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THE RELATION BETWEEN EPIDERMAL HYDRATION STATE AND THE ACTIVATION OF NEURONS IN THE PRIMARY SOMATOSENSORY CORTEX

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ABSTRACT

THE RELATION BETWEEN EPIDERMAL HYDRATION STATE AND THE ACTIVATON OF NEURONS IN THE PRIMARY SOMATOSENSORY CORTEX

By

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A major function of the epidermis is to inhibit the loss of fluids from the body. Although the epidermis is an effective water diffusion barrier, it is not impermeable, and is capable of absorbing and storing water. The epidermal hydration state is labile and determined by water diffusion from the dermis, sweat gland activity and ambient environmental conditions. Thermal, electrical and mechanical properties of intact skin depend on the epidermal hydration state. Moreover, it has been proposed that the activation of receptors at or below the dermo-epidermal junction depends on the energy transfer properties of the overlying epidermis. The purpose of this study was to test the effect of epidermal hydration state on the activation of cortical neurons which receive input from superficial glabrous skin mechanoreceptors.

The electrical sign of cortical neuronal activity was recorded extracellularly using a glass-insulated tungsten microelectrode and conventional neurophysiological recording techniques, while punctate stimuli of controlled force were delivered to the glabrous skin surface of the cat's (Felis catus) central footpad. Cats were used because of their

well-mapped somatosensory cortex and the thick epidermal layers of their central footpad. Epidermal hydration state was varied by either exposing the footpad to air streams of different relative humidities, or by soaking the skin in deionized water. Statistical analysis of experimental results was performed using an analysis of variance.

Data from these experiments show that the amount of force required to activate neurons in the primary somatosensory cortex varies with the epidermal hydration state of the glabrous skin. An inflection point occurs in this relationship, such that threshold forces are minimal for moist skin and are maximal for dry and water soaked skin. This study also shows that epidermal hydration has no significant effect on the spatial coupling of the stimulus to the response of single cortical neurons.

To my wife, Susan - whose love made it possible

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TABLE OF CONTENTS

PAGE

LIST OF TABLES

LIST OF FIGURES

FIGURE PAGE

INTRODUCTION

Skin is a nonhomogeneous and anisotropic tissue composed of several distinct layers, each with unique physical and chemical properties. The outer skin layers (the epidermis) are devoid of blood vessels and contain only a few bare nerve endings. In contrast, the lowest skin layer (the dermis) has a well developed vascular system, and is richly innervated. This nerve supply includes sympathetic fibers to sweat glands, blood vessels and hair follicles, as well as afferent nerves subserving the sensory modalities of touch, pain and temperature. Although the specialized endings of these afferent nerves are found throughout the dermis, they are more concentrated near the junction of the dermis and epidermis. The secretory portion and proximal tubules of atrichial (eccrine) sweat glands are also located in the dermis. These glands are found throughout human skin and in the glabrous, tactile skin of some furred animals, such as in the central footpad of the cat.

Because of the lack of a direct blood supply, the water content and hydration profile of the epidermis is extremely labile and dependent on at least three factors. These factors are: 1) diffusion of water from the underlying dermis, 2) absorption or desorption of water from the

environment, and 3) the lateral dboth salts and water from the helically coiled distal sweat gland tubules, which penetrate the epidermis. Although these factors have long been recognized to influence epidermal water content, little is known about their interactions, or of possible feedback mechanisms involved in controlling hydration in intact skin.

It has been clearly demonstrated that thermal and electrical energy transfer properties of the epidermis are affected by the amount of water stored in the skin. For example, both the thermal and electrical conductivity of the epidermis vary directly with skin hydration. Mechanical properties of skin, which have been indexed by stress-strain relationships or by coefficients of friction at the skin surface, also depend on water content. Both "free" water, which occupies the intracellular and intercellular spaces, and "bound" water, which exists in chemical combination with proteins and other water binding molecules, have been implicated in these hydration effects. It seems reasonable that hydration induced effects on epidermal mechanical properties might affect the three-dimensional distribution of forces applied to the skin surface, and consequently affect the activation of mechanoreceptive nerve endings located at the dermal-epidermal border.

At least three types of mechanoreceptive endings have been identified for glabrous skin. Two of these (Merkel Cells and Meissner Corpuscles) are located almost exclusively at the dermal-epidermal border, making them

particularly sensitive to epidermal hydration effects. The third type (Pacinian Corpuscles) are found deeper in the dermis and also in subdermal tissues. Primary afferent fibers arising from these receptors have been functionally classified as either rapidly or slowly adapting, according to their response to maintained skin surface indentations. Both of these receptor classes can be subdivided according to the relative size of their receptive fields. Individual rapidly adapting receptors show selectivity in the range of frequencies to which they respond when sinusoidal stimuli are given. It is generally assumed that the variation in primary afferent response is due to the topographical distribution of the fiber endings and to the viscoelastic and membrane electrical properties of the nerve ending. Some of the adaptation observed in the frequency of action potentials is likely due to the viscoelastic properties of the skin.

Primary afferent fibers which enter the spinal cord relay information to the brain through two parallel pathways. The larger diameter nerve fibers project directly to the brainstem via the dorsal columns of the spinal cord and synapse in the dorsal column nuclei of the medulla. Second order fibers cross over to the contralateral side of the brainstem in the medial lemniscus, and their axons terminate in specific relay nuclei of the thalamus. In contrast, the smaller diameter mechanoreceptive afferents cross over and synapse in the spinal cord, and the axons of

the second order neurons ascend to thalamic nuclei by way of the spinothalamic tract. Although the importance of the large fiber system in fine tactile discrimination is unquestionable, the relative contribution of the smaller diameter fibers remains controversial.

Axons of third order neurons which originate in the thalamus ascend through the internal capsule and terminate on the intrinsic neurons of the anterior parietal cortex. A high degree of organization and synaptic integrity is maintained throughout the mechanoreceptive projections, which results in a columnar, somatotopic organization in this cortical receiving area. Cells in a single cortical "column" have similar functional properties and similar receptive fields. Cells in adjacent "columns" represent either overlaping or adjacent peripheral receptive fields. Because of this somatotopic organization, a complete representation of the body surface exists in the somatosensory cortex. This representation or "homunculus" is somewhat distorted, because the area of cortical tissue devoted to input from a body region is directly related to the density of mechanoreceptive endings in that peripheral site. Accordingly, a relatively large proportion of the somatosensory cortex represents input from the hands and face, which have the greatest density of mechanoreceptive endings.

Recent evidence suggests that at least two and perhaps as many as three or four overlapping representations of the

body can be located in this cortical area. Each representation corresponds roughly with previously identified architectonic zones, and each exhibits different functional properties. In the cortical depiction of the cat central footpad, at least three separate representations exist. First, a population of neurons which slowly adapt to small, maintained displacements of the skin surface; second, a population which rapidly adapts to small, maintained skin displacements; and third, a population which responds to deep tissue stimulation by larger displacements of the skin or to movements of the limbs. The population of cortical neurons selected for study in this dissertation were located in the areas subserved by slowly and rapidly adapting cutaneous cells. These cells appear to receive input from the more superficial skin receptors, and are important in fine tactile discrimination. When activity is evoked by short duration (10 msec) square wave mechanical pulses, both types of cells respond similarly with single action potentials.

Results of previous experiments indicate that thresholds for perception of mechanical stimuli of controlled force or displacement correlate well with thresholds for the activation of primary afferent nerve fibers. Two types of thresholds were identified in these studies of primary afferents, an absolute threshold for initiating activity, and a higher threshold, where neuronal responses correspond in a nearly 1:1 fashion with the stimulus. This study

examines thresholds for activating cortical neurons over activating cortical neurons.

The hypothesis tested in this dissertation is: do the mechanical properties of the skin which depend upon epidermal hydration affect threshold response characteristics of rapidly or slowly adapting neurons in the primary somatosensory cortex, when punctate stimuli of controlled force are delivered to the glabrous skin surface?

LITERATURE REVIEW

Because numerous and voluminous reviews of skin and somatosensory anatomy and physiology have already been published, the scope of this literature review is limited to those aspects of skin anatomy, epidermal hydration, and mechanoreception judged crucial to this investigation. Textbooks by Rothman (1954), Montagna (1962), Tregar (1967), and Elden (1971), as well as review articles by Scheuplein and Blank (1971) and Newburgh and Johnston (1942) provide broad discussions of the structure and function of skin and its appendages. In addition, Holmes (1972) has reviewed the literature specific to the glabrous skin on the cat footpad. For comprehensive and detailed information on the somatosensory system, textbooks edited by Mountcastle (1980) and Iggo (1973), and review articles by Wall and Dubner (1972) and Dykes (1978) are recommended.

THE EPIDERMIS

The epidermis is a stratified tissue which ranges in thickness from 0.3mm (Conroy, 1964) to 0.9mm (Strickland, 1958). Early investigators identified two epidermal layers, the germinating layer (stratum germinativum) also known as

the Malpighian layer (Trautmann and Fiebiger, 1957), and a more superficial horny layer. Closer examination has revealed that there are at least five epidermal layers, which beginning with the deepest are: 1) the stratum basale, 2) the stratum spinosum, 3) the stratum granulosum, 4) the stratum lucidum, and 5) the stratum corneum (Creed, 1958; Andrew, 1959; Montagna, 1962; Conroy, 1964). All of these layers appear to be present in the glabrous skin of the cat central footpad (Holmes, 1972). Although the relative thickness of each layer varies over the body surface, the proportionality is characteristic for any one location (Rushmer et al., 1966).

Only cells in the deeper skin layers exhibit mitotic activity (Montagna, 1962). Cells formed in these layers migrate toward the skin surface while undergoing a gradual morphological transformation called cornification or keratinization. This process begins in the stratum germinativum and continues until cells typical of the stratum corneum are formed (Nicoll, 1972). Lavker and Matoltsy (1970) have identified two stages of keratinization called the "synthetic" and the "transformation" phases. During the "synthetic" phase, which occurs in the lower layers, three major intracellular constituents are formed. These constituents are: 1) the filamentous protein bundles, 2) the membrane coating granules, and 3) the keratohyalin granules. The "transformation" phase which occurs in the upper skin strata is characterized by the digestion of

intracellular constituents by proteolytic enzymes, sparing the filamentous proteins and the keratohyalin granules. Jerrett et al. (1965) proposed that these proteins are protected by extensive disulfide cross linkages and by phospholipid moieties with which they chemically interact. During the "transformation" phase, the membrane coating granules migrate toward the cell membrane and then extrude their contents into the interstitial space (Nicoll, 1972).

THE EPIDERMAL STRATA

The deepest epidermal layer, the stratum basale consists of a single layer of columnar cells which rests on the underlying dermis (Conroy, 1964). Microscopic cytoplasmic processes from the basal surface of these cells provide intimate contact with the dermis. Dick (1947) proposed that dermal collagen fibers attach to the cell membrane between these cytoplasmic processes, therby anchoring the epidermis. Medawar (1953) has shown a close relationship between the elastin fibers of the dermis and the basal layer cells. Trypsin, a proteolytic enzyme which readily attacks elastin, rapidly separates the dermis and epidermis (Medawar, 1953).

Basal cells have a large basophilic nucleus and a "scanty amount" of cytoplasm (Conroy, 1964). These cells have an abundance of nucleoprotein and a large proportion of sulfhydryl groups (Creed, 1958). Electron micrographs reveal both mitochondria and ribosomes in these cells.

(Montagna, 1962; Dellmann, 1971). It is generally accepted that the basal layer has a high degree of mitotic activity, and that its main functions are to anchor the epidermis (Montagna, 1962) and to provide for the regeneration of skin through cell division (Nicoll, 1972). Scattered among the basal layer cells are melanocytes, which under neuroendocrine control produce and disperse the pigment melanin and give skin its characteristic color (Conroy, 1964; Strickland, 1958; Strickland and Calhoun, 1963).

The stratum spinosum is superficial to the stratum basale; it is the thickest epidermal layer. This strata contains polygonal-shaped cells and has been reported to vary in thickness from 16 (Conroy, 1962) to 38 (Strickland, 1958) cell layers. As new cells are formed by the basal layer, the cells of the stratum spinosum migrate toward the skin surface and become progressivly more flattened (Andrew, 1959; Conroy, 1964; Dellmann, 1971).

The cells of the stratum spinosum contain small protein fibrils (tonofibrils), some of which are organized into microscopic structures called tonofilaments (Montagna, 1962). Although the protein fibrils are distributed throughout the cytoplasm, the filaments are organized into bundles which are generally directed from the cell nucleus out toward specialized structures on the cell membrane, called desmosomes. The desmosomes serve as loci for attachment of adjacent cells (Montagna, 1962; Selby, 1957; Dellmann, 1971). Although early investigators believed the

cells had cytoplasmic continuity with each other at these sites (Chambers and Renyi, 1925), electron micrographs by Selby (1955, 1956), Odland (1958, 1960) and others do not support this belief (Montagna, 1962). The cell membranes in these regions of contact are thickened. Rather than cytoplasmic bridges, the intercellular cleft contains fine protein filaments which join and separate adjacent cells. It is these filamentous tufts which give this layer its name (Montagna, 1962). Desmosomes are most evident in the stratum spinosum. Some can be identified in the stratum basale and stratum germinativum, but they appear to be absent from the stratum corneum (Montagna, 1962). Data by Wolff and Schreiner (1968) and Mercer et al. (1968) show the presence of a mucopolysaccharide in the intracellular space, which is especially concentrated near the desmosomes. They have concluded that the carbohydrate material represents an "intracellular cement" which binds together adjacent cells and limits the movement of fluids in the interstitial space. Mitochondria can be found in the stratum spinosum, but they become fewer and completely disappear as cells migrate toward the skin surface (Montagna, 1962).

The granular layer or stratum granularis lies superficial to the stratum spinosum and consists of flattened, diamond-shaped cells (Conroy, 1964). The granular cell membrane contains small villi, which give them a serrated appearance (Trautmann and Fiebiger, 1957). This layer has been reported to range in thickness from 3

(Conroy, 1964) to 8 (Strickland, 1958; Strickland and Calhoun, 1964) cell layers in the cat central footpad. In hairy skin, the cells of this layer are organized in vertical columns with the cells of adjacent columns interdigitating. Glabrous skin lacks this degree of columnar organization (Menton and Eisen, 1971).

Both the size and number of keratohyalin granules increase in the stratum granularis (Trautmann and Fiebiger, 1957). These granules contain phospholipids which are closely associated with the tonofibril protein moieties (Spearman, 1970). Electron micrographs reveal a close association of these granules with cytoplasmic tonofilaments (Dellmann, 1971). Selby (1957) has demonstrated a pronounced consolidation of the protein fibers in this layer. The tonofilament proteins contain a large proportion of cysteine residues, and therefore have a large number of sulfhydryl groups. The sulfhydryl groups of adjacent polypeptide chains are oxidized as the cells migrate toward the surface forming the disulfide bonds of cystine (Giroud and Leblond, 1951; Spearman, 1970). The extensive disulfide cross-linkages between polypeptide chains is characteristic of keratin, the primary structural protein of the epidermis (Giroud and Leblond, 1951). It is also in the granular layer that the digestion of intracellular organelles begins which results in the gradual reduction in size and number of ribosomes, mitochondria, and cell nuclei (Jarrett et al., 1965).

The stratum lucidum is a very thin cell layer interposed between the stratum granulosum and the stratum corneum. This layer is not present in skin covering the general body surface, but is distinct in the glabrous skin on the tactile surfaces (Andrew, 1959; Creed, 1958; Strickland, 1958; Strickland and Calhoun, 1963). This layer has been reported to vary in thickness between 28 and 40 microns in the cat central footpad (Strickland, 1958). The cells of the stratum lucidum are flat and closely packed (Andrew, 1959; Dellmann, 1971; Trautmann and Fiebiger, 1957). They have been described as "anuclear" (Conroy, 1964; Trautmann and Fiebiger, 1957) and appear to be devoid of organelles (Dellmann, 1971). Distinct cell membranes and intracellular spaces are difficult to discern with light microscopy (Trautmann and Fiebiger, 1971; Spearman, 1970), but have been demonstrated in electron micrographs (Selby, 1957). Electron microscopy also revealed closely packed intracellular protein filaments (Dellmann, 1971). Spearman (1970) demonstrated the presence of disulfide linkages and protein bound phospholipids. Selby (1957) and Hashimoto (1969) noted an abrupt change in the structure of the desmosome in this layer which forms dense intercellular bands. Spearman (1970) speculated that the stratum lucidum may serve as the primary barrier to water diffusion across the epidermis, but Scheuplein and Blank (1971) state that there are few data to support this hypothesis.

The most superficial epidermal layer is the stratum corneum. The cells in this layer are usually flattened (Andrew, 1959; Dellmann, 1971; Trautmann and Fiebiger, 1957). On the tactile surfaces, these cells overlap and lack the architectural organizaton found on the general body surface (Christophers, 1971; Menton and Eisen, 1971). On the cat footpad, the corneum is thicker than at other skin locations, varying between 15 (Strickland, 1958; Strickland and Calhoun, 1963) and 320 (Conroy, 1964) microns. Cells of this layer are devoid of organelles (Dellmann, 1971) and contain a "tightly packed" matrix of tonofibrils (Matoltsy and Balsamo, 1955) in close association with cytoplasmic granules (Dellmann, 1971).

Matoltsy and Balsamo (1955) reported that the dry mass of the stratum corneum is composed of 75% epidermal proteins (keratin), 10% amino acids, 7-9% lipids and 5% cell membranes. The sulfhydryl groups which are plentiful in the stratum granularis are fewer in the corneum, but a large number of disulfide bonds have been shown (Spearman, 1970). Giroud and Leblond (1951) demonstrated a pronounced decrease in the lipid and phospholipid content of the corneum. They proposed that the lipids that remained in the corneum were bound to proteins. Jerrett et al. (1965) give supportive evidence for this and attribute the reduction in lipid content to autolysis by epidermal hydrolytic enzymes. Spearman (1970) demonstrated the presence of protein-bound lipids in the stratum lucidum, but failed to find them in

the corneum. His observations also support the autolysis hypothesis.

The thickened and enzyme resistant cell membranes of the corneum have long foliated villi on their surfaces (Menton and Eisen, 1971). Concomitant with a condensation of the "intracellular cement" at the desmosomes (Wolff and Schreiner, 1968) is a reduction of the interstitial space (Selby, 1957). Mercer (1968) concluded that the desmosomal sites were the "main device holding the (stratum corneum) formation together" (Holmes, 1972).

THE DERMIS

Unlike the epidermis which is of ectodermal origin, the dermis is derived embryonically from mesoderm. Its major components are collagen, elastin, reticular fibers, hyaluronic acid, and chondroitin sulfate. The first three of these are structural proteins which provide the viscoelastic properties of the dermis, and the latter two are mucopolysaccharides which form the "ground substance" of skin (Holmes, 1972). The dermis of glabrous skin contains blood vessels, lymphatic vessels, both afferent and efferent nerves, and the secretory portions and proximal tubules of atrichial (eccrine) sweat glands. The dermis lies on top of the subcutaneous fat, but the border separating them is often indistinct (Andrew, 1959; Dellmann, 1971).

Unlike the avascular epidermis, the dermis has a well developed and unique vascular system. Blood flow to the skin greatly exceeds metabolic demand (Rushmer et al., 1966). Arterial blood brought to the skin in the arteriolar plexus located near the dermis-subcutaneous fat border is returned to the venous system through two routes (Newburgh, 1949). During cold stress, arterial blood can be shunted through communicating veins into venules which are closely opposed to the arterioles. This provides a counter-current heat exchange mechanism, which builds a steep thermal gradient across the skin and conserves body heat (Bazett, 1948). An alternate route for arterial blood is through long capillary loops, which extend into the dermal papillae at the dermal-epidermal junction. Blood flowing through these capillaries drains into venous plexuses located at two different levels of the dermis (Bazett, 1948). This circulatory scheme is an important thermoregulatory adaptation and provides the dermis with a direct source of water, while the avascular epidermis must rely on indirect sources.

Nervous system control of skin blood flow appears to be mediated solely by the sympathetic division of the autonomic nervous system (Hertzman, 1959). Adrenergic nerve fibers are responsible for maintaining a vasoconstrictor tone, while sympathetic cholinergic fibers can indirectly cause vasodilation by stimulating bradykinin formation (Barcroft, 1960). Kontos et al. (1967) demonstrated a profound, local

vasodilator effect of CO_2, which is unaffected by either alpha or beta adrenergic blockade.

The epidermis contains only a few, bare, afferent nerve endings which do not penetrate as far as the stratum corneum (Rushmer et al., 1966; Copenhaver, 1971). In contrast, the dermis has a rich supply of highly specialized afferent endings which are concentrated near the dermal-epidermal border, but are also found deeper in the dermis and in the subcutaneous fat (Rushmer et al., 1966; Janig, 1971; Malinovsky, 1966; Mountcastle, 1980). These afferent nerve terminals subserve the sensory modalities of touch-pressure, pain and temperature (Mountcastle, 1980) and will be discussed later in greater detail.

THE SWEAT GLANDS

The atrichial (eccrine) sweat gland is the only appendage of glabrous skin in both humans and cats (Conroy, 1964; Creed, 1958; Strickland, 1958; Strickland and Calhoun, 1963). Although the gross anatomy and histology of cat atrichial glands have been reported (Conroy, 1964; Munger and Brusilow, 1961; Sperling and Koppanyi, 1949), most of our knowledge of sweat gland blood supply, innervation and secretory processes comes from studies in primates including man (Holmes, 1972). Montagna et al. (1953) stated that the atrichial gland of the cat footpad is in no way comparable to the human eccrine gland. Using both light and electron

microscopy, Munger and Brusilow (1961) found the secretory portions of human and cat sweat glands to be identical. Electron micrographs by the same investigators, however, reveal the sweat gland tubule in cats to be "markedly different" from the human sweat duct. The osmolarity and composition of sweat collected at the skin surface is also different in cats and humans (Munger and Brusilow, 1961).

The secretory portions of the atrichial sweat glands are located deep in the dermis, or in the subcutaneous fat (Munger and Brusilow, 1961). This portion of the gland is highly coiled and intertwined with capillaries and small arterioles (Ellis et al., 1958). The secretory apparatus is innervated by small unmyelinated, sympathetic nerves (Langley, 1891), which release acetylcholine as a neurotransmitter (Langley, 1923; Patton, 1948; Chalmers and Keele, 1951, 1952; Foster and Weiner, 1970). Fujisawa (1959) reported the presence of three cell layers in this segment which are: 1) the glandular cells, 2) the myoepithelial cell layer, and 3) the basement membrane.

The glandular cells surround the lumen of the sweat gland secretory apparatus in a single cell layer (Montagna et al., 1953; Hibbs, 1958; Conroy, 1964). These cells have an abundant amount of cytoplasm, and can be classified as "dark" or "clear" according to their staining characteristics. The "dark" cells contain a prominent golgi apparatus, mitochondria, and secretory vacuoles containing mucopolysaccharides (Munger and Brusilow, 1961). These

cells also show a high degree of acid phosphatase activity (Kamamura, 1957). In contrast, the "clear" cells do not stain for mucopolysaccharides (Munger and Brusilow, 1961), and have little acid phosphatase activity (Kamamura, 1957). These cells contain glycogen granules (Hashimoto, 1971a), numerous mitochondria, and microvilli which project into the interstitial space (Munger and Brusilow, 1961). The interstitial spaces between adjacent tubular cells called intercellular canaliculi are greatly reduced in regions where adjacent cell membranes are closely opposed, where they are called tight junctions (Munger and Brusilow, 1961).

A layer of myoepithelial cells is interposed between the glandular cells and the basement membrane. These elongated, fibrillar cells are oriented either along the tubular axis (Sperling and Koppanyi, 1949), or spirally around the tubule (Hibbs, 1958). Sperling and Koppanyi (1949) reported that the myoepithelial cells are not contractile, but change their size by a reduction of their intracellular volume. This intracellular fluid contributes considerably to the initial secretion of the stimulated sweat gland.

Using Hashimoto's anatomical data, Dobson and Sato (1972) postulated that sweat is formed by the osmotic flow of extracellular water into the canaliculi which are made hypertonic through the action of an energy dependent sodium pump on the "clear" cell membrane. Fluid from the interstitial space then flows from the canaliculi into the

lumen of the sweat gland and is brought to the skin surface by volume displacement.

The dermal segment or proximal sweat gland tubule receives its blood supply from branches of the dermal capillary loops (Ellis et al.,1958). In both humans and cats, the dermal segment is composed of a superficial cell layer, a basal cell layer, and a basement membrane (Ellis and Montagna, 1962; Munger and Brusilow, 1961). In cats, however, there are far fewer mitochondria in the superficial cells then are found in human sweat gland tubules (Montagna et al., 1953; Munger and Brusilow, 1961).

The luminal side of the superficial cells has a dense border or cuticle which is thinner in cats than in humans (Munger and Brusilow, 1961; Hibbs, 1958; Holyoke and Lobitz, 1952; O'Brien, 1952). This cuticle is formed by a dense band of intracellular tonofilaments and granules which lie just below the cell membrane. The luminal membrane has microvilli which project into the lumen of the sweat duct (Hashimoto, 1971b; Munger, 1961; Munger and Brusilow, 1961). The cuticle has a large number of disulfide linkages which are similar to cells of the stratum corneum (Lobitz et al., 1954). Adjacent superficial cells are joined by tight junctions which are at or near well developed desmosomes (Hashimoto, 1971b; Ellis and Montagna, 1961). Ellis and Montagna (1961) postulated that the cuticle and desmosomes provide a rigid ring which prevent collapse and occlusion of the sweat duct.

