is produced by ionic movements associated with changes in membrane permeability (Diamond et al., 1958; Gray, 1959). In the crayfish

stretch receptor, the conductance changes responsible for the generator potential involved a non-specific increase in permeability to monovalent cations (Obera, 1968). It has been shown that the generator potential in the Pacinian corpuscle is mainly sodium dependent (Diamond, Gray and Inman, 1958).

2. Impulse Initiation

Hartline (1928) was the first to record a receptor potential in the visual system of Limulus. In the somatosensory system, Katz (1950b), studying the frog muscle spindle, was the first to observe directly a sensory generator potential, and he found a linear relationship between the size of the generator potential and the impulse frequency.

The site of impulse initiation may be separate from the site of the generator potential, or coincident with it. In the crustacean stretch receptor, the site of impulse initiation is located where the axon leaves the soma, while the generator potentials are formed in the dendrites adjacent to the muscle (Edwards and Ottoson, 1958). Early work in the Pacinian corpuscle, indicated that the site of impulse initiation is at the first node of Ranvier within the lamellar capsule and the generator potential originates in the non-myelinated portion of the nerve terminals (Diamond et al., 1956). However, subsequent studies (Hunt and Takeuchi, 1962; Ozeki and Sató, 1964) in which the outer lamellae of the capsule was removed, showed that impulses arose in the non-myelinated fiber within the corpuscle.

3. Adaptation

Adrian and Zotterman (1926) considered slowly adapting receptors to be those with position (static displacement) detection. Rapidly adapting receptors displayed little or no position response but responded to change of position velocity. Burgess and Perl (1973) considered that adaptation in mechanoreceptors occurs if the discharge frequency drops while the stimulus is constant. Most receptors do, in fact, decrease responsiveness with a continuing stimulus, but the mechanisms that cause this adaptation are not known. In touch receptors of frog skin (Lowenstein, 1956) and stretch receptors of crustacean muscle (Eyzaguirre and Kuffler, 1955) it appears that differences in the adaptation rate are due to mechanical properties of the tissues surrounding the sensory nerve endings. This hypothesis received direct support from studies on the Pacinian corpuscle, a very rapidly adapting touch/vibration receptor found in the mesentery and in the skin.

The structure of the Pacinian corpuscle resembles an onion bulb; there is a central thread of nerve surrounded by concentric lamellae. Hubbard (1958) studied the displacement of lamellae during compression at different distances from the center of the corpuscle using brief flash photomicroscopy. From measurements of the spacing of lamellae it appeared that the layers of the capsule progressively filter out all but transient disturbances. Hubbard's (1958) work suggested that the rapid adaptation of the receptor potential to a maintained stimulus might be due to mechanical properties of the corpuscle. This idea was confirmed by the work of Ozeki and Sato (1965) and Lowenstein and Mendelson

(1965), who removed the outer capsule of the corpuscle and were able to record a prolonged receptor potential in response to a prolonged stimulus.

An investigation of generator potentials was carried out by Nakajima and Onodera (1969b) on the crayfish slowly adapting stretch receptor neuron. They exerted different tensions on the muscle after abolishing spike potentials with tetrodotoxin. They suggested that 70% of the adaptation in the generator potential could be accounted for by changes in the viscoelastic properties of the surrounding tissue due to continued stretching.

Further, after stimulating receptors with a constant current, Nakajima and Onodera (1969a) found that changes in the spike triggering portion of the membrane itself may also contribute to characteristics of adaptation. They suggested that slow inactivation of sodium channels or an increase in potassium permeability of the membrane could be responsible for the observed characteristic adaptation. They concluded that differences in the properties of slowly and rapidly adapting stretch receptors of crayfish may be due both to plastic properties of the surrounding tissue and to differences in the properties of the spike generating portion of the membrane, with surrounding tissues playing a greater role in slowly adapting receptors.

In addition, previous ionic changes may contribute to adaptation. In the crayfish stretch receptor, the higher concentration of sodium ions in the cell as a result of action and generator potentials may trigger an electrogenic sodium pump which produces a hyperpolarizing

current that opposes the generator potential, thereby decreasing the likelihood of further activity (Nakajima and Takahashi, 1966).

In summary, it appears that adaptation may occur because of a decline in magnitude of the generator potential that may be due to specific elastic properties of the tissue surrounding the sensory nerve terminals (Nakajima and Onodera, 1969b). In addition to this mechanically-based adaptation, changes also take place in the properties of the spike generating part of the receptor membrane after the passage of current that may result in adaptation (Nakajima and Onodera, 1969a). Finally, the electrogenic sodium-pump, a metabolic process tied to ionic concentrations, may also contribute to adaptation (Nakajima and Takahashi, 1966).

2. Touch Domes

Transduction of mechanical stimulation in the skin is mediated, at least in part, by the transitory deformation of touch domes, which are Type I slowly adapting mechanoreceptors (Iggo, 1963a). Touch domes have been discovered and named, rediscovered and renamed several times (Pinkus, 1964). These structures were first described in hairy human skin by Pinkus in 1902, who named them haarscheiben and the structures were subsequently identified in several animals (Pinkus, 1905). Later, other workers also described haarscheiben in hairy human skin (Tamponi, 1938, 1939; Kawamura, 1954; Kawamura et al., 1964; Kamide, 1955). The touch dome has been described morphologically in rabbits (Straile, 1960; 1961), primates (Smith, 1970), cats (Iggo and Muir, 1969) and rodents (Smith, 1967; English, 1974; 1977a). In hairy skin,

the haarscheibe contains Merkel touch cells grouped into circular patches or elevated "touch domes" (Iggo, 1963a). They are often found at the base of large hairs, the tylotrich hairs of Straile (1960).

Unidentified slowly adapting Type I mechanoreceptors were studied electrophysiologically by several workers (Frankenhauser, 1949; Hunt and MacIntyre, 1960; Werner and Mountcastle, 1965) but the first associations of the "Haarscheiben" of Pinkus with Type I receptor properties were made independently by both Iggo (1963a) and by Tapper (1964). The touch dome has been characterized physiologically in rabbits (Brown and Iggo, 1967; Brown and Hayden, 1971), in primates, including man (Lindblom and Tapper, 1967; Perl, 1968; Harrington and Merzenich, 1970; Knibestol and Vallbo, 1970), in cats (Iggo and Muir, 1969; Burgess, Petit and Warren, 1968; Mann and Straile, 1965; Tapper, 1965) and in rodents (Smith, 1967). A similar slowly adapting receptor has been described in reptilian skin (Kenton, Kruger and Woo, 1971).

a. Touch Domes: Morphology

The touch dome is an elevated corpuscle 50-300 μm in diameter, 25-75 μm high, which has a tylotrich hair sprouting from its center in rats (Smith, 1967) or sprouting from its side in cats, rabbits and primates (Iggo and Muir, 1969; Smith, 1967, 1970; Straile, 1960, 1961). It contains keratinocytes above a flat disc of 30-50 Merkel cells at the epidermal/dermal border. The nerve twigs lose their myelin sheath a few microns from the expanded nerve terminal of the Merkel cell (Iggo and Muir, 1969). Around the circumference of the touch dome there is a ring of epidermodermal invaginations that may serve to

isolate the touch dome from skin stretching type of stimuli (Iggo, 1976). In Figure 1, a touch dome taken from adult rat skin and stained with toluidine blue is shown. Figure 2 is a touch dome stained with hematolxylin and eosin in which the tylotrich hair, which protrudes from the center of the touch domes, can be seen.

b. <u>Touch Domes: Descriptive Physiology</u>

Receptors are preferentially excited by specific stimuli, the "adequate stimulus," as described by Sherrington (1906). Three main classes of cutaneous mechanoreceptors can be distinguished: position, velocity, and rapid transient detectors (vibration). Adaptation, the previously discussed decline in the discharge of a receptor with time to a constant stimulus, may vary within classes of receptors (Burgess and Perl, 1973).

Touch domes are sensitive to both position and velocity of indentation, having a slowly adapting discharge (Iggo and Muir, 1969). The steady discharge of the receptor to the maintained stimulus is enhanced at cooler temperatures (Iggo and Muir, 1969). This increased firing in respose to cooling, however, is not the same type of response that cold receptors exhibit to temperature drops. Touch domes have been described as "spurious" thermoreceptors (Iggo, 1963a). Cold receptors have a high frequency burst of firing that is sensitive to temperature changes (Hensel, 1973).

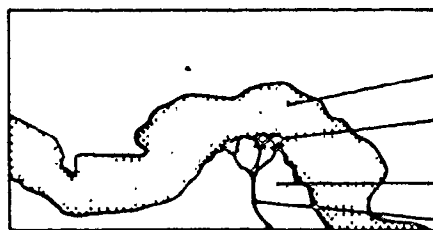
Touch domes are innervated by Type I afferent fibers with diameters in the A$\alpha$ range and are rapidly conducting (55-75 m/s in the cat; Iggo and Muir, 1969). The touch dome responds to a maintained

Figure 1A. Touch dome from the skin of a 200 gram adult rat, stained with toluidine blue.

Figure 1B. A diagram of touch dome structure.

1A

1B

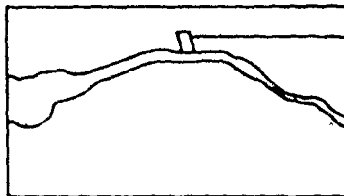



*not visible in photograph

Figure 2. Touch dome from a 2 week old rat pup, stained with hematoxylin and eosin.

2

30 $\mu m$



tylotrich hair

light touch with a characteristic "buzz" when monitored with an audio amplifier. This response represents a high frequency irregular pattern of discharge to the stimulus that levels off, or adapts, to a lower frequency irregular discharge that continues as long as the stimulus is maintained. Figure 3 is a typical response of a touch dome in the rat to prolonged stimulation. Figure 4 shows a touch dome which may be innervated by two axons in the rat. Smith (1967) also reported touch domes innervated by more than one axon in the rat. In the cat only one axon innervates a touch dome (Iggo and Muir, 1969).

Detailed descriptions of the receptor properties have been given by Iggo and Muir (1969), Tapper (1965) and Werner and Mountcastle (1965). Touch domes can be distinguished from other slowly adapting receptors in hairy skin by the irregular character of their maintained discharge which is designated as Type I. The Type II slowly adapting receptor in hairy skin has a very regular maintained discharge (Chambers et al., 1972) and may be structurally associated with the Ruffini ending (Chambers et al., 1972).

c. Input/Output Measurements

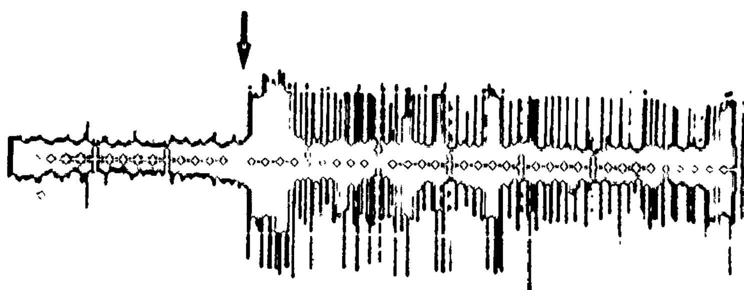
Type I receptors show an adapting position response with velocity sensitivity after a constant velocity displacement. Firing frequencies of such receptors are as high as 1000/s at high velocity stimulation. At low velocity, Type I receptors have linear frequency vs. displacement curves (Iggo and Muir, 1969), and at high velocity, frequency of firing is a power function of stimulation velocity (Tapper, 1965).

Figure 3. Typical response of an adult touch dome in the rat to prolonged mechanical stimulation recorded extracellularly from the dorsal cutaneous nerve. Prodder held in a micromanipulator. Stimulus begins at the arrow.

Figure 4. The response of a touch dome to prolonged mechanical stimulation recorded extracellularly from the dorsal cutaneous nerve. The touch dome may be innervated by two axons; note the difference in amplitude of the action potential of each unit.

3

4



20 μV

0.2 s

d. Spatial Directionality and Threshold of Touch Domes

Touch domes must be excited by direct contact with the dome; stretching the dome by pulling nearby skin does not provoke activity (Iggo and Muir, 1969). There is rarely a discharge in the absence of direct stimulation (Iggo and Muir, 1969; Smith, 1967). The tylotrich hair follicle is independently innervated from the touch dome; it is rapidly adapting, while the touch dome is slowly adapting (Brown and Iggo, 1967). Indeed, in the rat, dome firing is neither abolished nor even altered when the hair is removed (Leon, unpublished results), and the receptors do not respond to hair pushing or pulling (Iggo and Muir, 1969; Brown and Iggo, 1967). The threshold of touch domes in adult cats is 1-5 μm and usually consists of a single spike (Iggo and Muir, 1969).

e. Central Connections of Touch Domes

The central projections of the touch dome fibers in the spinal cord have been elegantly worked out by Brown, Rose and Snow (1978) using horseradish peroxidase as a cellular marker. Axon collaterals of touch dome fibers branch into lamina III, IV and the dorsal part of lamina V of the spinal cord. They form roughly ellipsoidal shaped arborizations in these lamina running 3-4 segments up and down from the level of entry. Evidence for the location of the ascending axons in the spinal cord is conflicting. Physiological evidence from the cat and rabbit indicates that ascending axons travel via the dorsal columns (Iggo and Ramsey, 1971; Brown, 1968; 1973; Uddenberg, 1968).

However, Petit and Burgess (1968) failed to antidromically activate slowly adapting Type I fibers in the sural nerve by stimulating the dorsal columns. If only a small proportion of Type I fibers in the sural nerve travel centrally in the dorsal columns, then it is possible to reconcile the results of Petit and Burgess (1968) with those of other workers (Iggo and Ramsey, 1971; Brown, 1968; 1973; Uddenberg, 1968). Although some of the physiological work on the dorsal column pathway of Type I fibers has been inferential (Iggo and Ramsey, 1971) since peripheral nerves were stimulated electrically, Brown (1968) has provided convincing evidence for the presence of Type I fibers in the dorsal columns. Brown (1968) stimulated identified touch domes mechanically and could record record responses in the dorsal columns.

Behavioral evidence from experiments using cats indicates touch dome axons may travel in the spinal cervical tract (Tapper and Van Scoy, 1967; Tapper, 1970), but Brown and Franz (1969) could elicit no response in the spinal cervical tract with physiological stimulation of touch domes.

Although there is not agreement in the literature as to the route the slowly adapting Type I fibers follow in the spinal cord, slowly adapting responses to cutaneous stimulation have been recorded electrophysiologically in the dorsal column nuclei (Dykes et al., 1982; Gordon and Jukes, 1964; Johnson, 1952; McComas, 1963; Kruger et al., 1971; Perl et al., 1962), thalamus (Dykes et al., 1981; Poggio and Mountcastle, 1963; Welker, 1973), and somatosensory cortex (Dykes et al., 1980; Dykes and Gabor, 1981; Mountcastle, 1947; Paul et al.,

1972; Rasmusson et al., 1979). Recent studies by Dykes and co-workers using multiunit recording in the dorsal column nuclei, thalamus and primary somatosensory cortex (Dykes et al., 1982; Dykes et al., 1981; Dykes et al., 1980; Dykes and Gabor, 1981; Rasmusson et al., 1979) have described neuronal segregation with regard to modality, depth of receptor, and rate of adaptation. However, discrimination of Type I slowly adapting responses (touch domes) from Type II slowly adapting responses is not possible with multiunit recording. No one thus far has systematically recorded central neuronal responses in the cortex, thalamus or dorsal column nuclei while stimulating identified touch domes mechanically.

Elegant studies of the central connections of an identified mechanoreceptor, the facial vibrissae of the rat, were carried out by Hand and co-workers (1977). When central neurons are active, they increase their uptake of glucose, and this feature of neurons has been examined using an analogue of glucose, radioactive 2-deoxyglucose ([$C^{14}$]-2DG) (Hand, 1981). After stimulating vibrissae singly and administering [$C^{14}$]-2DG, Hand and co-workers (1977) were able to localize peaks of radioactivity in the somatosensory cortex. The radioactive areas had a close correspondence to the functional cortical columns ('barrels') to which the vibrissae are known to project (Hand, 1981; Hand et al., 1979). This method of anatomical localization offers great potential as a research tool and has not yet been widely used in studying the central projections of touch domes.

f. Perception of Touch Dome Stimulation

In an awake human subject a slowly adapting touch receptor was stimulated but no subjective sensation was reported (Knibestol and Vållbo, 1970). Since this was in glabrous skin it is not clear whether the receptor was actually a touch dome since touch domes are typically found in hairy skin. Slowly adapting mechanoreceptors, consisting of a group of Merkel cells, have been reported in glabrous skin (Munger et al., 1971; Janig, 1971; Janig et al., 1968); however, it is unclear as to whether such structures were actually touch domes.

Although a conscious human subject reported "no specific sensation" after mechanical stimulation of a single touch dome in hairy skin (Harrington and Merzenich, 1970), Tapper and Van Scoy (1967) were able to condition the behavior of a cat using stimulation of a single touch dome. Recently, Jarvilehto and co-workers (1981) were able to record single unit activity from sensory nerves of conscious human subjects. They mechanically stimulated the hairy skin of the back of the hand and correlated conscious subjective evaluation of sensation with single unit nerve activity. They recorded responses from many Type I slowly adapting units with subjective and mechanical thresholds that were identical, although the reported sensation at subjective threshold was described as closer to itch than touch. At above threshold, the sensation described by subjects during stimulation of Type I slowly adapting units was touch. They concluded that in hairy skin, slowly adapting units are involved in the coding of sensation threshold (Jarvilehto et al., 1981).

g. Appearance of Touch Domes

In the present investigation, touch domes were chosen for study because they are discrete, easily identified, and single receptors can be marked on the skin *in vivo*. Receptors are visible on the rat's back after dipilatory has been applied to the skin (see Figure 5). Furthermore, the mechanosensory threshold of the identified receptor can be determined repeatedly with accuracy. Figure 6 shows oscillograph tracings of the threshold response of a touch dome, with the characteristic fluctuation in latency and action potential failures.

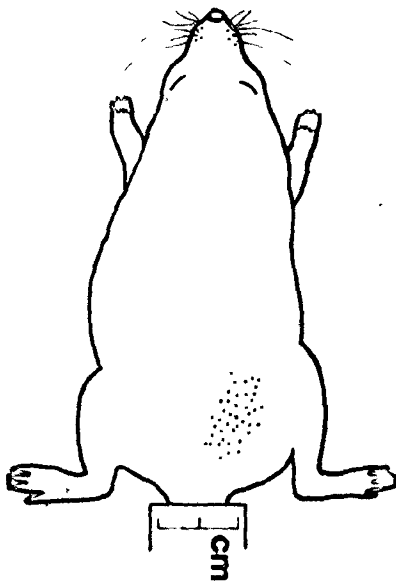
1. Merkel Cells: Specialized Cells Within the Touch Dome

Adult rat touch domes contain approximately 25 Merkel cells closely apposed to the neuronal terminal (Smith, 1967), although recent work (Diamond et al., 1981) suggests that adult touch domes may contain at least twice that number of Merkel cells. Merkel cells are morphologically specialized cells which differ from other epithelial cells and are found at the base of a touch dome. They are closely applied to the terminal expansions of sensory nerve fibers (see Figure 1b). Since it is probable that Merkel cells play an important role in the function of the touch dome as a mechano-electric transducer, various aspects of these cells will now be considered.

a. Distribution

Merkel (1875) first described "tastezellen," touch cells, in osmium-fixed skin specimens of goose skin, and then in the skin of dogs, oxen, pigs and man. Later authors (Bocke, 1932; Kadanoff, 1928)

Figure 5. Appearance of touch domes in the back of a 3 week old rat pup after depilation.


cm

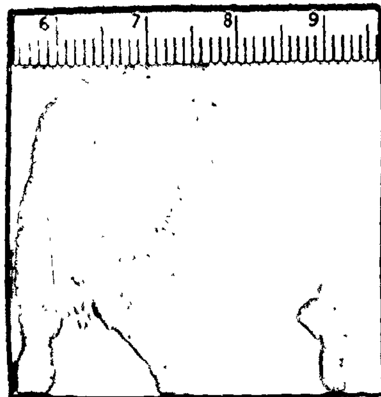
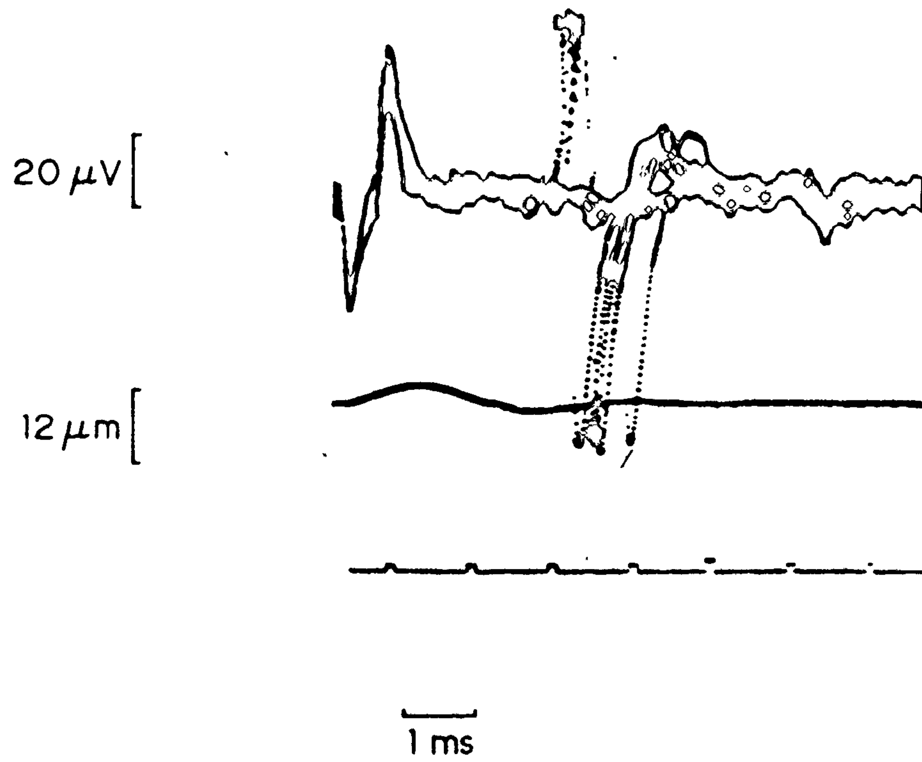

6
7
8
9

Figure 6. An adult rat touch dome response at threshold (2.7 μm) recorded extracellularly from a dorsal cutaneous nerve. Note the fluctuation in latency at threshold and occasional (5/10) failure to fire at threshold. Top trace: 20μV/Division. Middle trace: 12μm/Division. Bottom trace: 1ms/Division. 10 sweeps.


20 μV
12 μm
1 ms

used neurofibrillary stains to visualize Merkel "discs", the expanded nerve terminal closely apposed to the Merkel cell in the epidermis. Merkel cells are found in the skin of nearly all vertebrates that have been studied (Winkelman and Breathnach, 1973). Merkel cell receptor structures are numerous in glabrous skin of the snout of both opossum (Munger, 1965) and mole (Eimers organ) (Quilliam, 1966). Clusters of Merkel disc endings also have been described in rat vibrissal follicles (Patrizi and Munger, 1966). Merkel cells are found in the glabrous skin of finger and toe pads in humans, rodents and felines (Hashimoto, 1972; Kurosumi and Suzuki, 1971; Janig, 1971, Janig et al., 1968). Merkel cells are also found in reptiles, amphibians (Parducz et al., 1977; Fox and Whitear, 1978) and fish (Lane and Whitear, 1977). In amphibians, single Merkel cells are rapidly adapting touch receptors (Parducz et al., 1977).

Recent work by Bock (1980), Crowe and Whitear (1978) and Nurse, Mearow, Visheau, Holmes and Diamond (1981), demonstrating the fluorescence of Merkel cells after treatment with quinacrine, has lent support to the classification of Merkel cells as paraneurons (Fujita and Kobayashi, 1979; Fujita, 1976; Winkleman, 1977). All paraneurons are cells that contain membrane-bound granules, are suspected to be secretory and are derived from ectoderm. Glomus cells of the carotid body, chromaffin cells of the adrenal medulla and some secretory cells of the gut which also exhibit quinacrine fluorescence are considered to be paraneurons. Merkel cells are considered to fit some of the criteria of paraneurons (Winkelman, 1977).

b. Embryological Origin

There is some controversy about the embryological origin of Merkel cells. English (1980) considered Merkel cells to be derived from keratinocytes, epithelial cells of ectodermal origin, following electron microscopical examination of fetal and newborn rat skin. She made this claim on the basis of the existence of transitional cells which have features common to keratinocytes and Merkel cells. It is also possible that transitional cells are de-differentating Merkel cells and Merkel cells are not modified keratinocytes (English, 1977b).

Tweedle (1978) tested the possibility that Merkel cells were not ectodermal in origin but were derived from neural crest cells that migrated to epidermis. After he removed neural crest cells from the differentiating salamander larvae, however, the larvae epidermis still contained many Merkel cells, indicating that Merkel cells are not of neural crest origin.

Breathnach and Robbins (1970) claimed that the existence of dermal Merkel cells in electron micrographs from the human fetus may indicate a mesenchymal origin. However, direction of migration cannot be derived from electron micrographs. Avian Grandry cells, which resemble Merkel cells but are present in the dermis and have dense core vesicles scattered throughout their cytoplasm, are assumed to be of neural crest origin, on the basis of evidence from electron micrographs (Ide and Munger, 1978). Aside from Tweedle's salamander work (1978), the evidence for either neural crest or ectodermal origin of Merkel cells is based solely on electron microscopical

work. More experimental work is needed in the mammalian system and in the avian embryo before the issue is finally resolved.

c. Structure of Merkel Cells

When examined ultrastructurally, the Merkel cells have large polylobulated nuclei, osmophilic dense core vesicles and rod or finger-like extensions penetrating invaginations of the overlying epidermal cells (Andres, 1966; Iggo and Muir, 1969; English, 1977b). The Merkel cells contain dense core vesicles with a thickening of the cell membranes along the nerve/Merkel cell borders (English et al., 1980; Smith, 1970). This structural arrangement is suggestive of a synapse, the Merkel cell being pre-synaptic (Andres, 1966; Iggo and Muir, 1969).

Electron micrographs show that synapse-like thickenings with membrane-bound dense core vesicles near the thickenings do occur on Merkel cells and adjacent nerve terminals (Andres, 1966; Saxod, 1978; Fox and Whitear, 1978). Identification of the substance contained in the membrane-bound dense core granules of the Merkel cells, though, has been unsuccessful. For example, the substance does not exhibit endogenous catecholamine fluorescence after treatment with formaldehyde by the Falck method (Winkelmann, 1977; Smith, 1967; Smith and Creech, 1967). Likewise, reserpine, which acts to deplete catecholamines, did not deplete the granules of the Merkel cells or have a physiological effect on the mechanical response of touch domes (Smith and Creech, 1967).

Fox and Whitear (1978) observed, however, that Merkel cell granules are similar to granules of certain autonomic nerves which

contain ATP. Burnstock (1972) discussed the possibility that some nerves in the autonomic nervous system may use ATP as a neurotransmitter. The basis of quinacrine fluorescence is not known, but possibly it involves binding of purinergic compounds like ATP (Irvin and Irvin, 1954). Quinacrine causes Merkel cells to fluoresce (Crowe and Whitear, 1978; Bock, 1980; Nurse et al., 1981), and nucleoside triphosphatase occurs in nerve terminals on Merkel cells in mammals (Winkelmann, 1977). It is, therefore, possible that the fluorescence seen after quinacrine treatment originates from the Merkel dense core vesicles containing ATP (Crowe and Whitear, 1978), and that ATP alone or with some other substance may be released by the Merkel cell during neural transduction or as a trophic substance.

d. The Role of Merkel Cells in Transduction

The precise role the Merkel cell plays in sensory transduction in the touch dome or in other touch receptor structures has not as yet been elucidated. It is not known, for example, whether the Merkel cell releases some chemical substance from its dense core granules, depolarizing the adjoining neurite in response to stimulation.

The putative transmitter ATP remains an untested candidate for a sensory transducer substance in the touch dome. Several experiments could test this hypothesis. A combination of the use of tritiated adenine to form $H^3$ ATP and the subsequent use of microradioautography with quinacrine fluorescence in the same section could lead to cellular localization of the fluorescence and/or ATP. If fluorescence could be reliably attributed to the accumulation of ATP in the dense core

vesicles, then a combined study correlating measured changes in threshold with measured changes in fluorescence would provide indirect evidence that the Merkel cell plays a role in transduction chemically mediated by ATP. Alternatively, the effects of blocking ATP formation could be examined.

Many drugs have been applied to touch domes to test for a neurotransmitter in the Merkel cell-neurite complex. Smith and Creech (1967) tested numerous compounds topically to test for their effect on the touch dome response, but none evoked spontaneous activity. Nicotine alone enhanced the touch dome response briefly before depressing it, a response similar to that found in other mechanoreceptors (Gray, 1959).

Local anesthetics blocked the touch dome response without evoking any neural stimulation, probably simply blocking nerve conduction. Transient blockage of the receptor was achieved with $10^{-2}$ M potassium, calcium and magnesium. Obvious transmitter candidates like acetylcholine, epinephrine, norepinephrine, dopamine, serotonin, and gamma amino butyric acid, had no effect on touch domes. Bradykinin and histamine, compounds suspected to be involved in cutaneous pain responses, also had no effect on the domes (Smith and Creech, 1967).

Close arterial injection of suspected neurotransmitters into touch dome circulation may help to resolve the problem of possible chemical mediation of sensory transduction. Recently, Diamond and colleagues have succeeded in raising Merkel cells and neurons in culture (Mearow, Nurse, Visheau and Diamond, 1981). This preparation may lend itself to direct testing of chemical and other mechanisms of mechanosensory transduction. Alternatively, if measurement of synaptic delay

during sensory transduction could be made, it might help to clarify this issue. Unfortunately, generator potentials have not been recorded from the touch dome preparations as yet.

Electrophysiological evidence, however, indicated that the slowly adapting character of the touch dome response is dependent on the Merkel cells, for when the nerve supplying the dome is cut, the fibers lose their characteristic slowly adapting response (Brown and Iggo, 1963; Burgess and Horsch, 1973; Burgess et al., 1974). Thus, the regenerating axon tips of the cut nerves fire in response to touch, but without the normally slowly adapting characteristics. Regenerating axons cannot sustain this type of slowly adapting neural discharge until they make contact with the Merkel cells, again demonstrating the dependence of the neural response to touch on the Merkel cells.

