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THESIS – THÈSE

Title of Thesis – Titre de la thèse

Reclamation Suction in dorsal cutaneous receptors of the leopard frog, Rana pipiens

Degree for which thesis was presented Grade pour lequel cette thèse fut présentée	Year this degree conferred Année d'obtention de ce grade
M. Sc.	1985
University – Université	**Name of Supervisor – Nom du directeur de thèse**
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MECHANOTRANSDUCTION IN DORSAL CUTANEOUS RECEPTORS OF THE LEOPARD FROG, *Rana pipiens*.

by

ROBERT E. WATTS

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF Master of Science

Department of Physiology

EDMONTON, ALBERTA

spring 1985

THE UNIVERSITY OF ALBERTA

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Abstract

The dynamic properties of sensory transduction in some frog cutaneous mechanoreceptors were studied. The dorsal skin was excised along with an intact nerve trunk, the skin was stretched, and the activities of single receptors were recorded extracellularly. The frequency response function for transduction was obtained by stimulating the skin with a randomly moving probe and observing the resulting afferent action potentials. Transduction could be well characterized by a power-law or fractional differentiator model with two parameters: k, the fractional exponent and g, the gain at one radian per second.

Room temperature studies showed a significant variability in the exponent, k, and in the sensitivity, g, of the power laws fitted to the frequency responses. However, both the exponent and the sensitivity showed normal distribution curves. When the temperature was varied from 15-25 degrees celsius, there was little change in the fitted sensitivity, g, or exponent, k.

The external potassium concentration was increased by a factor of two in an attempt to depolarize the receptor cell membrane. The results from these experiments displayed three characteristic forms: (1) an increase in sensitivity, g, with a concomitant decrease in exponent, k, (2) no change in either sensitivity or exponent or, (3) a decrease in sensitivity with a concomitant increase in exponent. The effects of the increased extracellular potassium are

discussed in terms of the current ionic mechanisms of sensory adaptation.

The type of receptor which was stimulated during the experiments was not morphologically identified. However, a specific type of receptor, the 'free' nerve ending, was considered to be most likely to be responsible for the results obtained, based on the adaptation characteristics and afferent conduction velocities measured.

Acknowledgements

I would like to thank Dr. Andrew French for his inspiration, guidance and patience and to Rod Gramlich for his expert technical assistance.

Finally, I would like to dedicate this work to Helen, who was always there.

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I. Introduction

A. Vertebrate Mechanoreceptors

It is generally believed that the development of specialized lamellated encapsulated cutaneous receptive structures (Meissner's, Pacinian, Herbst and Golgi-Mazzoni corpuscles, Krause's endings and Eimer's organs) coincided with the evolution of animals from an aquatic to a terrestrial environment. At present, amphibians are the lowest group of animals on the phylogenetic tree to possess lamellated encapsulated cutaneous receptors (During & Seiler, 1974). Weakly encapsulated receptors or Merkel disks (Catton, 1970) have been reported in Teleost fish (Lane & Whitear, 1977) and touch corpuscles in the lips of the moray eels (*Gymnothorax vicinus* and *G. moringa*) (Bardach & Loewenthal, 1961), but true lamellated encapsulated cutaneous receptors have not been found.

Rapidly Adapting Receptors

The Pacinian corpuscle is a large lamellated encapsulated receptor of mammals, 0.5-2.0 mm in length by 0.7 mm in diameter. It is located in the joint capsules, tendons and fascia of muscles, on the periosteum, and in fatty tissue of the subcutaneous layers of both hairy and hairless skin (Schmidt, 1981). It is composed of a many layered perineural capsule and an inner layer of bilaterally

arranged Schwann cell lamellae surrounding a central unmyelinated nerve terminal.

The many layered perineural capsule of the Pacinian corpuscle is not involved in the receptor process of mechano-electric conversion directly (Loewenstein & Rathkamp, 1958) but behaves as a high pass filter of mechanical stimuli (Loewenstein & Skalak, 1966). When stimulated, the receptor potential's time course is the same whether it is elicited by a stimulus of long or short duration (Gray & Sato, 1953). Mendelson and Loewenstein (1964) showed that the removal of the lamellar layers disrupted this regular time course of the receptor potential (reduced receptor adaptation), nevertheless, only one action potential was still produced in response to a step deformation. These studies demonstrated that mechanical filtering can contribute to adaptation of the receptor potential, but that rapid adaptation of the Pacinian corpuscle occurs most probably at the level of impulse initiation (encoding process).

The Golgi-Mazzoni corpuscle is quite similar to the Pacinian corpuscle, and is located in the dermis, the periosteum, and articular structures (Sakada & Aida, 1971a). Its differences include smaller dimensions (150-250μm in diameter in man), a considerably thinner capsule, a different location in the dermis, the absence of close capillaries, and the frequent presence of two afferent myelinated nerve fibres (Chouchkov, 1973). The avian

homologue of the Pacinian corpuscle is the Herbst corpuscle, approximatley one thousand times smaller and located in the skin near the feather roots, the membranes covering and uniting the bones of the legs, and in the skin of the bill of aquatic birds (Quilliam & Armstrong, 1963) and hens (Andersen & Nafstad, 1968). A similar unnamed lamellated corpuscle is found in Eimer's organ of the mole and resembles a simplified version of a Pacinian corpuscle. It is composed of a central unmyelinated nerve terminal surrounded by an array of circumferentially disposed satellite nerve terminals (Armstrong & Quilliam, 1961). The Pacinian, Herbst and Golgi-Mazzoni corpuscles show striking sensitivity to vibration and are classified as rapidly adapting mechanoreceptors (brief discharge to a maintained stimulus) (Gray & Matthews, 1951a; Dorward & McIntyre, 1971; Sakada & Aida, 1971b; Gregory, 1973; Shen, 1983), while the Eimer's organ receptor is tentatively assumed to be rapidly adapting due to its morphological similarity with the other receptors (Armstrong & Quilliam, 1961).

Electrophysiological studies on frog skin have reported the presence of rapidly adapting mechanoreceptors (Dun & Finnley, 1938; Gray & Malcolm, 1951; Loewenstein, 1956; Catton, 1958; Lindblom, 1962). The similarity between the discharge patterns of these morphologically unknown mechanoreceptors and the Pacinian corpuscle of mammals was pointed out by Gray and Malcolm (1951). During and Seiler (1974) have revealed the presence of lamellated receptors in

the skin of *Rana esculenta* possessing similar morphological characteristics to the Pacinian corpuscle, and it is on this basis they suggest it to be the rapidly adapting receptor recorded by Loewenstein (1956). During (1973) has described three types of lamellated receptors in reptiles which are: (1) lamellated free endings in *Caiman* that resemble the lamellated corpuscle in Eimer's organ of the mole, (2) lamellated encapsulated endings in *Varan* and *Natrix* resembling the Herbst corpuscle and, (3) lamellated encapsulated endings possessing a capsular space in *Caiman* that is structurally similar to the core of the Pacinian corpuscle. Rapidly adapting receptors responding to 'on' and 'off' transients of rectangular displacements, similar to a Pacinian corpuscle response, have been reported in reptiles (Siminoff & Kruger, 1968) but correlated morphological studies were not performed.

There are two morphologically distinct Krause endings in mammals: the cylindrical end bulbs and the spherical end bulbs. The cylindrical end bulb is found in glabrous skin of non-primates just below the epidermis. They are lamellated and may contain unbranched, branched, or multiple nerve terminals. Iggo and Ogawa (1977) concluded from correlated electrophysiology and morphological experiments that these receptors were rapidly adapting. The spherical end bulbs have an intertwining network of nerve endings within the lamellated corpuscle, more or less completely filling the inner core. These receptors occupy a different location in

the glabrous skin of primates than the Meissner's corpuscles, and their functional characteristics have not been established (Iggo & Andres, 1982). Meissner corpuscles are located at the apex of the dermal papillae of the hairless skin of the primates. They are lamellated mechanoreceptors with sensory neurites that follow a winding path through the stacked lamellae, where more than one neurite may enter a single corpuscle (Cauna & Ross, 1960). Through electrophysiology and morphologically correlated experiments, Meissner corpuscles have been shown to be rapidly adapting (Munger et al., 1979). The impulse firing frequency of these receptors is dependent on the velocity of indentation of the skin and these are therefore known as velocity detectors (Schmidt, 1981). Velocity detector counterparts in the hairy skin of mammals are the hair follicle afferent units (lanceolate endings) first described by Brown and Iggo (1967) in cats and rabbits, which respond to hair movement but not to a maintained displacement and are also rapidly adapting. Genital end bulbs are morphologically similar to Meissner's corpuscles with a loosely coiled neurite tightly surrounded by thin lamellae of cytoplasm from associated lamellar cells (Pàtrizi & Munger, 1965). Their physiological properties have not been determined but the appearance of lamellar cell specializations may imply rapidly adapting characteristics.

Slowly Adapting Receptors

Merkel cells have been described in the order Anura: *Rana pipiens* (Nafstad & Baker, 1973), *Rana temporaria* (Whitear, 1974), larval *Xenopus laevis* (Fox, 1974), *Bufo bufo* (Budtz & Larsen, 1975) and, in the order Urodela: larval *Salamandra salamandra* (Whitear, 1977), and *Ambystoma tigrinum* (Parducz et al., 1977). These amphibian Merkel cells have similar morphological characteristics to those of mammals which are: (1) a small ratio of cytoplasm to nucleus, (2) the presence of membranous bound osmiophilic granules with the granules most abundant between the nucleus of the Merkel cell and the subjacent expanded nerve terminal, (3) the presence of finger-like processes which extend between adjacent cells and, (4) a possible synaptic association with nerve fibres (Parducz et al., 1977; Fox & Whitear, 1978). Mammalian Merkel disks are present in both the glabrous and hairy skin of mammals where one myelinated afferent fibre branches extensively to make contact with many Merkel cells. This is in contrast to the amphibians, where Merkel cells are isolated and nerve fibres supply individual Merkel cells (Parducz et al., 1977). In mammalian glabrous skin, Merkel disks are contained in rete pegs, and in hairy skin Merkel disks lie at the base of the epidermis, and beneath the glassy membrane and within the basal cell layer of the sinus hair follicles. In anurans, the Merkel cells lie immediately above the basal layer while in urodeles, Merkel cells are located between the basal layer

cells, only rarely in contact with the basement membrane of the epidermis.

Correlated electrophysiological and morphological studies have been completed on the salamander, *Ambystoma tigrinum*, and Parducz and co-workers (1977) have reported rapidly adapting characteristics for Merkel cells. This is in agreement with Cooper and Diamond (1977), who reported that all salamander cutaneous mechanoreceptors were rapidly adapting. Electrophysiological studies on frog skin have reported the presence of both slowly and rapidly adapting mechanoreceptors. However, morphological correlation with specific mechanoreceptors has yet to be performed. Iggo and Muir (1969) have classified Merkel cells in the cat as slowly adapting type I mechanoreceptors after correlated electrophysiological and morphological studies were performed. These receptors have no resting discharge, do not respond to skin stretch, and give a high frequency discharge with skin indentation. The high frequency discharge is followed by an irregular discharge rate when the stimulus is maintained. Merkel disks may be confused functionally with Ruffini endings which share several similar characteristics. Both receptors are: (1) slowly adapting, with the discharge from a maintained stimulus lasting at least several minutes, (2) innervated by myelinated axons with fast conduction velocities and, (3) responsive to vertical displacement of the skin with both dynamic (velocity) and static (displacement) components, thereby transmitting information

concerning the magnitude of cutaneous deformation.

The actual role of Merkel cells in the mechanoelectric transduction process is still unknown. Horch and co-workers (1974) have suggested that Merkel cells are the sensory elements of the type I receptor, the Merkel cells synaptically stimulating the nerve endings and, Chen, Gerson and Meyer (1973) have demonstrated Merkel cell granule fusion with specialized areas of membrane between the neurites and the Merkel cell. A met-enkephalin-like substance has also been demonstrated through immunohistochemistry (Hartschuh et al., 1979), adding evidence to the hypothesis. Gottschaldt and Vahle-Hinz (1981) have demonstrated that Merkel cells can respond in phase to a 1200 Hz vibratory stimulus. Therefore, they argue that it could not be mediated by a chemosynaptic transmitter, because an accumulation of released transmitter in the synaptic cleft would occur resulting in a phase jitter of the afferent impulses. Gottschaldt and Vahle-Hinz (1982) used naloxone a met-enkephalin antagonist, and saw no functional decreas[illegible]n Merkel cells were stimulated and therefore have postulat[illegible]hat the Merkel cells are trophic in nature. They hyp[illegible]hesize that the nerve endings themselves, abundant in m[illegible]ond[illegible]a and containing a fine granular axoplasmic struct[illegible]e whic[illegible] are characteristics of sensory nerve endings, may [illegible]e the actual mechanoelectric transducer elements.

Ruffini endings, unlike Merkel disks, are located in the dermis or corium of glabrous and hairy skin, and in joint capsules of mammals. The receptor is spindle shaped with collagenous fibres entering the capsule from both poles, providing a structurally rigid position within the skin and joints. The outer capsule consists of four to five layers of perineural cells, and inside the capsule, endoneural connective tissue is separated into an inner core and an outer envelope separated by fluid filled spaces. A single myelinated nerve fibre supplies the receptor which ramifies and follows a meandering course, until the end axons make direct contact with the collagenous fibre plexus of the inner core. Chambers and co-workers (1972) have classified this receptor as a slowly adapting type II cutaneous mechanoreceptor which characteristically shows a resting discharge, response to skin stretch, and a low frequency discharge rate to skin indentation followed by a regular discharge rate to a maintained stimulus. Electrophysiological studies performed on knee joint capsules of the cat have shown an increase in receptor activity upon rotation of the joint (Boyd & Roberts, 1953; Grigg & Hoffman, 1982) while dermal receptors have responded to skin stretch with the same patterns and dimensions as those produced by vertical displacement (Chambers et al., 1972). Morphological correlations in both cases have revealed Ruffini endings (Chambers et al., 1972; Grigg & Hoffman, 1982). Dermal and joint capsule Ruffini endings are

directionally sensitive to stretch, which can be accounted for in terms of the structural coupling between the external collagen fibrils and the collagenous matrix of the capsule. A possible functional role of joint capsule Ruffini endings are as limit detectors, providing information on joint angles through capsular stresses, and more importantly, to indicate when a joint is at or near the limit of its range of movement (Grigg & Hoffman, 1982). Dermal receptors, however, respond to both vertical displacement of the skin and skin stretch with equal activity so that both responses are accepted as Ruffini ending characteristics.

Slowly adapting reptilian mechanoreceptors show striking similarities to the slowly adapting receptors found in mammals. Slowly adapting type II (SAII) receptors are more subject to lateral stretching of the skin than slowly adapting type I (SAI), SAII have a higher probability of a resting discharge than SAI, and both receptors transmit information concerning the magnitude of cutaneous deformation (Kenton et al., 1971). Morphological identification of two distinct receptors in reptiles have not correlated these results.

Hair Cells

The mechanoreceptors involved in hearing and equilibrium in vertebrates and the lateral line organs of aquatic amphibia and fish are neuroepithelial cells called hair cells. These hair cells are morphologically similar in

that numerous closely packed microvilli, termed stereocilia, and a single true cilium, the kinocilium (except for mammalian cochlea where the hair cells have secondarily lost their kinocilium), extend from the apical surface and protrude into auxilliary structures specific to the individual organ.