The cells of the basal layer contain glycogen granules and numerous mitochondria. These cells also have desmosomes, but they are fewer and less pronounced than in the superficial layer (Ellis and Montagna, 1961). Munger and Brusilow (1961) reported that like the superficial layer, the basal layer in cat tubules has fewer mitochondria than in human sweat glands.

Human sweat collected at the skin surface is hypotonic; its osmolarity is directly related to the rate of sweat secretion (Lobitz and Dobson, 1961). Schwartz and Thaysen (1956) postulated that reabsorption of ions in the proximal sweat gland tubule might account for the hypotonicity. Brusilow and Munger (1962) noted that sweat collected from the skin surface of the cat footpad was hypertonic. They attributed this species difference to the shorter length and reduced number of mitochondria in the proximal tubule cells of the cat. Histological and enzymatic data from other investigators support this hypothesis (Hashimoto, 1971b; Sato and Dobson, 1970; Sato et al., 1971).

The epidermal segment or distal sweat gland tubule is a helically coiled duct which opens to the skin surface in a pore (Sperling and Koppanyi, 1949; Takagi and Tagawa, 1955; Wolf, 1968b). The lumen of the duct is lined with one (Zelickson, 1961) or more (Hashimoto, 1971b; Pinkus, 1939; Takagi, 1952; Wolf, 1968a) cell layers which are anatomically distinct from the surrounding tissue. Blair (1968) has reported as many as 16 tightly wrapped cell

layers. The luminal cells of the distal tubule have a cuticle (Conroy, 1964) which gradually disintegrates as it approaches the skin surface (Ellis and Montagna, 1961). These tubular cells become keratinized sooner than the epidermal cells which surround the duct (Takagi, 1952; Lobitz et al., 1954).

EPIDERMAL HYDRATION

Sanctio Sanctorious demonstrated in 1614 that humans continuously lose weight even when sleeping or resting quietly (Benedict and Benedict, 1927). Until the early 1900's, this weight loss was attributed to evaporation from the respiratory surfaces and low rates of sweat secretion. Fleischer in 1877 concluded that the intact skin of man was totally impermeable to all substances (Scheuplein and Blank, 1971). More recent studies by Buettner (1959a,1959b,1959c), Adams (1966) and Scheuplein and Blank (1971) indicate that the skin is permeable to water, ions and other substances.

Blank (1953) attempted to locate the "permeability barrier" of the epidermis by measuring evaporative water loss while successively removing thin layers of cells from the skin surface. He noted that the epidermis retained its semipermeable nature until the last few layers of stratum corneum cells were removed. This led him to the erroneous conclusion that a "thin permeability barrier" existed near the base of the stratum corneum (Scheuplein and Blank,

1971). Mackee et al. (1945) postulated that the stratum lucidum was the "thin barrier" to both ions and uncharged molecules. Spearman (1970), and Elias and Friend (1975) support the "thin barrier" hypothesis. Further studies by Blank and Gould (1962), Fredricksson (1962) and Matoltsy et al. (1962) present convincing arguments that the corneum is nearly uniformly permeable to water, and that its entire thickness provides the "permeability barrier".

Adult human skin contains a large quantity (about 9 liters) of water (Skelton, 1927). Blank (1952) calculated that the epidermis alone was able to absorb up to six times its weight when fully hydrated. Buettner (1959a) also demonstrated that the skin was hydroscopic and could either take up or lose water depending on the environmental conditions. He also proposed the existence of an energy dependent process which could cause the movement of water from the epidermis into the general circulation. Adams (1966) presented data which suggest the reabsorption of sweat water from the epidermis. No direct evidence of an active transport process for water has been presented (Scheuplein and Blank, 1971).

Laden and Spitzer (1967) summarized the factors which influence the hydration of the stratum corneum as follows: 1) the rate at which water reaches the stratum corneum from underlying tissues, 2) the rate at which water leaves the skin surface, 3) the ability of the corneum to hold water.

Water is brought to the corneum from underlying tissue by diffusion and through sweat gland activity. Diffusion into the corneum from the well hydrated dermis appears to be limited only by the permeability of the corneum itself (Laden and Spitzer, 1967). Sweat gland secretion brings a substantial amount of water to the upper skin strata. Experiments in cats (Adams, 1966) and raccoons (Steinmetz et al., 1977), in which sweat gland activity was precisely controlled by electrical stimulation of the sweat gland nerves and skin surface evaporative water loss rate was continuously measured, indicate a 5 to 10 fold increase in evaporation rate when the sweat glands were maximally stimulated. Sweat water can enter the epidermis either by absorption at the skin surface, or by lateral diffusion from the distal sweat gland tubule. Adams (1966) proposed a micro-circulation of water at low levels of sweat secretion as water diffusing laterally from the sweat ducts was reabsorbed across the dermal-epidermal border.

The rate of water loss across the skin surface depends in part on environmental conditions. Buettner (1959a) demonstrated that skin absorbed water when exposed to solutions of low osmolarity or to air at relative humidities above 90%. Goodman and Wolf (1969) examined the effect of ambient relative humidity on water loss from the stratum corneum. They noted a paradoxical increase in evaporation rate when the relative humidity was raised from 2% to 50%. Further increases in ambient relative humidity caused the

expected decrease in evaporation as the gradient between air and skin was reduced. Grice et al. (1972) investigated this phenomena further and concluded that the permeability of the epidermis varied with its water content.

There has been a great deal of discussion on the nature of substances in the skin responsible for its being able to hold water. Scheuplein and Morgan (1967) identified two "water pools" in the skin, which they identified as "free" and "bound" water. According to their data, "free" water accounts for about 20% of the total water stored. It is depleted in the first few minutes during exposure to a severely dehydrating environment. "Bound" water comprises 80% of skin water, and is removed slowly over a period of hours or days during dehydration. The ability of the corneum to hold water is impaired by its treatment with lipid solvents (Blank, 1953) or detergents (Blank and Shappirio, 1955). Blank (1953) showed that no change in water binding capacity occurred if the skin was exposed to lipid solvents or to water alone. If the skin was extracted with water after exposure to the lipid solvents, however, a significant reduction in its water binding capacity was noted. He concluded that the reduction in water binding capacity was due to the extraction of water soluble substances which are held in the skin by lipid-containing semipermeable membranes. Many of these hygroscopic substances have been identified. They include a mixture of amino acids, organic acids, urea and inorganic ions

(Bolliger and Gross, 1954; Laden, 1954). Sodium lactate (Fox et al., 1962) and 2-pyrrolidone-5-carboxylic acid (Laden and Spitzer, 1967) appear to be the most important of them (Middleton, 1968). Middleton (1968) showed that a disruption of cell membranes in the stratum corneum had the same effect as extraction with lipid solvents. His data are interpreted to show that either lipid extracted or membrane disrupted skin tissue retained about 59% of its water binding capacity. Park and Baddiel (1972b) suggested that intracellular and cell membrane proteins might account for the remaining water binding capacity.

HYDRATION EFFECTS ON SKIN THERMAL PROPERTIES

It has been well established that the thermal energy transfer properties of skin are related to its water content. After reviewing the literature reporting thermal conductivity coefficients (k) for skin and other tissue, Tregar (1966) speculated that heat conduction in skin was primarily through water molecules. Using data from the footpads of arctic canines (Henshaw et al., 1972), Holmes (1972) calculated k values and concluded that the k of wet skin was more than double that for dry skin. Holmes (1972,1975) investigated the effect of hydration on thermal conductivity of intact cat footpad epidermis through the simultaneous measurement of unidirectional heat flow and temperature gradient. Epidermal hydration was varied either

by electrical stimulation of nerves innervating sweat glands, or by exposure to a high ambient relative humidity. These data show conclusively the direct relationship between epidermal water content and thermal conductivity.

HYDRATION EFFECTS ON SKIN ELECTRICAL PHENOMENA

The effect of hydration on skin electrical properties has been demonstrated. Electrical properties of the skin are usually indexed either as its endogenous, transcutaneous electrical potential, or as some measure of its resistance to electrical current flow. The latter may be expressed in terms of resistance, conductance or impedance depending on the measurement technique (Steinmetz and Adams, 1980). The methodology, terminology and physiological basis of these electrical phenomena have been reviewed by Edelberg (1971) and Venables and Christie (1973).

An inverse relationship between sweat gland activity and skin electrical resistance was first demonstrated by Thomas and Korr (1957). A similar relationship was observed by Lloyd (1959a,1959b) between sweat gland activity and the electrical impedance of skin. Adams and Vaughan (1965) reported a strong correlation between skin electrical resistance and skin surface evaporative water loss when they were measured simultaneously in humans either resting quietly or engaged in provocative conversation. The implication of these data is that observed changes in

epidermal electrical characteristics are due either directly to the activity of sweat glands, or are a result of the consequent skin hydration.

In studies on the cat foot pad model, Stombaugh and Adams (1971) noted an exponential decline in both skin electrical potential (SEP) and skin electrical conductance (SEC) during progressive dehydration of a previously wetted skin. These investigators also recorded the amplitude and time course of transient changes in SEP and SEC evoked by a single stimulus to the sweat gland nerves at various times during progressive dehydration. A similar relationship between these electrical phenomena and epidermal hydration was observed by Adams et al. (1980) during progressive skin wetting. In addition these investigators noted changes in SEP and SEC baselines and evoked responses when the ion content of the epidermis was altered either by allowing salt deposited in previous sweating bouts to accumulate, or by exposing the epidermis to solutions of varying osmolarity. The conclusion reached from these studies is that both the water and ion content of the epidermis are important determinants of steady state and transient skin electrical characteristics (Steinmetz and Adams, 1981).

HYDRATION EFFECTS ON SKIN MECHANICAL PROPERTIES

Measurements of coefficients of friction between various materials and the skin surface have been used as indices of

skin mechanical properties. Naylor (1955) determined coefficients of friction between the tip of a moving plastic rod and intact human skin. His studies show that skin moistened with "trace" amounts of water or by moderate sweating has a higher coefficient of friction than either dry or thoroughly wetted skin. Similar results were obtained by Adams and Hunter (1969), using a plexiglass plate drawn across the central footpad skin of the cat. In this latter study, epidermal hydration was varied by controlled electrical stimulation of sweat gland nerves. In sequential experiments in the same footpads, these investigators observed and measured sweat gland activity while activating the peripheral nerves with the same stimulation parameters. Their studies demonstrate that the increase in friction measured at the moist skin surface and the subsequent decrease with further hydration both occur before liquid sweat reaches the skin surface. Comaish and Bottoms (1971) and Highley et al. (1977) demonstrated hydration effects similar to those of Naylor (1955) and Adams and Hunter (1971).

Epidermal hydration effects on skin mechanical properties have also been indexed by evaluation of stress-strain relationships. Blank (1952) first showed that the "pliability" of excised skin was directly related to its water content. By measuring the response of excised stratum corneum strips, Laden and Morrow (1970) determined that the relative "softness" and "flexibility" of the stratum corneum

were directly related to ambient relative humidity. Strips of corneum which were lipid solvent-water extracted exhibited a drastically reduced "flexibility", and were plasticized less by exposure to water vapor. Wildnauer et al. (1971) measured the strain (elongation) of excised stratum corneum strips uniaxially loaded, and the maximum stress (force) before fracture. They showed that the stress at any given strain is greater at high ambient relative humidities than at low ones. They observed that the stratum corneum cells were "fibrous protein bundles" covered with lipids. When the corneum was stretched to the breaking point, it fractured intercellularly rather than intracellularly. They concluded that water decreases interfibrillar protein interaction, lessening cohesion among corneal cells.

Park and Baddiel (1972a,1972b) examined the rheology of excised stratum corneum strips in uniaxial loading experiments. They showed that the elastic modulus (slope of the stress-strain curve) is inversely related to the ambient relative humidity in a manner predicted by models based on data from other keratinous substrates and polymers. They proposed that the increase in elasticity which accompanies lipid solvent-water extraction is due to increased "protein-protein" interaction. Christensen et al. (1977) devised a method for measuring uniaxially loaded stress-strain relationships of the intact human stratum corneum. Using sinusoidal stimuli they demonstrated rapid

and pronounced changes when moist air was blown across the skin. They interpreted their data to indicate that hydration influences the mechanical properties of intact stratum corneum.

There have been many other methods of indexing skin mechanical properties *in vitro*. Lanier and Fung (1974a,1974b) developed a computer controlled device for measuring stress-strain relationships in biaxially loaded, excised skin samples. Their data have contributed to our understanding of the nonlinearity and directional anisotropicity of skin. They have demonstrated the importance of temperature and pre-loading conditions on experimental results.

Many methods have been devised recently for measuring the mechanical properties of intact human skin. These techniques include methods in which the skin is loaded uniaxially (Burlin *et al.*, 1977), torsionally (Barbenel and Evens, 1977), by vertical extension (Pierard and Lapiere, 1977), suction (Cook *et al.*, 1977), ballistometry (Tosti *et al.*, 1977) or compression (Daley and Odland, 1979). Although the hydration state of human skin is difficult to control in these human experiments, it is recognized by these investigators as an important consideration.

GLABROUS SKIN MECHANORECEPTORS

Mechanoreceptors in the glabrous tactile skin of cats and primates appear to be similar in both structure and function among species (Burgess and Perl, 1973; Mountcastle, 1980). Early anatomists identified four types of receptors in the glabrous skin, which are: 1) bare nerve endings, 2) Merkel cells, 3) Meissner corpuscles, and 4) Pacinian corpuscles. The functional characteristics of each of these receptors have been determined by recording nerve cell activity from primary afferent fibers, while delivering controlled stimuli to the skin surface.

The bare nerve endings are distributed throughout the dermis and in the lower layers of the epidermis (Andres and During, 1973). These endings subserve the sensory modalities of pain and temperature, and are associated with small diameter myelinated (A-delta) and unmyelinated (C) primary afferent fibers (Mountcastle, 1980). Some nerve fibers of this size are associated with mechanoreceptors in hairy skin, but they do not appear to be important for mechanoreception in glabrous skin (Burgess and Perl, 1973; Mountcastle, 1980).

The Merkel cell receptors were first described by F. Merkel in 1875, as an example of an "epithelial cell-neurite" complex (Munger, 1977; Gottschaldt and Vahle-Hinz, 1981). The structure and function of these

receptors has been characterized in the opossum snout by Munger (1965) and in the cat by Iggo and Muir (1969) and Janig (1971). The Merkel cell is a specialized epithelial cell of the stratum basale, which is in intimate contact with other epidermal cells through tonofilament and desmosomal attachment (Andres and During, 1973: Munger, 1977). These cells contain secretory granules and are generally assumed to be the mechanoreceptive element (Munger, 1977).

The Merkel cells make contact with specialized structures (Merkel Disks) on the primary afferent nerve ending. There is an abundance of secretory granules both in the Merkel cell and in the neuron at these sites which suggests bidirectional information transmittal at this synapse (Andres and During, 1973). Recent data by Gottschaldt and Vehle-Hinz (1981) suggest that the primary afferent ending may be the mechanoreceptor, while the Merkel cell serves as an abutment for the nerve ending. Although the transduction process is still uncertain, there is little disagreement concerning its functional properties. This receptor is generally considered to be a slowly adapting mechanoreceptor, whose function is to detect both velocity and position. (Iggo and Muir, 1969; Burgess and Perl, 1973; Munger, 1977; Mountcastle, 1980; Gottschaldt and Vehle-Hinz, 1981). Stimulation of these receptors gives rise to the sensation of "touch-pressure" (Mountcastle, 1980).

Meissner corpuscles were identified in cat glabrous skin by Malinovsky (1966). These receptors like the Pacinian corpuscles are distinguished morphologically by concentric rings of lamellar cells which surround the nerve ending (Andres and During, 1973). In glabrous skin, these receptors are located in the dermis, just below the dermal-epidermal border. They are activated by small displacements of the epidermis which are transferred through the tonofilaments to dermal collagen fibers, which enter the upper portion of the receptor corpuscle. In contrast, displacements of the deeper dermis do not activate these receptors, because the collagen fibers of the lower dermal layers are not continuous with the lamellae of the receptor (Andres and During, 1973). Janig (1971) classified these receptors as rapidly adapting mechanoreceptors. They respond best to low frequency (30-40 Hz) sinusoids, and their stimulation evokes the sensation of contact and flutter (Mountcastle, 1980).

Pacinian corpuscles are found in the deeper dermis and subcutaneous tissue in glabrous skin (Mountcastle, 1980). The structure and function of these mechanoreceptors has been described in detail by Loewenstein (1971). Like the Meissner corpuscle, these receptors consist of a nerve ending surrounded by concentric layers of lamellae (Andres and During, 1973). Pacinian corpuscles are also classified as rapidly adapting mechanoreceptors; they are most sensitive to high frequency (250-300 Hz) sinusoids.

Activation of these receptors evokes the sensation of contact and vibration (Mountcastle, 1980). Loewenstein (1971) has shown that it is the viscoelastic properties of the concentric lamellae which gives the corpuscular nerve endings their characteristic response. Because of their larger size and deeper location, the cutaneous receptive fields of the Pacinian corpuscles are much larger than those of the Meissner corpuscles and Merkel cells. All three of these receptor types are associated with large diameter (A-alpha and A-delta) nerve fibers (Eyzaguirre and Fidone, 1975; Mountcastle, 1980).

THE MECHANORECEPTIVE PATHWAY

The cell bodies of the primary afferents are located in the dorsal root ganglia (Eyzaguirre and Fidone, 1975). After entering the spinal cord, the axons of the primary afferents divide, sending collateral branches to several locations within the spinal cord. Some collateral branches of the primary afferent axons enter ascending spinal pathways, while others synapse with spinal neurons. Rethelyi and Szentagothai (1973) have reviewed the literature describing the spinal ramifications of primary afferents. Perl and his co-workers more recently have described the location and morphology of the spinal terminations of functionally identified primary afferents, with special emphasis on A-delta and C fiber

mechanoreceptors and nociceptors (Rethelyi et al., 1979; Light and Perl, 1979a, 1979b; Light et al., 1979).

Rethelyi et al. (1979) demonstrated that some branches of the large diameter mechanoreceptive fibers terminated in the nucleus proprius (lamina III) of the spinal cord. Second order neurons which arise from this nucleus may either synapse with other neurons in the spinal cord, or project to the brain in any one of several parallel pathways. Other collateral branches of the primary afferents project directly to the brain in the dorsal columns of the spinal cord. Fibers are added to this tract in a highly organized fashion, so that a complete representation of the body surface can be mapped onto the dorsal columns at the base of the brain (Mountcastle, 1980). Brown (1973) reviewed the literature concerning the topographical organization and fiber content of the dorsal columnns. He concludes that: 1) the dorsal columns are the most direct and fastest ascending mechanoreception pathway, which relay information for fine tactile discrimination, 2) not all fibers which enter the dorsal columns terminate in the same location in the brain, and 3) some fibers other than the large diameter ones from mechanoreceptors also travel in this pathway. Wall and Dubner (1972) note that lesions of the dorsal columns do not always cause the sensory deficit that would be predicted on the basis of the fiber content of this tract. They suggest the possiblity of redundancy in the ascending projections which relay fine

tactile information. The small diameter mechanoreceptive fibers from skin project to the brain in several parallel pathways (anterolateral system), but few of these fibers originate in glabrous skin. The role of these small diameter fibers in fine tactile discrimination appears to be minimal (Burgess and Perl, 1973), but it may serve as the redundant pathway suggested by Wall and Dubner (1972).

The fibers of the dorsal column pathway terminate in the dorsal column nuclei (nucleus gracilis and nucleus cuneatus) which are located in the caudal medulla. Johnson et al. (1968) demonstrated that the cells of the dorsal column nuclei are arranged in an accurate somatotopic fashion. The cells of these nuclei have a high degree of modality specificity which closely mimics the discharge patterns of the primary afferents (Eyzaguirre and Fidone, 1975). Although the modality specificity is preserved at this synapse, cells in the dorsal column nuclei have larger receptive fields than do primary afferents. It has also been observed that stimulation of a single primary afferent can result in the activation of several dorsal column nuclear cells. This suggests that some degree of both convergence and divergence of primary afferent nerve endings occur at this level (Eyzaguirre and Fidone, 1975). Mountcastle (1980) cautions that these nuclei should be considered as integrating centers, rather than simply as relay nuclei.

The axons of the second order neurons decussate in the medulla and project in the medial lemniscus to the ventrobasal complex of the thalamus. This complex consists of two groups of cell bodies, the ventroposterolateral and ventroposteromedial nuclei. Welker and Johnson (1965) demonstrated that the third order neurons of these nuclei exhibit a complete somatotopic representation of the body. These third order neurons also retain the same functional properties as the primary afferents (Mountcastle, 1980). In addition to receiving input from the medial lemniscus, the thalamic nuclei receive projections from the anterolateral system and possibly from the contralateral lemniscal system (Ruch et al., 1965; Eyzaguirre and Fidone, 1975; Mountcastle, 1980). Axons from the third order neurons in the thalamus project to the cortex through the internal capsule. As in the other portions of this ascending system, the axons of the third order neurons remain highly organized, so the somatotopic representation remains preserved (Mountcastle, 1980).

THE PRIMARY SOMATOSENSORY CORTEX

The somatotopic organization of the somatosensory cortex was first suggested by Bard (1938). Complete somatotopic maps of the body were first described in the 1940's by Woolsey, and have since been described for many species (Dykes, 1980). These representations consist of

two-dimensional "sheets" of cortical neurons which are activated by small deformations of the skin or deeper body tissues (Jones and Powell, 1973). The cortical maps are distorted because the area devoted to input from a body region is proportional to the tactile acuity (Weinstein, 1968) or the number of fibers innervating that region (Mountcastle, 1980), rather than the surface area. Measurements of the response latency suggest that cutaneous mechanoreceptors activate the cortical neurons through a fast conducting pathway with little synaptic delay (Dykes, 1980). This and other anatomical data implicate the dorsal column-medial lemniscus as the major input pathway (Jones and Powell, 1973).

In addition to the primary somatosensory cortex (SI), a complete representation of the body has been located in a secondary (SII) cortical receiving area in many species. In general, the SII area is located posterior and ventral to SI in the parietal lobe (Werner and Whitsel, 1973; Dykes, 1978; Mountcastle, 1980). The somatotopic maps in SII differ from SI, in that either ipsilateral or contralateral stimuli will evoke SII cortical responses (Mountcastle, 1980).

Mountcastle (1957) showed that an electrode penetration normal to the cortical surface would encounter nerve cells of the same functional type (ie. rapidly or slowly adapting) and with the same peripheral receptive field. He described this organization as "columnar". Mountcastle (1957) also noted that different functional columns were arranged in a

mosaic, implying a mixing of functionally different columns within the same body representation.

Recent data by Dykes et al. (1980) suggest that at least three separate representations of the body occur in SI, each with different functional properties. One set of neurons receives inputs exclusively from "deep" receptors (joint and muscle), another set receives input from slowly adapting "cutaneous" receptors, and the third set is driven by rapidly adapting "cutaneous" receptors.

The cytoarchitecture of SI is not uniform. Four different architectonic zones have been identified. Dykes et al. (1980) have shown that the "deep" body representation corresponds with one of these zones, while the two "cutaneous" representations overlap somewhat within one of the other zones. He proposes that closer examination will reveal at least two more complete representations within SI, each with unique functional properties. Dykes concludes that, "the concept of modality segregation for separate but parallel processing of afferent input is in fact a major principle of cortical organization" (Dykes et al., 1980).

MATERIALS AND METHODS

SURGERY AND EXPERIMENTAL PREPARATION

Adult cats (Felis catus) of either sex, weighing between 2.4 and 6.5 kg were anesthetized by intravenous injection of sodium pentobarbital (25 $mg \cdot kg^{-1}$; Abbott Laboratories, Chicago, IL) into the cephalic vein and intubated with a tracheal cannula (4.0-5.5 mm ID.; American Hospital Supply, McGraw Park, IL). Cannulae (PE-90; Clay-Adams, Parsippany, NY) were inserted into the femoral artery and vein and advanced approximately 7 cm into the abdominal aorta and inferior vena cava, respectively. Systemic arterial pressure was monitored by means of a blood pressure transducer (Statham model P23Dc; Grass Instruments, Quincy, MA) and a low level DC preamplifier (model 5P1; Grass Instruments, Quincy, MA) and displayed continuously on a calibrated strip chart recorder (model 7100B; H-P Mosley, Pasadena, CA). Body fluids were supplemented with a slow saline (5% dextrose in lactated Ringer's solution; Cutter Medical Laboratories, Berkley, CA) drip infusion into the venous cannula. Body temperature was monitored by means of a rectal thermistor probe inserted approximatly 10 cm into the lower bowel and connected to a calibrated resistance

bridge and temperature scale (model 44TD; Yellow Springs Instruments, Yellow Springs, OH). A heating pad (120VAC/6A; General Electric, Cleveland, OH) placed under the animal was used to maintain body temperature between 38.5 and 39.5^{o}C. A diagram of the experimental preparation is shown in figure 1.