As previously discussed, touch domes characteristically display an irregular sustained discharge and a Poisson-like distribution of impulse intervals to a sustained stimulus. This response pattern suggests a random process in the generation of the dome response, since such a response can be generated by the random firing of multiple, independent spike generators (Iggo and Muir, 1969; Horsch et al, 1974) with the Merkel nerve disc abutting each Merkel cell assumed to be the spike generator (Iggo, 1976).

An alternative to the chemical hypothesis of transduction is that the Merkel cell, together with the physical structure of the touch domes, constitutes a coupling device that efficiently transfers deformation of the skin to the neurite where the impulse is then initiated

(Smith, 1977; Gottschaldt and Vahle-Hinz, 1981). Recent work by Gottschaldt and Vahle-Hinz (1981) suggests that Merkel cells may serve as passive mechanical link for nerve fibers, with sensory transduction occurring in the fine nerve terminals. They base this conclusion on electrophysiological and electron microscopic evidence. In the cat vibrissae, 50% of the Merkel cells made no contact with nerve fibers. The contacts that are made appear to be more similar to desmosomes than synapses. Electrophysiological evidence indicated that the time course of the nerve response to vibratory stimuli is not consistent with chemical transduction mediated by Merkel cells.

e. Other Functions of Merkel Cells

Merkel cells may serve a trophic function by providing a target for growing nerve fibers during development. In the salamander, there is evidence that the Merkel cell is a target for ingrowing nerve fibers (Scott, Cooper and Diamond, 1981). Merkel cells are present in the chronically denervated salamander limb (Cooper et al., 1977; Scott, Cooper and Diamond, 1981) and are present in rat skin prior to innervation (English et al., 1980). Independent of any role the Merkel cell may play in transduction, the Merkel cell may enable the nerve ending to differentiate into a fully functional receptor. Another alternative function of the Merkel cell suggested by Cauna (1966) is that Merkel cells may be metabolic reservoirs for the maintenance of sustained nerve activity, possibly by releasing ATP.

### 3. Development of Cutaneous Innervation

Several questions arise from the observation that receptor structures in the skin form a characteristic arrangement and density in different parts of the body and in different species. During development does invasion by sensory nerves trigger the differentiation of epithelial tissue? Alternatively, does the presence of already formed receptors attract nerve fibers to specific sites in the epidermis? What control is there of innervation density after maturation? It appears, on the basis of anatomical and behavioral studies in fetal material, that cutaneous sensibility and reflexes are established before specialized cutaneous receptors are seen (cf. review by Bradley and Mistretta, 1975).

#### a. Development of Merkel Cells

The presence of nerve fibers in contact with the basement membrane of the epithelium of the face was reported at 9 weeks postconceptional age in human fetuses (Humphrey, 1966). Using light microscopy, Hogg (1941) did not observe Merkel corpuscles in the lip until 12 weeks postconceptional age. Merkel cells are, however, difficult to identify on the basis of light microscopy alone. More recent studies using electron microscopy of rat fetal snouts have indicated the presence of Merkel cells in the skin prior to innervation (English et al., 1980). Other workers have found Merkel cells present in chronically denervated limb (Cooper et al., 1977; Scott, Cooper and Diamond, 1981) and in aneural salamander larvae (Tweedle, 1978), suggesting that Merkel cells may develop independent of innervation.

b. Development of Touch Domes

Postnatal development of touch domes has been studied ultrastructurally by English (1977b) in the newborn rat; these studies indicated the presence of innervated Merkel cells adjacent to tylotrich hair follicles on the belly. Small touch domes were present on the belly at birth but were not found on the back until 14 days of age. Groups of Merkel cells appeared in the skin before the touch domes were elevated (English, 1977b).

However, Kasprzak (1970) found that, in kittens, touch domes appeared before Merkel cells could be detected histochemically. The differences between English's and Kasprzak's results may be due to a species difference, or possibly, to difficulty in identifying Merkel cells in the kittens with light microscopyy. PAS (periodic acid-Schiff) activity was used as the marker for Merkel cells in the kitten domes, but since it is unknown whether PAS activity of Merkel cells varies during development, the cells may have been present but not PAS-reactive. Indeed, there is no PAS activity detectable in the touch domes of both rats and humans even though there are many Merkel cells present (Smith, 1967; 1970). Alternatively, if the PAS reaction was in the Merkel discs of the nerve fibers rather than in the Merkel cells themselves, the stain would not have been a reliable marker for the appearance of the cells. It should also be noted that the PAS reaction is not specific for any one compound (Drury and Wallington, 1980).

Kasprzak et al. (1970) attempted to correlate the development of the slowly adapting response of cat touch domes to the presence of

the histological PAS reaction. They assumed that the presence of Merkel cells in touch domes would be associated with a positive PAS reaction. Although, as observed above, this assumption may not be true, Kasprzak and colleagues were able to correlate the development of PAS activity with the development of the slowly adapting response. They also found that the ability of dome fibers to follow 100 Hz stimulation at adult displacement thresholds was not present until 16 days postnatally. The number of Merkel cells stained by the PAS reaction also reached the adult level of 4 per cross section at 16 days postnatally.

In contrast to the above results, Ekholm (1967) found that touch domes did not fire at the adult frequency and duration for a given constant stimulus until 6 weeks of age in the kitten. The domes of newborn and 3 week old kittens fired at lower frequencies and for shorter durations than did adult touch domes subjected to similar pressure. When direct electrical stimulation of the skin was used kitten domes fired at lower frequencies than adult touch domes for the same stimulus, indicating that the nerve endings of the former were less capable of high frequency discharges. However, a longer duration of firing could be achieved with an electrical stimulus than with a mechanical one in the kitten dome.

Young rats have touch domes and as the rats grow, the skin area is increased manyfold. The dome density on the skin may either be kept constant by the development of new domes, or the number of domes may remain constant, decreasing the dome density. Kazprak et al. (1970) suggested that the latter possibility is the case in the kitten but

since dome innervaton is different in the rat (see page 22), dome density was examined in the present study during both development and ageing. Since there has been no developmental study of the mechanical thresholds of touch domes, this was also investigated. The combination of these two factors, touch dome density and threshold, would determine the sensitivity of the rat skin to tactile stimulation at any given age.

c. Reaction of Touch Domes to Denervation

In adult salamanders, single Merkel cells survive denervation (Cooper et al., 1977; Tweedle, 1978), although denervated Merkel cells have fewer dense core vesicles than normal (Tweedle, 1978). Touch domes in adult cats and opossums atrophy after denervation or nerve crush and show both a loss of Merkel cells and degranulation of the Merkel cells (Brown and Iggo, 1963; Burgess and Horsch, 1973; Burgess et al., 1974; English, 1974, 1977a; Palmer, 1965). In other studies, however, no atrophy or extensive losses of dense core granules in Merkel cells were reported after denervation in rats (Smith, 1967) or in cats (Hartschuh and Weihe, 1977). The discrepancy in the published reports may have been due to species differences, inappropriate statistical analysis, or sampling error in the ultrastructural preparation. Alternatively, it is possible that touch domes might not atrophy or disappear in adult rats, but might do so in immature animals. In another type of mammalian mechanoreceptor, the muscle spindle, there is a critical period in development when sensory denervation causes de-differentiation and

even disintegration of the spindle. After this period is passed (14 days of age), denervation no longer has catastrophic effects on the spindle (Zelena, 1975).

Acute denervation studies also suggest that neural influences may play a role in the maintenance of the structural integrity of the Merkel cell of the touch dome. It was found that the threshold to light touch in rat touch domes increased 24-28 hours after denervation, and touch domes failed to respond to mechanical or electrical stimulation as early as 28 hours post-denervation even though axons were still excitable (see Appendix 4). Early changes in the structure of Merkel cell granules were reported 24 hours after denervation in the Merkel cells of the snout in the opossum (Palmer, 1965). There is no parallel ultrastructural study of early denervation changes in rat touch domes.

Other sense organs are dependent on nerve supply as well. In newt tadpoles the lateral line nerve must innervate the lateral line system for long term survival of the latter (Speidel, 1948; Wright, 1951), and in adult newts innervation by the dorsal root ganglion cell is necessary for the survival of the lateral line system (Jones and Singer, 1969). Taste buds atrophy and disappear after denervation (Zalewski, 1973).

4. Ageing of Sensory Receptors

A number of studies have shown that, in young animals, the development of neural systems may be greatly affected by sensory experience (cf. review by Mistretta and Bradley, 1978). However, there is also evidence of neuronal plasticity in older animals that have

been subjected to a changed experimental milieu. For example, Green, Schlumpf and Greenough (1981) showed that when aged rats (15 months old) were exposed to an enriched environment they developed a greater dendritic density in layer III and IV cells of the visual cortex than unenriched rats of the same age. Similar findings regarding increased cortical thickness were reported by O'Connor et al. (1980) and Cummins et al. (1973) using enriched environments and aged animals.

During ageing there are CNS changes which are manifested by a decrease in cortical thickness both in man (Brody, 1955) and in laboratory animals (Brizzee et al., 1968). Whether this decrease is due to neuronal death (Brody, 1955; 1973; Critchley, 1942; Johnson and Erner, 1972; Vernadakis, 1973a), glial changes (Brizzee et al., 1968) or changes in aspects of the dendritic tree (Bondareff and Geinisman, 1976; Feldman and Dowd, 1974) is not known and many ageing mechanisms, some pathological, may be responsible for cortical shrinkage. One possibility, however, is that decreased activity from the periphery is a relevant factor in central nervous system ageing by limiting sensory input to the brain. Perhaps the window on the environment provided by the peripheral receptors in aged animals is different from that of adults. This possibility was tested by measuring the densities, thresholds and firing patterns of individual touch domes in the present study.

## 5. The Effect of Vincristine on a Sensory System

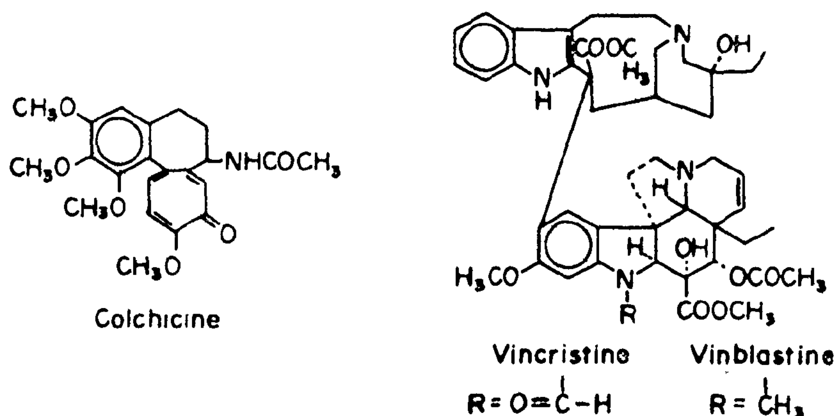
Having established certain features of touch dome density and threshold in the life cycle of the normal animal, I then examined what happens to touch sensitivity mediated by touch domes when a neurotoxic

drug, vincristine sulfate, is administered. Vincristine is one of the most effective antimitotic drugs available to clinicians and is widely used for the treatment of malignancies (Calabresi and Parks, 1980).

### a. Structure and Biological Action of Vincristine and Related Compounds

Two of the alkaloids isolated from Vinca rosea, the periwinkle plant, vincristine and vinblastine, are very similar structurally. In the diagram below it can be seen that vincristine and vinblastine differ in structure only in respect to one locus (Calabresi and Parks, 1980). Colchicine, an alkaloid obtained from Colchicum autumnale, the autumn crocus, differs greatly in structure from vinblastine and vincristine (Flower et al., 1980).

Molecular structure of Vincristine, Vinblastine and Colchicine



The three compounds, however, are quite simlar in their biological action, namely the ability to produce metaphase arrest during mitosis of cells (Creasey, 1975). The metaphase arrest is produced by the dissolution of the mitotic spindle and consequent dispersal of the chromosomes (Sentein, 1964).

b. Interaction of Vincristine and Related Compounds with Microtubules

Vincristine, vinblastine and colchicine all have been shown to cause the disappearance of microtubules from treated cells (Malawista and Bensch, 1967; Robbins and Gonatas, 1964) but only the *Vinca* alkaloids form cytoplasmic inclusions described as microtubule crystals (Bensch and Malawista, 1969; Marantz and Shelanski, 1970). The interaction of the anti-mitotic drugs with the microtubules present in the mitotic spindle apparatus is believed to be the basis for the observed dissolution of the mitotic spindle (Creasy, 1975).

c. Neurological Effects of Vincristine and Related Compounds

A significant effect of the anti-mitotic drugs on nerves is the inhibition of both fast and slow axoplasmic transport in nerve fibers. Colchicine interrupts fast and slow axoplasmic transport in mammalian nerves (Karlsson and Sjostrand, 1969; Komiya and Kurokawa, 1980), and in cat sciatic nerve vincristine and vinblastine interrupt fast axoplasmic transport (Ochs and Worth, 1975).

In addition to interrupting axoplasmic flow, the anti-mitotic drugs have other neurotoxic effects. Axonal degeneration and demyelination were reported after direct infusion of colchicine into the

sciatic nerve sheath in the mouse (Angevine, 1957). After a course of clinical treatment with vincristine or vinblastine, nerve biopsies from patients showed some demyelination (Gottschalk et al., 1968). After large doses of vincristine, the hind limbs of rats were paralyzed (Uy et al., 1967).

d. Vincristine Neurotoxicity

Since the development of a sensorimotor neuropathy is an invariable complication of vincristine treatment, and a limiting factor in the therapeutic use of vincristine (Calabresi and Parks, 1980), the study of the effect of vincristine on the touch domes has considerable clinical relevance. The clinical, pathological and EMG features of vincristine neuropathy have been well described. Vincristine produces a peripheral neuropathy characterized by numbness, a tingling sensation in the extremities, weakness, loss of reflexes, foot drop, ataxia, muscular cramps and neuritic pains (Tobin and Sandler, 1968; Sandler, Tobin and Henderson, 1969; Bradley, 1970; Casey et al., 1973).

The cellular action of vincristine both *in vivo* (Shelanski and Wisniewski, 1969; Schlaepfer, 1971) and *in vitro* (Wilson et al., 1974; Owellen et al., 1972) is to promote dissolution of axonal microtubules while encouraging proliferation of neurofilaments. In view of the suspected involvement of microtubules and neurofilaments in axoplasmic transport (Grafstein and Foreman, 1980), it was anticipated that transport would be affected by the administration of *Vinca* alkaloids. However, no interference was found in neurotoxic cats (Bradley and Williams, 1973), while slight and inconstant changes in fast axoplasmic

flow were noted in another study of neurotoxic cats (Green et al., 1977). In vitro blockage of fast axoplasmic transport by vincristine, however, has been reported (England et al., 1973; Ochs and Worth, 1975; Green et al., 1977).

McComas (unpublished observations) noted the onset of symptoms of a sensory neuropathy involving numbness and tingling sensations in the distal extremities in vincristine-treated patients when impulse conduction was normal in digital sensory axons. This observation raised the possibility of dysfunction in cutaneous mechanoreceptors or their associated nerve terminals. It was, therefore, of interest to follow the effect of vincristine administration on the threshold of a cutaneous mechanoreceptor and this was undertaken as part of this study.

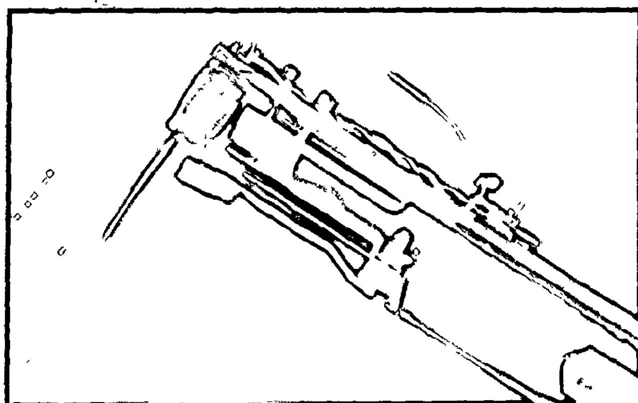
## II. METHODS

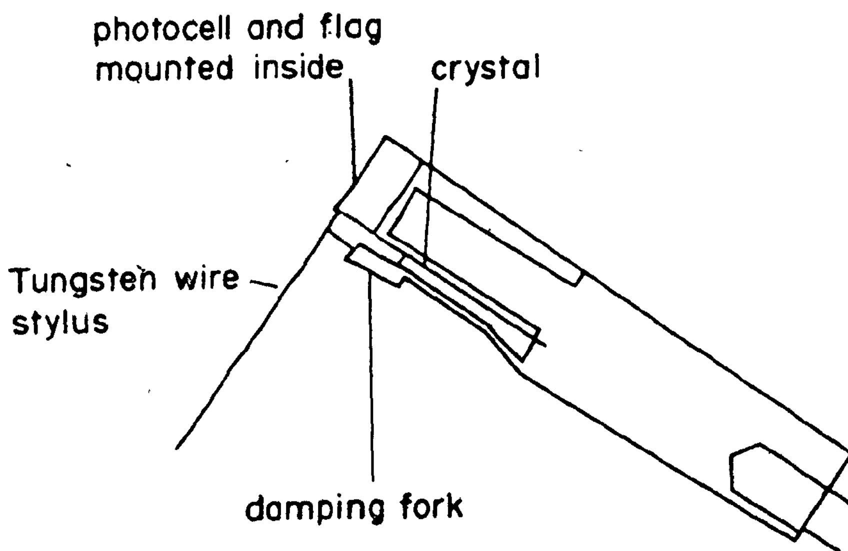
### A. Experimental Apparatus

#### 1. The Mechanical Prodder

The mechanical prodder consisted of a piezoceramic bender element mounted in a plastic holder. Figure 7 is a diagram of the prodder, with a photograph of the prodder on the right. The piezoceramic crystal (part R1025, Gulton Industries, Inc., Metuchen, New Jersey), 2.5 cm long by 0.6 cm wide, was used to convert an applied voltage to mechanical movement. The crystal was cantilever-mounted in a plastic holder. The holder could be attached to a micromanipulator (Eric Sobotka Co., Farmingdale, New York). The free end of the crystal was attached to a 1.0 cm x 0.5 cm x 0.1 cm rectangular aluminum sheet with a small aluminum tube at one end. The tube fitted tightly around the 2.5 cm long, 250 μm diameter, tungsten prodder stylus. Directly below the crystal and parallel to it was a 3.1 cm x 0.6 cm aluminum bar divided into two pieces; these ran on each side of the rectangular aluminum sheet and were separated from it by a gap of 1 mm. This gap could be filled with silicone grease for damping any oscillations of the crystal. A small aluminum sheet was fitted to the other side of the tube and served as a 'flag' for the photo coupled interrupter module (General Electric, model H13A1). The mechanical movement of the flag interrupted the electrical signal received from the photo cell; the modified signal was amplified and displayed on a dual beam storage oscilloscope (Tektronix model D13). The photocell output was calibrated by

Figure 7. Illustration of the mechanical prodder.




photocell and flag
mounted inside
crystal
Tungsten wire
stylus
damping fork

moving the prodder tip known distances with a micrometer held in a micromanipulator. Figure 8 is a graph of the photocell output (mV) resulting from a known movement of the prodder tip. The response of the photocell was linear and stable over time.

Voltage was applied to the crystal with a battery-powered stimulator (Devices Ltd. Isolated Stimulator MKIV) which was triggered by a digital timing unit (Devices Ltd. Digitimer type 3290). The digitimer also triggered a dual beam storage oscilloscope (Tektronix model D13). The photocell was powered by a 9 volt transistor battery.

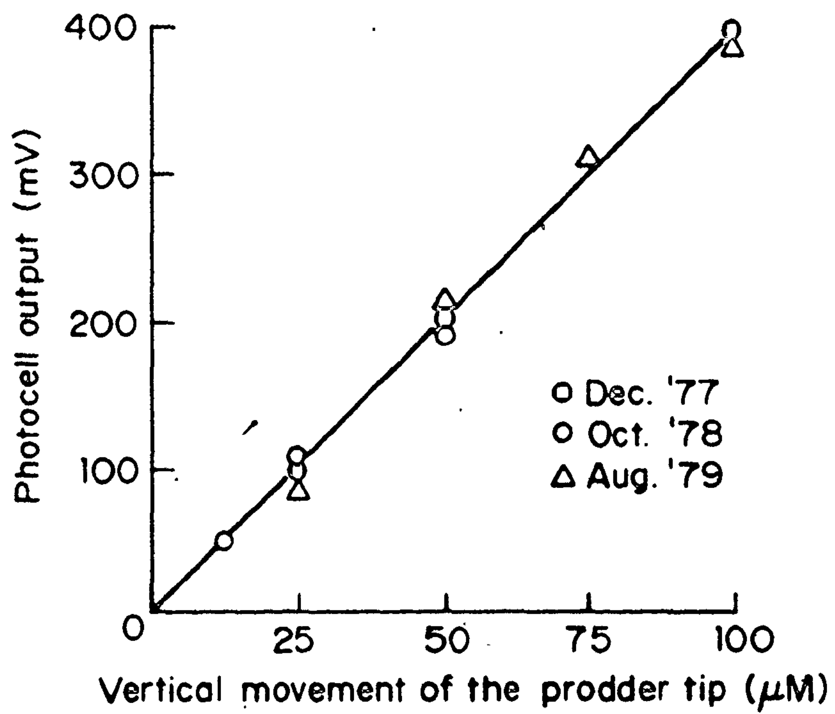
a. Characteristics of the Crystal

A square wave pulse of variable strength from 0-100 volt and of 1 millisecond duration was applied to the crystal. Figure 9 is an oscillograph trace of sample responses of the crystal to varying voltage. The time to peak remains 1 millisecond regardless of the strength of the voltage applied. All stimulus strength values used in this thesis were read directly from the on-line photocell trace during touch dome stimulation and are expressed in μm. Figure 10 is a graph of the vertical movement (μm) of the prodder tip with increasing stimulus strength (volts). The response of the crystal to increasing voltage was linear throughout the range of values used in these thesis experiments (2.5 μm-45 μm).

b. Electrical stimulation of touch domes

A dual high voltage electrical stimulator (Devices, Ltd. type 3072) was used to electrically stimulate touch domes. Brief electrical pulses, 50-200 microseconds in duration and 0-50 volts in amplitude, were applied to touch domes using a blunt 0.5mm diameter stainless steel pin.

Figure 8. Photocell output resulting from a known movement of the prodder tip.



400
300
200
100
0
25
50
75
100
Photocell output (mV)
Vertical movement of the prodder tip (μM)
Dec. '77
Oct. '78
Aug. '79

Figure 9. Sample responses of the crystal to applied voltages.

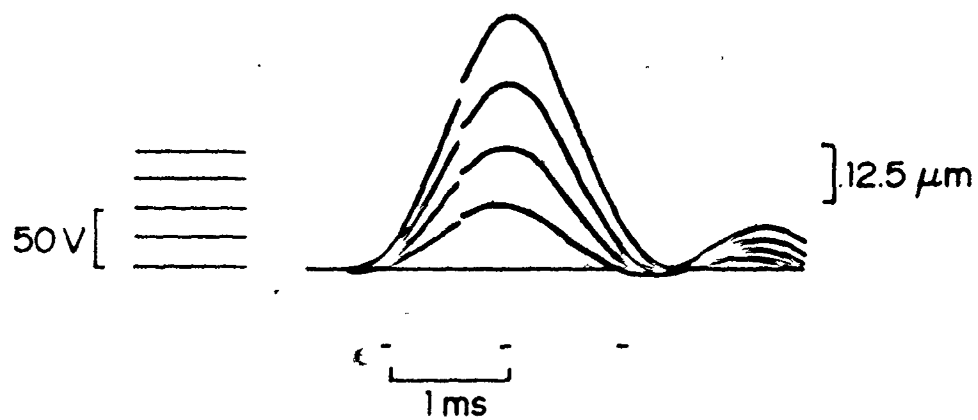
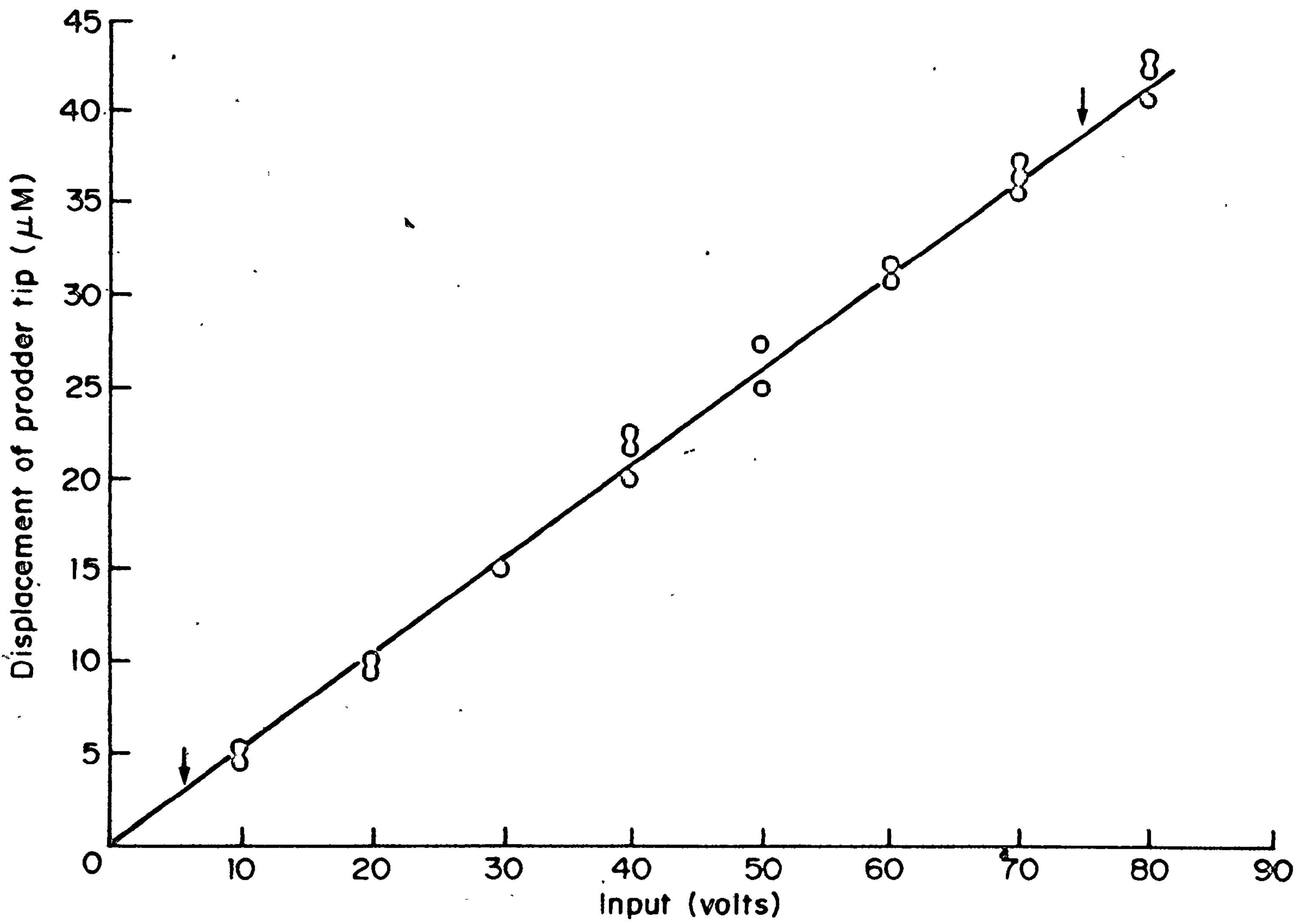
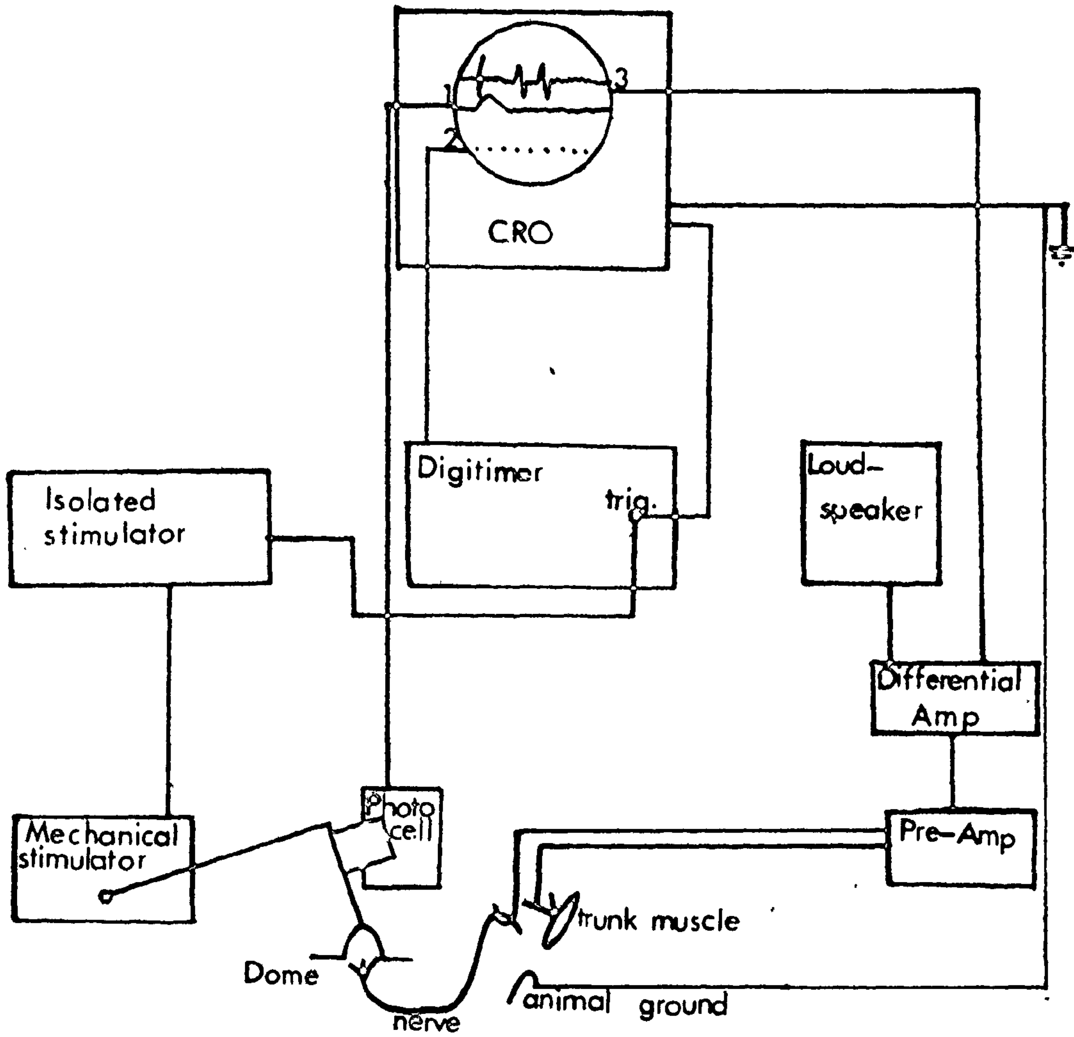

50 V
12.5 μm
1 ms

Figure 10. Displacement of the prodder stylus as a function of applied voltage.