The lateral line organs are a collection of sensory endings (neuromasts or canal organs) distributed systematically over the body of fishes, larval amphibians and adult urodeles and anurans which maintain an aquatic existence. Neuromasts, which contain 20-60 hair cells, are found in amphibians and are superficially located and distributed singly or in groups (stitches) while canal organs are primarily found in bony fish. The neuromasts are composed of three cells: (1) Mantle cells which surround the centrally located supportive and sensory cells, (2) Supportive cells which surround each sensory cell and extend from the basement membrane to the outer surface, and (3) Sensory, or Hair cells, located in the apical half of the neuromast. The stereocilia and the kinocilium of each hair cell protrude into an overlying gelatinous cupula that projects directly into the surrounding water in neuromasts or into the canal lymph in canal organs, which communicate with the surrounding water by way of pores. The hair cells are directionally sensitive to cupular movement, and in each neuromast, the kinocilium of adjacent hair cells are oppositely orientated. The lateral line organs of amphibians

are innervated by two large myelinated afferent nerve fibres, each of which divides and supplies one branch to each neuromast within a stitch, and by myelinated and unmyelinated efferent nerve fibres. The afferent nerve fibres, like the hair cells, have been shown to be directionally sensitive to water displacement (cupular movement), indicating that each afferent fibre innervates hair cells of only one orientation (Sand, 1937; Russell, 1976). The efferent neurons in amphibians are closely associated with movement, the fast motor neurons controlling voluntary muscles inhibit the lateral-line hair cells 10-20 ms before and during any movement, the extent of inhibition increasing the more vigorous the movement. The slow motor neurons responsible for muscle tone, however, do not inhibit the hair cells (Russell, 1971). This neuronal makeup may be protective in nature, the lateral-line receptors becoming less sensitive to stimulation, allowing the lateral-line system to be fully responsive to water displacements immediately when movement ceases (Russell, 1976).

The hair cells involved in spatial orientation and hearing in vertebrates are morphologically similar to the hair cells of the lateral line organs. The vestibular and auditory hair cells are separated from each other by supportive cells, to which they are joined by tight junctions on their apical surfaces. In the semicircular ducts, the hair cells are oriented in one direction and project into an overlying gelatinous cupula. In the saccule

and utricle, the hair cells are not oriented in a single direction but are oriented throughout 360°. The kinocilia are oriented either towards or away from a single curving landmark known as the striola, and the hairs project into an overlying gelatinous matrix in which crystals of calcium carbonate are embedded. The cochlear hair cells are arranged in rows along a basilar membrane with the apical stereocilia oriented in the same direction and projecting into an overlying tectorial membrane.

The hair cells are tonically active due to a steady current flow across the transducer membrane, accounting for approximately one-fifth of the maximum conductance value of the transduction channels. Mechanical displacement of the stereocilia is thought to alter this current flow (Davis, 1965). Displacement of the stereocilia towards the kinocilium causes an increase in membrane conductance and cell depolarization which increases the firing rate. Mechanical displacement in the opposite direction causes a decrease in firing rate by decreasing membrane conductance and hyperpolarizing the cells (Hudspeth & Corey, 1977; Hudspeth & Jacobs, 1979). Orthogonal displacements of the hair cells evokes little or no response (Hudspeth, 1983). Hair cells from the bullfrog sacculus (Hudspeth & Corey, 1977; Corey & Hudspeth, 1983a, 1983b), turtle and guinea-pig cochlear hair cells (Crawford & Fettiplace, 1981; Sellick, 1979) all show similar asymmetrical displacement-response curves which measure the hair cells mechanical sensitivity

to a stimulus. The displacement-response curve was seen to shift along the displacement axis in bullfrog saccular hair cells in response to a maintained step displacement, illustrating adaptive properties of the hair cells which are dependent on Ca^{2+} concentration at the apical hair cell surface (Eatock et al., 1979). When the cells are displaced out of their responsive range, the adaptive mechanism shifts the responsive range to the new bundle position. This allows the hair cells to be highly sensitive to small displacements while maintaining a large displacement range. The displacement response curve is also affected by a change in the Ca^{2+} concentration bathing the apical cell membrane. Increasing the Ca^{2+} concentration shifts the displacement response curve to the right and causes a decrease in maximum current while decreasing the Ca^{2+} concentration shifts the displacement response curve to the left and increases the peak current (Corey & Hudspeth, 1983b). The change in displacement-response curve with change in Ca^{2+} concentration may not be due to a direct effect of Ca^{2+} on the transduction mechanism, but may be explained by other factors. First, Ca^{2+} may screen the membrane surface, thereby reducing the negative surface charge. This reduction in negative surface charge will cause a subsequent decrease in the concentration of permeant monovalent cations at the membrane surface. Second, Ca^{2+} may act as a partial blocker of the transduction channels. Third, the extracellular K^+ was altered in the experiments performed by Hudspeth and

Corey (1983b) when Ca^{2+} was altered to maintain constant tonicity in the saline solution.

Frequency-response studies have been performed on *Xenopus laevis* lateral line organs with both small and large amplitude sinusoidal displacements (Kroese et al., 1978, 1980), the increasing stimulus amplitudes alter the response from a water velocity detector to a water acceleration detector. Frequency-responses of bullfrog saccular hair cells show a similar frequency-response function as the lateral line hair cells, with small stimulus displacements resulting in a phase lead of 90° at low frequencies (velocity detector), rolling off at high frequencies (similar to a high-pass filter roll-off) (Corey & Hudspeth, 1983a). The high-pass filter characteristics of the lateral line and bullfrog saccular hair cells are a result of the adaptive shifts in the hair cells at low stimulus amplitudes.

The transduction process is similar in the various acoustico-lateralis organs and commences with the application of a mechanical stimulus to the stereocilia. It is the overlying auxilliary structures of the individual organs which have been proposed to specify the adequate stimulus (water movement, linear or angular acceleration, auditory vibrations) for each organ (Lowenstein, 1956). It has long been postulated that the kinocilium was an integral component of the transduction process in hair cells (Hillman, 1969) because of other sensory systems which

utilize ciliary derivatives in sensory transduction (Wiederhold, 1976; Moran et al., 1977; Thurm et al., 1983). However, Hudspeth and Jacobs (1979) have shown in the bullfrog sacculus, by removal or deflection of the kinocilium, that the kinocilium is not required for sensory transduction to occur, but that the stereocilia mediate the transduction process. Hudspeth (1982) has also shown by measuring extracellular potentials of stimulated bullfrog saccular hair cells that there is a predominant current flow into the distal portions of the hair cells, suggesting that the transduction apparatus lies at or near the base of the stereocilia.

In mammalian cochlea and vestibular systems, an endolymph high in K^+ and Cl^- bathes the apical hair cell membranes, producing a potential difference of +50 mV between the apex and the base of the cell. It has been suggested that a K^+ current carries the receptor current in hair cells, the current flowing inward through the apical cell membrane and outwards through the basolateral membrane (Sellick & Johnstone, 1975; Corey & Hudspeth, 1979). In *Xenopus* lateral-line organs, the cupula close to the hair cells was found to possess a micro-environment high in K^+ and Cl^- ions similar to that of endolymph in mammals, and it is thought to be mantained by an electrogenic K^+ pump (Russell & Sellick, 1976). Russell and Sellick (1976) proposed that the receptor current was carried by K^+ ions and not Ca^{2+} ions as proposed by Sand (1975) in the

mudpuppy, and that Ca^{2+} may play an alternate role in the transduction process. Ca^{2+} may control the permeability of the apical hair cell membrane to K^+ ions (Russell & Sellick, 1976; Jorgensen, 1983), and is involved in the adaptive properties of the cell, since a change in apical hair cell membrane Ca^{2+} concentration shifts the displacement-response curve of the hair cell (Corey & Hudspeth, 1983b). Two K^+ conductances, one voltage-sensitive and the other calcium-dependent, have been described in the basolateral membrane of hair cells (Lewis, 1982). These conductances cause an increase in outward K^+ current which repolarizes the cell membrane and increases the receptor potential. A voltage-sensitive Ca^{2+} conductance in the basolateral membrane (Hudspeth & Corey, 1977; Lewis, 1982) is probably involved with the release of transmitter from the cell (Corey & Hudspeth, 1983a).

B. Crustacean Stretch Receptors

There are two distinct types of muscle stretch receptors in the Crustacea characterized by their rate of adaptation to a maintained stimulus. The first is the slowly adapting receptor, which ends on the lateral muscle bundle while the second is the rapidly adapting receptor which ends on the medial muscle bundle (Alexandrowicz, 1951; Wiersma et al., 1953). This sensory innervation is of particular interest because Kuffler (1954) showed through electrical stimulation of the efferent nerve supply to the receptor

muscles, that a fast, or twitch contraction occurs in the medial "fast" receptor muscle and a slow, or tonic contraction occurs in the lateral "slow" receptor muscle. Both muscle bundles are innervated by excitatory and inhibitory motor neurons. Kuffler and Eyzaguirre (1955) and Jansen et al. (1971) have shown that the dendrites of both stretch receptors receive inhibitory innervation.

The lateral and medial muscle bundles exhibit a peculiar feature in lobsters in that the muscle-fibres are replaced near the centre of the bundles by connective tissue, and it is in this region where the stretch receptor dendrites are distributed (Alexandrowicz, 1951). This non-contractile region is absent from the crayfish receptor muscles (Florey & Florey, 1955), however, excitation of the sensory nerves by passive stretch or active contraction of the receptor muscle infers the paucity of contractile elements from the dendritic region (Kuffler, 1954; Eyzaguirre & Kuffler, 1955). Nakajima and Onodera's (1969b) visco elastic model proposed that the dendritic terminals are located in a region of elasticity (non-contractile material) and that this region can be rapidly stretched while the contractile apparatus of the muscle (viscous element) stretches less rapidly. As the viscous elements stretch, the tension in the dendrites is reduced and the deformation is spread evenly along the fibre. Their experiments on the receptor potential of the slowly adapting receptor of the crayfish in response to a constant stretch

showed that the receptor potential rises rapidly to a peak and then declines to a steady level with time, but under tension clamping the form of the receptor potential closely approaches the square-wave of the induced tension. The peak of the receptor potential is associated with the initial rapid burst of action potentials which then declines to a sustained rate with a maintained stimulus. Nakajima and Onodera (1969b) estimated that approximately 50% of the adaptation in the slowly adapting stretch receptor involves the transformation of the stretch stimulus to an adequate stimulus for transduction. The remaining 50% of adaptation is involved in the transduction and encoding stages.

The membranes of the crayfish stretch receptor which generate the receptor potential are presumably the fine dendritic terminals. These dendritic terminals have dense clusters of mitochondria situated in areas where mechanical deformation is expected to be the greatest (Bodian & Bergman, 1962). Concentrations of mitochondria are observed in the dendritic terminals of vertebrate mechanoreceptors (Pacinian corpuscles (Quilliam & Armstrong, 1963), Meissner''s corpuscles (Cauna & Ross, 1960), Golgi-Mazzoni corpuscles (Chouchkov, 1973)) and therefore, provide evidence that these dendritic terminals are responsible for transduction in these receptors. Mechanical deformation of the dendrites causes the receptor membrane to depolarize (inward graded Na^+ current) (Edwards et al., 1963; Gestrelius et al., 1983) and this depolarization activates

subsequent channels leading to an outward K^+ current (Gestrelius & Grampp, 1983), an outward Ca^{2+} dependent K^+ current activated by Ca^{2+} influx (Gestrelius et al., 1981; Ottoson & Swerup, 1982) or intracellular Ca^{2+} inactivating the transducer channels (Swerup, 1983). An increased conductance for Cl^- does not appear to be involved in the receptor potential and the receptor membrane can be regarded as a cation-sensitive membrane (Obara, 1968). There are two stages of adaptation of the receptor potential in the slowly adapting stretch receptor of the crayfish: an early phase which is predominated by ionic factors and a late phase which is mainly associated with mechanical factors.

Brown, Ottoson and Rydqvist (1978) have shown that the Na^+ channels of the crayfish stretch receptor are not completely sensitive to Na^+ because when Na^+ is replaced by the larger cations tris and arginine, a slight depolarization of the receptor membrane occurs with stretching. The Na^+ channel is also permeable to the divalent cations Ca^{2+}, Mg^{2+}, Sr^{2+} and Ba^{2+} (Edwards et al., 1981) and increased intracellular Ca^{2+} may be responsible for the increased K^+ conductance similar to that found by Meech and Strumwasser (1970) in *Aplysia*. The proposal by Ottoson and Swerup (1982) that the adaptive characteristics of the receptor is a Ca^{2+} controlled outward K^+ current is based on experiments in which an increased intracellular Ca^{2+} concentration increased the adaptive decline and almost eliminated the static phase. Injecting a calcium chelator,

EGTA (Ethyleneglycol-bis(β-aminoethylether)-N,N'-tetraacetic acid), or a calcium blocking agent, D600, resulted in the opposite effect: the static phase increased in amplitude while the early adaptation was almost eliminated (Ottoson & Swerup, 1982).

A decrease in intracellular K^+ activity was noticed by Brown et al. (1978) when extracellular K^+ was removed and this accelerated when extracellular Ca^{2+} was concomitantly reduced or removed. This decrease in intracellular K^+ activity is similar to that which Brown and Ottoson (1976) found in the barnacle photoreceptor, except that they also found an increase in intracellular Na^+ activity, which was most likely due to inhibition of a Na^+/K^+ pump. The decreased intracellular K^+ activity would reduce the normal outward K^+ current contributing to adaptation in the receptors. Swerup (1983) also studied the removal of K^+ from the external solution and found the early adaptive decline of the receptor was abolished, the static phase (late adaptation) increased and the membrane potential slowly depolarized. Upon restoration of K^+ to the external environment, reactivation of the electrogenic pump would cause a rapid influx of K^+ ions and extrusion of Na^+ ions restoring the adaptive properties of the stretch receptors (Brown & Ottoson, 1976). The membrane potential only slightly hyperpolarized back to its normal value but the early adaptive decline returned and the static phase remained higher than in controls. The return of

extracellular Ca^{2+} to a K^{+} free solution resulted in a rapid return of adaptation. When the receptor was returned to normal saline a normal receptor potential returned but the static phase was smaller than control responses.

C. Frog Mechanoreceptors

The sensory innervation of frog skin arises from myelinated and non-myelinated axons entering the subepidermal layers, forming a deep and a superficial plexus. Some axons emerging from these plexuses end in association with skin glands, blood vessels, differentiated receptor endings (During & Seiler, 1974; Nafstad & Baker, 1973), and many penetrate and end freely in the epidermis (Whitear, 1955).

Catton (1958) has catalogued four types of receptors in frog skin, based upon their spike amplitudes, conduction velocities and adaptability to a mechanical stimulus. They are:

Type *a*, high amplitude (350-400 μV), rapidly conducting (20-30 m/s), rapidly adapting receptors with discrete areas of stimulation which correspond to Fessard and Segers (1943a) type A_1 receptors, Maruhashi, Mizuguchi and Tasaki's (1952) tactile endings, Loewenstein's (1956) touch fibres, Lindblom's (1962) very rapidly adapting receptors and, Ogawa, Moromoto and Yamashita's (1981) rapidly adapting type II units.

Type *b* are medium amplitude (200-300 μV), with a conduction

velocity of 10-15 m/s and more slowly adapting than the type *a* receptors. These receptors correspond to the type A_2 endings (slowly-adapting touch receptors) of Fessard and Segers (1943b), stretch fibres of Loewenstein (1956), less rapidly adapting receptors of Lindblom (1962) and, Ogawa, Morimoto and Yamashita's (1981) rapidly adapting type I and slowly adapting units which showed mean conduction velocities in the above range.

Type *c* are small amplitude (100-150 μV), conduction velocity of 5-10 m/s demonstrating a slower adaptation rate than the type *b* endings which readily responds to rapid repetitive stimulation (vibratory receptors).

Type *d*, non-myelinated fibres with small amplitude (<100 μV), conduction velocity of 0.1-0.3 m/s and correspond to Fessard and Segers (1943b) slowly conducting pain receptors.

The adaptive behavior of the two (type *a* and *b*) receptors were studied in a frog skin preparation by Loewenstein (1956) using stretch as the main parameter. The type *b* (stretch) fibre has a graded stationary discharge in response to a steadily maintained stretch and a period of depressed activity following sudden release of stretch. The type *a* (touch) fibre is readily excited by stretch, the application and release resulting in an 'on' and 'off' discharge, respectively. As stretch is increased, a logarithmic increase in the 'on' response occurs until at high degrees of stretch this receptor acquires all the properties of a slowly adapting stretch receptor.