ANESTHESIA SUPPLEMENTATION

Anesthesia was supplemented by intravenous injections of sodium pentobarbital (1.25-2.50 mg$\cdot$kg^{-1}) at approximately one hour intervals. The depth of anesthesia was monitored through periodic evaluations of spontaneous cortical nerve cell activity, respiratory frequency, systemic arterial pressure, and palpebral, corneal and paw pinch reflexes. In order to standardize the effect of pentobarbital on evoked nerve cell activity, each animal was given an intravenous injection of 1.25 mg$\cdot$kg^{-1} ten minutes prior to each footpad stimulation sequence, but not supplemented during the subsequent 45 min. testing period.

PREPARATION FOR CORTICAL RECORDING

The cat was placed in a laterally recumbent position on its right side, with its head fixed in a stereotaxic frame (model 1204; David Kopf Instruments, Tujunga, CA). A 5 to 8 cm midline incision was made in the scalp and the wound

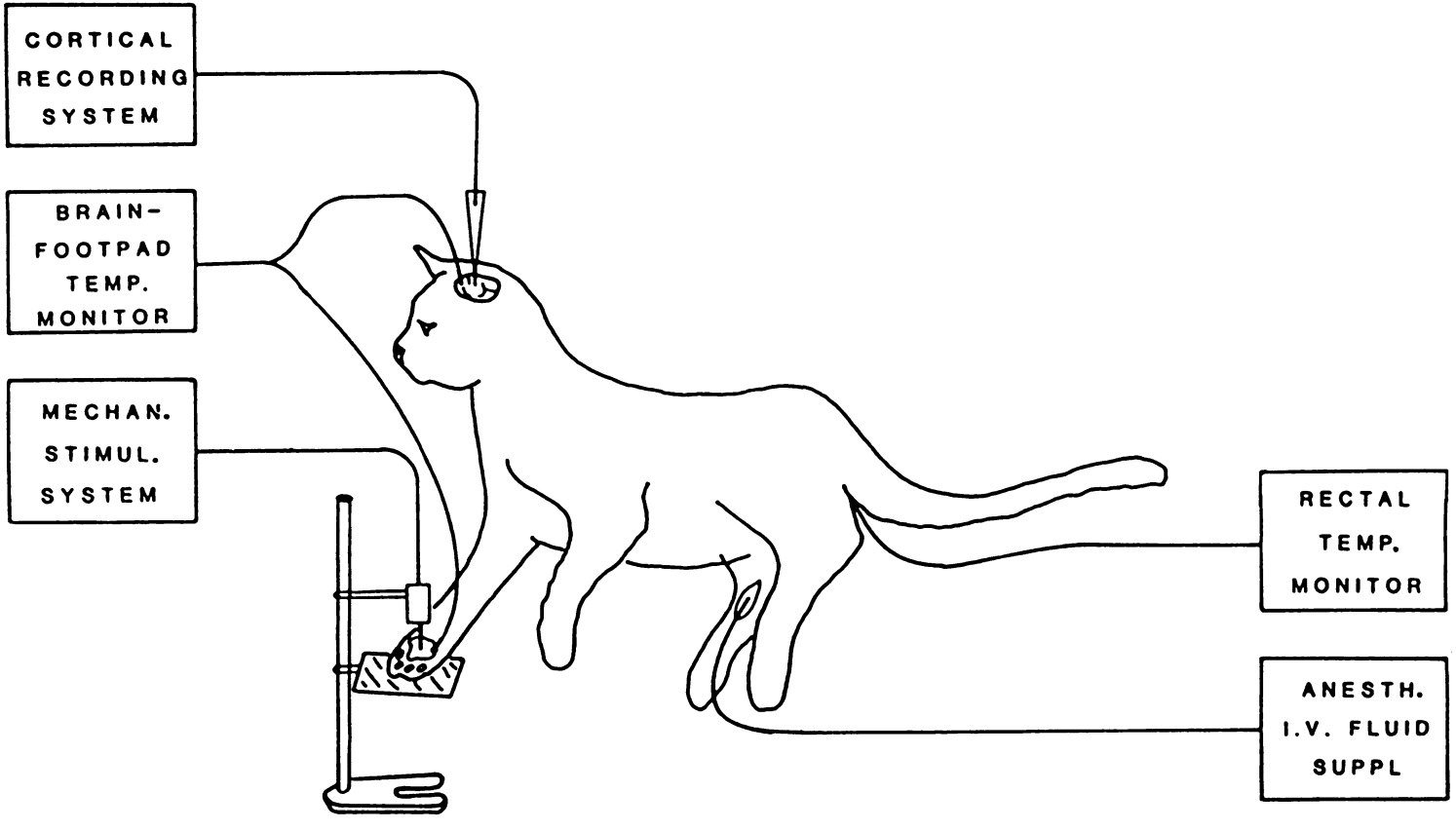

Figure 1. Diagram of experimental preparation. The anesthetized cat rested on a controlled heating pad (not shown) to maintain its body temperature. The animal's head was held in a stereotaxic frame (not shown). Its right paw was restrained against the padded surface of a mechanical support while the glabrous skin was stimulated and electro-physiological recordings were made from the animal's cerebral cortex.

edges retracted exposing the underlying tissue. The frontal bone covering the left cerebral hemisphere was cleared of muscle and connective tissue through blunt dissection. A circular piece of bone 2 cm in diameter was removed from the skull overlying the primary somatosensory cortex (SI) with a Galt trephine (Roboz Surgical Instruments, Washington DC) to expose the dura. Dental acrylic (New Weld; L.D. Caulk Company Milford, DE) was used to form a dam around the craniotomy, rising about 0.5 cm above the skull surface. The dura was carefully removed with the aid of a dissecting microscope (Stereozoom 7; Bausch and Lomb, Rochester, NY) using fine forceps and curved scissors (Geo. Tiemann and Co., Long Island, NY). Care was taken to avoid damage to the blood vessels on the brain surface or contact with brain tissue. After removing the dura, the cortical surface was kept moist with a warm (38-40^{o}C) mammalian Ringer's solution (Lactated Ringers; Cutter Medical Laboratoties, Berkley, CA). Cortical surface temperature was monitored with a thermopile (figure 2) constructed from 40 gauge copper-constantan thermocouple wire (Omega Engineering, Stamford, CT) and displayed on a calibrated strip chart recorder (model 7100B; H-P Mosley, Pasadena, CA). A narrow beam high intensity lamp (Dynalum; Cole Palmer Instruments, Chicago, IL) was used to maintain brain surface temperature between 38 and 40^{o}C during the experiment.

Brain surface pulsations owing to respiratory and cardiovascular oscillations were damped by covering the

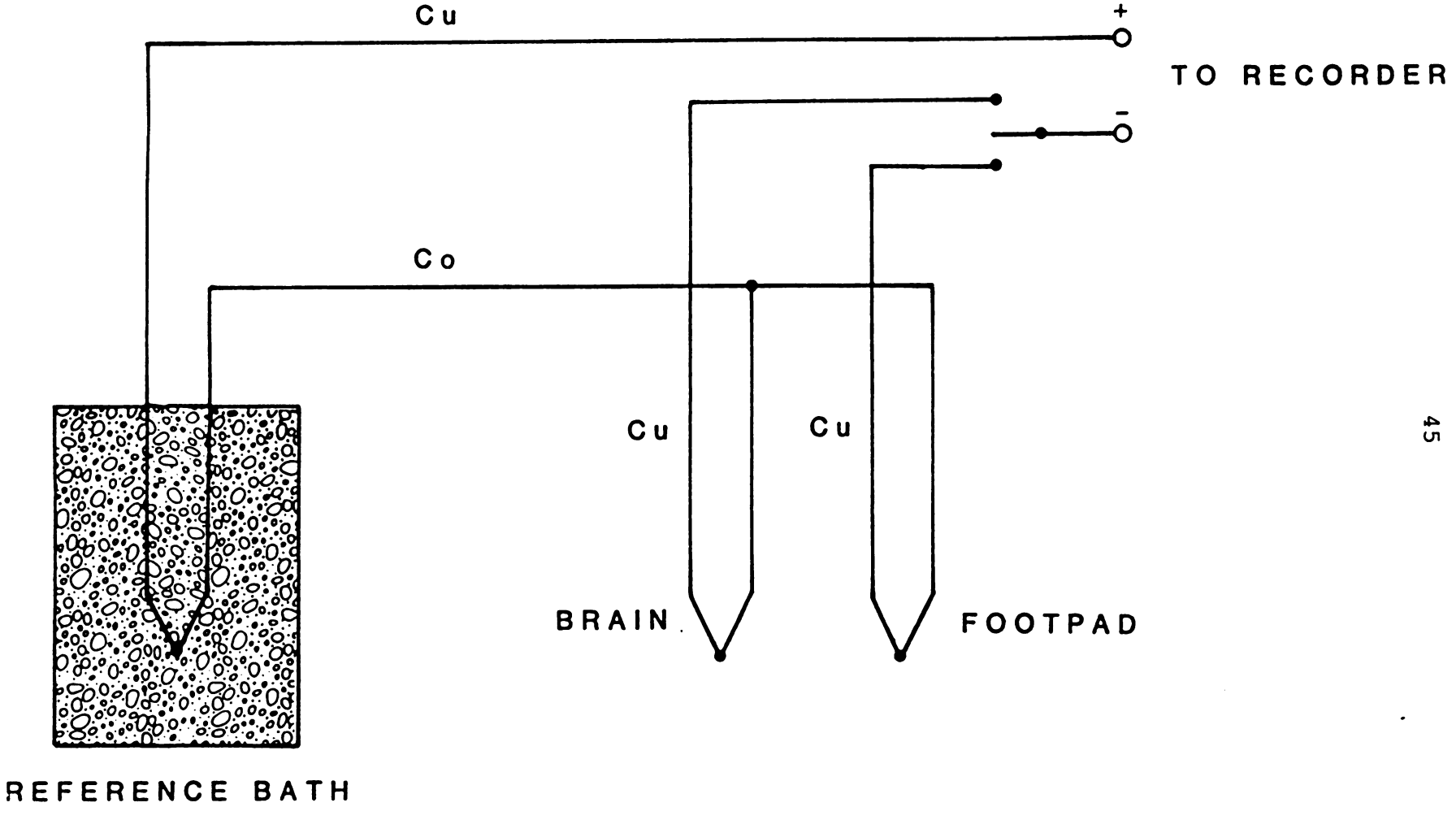

Figure 2. Copper-constantan thermocouples (40 gauge) referenced to the temperature of an unstirred, thermally insulated ice-in-water slurry were used for measuring brain and footpad temperatures.

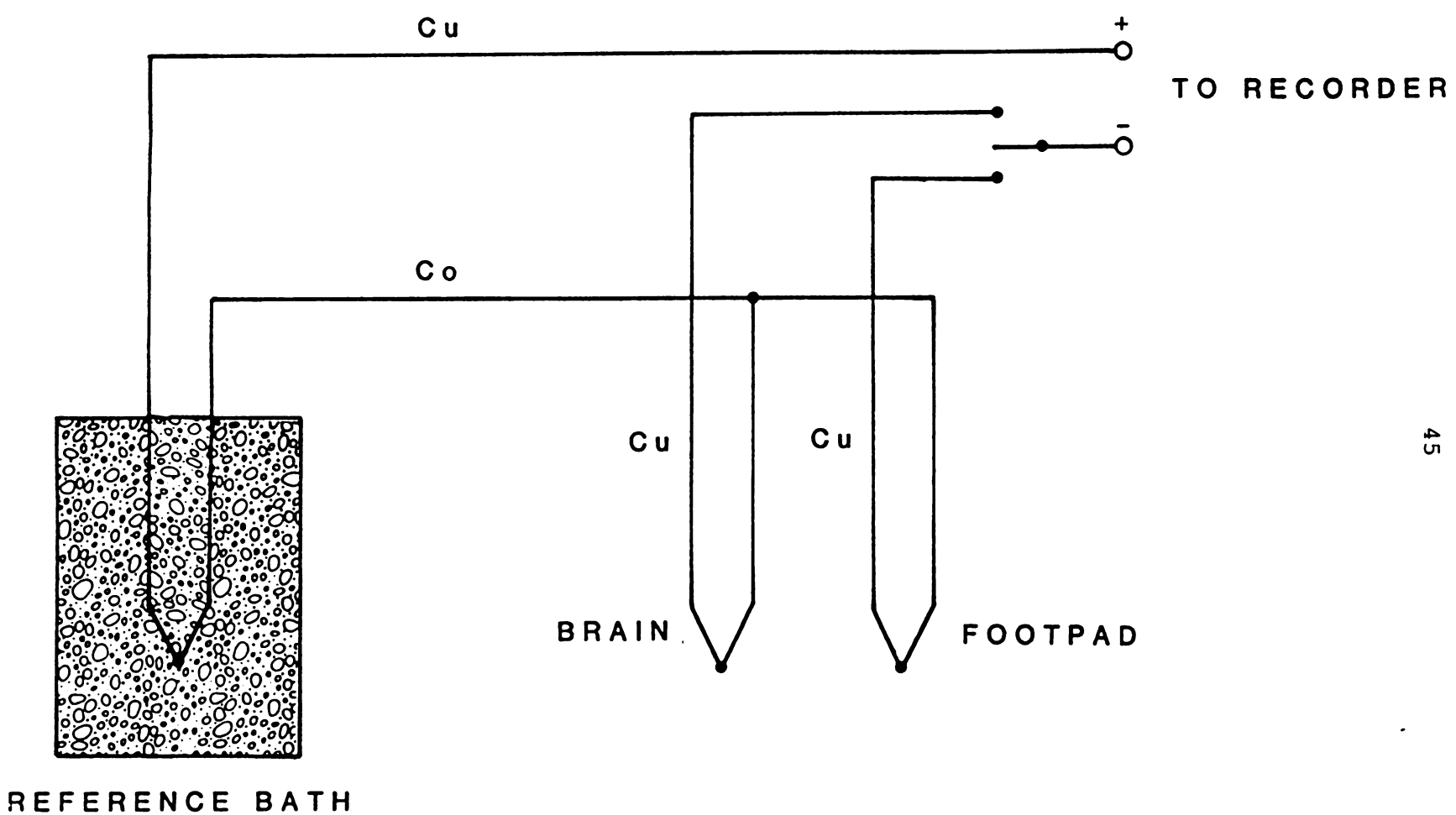

Figure 2. Copper-constantan thermocouples (40 gauge) referenced to the temperature of an unstirred, thermally insulated ice-in-water slurry were used for measuring brain and footpad temperatures.

cortex with an agar gelatin. The gelatin was prepared by boiling 0.7 mg of agar (Bacto-Agar; Difco Laboratories, Detroit, MI) in 10 ml of mammalian Ringer's solution (lactated Ringer's solution; Cutter Medical Laboratories, Berkley, CA) for about 2 min., or until the agar was completely dissolved. The solution was allowed to cool in a stirred water bath (Dubnoff Incubator; Precision Scientific, Chicago, IL) and then held at a temperature of 45^{o}C for 30 to 45 min. to allow air bubbles to escape. The cooled but still partially liquid agar solution was poured over the cortex to form a 0.5 to 1.0 cm thick layer within the dental acrylic dam, which served as a retainer and stabilized the gelatin after it had solidified. Although the gelatin layer was solid enough to effectively dampen pulsations of the brain surface, it was easily penetrated with a microelectrode.

MICROELECTRODES

Glass insulated, tungsten microelectrodes (see figure 3) were assembled using the technique of Johnson (Personal Communication). For straightening, a 5.5 cm length of 127u diameter tungsten wire was held taut between two vises, while 15VDC was applied across it, causing it to glow red. After it had cooled, the straightened wire was inserted into a 4.0 cm length of 26 gauge stainless steel tubing (Laboratory Accessories Inc., Millburn, NJ), which was then

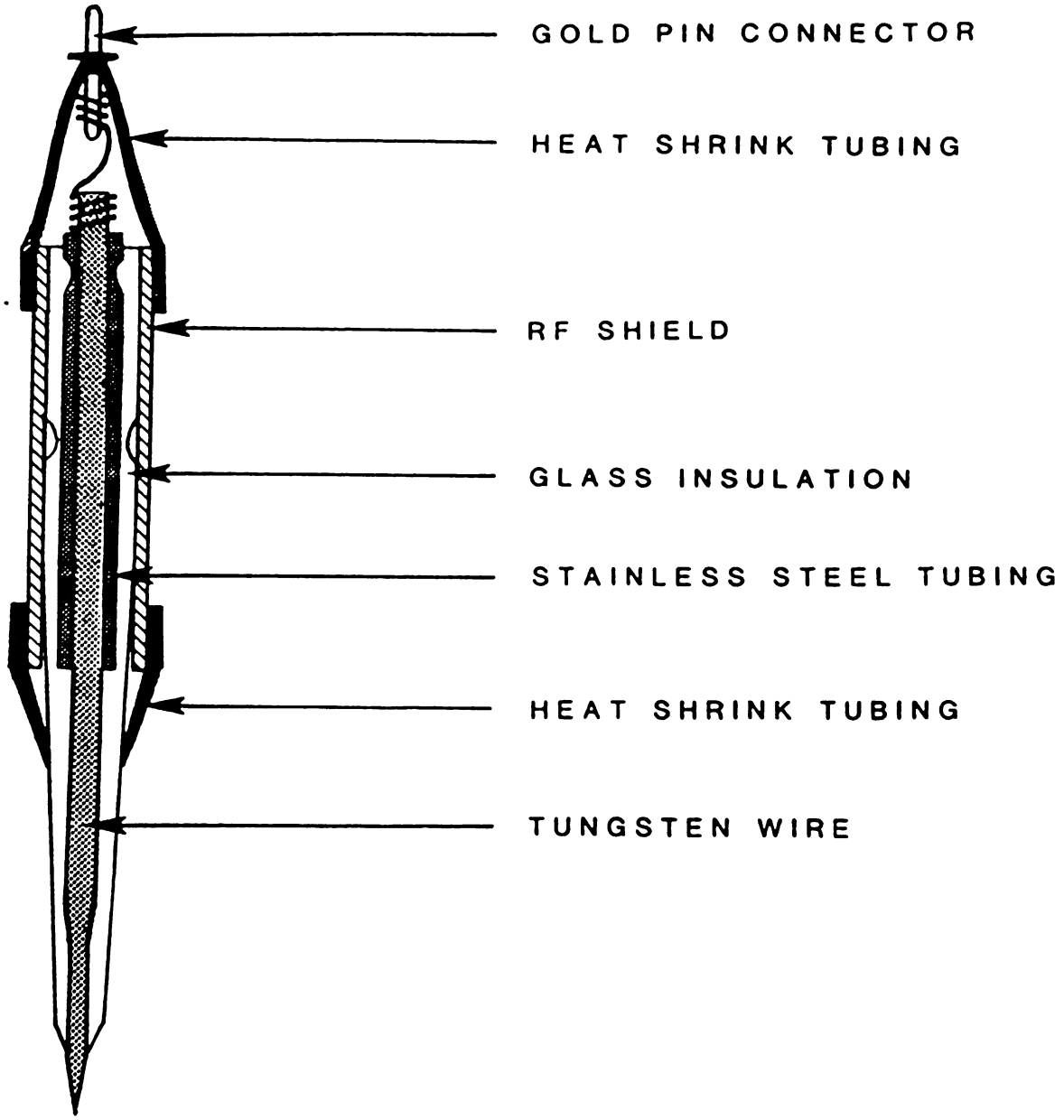

Figure 3. This sketch of the microelectrode for recording unit activity is not drawn to scale. Electrodes varied between 5 and 8 cm in length with uninsulated tips 10-50u in length, and tapering from 10-25u in diameter.

crimped at the butt end tight enough to insure good electrical connection. About 0.5 cm of the exposed tungsten wire was etched electrolytically in a saturated sodium nitrite (Mallinkrodt Inc., St Louis, MO) solution at about 20VAC (Powerstat; Superior Electric, Bristol, CT) to produce a stubby but sharp tip approximately 50u in diameter. Electrode tips were tapered further either by etching at 10VAC for 50 sec. or by repeatedly dipping the electrode in and out of the solution at 30VAC until the desired shape was obtained (figure 3). The possibility of sodium nitrite contamination of the electrode tip was eliminated by successive washings with deionized water, acetone, ethanol and ether.

The metal microelectrode was electrically insulated by drawing molten glass tubing over it using a vertical micropipet puller (model 700B; David Kopf Instruments, Tujunga, CA). This was accomplished in the following series of steps. First the electrode was inserted butt end down into a 6.0 cm length of 2 mm OD glass tubing which had been heat sealed at its lower end. The electode was postioned so that the middle of the stainless steel tubing was in the center of the heating coil. Allowing the lower clamp to drop about 1 cm while heating the glass tubing (24A) crimped the glass tightly around the steel tubing. The electrode was then inverted and positioned in the clamp with the tip 1 cm below the heating coil and then pulled again at a heater current of 24A, until the diameter of the glass tubing was

reduced about 50%. The electrode tip was lowered to a position slightly below the heating coil, and then the lower clamp was allowed to drop very slowly with a 20A heater current, drawing the molten glass over the electrode tip. In properly insulated electrodes, the glass was bound to the metal electrode along its entire length and covered the tip. The glass insulation was then removed from the very tip of the electrode, by etching in concentrated hydrofluoric acid (Mallinckrodt Inc., ST Louis, MO) under mineral oil (Fisher Scientific, Pittsburgh, PA). The acid-oil interface was viewed through a dissecting microscope (Stereozoom 7; Bauch and Lomb, Rochester, NY) in order to monitor the etching process. Electrodes produced in this fashion for extracellular recording of activity of small clusters of nerve cells, characteristically had conical tips 14.0 to 16.5u in diameter and 35.0 to 45.0u in height. A flexible wire lead with a gold pin connector was soldered to the butt end of the electrode after breaking off about 5 mm of the glass insulation. Electrodes chosen for use were radio-frequency shielded by securing them inside 12 gauge stainless steel tubing (Laboratory Accessories Inc.; Millburn, NJ) with heat shrinkable tubing (Newark Electronics, Milwaukee, WI).

CORTICAL RECORDINGS

A block diagram of the recording system is shown in figure 4. Microelectrode signals in reference to an indifferent electrode clipped to the skin were amplified with a differential input, AC preamplifer (type 122; Tektronix Inc., Beaverton, OR) having an approximate gain of 1000 and lower and upper filter cut off frequencies set at 0.8 Hz. and 1.0 KHz respectively. The preamplifier output was filtered with a 60 Hz. notch filter and further amplified by the circuit diagramed in figure 5. The gain of this amplifier was adjusted to give a maximum output of about 2.5 volts. Voltage spikes were monitored on a dual beam storage oscilloscope (model 564; Tektronix Inc., Beaverton, OR) or recorded on magnetic tape using a 7 track, FM tape recorder (model 2000; Sanborn-Ampex, Waltham, MA). A tape speed of 3 3/4 $in \cdot sec^{-1}$ was used for both recording and playback, with a resulting band width of 50Hz. to 6.25KHz (±3dB) and a 40dB signal to noise ratio. In addition, the amplifier output was used to drive an audio monitor (figure 5). The recording system was calibrated with 100uV square waves generated with a DC stimulator (model S8; Grass Instruments, Quincy, MA) and a voltage divider. A representative calibration signal is shown in figure 6.

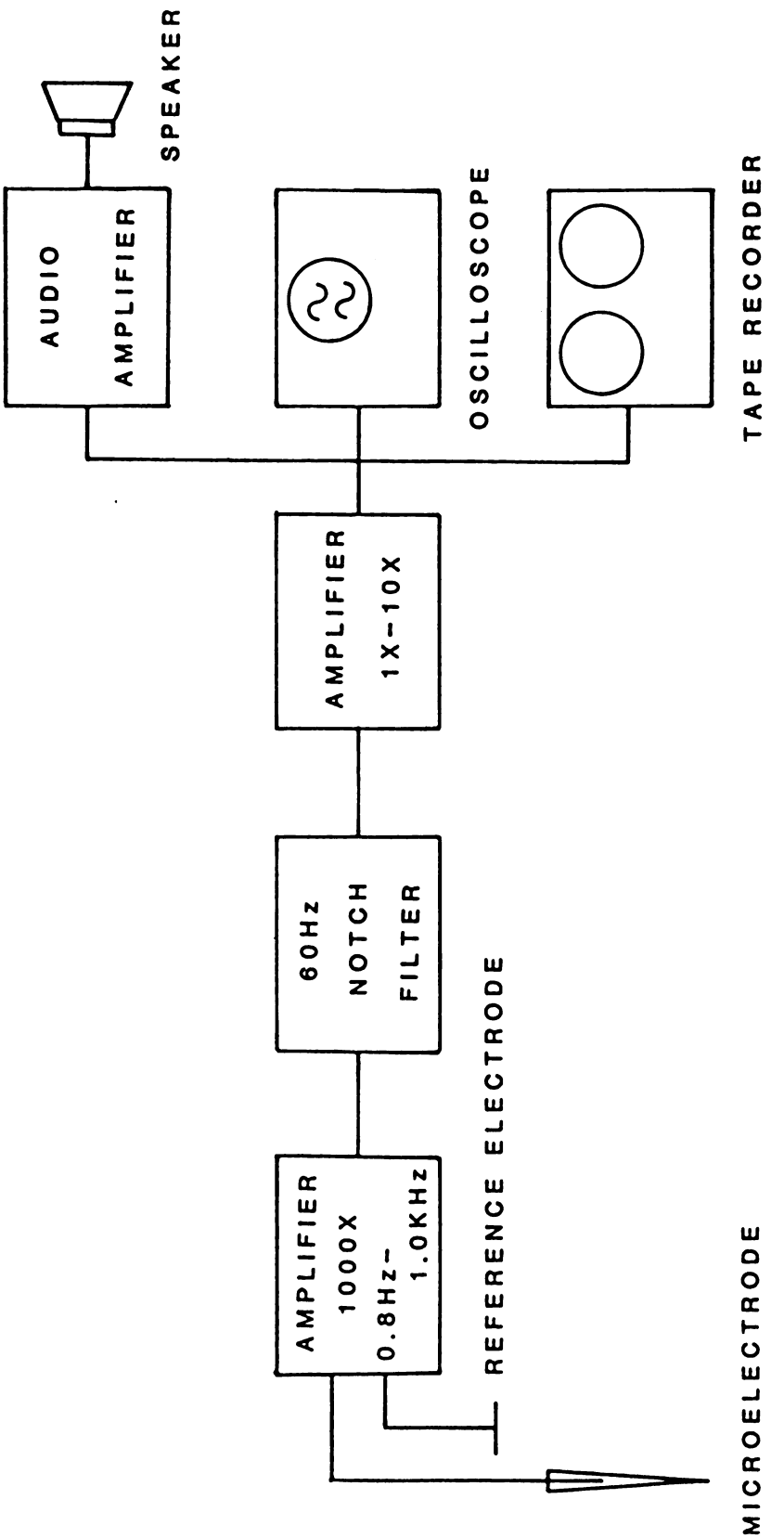

Figure 4. Block diagram of signal amplification and recording system.