Displacement of prodder tip (μM)
45
40
35
30
25
20
15
10
5
0
10
20
30
40
50
60
70
80
90
Input (volts)

Figure 11. Diagram of the recording set-up used to determine mechanical threshold.



CRO
1
2
3
Digitimer
trig.
Isolated stimulator
Loud-speaker
Differential Amp
Photo cell
Mechanical stimulator
Pre-Amp
trunk muscle
Dome
nerve
animal ground

2. Recording Apparatus

The recording set-up is represented diagramatically in Figure 11. The digital timing unit (Devices Ltd. Digitimer type 3290) triggered both the dual beam cathode ray storage oscilloscope (Tektronix D 13) and the isolated stimulator (Devices Ltd. MKIV) which was used to apply varying voltages to the prodder crystal. The photocell of the prodder was monitored on-line on the oscilloscope (trace 1 in diagram). The Digitimer provided a time signal, in the form of a ruler, which was displayed on a second trace (trace 2 in diagram). Touch domes were mechanically stimulated by the prodder, and the nerve branch innervating a dome was lifted onto a single platinum wire electrode (250 µm diameter) and covered with a drop of petroleum jelly (Vaseline). The reference electrode, a small alligator clip, was attached to the dorsal trunk muscles of the rat and a ground electrode, a similar clip, was inserted subcutaneously. The nerve signal was passed through a preamplifier (Grass P15 AC amplifier) and a Tektronix 5A22N differential plug-in amplifier (bandwidth 100 Hz-15 KHz). The signal was monitored on the oscilloscope (trace 3 in diagram) and also by an audio loudspeaker. The characteristic 'buzz' of a touch dome after prolonged stimulation was broadcast through the audio loudspeaker and aided in identification of touch domes.

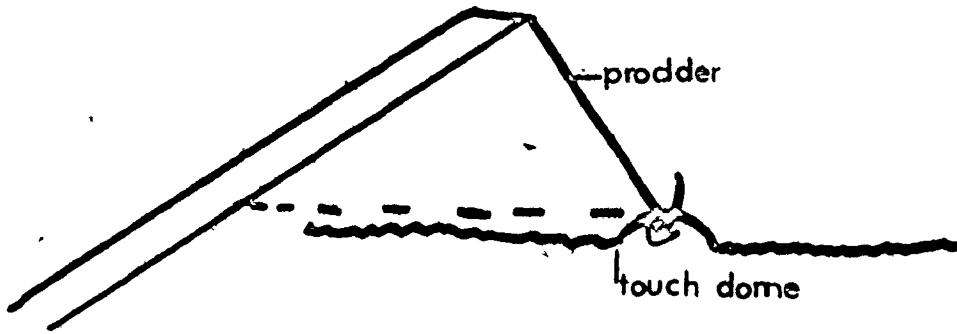
B. Threshold Measurements

In order to measure the threshold of a touch dome, the mechanical prodder had to be aligned properly. The position of the prodder was adjusted until an imaginary line joining the stylus tip to the

fulcrum of the crystal was parallel to the surface of the skin; this arrangement ensured that the displacement of the stylus was vertical in relation to the convex surface of the dome (Figure 12). An initial indentation of 0.07-2.00 µm was made (observable as a shift in the calibrated photocell baseline) and then the touch dome was tested with mechanical stimuli of increasing strength until threshold was determined. The threshold of a touch dome was defined as the smallest displacement capable of initiating an impulse in at least 5 out of 10 trials. Stimulus strength was then increased to up to four times threshold to record any additional response. In every case, the amplitude and wave form of the mechanical movement were monitored on-line with the photoelectric cell (see above). The latency of a nerve fiber impulse from the beginning of the mechanical stimulus was noted, as well as the number of impulses evoked by a single mechanical pulse. Usually no more than one or two impulses were evoked. Threshold measurements were repeatable; often the threshold of a single touch dome was measured three or four times during an experiment and values obtained were not significantly different.

The thresholds of touch domes reported by other workers were expressed in terms of amplitude (µm) (Aiken and Lal, 1982; Burgess et al., 1968; Iggo and Muir, 1969; Smith, 1967; Tapper, 1965). Threshold displacement of touch domes was independent of the rate of rise of the displacement (Tapper, 1965). This was an unusual finding, since after above-threshold stimuli were delivered, touch domes responded both to displacement amplitude and velocity (cf. Burgess and Perl, 1973).

Figure 12. The alignment of the prodder.


prodder
touch dome

Horch and Burgess (1976) addressed this problem specifically using touch domes in adult cats and confirmed that for brief, small amplitude stimuli near threshold touch domes responded only to amplitude. No mechanism was proposed by Horch and Burgess (1976) to explain the discrepancy in threshold and suprathreshold behavior of touch domes. In the following experiments, threshold was determined using a brief pulse with a fixed time to peak; therefore both velocity and amplitude of the pulse varied. However, amplitude, not velocity, was the relevant variable in determining the thresholds of touch domes, and the thresholds were therefore expressed in μm.

C. Dissection

All animals were anesthetized with sodium pentabarbital solution (Nembutal, Abbott Laboratories) injected intraperitoneally in a dose ratio of 0.039 mg/kg. The fur was clipped and the stumps removed with a depilatory (Neet). Touch domes were marked with India ink in the region extending from the 13th thoracic to the 5th lumbar dorsal cutaneous nerve fields and an incision was made to the right of the midline and parallel to it, in order to prevent inadvertent section of skin and subcutaneous nerve twigs within the area of study. In acute experiments, the appropriate dorsal cutaneous nerve was dissected free, tied with 6/0 surgical silk, and cut proximal to the tie. As much connective tissue as possible was removed from the nerve. The dorsal cutaneous nerve was left intact in experiments in which additional measurements were carried out at a late date.

## D. Histological Techniques

### 1. Fixation and processing of skin

After touch domes had been marked with a blue food coloring solution they were excised and placed on filter paper, dermis side down, and put into Bouin's solution (Drury and Wallington, 1980) for 6-12 hours. The specimens were taken through graded alcohols to dehydrate them, and were then cleared in toluene and embedded in Paraplast Plus. Alternatively, excised touch domes were quick-frozen in isopentane kept at -78°C in liquid nitrogen. Other excised touch domes were fixed in 2% glutaraldehyde, then postfixed in 1% osmic acid in cacodylate buffer, dehydrated and embedded in plastic.

Wax-embedded material was cut on the microtome at 6 µm. Frozen specimens were cut on the cryostat at 10 µm. Plastic-embedded sections were cut at 1 µm. Specimens embedded in wax or frozen sections were stained using hematoxylin and counterstained with eosin (Drury and Wallington, 1980). Plastic-embedded sections were stained with toluidine blue.

### 2. Fixation and processing of nerve

The dorsal cutaneous nerve was excised and fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer and post-fixed in 1% osmic acid in cacodylate buffer. Tissue was dehydrated through graded alcohols, cleared in propylene oxide and embedded in plastic. Sections were cut at 1 µm and stained with toluidine blue.

#### a. Counting of axons

Representative nerve cross sections of the dorsal cutaneous

nerves were photographed and printed. The number of large myelinated fibers in the cross-sections were counted from the photographs, the final enlargement (microscopic and photographic) being approximately 1300X.

b. Measurement of axon diameter

The photographs mentioned in the above section (2a) were used to trace the outline of the axon onto the $mm^2$ grid of the Zeiss MOP-3 Digital Analyzer. The digital analyzer was used to measure the maximum diameter of each nerve fiber including its myelin sheath.

E. Experiments

1. Experiment 1: The distribution of touch domes in the skin during development.

Sixty-three Wistar rats (47 females, 16 males; from Woodlyn Farms, Guelph, Ontario) of ages ranging from 11 days to 21 months were housed at 22°C with their mothers until 29 days of age. All rats had Purina Laboratory Chow (6 percent fat) and water available ad libitum Animals were kept in an environment having a 12:12 hour light/dark cycle, with the lights switched on at 0800 hour. The rats were anesthetized with pentabarbital (Nembutal, Abbott Laboratories), their hair was clipped, and touch domes were located visually by eye, without the aid of a dissecting microscope and marked with blue food coloring solution (McCormick). The touch dome map was then traced onto clear celluloid film and touch domes were counted after the map had been superimposed on a square centimeter grid. From 30 to 400 domes were identified on the back of each rat.

In addition to this, the diameters of 100 domes total were measured on ten live rats of various ages, using a dissecting microscope at 40X magnification. The resolution of this technique was ±10 μm.

2. Experiment 2: The development of touch dome density within the receptive field of a single peripheral nerve.

Ten female Wistar rats were obtained from Woodlyn Farms (Guelph, Ontario). Group one, consisting of six 23-26 day old rats, were anesthetized with sodium pentabarbital (Nembutal, Abbott Laboratories). Their hair was clipped and depilatory was applied to the skin to remove any remaining hair. In each rat touch domes in the region of the 13th thoracic to 5th lumbar dorsal cutaneous nerve fields were marked with a blue food coloring liquid (McCormick, Inc.). The medial branch of the T13 dorsal cutaneous nerve was dissected out, in continuity, and lifted onto a single 250 μm diameter platinum wire electrode; a reference electrode was clipped onto the muscle. Using a blunt 250 μm diameter tungsten wire, mounted in a holder and held by hand, all touch domes responding within the nerve field were located and counted. The characteristic 'buzz' of the touch domes in response to prolonged stimulation aided in identification of touch domes. The borders of the nerve field were marked with India ink and subsequently tattooed. The number of domes within the field were recounted at 40-43 days of age and again at 60 days of age, and the increase in the size of the field was recorded.

A similar procedure was followed on a second group of four 2.5 month old rats. In each animal the nerve field borders of the T13 medial

dorsal cutaneous nerve were tattooed and the number of touch domes within the tattoo marks were recorded at 2.5 months and then at 7.5 months of age, and the increase in the size of the field was recorded. In addition to this, nerve field areas and the numbers of touch domes in T13 medial nerve fields were noted in five rats of different ages.

3. Experiment 3: Spatial distribution of the touch domes.

The density of touch domes in the skin was determined as in experiment 1. A T-shaped grid of 1 $cm^2$ divisions was superimposed on the touch dome map, starting at the level of the last rib and at the midline. Touch dome densities of twelve square centimeters, six centimeters on each side of the midline, were averaged for the three rats (see Table 8B).

4. Experiment 4: Threshold of touch domes during development.

Touch dome responses were recorded extracellularly from fibres of the fourth and fifth lumbar dorsal cutaneous nerves of anesthetized rats, using a 250 μm diameter platinum wire electrode.

Seventy-eight domes on thirty-two rats were tested with a mechanical prodder constructed from a piezoceramic crystal mounted in a holder (see page 51). The movement of the prodder was monitored continuously by a photocell that was calibrated to detect movements of the stylus tip, a blunt tungsten wire 250 μm in diameter. The amplitude of the movement could thus be monitored with an accuracy of ±1 μm.

5. Experiment 5: The effect of suprathreshold stimuli on response latency.

Response latencies at threshold and at twice-threshold intensity were measured in seventeen adult rats, using thirty domes. Similar measurements were made in one nursing pup using five domes, and in six aged rats using eighteen domes. The stimulation and recording procedures were as in experiment 4.

6. Experiment 6: Touch dome densities in adult animals after administration of vincristine sulfate.

Each of six female Wistar rats (Woodlyn Farms, Guelph, Ontario), 2-3 months old, received one subcutaneous injection of vincristine sulfate Eli Lilly, Inc.), in a dose ratio of 0.75 mg/kg. Three similar control rats received injections of 2.0 cc saline subcutaneously. Three weeks later touch dome density counts were carried out on vincristine-treated and control animals, following the procedure outlined in Experiment 1.

7. Experiment 7: Threshold of touch domes after administration of vincristine sulfate.

Each of twenty-two 2-3 month-old rats were injected with 0.75 mg/kg vincristine sulfate subcutaneously. Groups of rats were sacrificed at 1, 7, 14, 21, 28 and 42 days post-injection and seventy-six dome thresholds were measured, following the method outlined in Experiment 4.

8. Experiment 8: The effect of suprathreshold stimuli on response latencies after the administration of vincristine sulfate.

Response latency at threshold and, if possible, at twice-threshold or at maximum stimulus strength was measured in twenty female rats 2-3 months old; sixty-one touch domes were investigated. There were six experimental groups, corresponding to 1, 7, 14, 21, 28 and 42 days post-injection. Stimulation and recording methods were as outlined in Experiment 4.

## III. EXPERIMENTS: Rationale and Results

### A. Experiment 1: The distribution of the touch domes in the skin during development.

#### 1. Rationale

Touch sensitivity should be mediated, at least in part, by the number of transduction structures responding to the tactile stimulus. Young rats have touch domes, and as they grow, dome density on the skin may either be kept constant by the development of new domes, or the number of domes may remain constant, decreasing the dome density during development. To discriminate between these two possibilities, domes in a standard unit of area were marked and counted on rats of different ages.

It is also possible that the size of individual domes changes during development. Therefore, the size of domes was measured over the course of development.

#### 2. Results

Table 3 and Figure 13 show that the number of touch domes per unit skin area decreases during growth. The reduction in touch dome density after weaning was found to be statistically significant (Students 't' test; $p < 0.005$) and the density continued to decrease with age. Figure 14 illustrates the dome density for each animal in relation to the developmental epochs of the animal's life. Table 4 (see Appendix 1) contains a summary of the data collected in this experiment.

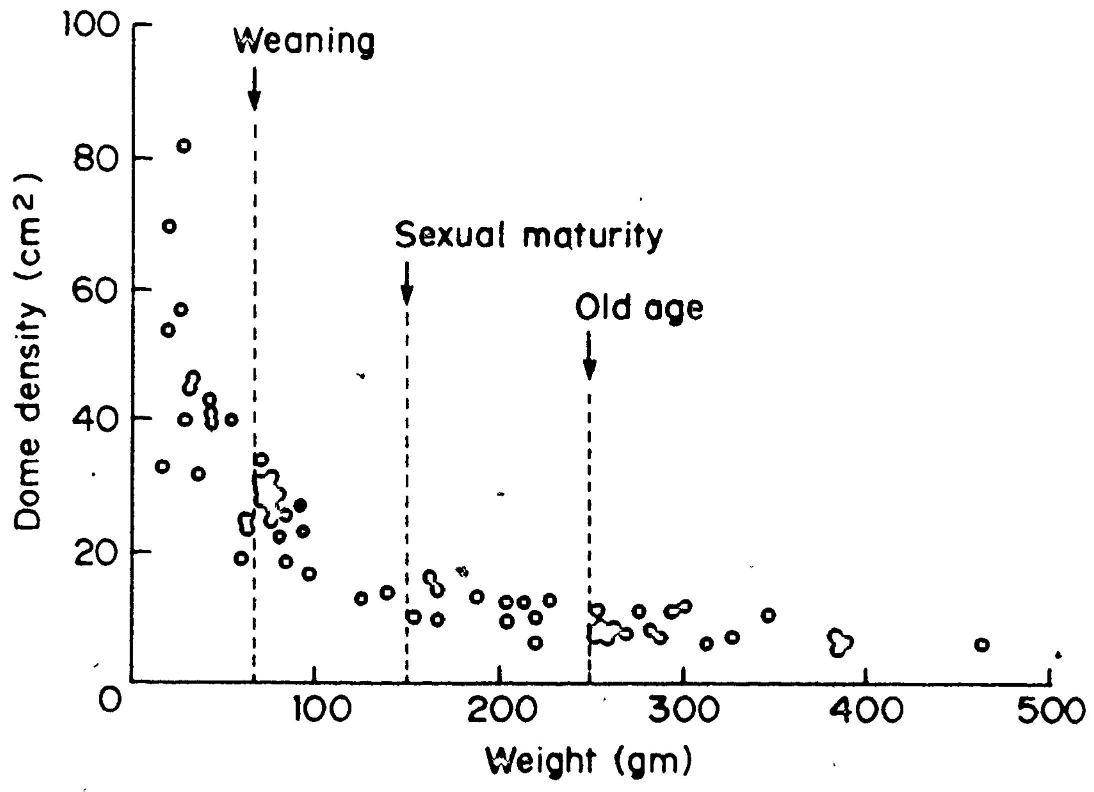
TABLE 3

TOUCH DOME DENSITY DURING DEVELOPMENT

| | Nursing Pups | Juvenile | Adult | Mature | Aged |
|---|---|---|---|---|---|
| Age | <29 days | 30-45 days | 45 days-6 months | 6 months-1 year | 1.5 years + over |
| Weight | <65 grams | 65-150 grams | 150-250 grams | 250-300 grams | 300 grams + over |
| $\bar{x} \pm$ sem | 43± 4 domes/cm$^2$ | 25± 1 domes/cm$^2$ | 12± 1 domes/cm$^2$ | 9± 1 domes/cm$^2$ | 8± 1 domes/cm$^2$ |
| | 16 rats | 18 rats | 12 rats | 9 rats | 8 rats |

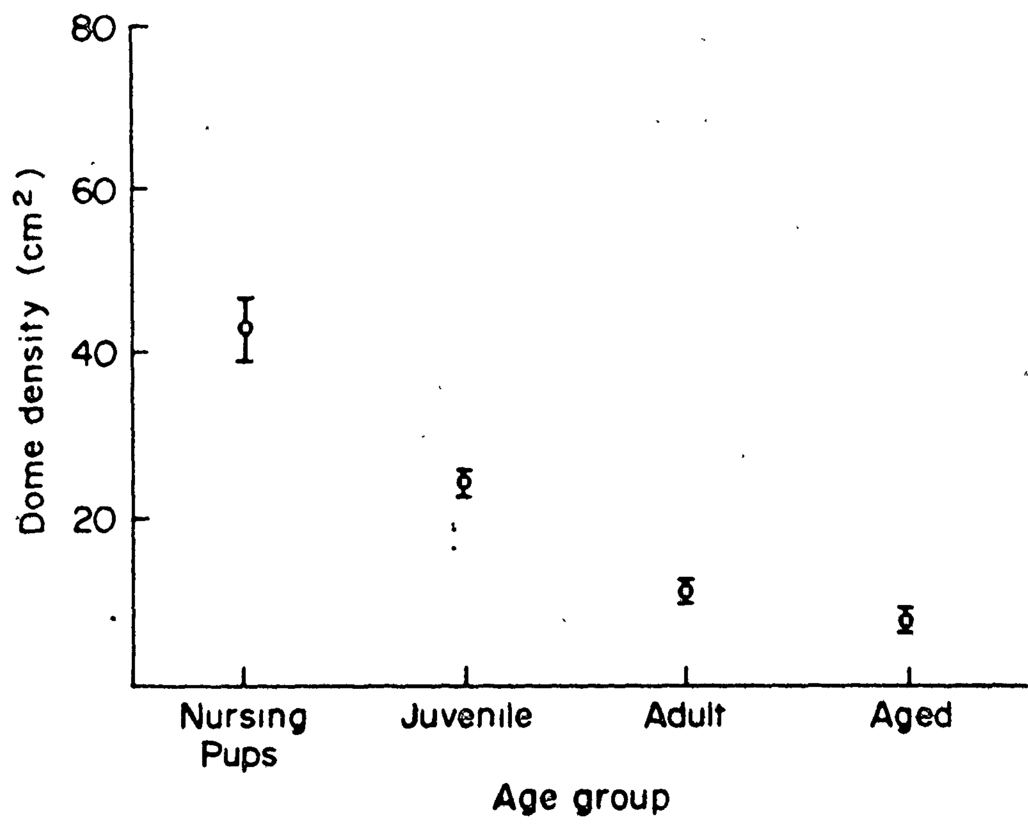
NOTE: Each group is significantly different from the next ($p < 0.0005$; $f < 0.005$) except the last two groups ($p < 0.10$).

Figure 13. Touch dome densities as a function of body weight.



100
80
60
40
20
0
Dome density (cm²)
Weaning
Sexual maturity
Old age
100
200
300
400
500
Weight (gm)

Figure 14. Touch dome densities during development. Pooled data for 63 animals.



80
60
40
20
Dome density (cm²)
Nursing
Pups
Juvenile
Adult
Aged
Age group

In addition to having the lowest dome density, adult animals had the largest domes. The mean diameter of the domes in the adult group was 50 percent larger than that of the juvenile group. These results are presented in Table 5. Table 6 (see Appendix 1) contains the data from this experiment, and the results are illustrated in Figure 15.

Approximately 1 $mm^2$ of skin is covered by touch domes within 1 $cm^2$ of skin from youth through adulthood. In the aged animal the area of skin covered by touch domes drops to approximately one-half of the "dome" mechanosensitive area of the adult, to 0.5 $mm^2$ within 1 $cm^2$ of skin. Although the number of transduction structures within a given area of skin declines during development, the size of the surface area of skin covered by those structures is maintained, chiefly by the growth in size of the receptors. The net result is that the "dome" mechanosensitive area within a given area of skin remains fairly constant through adulthood. In old age, however, the area available for tactile stimulation through domes is reduced by half (see Appendix 1, Table 7 for this calculation).

However, unless punctate stimuli are used, the size of a dome is unimportant in determining touch sensitivity; rather it is the spacing between domes which is critical. In B, only one dome is stimulated

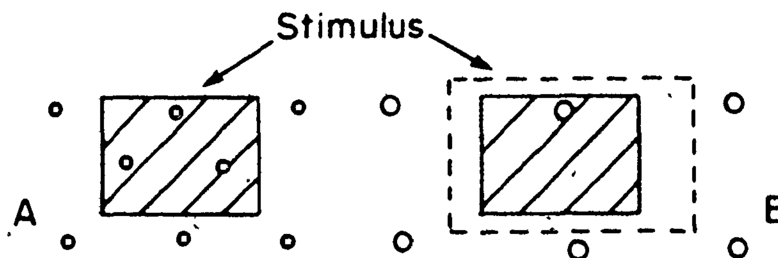
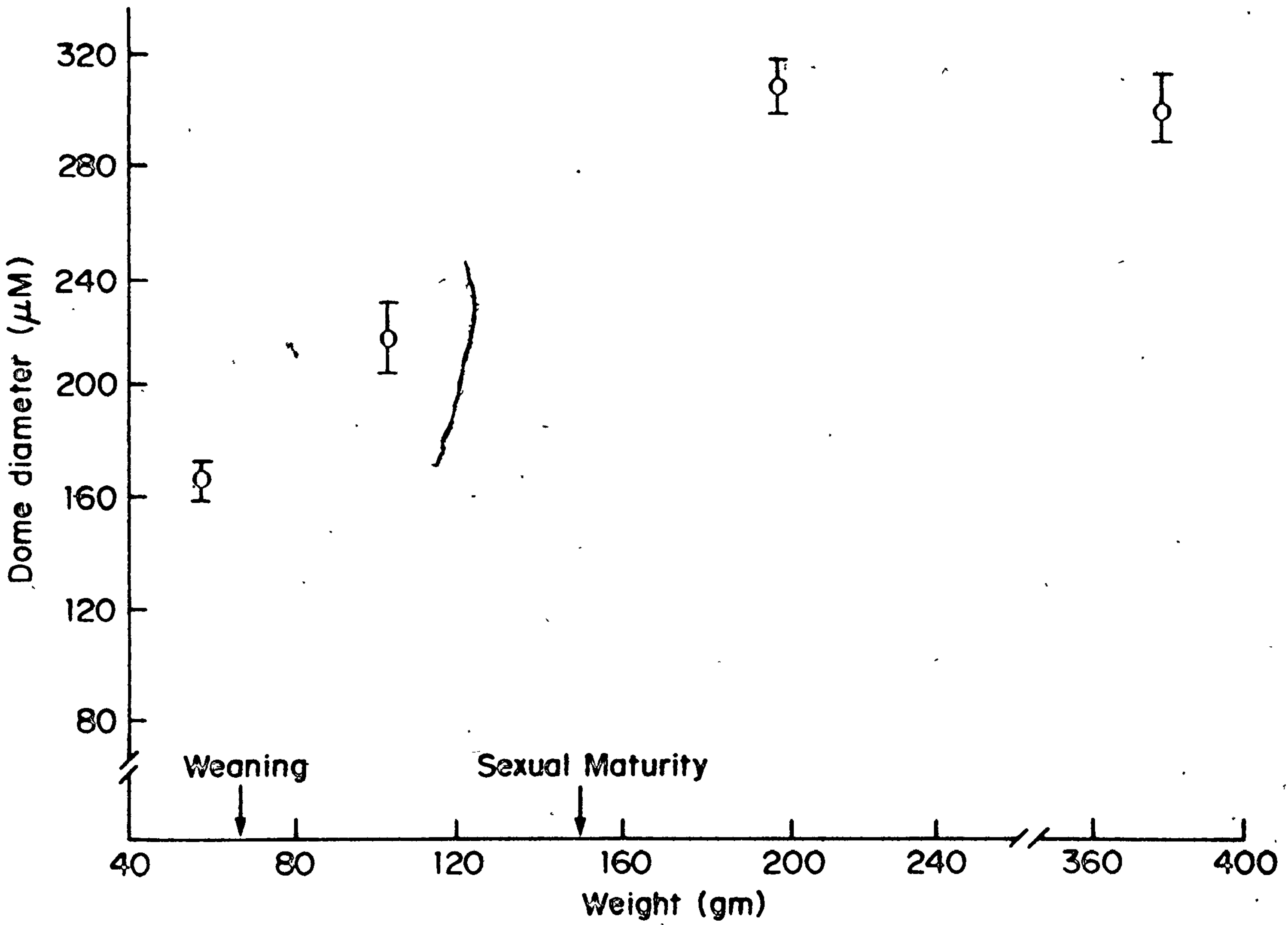


TABLE 5

TOUCH DOME SIZE DURING DEVELOPMENT

| | Nursing Pups | Juvenile | Adult | Aged |
|---|---|---|---|---|
| Age | <29 days | 30-45 days | 45 days-6 months | over 1.5 years |
| Weight | <65 grams | 65-150 grams | 150-250 grams | over 350 grams |
| Dome Diam. ($\mu m$; $\bar{X} \pm$ sem) | 170 ±8 | 219 ±12 | 314 ±9 | 302 ±12 |
| # of Domes | 20 | 21 | 39 | 20 |
| # of Rats | 2 | 2 | 4 | 2 |
| | $p < 0.005$ | $p < 0.005$ | N.S. | |

Figure 15. Touch dome diameters during development; values are means ± s.e.m.



Dome diameter (μM)
320
280
240
200
160
120
80
Weaning
Sexual Maturity
40
80
120
160
200
240
360
400
Weight (gm)

despite the domes being larger than A. The nervous system cannot know whether the stimulus is as small as a single dome or as large as the interrupted area in B. In A, however, because three domes are stimulated and their neighbors are not, much more precise information is given to the CNS. Indeed, Phillips and Johnson (1981) reported that the spacing between the monkey slowly adapting receptors in glabrous skin probably determines the resolution of fine spatial detail (two point discrimination). Thus, the increase in sizes of domes during development cannot compensate for reduced densities.

B. Experiment 2: The development of touch dome density within the receptive field of a single peripheral nerve.

1. Rationale

Given a constant number of domes, skin growth could account entirely for the decrease in dome density in developing rats, but it is also possible that the absolute number of domes decreases as well, particularly when the animals have reached adulthood. It was therefore necessary to determine whether the absolute number of domes declined during rat development. To that end, the size of the skin receptive field of an identified peripheral nerve was measured in 23-26 day old rats. In each rat the number of domes within the receptive field was then counted and the borders of the field were marked. The number of domes in that same receptive field was again counted at 40-43 days and at 60 days of age to observe changes in young, rapidly growing animals. Other rats were examined at 80 days and again at 7.5 months to determine

whether the number of domes in a sensory field changed in slowly growing adults and mature animals.

2. Results

The area of the nerve fields increased rapidly from day 20 to day 60, a period in which body growth was rapid. When body growth slowed down after 80 days of age, the sensory field size increased slowly. Moreover, the number of domes in each nerve field remained stable over early growth and in adulthood. These results are illustrated in Figures 16 and 17. The values for each animal are given in Tables 8, 9, and 10 of Appendix 1 and in Table 8A. There was no statistically significant change in the number of domes in the nerve field between 20 days, 40 days and 60 days of age when the values were compared with an F-test using an analysis of variance. The results of the analysis of variance can be seen in Table 11 of Appendix 1. Between 80 days of age and 7.5 months of age there was again no statistically significant difference in the number of touch domes in the T13m nerve field.

These data indicated that the growth of the rat spread a constant number of domes over the surface area of the animal, even within the sensory field of a single nerve, until the rat became aged. There was a suggestion in Figure 17 that the T13m field size did not continue to increase as rats continue to gain weight past 7.5 months of age; this was confirmed statistically, there being no significant difference between the mean field sizes at 7.5 months and 1.5 years of age. This finding implied that female rats added skin over the ventral but not

Figure 16. Size of the skin receptive field of T13M nerve during development; values are means ± s.e.m.; data pooled from 13 animals.