Loewenstein (1956) attributed these characteristics to the mechanical state of the receptors. Under relaxed conditions, the membrane of the stretch ending was in an expanded state, that of the touch ending in a folded state, with the assumption that the receptors fire in response to membrane unfolding. Catton and PeToe's (1966) theory of mechanoreceptor adaptation differed from this and was based on the coupling of forces between skin tissues and the receptor. The slowly adapting receptors were considered to have a high elastic modulus where little slip occurs between tissue and receptor, resulting in the terminal following and responding to the applied deflexion, predicting an amplitude threshold response. The main force acting on rapidly adapting receptors was thought to be viscous drag (low modulus), allowing the terminal to recover quickly at the end of a dynamic stimulus, and predicts variation of response-latency with velocity and the existence of a critical slope. Evidence for this theory was found when hyaluronidase, which temporarily decreases the viscosity of the cellular cement (Swinyard & Pathak, 1980), and collagenase, which digests collagen, shifted the slope-latency curves to the right and decreased the static phase in long conditioning pulses. The shift in slope-latency curve means that a higher stimulus slope (mm/s) was required to excite the receptor at the same latency used prior to enzyme treatment, while a decreased static phase means that a greater test pulse had to be added

to the conditioning pulse at various times to evoke a just threshold response. Both these results do demonstrate an increased slippage of the receptor and affect predominately the elastic coupling forces between skin tissues and receptor.

D. Objectives

The present study was initially undertaken to categorize sensory receptors in the skin of the frog, *Rana pipiens*, using linear systems analysis. Many sensory receptors respond to a sinusoidal driving function in a non-linear fashion. That is, the response is not a sinusoid differing only in amplitude and phase, but is a modulated train of action potentials. The use of random mechanical stimulation as the driving function can be used to categorize a receptor by estimating a describing function (frequency response function) and some measure (coherence function) of how completely the describing function relates the response (action potentials) to the driving function.

Linear systems theory has been used to describe many sensory receptors, and a large proportion of these receptors display fractional differentiation (Landolt & Correia, 1980; Bohnenberger, 1981; French & Kuster, 1981; Tomko et al., 1981; Kuster & French, 1983).

Once the receptors were categorized, experiments were designed to test theories which had already been put forward using other sensory systems. A strong temperature

sensitivity was observed in the Pacinian corpuscle (Ishiko & Loewenstein, 1961) and the cockroach tactile spine (French & Kuster, 1982), arguing that a high energy barrier was invariably necessary for mechanotransduction in these receptors. Temperature experiments were performed on frog mechanoreceptors to test if a similar energy barrier was present in the frog cutaneous tactile mechanoreceptors.

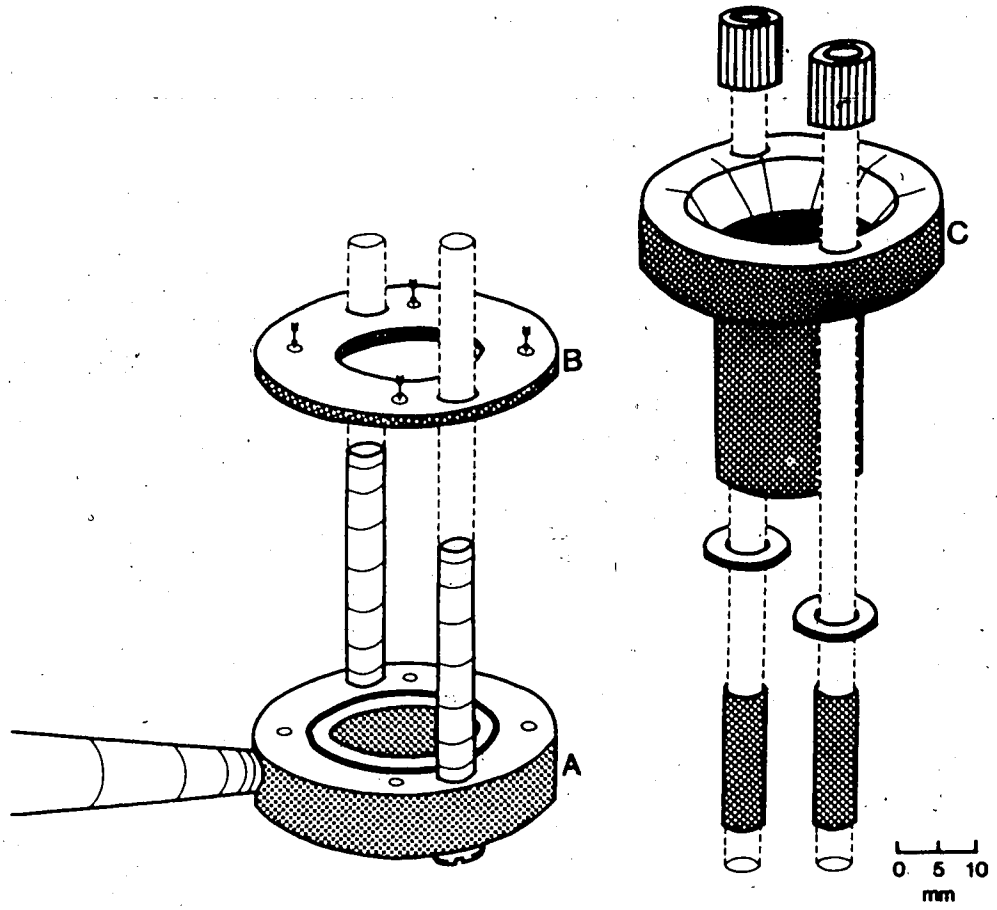
Recent electrical stimulation studies of the cockroach tactile spine (French, 1984c) have shown that membrane potential affects the adaptive properties of the receptor at the action potential initiation stage. Depolarizing the cell increases adaptation while hyperpolarizing the cell decreases adaptation. Similar responses could be obtained by altering the extracellular potassium concentration and stimulating the receptors mechanically. Increasing the extracellular potassium concentration will cause a depolarization of the receptor membrane potential while decreasing the extracellular potassium concentration will hyperpolarize the receptor membrane potential. Studies were therefore performed using a high external potassium concentration to see if the adaptive properties of the receptors could be altered, thereby allowing a prediction that either the transduction or action potential initiation stages were responsible for adaptation in these receptors.

II. Materials and Methods

A. Mounting and Stretching the Skin

All experiments were performed using dorsal skin preparations from adult frogs, *Rana pipiens*. All frogs were 90 mm or larger (body size) and were kept at room temperature. The frogs were double pithed and the dorsal skin was excised along with an intact dorsal cutaneous nerve trunk (*rami cutanei dorso mediales*) running from the spinal column through the dorsal lymphatic space to the skin. The skin was subsequently stretched and an isolated receptor located for study. All experiments were performed at room temperature (20±1°C), except where indicated. The stretcher (Figure 1), made from teflon was composed of three main ring shaped pieces: A) supportive base, B) clamp, and C) stretcher. The supportive base had two large 38x5 mm screws used for guiding the clamp and stretcher. A circular protrusion from the clamp inserted into an indentation in the supportive base. Four small screws (10x2 mm) were used for securing the clamp to the base. The dorsal skin was placed on the supportive base with the dermal side up and the dorsal nerve centered. The clamp was lowered on top of the skin and secured with the four screws. An 18 mm spring, a single washer and the stretcher, internal diameter 15 mm, were placed over the guiding screws and held taut by a large nut. The springs were placed between the clamp and the

Figure 1. Device used for holding and stretching the dorsal skin for each experiment. A sheet of skin was placed on the upper surface of base (A) and held in place with the clamp (B). The stretcher (C) was then lowered through the center of the clamp onto the skin and stretched the central region by attempting to pass down through the central hole of the clamp and base. A small amount of Ringers was then placed in the upper portion of the stretcher.


A
B
C
0 5 10
mm

stretcher to provide resistance to the stretcher, and the locking nuts were used to increase the stretch by moving the stretcher downward. A 200 mm long, 12.5 mm diameter brass rod, tapered and threaded at one end to screw into the supportive base, was used to support the stretching apparatus.

The degree of circumferential stretch was slowly increased in the preparation until it was free of obvious wrinkles and the receptor under investigation continued firing for the time required to gather data for a frequency response. The degree of stretch was calculated at the end of each experiment by measuring with a microscope graticule the amount of skin which had been displaced by the stretcher. The skin which had been compressed between the stretcher and the wall of the base was rendered pale in colour and this facilitated estimation of the degree of stretching. No attempt was made to measure the tension in the skin, however, the tension could be increased to the point of tearing the skin.

B. Stimulating and Recording

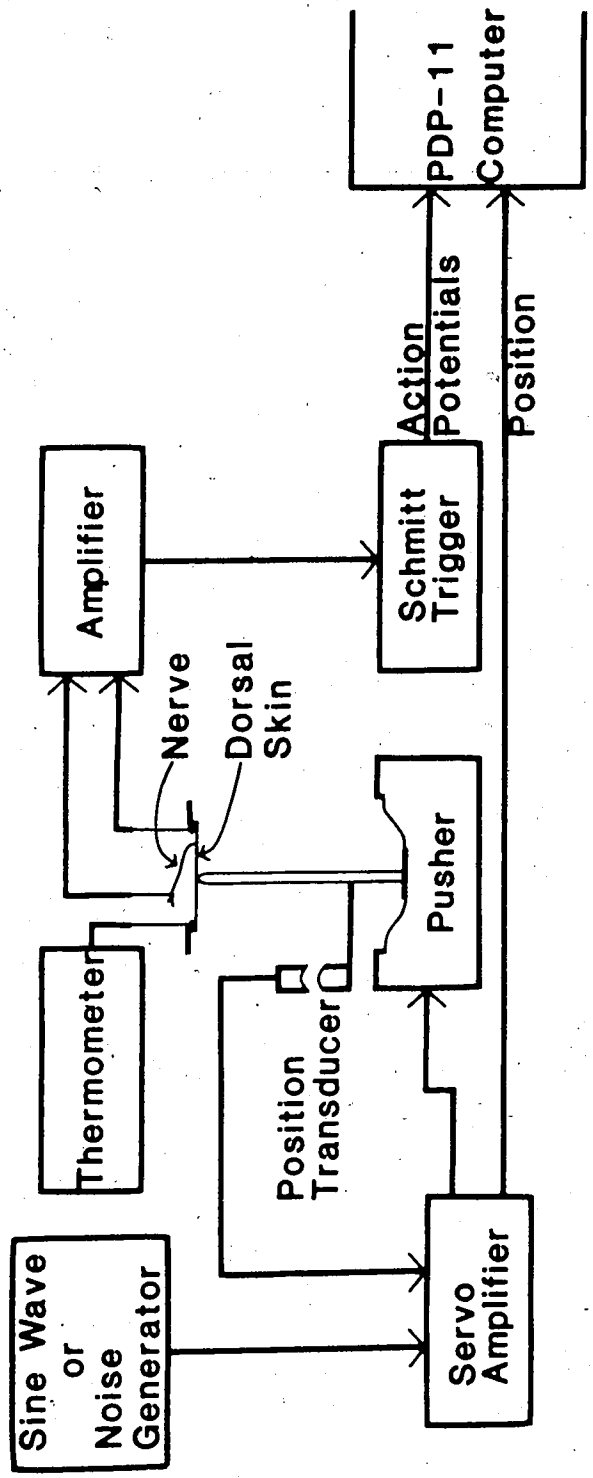
The stretcher was positioned above a three dimensionally adjustable glass rod stimulator. Electrical activity in the nerve was recorded using a fine silver wire hook electrode. The electrode and nerve were lifted from the bathing solution and coated with petroleum jelly or oil to prevent desiccation. The skin was bathed in a Ringers

solution, continuously bubbled with room air, and was replaced at regular intervals. This solution consisted of: (mM) NaCl 115, KCl 2.5, $CaCl_2$ 1.0, $MgSO_4$ 1.0, $NaHCO_3$ 2.5, NaH_2PO_4 0.3, Na_2HPO_4 0.7, $C_6H_{12}O_6$ 5.0, pH 7.2. The experimental arrangement is illustrated in Figure 2.

The stimulating glass rod was a 1.6-1.8 mm capillary tube with a hemi-spherical tip (KIMBLE PRODUCTS #34505). The area exposed for mechanical stimulation was approximately 175 mm^2, which greatly exceeded the receptive field size of any receptor studied. Comparable receptive fields in toads varied from 2-35 mm^2 (Lindblom, 1958) and for individual receptive units in dorsal skin of *Rana pipiens*, 3-7 mm^2 (Verveen, 1963).

The glass rod stimulator was fixed to the center of a small loudspeaker cone, 7.6 cm in diameter. The stimulator position was sensed by an infrared position transducer, consisting of an infrared light emitting diode fixed to the glass rod and an infrared phototransducer held stationary approximately 1 mm away, with the light shining directly from one to the other. This setup gave good linearity with position and signal-to-noise ratio for the movements required in these experiments. Transducer calibration was performed by direct observation of glass rod movement using a microscope graticule at a series of output voltages. The position transducer was used to complete a servo loop control of loudspeaker position with second order compensation. The loudspeaker position signal was viewed on

Figure 2. The experimental arrangement employed for stimulation and recording of dorsal cutaneous mechanoreceptors. A sine-wave or pseudo-random noise generator was used to drive a speaker cone with an attached glass rod stimulator. The stimulator position was monitored by a position transducer consisting of an infrared light emitting diode and infrared phototransducer mounted to detect speaker movement. Action potentials were recorded from a dorsal nerve by a hook electrode and fed simultaneously into a PDP-11 computer with the stimulator position.


PDP-11 Computer
Action Potentials
Position
Amplifier
Schmitt Trigger
Nerve
Dorsal Skin
Pusher
Thermometer
Position Transducer
Sine Wave or Noise Generator
Servo Amplifier

a Tektronix 5110 oscilloscope and fed to a PDP-11 digital computer via a 12-bit analog to digital converter.

The electrical recording from the dorsal nerve was amplified with an AC amplifier (GRASS P15D) and fed into the oscilloscope for visualization. The signal was then used to supply an adjustable audio amplifier and a Schmitt trigger circuit to detect action potentials. Single unit recordings could be reliably discriminated on the basis of action potential amplitude. Electrical pulses from the Schmitt trigger were fed into a PDP-11 computer and to an electronic counter which gave a visual display of the rate of action potentials at 10 second intervals. For deterministic inputs the data was analysed on-line. For random inputs the action potentials and the position signal were stored on magnetic discs in digital format, to be processed later.

The command signal to the servo amplifier was one of the following:

1. Sinusoidal stimulation or a repeated step deformation from a Hewlett Packard 3310A function generator.
2. Pseudo random noise generated by a 33 bit binary shift register clocked at 1000 Hz and subsequently fed through a 9 pole active low pass filter to produce a band limited signal of 0-125 Hz. This band limited signal was then passed through a two pole passive low pass filter (30 Hz) to increase the low frequency spectrum of the stimulus.
3. Any linear sum of (1) and (2).

To reduce contamination of the driving signal by external vibrations, the stretcher, loudspeaker pusher, position transducer, AC amplifier and recording electrode were all mounted on an air driven vibration isolation table (Micro-g, Technical Manufacturing Corporation).

C. Data Processing

The responses to cyclic stimuli were processed using the program PULSE (French, 1970). This program constructs histograms of impulse trains by counting the number of impulses arriving in fixed time intervals, or bins. In these experiments the trigger signal from the function generator was used to initiate each sweep through the histogram and pulses produced by action potentials were counted. Normally histograms of 100 bins were used with the bin width adjusted to give a total histogram width corresponding to one cycle of the stimulus. Histograms from sinusoidal stimuli were fitted to a sinusoid with a minimum mean square error procedure. Histograms to step stimuli were similarly fitted with a fractional power-law decay.

For random stimulation experiments the analog signal of the position transducer was sampled at regular intervals of 4 ms and the action potentials were recorded at their time of occurrence with a resolution of 100 μsecs. The action potential signals were band-limited by digital convolution with a Sinc function (French & Holden, 1971a) to give a regularly spaced time series. The two time series (position

and action potentials) were then processed in the frequency domain after conversion by the fast Fourier transform (French, 1973). The data was processed in segments of 512 samples of each signal and more than 200 such segments were used to compute average spectra. Frequency response functions to band limited white noise stimulation were plotted as Bode diagrams of phase lead and log gain versus log frequency. Log gain versus log frequency results were linearly fitted to a fractional power-law by a minimum square error procedure. Most data processing was carried out by programs written in the high level language MOTLEY.