Figure 5. Circuit diagram of second stage amplifier, filter and audio monitor. Bandwidth, filtering quality and gain of the switchable 60 Hz filter were adjusted with the "Wo", "Qn" and "gain" potentiometers to provide optimal filtering of a 60 Hz sine wave. The audio monitor could be switched in order to monitor amplifier output ("mon") or magnetic tape output ("r/p"). The audio limiter provided filtering of the FM tape recorder carrier frequency.

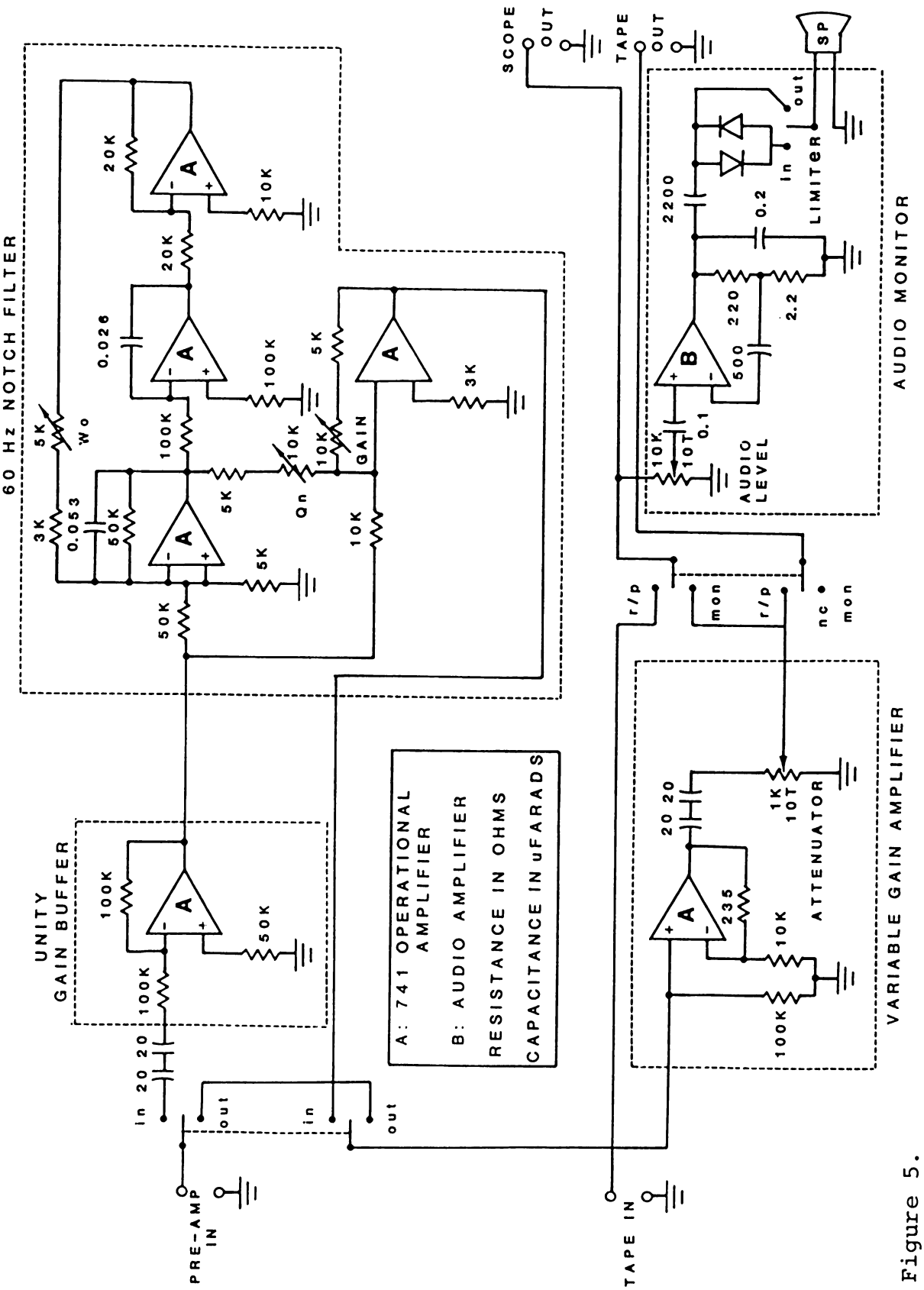
60 Hz NOTCH FILTER
UNITY GAIN BUFFER
VARIABLE GAIN AMPLIFIER
AUDIO MONITOR
A: 741 OPERATIONAL AMPLIFIER
B: AUDIO AMPLIFIER
RESISTANCE IN OHMS
CAPACITANCE IN uFARADS
PRE-AMP IN
TAPE IN
SCOPE OUT
TAPE OUT
SP
LIMITER
AUDIO LEVEL
ATTENUATOR
GAIN
Wo
Qn
r/p
mon
nc

Figure 5.

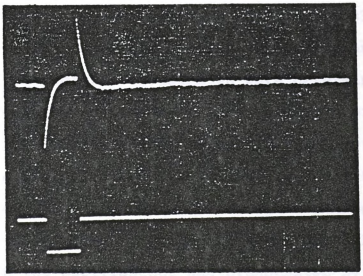

Figure 6. In this recording system calibration the upper trace shows tape recorder output, and the lower trace shows -100uV input signal. Calibrations: Upper trace= 0.5V/cm div.; Lower trace= 100uV/cm div.; Sweep= 10msec/cm div.

A shielded microelectrode, mounted in a micromanipulator (Narishige model 2138; Eric Sobotka Co., Farmingdale, NY) was positioned over probable sites for recording extracellular activity from small clusters of cortical neurons having receptive fields on the central footpad. Experience gained from preliminary experiments and data from experiments by Mountcastle (1957) and Dykes et al. (1980) were used to locate central footpad units. Cortical sulci and postcentral gyrus topography served as visual landmarks. Prior to covering the brain surface with the translucent agar layer, stereotaxic coordinates were determined for probable recording locations, taking care to avoid surface blood vessels. Once the agar had solidified over the brain surface, the microelectrode was slowly advanced through the gelatin layer until contact with the brain surface was verified by distinctive reductions in the noise levels displayed on the oscilloscope and broadcasted by the audio monitor. The microelectrode was then advanced in 50 to 200u steps, to a maximum depth of 1.0-2.0 cm from the brain surface, or where evoked responses were no longer recordable. After pausing at each site long enough for spontaneous activity induced by electrode movement to subside, the boundaries of the peripheral receptive field for that unit were determined by lightly stimulating the skin with a sharp tipped wooden rod, a fine paintbrush or with the mechanical stimulator set for a midscale force. After determining the locations of the receptive field and

considering receptive fields encountered during previous penetrations, the electrode was withdrawn and repositioned for a new penetration. A small area of the primary somatosensory cortex was systematically explored in this manner until a cortical unit was located which responded to light tactile stimulation of the glabrous skin on the central footpad. A representative neuronal response is shown in figure 7. Once a unit of this type was located, no further movements of the microelectrode were made, and the preparation was allowed to stabilize for 30 to 45 min. before data were recorded.

FOOTPAD PREPARATION

Fur was removed from the right foreleg and paw by clipping (model A2; Oster, Milwaukee, WI) and from the central foot pad area with a depilatory cream (Neet; Whitehall Laboratories, New York, NY). Caution was exercised so that the depilatory agent did not come in contact with the glabrous skin. The forepaw was positioned and gently restrained against a padded platform, with the plantar surface upward and parallel to the table surface as shown in figures 1 and 8. Rubber dental dam (Light-Thin; Hygienic Corporation, Akron, OH) with holes cut to expose the central glabrous footpad and toepad skin was used to restrain the paw. The limb was carefully positioned at or slightly below the level of the heart in order to keep

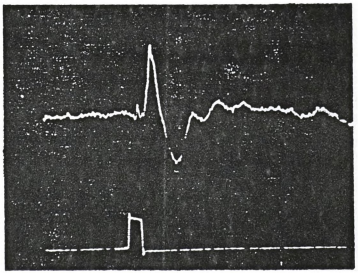

Figure 7. In this recording of a cortical unit response, the upper trace shows a single biphasic cortical unit action potential with negative polarity in an upward direction, and the lower trace shows the stimulus event marker. Calibrations: Upper trace= 100uV/cm div.; Lower trace= 1.0V/cm div.; Sweep= 20msec/cm div.

Figure 8. Mechanical stimulator and hydration capsule were held in a micromanipulator (not shown) which allowed them to be positioned above the footpad locations to be tested. Conditioned air flowed through the inlet tube and out around the edges of the clear plastic hood.

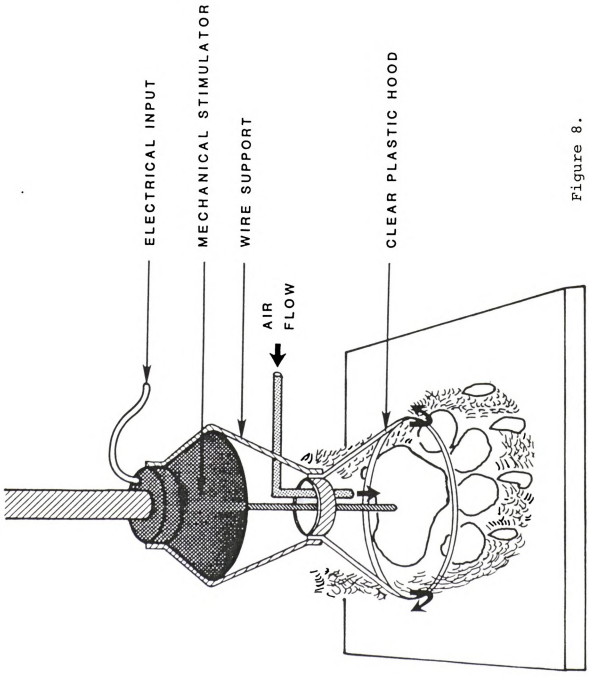
ELECTRICAL INPUT
MECHANICAL STIMULATOR
WIRE SUPPORT
AIR FLOW
CLEAR PLASTIC HOOD

Figure 8.

footpad blood flow unobstructed. Footpad temperature was monitored with a thermopile (figure 2) constructed from 36 gauge copper constantan thermocouple wire (Omega Engineering; Stamford, CT) and displayed on a calibrated strip chart recorder (model 7100B; H-P Mosley, Pasadena, CA) and served as an index of an uncompromised circulation. A footpad temperature of 34-38^{o}C was accepted as indicating that the extremity had an unimpeded circulation.

MECHANICAL STIMULATION

A device capable of delivering small displacement stimuli of varying force was assembled (figure 9), using the design of Brown (1974). This stimulator was constructed by cutting away the cone and support bracket from an 8 ohm, 2.25 in. transistor radio speaker (Philmore Manufacturing, Inwood, NY) leaving intact only its permanent magnet, central cylinder and support baffle. Punctate stimuli were delivered to the skin through a brass rod cemented with epoxy to the center of the speaker cylinder. The stimulating rod was tapered using a jeweler's lathe to a tip diameter of 0.8 mm The mechanical stimulator was driven with a DC stimulator (model S9; Grass Instruments, Quincy, MA) capable of producing 1.0 to 7.0VDC square waves pulses, 10 msec. in duration and at a frequency of 1 Hz. These pulses were used as input to the circuit diagramed in figure 10, which provided the current necessary to drive the

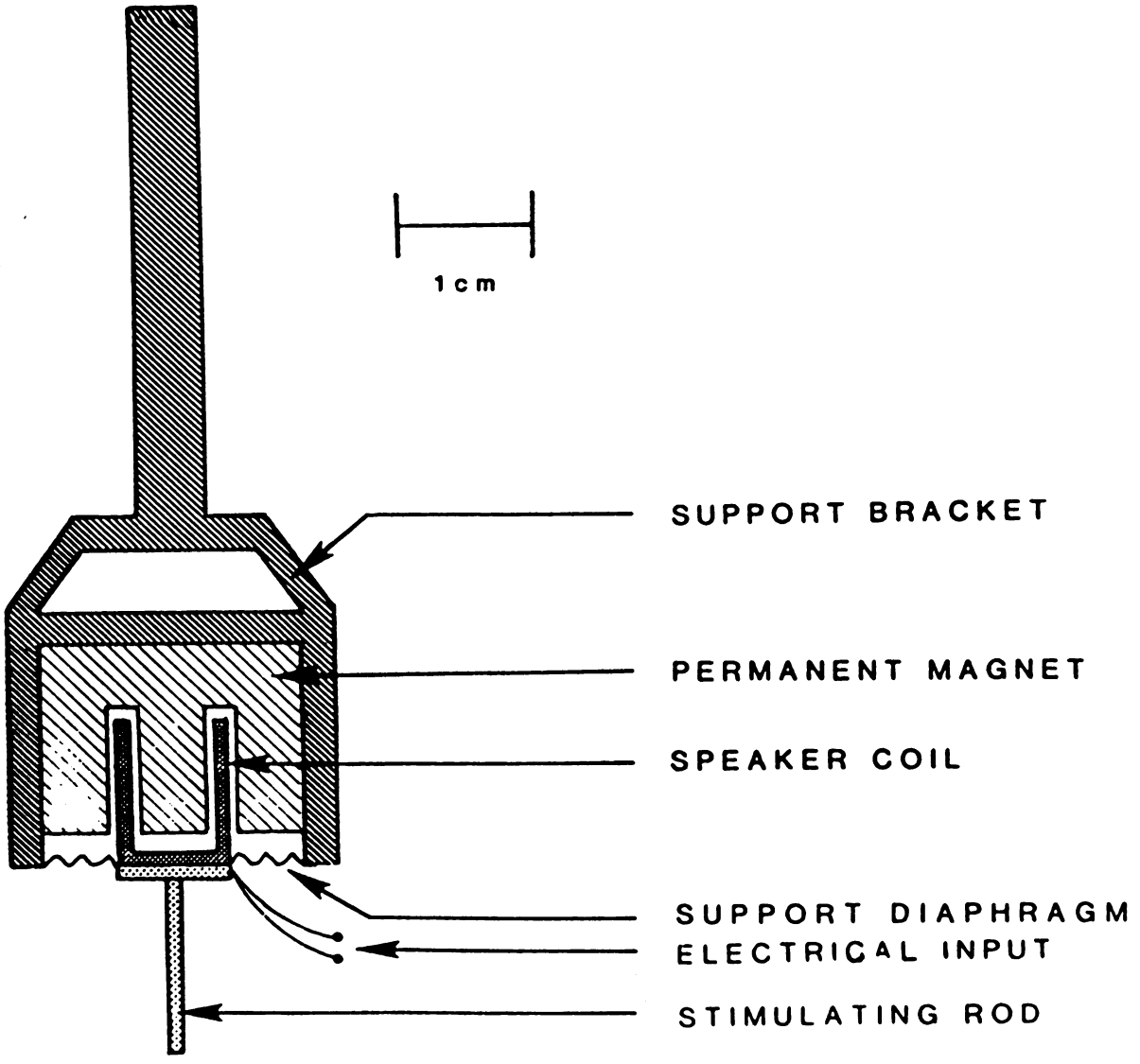

Figure 9. This drawing is a cross section of the mechanical stimulator. Displacements of the stimulating rod were obtained by applying a current pulse to the electrical inputs of the speaker coil.

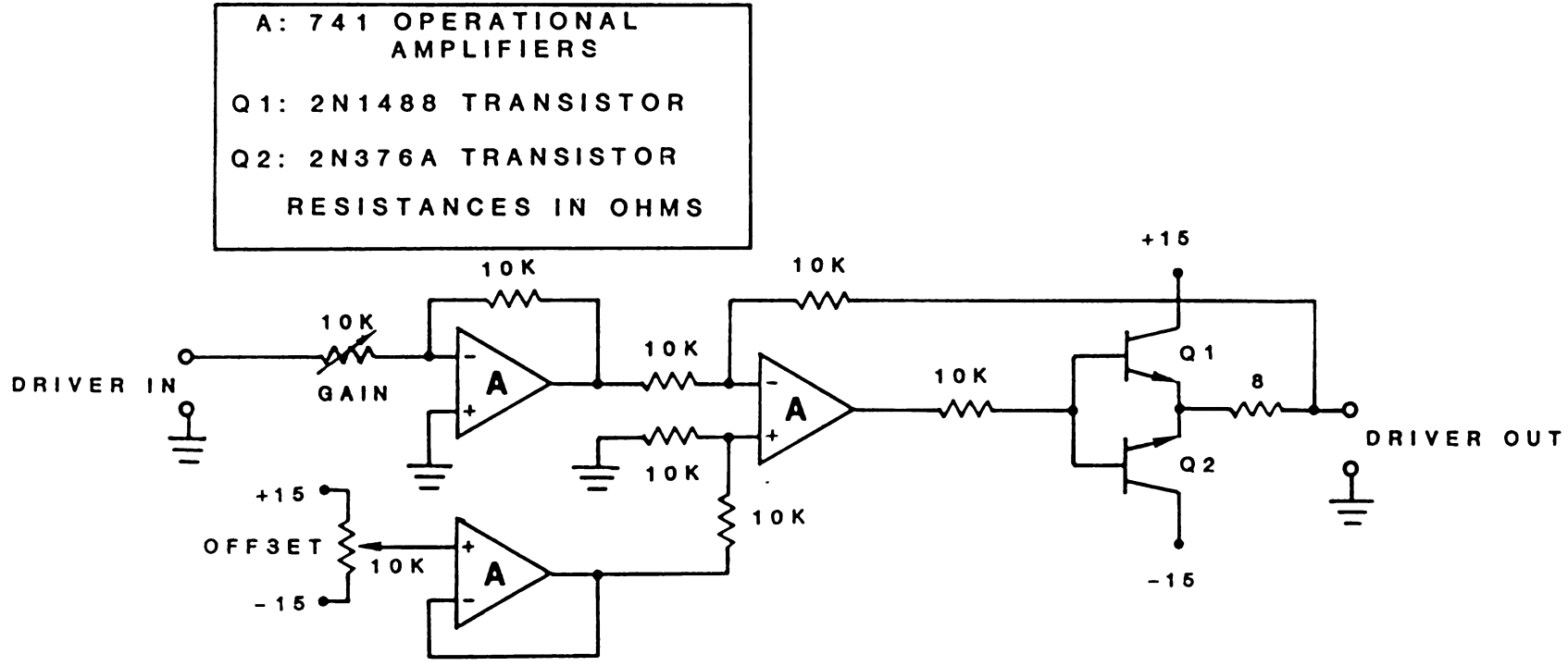

Figure 10. This schematic shows the circuit diagram of the mechanical stimulator current source. Current pulses for the mechanical stimulator were obtained at "driver out" in response to voltage pulses presented at "driver in". See figure 11 for calibrations.

mechanical stimulator. The gain of this circuit was adjusted during preliminary experiments so that a force just great enough to evoke cortical nerve cell activity was produced with a midscale input. A calibration of the stimulating system is shown in figure 11.

The model S9 DC stimulator output was also used as parallel input to the circuit diagramed in figure 12, which provided an impulse to be used as an event marker for mechanical stimulation of the skin. This event marker was monitored on a second channel of the dual beam oscilloscope, and recorded on a second channel of the magnetic tape recorder. Cortical neuronal responses and the stimulus events which evoked them could be displayed simultaneously as shown in figures 7 and 13.

Stereotaxic coordinates for 7 colinear, equidistant skin locations were mapped using the mechanical stimulator mounted in a micromanipulator (Narishige model 2138; Eric Sobotka Co., Farmingdale, NY). The first and seventh locations were chosen on the medial and lateral edges of the glabrous central footpad skin. These were the most peripheral locations on the central footpad which allowed the mechanical stimulator to apply forces normal to the skin surface. The remaining 5 skin locations were spaced equally between these limits. This medial-lateral transect across the skin was oriented to include the area on the footpad which appeared to be most sensitive to low level stimulation of the skin, as evidenced by cortical evoked potentials.

Figure 11. Mechanical stimulator driver calibration. This figure shows the current output of the circuit diagramed in figure 10 in response to controlled voltage inputs, and demonstrates the linearity of the output over the range of inputs used in these experiments.

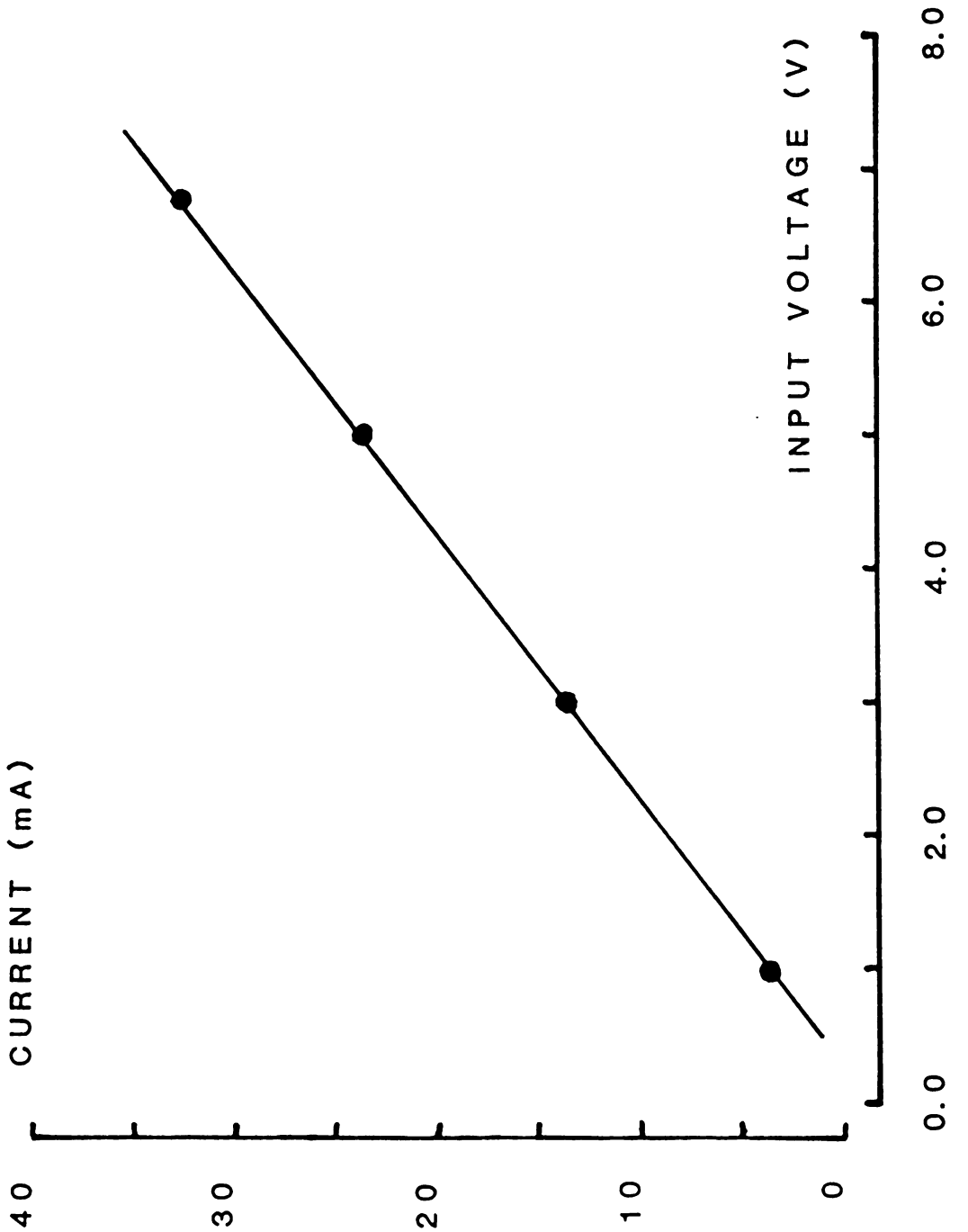

Figure 11.