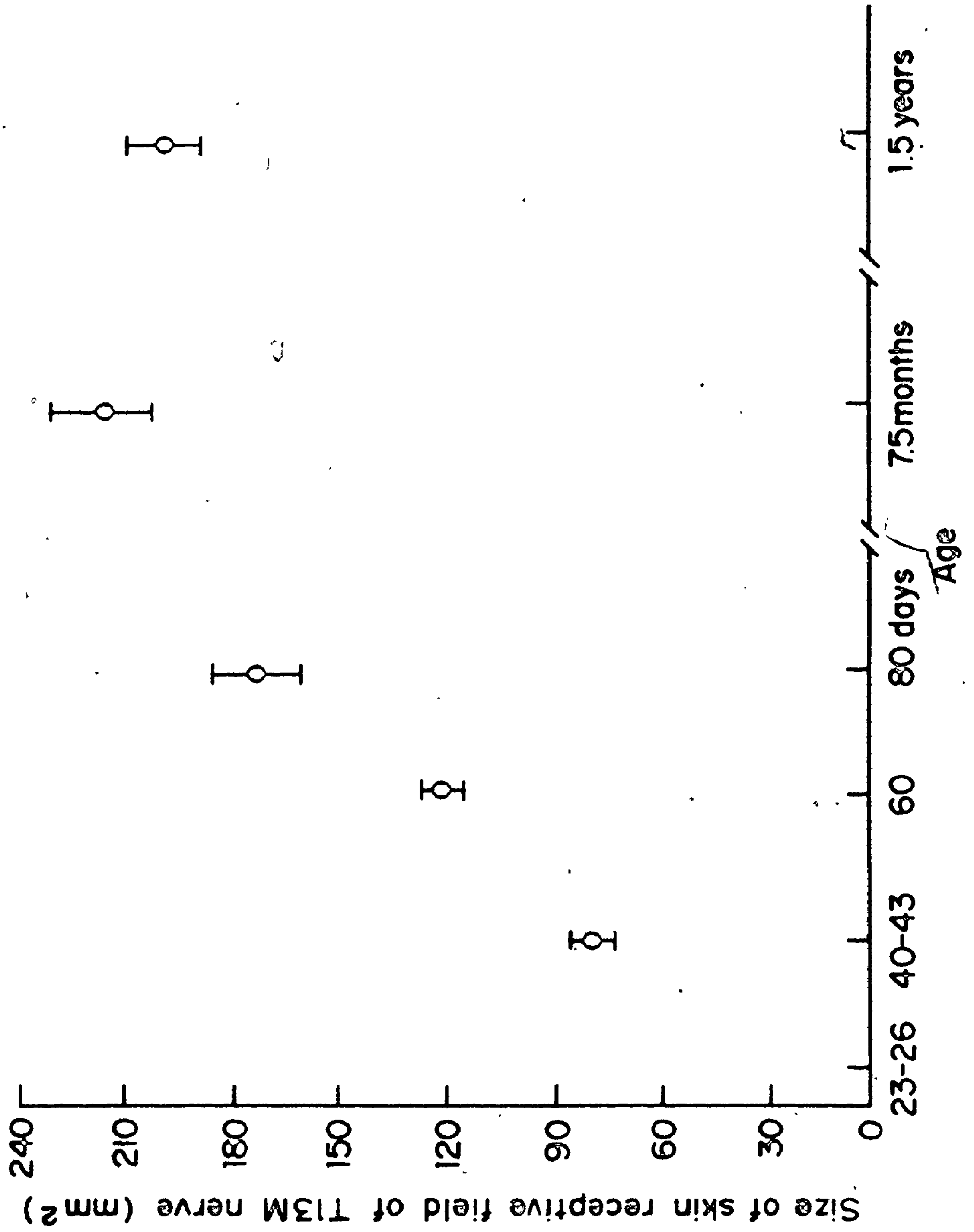

Size of skin receptive field of T13M nerve (mm²)
240
210
180
150
120
90
60
30
0
23-26
40-43
60
80 days
7.5 months
1.5 years
Age

Figure 17. Size of the skin field of T13M nerve as a function of body weight.

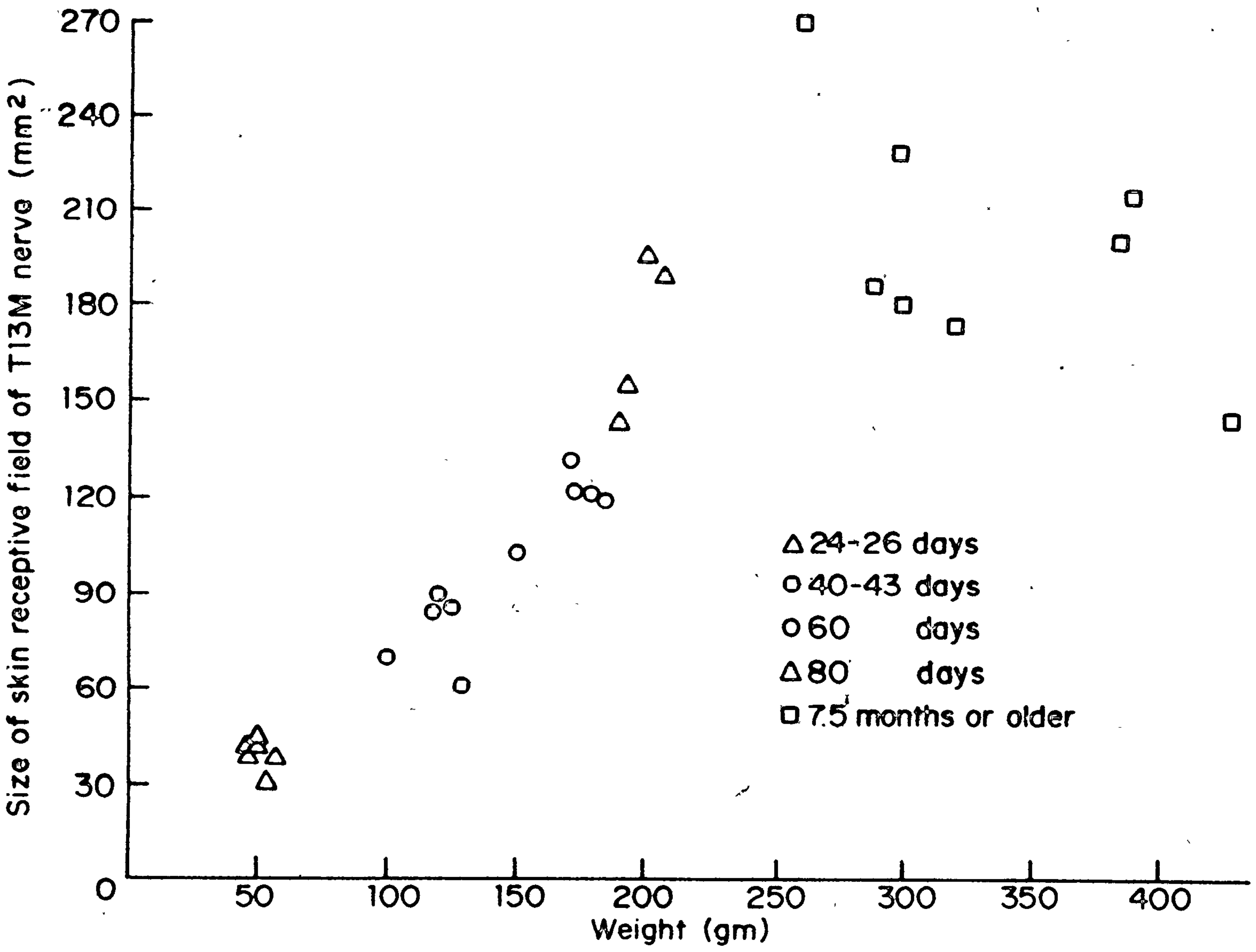

Size of skin receptive field of T13M nerve (mm²)
270
240
210
180
150
120
90
60
30
0
50
100
150
200
250
300
350
400
Weight (gm)
Δ 24-26 days
o 40-43 days
o 60 days
Δ 80 days
□ 7.5 months or older

TABLE 8A

THE NUMBER OF TOUCH DOMES WITHIN THE T13M NERVE RECEPTIVE FIELD DURING DEVELOPMENT

| RAT | AGE (days) | NUMBER OF TOUCH DOMES |
|---|---|---|
| 1 | 24 | 24 |
| | 40 | 24 |
| | 60 | 23 |
| 2 | 24 | 24 |
| | 43 | 17 |
| | 60 | -- |
| 3 | 25 | 32 |
| | 43 | -- |
| | 60 | 29 |
| 4 | 26 | 23 |
| | 43 | -- |
| | 60 | 18 |
| 5 | 26 | 20 |
| | 43 | 18 |
| | 60 | 18 |
| 6 | 80 | 14 |
| | 7.5months | 14 |
| 7 | 80 | 14 |
| | 7.5months | 14 |
| 8 | 80 | 19 |
| | 7.5months | 20 |
| 9 | 80 | 23 |
| | 7.5months | 27 |

the dorsal aspects of the trunk during growth past maturity. Measurements of length and circumference on rats of different ages indicated that this was indeed the case (Leon, unpublished observations).

C. Experiment 3: Spatial distribution of the touch domes.

1. Rationale

Since growth spreads a constant number of domes over the surface area of the animal (see above), one might have expected the domes to be distributed evenly over the surface area of the back, given equal growth of all areas of skin and an even distribution of receptors on the skin at birth. To test this prediction, dome density was compared on three adult female rats using a standardized $cm^2$ grid. (See illustration in Table 8B.)

2. Results

There was no side-to-side variation in dome density on the back. However, when touch dome densities were compared in the medial and lateral halves of each dorsal skin field, it was evident that density was significantly higher medially. (See Table 12 of Appendix 1 for these calculations, and summary in Table 8B.)

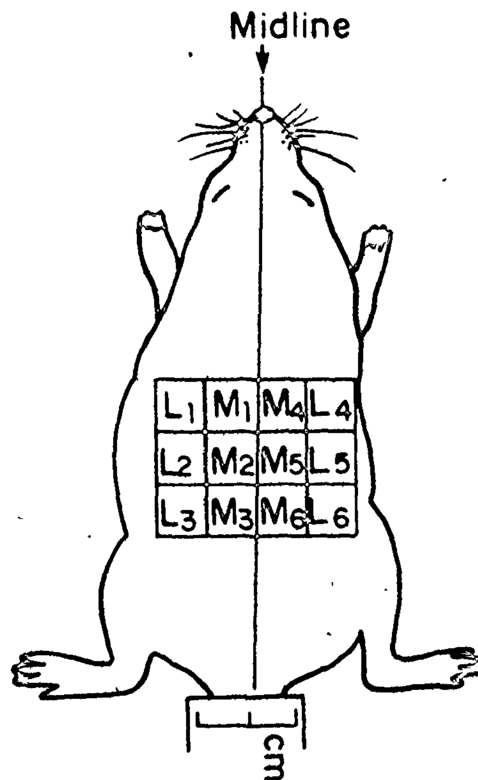
D. Experiment 4: Thresholds of touch domes during development.

1. Rationale

It was possible that, despite the declining dome density, punctate skin sensitivity was enhanced in rats by a decrease in the threshold of individual domes. To test this possibility a more precise

TABLE 8B

SPATIAL DISTRIBUTION OF TOUCH DOMES




$M_1+M_2+M_3+L_1+L_2+L_3 = M_4+M_5+M_6+L_4+L_5+L_6$; There is no side to side variation.

$M_1+M_2+M_3 > L_1+L_2+L_3$; $M_4+M_5+M_6 > L_4+L_5+L_6$; The more medial areas have a higher touch dome density.

method for mechanical stimulation of touch domes was designed and used to test dome thresholds during development.

2. Results

The touch domes in nursing pups were found to be more sensitive to mechanical stimulation than those in older animals. Thus, the mean threshold was significantly higher ($p < 0.025$) post-weaning, as seen in the juvenile group. There was no significant change in threshold as the animals matured until old age was reached; the mean threshold then became significantly higher ($p < 0.005$) than that of younger adult rats. These data are summarized in Table 13 and illustrated in Figure 18; Table 14 in Appendix 1 contains the individual values for each rat. Typical oscilloscope records of touch dome responses to threshold stimuli in pup, adult, and aged rats can be seen in Figure 19. In summary, touch dome threshold did not decrease to provide partial compensation for decreasing receptor density during development; in fact there was a significant increase in dome threshold over the course of the animal's life.

E. Experiment 5: The effect of suprathreshold stimuli on response latency.

1. Rationale

It is a general property of mechanoreceptors studied in mature animals that an increase in stimulus strength shortens response latency (Katz, 1950b; Gray, 1959). The question now asked is whether this relationship holds both for young and for aged rats. The mechanical

TABLE 13

TOUCH DOME THRESHOLDS (μm)

| | Group I Nursing Pups | Group II Juveniles | Group III Adults | Group IV Aged |
|---|---|---|---|---|
| Age | <28 days | 5 weeks-1.5 months | 1.5 months-6 months | 6 months-21 months |
| Weight | <65 grams | 65-150 grams | 150-250 grams | >250 grams |
| $\bar{x}$ Threshold | 3.21 | 5.05 | 5.23 | 9.19 |
| sd | ±1.96 | ±0.83 | ±2.04 | ±5.88 |
| sem | ±0.65 | ±0.26 | ±0.33 | ±1.28 |
| n | 9 domes<br>3 rats | 10 domes<br>3 rats | 38 domes<br>19 rats | 21 domes<br>7 rats |
| | $p < 0.025$ | N.S. | $p < 0.005$ | |

Figure 18. Mechanical stimulus threshold of touch domes during ageing; values are means ± s.e.m.

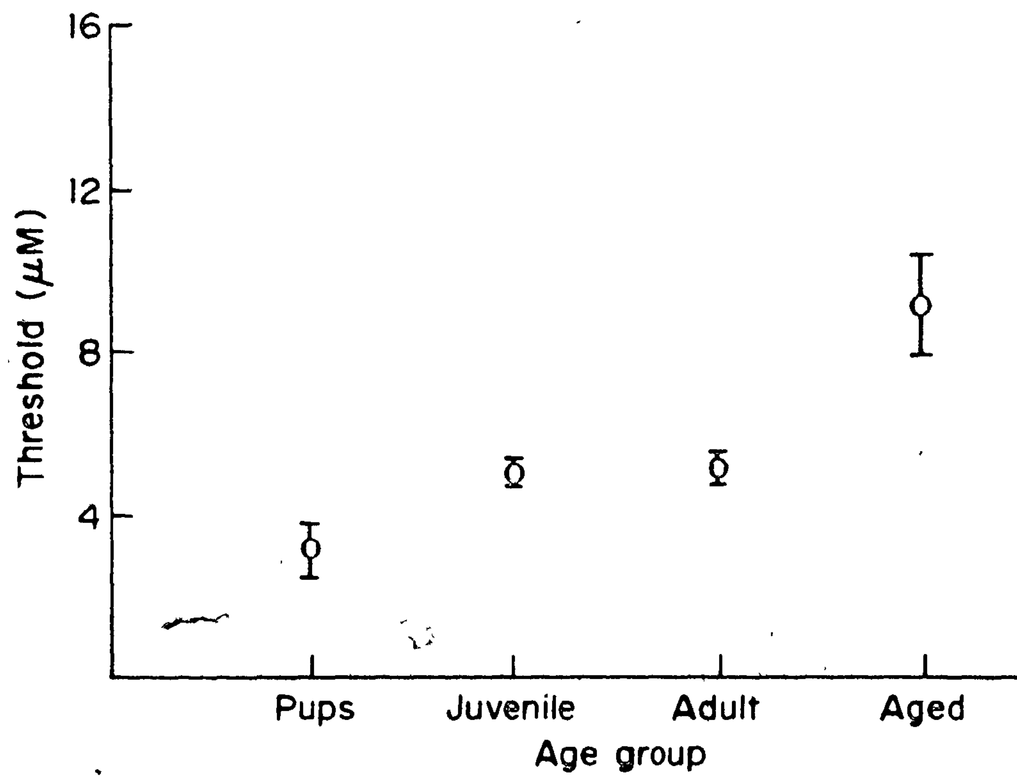
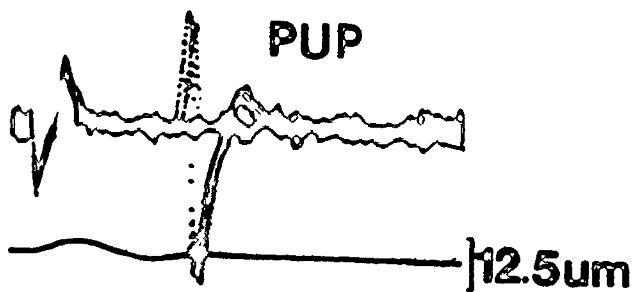
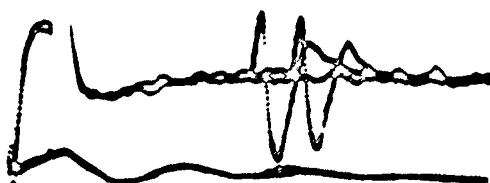
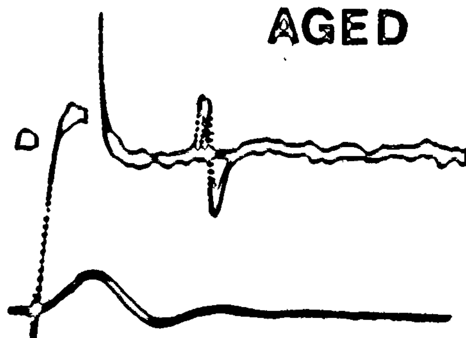

16
12
8
4
Threshold (μM)
Pups
Juvenile
Adult
Aged
Age group

Figure 19. Typical touch dome responses at threshold in pup, adult, and aged animals. Note the larger stimuli required to elicit responses in the adult and 'aged' animals.




1ms



stimulus was therefore increased to twice threshold and the response latency was measured in animals of different ages.

2. Results

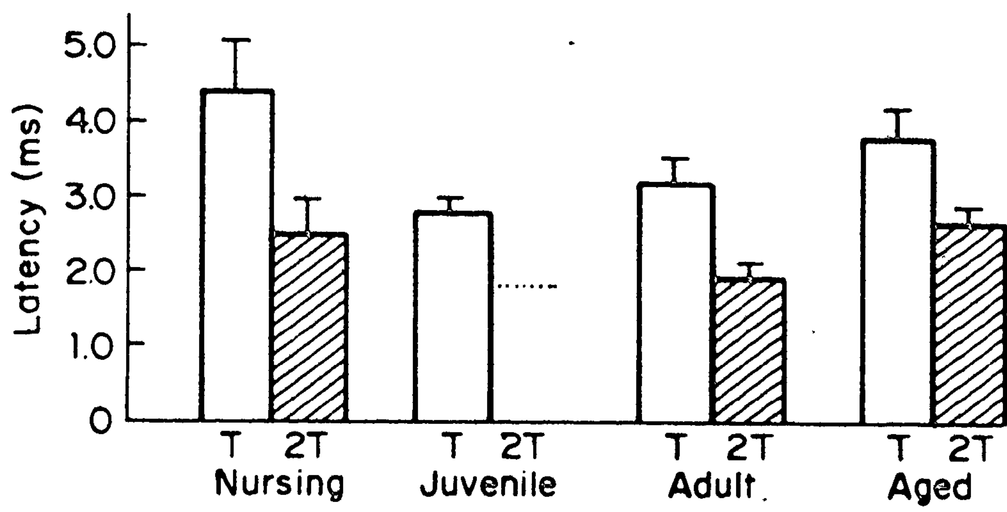
The touch domes of nursing pup, adult, and aged animals all exhibited decreased response latencies when stimuli were increased to twice threshold amplitude; this finding suggested that the receptor mechanism was similar in rats at all ages. These results can be seen in Figure 20 and Table 15. The trend towards an increased latency at threshold as the animals grew was probably a result of nerve elongation during body growth. The long latency at threshold seen in the nursing pups may have reflected a lower impulse conduction velocity in the sensory nerve fibers of those animals. Nerve lengths were not measured in young and old animals because it was difficult to make reliable measurements, the paths of nerve fibers to domes being irregular. However, certain observations in the cat, reported by Eckholm (1967), appear relevant. This author found that, in newborn kittens, the maximal impulse conduction velocity in sural nerve fibers was 8-12 m/sec and increased by 0.6 m/sec per day during development (Eckholm, 1967).

F. Experiment 6: Touch dome density in adult animals after administration of vincristine sulfate.

1. Rationale

Vincristine is widely used for the treatment of malignancies (Calabresi and Parks, 1980). The limiting factor in its therapeutic

Figure 20. Touch dome response latencies to stimuli of threshold and twice threshold strength; values shown are means ± s.e.m.



Latency (ms)
5.0
4.0
3.0
2.0
1.0
0
T
2T
Nursing
T
2T
Juvenile
T
2T
Adult
T
2T
Aged

TABLE 15

TOUCH DOME RESPONSE LATENCIES (ms)

| | At Threshold | At Twice Threshold |
|---|---|---|
| Nursing Pup ($\bar{x}$ ± sem) | 4.4 ± 0.7 (n = 5) | 2.5 ± 0.5 (n = 3) |
| Juvenile ($\bar{x}$ ± sem) | 2.8 ± 0.1 (n = 8) | ........... |
| Adult ($\bar{x}$ ± sem) | 3.3 ± 0.2 (n = 30) | 2.0 ± 0.1 (n = 24) |
| Aged ($\bar{x}$ ± sem) | 3.9 ± 0.3 (n = 18) | 2.7 ± 0.2 (n = 10) |

use is the development of sensori-motor neuropathy (Creasy, 1975) that involves loss of tendon reflexes, followed by parasthsiae and, in severely affected patients, by weakness (Casey et al., 1973; Bradley et al., 1970; Sandler et al., 1969; Tobin and Sandler, 1968).

Electromyographic studies of patients suffering from this neuropathy have confirmed the presence of sensory and motor denervation; however, impulse conduction velocities were little changed in surviving fibers, indicating that the neuropathy is of the dying-back type and not associated with primary demyelination (Casey et al., 1973; McComas, 1977). Since sensory loss involving parasthesiae is apparent after vincristine therapy, we examined the effect of vincristine on an identified cutaneous receptor, the touch dome, having already established certain features of the normal structure and function of mammalian touch domes.

In preliminary experiments using 0.01-1.00 mg/kg of vincristine sulfate injected subcutaneously, it was found that a single injection of 1.0 mg/kg produced death within 24 hours of administration and lower doses produced various degrees of toxicity manifested by hind limb paralysis, alopecia and diarrhea. A single subcutaneous dose of 0.75 mg/kg vincristine sulfate was used in the following experiments since it was the highest single dose of the drug that could be administered subcutaneously to rats without obvious toxicological effects. The clinically used dose of vincristine sulfate is substantially lower 0.01-0.05 mg/kg in adults (Calabresi and Parks, 1980). This dose of the drug is administered intravenously to cancer patients

usually once weekly over a period of several weeks. The earliest signs of neurotoxicity in patients are sensory; patients experience a tingling and numbness in the extremities.

Is the sensory neuropathy induced by vincristine sulfate due due to a loss of receptors? After vincristine sulfate had been administered to rats, touch dome density was measured to answer this question.

2. Results

Administration of vincristine sulfate had no effect on the density of the receptors in the skin, there being no statistically significant difference in touch dome density between control and treated animals. The data analysis summary can be seen in Table 17, and individual values are in Table 18 of Appendix 1.

G. Experiment 7: Threshold of touch domes after administraton of vincristine sulfate.

1. Rationale

Since there was no loss of touch domes in rats after the administration of vincristine sulfate, it was possible that the subjective complaint of numbness in patients treated with this drug might have been the result of increased thresholds of individual receptors. Receptor thresholds were therefore measured in rats to determine if vincristine was able to influence thresholds.

TABLE 17

TOUCH DOME DENSITY

AFTER ADMINISTRATION OF VINCRISTINE SULFATE

| Dome Density | Vincristine-treated | Control |
|---|---|---|
| $\bar{x}$: | 11.17 domes/$cm^2$ | 10.17 domes/$cm^2$ |
| sd: | ± 2.52 | ± 0.67 |
| sem: | ± 1.03 | ± 0.39 |
| n (rats) | 6 | 3 |
| n domes | 763 | 343 |

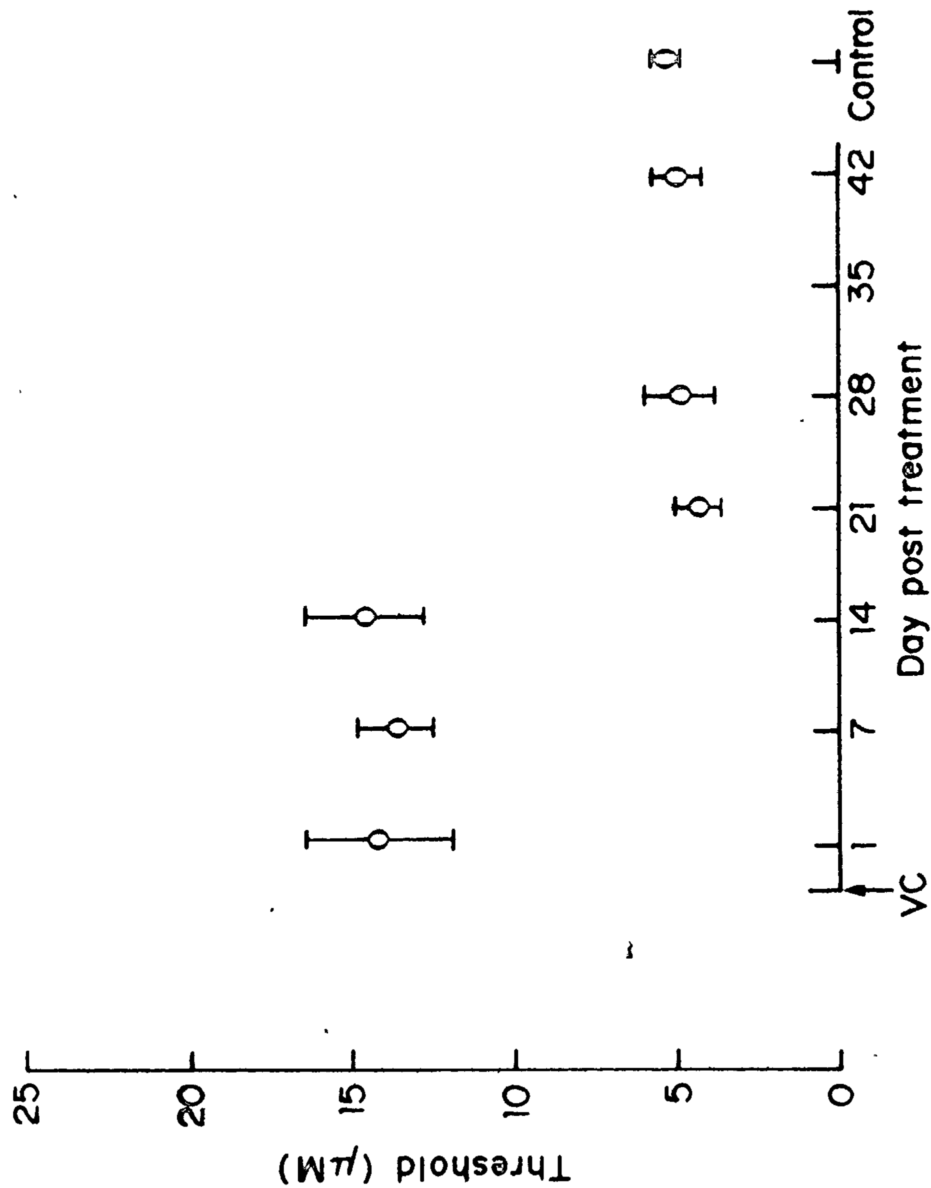
N.S.

## 2. Results

In preliminary experiments (Leon and McComas, 1980), touch dome threshold changes were produced with single subcutaneous 0.25-0.75 mg/kg doses of vincristine sulfate without obvious toxicological effects. A single subcutaneous dose of 0.75 mg/kg vincristine was then used in the definitive experiments. Touch thresholds were found to increase dramatically within 24 hours of administration of vincristine sulfate. The rapid onset of the high threshold state was maintained until fourteen days post-treatment. Thresholds then recovered to the control level by twenty-one days post-treatment and were normal thereafter. These results are illustrated in Figure 21 and individual values are given in Table 19 of Appendix 1. The mean values of threshold for each group post-treatment are shown in Table 20. When compared to control values, the mean threshold became significantly higher ($p < 0.005$) between 1 and 14 days following vincristine treatment. There was no significant difference between control values for threshold and thresholds at 21, 28 and 35 days post-treatment.

Typical oscilloscope records of touch dome responses obtained from vincristine-treated animals can be seen in Figure 22. In addition to having increased thresholds, vincristine-treated touch domes fired at lower frequencies than touch domes in control animals for a given stimulus, as can be seen in Figure 22. These results showed that a high threshold state could be produced in touch domes by administering vincristine sulfate and that this effect was reversible. Occasionally, (3/37), a touch dome in a treated animal was inexcitable both mechanically and electrically.

Figure 21. The effect of subcutaneous injection of 0.75 mg/kg vincristine sulfate on the threshold of touch domes.