The use of random mechanical stimulation allowed the response to each frequency component to be determined by spectral analysis. The frequency response, $G(\omega)$, was obtained by the relationships:

$$G(\omega) = Sxy(\omega)/Sxx(\omega) \qquad (1)$$

and the coherence function, $\gamma^2(\omega)$, from:

$$\gamma^2(\omega) = |Sxy(\omega)|^2/Sxx(\omega)\,Syy(\omega) \qquad (2)$$

where $Sxx(\omega)$ and $Syy(\omega)$ are the power spectral densities of the input, $x(t)$, and output, $y(t)$, while $Sxy(\omega)$ is the forward cross-spectral density, respectively.

D. Temperature Measurement

Experiments were conducted at three different temperatures: 15°C, 20°C, 25°C. The temperature of the skin was monitored by a fine thermocouple connected to an electronic thermometer (Bailey Instruments BAT-4) and placed in contact with the skin and within the Ringers solution. The thermocouple was made from 40 gauge Copper/Constantan and was electrically isolated from the skin by a thin coating of varnish. To lower the temperature, a KOLDWAVE air conditioner was used to cool the skin and surrounding area to the desired temperature. To raise the temperature, an infrared incandescent bulb was held at a suitable distance from the preparation. Each procedure held the temperature constant (±1°C) for long periods.

E. The Manipulation of Extracellular K^+ Concentration

Experiments on the effects of potassium concentration were performed with the same protocol as room temperature experiments but the normal solution was replaced by a high potassium solution after the preparation had stabilized in the normal solution. The high potassium solution consisted of: (mM) NaCl 112.5, KCl 5.0, $CaCl_2$ 1.0, $MgSO_4$ 1.0, $NaHCO_3$ 2.5, NaH_2PO_4 0.3, Na_2HPO_4 0.7, $C_6H_{12}O_6$ 5.0, pH 7.2, and was replaced at regular intervals.

Membrane potentials were calculated using the Goldman constant-field equation:

$$Vm = RT \cdot \ln[[K^+]_o + q[Na^+]_o/[K^+]_i + q[Na^+]_i]/F \quad (3)$$

where q is the ratio of the permeability of Na^+ ions to K^+ ions (Stein, 1980) and can be calculated from equation (3). At high external potassium concentrations, the membrane potential approaches the equilibrium potential (E_k) for potassium, while at low external potassium concentrations, the membrane potential (Vm) approaches a constant, therefore, assuming $Vm=E_k$:

$$q = [K^+]_o[K^+]_i/[Na^+]_o[K^+]_i - [Na^+]_i[K^+]_o \quad (4)$$

When q is sufficiently large, equation (3) can be simplified to the Nernst equation for Na^+ ions and when q is sufficiently small it simplifies to the Nernst equation for K^+ ions.

III. Results

The responses of the receptors to mechanical displacement and stretching of the skin allowed them to be classified into three groups.

Group I. Under lightly stretched conditions small amplitude (<50 μV) action potentials would fire tonically or could be elicited by small amplitude (<12 μm) mechanical displacement of the skin. This group corresponded to the type *d* discharge patterns seen by Catton (1958).

Group II. Larger amplitude (50-250 μV) action potentials were seen when: i) higher amplitude (13-22 μm) mechanical displacements were given or, ii) the skin was stretched by approximately 10-20% of original diameter. These receptors responded to high frequency sinusoidal stimulation (>300/sec) for short periods of time (<30 sec). The high frequency response lasted longer under the stretched condition than with higher amplitude displacements. These receptors also responded for the duration of data gathering (up to 10 minutes) without a decrease in firing frequency. These receptors correspond to the type *b* and *c* discharge patterns of Catton (1958).

Group III. The type *a*, high amplitude action potential (>300 μV) discharge seen by Catton (1958) were only observed under large degrees of stretch (greater than 25% of

original diameter). These receptors responded to mechanical displacements but only fired regularly if the displacement amplitude (>25 μm) was regularly increased.

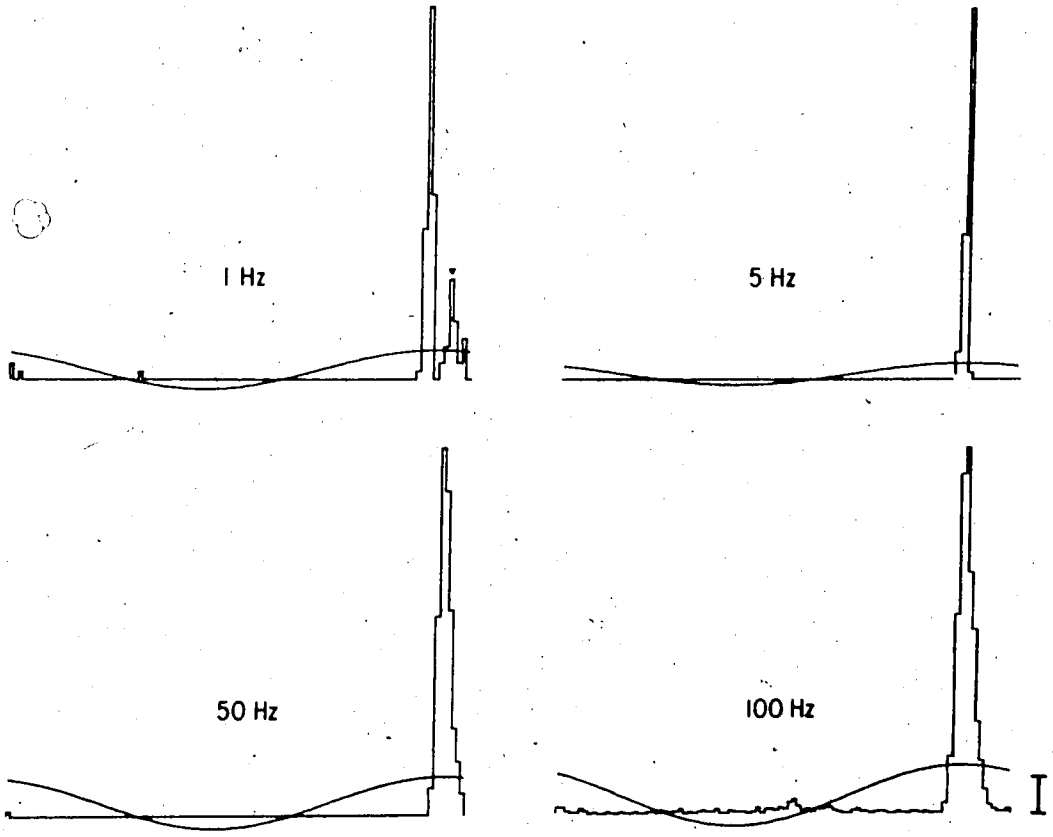
The three groups of receptors observed during these experiments also correspond to those observed by Loewenstein (1956).

The group II receptors were the only ones studied further, and each experiment used a single unit recording based on spike amplitude and firing frequency during data gathering. The group I receptors were not studied further because of their tonic firing properties and small amplitudes, while the group III receptors were not studied because with the data gathering techniques employed, mechanical displacement adjustment was not possible, so that most receptors studied in this group adapted before completion of data gathering.

A. Sinusoidal and Step Stimulation

Cycle histograms of the responses of tactile mechanoreceptors to sinusoidal driving frequencies at 1, 5, 50 and 100 Hz are shown in Figure 3. The receptors displayed phase-locking behavior over the entire frequency range and were most sensitive to high frequency stimulation. The response to low frequencies (0.1, 1 Hz) was often difficult to obtain. The receptors showed a strong tendency to fire once per cycle, except at low frequencies (0.1, 1 Hz) where

Figure 3. Cycle histograms of action potentials from dorsal cutaneous receptors showing their characteristic phase-locked response to sinusoidal driving functions at 1, 5, 50 and 100 Hz. Mean rates respectively were: 1.21, 3.95, 44.75 and 84.75 impulses/sec. Arrowhead indicates second spike which fired at a slightly different phase. Each histogram consists of 100 bins with 100 sweeps for 1 and 5 Hz, 1000 sweeps for 50 Hz, and 1500 sweeps for 100 Hz. The number of action potentials per bin indicated by the scale bar are 4.3, 5.2, 25 and 26 for 1, 5, 50 and 100 Hz, respectively. The time period of each histogram in this figure and figures 6 & 7 represents one cycle of the stimulating frequency. Each histogram was triggered at a phase of zero degrees (the peak of a cosine wave).


1 Hz
5 Hz
50 Hz
100 Hz

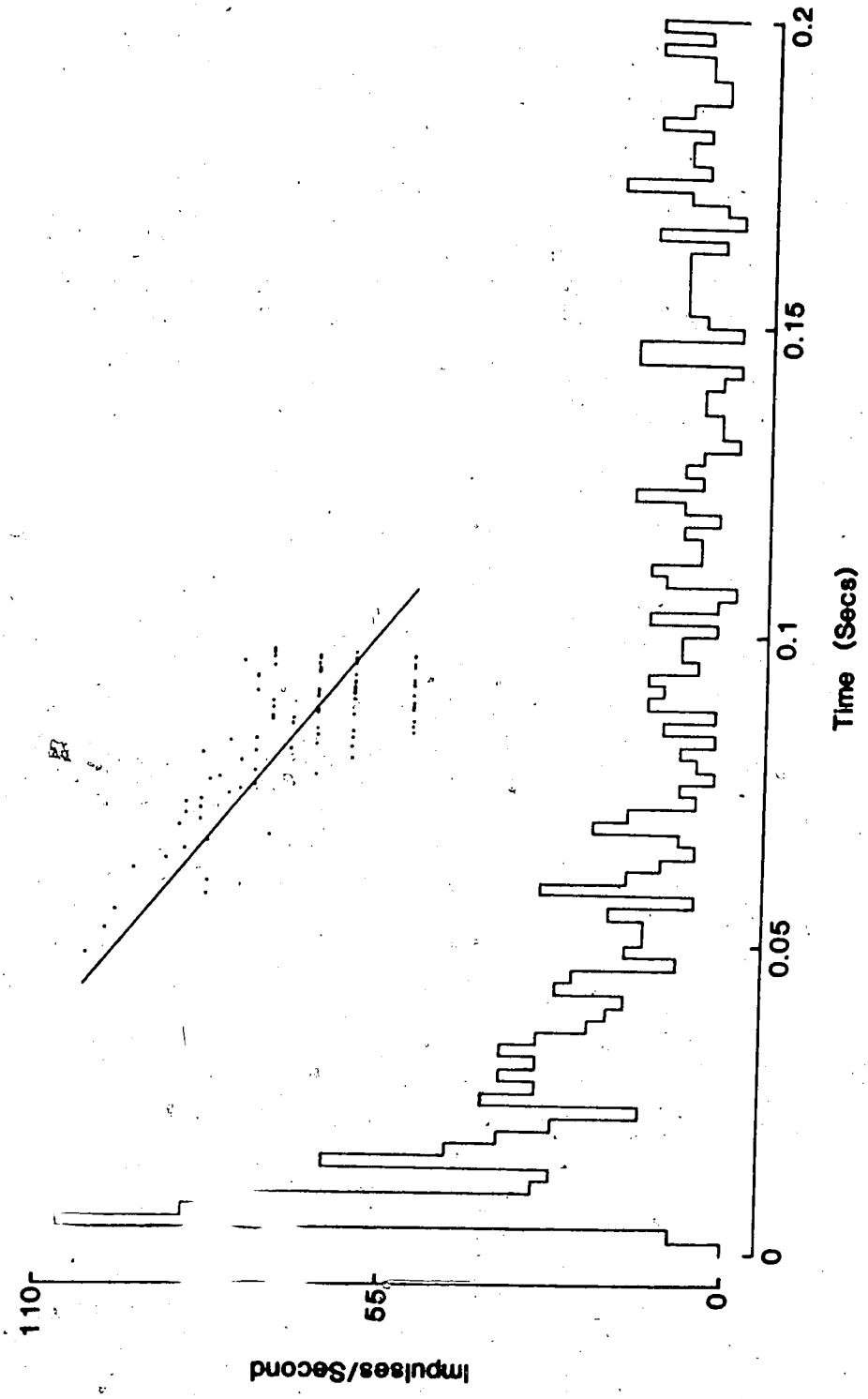
a second spike was usually seen. The mean rate of firing in spikes/second was slightly lower than would be predicted from a one-to-one entrainment due to adaptation of the receptors. They responded initially with a one-to-one entrainment to the stimulation but this decreased and the receptor eventually adapted completely to the mechanical stimulus. Phase-locking has been observed before in frog dorsal-cutaneous receptors, where the neural response was synchronized to the sinusoidal stimulus up to a frequency of 300 Hz (Keidel, 1968), as well as in other sensory neurons (Matthews & Stein, 1969; French et al., 1972).

The response of one tactile mechanoreceptor to a 1 Hz step function is shown in Figure 4. The histogram could not be fitted by a single exponential decay but a log-log plot of the histogram (Figure 4) was fitted with a straight line by a least mean square error procedure. The slope of the line was -0.80 with a linear correlation coefficient of 0.75. The histogram was therefore fitted by a power function decay of the form:

$$y(t) = At^{-k} \qquad (5)$$

where t is time, $y(t)$ is the rate of firing in spikes/second, A is the rate of firing after 1 second and k is a fractional exponent of time. This relationship agrees well with the data from the femoral tactile spine of the cockroach (Chapman & Smith, 1963; Holden, 1971; French et

Figure 4. Cycle histogram of a single mechanoreceptors' response to a step function with peak-to-peak amplitude of 40 μm.
Insert: log-log plot with best fitting linear regression line (first 2 points of histogram were omitted in the log-log plot).


Impulses/Second
110
55
0
0
0.05
0.1
0.15
0.2
Time (Secs)

al., 1972), where similar step functions were used.

A Bode plot of the logarithm of the average response amplitude versus logarithm of frequency was plotted for all responses to sinusoidal driving functions (Figure 5). The predicted dashed line (slope of k) was estimated from the step response of Figure 4, and fits the data points well over the entire frequency range. The phase constant predicted by equation (7) has been plotted along with the average phase responses to sinusoidal stimulation. The phase data was consistently below the predicted result and rolled off with higher frequencies until about 50 Hz where it remained constant. The phase data is also compared to the phase data of Figure 8 and matches well over most of the frequency range.

When a band-limited white noise auxiliary signal was added to a sinusoidal driving function, the phase-locking response of the receptor was broken-up and the histogram appeared sinusoidal, as is illustrated in Figure 6. This auxiliary signal, which was not correlated with the driving function, acted to generate a random carrier rate of spike discharge. This technique has been used before to obtain a smooth response to deterministic signals (Spekreijse & Oosting, 1970; French et al., 1972). A sinusoidal driving function with added band-limited white noise modulated the frog tactile receptors studied in three characteristic ways depending on the mode of stimulation.

1. When the stimulator was positioned so it was initially

Figure 5. Bode plot of the logarithm of response amplitude versus logarithm of frequency to sinusoidal driving functions. The dashed lines are the predicted responses from Figure 4 with its corresponding phase value. The filled circles are data from sinusoidal driving functions and the filled squares are the phase values obtained from Figure 8 for comparison.

Vertical lines: standard deviations around the means.