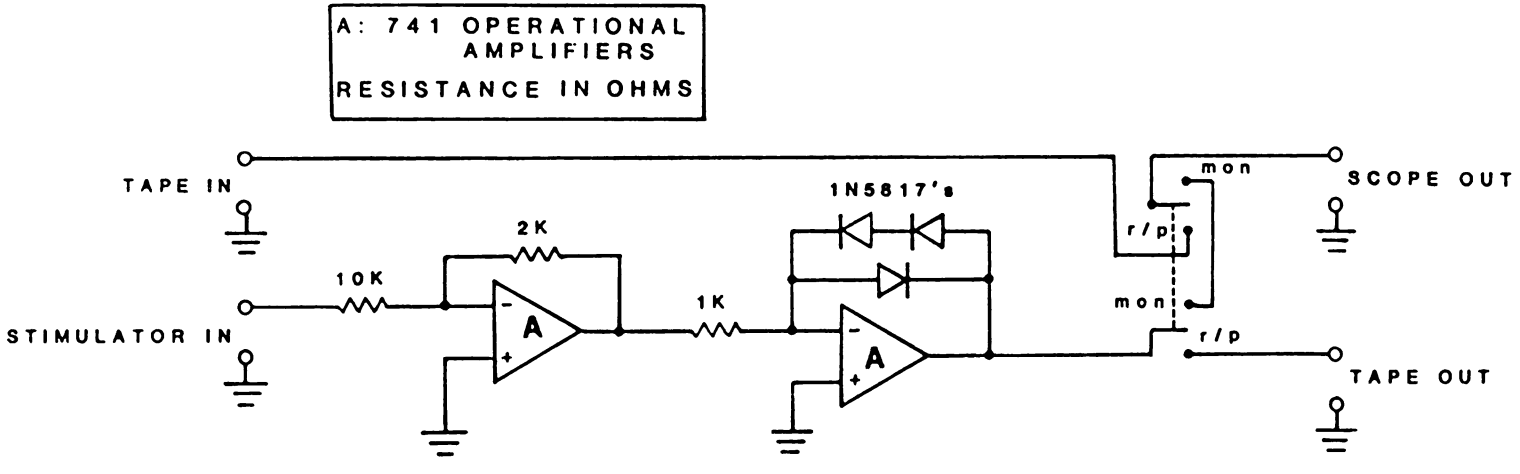

Figure 12. Circuit diagram of the stimulus event marker. Stimulus event marks shown in figures 7 and 13 were provided by the circuit diagrammed in this figure. Parallel output of the voltage source driving the circuit shown in figure 10 provided input to the event marker circuit. Output from this circuit could be switched in order to monitor it directly ("mon") or to record and monitor it from magnetic tape ("r/p").

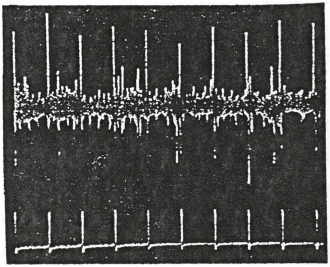

Figure 13. Cortical neuronal responses to ten successive mechanical stimuli. Upper trace shows cortical unit responses to mechanical stimuli delivered to the footpad surface. Lower trace shows the stimulus event markers. Calibrations: Upper trace= 100uV/cm div.; Lower trace= 1.0V/cm div.; Sweep= 1sec/cm div.

Once stereotaxic coordinates were determined, each of the 7 skin locations was stimulated 10 times at a frequency of 1 Hz. for each of 31 stimulator driving voltages progressing from 1.0 to 7.0VDC in 0.2VDC increments. This resulted in 310 stimulations of the footpad for each of the 7 footpad locations. For each hydration treatment, the progression of stimulator driving voltages and skin locations was repeated in the same sequence.

SKIN HYDRATION

Four skin hydration conditions, "room air" (RA), "wet air" (WA), "dry air" (DA) and "liquid water soaked" (WS) were established sequentially in each of six experimental animals. Evoked responses to mechanical stimulation of the skin were recorded as described previously for all hydration conditions in each animal. In addition, the pliability of the skin was subjectively evaluated for the six footpads used for the analysis of variance. The hydration treatment sequence was the same for all animals.

The first condition (RA) for which the response to mechanical stimulation was tested was with the central footpad exposed to room air and with no forced air flow across the footpad surface. After stimulating all seven skin locations, a second hydration condition (WA) was established by exposing the footpad to a water saturated air stream flowing at 0.5 to 1.0 liter$\cdot$min^{-1} for 15 to 30 min.

The water vapor stream was produced by bubbling air through an airstone (Mist-Air; Kordon, Haywood, CA) immersed in an erlenmeyer flask containing water heated on a hotplate (model HP-A1915B-13; Thermolyne, Dubuque, IA). The air stream was cooled to room temperature (nom. 25^{o}C) before being blown across the skin surface. After the initial wetting period, the air flow was reduced to 0.1 to 0.3 liter$\cdot$min^{-1} and held at that level during the mechanical stimulation of the skin. For the third hydration condition (DA), the skin water was reduced by exposure of the footpad to a dried air stream flowing at 5 to 10 liters$\cdot$min^{-1} for 15 to 45 min. The air flow rate was again reduced to 0.1 to 0.3 liter$\cdot$min^{-1} during the mechanical stimulation period. The fourth skin hydration condition (WS) was produced by spraying deionized water on the skin surface and then covering the central footpad with water soaked gauze sponges for 15 to 45 min. In order to keep the skin water content high during the subsequent mechanical stimulation of the skin, a water saturated air flow of 0.1 to 0.3 liter$\cdot$min^{-1} was blown across the footpad. Air flows during the WA, DA and WS hydration treatments were directed across the footpad as shown in figure 8.

EXPERIMENTAL PROTOCOL AND DATA REDUCTION

All animals were identically treated using the following protocol. The somatosensory cortex was probed with a

microelectrode until a neural unit responding to mechanical stimulation of the central footpad was located. Evoked responses were recorded from this unit during the sequential hydration conditions of room air (RA), wet air (WA), dry air (DA) and liquid water soaked (WS) exposure. During each hydration condition, seven preselected skin locations were mechanically stimulated with ten repetitions of each of 31 stimulus strengths. The resulting evoked action potentials and a corresponding stimulus event marker were recorded on magnetic tape, along with a voice commentary of stimulus strength, skin location and hydration condition. After completing the experiment, the data were replayed through a dual trace oscilloscope and all evoked action potential amplitudes were read from the calibrated oscilloscope screen and their values entered by keyboard into a digital computer (PDP 11/34A; Digital Equipment Corporation, Maynard, MA). The computer was used for both data storage and reduction, with outputs printed on a high speed line printer (model 300; Printronix, Irvine, CA).

Histograms of the number of observations of each action potential amplitude were plotted for each skin location and each hydration condition. These histograms were used to set upper and lower amplitude windows, which defined "unit" response. First the most sensitive skin location was identified. This was the site for which the action potential amplitude histograms were skewed most toward the larger amplitude responses during the RA treatment. From

the range of responses for this skin location and hydration condition, an upper window was chosen as the largest amplitude which was observed at least five times. The lower amplitude limit for this unit was chosen as twice the peak-to-peak background noise level. Only neuronal responses within this amplitude window were considered as responses of that unit. The same window was used for all skin locations and hydration conditions in any one experimental animal. Although an amplitude window defined by this method does not guarantee that unit responses correspond to activity in a single neuron, it does represent a clearly definable and detectable response from one or a small number of nerve cells which can be compared while stimulating other skin locations under different hydration conditions.

Once amplitude window limits were chosen, the number of unit responses resulting from ten consecutive mechanical stimulations were plotted as a function of the stimulus strength for all hydration conditions at each skin location. In addition, threshold levels (T10,T20,T30,...,T100) defined as the minimum stimulus strength required to evoke 1,2,3,...,10 unit responses respectivly in 10 trials were computed, for each hydration condition and skin location. These threshold levels (T10-T100) represent the minimum stimulus strength required to achieve a 10, 20, 30, ..., 100 percent probability of unit response.

STATISTICAL ANALYSIS

A split-plot analysis of variance (Steel and Torrie, 1960) was chosen to evaluate statistically the threshold values (T10-T100) determined in these experiments. The skin hydration condition was designated as the main treatment, five skin locations were selected from each animal as the subplots and each of the six animals was considered to be an individual block. Five adjacent skin locations were chosen as subplots so that "location 1" corresponded to the skin location having the lowest T50 value during the RA exposure, and "locations 2 thru 5" represented adjacent skin sites progressively more distant from the low threshold position. Only five locations were used in the statistical analysis, so that in all cats, each of the subplot locations (2-5) maintained the same geometric relationship to the most sensitive skin location (1), regardless of their actual position on the footpad. If the most sensitive site was located on the periphery of the central footpad, it and the next four locations in sequence were chosen for analysis. If, however, the second or third skin location was the most sensitive site, the first one or two sites respectively were omitted and the next five were used in the analysis.

Duncan's Multiple Range Test (Steel and Torrie, 1960) was used for multiple comparisons of treatment (hydration condition) and subplot (skin location) means. A type I

error probability of five percent (a=.05) was used to judge statistical significance in all tests.

RESULTS

During this investigation approximately 230 electrode penetrations were made into the primary somatosensory cortex of 28 anesthetized cats. The amplitude of action potentials recorded varied between 50 and 350uV. Figure 7 shows an example of a unit response typical of those recorded during this study. Although some units had initially positive waveforms, only those with initially negative waveforms were chosen for study. The neurons encountered in these penetrations varied greatly in their response characteristics. Some cortical neurons responded only to deep pressure or vigorous tapping of the skin surface, while others responded to light stroking of the skin with either a fine paint brush or the sharpened tip of a wooden rod. Only the latter were examined for their response to controlled mechanical stimulation of the skin.

In initial experiments the position of cortical neurons which responded to small deformations of the glabrous central footpad skin was located with reference to brain surface landmarks. This was accomplished by superimposing somatotopic maps obtained during systematic microelectrode exploration of the somatosensory cortex onto drawings of the cortical surface. Experience gained from these explorations

and information from previously published somatotopic maps (Mountcastle, 1957; Dykes et al., 1980) allowed us to locate routinely cortical neurons activated by light tactile stimulation of the central footpad.

Two types of neurons responded to light tactile stimulation of the skin. One group chacteristically had large receptive fields, often including one or more toe pads in addition to the central footpad. They were usually extremely sensitive to vibration and could be easily activated by slight vibrations of the table or footpad support stand. A second type of cortical nerve cells typically had much smaller receptive fields, which were usually restricted to the central footpad. These were much less sensitive to vibrations, but could be easily driven by light mechanical stimulation of the glabrous central footpad skin. Eleven neurons of this type were located and examined for their response to controlled mechanical stimulation of the skin during different epidermal hydration states.

Once a neuron with the second type of response characteristics was located and the preparation allowed to stabilize, the response of that unit to 10 repetitions of each of 31 mechanical stimulus strengths was recorded on magnetic tape. This same stimulation sequence was repeated and the neuronal response recorded for 7 equidistant and colinear stimulation sites on the central footpad surface. A representative record of the response of a cortical neuron to 10 repetitive stimulations at the same stimulus strength

is shown in figure 13. Two noteworthy features of this record are the variation in the amplitude of the unit response, and the presence of spontaneous discharges of neurons which were more distant from the electrode and therefore smaller in amplitude.

After an experiment was completed, the magnetic tapes were replayed and the amplitude of any neuronal response which coincided with the presentation of a mechanical stimulus was entered into the computer along with identification of the skin location, hydration condition and the stimulus strength. Histograms of the frequency of occurence of each action potential amplitude observed were plotted by the computer. Figures 14 and 15 show representative data from two different animals. Each of these histograms represents all action potential amplitudes observed when stimulating a single skin location during one epidermal hydration state. Multimodal distributions such as those in figure 14 are interpreted to indicate that the response of more than one neuron is being detected by the electrode. Distributions similar to the one shown in figure 15 were more typical.

Amplitude limits were chosen as previously described in order to discriminate the activity of the neuron or small cluster of neurons closest to the electrode from that of more distant nerve cells. Once these limits were chosen, the number of neuronal responses within the upper and lower amplitude limits was determined for each stimulus strength.

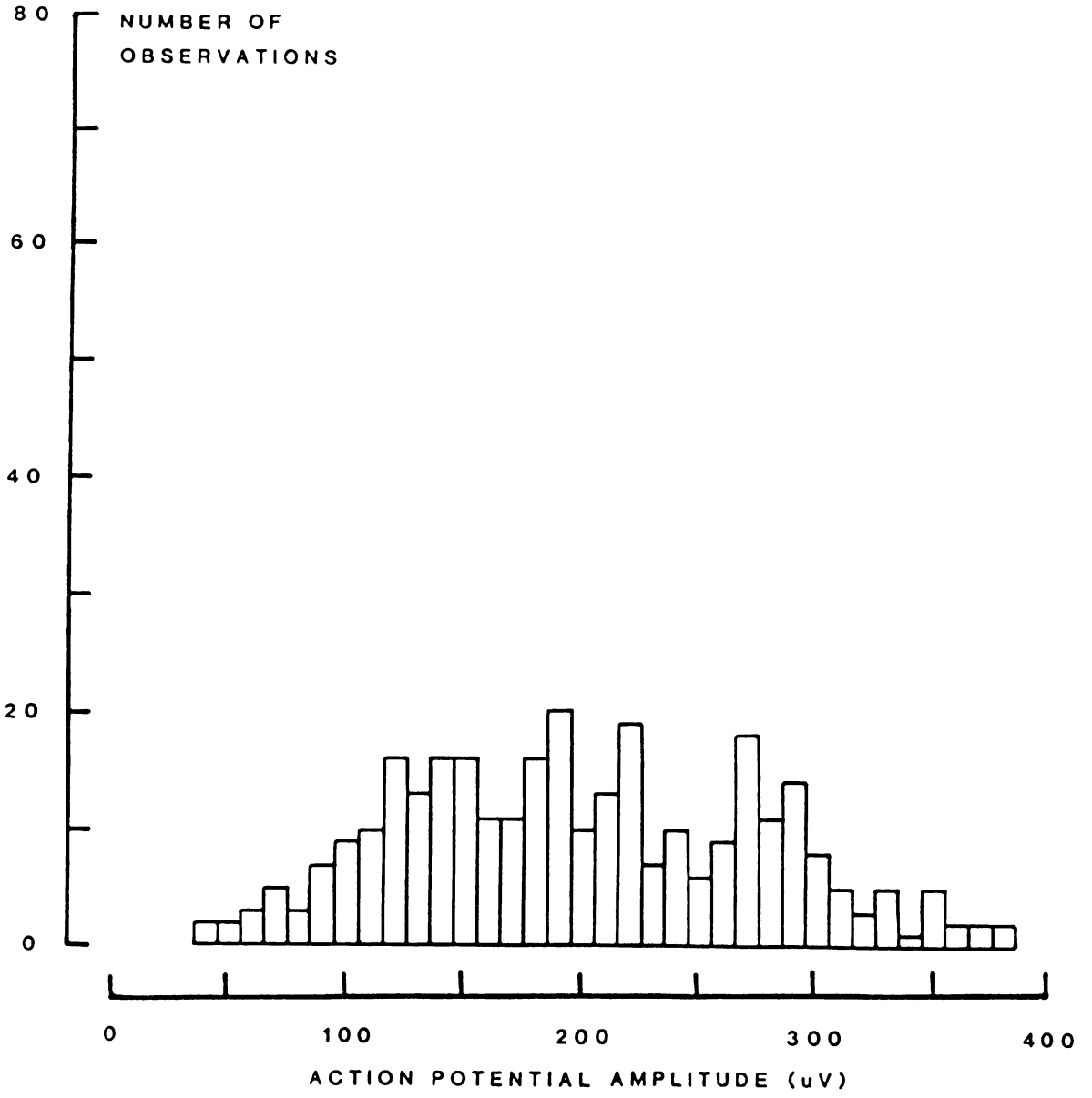

Figure 14. Action potential amplitude histogram - cat BC01WA.084.

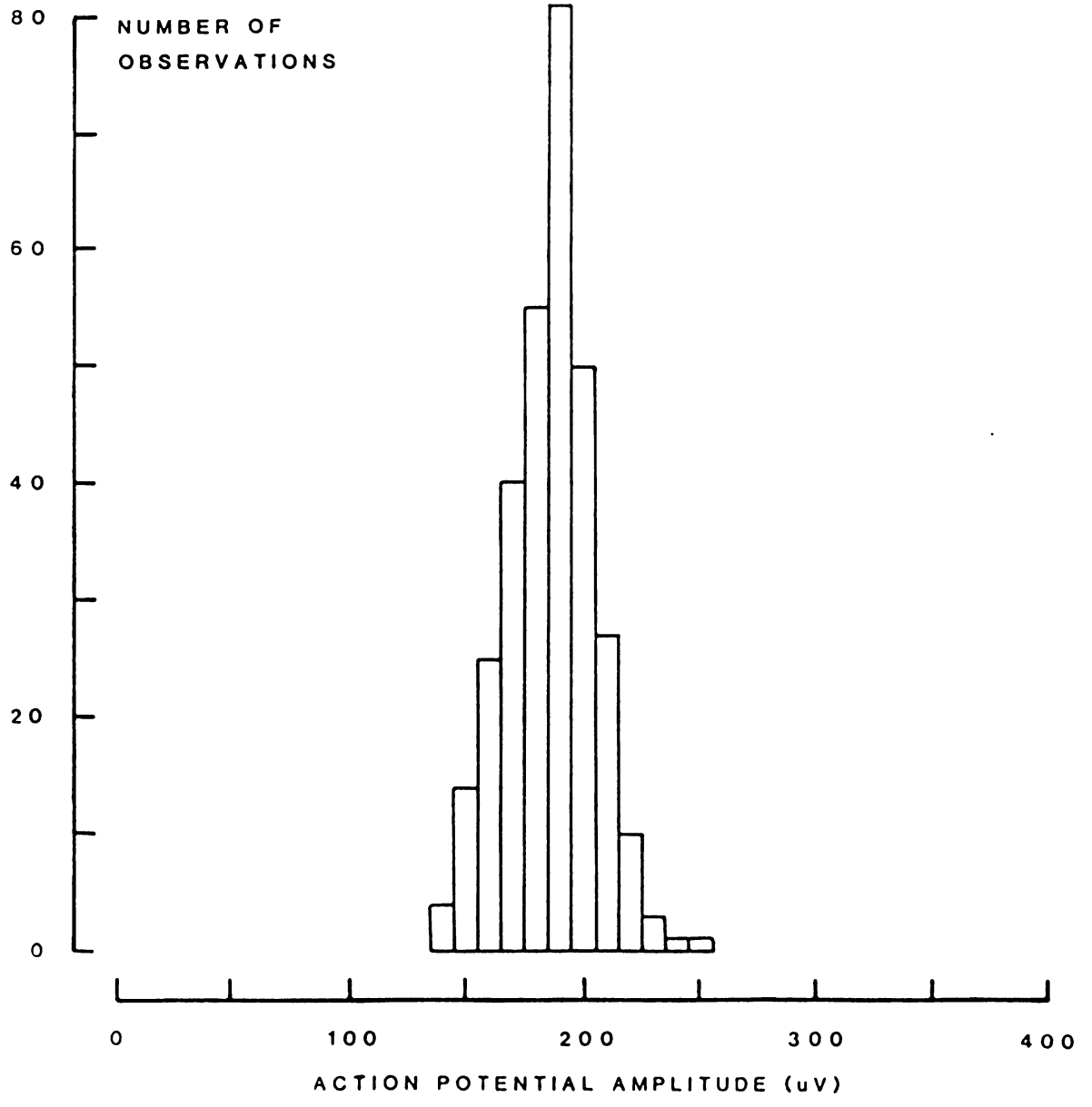

Figure 15. Action potential amplitude histogram - cat BC07LW.125.

From these data, the probability of activating the cortical neuron at any given stimulus strength was determined. The graph in figure 16 shows data from one skin location in an experiment in which the skin was first stimulated during exposure to a dry air stream, and then re-tested during exposure to an air stream saturated with water vapor. The leftward displacement of the curve obtained in the wetted skin indicates a higher probability of activating this cortical neuron at any given stimulus strength when the skin is exposed to the wet air stream.

Figure 17 shows similar data from an experiment in which the skin was first tested during exposure to room air, re-tested during exposure to dry air, and then tested again during exposure to a wet air stream. As in the previous example, there is a rightward displacement of the probability curve obtained when the skin was tested while exposed to the dry air stream, which returned to the left when the skin was tested during exposure to the wet air stream.

Figure 18 shows data from another experiment in which the hydration sequence was reversed so that after testing the skin during exposure to room air, the response was examined next during exposure to wet air, and then re-tested during exposure to the dry air stream. The leftward displacement of the curves obtained in the wetted skin, and the rightward shift observed in the dried skin is consistent with the results shown in the previous two figures.

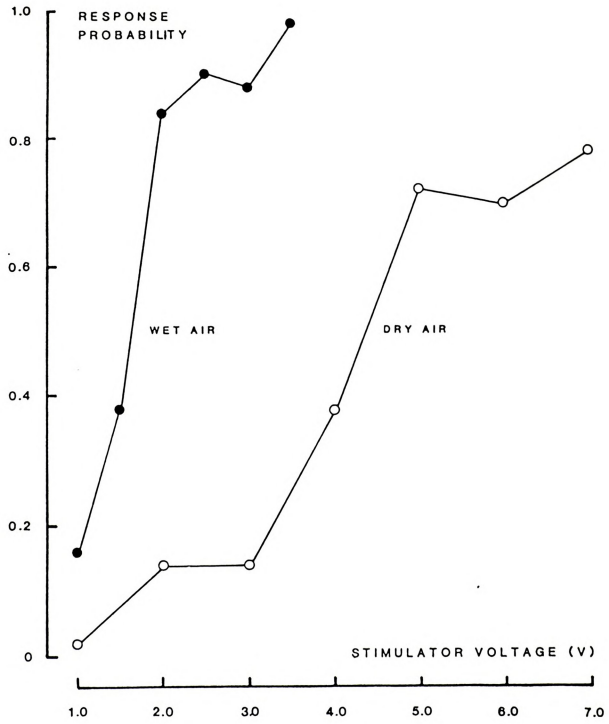

Figure 16. Representative effect of hydration state on cortical unit response probability - cat PC17.241. Abscissa units are given as voltages driving the mechanical stimulator which are proportional to the amount of force delivered to the skin surface. In this experiment the dry air sequence preceded the wet air sequence.

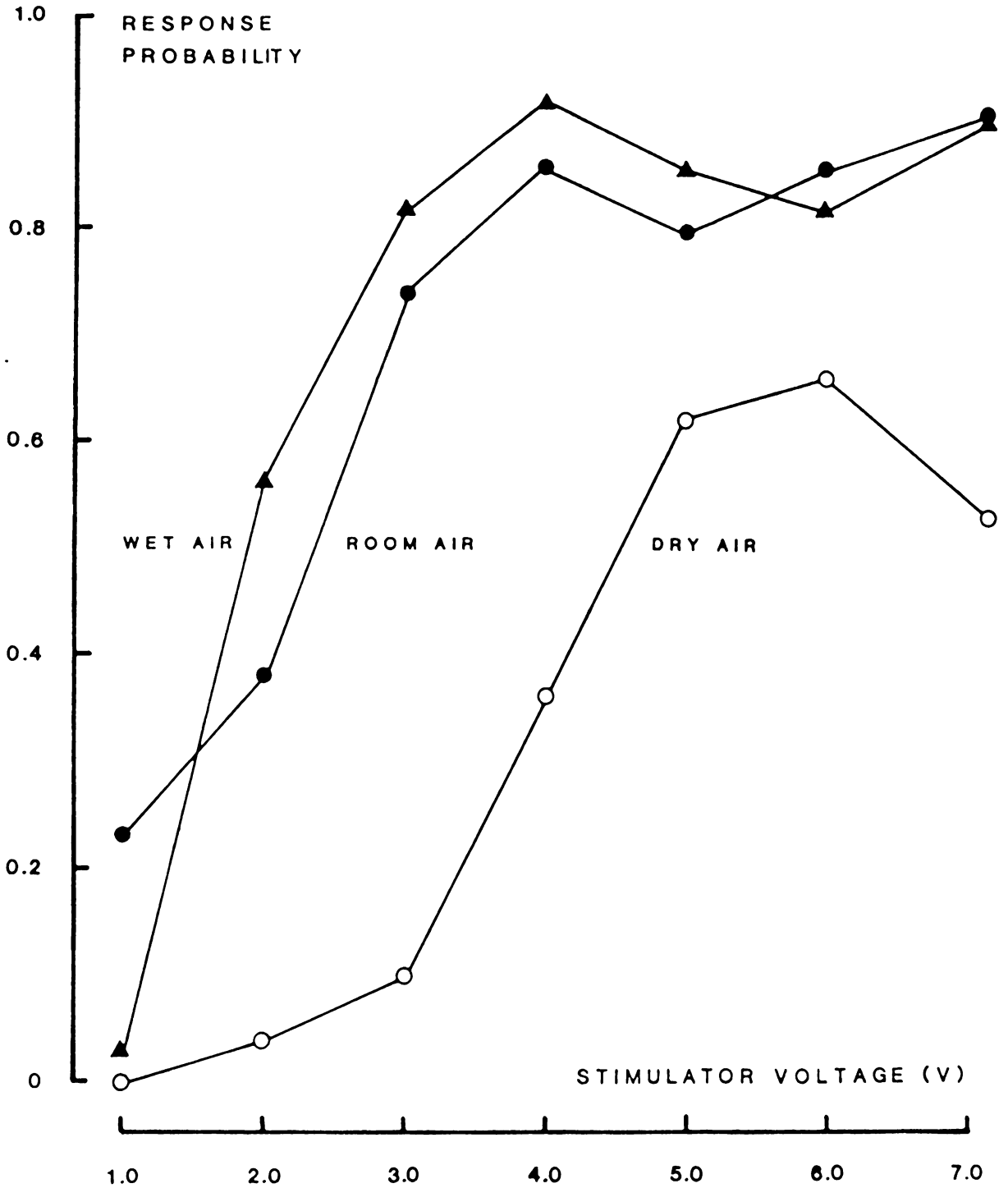

Figure 17. Representative effect of hydration state on cortical unit response probability - cat PC04.022. In this experiment the dry air sequence was preceded by the room air sequence and followed by the wet air sequence.