Threshold (μM)
25
20
15
10
5
0
VC
1
7
14
21
28
35
42
Control
Day post treatment

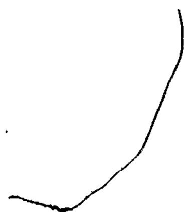


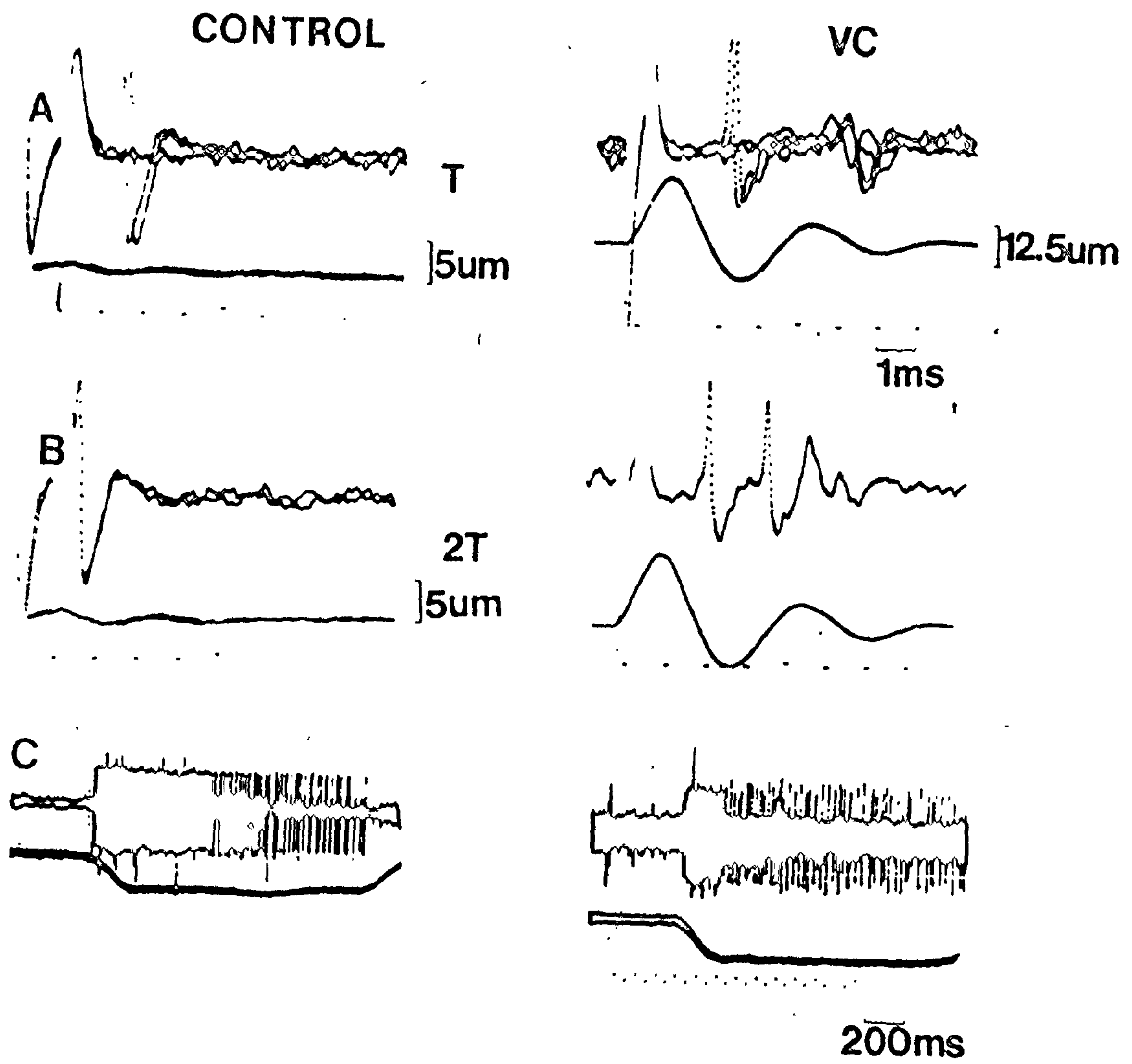
TABLE 20

THRESHOLD VALUES (μm) FOR TOUCH DOMES AFTER TREATMENT WITH 0.75 mg/kg VINCRISTINE SULFATE

| | Day Post-treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day 1 | Day 7 | Day 14 | Day 21 | Day 28 | Day 42 | Control |
| $\bar{x}$: | 14.15 | 13.62 | 14.73 | 4.25 | 5.05 | 5.00 | 5.23 |
| sd: | 8.80 | 4.73 | 6.37 | 1.89 | 3.04 | 2.04 | 2.04 |
| sem: | 2.13 | 1.18 | 1.77 | 0.60 | 0.96 | 0.65 | 0.33 |
| n domes: | 17 | 16 | 13 | 10 | 10 | 10 | 38 |
| n rats : | 5 | 4 | 4 | 3 | 3 | 3 | 19 |

Figure 22. Typical oscilloscope records obtained from touch domes after treatment with vincristine sulfate. On the left are records obtained from control animals. Note the increased stimulus strength (middle trace of each set) needed to elicit a response after treatment with vincristine sulfate. (top trace: extracellular recording from the dorsal cutaneous nerve innervating the touch dome; middle trace: photocell output monitorinng the movement of the 'prodder' stylus tip; bottom trace: time increments)

A: Responses recorded from control and vincristine-treated animals at touch dome threshold.
B: Responses recorded at twice threshold.
C: Responses recorded during prolonged mechanical stimulation.


CONTROL
VC
A
T
]5um
]12.5um
1ms
B
2T
]5um
C
200ms

## H. Experiment 8: The effect of suprathreshold stimuli on response latency after the administration of vincristine sulfate.

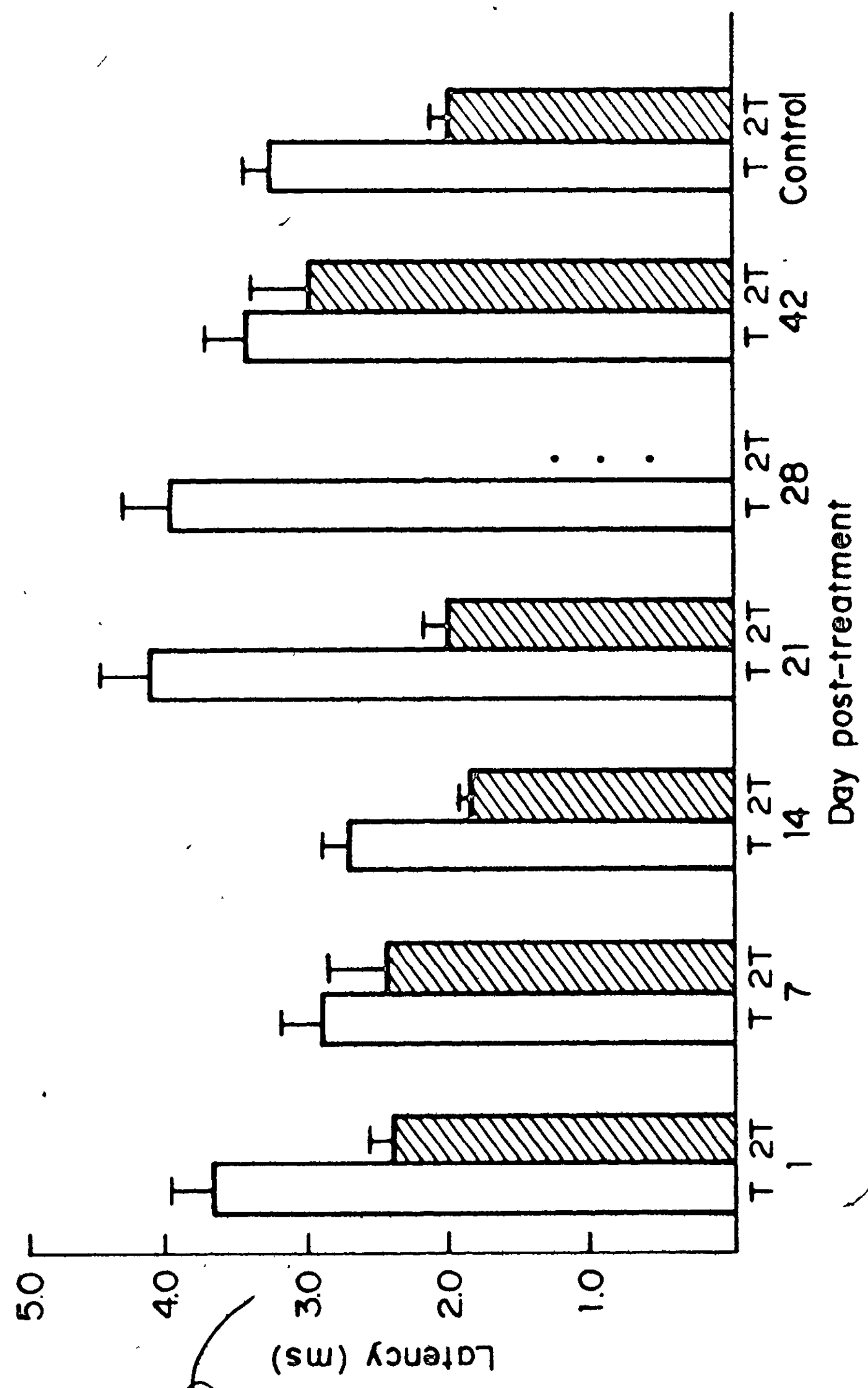
### 1. Rationale

As shown in the previous section, parenteral administration of vincristine to rats rapidly induced a temporary high-threshold state in touch domes; recovery of normal threshold occurred within three weeks of treatment (see experiment 7). It could be postulated that treatment with vincristine temporarily disrupted the mechano-electric transducer mechanism that generates the receptor potential. In control adult animals increasing the stimulus strength shortens mechanoreceptor response latency (cf. Gray, 1959) and this was shown to be true for rat touch domes (Section E, Experiment 5). To test whether this relationship held for rats treated with vincristine, mechanical stimuli were increased to twice-threshold strength and response latencies were measured in animals who had been treated with vincristine. If the receptor potential was generated in a manner similar to that in normal animals then increasing the stimulus strength would likewise have shortened the response latency.

### 2. Results

In all experimental animals the touch dome responses had decreased latencies following an increase of stimulus strength to twice threshold; these observations suggested that the basic receptor mechanism remained similar to that of touch domes in control animals. These results are plotted graphically in Figure 23 and presented in Table 21. For further comparison with control responses, all measurements

Figure 23. Touch dome response latencies to stimuli of threshold and twice threshold strength at various times after administration of vincristine sulfate subcutaneously (0.75 mg/kg). Values shown are means ± s.e.m.



Latency (ms)
5.0
4.0
3.0
2.0
1.0
T 2T
1
7
14
21
28
42
Control
Day post-treatment

TABLE 21

RESPONSE LATENCIES TO STIMULI OF THRESHOLD AND TWICE THRESHOLD STRENGTH AFTER TREATMENT WITH VINCRISTINE SULFATE

| | LATENCY AT THRESHOLD | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day Post-treatment | | | | | | |
| | Day 1 | Day 7 | Day 14 | Day 21 | Day 28 | Day 42 | Control |
| $\bar{x}$: | 3.70 ms | 2.92 ms | 2.74 ms | 4.18 ms | 4.07 ms | 3.52 ms | 3.29 ms |
| sd: | 1.04 | 0.84 | 0.53 | 1.01 | 0.95 | 0.86 | 0.87 |
| sem: | 0.29 | 0.27 | 0.17 | 0.36 | 0.30 | 0.27 | 0.16 |
| n domes: | 13 | 10 | 10 | 8 | 10 | 10 | 30 |
| n rats : | 4 | 3 | 4 | 3 | 3 | 3 | 17 |

| | LATENCY AT TWICE THRESHOLD | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day Post-treatment | | | | | | |
| | Day 1 | Day 7 | Day 14 | Day 21 | Day 28 | Day 42 | Control |
| $\bar{x}$: | 2.40 ms | 2.44 ms | 1.84 ms | 2.03 ms | | 3.00 ms | 2.05 ms |
| sd: | 0.55 | 1.05 | 0.17 | 0.41 | | 1.10 | 0.54 |
| sem: | 0.15 | 0.47 | 0.07 | 0.21 | | 0.49 | 0.11 |
| n domes: | 13 | 5 | 5 | 4 | | 5 | 24 |
| n rats : | 4 | 2 | 2 | 2 | | 2 | 14 |

have been assigned to two groups. Group I contains data from vincristine-treated high-threshold touch domes (1, 7 and 14 days post-injection) and Group II contains the results from all 'recovered' touch domes (21, 28 and 42 days post-injection). Figure 24 shows, in histographic form the mean latency values for these two groups and for pups, control adult and aged animals, and Table 22 contains the raw data presented in the graph. Individual values can be found in Table 19 of Appendix 1. Figure 25 is a plot of response latency at different stimulus strengths for all groups of animals. Since the rate of the decrease in latency with increasing stimulus strength (the slope of the lines in Figure 25) may give information about the efficiency of the transducer mechanism in general, it is of interest to examine this parameter in more detail.

The nursing pup group, in which touch domes have the lowest thresholds, (see page 121), had the steepest rate of decline in latency of all the groups examined, approximately 3 ms reduction in latency for each 5 μm increase in stimulus strength. This finding suggested that the rate of rise of receptor potential had its greatest dynamic responsiveness in the nursing pup group.

The adult control and 'recovered' vincristine groups had similar rates of decline in latency, approximately 1-1.25 ms for each 5 μm of increased stimulus strength. This observaton was interpreted as indicating that in recovered vincristine touch domes, the dynamic responsiveness of the transducing mechanism was similar to that of the control group.

In the aged group of rats there was a slower rate of decline in

Figure 24. Mean (± s.e.m.) touch dome response latencies in different groups of experimental animals, using stimuli of threshold and twice threshold strength.

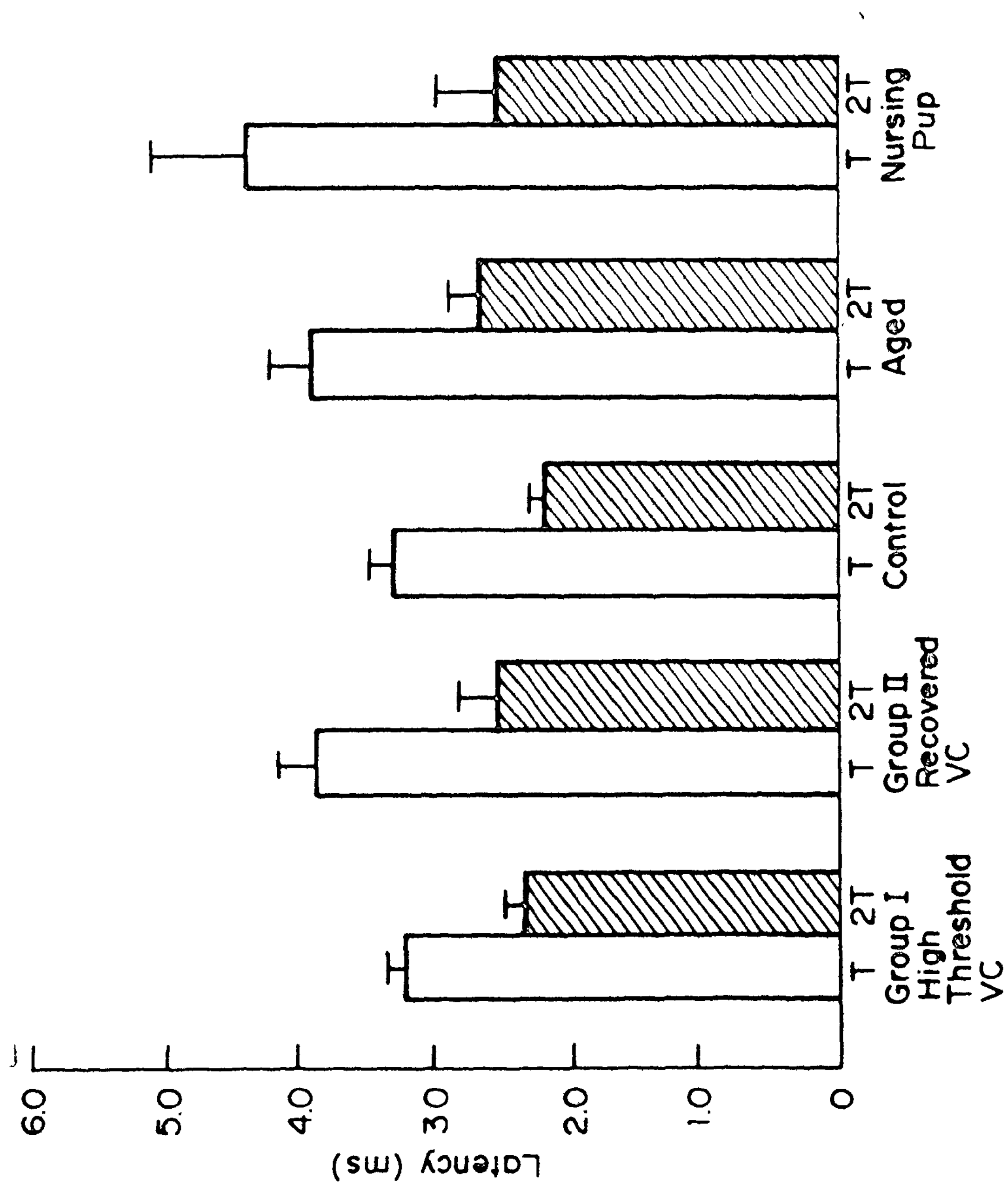

Latency (ms)
6.0
5.0
4.0
3.0
2.0
1.0
0
T 2T
Group I
High
Threshold
VC
T 2T
Group II
Recovered
VC
T 2T
Control
T 2T
Aged
T 2T
Nursing
Pup

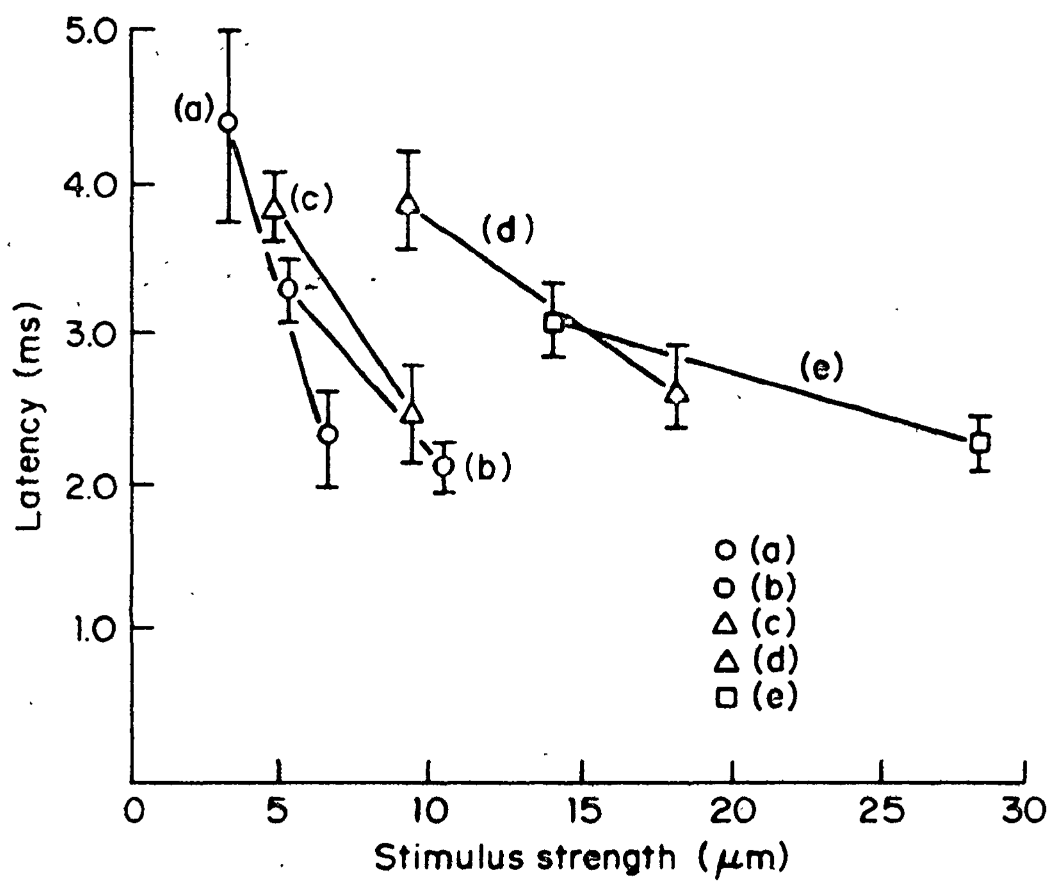
TABLE 22

RESPONSE LATENCIES AFTER TREATMENT WITH VINCRISTINE AND AT DIFFERENT STAGES OF DEVELOPMENT

| | LATENCY AT THRESHOLD (ms) | | | | |
|---|---|---|---|---|---|
| | Vincristine High Threshold | Vincristine Recovered | Control Adult | Aged | Pup |
| $\bar{x}$: | 3.17 | 3.90 | 3.29 | 3.89 | 4.39 |
| sd | 0.94 | 0.95 | 0.87 | 1.30 | 1.53 |
| sem | 0.16 | 0.18 | 0.16 | 0.30 | 0.68 |
| n domes. | 33 | 28 | 30 | 18 | 5 |
| n rats : | 11 | 9 | 17 | 6 | 1 |

| | LATENCY AT TWICE THRESHOLD (ms) | | | | |
|---|---|---|---|---|---|
| | Vincristine High Threshold | Vincristine Recovered | Control Adult | Aged | Pup |
| $\bar{x}$ | 2.29 | 2.51 | 2.05 | 2.67 | 2.50 |
| sd | 0.65 | 0.88 | 0.54 | 0.73 | 0.87 |
| sem | 0.14 | 0.27 | 0.11 | 0.20 | 0.50 |
| n domes | 23 | 11 | 24 | 10 | 3 |
| n rats : | 8 | 5 | 14 | 4 | 1 |

Figure 25. Touch dome response latency relative to stimulus strength in different groups of experimental animals; values are means ± s.e.m. (a) nursing pups; (b) adult control rats; (c) rats treated 21-42 days previously with vincristine; (d) aged rats; (e) rats treated 1-14 days previously with vincristine.



5.0
4.0
3.0
2.0
1.0
Latency (ms)
(a)
(c)
(d)
(e)
(b)
○ (a)
○ (b)
△ (c)
△ (d)
□ (e)
0
5
10
15
20
25
30
Stimulus strength (μm)

response latency, approximately 0.25 ms for 5 μm of increased stimulus strength. The vincristine-treated group of animals had the slowest rate of decline in response latency of all the groups tested. The longer latencies of the responses in the aged group could not have been due to longer lengths of nerve between touch domes and recording electrodes since the nerve field did not increase between 7.5 and 18 months (e.g., Figure 16). More plausible explanations for the longer response latencies in the aged group and the vincristine high-threshold group were that impulse conduction was slowed in the group I fibers or that there was delay in transduction at the receptor. A lower conduction velocity may have been the result of decreased fiber diameter, demyelination or loss of large diameter fibers in aged and vincristine-treated animals (Buchthal and Rosenflack, 1966; Arnold and Harriman, 1970; O'Sullivan and Swallow, 1968).

A delay in transduction in the touch dome could have occurred if there was greater mechanical compliance between the surface of the touch dome and the cells (Merkel) responsible for the receptor potential. Alternatively, the change in membrane conductance of the transducing cells could have had a slower time course than normal. Finally the thresholds of the group I nerve terminals could have been elevated so that the receptor potential would have to rise for a longer time before an impulse could be initiated. These possibilities could be resolved by recording impulses closer to the touch domes or by analyzing the receptor potential directly.

# I. Histological results

## 1. Appearance of the dorsal cutaneous nerve during development and ageing.

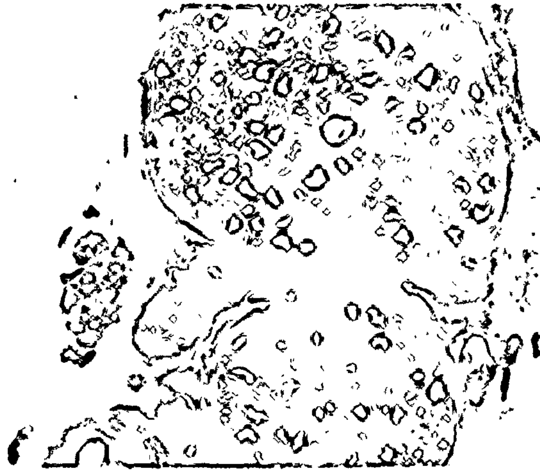
Since there may be a decrease in sensitivity of touch domes in adults, it is possible that a loss of low threshold axons after weaning accounts for the decrease in sensitivity observed in adults. However, the data presented here do not rule out the possibility that there is a switch from low to high threshold in one receptive unit and more quantitative histological work is needed.

The number of large myelinated axons in the T13 medial nerve was found to vary between 326-405 in pup, juvenile and adult rats and then to plunge to 195 in an aged animal. This data indicate that in the aged animal almost 50% of the large myelinated fibers in the purely cutaneous sensory nerve is lost. Extensive quantitative histology is necessary to determine if changes in the axon number occur during development and to confirm the extensive loss of axons observed during ageing. The axon silhouettes in these cross sections were measured on enlarged (x1300) photographs, and a digitizer was used to measure the maximum diameter of each axon, including the myelin sheath. The set of axon diameters for each nerve section was then converted into a frequency histogram. The nerve cross section and corresponding histogram can be seen in Figure 26. The frequency distribution of axon diameters is bimodal in the adult nerve and is unimodal and skewed towards smaller diameters in the pup and juvenile nerves. This is similar to data collected by other workers on axons diameters of developing cutaneous nerves (Ecklund, 1967; Romero and Skolund,1965).

Figure 26. Cross sections of the dorsal cutaneous nerves in pup, juvenile, adult and aged rats. Sections A-D stained with toluidine blue and shown at a magnification of 500X. Below the nerve cross section is a frequency histogram of axon diameter.

A. Pup: 376 myelinated axons were present.

A



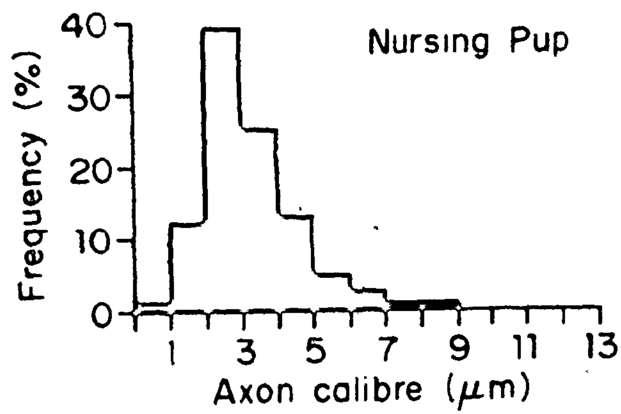
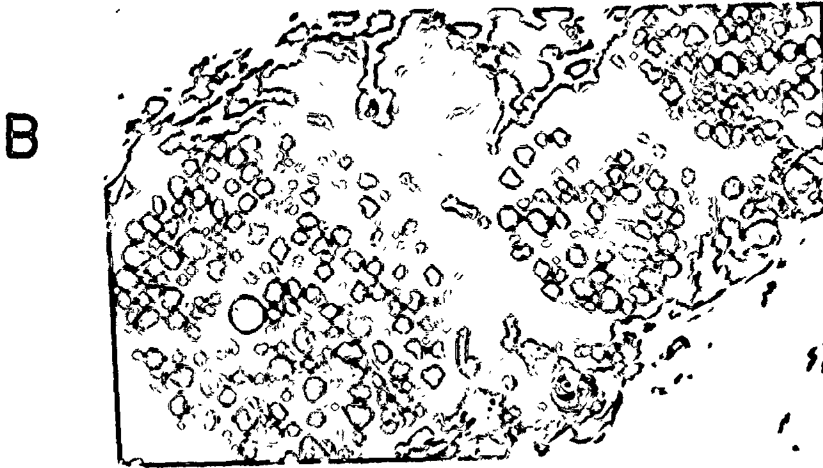


Figure 26.

B. Juvenile: 405 myelinated axons were present.

B



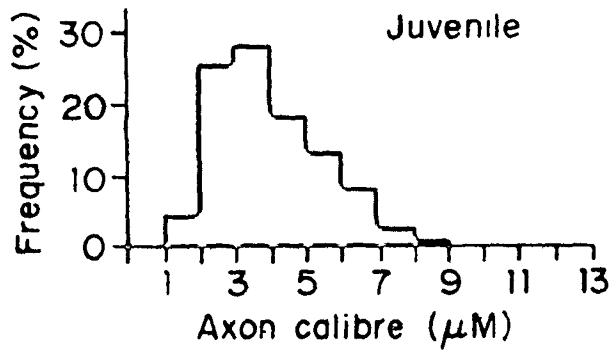
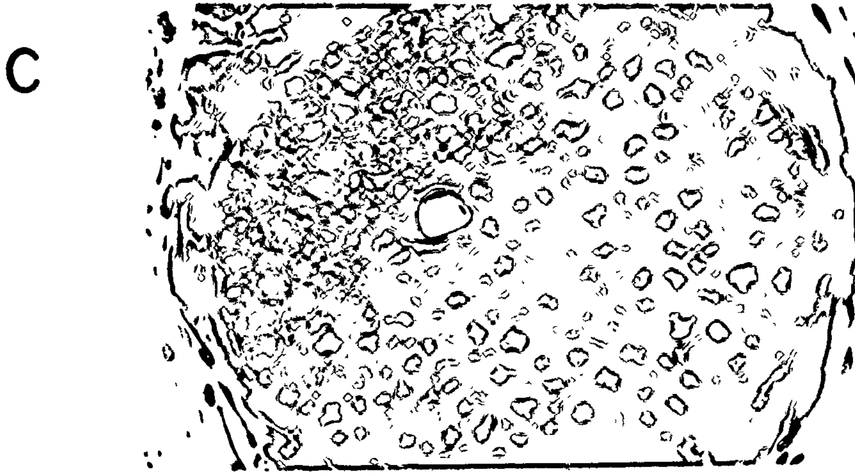


Figure 26.

C. Adult: 326 myelinated axons were present.

C



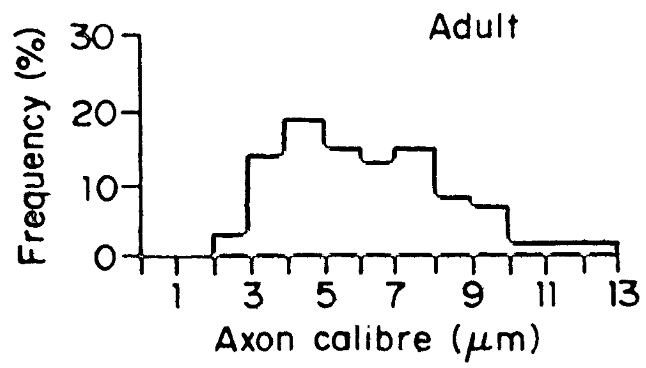
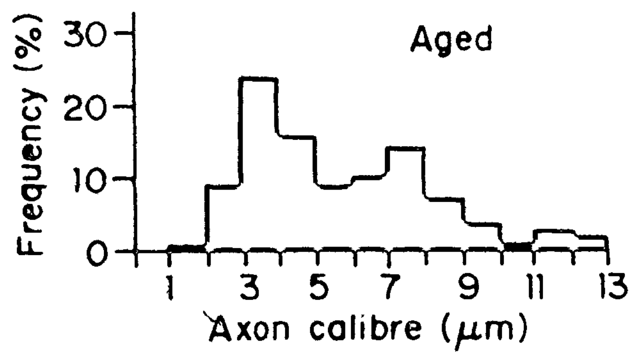


Figure 26.