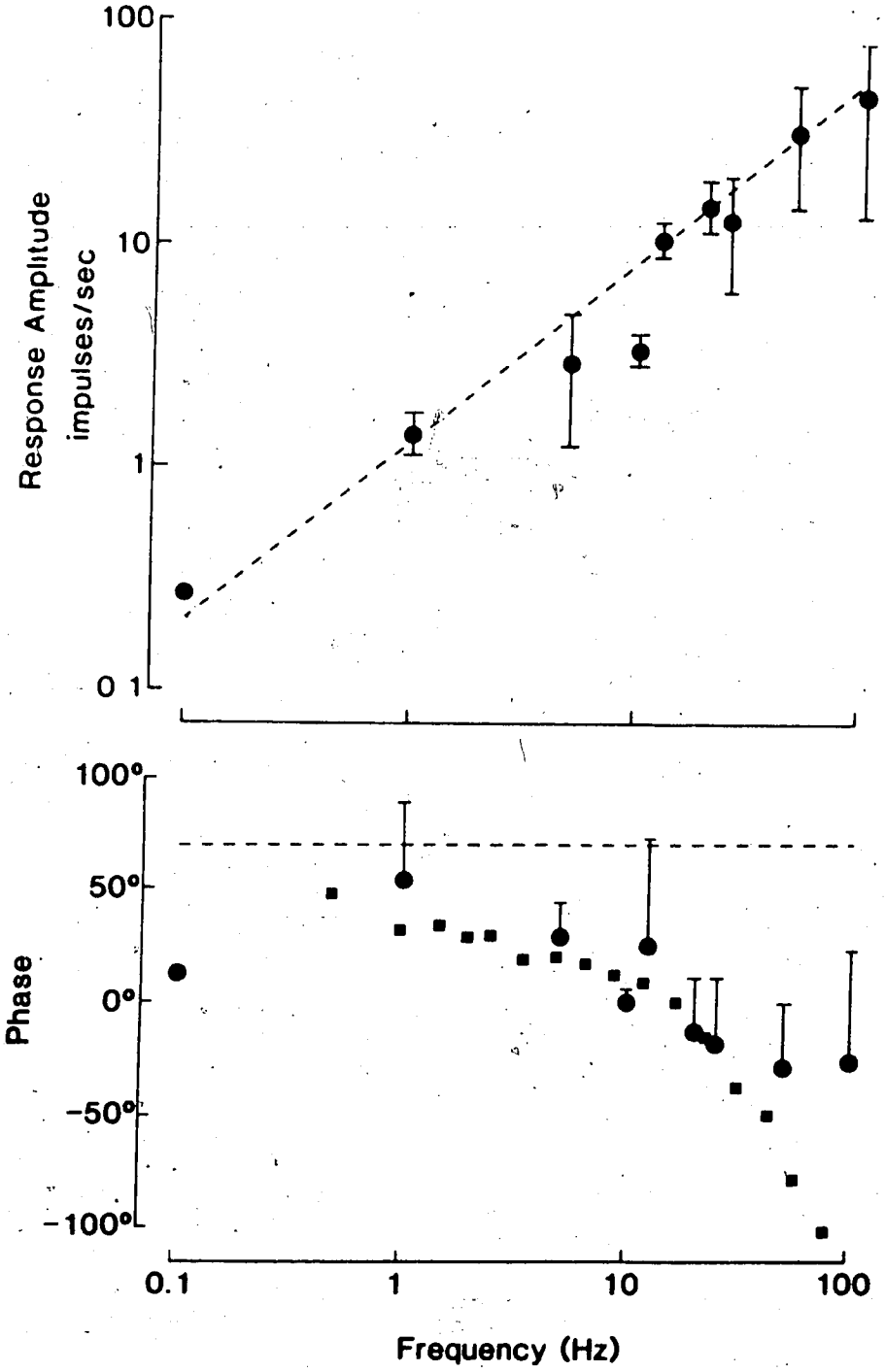
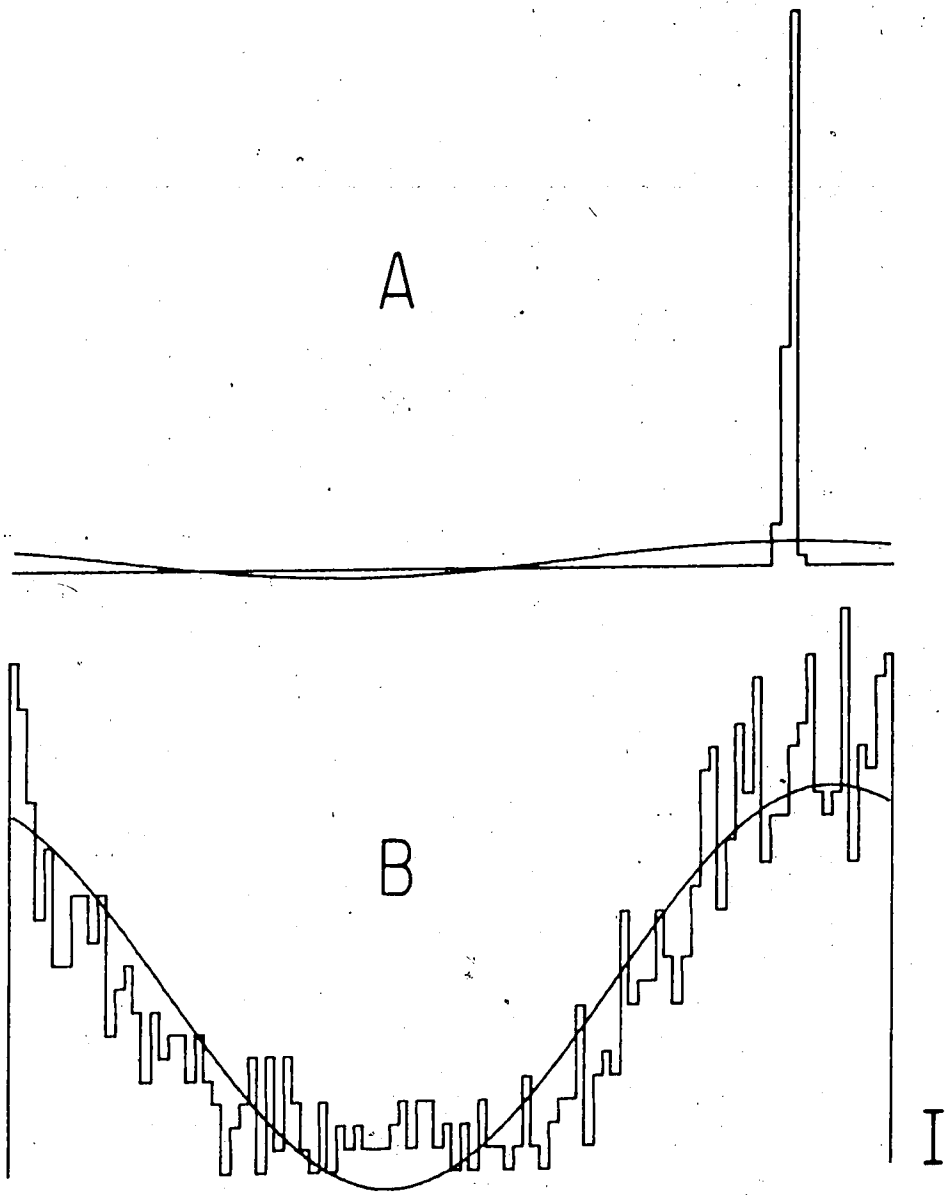

100
10
1
0 1
Response Amplitude
impulses/sec
100°
50°
0°
-50°
-100°
Phase
0.1
1
10
100
Frequency (Hz)

Figure 6. Characteristic responses of receptors to a 5 Hz sinusoidal driving function in A and band-limited white noise (0-100 Hz) added to a 5 Hz sinusoidal driving function in B. Each histogram consisted of 100 bins. A. A total of 100 sweeps were taken with a mean rate of 3.95 impulses/sec. B. A total of 1000 sweeps were taken with a mean rate of 3.90 impulses/sec. Number of action potentials per bin indicated by the scale bar are 5.2 and 2.4 for A and B respectively.


A
B
I

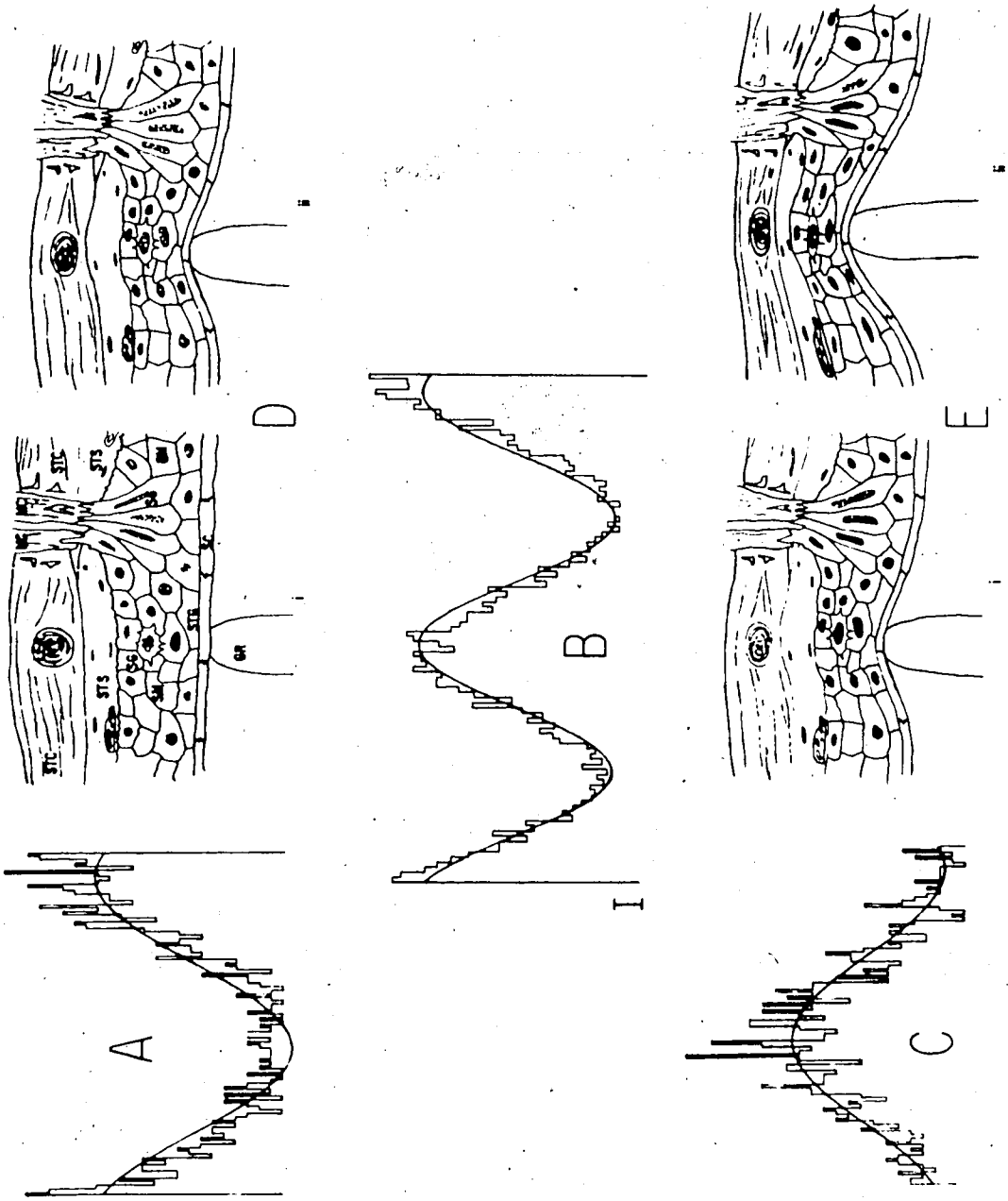
just touching the skin, the response was sinusoidal with the receptor responding most to the peak of stimulator movement (inward movement) (Figure 7A).

2. As the stimulator was pushed further into the skin, a second harmonic response was obtained where the receptor responded to both the peak and the trough of stimulator movement (Figure 7B).
3. When moved past this position the response was again sinusoidal but was now 180° out of phase to the stimulator movement, (Figure 7C).

A suggested model of epithelial and receptor movement is also represented in Figure 7. Merkel cells, which have been morphologically identified in the epidermis of *Rana pipiens* (Nafstad & Baker, 1973; Whitear, 1974), and lamellated receptors in the dermis of *Rana esculenta* (During & Seiler, 1974) are represented in the drawings. Both receptor types are considered rapidly adapting and could be responsible for the activity observed in these studies.

Figure 7D(i) illustrates most of the cell types in the dorsal epithelium under resting conditions and shows the starting position of the glass rod stimulator, while Figure 7D(ii) shows the stimulator at the peak of its inward sinusoidal movement and the point where the receptor was maximally stimulated. Figure 7E(i) is the same diagram as 7D(ii) but now represents the initial stimulator position while Figure 7E(ii) shows the greatest inward sinusoidal movement of the stimulator. For a second harmonic response

Figure 7. Characteristic responses to three different modes of stimulation with a 5 Hz sinusoidal displacement plus band-limited white noise. A. Response to inward movement of stimulator. B. Response to both inward and outward movement of stimulator. C. Response to outward movement of stimulator. Sections D and E demonstrate the probable situation in the epithelium at the extremes of stimulator movement (detailed description in text p. 51). Note that the stimulus used in 7B was identical to that of 7A and 7C so that the response was twice the stimulating frequency. GR glass rod stimulator, LER lamellated epithelial receptor, M Merkel cell, MC muscle cells, N nerves, SC stratum corneum or keratinised layer, SG stratum germinativum or basal cell layer, SM stratum mucosum, STC stratum compactum, STG stratum granulosum, STS stratum spongiosum. The number of sweeps were: 1000, 10000 and 700, rates of firing were: 3.95, 3.65 and 5.45 impulses/sec, and the scale bar indicates: 2.4, 15.8 and 2.2 action potentials per bin in A, B and C, respectively.


A
B
C
D
E

to occur (Figure 7B) the stimulator position would be intermediate to the two extremes of the sinusoidal responses.

B. Random Stimulation

Frequency response functions for transduction could generally be well fitted by the relationship:

$$G(\omega) = g\omega^k \qquad (6)$$

and

$$P(\omega) = k\pi/2 \qquad (7)$$

where $G(\omega)$ is the gain and $P(\omega)$ is the phase of the output relative to the input as a function of radial frequency ω, g represents the gain at ω=1 radian/sec, and k is the exponent of frequency. Random mechanical stimulation was used because it is much more efficient than sinusoidal stimulation and it significantly reduces the effects of several nonlinear processes, such as phase-locking and rectification, which occurred with sinusoidal stimulation at frequencies as low as 0.05 Hz. Along with every frequency response function, the coherence function, $\gamma^2(\omega)$, was also measured. The coherence function measures the degree of linearity of the input-output relationship (Bendat & Piersol, 1968; French & Holden, 1971b; Stein et al., 1972). A perfectly linear and noise free system has a coherence of one, while values less than one can be due to one or more of three possible

situations:

1. An intrinsic noise source is present in the system.
2. The system is nonlinear.
3. There are external inputs present not related to the driving function.

Figure 8 shows a typical frequency response function for mechanical stimulation up to 100 Hz. The gain data is the best fitting linear relationship using the minimum mean square error criterion and corresponds to a value of k=0.268. The phase advance predicted by equation (7) was 24.133° at all frequencies and is plotted as a solid line that fits the data well at frequencies below 10 Hz. However, the experimental phase lagged progressively more behind the predicted phase at frequencies above 10 Hz. This probably arose from the delay due to action potential conduction between the receptor and the recording electrodes. The coherence measurement was significantly lower than one, but was similar to values obtained in other receptor studies (French & Wong, 1976; French & Kuster, 1981; Kuster & French, 1983). Figure 9 is a linear frequency plot of the difference between the experimental phase data and the predicted phase constant of Figure 8 and is well fitted by a straight line which corresponds to a pure time delay of 5.066 ms. This pure time delay does not affect the gain of a linear system but causes a phase lag which is directly proportional to the length of the delay and to the frequency:

Figure 8. A typical frequency response function for sensory transduction by a frog dorsal cutaneous mechanoreceptor. The stimulus was pseudo-random noise with a bandwidth from 0-125 Hz. The best fitting linear relationship gives k=0.268 and g=0.111 impulses/sec·μm. The maximum value of the coherence function was 0.225 at 8.79 Hz.