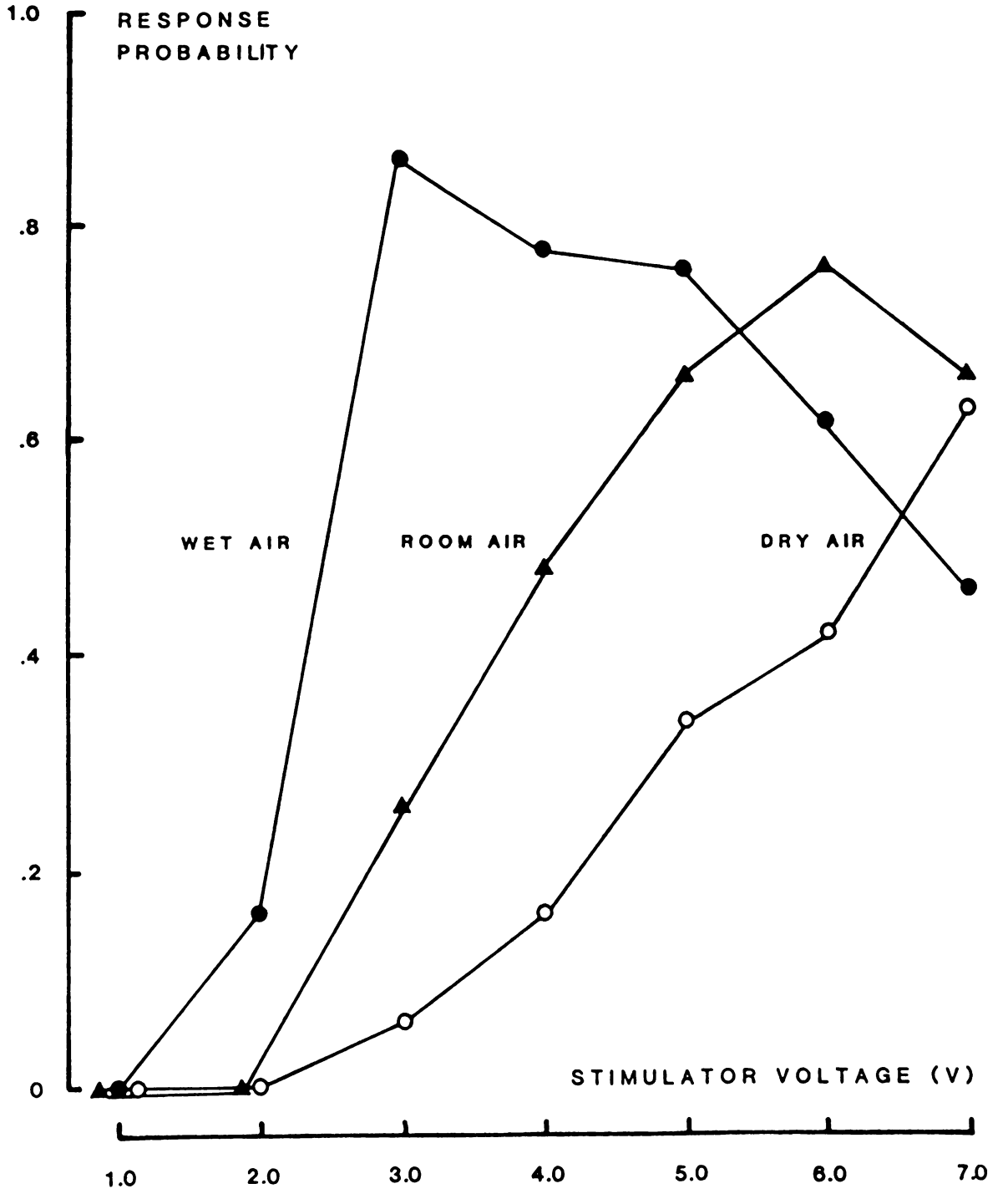

Figure 18. Representative effect of hydration state on cortical unit response probability - cat PC01.084. In this experiment the wet air sequence was preceded by the room air sequence and followed by the dry air sequence.

The effect of the location of the stimulus on the footpad surface on the probability of activating a cortical neuron is shown in figure 19. This graph shows probability of response curves for four equidistant skin locations under the same epidermal hydration condition. In this example, the cortical neuron was more readily activated by stimulation of skin location 2 than the other 3 skin locations. The minimum stimulus strength required to achieve a given probability of response was greater as the distance in either direction from skin location 2 was increased.

An alternate means of demonstrating the epidermal hydration effects on the activation of cortical neurons is to compare the minimum stimulus strength required to achieve a given probability of activating that nerve cell under the different hydration conditions. These minimum stimulus strengths are defined as thresholds (T10, T20, T30, ... , T100), which correspond respectively to a 10, 20, 30, ... , 100 percent probability of evoking activity in the cortical neuron under study. The advantage of this method of examining the data is that it provides indices for curves obtained under different hydration conditions so that they can be statistically compared.

The histogram in figure 20 compares the minimum stimulus strength required to achieve a 50 percent probability (T50) of activating a cortical neuron under both wet and dry ambient conditions. These threshold values are calculated from the same data described in figure 16, and they

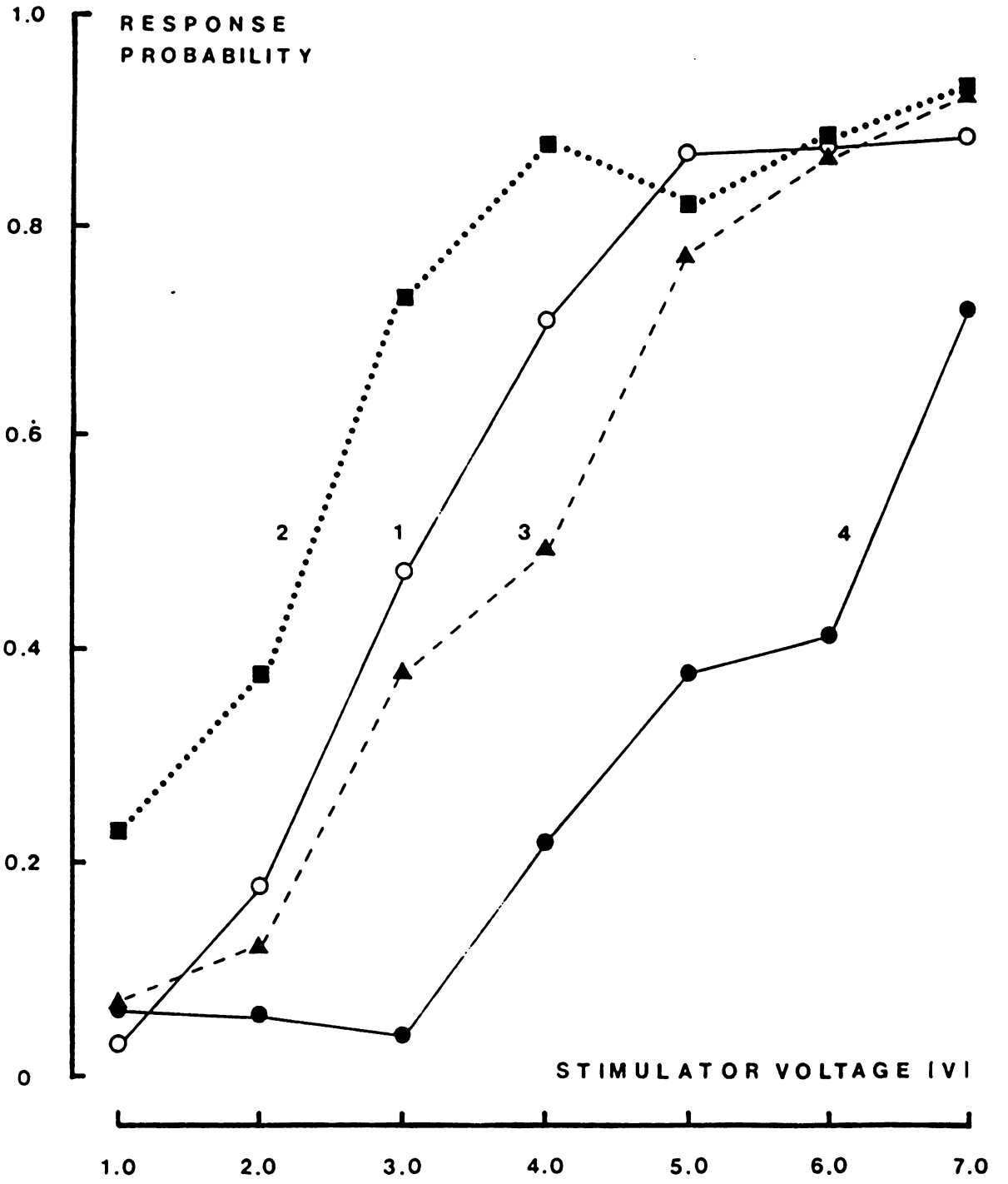

Figure 19. Representative effect of stimulus location on cortical unit response probability - cat BC04RA. Response probabilities are shown for a series of stimulations of adjacent footpad locations (1-4). Force requirements were lowest for skin location 2, higher for adjacent locations (1 and 3), and highest for location 4.

Figure 20. Representative effect of hydration state on T50 threshold - cat PC17.241. This histogram corresponds to the 0.5 probability level in figure 16. Threshold units in this and subsequent figures are given in stimulator driver voltages which are proportional to the force applied at the skin surface.

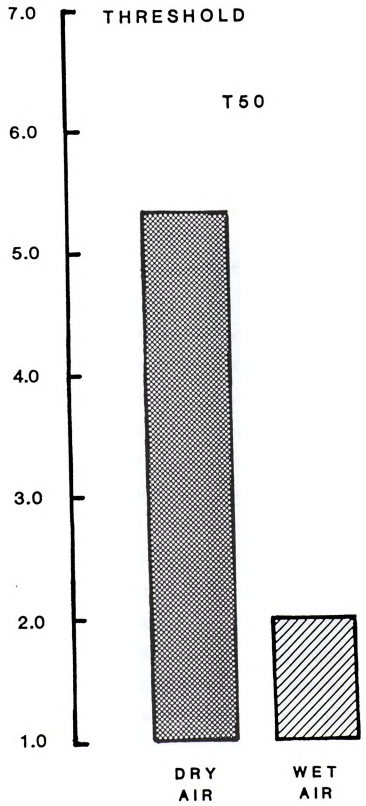

Figure 20.

approximate the 50 percent probability level in the curves presented in that figure. The histograms in figures 21 and 22 show similar T50 comparisons calculated from the data presented in figures 17 and 18 respectively. The threshold values shown in figures 20, 21 and 22 are consistent with the leftward displacement of the curves obtained in the wetted and normally hydrated skin shown in figures 16, 17 and 18. They indicate that less force is required to activate a given cortical neuron under moistened skin conditions.

In order to evaluate statistically the effects of both stimulus location and epidermal hydration state on thresholds of cortical neurons, a split-plot analysis of variance was performed on data from 6 cats, in each of which 7 skin locations were tested under 4 different hydration conditions as previously described. An assumption of this analysis is that the excitability of the cortical neuron being tested has not significantly varied independently of the induced changes in epidermal hydration during the 4 hours required to collect a complete data set. This assumption was tested in a preliminary experiment, in which 3 sequential series of stimulations were conducted on the same footpad locations over a 4 hour period, without experimentally varing the epidermal hydration state. Each series included all 31 stimulus strengths at each of the 7 skin locations. Table 1 summarizes all 10 threshold values (T10-T100) for this experiment. The T50 values from table 1

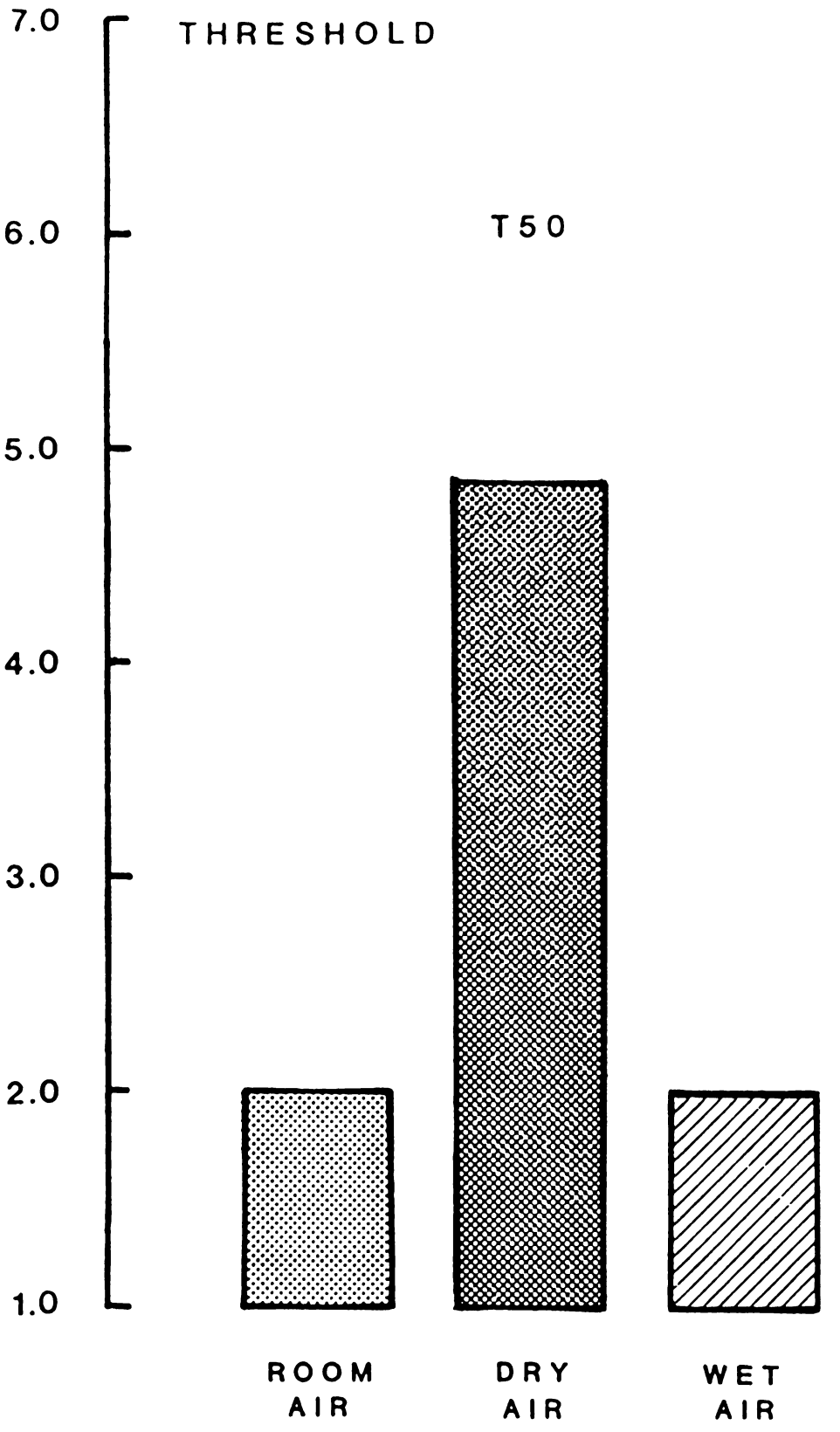

Figure 21. Representative effect of hydration state on T50 threshold - cat PC04.022. This histogram corresponds to the 0.5 probability level in figure 17.

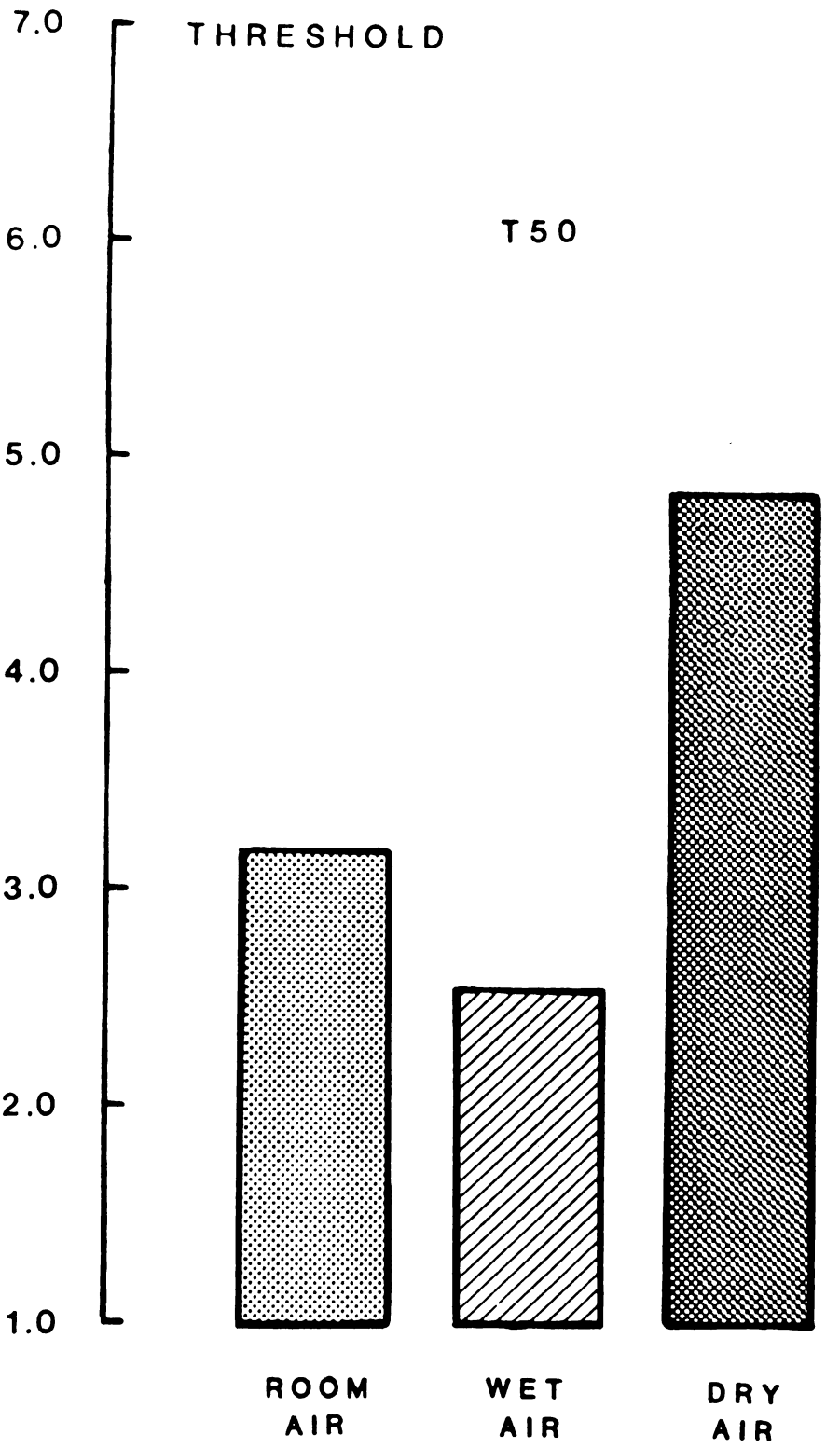

Figure 22. Representative effect of hydration state on T50 threshold - cat PC01.084. This histogram corresponds to the 0.5 probability level in figure 18.

Table 1. Threshold Values For Cat PC06. Missing values indicate thresholds which were not reached.

SITE	1			2			3			4			5			6			7		
SERIES	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
T10	1.4	1.2	1.2	1.0	2.0	1.0	1.8	1.4	1.6	2.0	3.2	1.6	1.0	3.2	1.8	1.2	5.2	1.4	1.2	3.8	1.0
T20	1.4	1.4	1.2	1.8	2.2	1.2	2.6	2.6	1.6	2.0	3.6	2.6	3.4	4.0	2.8	3.6	6.0	3.0	1.6	4.4	2.4
T30	1.6	1.6	1.4	1.8	2.4	1.6	2.6	2.6	3.6	3.2	4.4	3.2	3.4	4.4	2.8	4.0	---	4.6	2.4	4.4	3.0
T40	1.8	1.8	1.6	1.8	2.6	1.6	2.8	3.0	3.8	3.2	4.4	3.2	3.4	4.4	2.8	4.0	---	4.6	2.4	4.4	3.0
T50	1.8	2.2	1.6	1.8	3.0	1.6	2.8	3.0	3.8	3.2	5.0	2.4	4.0	4.4	3.6	5.4	---	4.6	3.2	4.4	3.0
T60	1.8	2.6	1.6	1.8	3.0	1.6	3.4	3.0	4.8	3.2	5.2	3.6	4.8	7.0	3.6	5.4	---	4.6	3.2	4.8	3.0
T70	2.2	2.8	1.6	1.8	3.4	1.6	4.8	5.0	5.0	3.8	5.2	4.0	5.4	7.0	3.6	6.6	---	4.6	3.2	5.4	3.4
T80	4.0	4.2	2.0	2.8	3.4	2.4	5.0	5.4	5.8	4.6	6.6	4.0	5.8	---	4.8	7.0	---	4.6	3.2	5.4	3.4
T90	4.2	5.0	2.4	4.2	4.8	3.2	5.2	5.4	6.8	5.0	6.6	4.0	5.8	---	5.2	---	---	5.2	3.8	5.4	3.8
T100	4.4	5.2	4.8	6.2	7.0	3.4	5.2	5.4	---	---	6.6	4.6	---	---	5.4	---	---	5.8	3.8	5.4	3.8

are plotted as a function of the skin location in figure 23. The variability in response associated with the stimulus location appears to be consistent among these 3 series of stimulations, and no consistent variation in the response associated with the stimulation sequence is observed. The lack of a response from skin location 6 during the second series of stimulations was most likely the result of poor contact of the stimulator probe with the skin surface.

In cats in which the epidermal hydration state was experimentally varied, the initial mechanical properties of the skin during exposure to room air, and the effect of the wet air, dry air and liquid water exposures on these mechanical properties were quite variable. Subjective evaluations of the pliability of the skin were made during each of the hydration states. Table 2 summarizes these subjective evaluations for the 6 animals which are examined in the analysis of variance.

In general, skin exposed to the wet air stream appeared to be either slightly softer or not different from the room air exposed skin. In all cases, the skin exposed to dry air appeared to be less pliable than either the room air or wet air exposed skin. The effect of liquid water soaking on skin pliability was the most variable. Although in some animals the liquid water soaked skin appeared to be softer than the dry air exposed skin, the most common effect was a pronounced swelling and stiffening of the skin.

Figure 23. T50 thresholds for cat BC06RA. This figure shows thresholds for 3 series of stimulations (1-3) on seven footpad locations (abscissa).