D. Aged: 195 myelinated axons were present.

D



2. Appearance of the dorsal cutaneous nerve after the administration of vincristine sulfate.

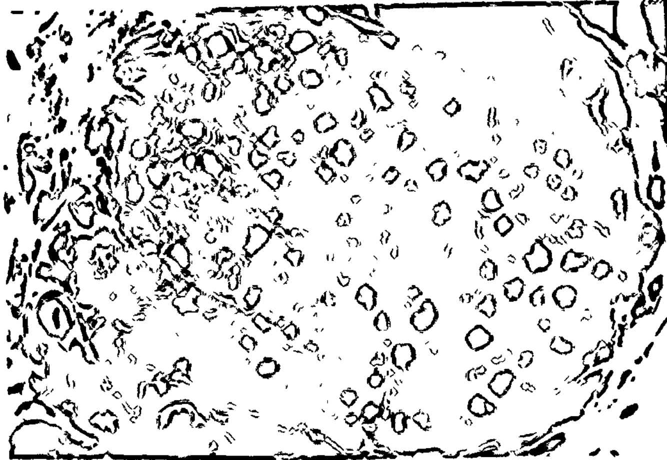
Cross sections of the T13 medial nerve were taken from vincristine-treated and control animals and can be seen in Figure 27. At 24 hours after vincristine treatment, a time when the thresholds of the touch domes were elevated, (see page 106), the axons appeared normal and no loss of fibers could be demonstrated. These morphological data, taken together with the physiological data presented in Appendix 3, indicated that vincristine was modifying transduction at the level of the touch domes themselves or the fine nerve terminals abutting the touch domes.

By 6 days after vincristine treatment, the nerve appeared abnormal. The axons were no longer tightly packed, suggesting that there had been loss or shrinkage of fibers (Figure 27B); quantitative histological measurements suggested the latter was the more important factor. When examined under the light microscope, however, the largest diameter fibers retained normal appearances, at least in relation to their myelin ensheathment. At 12 days after vincristine treatment, when thresholds were still elevated, the impression of abnormality persisted. Although axon counts were similar to those of control animals, there was a greater proportion of small diameter fibers with relatively thin myelin sheaths (Figure 27C); some of these may have been regenerating fibers (Spencer and Schaumberg, 1978). In contrast, the larger fibers were more irregular in outline and in some there was

Figure 27. Cross sections of the dorsal cutaneous nerves after treatment with vincristine sulfate injected subcutaneously (0.75mg/kg). Sections A-D stained with toluidine blue and shown at a magnification of 500X. Below the nerve cross section is a frequency histogram of the axon diameters.

A. Vincristine, 24 hours: 308 myeliinated axons were present.

A



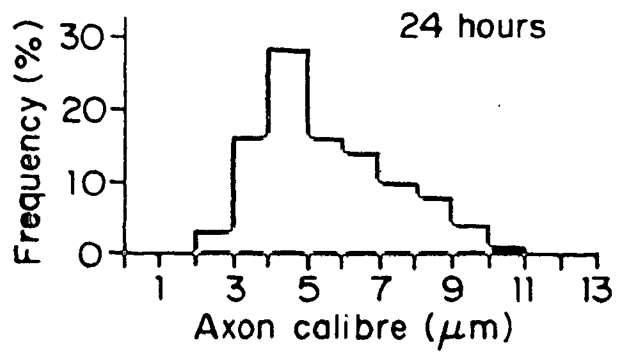
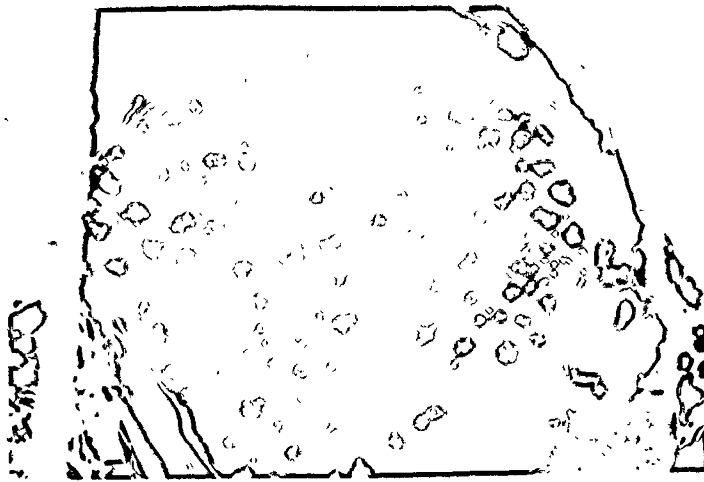


Figure 27.

B. Vincristine, 6 days: 300 myelinated axons were present.

B



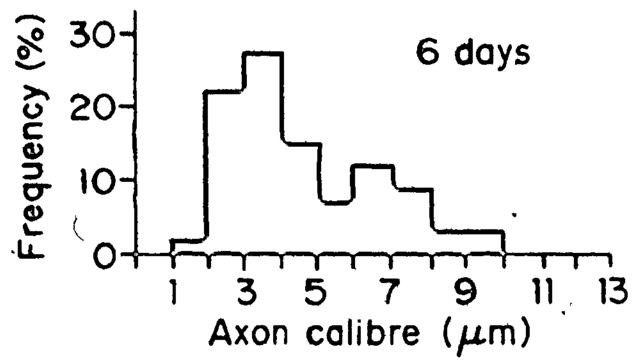
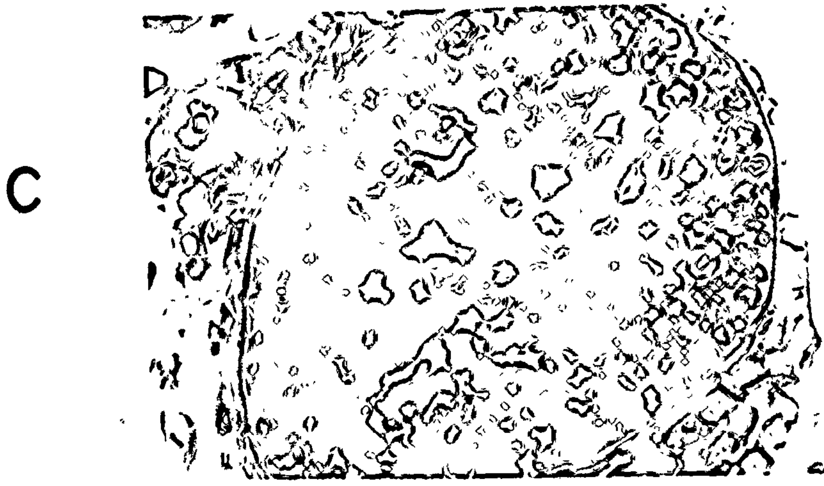


Figure 27.

C. Vincristine, 12 days: 356 myelinated axons were present.

C



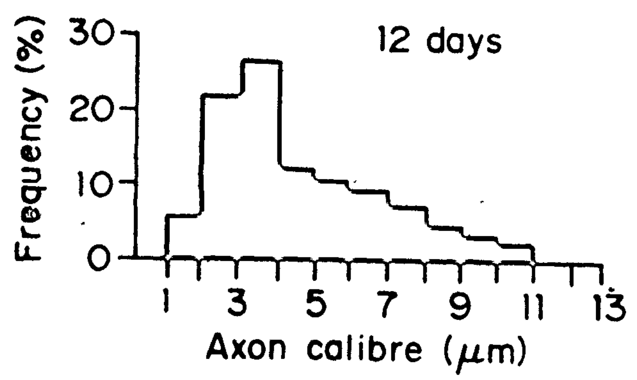
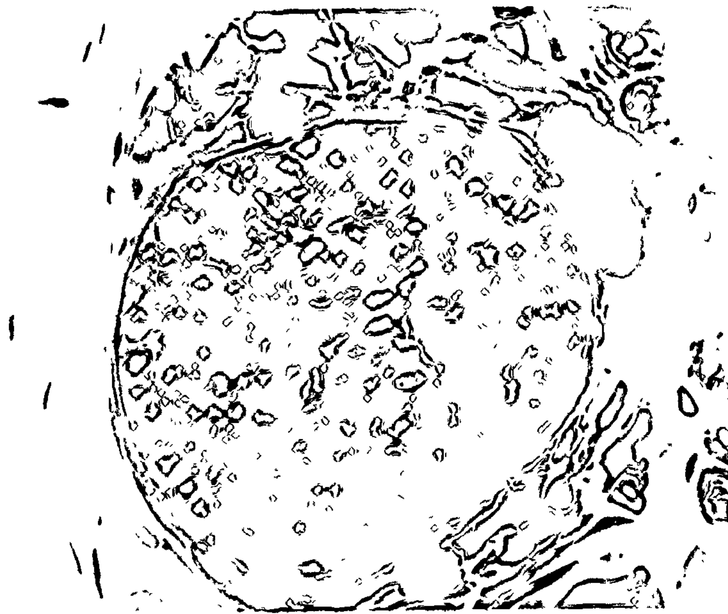
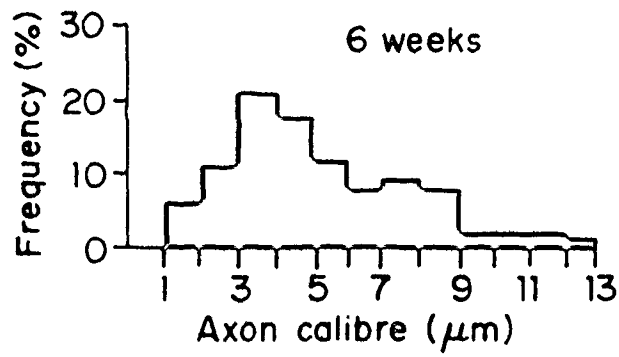


Figure 27.

D. Vincristine, 6 weeks: 327 myelinated axons were present.

D





an appearance as if the myelin sheath was 'collapsing' inwardly. At 6 weeks after vincristine treatment the nerve still maintained the appearance of degeneration and regeneration, although the touch threshold had returned to normal values and the frequency histogram (Figure 27D) of nerve fiber diameters had a normal shape.

In summary, the morphological studies suggest that, following vincristine administration, there may be some degeneration and regeneration of myelinated axons, including those innervating touch domes. These changes were not evident at 24 hours, however, at a time when the mechanical thresholds were elevated. For this reason, the rise in threshold could not have been due to early degeneration of low-threshold fibers, but rather to some change in the sensory nerve terminal or in the cellular transducing elements themselves.

## IV. DISCUSSION

### A. Touch dome function in developing and ageing rats

This study has shown that there is a decrease in touch dome density in the skin throughout the life cycle of the rat. From 3 weeks of age to 7-1/2 months of age the growth of the skin of the T13 medial dorsal cutaneous nerve field accounts for the decreased touch dome density. The absolute number of touch domes in the skin of the T13 medial nerve receptive field remains constant.

During this epoch in the animal's life the diameter of each individual touch dome increases in size, thereby maintaining a constant touch dome-sensitive area of skin of about 1 $mm^2$ over 1 $cm^2$ of skin. After 7.5 months of age touch dome density continues to decrease slowly (see Table 24), although the difference in touch dome density is not highly statistically significant between 7.5 months and 1.5 years of age.

---

TABLE 24

| | | Mature (7-1/2 months) | Aged (1-1/2 years) |
|---|---|---|---|
| Touch Dome Density | x: | 8.8 | 7.6 |
| | sd: | ±1.7 | ±2.2 |
| | n: | 9 | 8 |

$p < 0.25$
$t = 1.2850$

---

At the same time the size of the skin receptive field of T13 medial nerve does not continue to increase (see Figure 16; $t = 0.95$, $p > 0.05$). These data suggest that there may be an actual loss of touch domes in the nerve field during ageing. Because of the decreased touch dome density in aged animals, the estimated touch dome-sensitive skin area is 0.5 $mm^2$ in 1 $cm^2$ of skin, a loss of about 50% of the adult value.

A decrease in touch dome density in early development along with an increased touch dome diameter was noted by Kasprak and Tapper (1970) in the cat. Histological studies of Meissner's corpuscles, an encapsulated touch receptor in glabrous skin in humans, have revealed a decrease in density of receptors from birth to old age (Bolton et al., 1966; Dickens et al., 1963; Cauna, 1965; Hunter et al., 1969; Martinez, 1931; Quillam and Ridly, 1971; Ridley, 1968; Ronge, 1943b).

The experiments presented thus far show that both touch dome density and touch dome sensitivity decline over the course of a rat's life. Since touch domes have low thresholds in young rats, these animals will be able to detect stimuli which would not be perceived in older animals. Since touch domes move further apart from each other during growth, the spatial extent of a cutaneous deformation will be coded differently in young and in old animals. The same stimulus will excite more touch domes in younger than in older animals. Possibly the net effect of this enlarged peripheral window is to provide greater stimulation at a time when central circuitry is forming.

If the mechanical stimulus is a punctate one, however, and is applied randomly, the mature animal will be as successful as a younger

one in detecting cutaneous deformation. This is because, in animals up to 7.5 months of age, the increase in diameter of individual touch domes compensates for reduction in touch dome density.

Threshold values for adult rats were in agreement with those found for adult cats, 1-5 μm (Iggo and Muir, 1969). There is an increase in threshold to a light mechanical stimulus after weaning and again in old age. The increased touch dome threshold after weaning may be due to a loss of low threshold axons innervating the touch domes, but an adequate, quantitative histological study of the number of axons innervating a touch dome is needed to evaluate such a hypothesis. Alternatively, the increase in threshold at weaning might be the result of changes that occur in the non-neural touch dome structure at this time.

Using electron microscopy, English (1977b) has found clear morphological differences in the non-neural aspects of touch domes of nursing pups and adults that may underlie the physiological findings presented here. There are flattened keratinocytes in the epidermis of adult touch domes, but not in twenty-three day old pups. These flattened keratinocytes may act to dampen mechanical stimuli, making the adult touch dome less sensitive than the touch domes of the nursing pups.

English (1977b) also found that there are many more clear-core vesicles present in nerve terminals abutting the Merkel cells of the touch domes of nursing pups relative to the number of such vesicles found in adults. Their function, however, is not known. Dense core vesicles are present in the Merkel cells of touch domes of adult rats

in abundance, whereas in the nursing pup they are less plentiful (English, 1977b; English et al., 1980). Again, it is unclear as to their physiological role. It is possible the contents of the vesicles may act as a messenger between Merkel cell and sensory nerve terminal, either as a transmitter during sensory transduction or as a trophic factor during development (English et al., 1980).

Although the experiment described in Appendix 2 suggests there is no loss of axons innervating an individual touch dome from adulthood to old age, histological studies in man suggest that the total number of myelinated sensory fibers decreases in old age (Ochoa and Mair, 1969). Preliminary data on the number of large myelinated axons in the T13 medial nerve, the purely sensory cutaneous nerve innervating the medial 13th thoracic dermatome in the rat, suggest that there is a loss of 50% of the large myelinated fibers in aged rats. It is possible that in aged animals the axons may be lost as a consequence of receptor loss. This hypothesis could be tested directly by cauterizing all the touch domes in the T13 M field and then using histological stains that detect axon degeneration to trace the extent of axon loss and transsynaptic degeneration, if any, in both young and aged animals.

A loss of axons after dome extirpation might be due to deprivation of trophic factors secreted by non-neural receptor tissue within touch domes or a loss of electrical activity in the axon. There is a loss of sensory neurons after nerve section or amputation (Sica and McComas, 1978).

There is a discordance, however, in the estimated number of

touch dome receptors lost during ageing and the decrease in the estimated number of myelinated axons in the sensory nerve. Far more nerve fibers are lost than touch receptors, assuming that other touch receptors are lost at the same rate as touch domes. The effect of ageing on other touch receptors in the rat skin must be investigated to determine the source of the estimated loss of sensory axons in the sensory nerve.

An increase in threshold to touch with ageing has been reported by several clinicians (Axelrod and Cohen, 1961; Dyck et al., 1972; Ghent, 1971; Fagius and Wahren, 1981). This increase, though, cannot be correlated with a change in threshold in any one receptor or attributed unequivocally to a decrease in receptor density because individual receptors could not be identified in subjects and threshold was a subjective measurement in these studies. A recent clinical study by Fagius and Wahren (1981) found touch threshold in the forefinger to go from a mean value of 5.6 μm to 13.2 μm indentation from 21 years to 70 years of age. Individual receptors were again not identified. Subjective perceptual threshold was measured, and much variation in the responses to repeated stimulation of the same subject was found, probably due to changes introduced by the psychological state of the subject. In studies of the human cornea, an area with free nerve endings subserving touch, threshold to touch also increased beyond 50 years of age (Zobel, 1938; Jalavisto et al., 1951).

In recent experiments, Jarvilehto and co-workers (1981) were able to stimulate and record from single touch dome axons in man. The threshold of touch domes in hairy skin on the back of the hand was well

correlated with the perceptual threshold to touch that was reported. This work raises the possibility of performing psychophysical experiments with elderly subjects. The effects of different experimental manipulations, such as increased stimulation, on the threshold of touch domes could be measured.

Ronge (1943a) found a decrease in threshold on the wrists of elderly subjects which he attributed to a thinning of the skin on this part of the upper limb. This study, however, used a very small sample size of only 4 aged subjects, measured threshold subjectively, and did not use a statistical analysis. From the table (p. 347) in Ronge's report (1943a) it appears that the smallest stimulus (1.4 g/mm) elicits the most positive responses in the youngest group. It is possible that the threshold for pain (up to 6.3 g/mm) is lower in older subjects as Ronge argues but that the light touch threshold is lowest in the younger subjects. These results should be reexamined with a classification of the stimuli into intensities responsible for sensations of touch or pain. Possibly there is a decreased sensitivity to light touch simultaneously with an increased sensitivity to pain in old age.

Unfortunately, there are no studies of touch dome morphology in aged animals that could suggest a basis for the change seen in thresholds of aged rat touch domes. While Jackson (1977) reported that the dermis of aged human skin is thinned, Montagna (1965) and Whitton (1973) reported no consistent thinning of epidermis or dermis in old age. There are decreased amounts of elastin (Ma and Cowdry, 1950) and collagen (Pearce and Gummer, 1972) in aged skin.

The aged neural receptor mechanism appears to be less efficient at transducing a given stimulus than a young receptor. What could bring this about? One possibility is that the structure of the fine nerve terminal disc abutting the Merkel cell may degenerate, making the Merkel cell less capable of playing its role, if any, in transduction. A quantitative histological study of aged touch domes should be done to investigate this possibility.

On the cellular level many factors may contribute to the setting of the touch threshold. In a high threshold touch dome a large mechanical deformation may be needed because the magnitude of the generator potential produced by a given stimulus may be relatively small due to structural changes in the touch dome or changes in the transduction process. Alternatively, a larger generator potential might be needed to initiate an action potential in the zone of spike initiation at the nerve endings. This situation could be due to a change in the density of Na+ conductance channels in the membrane of the axon at the site of spike initiation. These possibilities are difficult to test due to the difficulty in recording from nerve endings in the touch domes. Whether the threshold change observed in the touch dome is due to changes in the Merkel cell or in the nerve ending is difficult to distinguish.

Perhaps the threshold changes observed in old age are a consequence of altered axoplasmic transport. The effects of ageing on axoplasmic transport have not been extensively investigated, though there are reports of a decreased transport of acetylcholinesterase (McMartin and O'Connor, 1979) and of glycoproteins in the nerves of

aged rats (Geinisman et al., 1977). There is also a slowing of slow transport in the axons of dorsal root ganglion cells of the rabbit (Komiya, 1980).

As the animal grows, fewer and fewer receptors are excited by a given stimulus and the threshold for excitation of those receptors is increased. As a consequence of growth and a fixed number of receptors there may be a graded functional deafferentation during ageing. The functional deafferentation initiated by body growth acts to decrease the net amount of electrical activity in the sensory nerve. Perhaps subsequent trophic interactions between touch dome receptor and primary sensory neuron may also be reduced as a result of decreased activity. The reduced interactions, electrical and trophic, may act to produce the degeneration in the sensory system seen during ageing.

In adults, deafferentation produces atrophic changes in the central nervous system. There is evidence in the olfactory system that atrophic changes centrally in the olfactory bulb may be secondary to degenerative changes in the peripheral receptors of the olfactory epithelium as the rat ages (Hinds and McNelly, 1980). In adult cats, visually deprived by bilateral eyelid suture, there was a shrinkage of the visual cortex of approximately 9%. This loss was of the same extent whether cats were visually deprived by eye-lid suture or by enucleation, indicating that lack of neuronal activity from the periphery may be a significant factor in cortical losses (Cragg, 1975). In the adult rat visual deprivation produced a reduction in neuronal numbers in visual cortex (Fifkova and Hassler, 1969).

Could the central changes in ageing be due to a loss of receptors, and of receptor activity peripherally? O'Connor et al. (1980) provide an indirect, yet intriguing answer to this question. They found an increased amount of dendritic branching in the somatosensory cortices of 660 day old rats who were housed socially. This result is in disagreement with others who report losses in dendritic branching in ageing, so O'Connor and co-workers (1981) suggest that the discrepancy between their results and others may be due to deprived versus enriched environments. Green et al. (1981) confirmed the above work by reporting increased dendritic density in aged rats exposed to an enriched environment.

Jacobson (1978) notes that the number and density of receptors in any particular area of the body is genetically determined and characteristic for that area. Indeed, the rat has a constant number of touch domes in the receptive fields of a single nerve at least until 7.5 months of age. The questions to be answered in the future are:

(1) To what extent is the loss of receptors and raised receptor thresholds in old age genetically determined and to what extent does environment play a role in these processes?

(2) What role does the loss of receptors in the periphery play in central losses seen in the aged nervous system?

(3) Will an increase in peripheral sensory activity prevent central losses?

B. Touch dome function after vincristine administration

Since the research for this study was to be conducted in the Faculty of Health Sciences at McMaster, it seemed appropriate at the outset to include some aspect which had significance for the understanding or management of illness. The choice of vincristine, as an agent which might be used to modify touch dome function, was made for the following reasons. This drug, and the related compound, vinblastine, were introduced into clinical medicine because of their effectiveness in the treatment of neoplasms. Soon after their introduction, however, it was recognized that a potentially serious neuropathy was an invariable complication of therapy. The neuropathy is the limiting factor in the clinical use of this otherwise very effective anti-neoplastic drug (Calabresi and Parks, 1980; Sterman and Schaumberg, 1980).

In patients treated with vincristine, the first evidence of neuropathy could be found within two weeks of starting treatment and consisted of a loss of tendon reflexes, followed by parasthesiae and, in severely affected patients, by weakness. Electromyographic studies have confirmed the presence of sensory and motor denervation; the fact that the impulse conduction velocities are little changed in surviving fibres indicates that the neuropathy is of the dying-back type and is not associated with primary demyelination. After cessation of therapy clinical recovery from the neuropathy was more complete than the restoration of electrophysiological function (Tobin and Sandler, 1968; Sandler et al., 1969; Bradley, et al.,1970; Casey et al., 1973).

In the present study, it was shown that, after the administration

of vincristine sulfate, touch dome density is unaffected, just as is the case after cutting the dorsal cutaneous nerve (Smith, 1967). Both nerve section (Zalewski, 1973) and colchicine administration (Sloan et al., 1980), however, caused fungiform taste buds to disappear. The reason for this difference in receptor behavior is not apparent. Possibly, structural changes in the Merkel cells of the touch dome occur as they do in the cat after denervation (English, 1974;1977a) but were not detected because quantitative electron microscopy was not performed in the rat.

There is a marked rise in the thresholds of the touch domes at a time when impulse propagation is unaffected. This change occurs much sooner than might have been anticipated on the basis of clinical observations and is fully reversible. As early as 24 hours after the administration of vincristine, the behavior of touch domes was clearly abnormal. Prolonged stimulation of the touch domes, instead of provoking well-sustained regular discharges, induced less regular impulse trains of lower frequency ( See Figure 22; p.110) Some touch domes could only discharge during the deforming transient and gave no response to the steady indentation. A few touch domes (3/37) were inexcitable both to mechanical pulses and to electrical stimuli applied directly to the touch dome. The elevation in threshold was maintained for at least two weeks but normal sensitivity was regained by third week after injection.

Measurements were also made of response latency, this being defined as the time elasping between the onset of the mechanical pulse and the arrival of the impulse at the recording electrode. The length

of nerve between the touch dome and electrode was not determined as the fine branches could not be visualized with certainty; the touch domes lay between 10 to 30 mm from the recording point and the nerve. It was found that as the amplitude of the mechanical pulse was increased, the response latency shortened. Despite the considerable change in threshold no significant difference in latency-shortening could be demonstrated between the domes in normal and vincristine treated animals when stimuli of threshold and twice threshold intensity were applied.

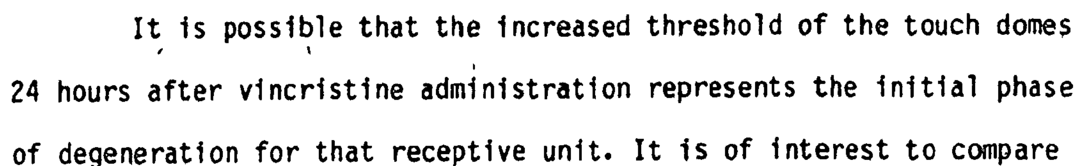
The ability of the Vinca alkaloids to produce degeneration of nerve fibers both in man and in experimental animals is well established (Bradley et al., 1970; Bradley and Williams, 1973; Gottschaldt et al., 1968; Schlaepfer, 1977; Uy et al., 1967). The present study has extended this work by showing that vincristine may cause a three-fold rise in threshold of touch domes without any significant increase in response latency. The interpretation of these observations is that the drug is capable of affecting the function of a sensory receptor before there is interference with impulse propagation in the associated nerve fibers.

The possibility exists that there are changes in the morphology of the nerve fiber 24 hours after vincristine administration that may not be detectable with light microscopy; further studies using electron microscopy would be necessary to resolve this question. Large doses of vincristine produced Wallerian degeneration of nerve fibers (Uy et al., 1967); however this degeneration was not detectable, using light microscopy, in the nerve fiber 24 hours after vincristine administration.

Although in another study, chromatolysis was present in the cells of the dorsal root ganglion 24 hours after vincristine administration (Uy et al., 1967).

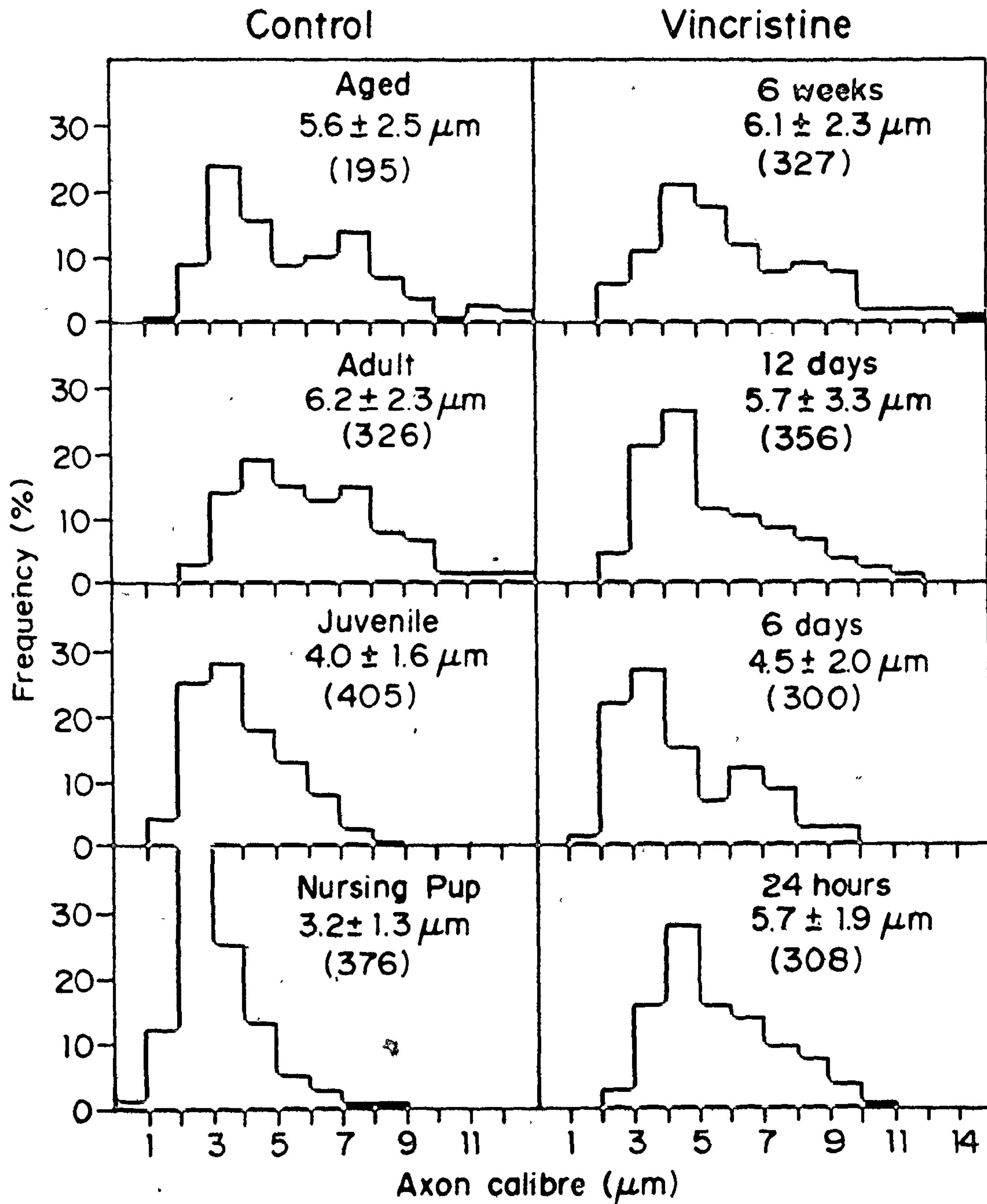
The only finding of the present study that is suggestive of an altered nerve structure at 24 hours after vincristine administration is the altered distribution of the nerve fiber diameters 24 hours after vincristine administration. In the adult and aged nerves the distribution of fiber diameters in the dorsal cutaneous nerve was bimodal (see Figure 28; P. 156). A bimodal distribution of nerve fiber diameters was reported for cutaneous nerves of the cat, (Eckholm, 1967; Skoglund and Romero, 1965) and human (Rexed, 1944). In contrast to the control nerve fiber diameters, the vincristine treated nerve fiber diameters were in a unimodal distribution. Possibly some alteration had occurred in the overall distribution of nerve fibers; however more nerves have to be examined using quantitative electron microscopy before any conclusions can be drawn.

The morphology of the nerve 24 hours after vincristine treatment was not pathological. Similar findings were reported in another study of the neurological effects of vincristine on rats (Uy et al., 1967). Both the physiological and the morphological data presented in the present study indicated that at 24 hours after vincristine administration the nerve is comparable to control nerve.