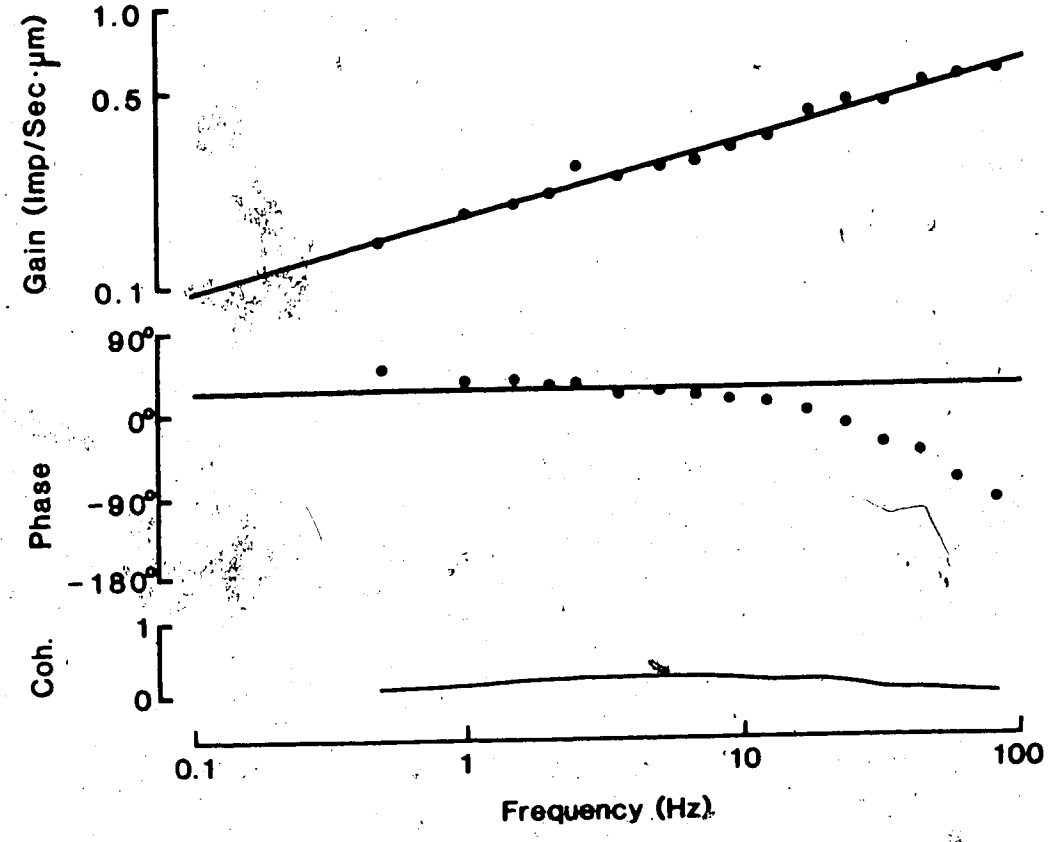
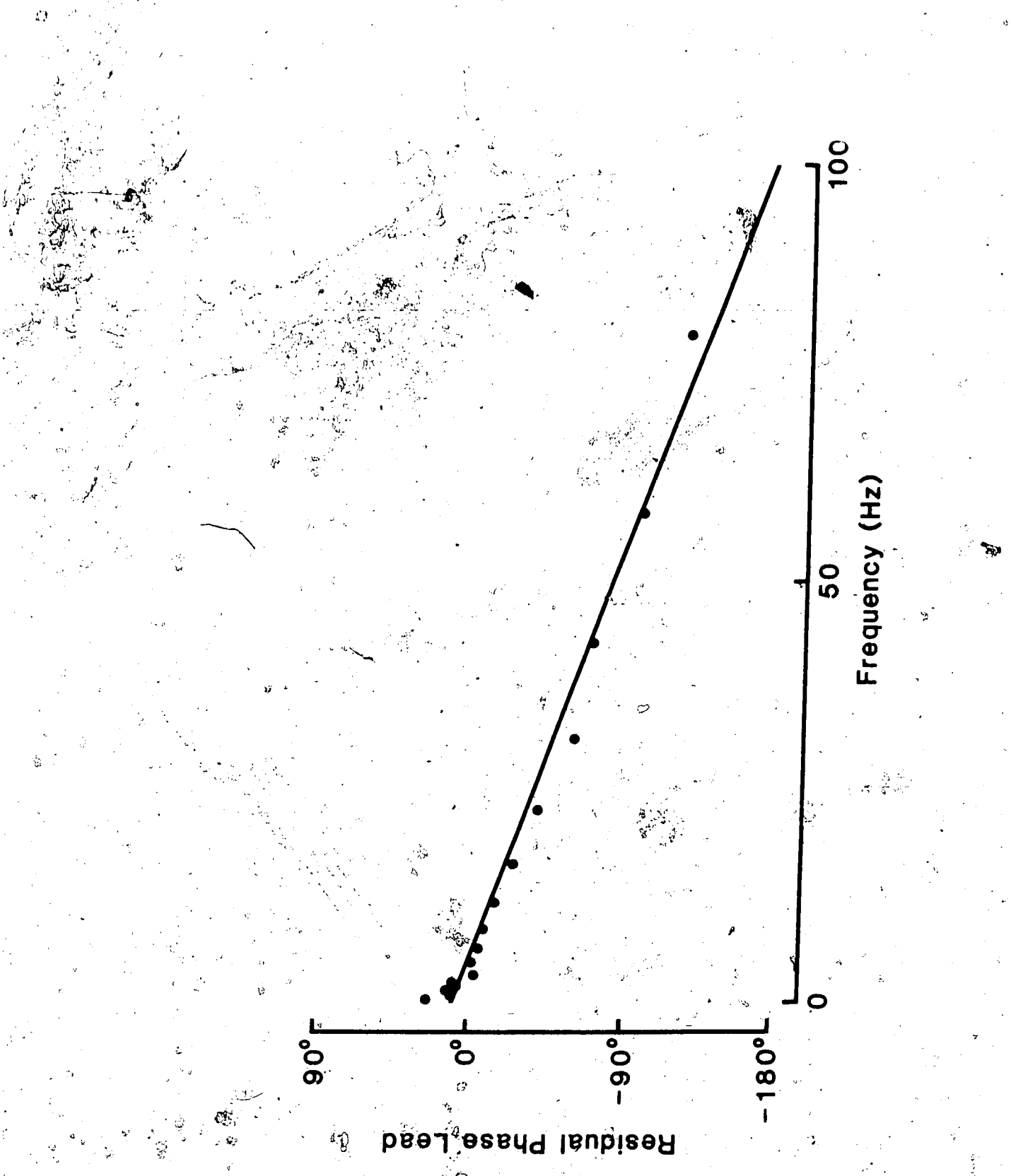

Gain (Imp/Sec·µm)
1.0
0.5
0.1
Phase
90°
0°
-90°
-180°
Coh.
1
0
0.1
1
10
100
Frequency (Hz)

Figure 9. The difference between the experimental phase data of Figure 8 and the predicted phase values of Figure 8 have been replotted on a linear frequency axis. The solid line corresponds to a pure time delay of 5.066 ms and is the minimum mean square error linear relationship.


Residual Phase Lead
90°
0°
-90°
-180°
0
50
100
Frequency (Hz)

$$P(\omega) = -\omega\Delta t \qquad (8)$$

The distance between the sending and the recording electrode was estimated to be 8.56 mm for this preparation giving a conduction velocity of 1.69 m/s.

Figure 10 demonstrates the large variabilities in gain (sensitivity) and exponent (dynamic behavior) observed in frog skin mechanoreceptors (n=128 single unit recordings) at room temperature, demonstrating the mean, upper and lower extremes. All experiments except DEC12M showed phase lags similar to Figure 8, with a conduction velocity of 2.84±1.01 m/s (mean±standard deviation). The large variabilities seen do not appear to arise from several different populations of receptors because both the gain and exponent populations showed normal distributions about the mean (Figure 11).

C. Temperature Experiments

Figure 12 shows the gain, g, at ω=1 radian/sec and the exponent of frequency, k, as functions of temperature. The parameter, g, decreased slightly between 15°C and 20°C where it remained constant up to 25°C and represented the normal sensitivity of the receptor. These results are in marked contrast to those seen in other mechanoreceptors for the same temperature range (Ishiko & Loewenstein, 1961; French & Kuster, 1982). The exponent, k, was also unaffected by the change in temperature as has been observed in the cockroach tactile spine (French & Kuster, 1982). The firing rate of

Figure 10. Three frequency response functions for mechanotransduction in frog dorsal cutaneous receptors showing the variability in gain, g (Top graph) and exponent of j-omega, k (Bottom graph) observed. Top. The circles represent the mean gain while the triangles and squares represent the extreme high and low gains, respectively.

• - MAR21B 84, g=0.111 impulses/sec·μm., k=0.268, delay=5.066 ms, conduction velocity=1.69 m/s.

▲ - NOV7B 83, g=0.539 impulses/sec·μm., k=0.227, delay=3.764 ms, conduction velocity=2.25 m/s.

■ - JUN21B 83, g=0.041 impulses/sec·μm., k=0.458, delay=2.592 ms, conduction velocity=2.46 m/s.

Bottom. The filled circles represent the mean exponent recorded while the filled triangles and squares represent the extreme low and high exponents respectively.

• - MAY8F 84, k=0.274, g=0.194 impulses/sec·μm, delay=1.541 ms, conduction velocity=3.01 m/s.

▲ - DEC12M 83, k=0.083, g=0.369 impulses/sec·μm.

■ - DEC7L 83, k=0.586, g=0.041 impulses/sec·μm, delay=2.936 ms, conduction velocity=2.94 m/s.

The difference between the experimental phase and the predicted phase values were computed, except for DEC12M 83, and showed similar time delays for the three receptors. Solid lines are the best fitting linear relationships using a minimum mean square error criterion.

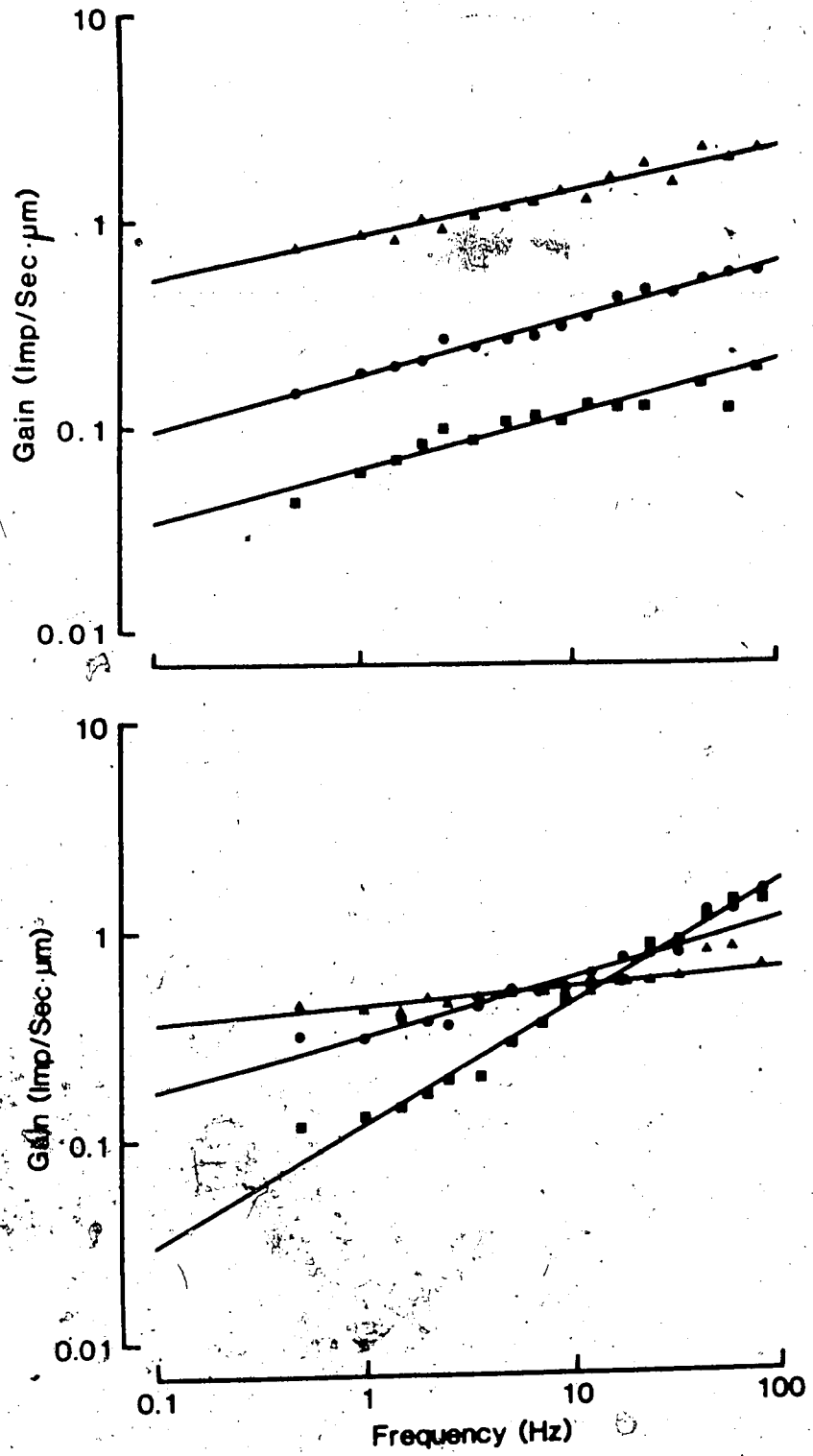
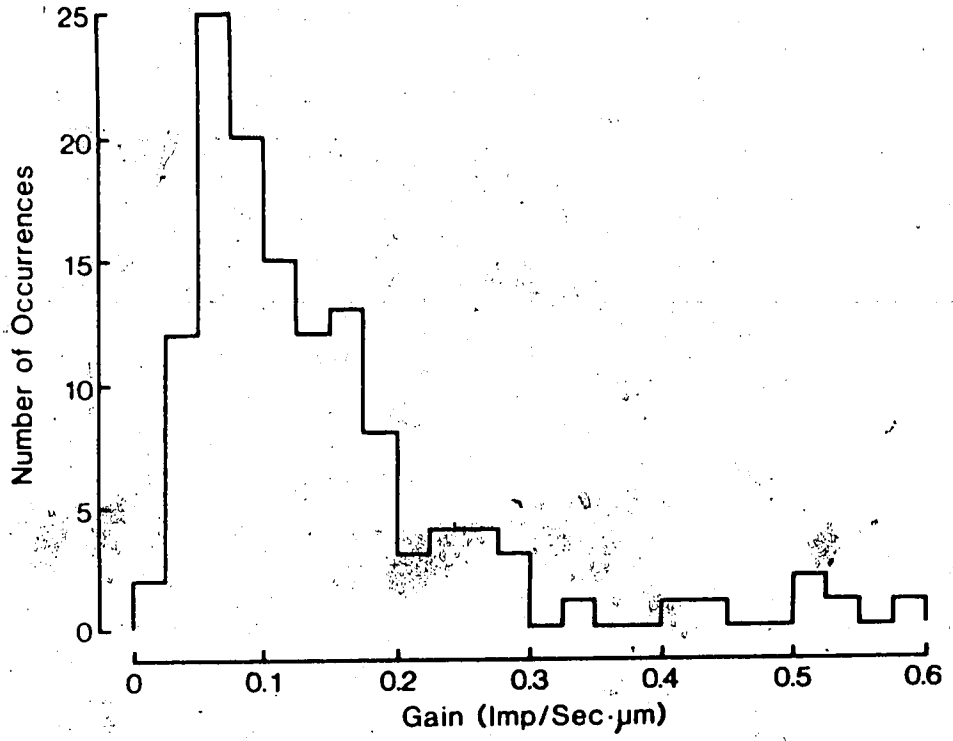

10
1
0.1
0.01
Gain (Imp/Sec·μm)
10
1
0.1
0.01
Gain (Imp/Sec·μm)
0.1
1
10
100
Frequency (Hz)

Figure 11. Histograms of the number of occurrences vs. gain, g (A) and exponent of j-omega, k (B).