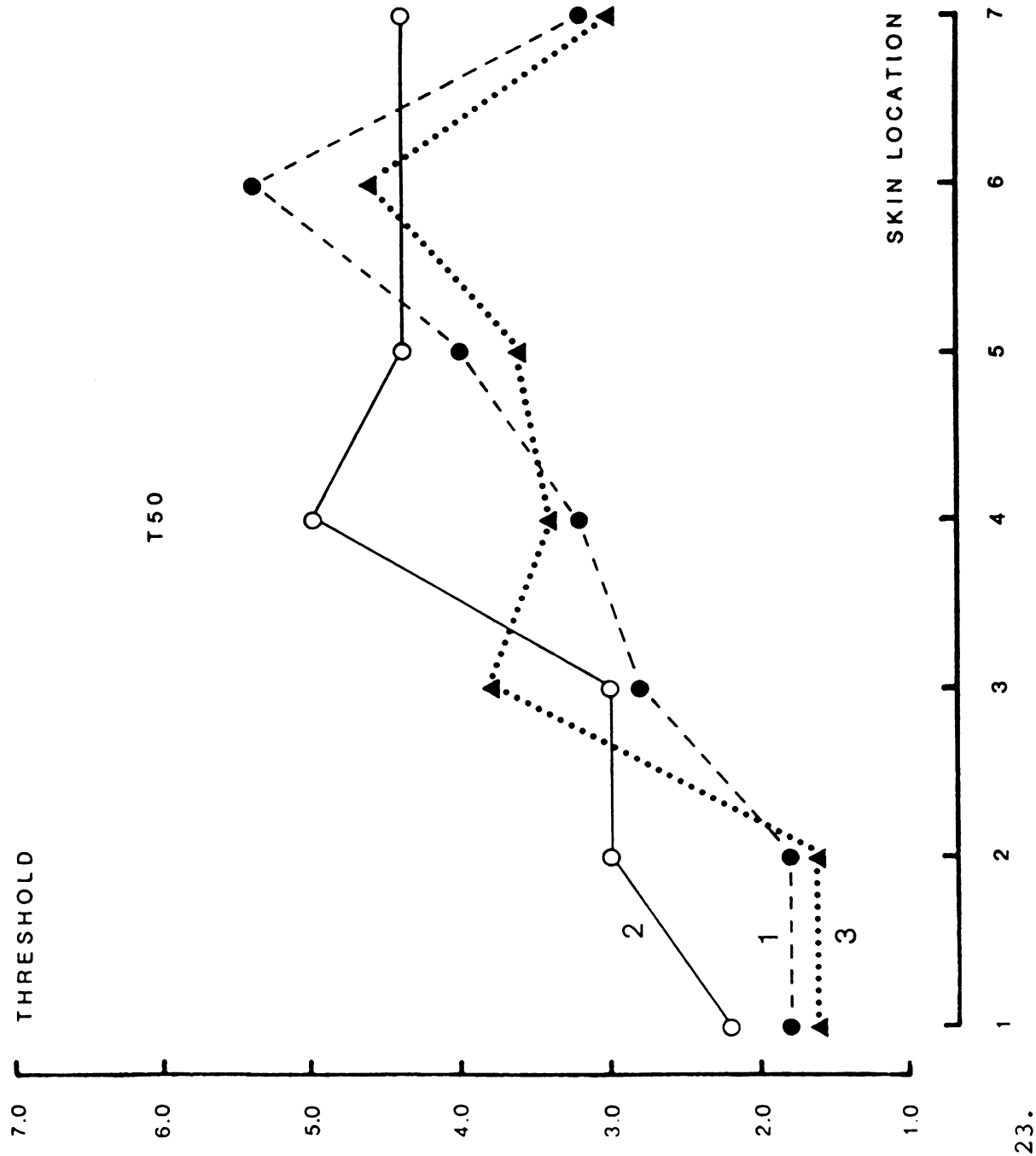

Figure 23.

Table 2. Hydration Effects On Skin Pliability.

HYDRATION CONDITION	Water Soaked	Wet Air	Room Air	Dry Air
Cat 1	-	-	+	-
Cat 2	Ø	++	+	Ø
Cat 3	+	Ø	+	Ø
Cat 4	--	+	Ø	-
Cat 5	Ø	++	+	-
Cat 6	--	-	Ø	--

++ = very pliable + = pliable Ø = neutral
-- = very stiff - = stiff

In order to evaluate statistically the effect of the location of the stimulus on the skin surface (ie. the subplot effect in the analysis of variance) and the possibility of an interaction between the hydration condition and the skin location, it was necessary to re-number the skin locations so that the subplot numbers correspond to the same position on the footpad relative to the center of the receptive field of the cortical neuron under study, independent of its actual geographical location on the footpad surface. This was accomplished as previously described, so that subplot 1 in all cats represents the footpad location most sensitive to mechanical stimulation, and subplots 2-5 represent 4 adjacent and equidistant skin locations progressively more distant from the skin location in subplot 1.

Table 3 shows the analysis of variance tables for all 10 threshold values (T10-T100). Statistically significant F values ($p<0.05$) are marked with asterisks. F values in this table indicate that the hydration condition has a significant effect on all threshold values. Except for the T10 values, the effect of stimulus location is also significant for all threshold values. The hydration condition and skin location interaction is not significant for any of the threshold levels. Duncan's multiple range test was used to detect statistically significant differences ($p<0.05$) between treatment and location mean values. Because of the lack of significance in the

Table 3. Anova Tables. Asterisks (*) mark significant F ratios ($p<0.05$).

		T10			T20		
SOURCE OF VARIATION	DF	SS	MS	F	SS	MS	F
BLOCKS (CATS)	5	8.07	1.61	1.64	16.31	3.26	2.25
TREATMENT (HYDRATION)	3	14.58	4.86	4.95*	19.55	6.52	4.50*
TREATMENT ERROR	15	14.72	0.98	---	21.70	1.45	---
SUBPLOTS (LOCATION)	4	4.46	1.12	2.22	18.74	4.68	5.44*
TREATMENT X SUBPLOT	12	3.43	0.29	0.57	5.94	0.49	0.57
RESIDUAL ERROR	80	40.26	0.50	---	68.83	0.86	---
TOTALS	119	85.53			151.07		

		T30			T40		
SOURCE OF VARIATION	DF	SS	MS	F	SS	MS	F
BLOCKS (CATS)	5	35.74	7.09	4.33*	43.81	8.76	5.19*
TREATMENT (HYDRATION)	3	43.24	14.41	8.81*	65.01	21.67	12.84*
TREATMENT ERROR	15	24.55	1.64	---	25.31	1.69	---
SUBPLOTS (LOCATION)	4	16.38	4.10	4.23*	26.97	6.74	7.07*
TREATMENT X SUBPLOT	12	12.27	1.02	1.06	11.84	0.99	1.03
RESIDUAL ERROR	80	77.44	0.97	---	76.28	0.95	---
TOTALS	119	209.35			249.23		

		T50			T60		
SOURCE OF VARIATION	DF	SS	MS	F	SS	MS	F
BLOCKS (CATS)	5	55.91	11.18	4.52*	61.65	12.33	3.72*
TREATMENT (HYDRATION)	3	98.65	32.88	13.29*	107.10	35.70	10.76*
TREATMENT ERROR	15	37.10	2.47	---	49.76	3.32	---
SUBPLOTS (LOCATION)	4	34.48	8.62	9.03*	35.45	8.86	8.44*
TREATMENT X SUBPLOT	12	13.12	1.09	1.14	9.97	0.83	0.79
RESIDUAL ERROR	80	76.34	0.95	---	83.98	1.05	---
TOTALS	119	315.59			347.92		

Table 3 (cont'd.).

		T70			T80		
SOURCE OF VARIATION	DF	SS	MS	F	SS	MS	F
BLOCKS (CATS)	5	82.43	16.49	4.56*	79.41	15.88	4.10*
TREATMENT (HYDRATION)	3	151.86	50.62	14.01*	149.97	49.99	12.90*
TREATMENT ERROR	15	54.19	3.61	---	58.13	3.88	---
SUBPLOTS (LOCATION)	4	37.45	9.36	8.43*	33.31	3.33	6.76*
TREATMENT X SUBPLOT	12	7.70	0.64	0.58	9.86	0.82	0.67
RESIDUAL ERROR	80	88.86	1.11	---	98.59	1.23	---
TOTALS	119	422.50			429.28		

		T90			T100		
SOURCE OF VARIATION	DF	SS	MS	F	SS	MS	F
BLOCKS (CATS)	5	65.26	13.05	3.66*	70.83	14.16	3.51*
TREATMENT (HYDRATION)	3	136.94	45.64	12.80*	118.24	39.41	9.77*
TREATMENT ERROR	15	53.51	3.57	---	60.51	4.03	---
SUBPLOTS (LOCATION)	4	34.10	8.52	6.38*	30.36	7.59	6.43*
TREATMENT X SUBPLOT	12	11.17	0.93	0.70	12.40	1.03	0.88
RESIDUAL ERROR	80	106.87	1.34	---	94.39	1.18	---
TOTALS	119	407.84			386.73		

treatment-location interaction, treatment means at individual skin locations or location means for individual hydration states were not compared.

Table 4 summarizes treatment means, standard errors and statistical comparisons for the four hydration conditions. The histogram in figure 24 of the T50 treatment means is representative of the other threshold levels. Data in table 4 and figure 24 indicate that there is no statistical difference between the responses of room air and wet air exposed skin. Thresholds for dry air exposed and liquid water soaked skin are significantly greater than the both wet air and room air exposed skin. Thresholds for liquid water soaked and dry air exposed skin are statistically different from each other at the T30, T40, T50, and T60 levels, but higher (T70-T100) or lower (T10-T20) threshold values are not different.

Skin location means, standard errors and statistical comparisons are shown in table 5. Although there is some variability in the statistical comparisons at the different threshold levels, the histogram of T50 values shown in figure 25 is representative of the general pattern. Data in table 5 and figure 25 indicate that the threshold is significantly lower in skin location 1 than in the other skin locations. Threshold values at skin locations 2-4 are not significantly different from each other. The threshold at skin location 5 is significantly greater than the thresholds at the other skin locations.

Table 4. Treatment Means And Statistical Comparisons. For comparison, means are ranked from lowest to highest. Those means underscored by the same line are not significantly different from each other ($p<0.05$).

HYDRATION CONDITION	Water Soaked	Wet Air	Room Air	Dry Air	STATISTICAL DIFFERENCES
T10	2.07(.25)*	1.33(.09)	1.14(.05)	1.55(.10)	RA WA DA WS
T20	2.51(.28)	1.73(.17)	1.42(.11)	2.07(.18)	RA WA DA WS
T30	3.36(.30)	2.00(.20)	1.80(.15)	2.40(.19)	RA WA DA WS
T40	3.89(.32)	2.17(.19)	2.02(.17)	2.85(.21)	RA WA DA WS
T50	4.47(.33)	2.37(.20)	2.19(.19)	3.35(.25)	RA WA DA WS
T60	4.89(.32)	2.67(.22)	2.53(.22)	3.70(.28)	RA WA DA WS
T70	5.39(.33)	2.89(.23)	2.77(.21)	4.63(.32)	RA WA DA WS
T80	5.61(.32)	3.15(.24)	3.00(.23)	4.87(.32)	RA WA DA WS
T90	5.81(.29)	3.64(.27)	3.31(.24)	5.33(.31)	RA WA DA WS
T100	6.19(.26)	4.36(.28)	3.82(.30)	5.85(.26)	RA WA DA WS

*(s.e.m.)

Table 4. Treatment Means And Statistical Comparisons. For comparison, means are ranked from lowest to highest. Those means underscored by the same line are not significantly different from each other ($p<0.05$).

HYDRATION CONDITION	Water Soaked	Wet Air	Room Air	Dry Air	STATISTICAL DIFFERENCES
T10	2.07(.25)*	1.33(.09)	1.14(.05)	1.55(.10)	RA WA DA WS
T20	2.51(.28)	1.73(.17)	1.42(.11)	2.07(.18)	RA WA DA WS
T30	3.36(.30)	2.00(.20)	1.80(.15)	2.40(.19)	RA WA DA WS
T40	3.89(.32)	2.17(.19)	2.02(.17)	2.85(.21)	RA WA DA WS
T50	4.47(.33)	2.37(.20)	2.19(.19)	3.35(.25)	RA WA DA WS
T60	4.89(.32)	2.67(.22)	2.53(.22)	3.70(.28)	RA WA DA WS
T70	5.39(.33)	2.89(.23)	2.77(.21)	4.63(.32)	RA WA DA WS
T80	5.61(.32)	3.15(.24)	3.00(.23)	4.87(.32)	RA WA DA WS
T90	5.81(.29)	3.64(.27)	3.31(.24)	5.33(.31)	RA WA DA WS
T100	6.19(.26)	4.36(.28)	3.82(.30)	5.85(.26)	RA WA DA WS

*(s.e.m.)

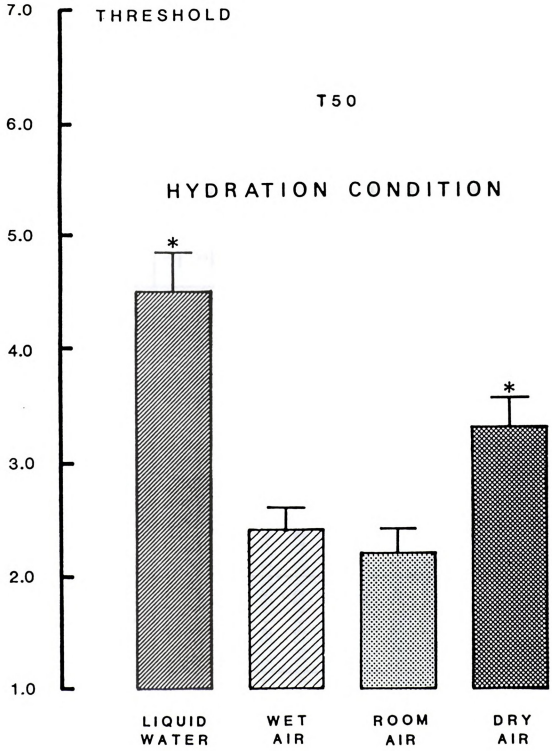

Figure 24. Effect of hydration condition on T50 threshold. Mean values and standard errors for 4 hydration conditions are from table 4. Bars marked with an asterisk are significantly different ($p<0.05$) from all other hydration condition means.

Table 5. Skin Location Means And Statistical Comparisons. For comparison, means are ranked from lowest to highest. Those means underscored by the same line are not significantly different from each other ($p<0.05$).

SKIN LOCATION	1	2	3	4	5	STATISTICAL DIFFERENCES
T10	1.29(.07)*	1.58(.20)	1.38(.10)	1.52(.19)	1.85(.24)	1 3 4 2 5
T20	1.65(.15)	1.81(.21)	1.72(.15)	1.77(.20)	2.72(.33)	1 3 4 2 5
T30	1.92(.18)	2.32(.24)	2.41(.27)	2.26(.27)	3.05(.34)	1 4 2 3 5
T40	2.04(.22)	2.60(.25)	2.76(.32)	2.74(.26)	3.52(.34)	1 2 4 3 5
T50	2.18(.23)	3.15(.32)	3.13(.36)	3.15(.34)	3.86(.33)	1 3 2 4 5
T60	2.52(.27)	3.43(.32)	3.50(.37)	3.58(.37)	4.22(.34)	1 2 3 4 5
T70	3.03(.35)	3.79(.35)	3.88(.39)	4.15(.40)	4.76(.37)	1 2 3 4 5
T80	3.37(.40)	3.97(.33)	4.13(.39)	4.37(.40)	4.98(.36)	1 2 3 4 5
T90	3.65(.40)	4.45(.34)	4.44(.38)	4.81(.36)	5.28(.35)	1 3 2 4 5
T100	4.19(.41)	5.04(.34)	4.98(.36)	5.38(.35)	5.69(.33)	1 3 2 4 5

*(s.e.m.)

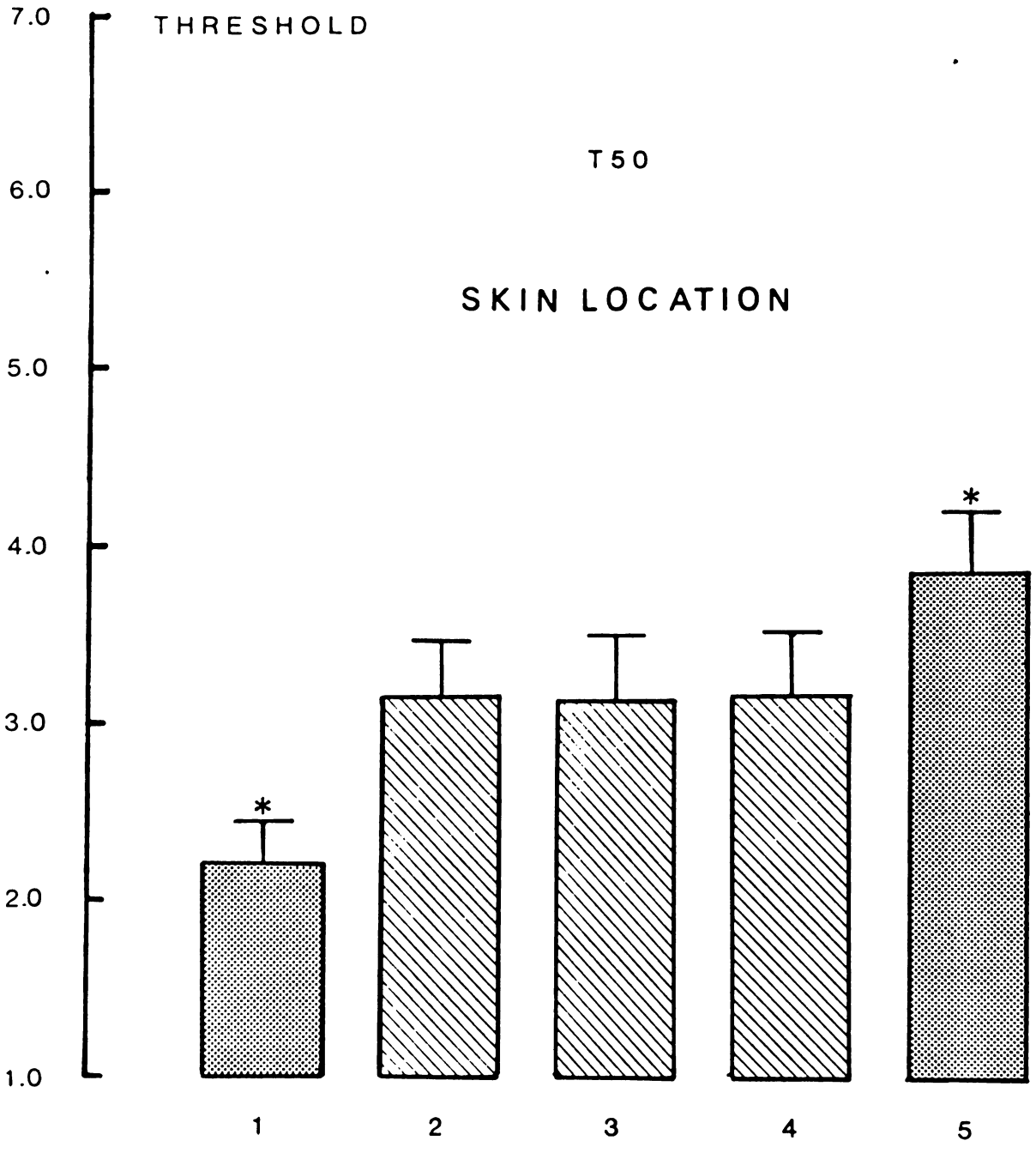

Figure 25. Effect of stimulus location on T50 threshold. Mean values and standard errors for 5 skin locations are from table 5. Bars marked with an asterisk are significantly different ($p<0.05$) from all other skin location means.

DISCUSSION

EPIDERMAL PERMEABILITY

A major function of skin is to minimize the loss of body water. The marked increase in evaporation rate which occurs after either partial or complete removal of skin clearly demonstrates its water barrier function. Many early studies showed that a diffusion barrier was located within skin epidermal layers. This is not surprising considering the desmosomal attachments and tight interstitial spaces among cells in these layers. The identification of a single epidermal layer serving as a water diffusion barrier, however, is unresolved. Studies in which epidermal layers were serially removed by stripping them at the skin surface demonstrated that skin surface evaporation rate changes little until a thin layer of cells near the base of the stratum corneum is removed (Blank, 1952). This was initially interpreted by these investigators to indicate that a "thin barrier" at the base of the stratum corneum functions as the primary impediment to water diffusion, although it was concluded later that the stratum corneum is a nearly uniform "thick barrier" to transcutaneous water loss.

Other studies show, however, that the rate of transcutaneous water loss from "nonsweating" skin is greater on the tactile surfaces of the hands and feet than it is from skin on the general body surface. This occurs despite the greater thickness and intercellular cohesion of the stratum corneum on the tactile surfaces (King et al., 1981). One explanation for this apparent contradiction to the "thick barrier" hypothesis is related to humans serving as experimental subjects for these studies. The distribution of eccrine sweat glands in humans is not uniform over the body surface. Kuno (1956) and others have shown a greater density of these sweat glands in skin on the tactile surfaces than in other regions of the body.

Lateral diffusion of water from distal sweat gland tubules provides a mechanism for "short circuiting" the epidermal water barrier. Studies in animal models other than humans (Adams, 1966; Adams and Hunter, 1969; Steinmetz et al., 1977) in which sweat gland activity was precisely controlled indicate that water vapor loss from the skin surfaces is increased by electrical stimulation of sweat gland nerves. This increase in skin surface evaporation occured at levels of sweat gland stimulation not strong enough to bring liquid sweat to the skin surface, so that low levels of sweat gland activity undetectable as such at the skin surface may, nonetheless, contribute to the relatively higher skin surface evaporation rate from the tactile skin.

There are at least three additional considerations which make intact human skin a difficult experimental model. First, the sympathetic nervous system differentially activates sweat glands in different body regions. For example, hyperhidrosis is characterized by profuse sweating that is generally restricted to the tactile surfaces of the hands and feet. Secondly, sweat secretion is activated reflexly by both ipsilateral and contralateral stimuli. Thirdly, because sweat gland activity is difficult to control in humans, the cat footpad or some other experimental model in which eccrine sweating is more precisely regulated is more appropriate for quantitative studies of water movement and storage in skin.

EPIDERMAL WATER STORAGE

Because the stratum corneum is an effective barrier to transcutaneous water movement, some investigators have concluded that it absorbs little if any water. Precise measurements from skin when sweat glands are denervated, show a persistent low rate of transcutaneous water movement (Adams, 1966; Steinmetz et al., 1977). Buettner (1959a, 1959b, 1959c) showed that not only is the epidermis permeable to water, but also that it absorbs water readily when exposed to either liquid water or to air with a high ambient relative humidity. Laden and Spitzer (1967) also demonstrated that the stratum corneum is hygroscopic and

that it can absorb both water and ions. These studies indicate that although the epidermis is an effective barrier to the loss of body water, it is not only permeable, but it is also capable of absorbing and storing significant amounts of water.

The amount of water stored in the epidermis is variable and depends on environmental conditions, sweat gland activity and the rate of diffusion of water from lower skin layers. Adams proposed a hydration gradient across the epidermis, the profile of which depends on these factors and the horizontal and vertical permeability coefficients within the skin (Adams et al., 1981; Steinmetz and Adams, 1981). Photoacoustic spectroscopy provides direct evidence for this hypothesis. Using this technique, Pines and Cunningham (1981) showed a variable water content across the thickness of the intact stratum corneum. They also showed that the amount of water stored in the skin is a nonlinear function of the distance from the skin surface, and that the shape of this curve depends on the total amount of water in the epidermis. These investigators concluded that normal and delipidized epidermis are "vastly different" in their ability to store water. They also concluded that "the uppermost skin layers are more sensitive to changes in water content" than are the lower skin layers, and therefore are "very important in the clinical conditions characterized by dry skin."

It is a common observation that when hands are immersed in warm water containing a detergent, there is a pronounced wrinkling of the skin, particularly of that of the fingers. This phenomenon is the result of cutaneous water absorption and a concomitant swelling of the epidermis (Bull and Henry, 1977). Skin wrinkling under these conditions appears to be related to the activity of sympathetic nerves innervating the hands (Lewis and Pickering, 1935). Patients suffering from cystic fibrosis or hyperhidrosis show a propensity for such skin wrinkling (Braham et al., 1979; Bull and Henry, 1977; Moynahan, 1974). Both of these conditions are also characterized by production of large volumes of hypertonic sweat. Peripheral nerve lesions, sympathectomy and peripheral nerve blockade with local anesthetics prevent wrinkling in patients suffering from these disorders which may provide a simple test of peripheral nerve integrity (Braham et al., 1979, Bull and Henry, 1977).

One explanation for this phenomenon is that the increased turgor of the dermis owing to the peripheral vasodilation which accompanies sympathetic nervous system lesions may prevent swelling of the epidermis. An alternate explanation is that ions deposited in the epidermis as a result of normal or hyperactive sweat secretion increase water absorbtion by the epidermis. This wrinkling is prevented by either sympathectomy or peripheral nerve lesions which preclude sweat gland function and thereby

decrease skin osmolarity. These explanations are not mutually exclusive and both mechanisms may be involved.

There is substantial evidence that the epidermis is capable of varying its hydration. The factors which influence the epidermal hydration state have been identified as being related to environmental conditions, diffusion of water from the dermis and sweat gland activity. Adams (1966) proposed a mechanism whereby water and salts deposited in the epidermis may be reabsorbed across the dermo-epidermal junction. Although several lines of indirect evidence support such a mechanism, there have been no attempts to demonstrate directly such an active process in mammalian skin.

The epidermal hydration state of normal skin remains relatively constant except during extreme changes in environmental conditions or sympathetic nervous system activity. Sweat gland secretion rates and the ionic content of sweat are undoubtedly important factors in the control of epidermal hydration. Because of a paucity of related information, feedback mechanisms remain speculative. Several investigators (Janig and Spilok, 1978; Janig and Rath, 1980) have demonstrated reflex activation of sweat glands when mechanoreceptors, thermoreceptors or nociceptors are stimulated which may be important in the normal control of skin hydration. Specific receptors for sensing skin hydration have not been described. Bare nerve endings which

penetrate the epidermis and for which no known function has been ascribed might be involved.