It is possible that the increased threshold of the touch domes 24 hours after vincristine administration represents the initial phase of degeneration for that receptive unit. It is of interest to compare

Figure 28. Histographic representations of the distribution of nerve fiber diameters of the T13M dorsal cutaneous nerve during development and after administration of vincristine sulfate. The upper right quadrant of each histogram contains the mean nerve fiber diameter ± 1 s.d. and the total number of fibers measured.


Control
Vincristine
Aged
5.6 ± 2.5 μm
(195)
6 weeks
6.1 ± 2.3 μm
(327)
Adult
6.2 ± 2.3 μm
(326)
12 days
5.7 ± 3.3 μm
(356)
Juvenile
4.0 ± 1.6 μm
(405)
6 days
4.5 ± 2.0 μm
(300)
Nursing Pup
3.2 ± 1.3 μm
(376)
24 hours
5.7 ± 1.9 μm
(308)
Frequency (%)
Axon calibre (μm)

the increased threshold after vincristine administration to the acute effects of denervation. When a touch dome is denervated by the cutting of the dorsal cutaneous nerve, threshold rises 24-28 hours post-denervation and nerve impulse transmission fails shortly after this (see Appendix 4). These results resemble the effects of denervation of a motor nerve in that the earliest effects are at the most peripheral points in the system (Miledi and Slater, 1967). A longitudinal study of individual high threshold touch domes after the administration of vincristine would be necessary to find out if the rise in threshold was the initial sign of fiber degeneration.

It is not possible from the present observations to attribute the rise in threshold to an alteration in the presumptive transducer action of the Merkel cells as opposed to one involving the impulse generating mechanisms in the adjacent terminal expansions of the sensory fibers. Electronmicrographs of touch domes, removed within one day of vincristine administration, might help to resolve this matter and will be the subject of a further study. Under the light microscope, however, the touch domes appeared normal at a time when thresholds were raised.

If it proves that changes in the terminal nerve plates are at least partly responsible for the raise in threshold, the the present findings would conform to those expected of a 'dying-back' type of neuropathy (Cavanaugh, 1964; Spencer and Schaumberg, 1974). The 'dying-back' type of neuropathy is a peripheral neuropathy characterized by degenerative changes that initially appear distally and progress in a proximal direction.

Inasmuch as the integrity of the nerve axon, including its terminal arborization, is dependent on the maintenance of axoplasmic transport (Grafstein and Forman, 1980), the present findings are not unexpected. Thus the Vinca alkaloids have been shown to promote the dissolution of microtubules following combination of the drug with tubulin protein; at a later stage the drug appears to encourage the proliferation of neurofilaments ( Creasy, 1975; Owellen et al.,1972; Wilson et al., 1974). Both structures, microtubules and neurofilaments, are considered to underly axoplasmic transport (Grafstein and Foreman, 1980) and interference of this process by vincristine might affect nerve endings before more proximal regions of the axons. Indeed this is a mechanism to explain the pathogensis of the 'dying-back' neuropathy caused by acrylamide (Spencer and Schaumberg, 1974). Cavanaugh (1964) advanced a similar explanation for tri-orthocresyl phosphate neurotoxicity.

Acrylamide, a neurotoxic chemical that produced a neuropathy of the 'dying-back' type, has been shown to affect the functioning of the Pacinian corpuscle, a mechanoreceptor present in mesentery (Spencer and Schaumberg, 1974; Spencer et al., 1977). Six hours to ten days after the administration of multiple injections of acrylamide, sensory transduction, represented by the presence of a generator potential after mechanical stimulation, had failed in 30/51 Pacinian corpuscles removed from feline mesentery. Acrylamide caused sensory transduction failure in Pacinian corpuscles at a time when no ultrastructural alterations could be observed in the features of the sensory nerve terminals or the corpuscle (Spencer et al., 1977).

Recent work by Sloan and co-workers (1980) has shown that colchicine has an effect similar to vincristine on taste receptors of the tongue, in terms of receptor threshold. If the nerve is left intact and a colchicine cuff is placed around it, there is an ipsilateral loss of fungiform taste buds and a decrease in taste responses. When radioactively labeled colchicine is administered, similar amounts of radioactivity are found in the ipsilateral and contralateral tongue, indicating that colchicine is acting on the nerve trunk and not directly on taste receptors to produce the effects seen (Sloan et al.,1980). Colchicine has been shown to interrupt axoplasmic transport (Karlsson and Sjostrand, 1969).

Vincristine is like colchicine and vinblastine, in that it inhibits mitosis and blocks components of fast transport (Hanson and Edstrom, 1978; Creasy, 1975). It is important to note that although vincristine reduced fast axoplasmic transport in cultured sympathetic neurons (England et al., 1973) and in cat vagus and sciatic nerve *in vivo* (Green et al., 1977; Ochs and Worth, 1975), the rates of fast and slow axoplasmic flow were not significantly altered by vincristine in a study done in sciatic nerves of neurotoxic cats (Bradley and Williams, 1973). While Bradley and Williams (1973) reported very little change in fast or slow axoplasmic flow, Green et al. (1977), using a slightly different method to measure transport, reported a change in fast transport in neurotoxic cats; they observed a partial blockage of fast transport and the appearance of a new fast-moving peak. Thus, vincristine can be shown to block fast transport *in vitro* but not reliably *in vivo*.

In morphological studies, vincristine has been shown to produce a disruption of neurotubules and an appearance of microtubular crystals within the axons when injected endoneurially into sural nerves of rats (Schlaepfer, 1977) or systemically into vagus nerve of cats (Green, 1977). It also caused accumulation of axoplasmic organelles and acetylcholinesterase activity, both above and below the site of injection (Schlaepfer, 1971). Similarly, vincristine therapy in patients has been shown to produce neurofibrillary degeneraton in nerve fibers (Shelanski and Wisniewski, 1969).

Apparently certain aspects of fast axoplasmic transport are affected by treatment with _Vinca_ alkaloids. If vincristine interfered with fast axoplasmic flow in the present study, it would have taken a minimum of 2-6 hours for the earliest effects to be observed in the touch domes; the changes in threshold were observed 24 hours after treatment. It is possible that there were effects before 24 hours had elapsed, this will be the subject of a further study. (Fast axoplasmic flow proceeds at a rate of 400 mm/day (Ochs, 1972) and the touch dome is 20-100 mm from the dorsal root ganglion cells).

Another _Vinca_ alkaloid, vinblastine, decreased the amounts of tubulin, the neurofilament protein triplet, and actin, transported by slow axoplasmic flow (Komiya and Kurokawa, 1980). During ageing the rates of slow axoplasmic flow of these same compounds are likewise affected (Komiya, 1980). Perhaps the threshold changes observed after vincristine treatment and ageing are both produced by changes in slow axoplasmic flow. If, in the present study, vincristine interfered with slow axoplasmic flow then the earliest changes would be expected

to occur by 4-6 days after administration of the drug . (Slow flow proceeds at a rate of 1-5 mm/day (Komiya and Kurokawa, 1978; Komiya, 1980) and the distance from the dorsal root ganglion cell to the touch domes was 20-100 mm.)

The threshold changes produced by vincristine were comparable to the increase in threshold seen in the aged animal. The high threshold state may be ascribed to an indirect effect on the touch dome or the fine nerve terminals abutting it mediated through axoplasmic transport or to a direct effect on these structures. The vincristine effect was reversible. The next step is to test for the reversibility of the aged effect, perhaps by increased stimulation of the receptor.

Alternatively it is possible that receptor dysfunction following vincristine treatment is due to the lack of an essential metabolite normally taken up from target tissue. In keeping with this possiblity are the recent observations that degeneration of sympathetic ganglia in developing rats following vinblastine administration may be prevented by the simultaneous administration of nerve growth factor. Normally nerve growth factor is transported to sympathetic neurons from target tissues by retrograde axoplasmic transport. When vinblastine was administered, a blockade of retrograde axoplasmic transport may have occurred and sympathetic ganglia atrophied. If nerve growth factor was administered concurrently with vinblastine the sympathetic ganglia were of normal size (Johnson, 1978; Menesini Chen et al., 1977).

The rapidity of the rise in touch dome threshold also deserves comment in view of the much later onset of the clinical features of a

peripheral neuropathy in patients receiving vincristine; typically 2-3 weeks elapse before a loss of tendon reflexes can be demonstrated (Casey et al., 1973). This discrepancy between human and animal findings is probably related to the different dose ratios; the therapeutic dose is usually 0.01-0.05 mg/kg in each weekly injection, with an increase of 0.01 mg/kg each week if necessary (Calabresi and Parks, 1980). The clinical neuropathy usually develops after a total dosage of 0.10-0.15mg/kg. The rats in this study received 0.75 mg/kg in a single dose. In a preliminary study, doses which were smaller (0.25 mg/kg), though larger than the total clinical dosage that produces a neuropathy, also gave rapid rises in receptor threshold (Leon and McComas, 1980).

It may also be observed that the change in receptor sensitivity probably occurred too soon to be explained by factors associated with the general debilitation of the vincristine treated animals. Despite the large dosage of vincristine in these experiments, it was usual for full recovery of receptor sensitivity to take place between the second and third weeks post-treatment. Uy and co-workers (1967) found that a similar amount of time was necessary for the morphological recovery of the dorsal root ganglion cells of rats receiving a similar dosage of vincristine. The clinical neuropathy is usually reversible in adults although the time course is variable (Calabresi and Parks, 1980).

Children treated with vincristine, however, may not experience a complete reversal of the neuropathy. The deep tendon reflexes remain absent and there is a prolongation of the sensory nerve conduction velocity that continues for months after vincristine treatment.

Sensation, however, was not permanently impaired (Lowitzsch et al.,1978). Perhaps the immature nerve fibers are more suseptible to permanent damage. Clearly, studies must be performed using immature animals to examine this problem.

The touch dome provides a very simple, yet sensitive system in which one can study, and ultimately, prevent, degenerative and neurotoxic changes in the peripheral nervous system. Animal experimentation can provide one key to understanding neural processes; companion, non-invasive clinical studies are also possible. The touch dome is exquisitely sensitive to mechanical deformation and this sensitivity reflects the integrity of neural function at a time when other measures do not. In view of the suseptibility of the touch dome to changes in threshold, following vincristine administration, it is possible that measurements of mechanical threshold made on identified touch domes in human skin could be useful in the monitoring of vincristine neurotoxicity. This aspect of touch dome function has not yet been exploited; it should be of use in future studies of neurotoxicity.

# V. CONCLUSIONS

1. An electrophysiological study has been made of mechanoreceptor function in touch domes (Type I mechanoreceptors) of rats during normal development. The receptors were studied in the hairy skin of the back using quantitative mechanical stimuli.

2. The number of touch domes within a nerve field remained constant from the pup (4 week old) stage to the adult animal (1½-6 months old) Since the animals grew during this period the density of the touch domes within an area of skin would be expected to decrease and this was shown experimentally.

3. The thresholds of touch domes were lowest in the youngest animals and highest in the aged animals (6-21 months old) .

4. During development the sizes of the touch domes increased such that the diameters were enlarged 1 3/4 times.

5. Because of the changes in size, density, and threshold of touch domes during development, the spatio-temporal pattern of impulse activity evoked in a cutaneous nerve by a given mechanical stimulus will differ between young, adult, and aged animals.

6. Touch dome function was also studied after the administration of the neurotoxic drug vincristine. A single dose of this drug resulted in increased thresholds of touch domes to mechanical stimulation within 24 hours. Within a further three weeks the touch dome thresholds had returned to control values. The density of the touch domes in the skin

was unaffected by vincristine treatment.

7. Twenty-four hours after vincristine administration the dorsal cutaneous nerve had a morphological appearance similar to control nerve, although the threshold to mechanical stimulation of the touch dome was raised. Measurements of response latency 24 hours after vincristine treatment indicated that nerve conduction was not impaired. Changes in threshold to mechanical stimulation could be explained by changes in the touch domes or the nerve terminal expansions abutting them.

8. Six days after vincristine treatment Wallerian degeneration was present in the nerve fibers, at a time when thresholds of touch domes were elevated.

9. The change in mechanical thresholds of touch domes induced by vincristine were completely reversed by three weeks after treatment although the morphological appearance of the nerve fibers at this time continued to be abnormal.

10. If changes in the touch dome or the nerve terminals a were responsible for the increased thresholds observed then the data collected support the hypothesis that vincristine produces a neuropathy of the 'dying-back' type.

# VI. APPENDIX

TABLE 4

RAW DATA OF EXPERIMENT 1; TOUCH DOME DENSITY

| Rat No. | Sex | Age | Weight | Domes/ $cm^2$ | $cm^2$ | No. of Domes Counted |
|---|---|---|---|---|---|---|
| Juvenile Group I Pre-Weaning (11-29d; 17g-65g) | | | | | | |
| 2 001 | F | 11d | 17.6g | 33.0 | 1 | 33 |
| 3 001 | F | 11d | 19.4g | 53.7 | 2 | 233 |
| 10 001 | F | 11d | 20.0g | 70.0 | 1 | 70 |
| 8 001 | M | 11d | 26.5g | 57.0 | 1 | 57 |
| 8 001 | F | 16d | 28.1g | 82.0 | 2 | 164 |
| 3 001 | M | 19d | 43.0g | 40.5 | 4 | 162 |
| 1 001 | F | 20d | 43.0g | 40.0 | 1 | 40 |
| 7a 001 | F | 20d | 29.7g | 40.0 | 2 | 80 |
| 8 001 | F | 20d | 32.7g | 46.0 | 2 | 92 |
| 11 001 | F | 20d | 31.6g | 45.0 | 4 | 180 |
| 5 001 | M | 22d | 54.3g | 40.0 | 1 | 40 |
| 1 Cont | M | 22d | 42.0g | 43.0 | - | --- |
| 19 E-1 | F | 26d | 63.2g | 24.8 | 4 | 99 |
| 4 Cont | F | 27d | 60.0g | 19.0 | 8 | 152 |
| 5 Cont | F | 29d | 65.0g | 23.8 | 16 | 380 |
| 9 Cont | F | 23d | 36.0g | 31.6 | 6 | 190 |
| $\bar{x}$ density = 43.1 domes/$cm^2$; sd = ±16.6; n = 16 | | | | | | 2,031 |
| Juvenile Group II Weaning to Maturity (30-45d; 66g-150g) | | | | | | |
| 2 16E | F | --- | 68.5g | 29.5 | 2 | 59 |
| 5 001 | M | 30d | 93.0g | 27.0 | 2 | 54 |

TABLE 4

| Rat | No. | Sex | Age | Weight | Domes/$cm^2$ | $cm^2$ | No. of Domes Counted |
|---|---|---|---|---|---|---|---|
| 13 | 001 | M | 31d | 77.8g | 29.3 | 3 | 88 |
| 14 | 001 | M | 31d | 76.4g | 31.0 | 2 | 63 |
| 15 | 001 | M | 31d | 73.7g | 29.5 | 2 | 59 |
| 16 | 001 | M | 31d | 66.3g | 34.0 | 4 | 136 |
| 17 | 001 | M | 31d | 84.7g | 25.6 | 3 | 77 |
| 3 | 001 | M | 30d | 93.8g | 23.0 | 7 | 161 |
| 7 | 001 | F | 32d | 69.8g | 30.5 | 4 | 122 |
| 16 | 001 | F | 32d | 69.5g | 28.0 | 6 | 168 |
| 17 | 001 | F | 32d | 75.1g | 26.5 | 4 | 106 |
| 18 | 001 | F | 32d | 74.4g | 29.0 | 6 | 174 |
| 6 | Cont | F | 33d | 84.0g | 18.3 | 24 | 439 |
| 10 | Cont | F | 35d | 80.0g | 22.5 | 13 | 293 |
| 11 | Cont | F | 37d | 96.0g | 16.7 | 13 | 217 |
| 2 | Cont | M | 32d | 75.0g | 24.4 | 5 | 122 |
| 11 | 001 | M | 40d | 126.3g | 13.0 | 2 | 26 |
| 9 | 001 | M | 40d | 142.4g | 14.0 | 3 | 42 |
| | $\bar{x}$ density = 24.8 domes/$cm^2$; sd = 6.2; n = 18 | | | | | | 2,346 |
| | Adult Group III (45d-6 months; 150g-250g) | | | | | | |
| A1 | Cont | F | 7W | 155.0g | 10.2 | 11 | 112 |
| 27 | 001 | F | 7W3d | 163.1g | 16.4 | 21 | 344 |

TABLE 4

| Rat No. | Sex | Age | Weight | Domes/ $cm^2$ | $cm^2$ | No. of Domes Counted |
|---|---|---|---|---|---|---|
| 8b Cont | F | 6W4d | 167.0g | 9.9 | 19 | 188 |
| 8c Cont | F | 7W3d | 167.0g | 14.2 | 6 | 85 |
| A8 Cont | F | 8W | 189.0g | 13.0 | 22 | 286 |
| 15 001 | F | 8W6d | 204.0g | 12.2 | 6 | 73 |
| 3 001 | F | 8W5d | 213.2g | 12.4 | 7 | 87 |
| 15 Cont | F | 14W5d | 220.0g | 10.0 | 18 | 180 |
| 23 E1 | F | 9W6d | 229.0g | 13.0 | 20 | 260 |
| 3 E1 | F | 8W4d | 205.3g | 9.7 | 6 | 58 |
| 1 Cont | F | 22W4d | 220.0g | 6.5 | 6 | 39 |
| 16 Cont | F | 15W5d | 250.0g | 7.5 | 2 | 15 |
| x density = 11.6 domes/$cm^2$; sd = 2.8; n = 12 | | | | | | 1,727 |
| Mature Group IVa (>6 months-1 year; >250-300 grams) | | | | | | |
| 2 Cont | F | 22W6d | 260.0g | 7.2 | 29 | 209 |
| 3 Cont | F | 23W1d | 270.0g | 7.8 | 18 | 140 |
| 12b Cont | F | 24W | 277.0g | 11.0 | 3 | 33 |
| 17 Cont | F | --- | 283.0g | 8.0 | 16 | 128 |
| 1 ES | F | --- | 258.0g | 8.3 | -- | --- |
| 2 DCN | F | --- | 255.0g | 11.0 | -- | --- |
| 2 ESZ | F | --- | 263.0g | 8.3 | -- | --- |
| 2 OLD | F | --- | 288.2g | 7.0 | 15 | 105 |
| 2 E1 | F | --- | 295.1g | 10.9 | 20 | 218 |

x = 8.8; sd = 1.7; s.e.m. 0.6; n= 9 (250-300 gm)

TABLE 4

| Rat No. | Sex | Age | Weight | Domes/ $cm^2$ | $cm^2$ | No. of Domes counted |
|---|---|---|---|---|---|---|
| Aged: Group IVb (1.0 year-1.75 years, 300-463 grams) | | | | | | |
| 1 E1 | F | | 300.0g | 11.5 | 20 | 230 |
| 21 E1 | F | | 328.0g | 7.4 | 30 | 222 |
| 1 OLD | F | 1yr9mo | 315.2g | 6.3 | 20 | 126 |
| 12 E-1 | F | | 347.9g | 10.4 | 20 | 209 |
| 1 OLD | F | 18mo | 384.5g | 6.7 | 12 | 81 |
| 11 001 | M | | 384.2g | 5.5 | 2 | 11 |
| 9 001 | M | | 462.5g | 6.2 | 12 | 74 |
| 3 OLD | F | 1yr9mo | 388.2g | 6.4 | 12 | 77 |
| x density = 7.6 domes/$cm^2$; sd = 2.2; n = 8 | | | | | | 1,907 |

NOTE: Total - 63 rats; 7,996 domes

TABLE 6

RAW DATA OF EXPERIMENT 1; TOUCH DOME SIZE

| Rat No. | | Age | Weight (gm) | Dome size (μ) (± s.d.) | No. of Domes |
|---|---|---|---|---|---|
| 19 *F* E1 | pup | 26d | 63.2g | 147μ ± 26 | 10 |
| 26 *F* E14 | pup | 26d | 53.6g | 194μ ± 29 | 10 |
| 16 *F* E1 | juvenile | 43d | 95.4g | 223μ ± 40 | 10 |
| 17 *F* E1 | juvenile | 44d | 118.7g | 216μ ± 69 | 11 |
| 14 *F* E1 | adult | 60d | 184.0g | 345μ ± 60 | 10 |
| 15 *F* E1 | adult | 61d | 216.1g | 336μ ± 57 | 9 |
| 23 *F* E1 | adult | 69d | 229.0g | 283μ ± 29 | 10 |
| 25 *F* E1 | adult | 70d | 193.1g | 294μ ± 43 | 10 |
| 1 *F* OLD | aged | 18mos. | 384.5g | 280μ ± 51 | 10 |
| 2 *F* OLD | aged | 21mos. | 379.2g | 326μ ± 54 | 10 |
| | | | | | 100 |

NOTE: *F* = Female.

TABLE 7

CALCULATION OF THE DOME SENSITIVE AREA WITHIN A SQUARE CENTIMETER OF SKIN

| Group | Nursing Pup | Juvenile | Adult | Aged |
|---|---|---|---|---|
| Dome diameter ($\bar{x} \pm$ sem) | $170\mu \pm 8\mu$ | $219\mu \pm 12\mu$ | $314\mu \pm 9\mu$ | $302\mu \pm 12\mu$ |
| Domes/cm$^2$ ($\bar{x} \pm$ sem) | $43 \pm 4$ | $25 \pm 1$ | $12 \pm 1$ | $8 \pm 0.5$ |
| Sensitive area | $976,014\mu^2$ | $941,700\mu^2$ | $929,244\mu^2$ | $573,048\mu^2$ |

1. Total area of domes within 1 cm$^2$ = sensitive area.

2. [Number of domes/cm$^2$] x [Surface area of domes] = sensitive area.

3. [Number of domes/cm$^2$] x [$\pi r^2$] = sensitive area.

For Nursing Pups:
4. [43] x [$\pi$][85]$^2$ = $976,014\mu^2$

$\approx 10^6\mu^2$ = 1 mm$^2$ dome sensitive area on 1 cm$^2$ of skin

For Juveniles:
5. [25] x [$\pi$][109.5]$^2$ = $941,700\mu^2$

For Adults:
6. [12] x [$\pi$][157]$^2$ = $929,244\mu^2$

For Aged:
7. [8] x [151]$^2$ = $573,048\mu^2$

$\approx 0.5 \times 10^6\mu^2$ = 0.5 mm$^2$ dome sensitive area on 1 cm$^2$ of skin

TABLE 8

REPEATED MEASURES OF THE SKIN RECEPTIVE FIELD AREA OF T13M NERVE

| Animal # | Age | Weight | # of Domes | Area of T13 M Field |
|---|---|---|---|---|
| | | GROUP I | | |
| 1 | 23d | 45 grams | 38 | 65.9 $mm^2$ * |
| | 43d | 117 | 30 | 138.8 $mm^2$ * |
| | 60d | 162 | 36 | 177.0 $mm^2$ * |
| 2 | 24d | 46 | 24 | 40.8 $mm^2$ |
| | 40d | 120 | 24 | 88.2 $mm^2$ |
| | 60d | 173 | 23 | 123.0 $mm^2$ |
| 3 | 24d | 51 | 24 | 44.7 $mm^2$ |
| | 43d | 123 | 17 | 87.5 $mm^2$ |
| | 60d | 184 | -- | 119.7 $mm^2$ |
| 4 | 25d | 50 | 32 | 42.1 $mm^2$ |
| | 43d | 119 | -- | 87.5 $mm^2$ |
| | 60d | 171 | 29 | 131.6 $mm^2$ |
| 5 | 26d | 57 | 23 | 38.2 $mm^2$ |
| | 43d | 129 | -- | 62.5 $mm^2$ |
| | 60d | 179 | 18 | 122.4 $mm^2$ |
| 6 | 26d | 47 | 20 | 39.5 $mm^2$ |
| | 43d | 100 | 18 | 70.4 $mm^2$ |
| | 60d | 150 | 18 | 103.3 $mm^2$ |

mean ± sd (n) T 13 M field size at 23-26d = 41.1 $mm^2$ ± 2.5 (n = 5)
field size at 40-43d = 79.2 $mm^2$ ± 12.0 (n = 5)
field size at 60d = 120 $mm^2$ ± 10.3 (n = 5)

| Animal # | Age | Weight | # of Domes | Area of T13 M Field |
|---|---|---|---|---|
| | | GROUP II | | |
| 7 | 80d | 192 grams | 14 | 155.9 $mm^2$ |
| | 7.5 mos. | 320 | 14 | 175.7 $mm^2$ |
| 8 | 80d | 189 | 14 | 145.4 $mm^2$ |
| | 7.5 mos. | 299 | 14 | 182.2 $mm^2$ |
| 9 | 80d | 206 | 19 | 190.8 $mm^2$ |
| | 7.5 mos. | 297 | 20 | 230.9 $mm^2$ |
| 10 | 80d | 200 | 23 | 197.4 $mm^2$ |
| | 7.5 mos. | 260 | 27 | 271.7 $mm^2$ |

($mean_n$ ± sd) field size at 80 days = 172.3 $mm^2$ ± 25.7 (n = 4)
field size at 7.5 months = 215.1 $mm^2$ ± 45.0 (n = 4)

Table 8

| Animal # | Age | Weight | # of Domes | Area of T13 M Field | |
|---|---|---|---|---|---|
| | | GROUP III | | | |
| 11 | 1 yr. 8 mos. | 288g | 15 | 188.2 $mm^2$ | Rats over 250 gm |
| 12 | 1 yr. 10 mos. | 388g | 24 | 216.5 $mm^2$ | |
| 13 | --- | 383g | 20 | 203.3 $mm^2$ | |
| 14 | --- | 427.6g | 18 | 146.7 $mm^2$ | |
| 15 | --- | 54.5g | 12 | 30.3 $mm^2$ | |

mean ± sd (n) field size in rats over 250 gm = 188.7 $mm^2$ ± 30.3 (n = 4)

*T13 M + L

TABLE 9

SIZE OF T13 MEDIAL FIELD AT DIFFERENT AGES

| 23-26 Days | 43 Days | 60 Days | 80 Days | 7.5 Months/ Over 250 gm | 1.5 years |
|---|---|---|---|---|---|
| 40.8 | 88.2 | 123.0 | 155.9 | 175.7 | 188.2 |
| 44.7 | 87.0 | 119.7 | 145.4 | 182.2 | 216.5 |
| 42.1 | 87.5 | 131.6 | 190.8 | 230.9 | 203.3 |
| 38.2 | 62.5 | 122.4 | 197.4 | 271.7 | 146.7 |
| 39.5 | 70.4 | 103.3 | --- | --- | --- |
| x=41.1 | x=79.2 | x=120.0 | x=172.3 | x=215.1 | x=188.7 |
| sd= 2.5 | sd=12.0 | sd= 10.3 | sd= 25.7 | sd= 45.0 | sd= 30.3 |
| n= 5 | n= 5 | n= 5 | n= 4 | n= 4 | n= 4 |
| $p < 0.005$ | $p < 0.005$ | $p < 0.005$ | $p < 0.1$ | n.s. | |
| $t = 6.45$ | $t = 6.30$ | $t = 6.0$ | $t = 1.6$ | $t = 0.974$ | |

TABLE 10

SIZE OF T13 MEDIAL NERVE FIELD VERSUS WEIGHT OF ANIMAL
(N = 28)

| Age | Weight (grams) | Field Size ($mm^2$) |
|---|---|---|
| 24d | 46.9 | 40.8 |
| 26d | 47.5 | 39.5 |
| 25d | 50.5 | 42.1 |
| 24d | 51.0 | 44.7 |
| --- | 54.5 | 30.3 |
| 26d | 57.7 | 38.2 |
| 43d | 100.2 | 70.4 |
| 43d | 119.3 | 87.5 |
| 40d | 120.0 | 88.2 |
| 43d | 123.8 | 87.5 |
| 43d | 129.0 | 62.5 |
| 60d | 150.0 | 103.3 |
| 60d | 171.0 | 131.6 |
| 60d | 173.5 | 123.0 |
| 60d | 179.0 | 122.4 |
| 60d | 184.8 | 119.7 |
| 80d | 189.0 | 145.4 |
| 80d | 192.1 | 155.9 |
| 80d | 200.0 | 197.4 |
| 80d | 206.3 | 190.8 |
| 7½ mos. | 260.0 | 271.7 |
| 1 yr. 8 mos. | 288.0 | 188.2 |
| 7½ mos. | 299.0 | 182.2 |
| 7½ mos. | 297.3 | 230.9 |
| 7½ mos. | 320.0 | 175.7 |
| --- | 383.0 | 203.3 |
| 1 yr. 10 mos. | 388.0 | 216.5 |
| --- | 427.6 | 146.7 |

TABLE 11

RESULTS OF ANALYSIS OF VARIANCE ON REPEATED MEASURES ON THE SAME SUBJECT

| RAT # | 23-26 Days | 40-43 Days | 60 Days |
|---|---|---|---|
| 5 | 38 domes | 30 | 36 |
| 6 | 24 | 24 | 23 |
| 10 | 20 | 18 | 18 |

| SOURCE | SUM OF SQUARES | DEGREES OF FREEDOM | MEAN SQUARE | F | P |
|---|---|---|---|---|---|
| Total | 440 | 8 | / | ...... | ... |
| subjects | 402 | 2 | / | ...... | ... |
| treatments | 16.7 | 2 | 8.3 | 1.5625 | NS* |
| error | 21.3 | 4 | 5.3 | ...... | ... |

*There is no statistically significant difference in the number of domes counted in the nerve field of a given rat at 23-26 days of age, 40-43 days of age, and 60 days of age.

TABLE 12

SYMMETRY IN TOUCH DOME DENSITIES

Dome density per $cm^2$ was measured on three female rats from 1.0 cm below the level of the last rib. An area of 12 $cm^2$ on each rat was tested, 6 $cm^2$ on each side. The grids below are a graphic representation of the densities obtained from each rat's back. The variation in dome density from the left side of the back to the right side of the back was compared after a mean value for the right and left sides of each rat was obtained.