A. Mean R.M.S. value of g=0.137±0.104, n=128.

B. Mean R.M.S. value of k=0.270±0.144, n=128.


25
20
15
10
5
0
Number of Occurrences
0
0.1
0.2
0.3
0.4
0.5
0.6
Gain (Imp/Sec·μm)

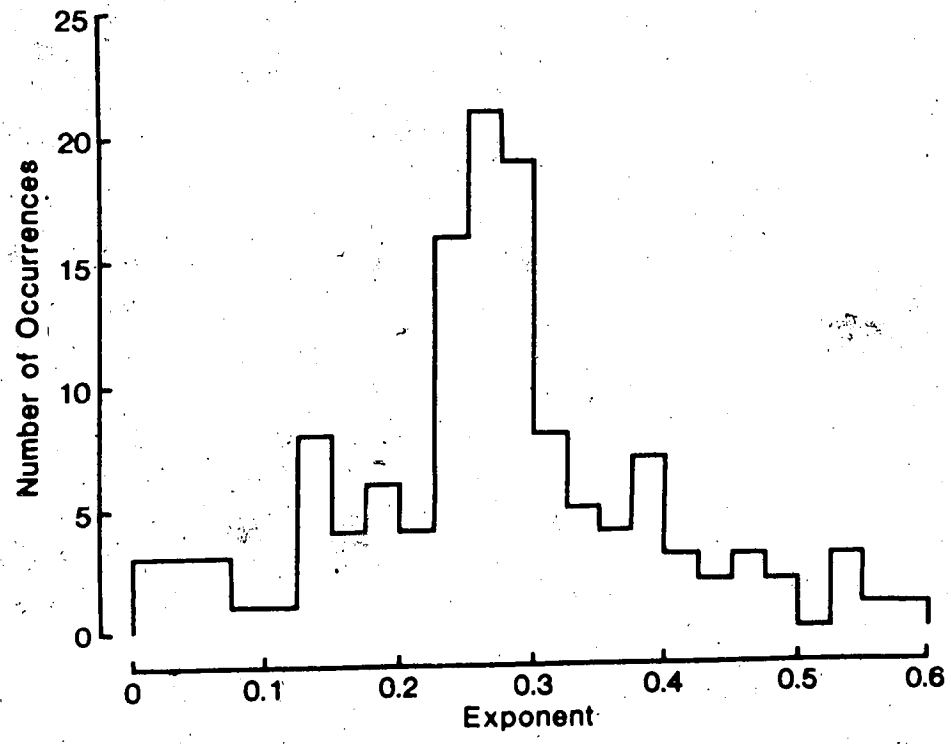
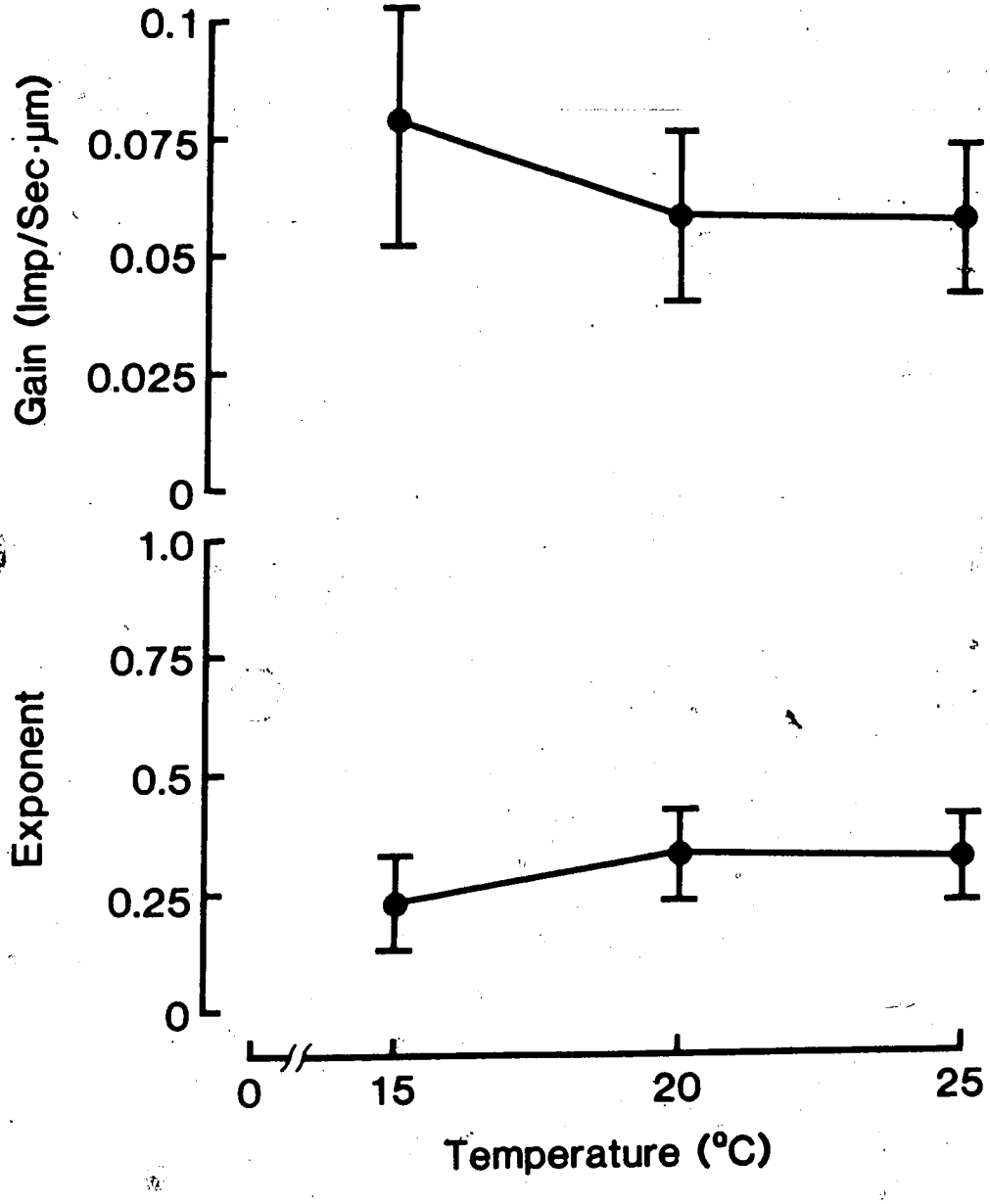

25
20
15
10
5
0
Number of Occurrences
0
0.1
0.2
0.3
0.4
0.5
0.6
Exponent

Figure 12. Mean values for the gain, g, at 1 radian/sec and the exponent of frequency, k, as functions of temperature. Vertical lines: standard deviations around the means. Mean R.M.S. displacement values in micrometers were: 15°C-19.52, 20°C-20.05, 25°C-19.79.


0.1
0.075
0.05
0.025
0
Gain (Imp/Sec·µm)
1.0
0.75
0.5
0.25
0
Exponent
0
15
20
25
Temperature (°C)

the receptor increased slightly with temperature from 1.88 imp/sec at 15°C to 2.33 imp/sec at 25°C (means), but these two values were not significantly different (Student's t-test, 95% confidence level, Figure 13).

D. High Potassium Experiments

Figure 14 shows three experiments in which the concentration of potassium ions in the extracellular fluid was increased above the normal. The results are presented in relative units of gain, g, and exponent, k, versus potassium concentration so that any change in the two parameters can be clearly seen. Increasing the extracellular potassium concentration by a factor of 2 would be expected to cause a depolarization of approximately 10 mV in the membrane potential of the cells. Not all receptors changed their frequency response function upon application of 5.0 mM potassium, but when a change did occur, it was complete within the first five minutes after application. The change lasted for as long as the 5.0 mM potassium was maintained. On several occasions, the preparation was returned to a normal Ringers solution and a gradual recovery to the original frequency response function occurred, taking place over a period of 4 to 6 hours.

A total of nine single unit recordings in high K^+ solution were performed. (i) four showed no change in either gain or exponent, (ii) three showed an increase in gain with a decrease in exponent and (iii) two showed a decrease in

Figure 13. Mean firing rate of the receptors at the three temperatures studied.

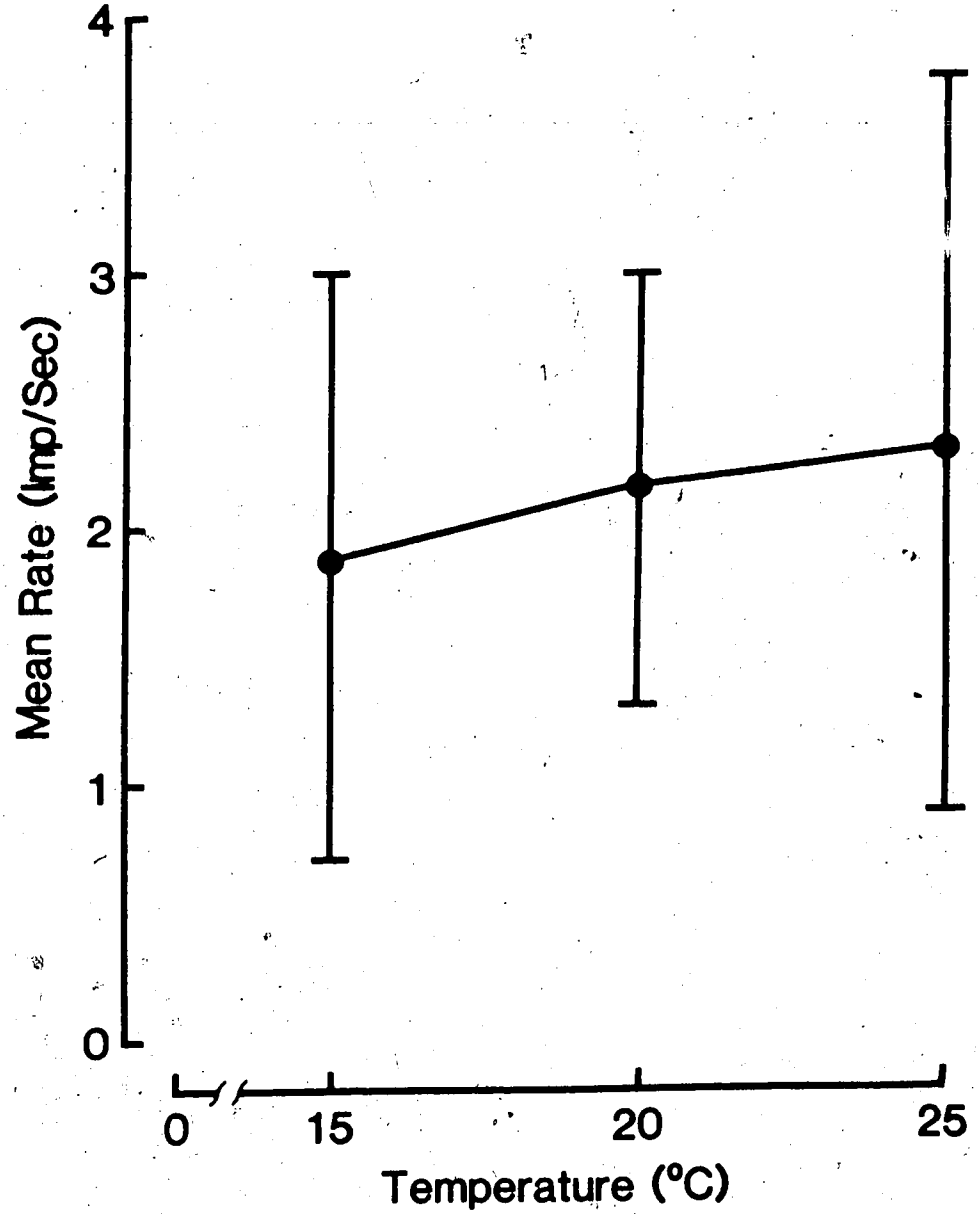
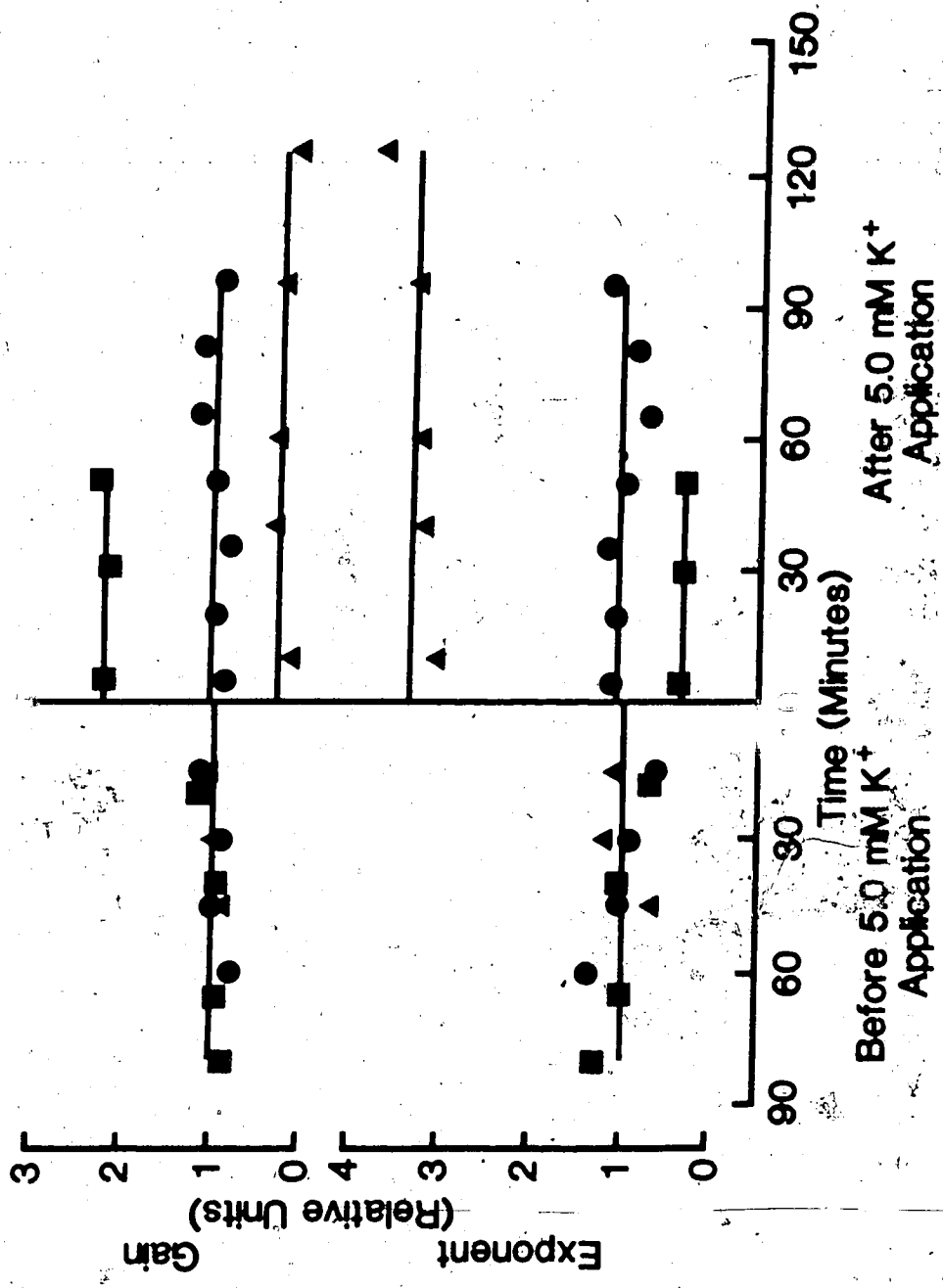

Mean Rate (Imp/Sec)
4
3
2
1
0
0
15
20
25
Temperature (°C)

Figure 14. Three different single unit recordings demonstrating frequency response functions observed in frog cutaneous mechanoreceptor(s) to an increase in extracellular potassium. Gain, g, and exponent, k, values are in relative units (ordinate) with a value of one in normal Ringers solution. The abscissa shows time relative to the replacement of the normal Ringer solution by a high potassium Ringer solution. Circles denote a receptor which was not different in the high potassium ringer compared to the normal ringer, and the triangles and squares represent two receptors which were different.

● - NOV30 83, g(before)=0.153 impulses/sec·μm, g(after)=0.165 impulses/sec·μm, k(before)=0.198, k(after)=0.209.

▲ - NOV4 83, g(before)=0.164 impulses/sec·μm, g(after)=0.055 impulses/sec·μm, k(before)=0.105, k(after)=0.353.

■ - DEC12 83, g(before)=0.159 impulses/sec·μm, g(after)=0.363 impulses/sec·μm, k(before)=0.232, k(after)=0.086.


Gain
Exponent
(Relative Units)
3
2
1
0
4
3
2
1
0
90
60
30
Before 5.0 mM K+ Application
Time (Minutes)
30
60
90
120
150
After 5.0 mM K+ Application

gain with an increase in exponent. The mean gain and mean exponent of (ii) and (iii) (above) were statistically different to the mean gain and exponent of (i) (above) after application of the 5.0 mM potassium (Student's t-test, 95% confidence level).