EPIDERMAL HYDRATION AND SKIN THERMAL PROPERTIES

Holmes (Holmes, 1972; Holmes and Adams, 1975) showed that the thermal conductivity of the epidermis increases 2-3 fold with an increase in epidermal water content due either to sweat gland activity, or exposure to a high ambient relative humidity. These authors suggested that the temperature at the dermo-epidermal junction where skin thermoreceptors are located is closer to environmental temperature when the skin is hydrated. Holmes proposed also that this may account at least in part for differences in thermal perception in environments with different relative humidities. Although trained human observers are incapable of detecting changes in temperature of 6-7 ^{o}C if they occur slowly over a 20-30 min period, they are able to identify correctly the larger of two temperature changes which differ by as little as 0.05 ^{o}C if they occur within a few seconds (Darian-Smith and Johnson, 1977). These rapid changes are similar to those one would expect were the skin to come in contact with a warm metallic block, for example. It appears that epidermal hydration state is an important variable in thermal sensibility.

Stoll and co-workers (Stoll, 1977; Stoll *et al.*, 1981) used the heat transfer properties of the epidermis as

an indirect method for measuring epidermal thickness. They showed that the time to reach a thermal pain threshold after the skin touched a hot object is related to the thickness of the epidermis, as well as to the temperature and heat transfer properties of the object itself. Considering the influence of hydration on epidermal thermal conduction, the water content of the skin may be an important consideration in these and other measures which depend on skin thermal transfer properties.

Perceived differences in skin temperature can be used as an important clinical sign in palpatory physical diagnosis. Both absolute and bilateral differences in regional skin temperature are used by some physicians as clinical indices. Adams et al. (1982) recently discussed the interaction of variables which might influence a physician's temperature perception in such an examination. Although these tests are important and useful diagnostic aids, the epidermal hydration states of both the patient and the examiner are important variables, and the results from such examinations require cautious interpretation.

EPIDERMAL HYDRATION AND SKIN ELECTRICAL PROPERTIES

The endogenous transcutaneous DC electrical potential and measures of resistance to electrical current flow in the skin are commonly used as indices of sweat gland activity

and sympathetic nervous system tone (Janig and Spilok, 1978; Janig and Rath, 1980). For example, a change in skin electrical resistance is the basis of the commonly used so called "lie detector" examination. Despite their common use, the underlying physiological mechanisms for these electrical phenomena are poorly understood. Epidermal hydration and the ionic content of the skin have been shown by Adams and co-workers to be important variables in all measures of skin electrical properties (Adams and Vaughan, 1965; Stombaugh and Adams, 1971; Adams et al., 1980; Steinmetz and Adams, 1981). Presumably these factors influence skin electical properties through alterations in the electrical volume conductor characteristics of the epidermis.

The measurement of biopotentials from the skin surface is important in examination of the electrical activity of both the heart (EKG) and brain (EEG). In both of these skin surface measures of internal electrical events, artifacts associated with the attachment and movement of electrodes, as well as changes in skin electrical properties may restrict the usefulness of these techniques, although some of these problems currently are minimized through the use of special electrodes, electrode pastes and electrode attachment techniques. A better understanding of the physical and physiological basis for skin electrical phenomena may provide new diagnostic techniques for evaluating sweat gland activity, skin blood flow and sympathetic nervous system activity. In addition, a better

understanding could result in the reduction of artifacts associated with the measurement of biopotentials from the skin surface, and might extend the usefulness of these techniques. The cat footpad model may be a more appropriate model for quantitative studies of skin electrical properties than human skin in which sweat gland activity and peripheral circulation are difficult to control.

EPIDERMAL HYDRATION AND SKIN MECHANICAL PROPERTIES

The importance of hydration in the evaluation of the mechanical properties of whole skin (dermis and epidermis) is well documented. The "pliability" of excised whole skin samples is directly related to its water content (Blank, 1952; Lanier and Fung, 1974a, 1974b). Most of the effect of hydration on skin mechanical properties has been attributed to changes in the dermis. The importance of the epidermis in this regard has been disputed.

Laden and Morrow (1970) and Park and Baddiel (1972a, 1972b) showed that the "softness" and "flexibility" of samples of excised stratum corneum are also directly related to their water content. Christensen et al. (1977) demonstrated rapid and significant changes in the mechanical properties of intact human skin when moist air was blown across its surface. Because of the methods used in these tests and the rapid time course of the hydration effects, these investigators attributed the observed effects to an

alteration of epidermal mechanical properties especially involving the stratum corneum.

The relative contributions of the dermis and the epidermis to the overall mechanical properties of intact skin is unclear. This is largely because of the wide range of methodological differences used to evaluate skin mechanical properties. For this reason, there is now a major effort to establish standard nomenclature, techniques and procedures for evaluating the mechanical properties of intact skin (Payne et al., 1981).

The hydration state of the epidermis appears to be more labile than that of the dermis. Hydration has been shown to have significant effects on the mechanical properties of both excised and intact epidermis. For these reasons, several investigators have recently concluded that epidermal mechanical properties are important to consider when evaluating either overall skin mechanical properties, or pathological conditions which are characterized by dry skin (Christensen et al., 1977; Pines and Cunningham, 1981; Steinmetz and Adams, 1981).

The mechanism for hydration-induced effects on skin mechanical properties appears to be related to hydrogen bonding within and among protein filaments. Park and Baddiel (1972a, 1972b) showed that the decrease in elasticity which occurs in hydrated samples of excised stratum corneum follows patterns predicted by experiments in other keratinous substrates and polymer models. They concluded

that the decrease in elasticity which accompanies increased water content is owed to decreased protein-protein interaction. In dry skin, intrafibrillar hydrogen bonding produces tightly coiled three-dimensional protein configurations and extensive interfibrillar cross-linking. As the corneum is hydrated, the three-dimensional structure of proteins relaxes and interaction between adjacent protein fibers decreases which allows stretching and sliding of protein filaments (decreased elasticity) within the skin. Takahashi et al. (1981) demonstrated that in human skin, the most drastic decreases in elasticity occur when the skin in equilibrated at relative humidities above 60 percent. Their results also indicate that it is at this relative humidity that "free" or "loosely bound" water increases in the skin. These same investigators have shown that substances such as urea and lithium bromide which are known to interfere with hydrogen bonding in proteins, produce a plasticizing effect in skin similar to that produced by water.

In the present study, both the initial mechanical properties of the cat central footpad skin and those induced by hydration were quite variable (table 2). Animals were preselected to have relatively soft, pliable skin free from abrasions and lacerations. Exposure of the footpad skin to an air stream saturated with water vapor had little or no effect on skin mechanical properties. One explanation for this may be that skin exposed to room air retained water

from sweat gland activity caused by restraint of the animal prior to anesthetization, and also from exposure of the skin to water during the footpad preparation procedures. Exposure of the footpad to the dry air stream caused a significant decrease in skin pliability (table 2).

The effect of soaking the footpad in deionized water, however, was quite variable. In some animals, this had a plasticizing effect on the skin which made it softer than the skin exposed to dry air. More commonly, it resulted in a significant swelling and stiffening of the skin (table 2). A similar result has been described in human skin (Dikstein and Hartzshtark, 1981), where there was a decrease in compressibility of the forehead skin when it was soaked in water. One explanation for this effect is that absorption of a large amount of water fills the intracellular and interstitial spaces of the skin with an incompressible liquid, which overwhelms the plasticizing effect that accompanies the breaking of protein hydrogen bonds. Soaking skin in deionized water may potentiate this effect, especially at those sites where the stratum corneum is thin, as it is on the forehead.

CORTICAL UNIT ACTIVITY

Much of the variability in action potential amplitudes recorded in these experiments (fig 13, 14 and 15) is related to the size of the microelectrode tip, brain pulsations and

baseline noise of the recording system. The effect of external noise sources was minimized by RF shielding of the electrodes and the experimental preparation. Brain surface pulsations were effectively damped by covering the cortical surface with a layer of agar. Only cortical units which were characterized by sharp initially negative biphasic waveforms were chosen for study. Neurons with these characteristics are more distant from the microelectrode tip and are less damaged than those which have initially positive waveforms. Experiments were immediately terminated if sudden changes in spike amplitudes or waveforms were detected. Using these criteria and amplitude window discrimination gives reasonable assurance that the responses detected represent changes in firing characteristics of single cortical units.

Variations in cortical neuron responses may also have been related to the effect of anesthesia since pentobarbital has been shown to depress their activity (Richards, 1972; Tsuchiya and Kitagawa, 1976; Harding et al., 1979; Collins and Roppolo, 1980). Collins and Roppolo (1980) demonstrated there were decreases in both spontaneous and evoked firing rates of neurons in the primary somatosensory cortex in awake monkeys given increasing doses of pentobarbital. In this study, S1 neurons were driven to high rates of activity (150 impulses per second) by a stimulus moving across the skin surface. Their results indicated an exponential decrease in both spontaneous and evoked activity which approached an asymptote 20 to 30 percent below control

levels at an anesthetic concentration of 16 to 20 mg per kg of body weight. This maximum dose of pentobarbital was less than that required for surgical anesthesia because animals could still be aroused by noxious stimuli.

Animals used in the present study were anesthetized to a surgical plane, and then maintained at that level as described in section III. Although the cortical cells of deeply anesthetized animals are certainly less responsive than they are in the waking state, small variations in circulating anesthetic are likely to have only a minimal effect on excitability, once a surgical plane of anesthesia has been reached.

Richards (1972) examined the mechanism for the depression of cortical activity by barbiturates and found that pentobarbital depresses the amplitude of EPSP's, but does not alter either axonal conduction or postsynaptic responsiveness to neurotransmitters. He concluded that pentobarbital depresses neurotransmitter release without affecting postsynaptic excitability. His data show an exponential decrease in EPSP amplitudes as a function of increasing anesthetic concentration which reaches an asymptote at subanesthetic concentrations. This result was similar to that demonstrated by Collins and Roppolo. Richards (1972) also showed that EPSP amplitudes in repetitively firing neurons were indistinguishable from control values at firing rates less than 2 to 5 impulses per second.

Small changes in circulating anesthetic are therefore unlikely to have a major effect on the responsiveness of cortical neurons in already deeply anesthetized animals, especially if their firing rate is less than 2-5 impulses per second, as they were in this study. In order to test this hypothesis, 3 series of mechanical stimulations on seven footpad locations were conducted over four hours, without varying the skin hydration (table 1 and figure 22). The time course for data collection and anesthesia supplementation was the same as in other experiments. The results from these tests show that the changes in thresholds as a function of time were minimal and without any consistent trends.

STIMULUS INTENSITY AND CORTICAL NEURONAL RESPONSE

Freeman and Johnson (1982a, 1982b) have recently developed a mathematical model which accurately predicts discharge patterns in primary mechanoreceptive afferents when sinusoidal stimuli are delivered to the skin surface. Data recorded from cutaneous afferents show there is a sigmoidal relationship between the response probability and the stimulus amplitude with low frequencies of mechanical stimulation (1982b). Their model predicts such a relationship only if the effect of randomly distributed membrane noise is introduced into their calculations. One explanation for this is that at low stimulus frequencies,

receptor depolarization is superimposed on random temporal variations in the receptor resting membrane potential. At low stimulus frequencies, the probability of evoking a neuronal response in the primary afferent depends on both the stimulus amplitude and the temporal variation in the resting membrane potential. As the frequency of stimulation is increased, the stimulus-response relationship is further complicated by the time constant for membrane repolarization following the preceding stimulus.

There was a curvilinear relationship between the probability of cortical neuronal response and the strength of the stimulus in the present study (figures 16-19). One explanation for this is that punctate stimuli were delivered at a low frequency (1 Hz), and the curvilinear response of the cortical neurons reflects the sigmoidal relationship expected in primary afferents when so stimulated. This explanation is supported by the results of previous studies which have shown that the subjective evaluation of stimulus intensity, as well as activity in neurons of the primary somatosensory cortex and ventrobasal nucleus of the thalamus, closely parallels the activity in primary mechanoreceptive afferents and are linear with respect to the stimulus amplitude (Mountcastle, 1980). This implies that the transfer functions at lemniscal system synapses are linear operators, and that cortical activity will be an accurate reflection of the activity in primary afferents.

Another explanation may be that the curvilinear relationship is the result of nonlinear skin mechanical properties. The stimulus characteristic controlled in this study was the amount of force (stress) delivered to the skin surface. It is well known that mechanoreceptors in skin are activated by the amount of displacement (strain) rather than the amount of stress (Lindblom, 1966). It is possible that the curvilinear relationships are due to nonlinear stress-strain characteristics of the skin.

Although many studies have shown that skin mechanical properties are nonlinear, this appears to be true only for relatively large stresses and strains. Over the range of displacements which were used in this study, and for skin which is separated from underlying bone structure by a thick layer of subcutaneous fat such as that found in the cat footpad, the stress-strain relationship is linear as predicted by Hook's law (Phillips and Johnson, 1982).

EPIDERMAL HYDRATION EFFECTS ON CORTICAL NEURONAL RESPONSE

Epidermal hydration state has a significant effect on the amount of force required to activate neurons in the primary somatosensory cortex when punctate stimuli are delivered to the skin surface (figures 16-18, 20-23, tables 3,4). One explanation is that the slope of the stress-strain relationship in the epidermis has been affected so that more

force is required to achieve any given amount of skin indentation in the dry skin.

There are at least two observations which support this explanation. First, data from studies of skin mechanical properties indicate that the elastic modulus (slope of the stress-strain relationship) is decreased in moistened skin. Second, comparison of threshold values for skin exposed to wet and dry air (table 4) show no statistical difference between the thresholds for low amounts of force (T10-T40). The difference between mean values becomes greater and significantly different, however, as the stress increases (T50-T100). The proposed stress-strain relationship in figure 26 is consistent with both of these observations.

The effect of water soaking the skin appears to be much more complicated. Data reported in table 4 show that thresholds for water soaked skin are statistically different from those for wet air exposed skin over the whole range of applied stresses. The thresholds from water soaked skin are statistically different from those obtained from dry skin at low (T10-T20) and high (T70-T100) stresses, but are not different within the intermediate range (T30-T60). One explanation for this effect is that swelling of the epidermis with water soaking drastically alters skin mechanical properties which results in a curvilinear stress-strain relationship (figure 26). The stress-strain relationships shown in figure 26 are presented as a working

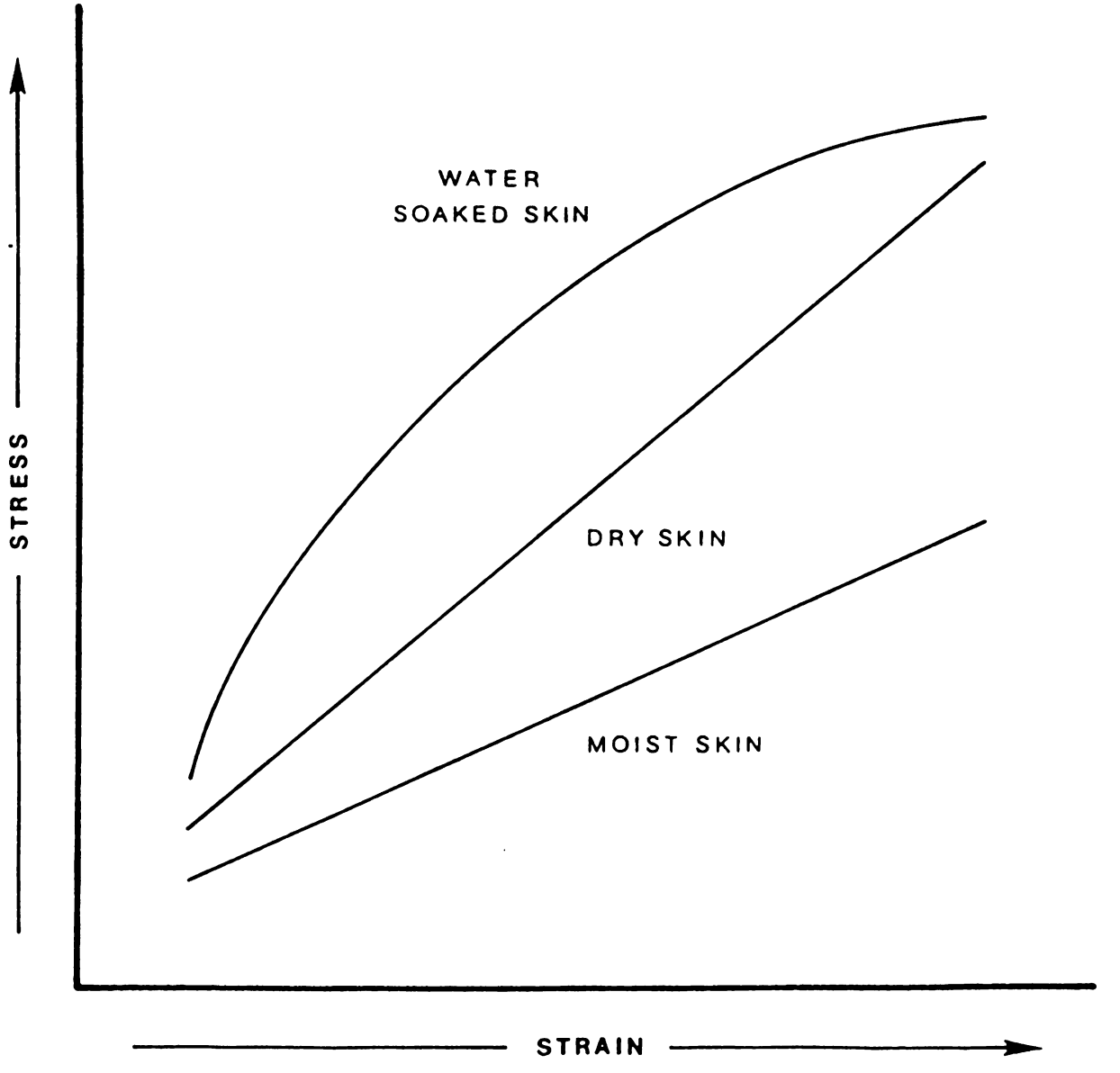

Figure 26. Proposed stress-strain relationships in the epidermis.

hypothesis to explain the results of the present study and can be tested directly in the cat footpad skin.

Results from skin tested during room air exposure indicate that thresholds are not different from those in wet air-exposed skin over the entire range of applied stresses (table 4). For low stresses (T10-T30), threshold values from room air-exposed skin are not statistically different from those obtained from dry skin, but are significantly different for higher stresses (T40-T100). These results are consistent with the hypothesis that under the conditions of this experiment, the mechanical properties of normal skin are very similar to those of the wet air-exposed skin.

Data shown in table 4 and figure 27 suggest a general relationship for thresholds of cortical mechanoreceptive neurons. It is proposed that there is an inflection point in the relationship between cortical neuronal thresholds and epidermal water content at or near the point of the normal hydration state (figure 27). Either absorption or desorption of large amounts of water from the epidermis causes an increase in the amount of force required to activate cortical neurons. In vivo measurements of the frictional porperties of skin (Adams, 1969) show a similar inflection point at intermediate hydration states. A better understanding of a control system for maintaining epidermal hydration within the range of the lowest threshold levels may yield information critical to the understanding of

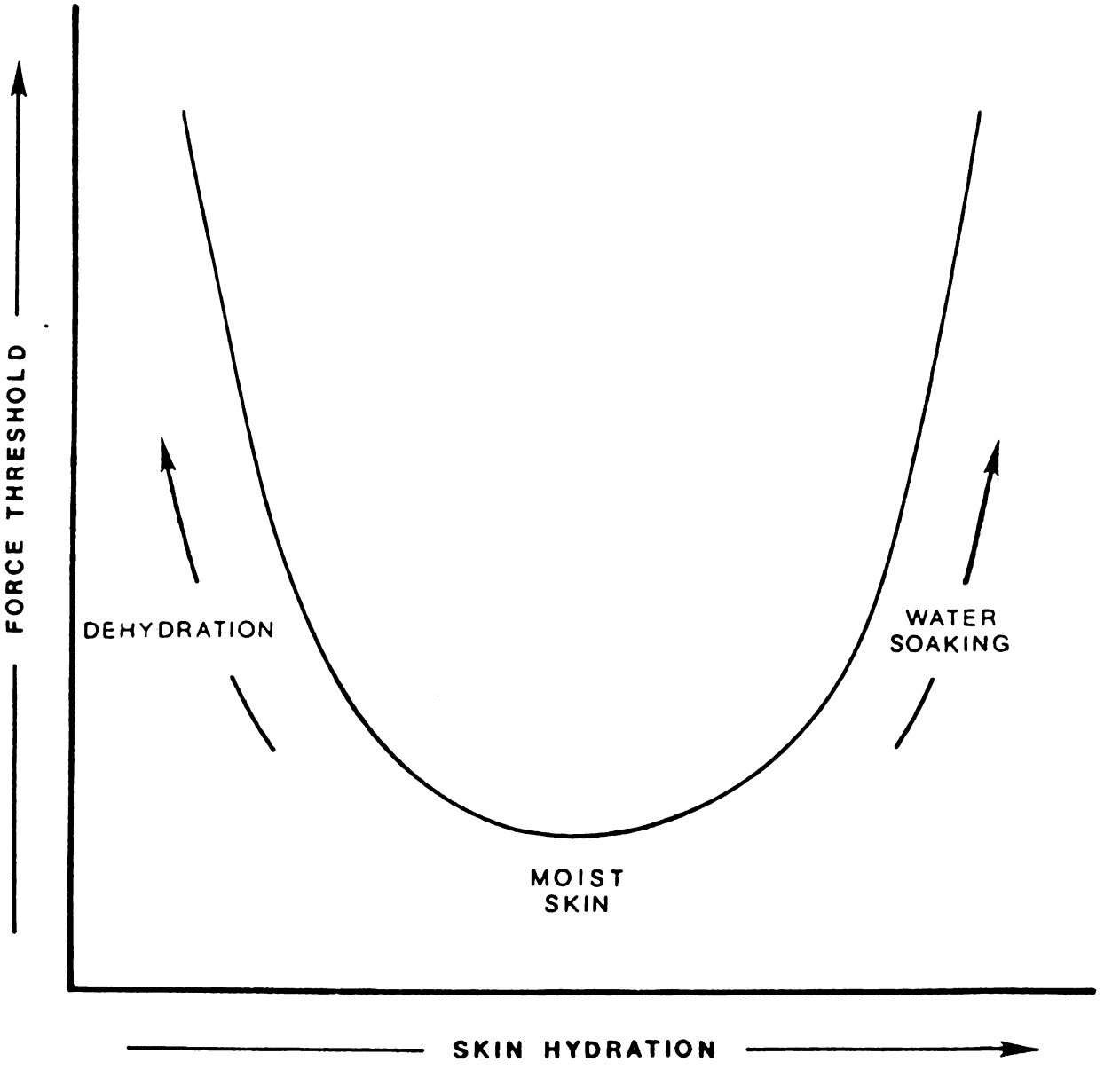

Figure 27. Summary of hydration state effects on cortical neuronal force thresholds.

pathological conditions characterized by extremes in the cutaneous hydration state.

STIMULUS LOCATION EFFECTS ON CORTICAL NEURONAL RESPONSES

The location of the mechanical stimulus on the footpad surface has a significant effect on the threshold for activating cortical neurons (figures 19, 25, tables 3, 5). Although there are some slight differences in the statistical comparisons for very low and very high stresses, data presented in figure 25 are representative of the observed relationships. These data show that there is a region (locations 2-4) between 4 and 12 mm from the most sensitive skin site (location 1) where the amount of stress required to activate a single cortical neuron does not change as a function of distance. Previous studies have shown that the threshold for single mechanoreceptive afferent fibers increases as a function of the square of the distance from the center of the receptive field (Johnson, 1974). This suggests that the region of equal thresholds in the present study is not due to the characteristics of the receptive field of a single primary afferent, but is more likely due to processing within the ascending mechanoreceptor pathway or the cerebral cortex. This is supported by the lack of a significant treatment-location interaction (table 3) which would be expected if spatial coupling were through the skin.

A general pattern of synaptic connections whereby a single cell receives its major input from one cell, and minor inputs from many cells with surrounding and overlapping peripheral receptive fields occurs at all levels of the lemniscal system (Mountcastle, 1981). The results of the present study are consistent with this known pattern of convergence within the ascending pathway. A study of the response of single primary afferents to closely spaced stimuli would be more appropriate to describe accurately the effect of epidermal hydration on spatial coupling of stimulus and response in the skin.

CONCLUSIONS

1. Epidermal hydration state affects the amount of force required to activate S1 cortical neurons when punctate stimuli are delivered to the skin surface.

2. There is an inflection point in the relationship between epidermal hydration state and force thresholds for activating cortical neurons so that force thresholds are minimal in moist skin, and maximal in dry or water soaked skin.

3. How skin hydration affects cortical neuronal thresholds is explained on the basis of epidermal elasticity.

4. There is no hydration effect on the spatial coupling of mechanical stimulation of the glabrous skin and response characteristics of cortical somatosensory neurons.

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