DOME DENSITY/$cm^2$

*[Rostral]*

↑

*Midline of back*

Rat #1 E-1 Weight: 300gm

Right $\bar{x}$ dome density = 11.5 vs Left $\bar{x}$ dome density = 11.0

| Left | | Right | |
|---|---|---|---|
| ... | ... | ... | ... |
| 9 | 14 | 12 | 11 |
| 9 | 13 | 11 | 10 |
| 12 | 12 | 12 | 10 |

→ *Last rib*

*[Caudal]*

(Each square = 1 $cm^2$)

Rat #12 E-1 Weight: 347.9gm

Right $\bar{x}$ dome density = 10.8 vs Left $\bar{x}$ dome density = 11.3

| Left | | Right | |
|---|---|---|---|
| ... | ... | ... | ... |
| 13 | 15 | 12 | 12 |
| 8 | 12 | 12 | 12 |
| 9 | 8 | 10 | 10 |

Rat #21 E-1 Weight: 328gm

Right $\bar{x}$ dome density = 7.5 vs Left $\bar{x}$ dome density = 9.2

| Left | | Right | |
|---|---|---|---|
| ... | ... | ... | ... |
| 4 | 10 | 11 | 8 |
| 5 | 10 | 9 | 8 |
| 7 | 9 | 12 | 7 |

TABLE 12

SUMMARY

The mean dome density per $cm^2$ for each side for each rat was averaged and the mean value for right and left dome density per $cm^2$ for all the rats was compared. There is no right side to left side variation in dome density, since the value for Student's $t$ was not significant.

DOME DENSITY PER $cm^2$: LEFT SIDE TO RIGHT SIDE VARIATION

| | $\bar{x}$ Right vs | $\bar{x}$ Left |
|---|---|---|
| Rat #1 | 11.5 | 11.0 |
| Rat #12 | 10.8 | 11.3 |
| Rat #21 | 7.5 | 9.2 |

AVERAGE FOR ALL RATS

| $\bar{x}$ Right | $\bar{x}$ Left |
|---|---|
| $\bar{x}$ = 9.9 | $\bar{x}$ = 10.5 |
| sem = 1.24 | sem = 0.66 |
| # $cm^2$ = 18 | # $cm^2$ = 18 |

$t$ = 1.64
df = 34
[NS]

For each rat, the 3 $cm^2$ abutting midline on the left and right sides was used to obtain the average <u>medial</u> dome density. This average medial dome density included the medial 6 $cm^2$ on each rat. The mean <u>lateral</u> dome density for each rat was obtained from the lateral 3 $cm^2$ on each side. Each mean lateral dome density included a total of 6 $cm^2$. The average medial dome density for all the rats was compared to the average lateral dome density for all the rats. Dome density is significantly higher medially ($p < 0.025$).

DOME DENSITY PER $cm^2$

| | Lateral | Medial |
|---|---|---|
| $\bar{x}$: | 9.1 | 11.3 |
| sem: | 0.576 | 0.419 |
| # $cm^2$: | 18 | 18 |

$t$ = 3.7371
df = 34
$p < 0.025$

TABLE 14

TOUCH DOME THRESHOLDS

| Rat # | Age | Weight | Threshold | Latency @ Threshold | Latency @ Twice Threshold | # of Units |
|---|---|---|---|---|---|---|
| GROUP I *Nursing Pups* <65 grams; <28 days; $\bar{x}$ = 3.21μ/ms sd = ±1.96μ/ms sem = ±0.65μ/ms n = 3 rats n = 9 domes | | | | | | |
| 20 E1 | 26d | 55.1g | 1.1 μ/ms | | | |
| | | | 2.2 | | | |
| 5 E1 | 27d | 65.0g | 5.5 | | | |
| | | | 7.2 | | | |
| 26 E14 | 26d | 53.6g | 3.8 | 6.0ms | 2.0ms | 1 |
| | | | 2.6 | 2.8-3.1 | | 3 |
| | | | 2.0 | 3.0 | 2.0 | 3 |
| | | | 2.5 | 4.0-8.0 | 3.5 | 5 |
| | | | 2.0 | 4.0 | | 5 |
| GROUP II *Juvenile* 65-150 grams; wks-1½ mos; $\bar{x}$ = 5.05μ/ms sd = ±0.83μ/ms sem = ±0.26μ/ms n = 3 rats n = 10 domes | | | | | | |
| 16 E1 | 43d | 95.4g | 5.2μ/ms | 2.0-3.0ms | | 3 |
| | | | 5.6 | | | 1 |
| | | | 6.0 | | | 1 |
| 1 J31 | 39d | 138.0g | 5.5 | 2.5-3.5 | | |
| | | | 6.2 | 2.1-2.3 | | |
| | | | 4.0 | 3.0-3.2 | | |
| | | | 5.0 | 2.4-2.6 | | |
| 2 J31 | 39d | 109.0g | 5.0 | 3.0-3.4 | | |
| | | | 3.8 | 2.9-3.1 | | |
| | | | 4.2 | 2.8-3.2 | | |

TABLE 14

THRESHOLDS--Continued

| Rat # | Age | Weight | Threshold | Latency @ Threshold | Latency @ Twice Threshold | # of Units |
|---|---|---|---|---|---|---|
| GROUP III *Adult* 150-250 grams; 1½-6 months; $\bar{x}$ = 5.23μ/ms sd = ±2.04μ/ms sem = ±0.33μ/ms n = 19 rats n = 38 domes | | | | | | |
| 8 DD1 | 45d | 150.0g | 4.1 μ/ms | 3.0-4.2ms | | 1 |
| 16 DD1 | 68d | 191.8g | 3.3 | 3.0 | 2.2ms | 4 |
| 3 DD1 | 70d | 212.7g | 4.6 | 4.5-5.5 | 2.8 | 6 |
| | | | 4.4 | 3.0-6.0 | | 2 |
| 2 E1 | 52d | 191.3g | 2.7 | 2.0.3.0 | | 1 |
| 14 E1 | 60d | 184.7g | 5.6 | 1.8-2.2 | 1.5-2.2 | 2 |
| | | | 5.5 | 2.5-3.0 | | 2 |
| | | | 3.8 | 2.0-3.0 | 2.0 | 2 |
| 15 E1 | 61d | 216.1g | 6.0 | 2.0-3.0 | 3.0 | 3 |
| | | | 5.5 | 2.0-2.5 | | 3 |
| | | | 9.2 | | | 1 |
| 23 E1 | 69d | 229.0g | 3.5 | 1-10.0 | 1.0 | 1 |
| 24 E1 | 70d | 173.0g | 5.0 | | 1.0 | 1 |
| 24 E1PM | 70d | 173.0g | 3.0 | | 1.6 | 2 |
| | | | 3.2 | | 2.4 | 1 |
| | | | 3.3 | 3.0 | 1.8 | 1 |
| | | | 4.4 | 3.0 | 2.0 | 1 |
| | | | 3.3 | 3.4 | 2.0 | 2 |
| | | | 3.3 | | | 1 |
| 25 E1 | 70d | 193.0g | 6.4 | | | 1 |
| 2 | | 233.0g | 7.0 | 3.0 | | 3 |
| | | | 7.8 | | | |

TABLE 14

THRESHOLDS-- Continued

| Rat # | Age | Weight | Threshold | Latency @ Threshold | Latency @ Twice Threshold | # of Units |
|---|---|---|---|---|---|---|
| N1#1 | | 250.0g | 5.0 μ/ms | 3.2ms | 2.0ms | 4 |
| #1 NL | | 239.0g | 7.8 | 2.8-3.8 | 2.3-3.0 | 3 |
| 1 J13 | | 163.0g | 5.0 | 3.0-3.6 | | 3 |
| | | | 5.6 | | | |
| J19#1 | | 177.3g | 6.2 | 3.2 | 2.2 | 3 |
| | | | 3.7 | 2.8-3.1 | 2.0 | |
| | | | 7.5 | 3.6-4.7 | | |
| J15N15 | | 235.0g | 1.3 | 2.4 | 1.4 | 2 |
| | | | 6.2 | 2.4 | 2.1 | |
| | | | 10.0 | 3.4 | 1.6 | 2 |
| J14N13 | 2mos | 159.0g | 7.8 | 4.0-4.5 | 2.9 | |
| | | | 3.7 | 2.9 | 2.5 | |
| 3N1J14 | | 239.0g | 3.7 | 4.0-6.0 | 2.4 | |
| | | | 10.0 | 4.0 | 1.5 | |
| 13J2N1 | | 165.0g | 5.0 | 3.0 | | |
| | | | 5.0 | 3.0 | 2.4 | |

GROUP IV *Aged* >250 grams; >6 months; $\bar{x}$ = 9.19μ/ms; sd = ±5.88μ/ms sem = ±1.28μ/ms n = 7 rats n = 21 domes

| Rat # | Age | Weight | Threshold | Latency @ Threshold | Latency @ Twice Threshold | # of Units |
|---|---|---|---|---|---|---|
| 6 E1 | | 319.0g | 6.4 μ/ms | 2.0-4.0ms | | 1 |
| 10 E1 | | 303.1g | 14.6 | 3.0-5.0 | 3.0ms | 2 |
| 13 E1 | | 376.8g | 13.7 | 3.0-4.0 | 2.0 | 4 |
| | | | 13.4 | 2.5-4.0 | 2.2 | 2 |
| 1 OLD | 18mos | 384.59 | 7.0 | | | 1 |
| | | | 9.5 | | | 1 |

TABLE 14

THRESHOLDS--Continued

| Rat # | Age | Weight | Threshold | Latency @ Threshold | Latency @ Twice Threshold | # of Units |
|---|---|---|---|---|---|---|
| 2 OLD | 21mos | 379.2g | 5.9 | 2.4ms | 1.5ms | 3 |
| | | | 6.0 | 8.0 | 2.6 | 1 |
| | | | 11.0 | 3.6 | 3.2 | 1 |
| | | | 2.6 | 2.8 | | 3 |
| | | | 9.2 | 2.3 | | 4 |
| | | | 3.5 | 4.6 | | 2 |
| | | | 4.4 | 3.0-3.2 | 2.6 | 2 |
| | | | 5.3 | 4.0-5.0 | 4.2 | 1 |
| | | | 5.0 | | | 3 |
| 1 | | 287.0g | 6.6 | 5.0 | | 5 |
| | | | 8.5 | 4.0 | | 4 |
| | | | 10.3 | 5.0 | | 4 |
| 30#1OLD | | 328gm | 12.5 | 3.5-3.6 | 2.6 | 4 |
| | | | 30. | 3.5-4.5 | | |
| | | | 7.5 | 3.5 | 2.8 | 5 |

TABLE 18

TOUCH DOME DENSITY AFTER ADMINISTRATION OF VINCRISTINE SULFATE

| Rat # | Weight (grams) | Dome Density (per $cm^2$) | # of Domes Counted |
|---|---|---|---|
| VC 1 | 237g | 15.5 | 130 |
| VC 2 | 253g | 11.9 | 189 |
| VC 3 | 218g | 9.2 | 112 |
| VC 4 | 258g | 8.9 | 120 |
| VC 5 | 232g | 9.5 | 115 |
| VC 6 | 229g | 12.0 | 97 |
| Control 7 | 245.5g | 10.6 | 111 |
| Control 8 | 253g | 10.5 | 105 |
| Control 9 | 234g | 9.4 | 127 |

VC = Vincristine

TABLE 19

TOUCH DOME THRESHOLDS AFTER ONE (0.75mg/kg) INJECTION OF VINCRISTINE

| Rat # | Weight | Threshold | Latency @ Threshold | Latency @ Twice Threshold* | Number of Units |
|---|---|---|---|---|---|
| | | GROUP I One Day Post-Injection | | | |
| 6 F 3 | 193g | 16.2μ/ms | | | 1 |
| 13 C 6 | 205g | 12.1μ/ms | 2.2-3.2ms | 2.5ms | 2 |
| | | 7.8μ/ms | 3.0-3.2ms | 1.8ms | 2 |
| | | 12.1μ/ms | 2.0-2.4ms | 2.0ms | 4 |
| VCTD 1 | 185g | 20.0μ/ms | 2.5-2.7ms | 2.0ms | 3 |
| | | 45.0μ/ms | 3.8ms | 3.6ms | 2 |
| | | 15.0μ/ms | 2.2-2.4ms | 2.0ms | 2 |
| VCTD 1A | 213g | 17.5μ/ms | 3.0-5.0ms | 2.2ms | 2 |
| | | 7.5μ/ms | 5.0-5.5ms | 2.8ms | 2 |
| | | 8.75μ/ms | 4.2-5.6ms | 2.1ms | 2 |
| VCTD 1B | 151g | 7.5μ/ms | 4.2-5.2ms | 2.8ms | - |
| | | 12.5μ/ms | 3.0-5.0ms | 2.0ms | 1 |
| | | 12.5μ/ms | 4.8ms | 3.2ms | 3 |
| | | 8.8μ/ms | 3.0-4.5ms | 2.2ms | 3 |
| | | 13.8μ/ms | --- | --- | - |
| | | 15.0μ/ms | --- | --- | - |
| | | 8.8μ/ms | --- | --- | - |
| | $\bar{x}$ = | 14.2μ/ms | 3.7ms | 2.4ms | 2.2 |
| | sd = | 8.8μ/ms | 1.0ms | 0.5ms | 0.8 |
| | sem = | 2.1μ/ms | 0.3ms | 0.2ms | 0.2 |
| | n = | 17 domes | 13 domes | 13 domes | 13 domes |
| | n = | 5 rats | 4 rats | 4 rats | 5 rats |
| | | GROUP II Seven Days Post-Injection | | | |
| 8 F 3 | 132g | 13.4μ/ms | | | |
| | | 8.8μ/ms | | | |
| | | 22.0μ/ms | | | |
| | | 18.0μ/ms | | | |
| | | 21.5μ/ms | | | |
| | | 10.5μ/ms | | | |

*Latency at twice threshold or at maximum stimulus, 30 μ/ms.

TABLE 19

| Rat # | Weight | Threshold | Latency @ Threshold | Latency @ Twice Threshold* | Number of Units |
|---|---|---|---|---|---|
| VCD 7 | 158g | 15.0μ/ms | 2.9ms | 2.2ms | 2 |
| | | 6.2μ/ms | 4.2-5.2ms | 4.2ms | 3 |
| | | 12.5μ/ms | 4.0ms | 2.3ms | 3 |
| | | 17.5μ/ms | 3.0ms | 2.1ms | 3 |
| VC 8 | 185g | 12.5μ/ms | 2.0-3.2ms | | |
| | | 10.0μ/ms | 2.0-3.2ms | | |
| | | 10.0μ/ms | 2.5-3.0ms | | |
| VC 9 | 158g | 8.8μ/ms | 1.8-2.0ms | 1.4ms | 3 |
| | | 18.8μ/ms | 2.4-2.9ms | | |
| | | 12.5μ/ms | 1.8-2.4ms | | |
| | $\bar{x}$ = | 13.6μ/ms | 2.9ms | 2.4ms | 2.8 |
| | sd = | 4.7μ/ms | 0.8ms | 1.0ms | 0.4 |
| | sem = | 1.2μ/ms | 0.3ms | 0.5ms | 0.2 |
| | n = | 16 domes | 10 domes | 5 domes | 5 domes |
| | n = | 4 rats | 3 rats | 2 rats | 2 rats |
| GROUP III Fourteen Days Post-Injection | | | | | |
| 10 C 5 | 227g | 13.4μ/ms | 2.0-5.2ms | | 2 |
| | | 13.4μ/ms | 1.8-2.5ms | | 2 |
| | | 9.8μ/ms | | | 1 |
| | | 25.0μ/ms | | | |
| VCD 14 | 178g | 12.5μ/ms | 2.4ms | 1.6ms | 3 |
| | | 6.3μ/ms | 2.0-3.0ms | 1.8ms | |
| | | 7.5μ/ms | 3.0ms | 1.8ms | |
| | | 8.8μ/ms | 2.6-2.9ms | 2.0ms | |
| VC 15 | 186g | 11.9μ/ms | 2.8-3.8ms | 2.0ms | |
| VC 16 | 233g | 25.0μ/ms | | | |
| | | 18.8μ/ms | 2.2ms | | |
| | | 17.5μ/ms | 2.2ms | | |
| | | 21.9μ/ms | 3.3ms | | |
| | $\bar{x}$ = | 14.7μ/ms | 2.7ms | 1.8ms | 2.0 |
| | sd = | 6.4μ/ms | 0.5ms | 0.2ms | 0.8 |

*Latency at twice threshold or at maximum stimulus.

TABLE 19

| Rat # | Weight | Threshold | Latency @ Threshold | Latency @ Twice Threshold* | Number of Units |
|---|---|---|---|---|---|
| | | sem = 1.8μ/ms | 0.2ms | 0.07ms | 0.4 |
| | | n = 13 domes | 10 domes | 5 domes | 4 domes |
| | | n = 4 rats | 4 rats | 2 rats | 2 rats |
| GROUP IV Twenty-one Days Post-Injection | | | | | |
| VCTD 21 | 195g | 3.1μ/ms | 4.0ms | | 1 |
| | | 5.0μ/ms | 3.0-4.5ms | 2.0ms | |
| VC 22 | 195g | 5.0μ/ms | 2.7-3.2ms | 2.1ms | 3 |
| | | 1.6μ/ms | 4.2ms | 1.5ms | 2 |
| | | 6.3μ/ms | 6.0ms | 2.5ms | 1 |
| | | 2.5μ/ms | 5.0ms | | |
| | | 6.3μ/ms | 4.5ms | | |
| VC 23 | 200g | 5.0μ/ms | 2.5-3.5ms | | |
| | | 1.5μ/ms | | | |
| | | 6.25μ/ms | | | |
| | | $\bar{x}$ = 4.2μ/ms | 4.2ms | 2.0ms | 1.8 |
| | | sd = 1.9μ/ms | 1.0ms | 0.4ms | 1.0 |
| | | sem = 0.6μ/ms | 0.4ms | 0.2ms | 0.5 |
| | | n = 10 domes | 8 domes | 4 domes | 4 domes |
| | | n = 3 rats | 3 rats | 2 rats | 2 rats |
| GROUP V Twenty-eight Days Post-Injection | | | | | |
| VCTD 28 | 210g | 2.5μ/ms | 5.0ms | | |
| | | 3.8μ/ms | 4.0ms | | |
| | | 1.5μ/ms | 3.0ms | | |
| VC 29 | 228g | 12.5μ/ms | 3.8-4.5ms | | |
| | | 6.5μ/ms | 4.0-5.2ms | 2.5ms | 2 |
| | | 5.0μ/ms | 2.8-3.5ms | 2.0ms | 1 |
| | | 3.8μ/ms | 5.0-6.0ms | | 1 |
| | | 5.0μ/ms | 4.0-6.0ms | | 1 |
| VC 30 | 200g | 6.3μ/ms | 3.0-4.0ms | | 1 |
| | | 3.8μ/ms | 2.5-3.0ms | | 1 |

*Latency at twice threshold or at maximum stimulus.

TABLE 19

| Rat # | Weight | Threshold | Latency @ Threshold | Latency @ Twice Threshold* | Number of Units |
|---|---|---|---|---|---|
| | $\bar{x}$ = | 5.0µ/ms | 4.1ms | 2.3ms | 1.2 |
| | sd = | 3.0µ/ms | 0.9ms | | 0.4 |
| | sem = | 1.0µ/ms | 0.3ms | | 0.2 |
| | n = | 10 domes | 10 domes | 2 domes | 6 domes |
| | n = | 3 rats | 3 rats | 1 rat | 2 rats |
| GROUP VI Forty-two Days Post-Injection | | | | | |
| VCD 42 | 244g | 3.8µ/ms | 2.5-3.0ms | 2.0ms | 2 |
| | | 5.0µ/ms | 3.0-5.0ms | 3.0ms | 2 |
| | | 5.0µ/ms | 2.0-5.0ms | 3.0ms | 1 |
| VC 43 | 246g | 5.0µ/ms | 2.5-3.2ms | 2.2ms | 3 |
| | | 10.0µ/ms | 5.2-5.5ms | 4.8ms | 2 |
| VC 44 | 200g | 5.0µ/ms | 3.0-6.0ms | | |
| | | 2.5µ/ms | 2.5-4.5ms | | |
| | | 3.8µ/ms | 2.0-4.0ms | | |
| | | 6.3µ/ms | 2.0-4.0ms | | |
| | | 3.8µ/ms | 2.5-3.0ms | | |
| | $\bar{n}$ = | 5.0µ/ms | 3.5ms | 3.0ms | 2.0 |
| | sd = | 2.0µ/ms | 0.9ms | 1.1ms | 0.7 |
| | sem = | 0.6µ/ms | 0.3ms | 0.5ms | 0.3 |
| | n = | 10 domes | 10 domes | 5 domes | 5 domes |
| | n = | 3 rats | 3 rats | 2 rats | 2 rats |

*Latency at twice threshold or at maximum stimulus.

TABLE 25

STATISTICAL COMPUTATIONAL FORMULAS

$$\overline{X} = \frac{\Sigma X}{N}$$

$$s.d. = \sqrt{\frac{\Sigma X^2 - \frac{(\Sigma X)^2}{N}}{N-1}} \qquad s.e.m. = \frac{s.d.}{\sqrt{N}}$$

$$t = \frac{\overline{X}_1 - \overline{X}_2}{\sqrt{\frac{1}{N_1} + \frac{1}{N_2} \cdot \frac{sd_1^2(N_1 - 1) + sd_2^2(N_2 - 1)}{N_1 + N_2 - 2}}}$$

$$df = N_1 + N_2 - 2$$

Appendix 2

## VII. Number of impulses evoked in single touch domes during development.

### A. Methods

Fifty-five touch domes in 25 rats were tested to obtain an estimate of the number of axons innervating a single touch dome. In Group I there were 5 touch domes tested in one nursing pup. In Group II 30 touch domes were tested in 17 juvenile and adult rats. In Group III there were 20 touch domes which had been tested in 7 aged rats. Stimulation and recording methods were the same as in Experiment 4.

In early experiments, after determining the number of units innervating a touch dome, the skin around and below the stimulated touch dome was cut to denervate the touch dome. The abolition of the response indicated that the earlier discharges had been recorded from the stimulated touch dome and not from surrounding receptors, through a traveling mechanical wave in the skin. Unfortunately the circumcision of the touch dome made the skin unfit for further stimulation. Therefore, in later experiments, after determining the number of units innervating a touch dome, the maximum stimulus used for that touch dome was applied to the surrounding skin to verify that responses had not been elicited from neighboring receptors. In all cases no stimulus greater than 30 μm was used.

### B. Rationale

In the cat each touch dome is usually innervated by only one axon (Iggo and Muir, 1969), but other studies have shown that the touch

domes of rats are often innervated by only one axon (Smith, 1967).

Physiological studies are obviously important in determining whether all the axons innervating a touch dome in a mature rat are functional or whether, during development, only one axon remains operational. In mammals, fetal and neonatal muscle fibers are hyper-innervated and during development lose all but one axon (Bagust et al., 1973; Bennett and Pettigrew, 1974; Redfern, 1970). During the development of the cerebellum of the rat , Crepel and co-workers (1976) have shown that there is hyperinnervation of Purkinje cells by climbing fibers; in the adult, however, only one climbing fiber contacts a Purkinje cell (O'Leary, 1971).

It is possible that the increase in threshold observed during development is the result of a loss of low threshold axons from the innervation of a touch dome rather than a "switch" from low to high threshold in one axon. To test this possibility it was first necessary to demonstrate physiological evidence that any touch domes in the rat were multiply innervated. Such evidence came from two observations. First, when touch domes in the boundary region separating two adjacent nerve skin fields were stimulated, it was possible to show that one axon in each of the two peripheral nerves supplied the same touch dome. Secondly, during maintained deformation of a touch dome, it was sometimes possible to record a train of impulses exhibiting two clearly different but constant amplitudes, as in Figure 4 (see page 24). In other neurophysiological situations (e.g., recordings from motor units or ventral root filaments; Adrian, 1928) this observation would be

interpreted as indicating the presence of two axons. The situation for touch domes in the centers of nerve fields and for touch domes generating impulses with similar amplitudes is more difficult to analyze. However, a third discriminative technique has recently been described for touch dome recordings by Horch (1979), but after completion of the present experiments. In this technique the oscilloscope is triggered by a spike during maintained pressure on a touch dome. If only one axon innervates a touch dome there will be a silent period after the triggering impulse due to the refractory period of the axon. This technique can show whether or not a touch dome is innervated by one or more than one axon.

In the present study an attempt to study the degree of touch dome innervation during development was made using brief mechanical pulses applied by the 'prodder'. If additional impulses were evoked as the stimulus was increased, the possiblity of polyneuronal innervation of a touch dome was raised. In practice, it was necessary to make sure that the results were not due to repetitive firing of a single axon. This last possibility became increasingly likely as the stimulus was raised due to the presence of mechanical oscillations in the movement of the prodder stylus, despite critical damping (Figure 9). The case for innervation by at least two axons was strong, however, if the initial impulse in the burst was smaller than one or more of the subsequent ones. The reverse situation, of a smaller second impulse, could be explained by partial inactivation of sodium channels following the first impulse in the same axon. Multiple innervation was also indicated

if two impulses sometimes summated or fell within 1 ms of each other (the approximate absolute refractory period of a mammalian axon). In many instances, however, evidence of multiple innervation of touch domes was not forthcoming. The values cited in Table 16 (and Table 14 of the Appendix) must therefore be interpreted with considerable caution, as the numbers of axons innervating touch domes could have been overestimated.

C. Results

Touch domes in nursing pups (Group I) had a significantly higher number of axons ($p < 0.05$) innervating them than adult touch domes. This data is summarized in Table 16. Table 14 of the Appendix contains the individual values. The data indicate possible hyperinnervation of touch domes in young animals. Alternatively, there may have been more spread of stimulus in the smaller, younger animals, accounting for the observed increases in the number of excited axons. More detailed analysis is needed to resolve the question of early hyperinnervation.

TABLE 16

NUMBER OF IMPULSES EVOKED AFTER STIMULATING A SINGLE TOUCH DOME

| | Group I Nursing Pups | Group II Juvenile and Adult | Group III Aged |
|---|---|---|---|
| Age | <28 days | 4 weeks-6 months | 6 months-1.5 years |
| Weight | <65 grams | 65-250 grams | >250 grams |
| No. of units | | | |
| $\bar{x}$: | 3.40 units | 2.03 units | 2.65 units |
| sd: | ±1.67 | ±1.22 | ±1.42 |
| sem: | ±0.75 | ±0.22 | ±0.32 |
| n: | 5 domes | 30 domes | 20 domes |
| | $p < 0.05$ | NS | |

| I Nursing Pups | II, III Juvenile, Adult and Aged |
|---|---|
| 3.40 | 2.32 |
| ±1.67 | ±1.32 |
| 0.74 | 0.19 |
| 5 domes | n = 50 |

NS

$p < 0.10$

Appendix 3

## VIII. Number of Impulses evoked in single touch domes after administration of vincristine sulfate.

### A. Methods

Thirty-seven touch domes in fifteen rats were tested using the stimulation and recording methods outlined in Experiment 4 and Appendix 2. Rats were tested 1, 7, 14, 21, 28 or 42 days after subcutaneous injection with 0.75 mg/kg vincristine sulfate. After estimating the number of units innervating a touch dome, the maximum stimulus used for that touch dome was applied to the skin on either side and above and below the touch dome to ensure that responses were not elicited from other receptors in the skin near the touch dome. In all cases no stimulus greater than 30 μm was used.

### B. Rationale

Touch domes in rats are frequently innervated by more than one axon (Smith, 1967) and this has been confirmed for touch domes in the boundary regions between adjacent nerve fields (see Appendix 2). It was possible, therefore, that the increase in threshold and the increase in latency observed after treatment with vincristine sulfate was the result of a selective loss of those axons with large diameters and low thresholds. Unfortunately, in the present experiment it was not usually possible to distinguish between repetitive firing of a solitary axon and single discharges in several axons, when multiple impulses were recorded following a single brief mechanical stimulus to a touch dome. With this proviso in mind, the number of impulses per stimulus was counted

as the intensity of the latter was increased.

C. Results

Table 23 shows that the mean number of impulses evoked by each stimulus did not change significantly during the period of high threshold following vincristine administration; the low value at day 28 may have been a reflection of the small number of touch domes examined. Individual values for touch domes can be found in Table 19 of Appendix 1. Overall, however, the physiological results lent no support to the possibility of a reduction in the number of functioning axons in those touch domes which were mechanically excitable.

TABLE 23

NUMBER OF IMPULSES EVOKED IN SINGLE TOUCH DOMES AFTER TREATMENT WITH VINCRISTINE SULFATE

| | Day 1 | Day 7 | Day 14 | Day 21 | Day 28 | Day 42 | Normal |
|---|---|---|---|---|---|---|---|
| # units: $\bar{x}$: | 2.23 | 2.80 | 2.00 | 1.75 | 1.17 | 2.00 | 2.15 |
| sd: | 0.83 | 0.45 | 0.82 | 0.96 | 0.41 | 0.71 | 1.23 |
| sem: | 0.23 | 0.20 | 0.41 | 0.48 | 0.17 | 0.32 | 0.24 |
| n domes | 13 | 5 | 4 | 4 | 6 | 5 | 27 |
| n rats | 5 | 2 | 2 | 2 | 2 | 2 | 16 |

Analysis of Variance: $F = 1.4413$, P is not significant.

# Appendix 4

## IX. Thresholds of touch domes after denervation

### A. Methods

The methods for stimulation and recording were as described in experiment 4. Three touch domes were denervated by cutting the appropriate dorsal cutaneous nerve at the point of emergence from the latissimus dorsi muscle, and recordings were made 24-28 hours after denervation.

### B. Rationale

When the nerve to a muscle is cut, neuromuscular transmission at the motor endplate fails before nerve conduction in the peripheral nerve stump is abolished (Miledi and Slater, 1967). The time elapsed before neuromuscular failure is positively correlated with the length of the periperal nerve stump. These data were interpreted as suggesting that some depletable factor present in the nerve fiber maintains neuromuscular function (Miledi and Slater, 1967). Would the earliest effects of denervation of a sensory receptor likewise be in the most peripheral structure, the touch dome ?

### C. Results

The results (see Table 26) show that touch dome thresholds did rise before conduction failure occurred in the nerve. At 24-28 hours after denervation thresholds rose to 13.4 - 13.6 μm (control values are 5.2 μm ± 2.0μm; mean ± s.d.). At 28 hours after denervation the nerve failed to respond to mechanical or electrical stimulation.

TABLE 26

Thresholds of touch domes after denervation

| Time after Denervation (hours) | Rat | Weight (grams) | Threshold (μm) | Latency at T (ms) | Latency at 2T (ms) |
|---|---|---|---|---|---|
| 28.0 | 1 | 292.5 | 13.6 | 4.5 | 2.5 |
| 24.0 | 2 | 235.0 | 13.4 | 2.0 | -- |
| 27.5 | 3 | 233.0 | No response to mechanical or electrical stimulation | | |

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