Preliminary studies were performed using a four fold increase in extracellular potassium concentration (10 mM), however, the receptors stopped firing altogether within 15 minutes of application and would not resume fi even with additional stretch applied to the skin. A four increase was expected to cause a membrane depolarization of approximately 23 mV.

IV. Discussion

A. Types of Receptive Afferent Units

The major set of receptors studied in these experiments (Group II) were considered rapidly adapting because their response to a step displacement of an unstretched skin preparation was a single, or at most, few action potentials. The receptors were considered to be similar to Loewenstein's (1956) tactile fibers in *Rana pipiens*, to Catton's (1958) type *b* fibers in *Rana temporaria*, and to Ogawa, Morimoto and Yamashita's (1981) rapidly adapting type I units in the plantar foot of the frog, *Rana catesbeiana*. Catton's (1958) interpretation of Loewenstein's (1956) tactile fibers as a type *a* fiber differs slightly from these findings since Loewenstein's (1956) tactile fibers were considered to be type *b* fibers. This discrepency in classification may be due to the stimulus parameters used relative to Loewenstein's (1956) experiments, where stretch was used as the main parameter for classification. In these experiments, stretch and mechanical displacement were the two parameters used to classify receptors and still larger (type *a* of Catton, 1958) action potentials were encountered with greater degrees of stretch. The rapidly adapting properties of the receptors were altered when lateral stretch was applied to the skin (Loewenstein, 1956) so that they adapted with a slower time course. During actual stretch of the skin, the receptors

fired with an initial rapid burst that always adapted completely within a couple of minutes. This again disagrees with the findings of Loewenstein (1956) who observed tactile receptors firing tonically for over 25 minutes after a 16% stretch of the skin. Loewenstein (1956) did not indicate the sizes of the action potentials recorded in his experiments, making comparisons between the different receptor categories extremely difficult.

The Group I receptors were slowly adapting and corresponded to Catton's (1958) type *d* fibers and Fessard and Segers (1943b) slowly conducting pain receptors. The Group II action potentials were higher amplitude, more rapidly adapting than type *a* receptors of Catton (1958), and corresponded to Catton's (1958) type *b* and *c* fibers and to Ogawa, Morimoto and Yamashita's (1981) rapidly adapting type II receptors which adapted completely after the first cycle of a sine wave.

Several types of receptors have been identified morphologically in amphibian cutaneous tissue by light and electron microscopy: Merkel cells (Nafstad & Baker, 1973; Fox & Whitear, 1978), lamellated receptors (During & Seiler, 1974), sensory nerve endings (Roberts & Blight, 1975), and 'free' nerve endings (Roberts & Hayes, 1977; Roberts, 1980; Hayes & Roberts, 1983). There are two types of 'free' nerve endings innervating the head skin of *Xenopus laevis*, *Rana temporaria*, and *Triturus helveticus*: 'movement detectors' which have a tonic discharge to movement of the skin and

'rapid transient detectors' which give a brief phasic discharge to rapid local indentations (Kitson & Roberts, 1983). The latter are sensory terminals of Rohon-Beard cells. Rohon-Beard cells have been located in embryonic frog (*Rana pipiens*) spinal cord and send nerve terminals to the periphery. In *Rana pipiens*, the Rohon-Beard cells are functionally replaced at later stages by the spinal ganglia (Bacher, 1973). The innervation pattern of Rohon-Beard neurites observed in *Xenopus laevis* is similar to that observed in adult frog skin (Whitear, 1974), so that the Group II receptors could possibly be the 'rapid transient detectors' observed by Roberts (1980).

Merkel cells have been identified by electron microscopy in the epithelium of several orders of Apoda, Urodela and Anura (Nafstad & Baker, 1973; Fox & Whitear, 1978). Merkel rete papillae and touch domes of mammals, which contain clusters of Merkel cells, have been shown to be slowly adapting mechanoreceptors (Iggo & Muir, 1969; Munger et al., 1979). Merkel cell granules have also been seen to fuse with the specialized area of membrane between the neurite and Merkel cell, an indication of synaptic transmission (Chen et al., 1973), and it is observations like these which have implicated Merkel cells in mechanosensory transduction. Parducz and co-workers (1977) provided evidence that Merkel cells in amphibians were rapidly adapting and were associated with a single mechanoreceptive nerve fiber. However, the correlation

between low threshold areas and Merkel cells were not perfect. If the Merkel cells are transducers of mechanical stimuli, the microvillar processes evident on Merkel cells could be involved in detecting deformations of the surrounding tissue. Other investigators (Munger, 1971; Gottschaldt & Vahle-Hinz, 1981) have postulated that the Merkel cells may be trophic in nature and that the nerve endings themselves may be the mechanoelectric transducer elements, and Fox and Whitear (1978) have postulated that the microvillar processes provide a route for diffusion of a secretory product into the surrounding epithelium. Roberts and Hayes' (1977) rapidly adapting 'free' nerve endings were observed at a stage when Merkel cells were not as yet differentiated and may indicate that Parducz and co-workers (1977) were stimulating 'free' nerve endings which were situated close to Merkel cells. Fox and Whitear (1978) have shown that nerve fibers associated with Merkel cells pass beyond the points of synapse and ramify freely in the epidermis.

Lamellated encapsulated receptors have also been observed in the frog, *Rana esculenta* by light and electron microscopy (During & Seiler, 1974) while three distinct types of lamellated endings have been observed in reptiles (During, 1973; During & Miller, 1979). Combined physiological and morphological studies have not been performed, but During and Seiler (1974) have suggested that the lamellated receptor is Loewenstein's (1956) rapidly

adapting receptor based on its structural similarity to mammalian Pacinian corpuscles. Loewenstein and Skalak (1966) have demonstrated that the Pacinian corpuscle is a mechanical filter of high-pass characteristics and Hubbard's (1958) experiments showed that only transient movements of the inner core occurred when the Pacinian corpuscle was compressed and released. When the outer lamellae were removed, the mechanical filtering was reduced and adaptation was slower in the receptor potential (Loewenstein & Mendelson, 1965; Ozeki & Sato, 1965). A decreased adaptation may occur when the skin is stretched experimentally because under normal physiological conditions the location of the receptor in the skin prevents stretching of the receptor (During & Seiler, 1974).

The conduction velocities obtained in these experiments was 2.84±1.01 m/s (mean±standard deviation, number of measurements=29), which corresponds to an unmyelinated nerve fiber of approximately 1.0-1.2 μm in diameter (Ritchie, 1982) or a myelinated nerve fiber of 1.0 μm in diameter (Hutchinson et al., 1970; Ritchie, 1982). During & Seiler (1974) have shown through serial sections that myelinated axons with a diameter of 8-12 μm innervate the lamellated receptors and would have a conduction velocity of 25 m/s or more (Hutchinson et al., 1970).

Neurites located next to Merkel cells in amphibians were variable in size, ranging from 1-4 μm in diameter (Fox & Whitear, 1978). If Merkel cells were indeed the

transducers of mechanical stimuli, a synaptic delay of approximately 1 ms would probably be present. The average conduction velocities in the nerves would then be 3.84±1.01 m/s (mean ±standard deviation, number of measurements=29). This higher conduction velocity would require a mean unmyelinated fiber diameter of approximately 4 μm (Ritchie, 1982), in the upper extremes of the range observed in close proximity to amphibian Merkel cells.

[redacted]ectron micrographs of 'free' nerve endings (Roberts & [redacted], 1977) showed diameters of 0.2-1.0 μm and Whitear (1974) showed unmyelinated nerve fibers in *Rana temporaria* with similar diameters. Thus, of the three types of possible sensory receptors, the 'free' nerve endings appear to be the receptor type which is most likely to be responsible for the results obtained here on the basis of their adaptation characteristics and their conduction velocities.

B. The Dynamic Behaviour of the Tactile Receptors

Random noise stimulation is a superior method of measuring dynamic behaviour to sinusoidal stimulation for several important reasons:

1. Estimating the frequency response function with random stimulation requires relatively short periods of experimental data whereas sinusoidal stimulation requires longer recording times and therefore, a greater opportunity for non-stationary behaviour.
2. Random noise reduces or prevents non-linearities such as

phase-locking (Spekreijse & Oosting, 1970).

3. The estimate of the coherence function, which is not possible when using sinusoidal stimulation, provides a measurement of how well the frequency response function describes the input-output relations of the receptor studied. The coherence function can only be obtained from estimates of the input, output and cross-spectra of the system (equation (2)).

The dynamic properties of the frog tactile receptors studied here could be well represented by the power law of equation (6). However, there was considerable variability in their sensitivities, g, and exponents, k. Frequency response functions determined by random noise or sinusoidal stimulation have not been described before for frog cutaneous receptors. However, step responses which demonstrate fractional exponents of frequency have been reported for slowly adapting receptors in the frog (Keidel, 1968; Ogawa & Yamashita, 1982). Sinusoidal stimuli showed phase-locking responses (Keidel, 1968; Ogawa et al., 1981), and a secondary harmonic was also observed with an increase in stimulus intensity (Keidel, 1968), similar to those observed in these experiments.

The receptors studied here showed similar fractional exponent values to those observed with the tactile spine of the cockroach (French, Holden & Stein, 1972; French & Kuster, 1981), locust multipolar receptors (Kuster & French, 1983), campaniform sensilla (Chapman et al., 1979), and slit

sensilla of the spider (Bohnenberger, 1981). The sensitivity of the frog cutaneous receptors is less than the cockroach tactile spine by a factor of 10 (French & Kuster, 1981) and to that of the locust multipolar receptors by a factor of 3 (Kuster & French, 1983). Step and frequency response functions which demonstrate fractional exponents of frequency have been reported in a wide range of other receptors including the cercal hair sensilla of the cockroach (Buno, Monti-Bloch and Crispino, 1981; Buno, Monti-Bloch, Mateos and Handler, 1981), crustacean stretch receptors (Brown & Stein, 1966), primate touch receptors (Mountcastle et al., 1972; Mei et al., 1983), Pacinian corpuscles (Bolanowski & Zwislocki, 1984a, 1984b), and vestibular afferents in mammals (Tomko et al., 1981) and birds (Landolt & Correia, 1980).

The process where a mechanical stimulus is transduced into a train of action potentials in a mechanosensory organ (mechanotransduction) can be considered to occur in a stepwise fashion (Eyzaguirre & Kuffler, 1955; Loewenstein, 1959):

1. an external mechanical stimulus is coupled to an internal mechanical displacement or force;
2. the displacement or force produces a graded generator potential (transduction); and
3. the generator potential causes a train of action potentials (encoding).

Adaptation has been observed in receptors during the

encoding stage (Mendelson & Loewenstein, 1964; Nakajima & Onodera, 1969a), and recent studies on the cockroach tactile spine (French, 1984a, 1984b), have demonstrated that fractional differentiation may be in fact a function of the nonlinear encoding process rather than the process of transduction as was earlier assumed (French & Sanders, 1981).

The experiments conducted here included all three stages of transduction and therefore could not add any evidence to this debate. However, the most likely type of receptor involved was the 'free nerve ending' which lacks any elaborate transduction structure. This makes it unlikely that the dynamic behaviour arose from complex mechanical structures and would agree with the action potential encoder being responsible for rapid adaptation.

C. Effects of Temperature

The sensitivity, g, and fractional exponent, k, of the receptors studied in these experiments did not change over the range of temperatures studied (15°-25°C). Kerkut and Taylor (1956, 1958) and Burkhardt (1959) have demonstrated a strong compensation for temperature change in the mechanoreceptors of cold-blooded animals, where a transient increase in activity occurs with a decrease in temperature, and a transient decrease in activity occurs with an increase in temperature. This temperature compensation may be very important in cold-blooded animals because their receptors

will be subject to environmental fluctuations in temperature. In the frog, *Rana pipiens*, which lives both in and out of water, temperature changes of the magnitude studied here should occur quite frequently, and one could expect a mechanism which will try to maintain the receptor sensitivity at a fairly constant level.

These results can be contrasted with those obtained in the cockroach (French & Kuster, 1982), where the sensitivity of the receptor changed dramatically between 15° and 25°C. The strong temperature sensitivity in the cockroach tactile spine and the Pacinian corpuscle (Ishiko & Loewenstein, 1961) was used to argue that a high energy step is invariably necessary in mechanotransduction. The lack of temperature sensitivity found here makes this less likely. However, it is possible that a compensating change in sensitivity with temperature is present at another stage, such as the encoder.

The effects of temperature on excitable cell membranes cannot easily be generalized because of the rather mixed results obtained from experiments with different preparations. Increasing the temperature has caused both hyperpolarizations (Heitler, Goodman & Rowell, 1977; Stephens & Atwood, 1982) and depolarizations (Moser, Ottoson & Rydqvist, 1979). With membrane potential, increases (West & Lent, 1974; Stephens & Atwood, 1982) and decreases (Moser et al., 1979; Abrams & Pearson, 1982) have been reported with temperature. Also, with membrane resistance and firing

rates, both increases and decreases have been reported with temperature (Murphy & Heath, 1983).

D. Effects of Potassium

Raising the external potassium concentration would be expected to depolarize the receptors, and this membrane depolarization could affect a variety of membrane processes: including the inactivation of Na^+ current, the activation of an electrogenic pump or an increase in calcium-dependent K^+ current, all of which have been reported to cause adaptation in other receptors in response to membrane depolarization (Nakajima & Onodera, 1969a; Gestrelius et al., 1981; Ottoson & Swerup, 1982; Gestrelius & Grampp, 1983). Elevated external potassium concentration could also directly influence a sodium-potassium pump or a potassium current. French (1984c) showed an increase in the adaptation of the cockroach tactile spine by depolarizing the receptor membrane with electrical current or by increasing the external potassium concentration, and earlier studies on frog skin by Feng (1933) and Hoagland (1934) suggested that an increased adaptation rate was a result of an increased extracellular potassium concentration through liberation from injured epithelial cells. An increased power-law adaptation observed in several of the receptors in these experiments is likely to be caused by some combination of these effects.

Hyperpolarization has been shown to cause slower adaptation in receptors (French, 1984c) and it was shown by Nakajima and Takahashi (1966) that the electrogenic sodium pump in the slowly adapting crayfish stretch receptor neurone had the same result as passing a constant hyperpolarizing current through the membrane. Gage and Hubbard (1964) also showed that a post-tetanic hyperpolarization, which lasted for as long as one minute, was due to a long lasting potassium permeability increase, and these results may be responsible for the decreased adaptation rates observed in several receptors studied.

Several receptors studied here displayed no change in their adaptation rate with an increase in extracellular potassium concentration. This could have been caused by a failure of the potassium to reach the receptor site or simply that this receptor was not affected by an increase in extracellular potassium. The exact mechanisms involved in receptor adaptation due to increased extracellular potassium are unknown, but changing the external potassium concentration might provide a mechanism for distinguishing between differing receptors in the skin of the frog, *Rana pipiens*.

E. Conclusions and Suggestions for Future Work

The frog skin preparation offers a relatively simple means of examining transduction in a vertebrate sensory system, and the power law response observed here is similar

to the behavior of a range of other invertebrate and vertebrate mechanoreceptors. An advantage of the system is the ability to change the bathing solution quickly and completely, which is not possible in some other preparations such as the widely used insect mechanoreceptors.

A major disadvantage at present is our ignorance about the relationship between the morphological types of receptors present and the different classes of electrophysiological units which can be identified. This question should probably be addressed before other studies are attempted. During the course of this work some initial attempts were made to identify the morphology of the sensory structures by back-filling the nerves with various stains. However, these experiments were not successful and illustrated how difficult this problem is.

A major problem in mechanoreception is to identify the location and mechanism of the power law adaptation which is a common feature. The evidence presented here suggest that free nerve endings are responsible for the Group II responses, so that any specialized mechanical structures would have to be very small. It seems more likely that the power law adaptation occurs during encoding of action potentials, so experiments to directly stimulate the sensory endings with electrical current might reasonably be attempted. The ease of changing the bathing solution in this preparation might also make it useful for testing specific channel blocking agents when more is known about the

channels involved in transduction or adaptation from other systems.

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