

**CLASSIFICATION OF TACTILE UNITS
OF FROGS BY USING VON FREY MONOFILAMENTS**

by

KORCAN UÇAR

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ABSTRACT

CLASSIFICATION OF TACTILE UNITS OF FROGS BY USING VON FREY MONOFILAMENTS

Twenty-four mechanoreceptor afferent units with fast conducting axons in the sciatic nerve innervating the hind foot were isolated for electrophysiological recording in pithed frogs. Ten rapidly adapting (RA) units and fourteen slowly adapting (SA) units were differentiated from each other mainly based on their discharge patterns. In the neural response to von Frey indentation, RA units had a rapid transient discharge with the maximal five sequential action potentials. SA units had longer and sustained activity during steady pressure on the receptive field. Two kinds of SA units were distinguished; one group of SA units (SA type I) generated irregular discharge pattern at a gradually decreasing rate when was applied ramp stimulus, while the other group (SA type II) generated fewer transient discharges followed by sustained regular discharges lasting longer. SA I and SA II units were differentiated on the basis of several features: i.e, spontaneous firing, transient response and interspike interval histograms. The property of regularity was determined quantitatively. SA units discharged with higher latencies than of RA units. RA units differed from SA units by their higher conduction speeds. The average latency of the first spike was recorded as 33 ms for RA units and 45 ms for SA units. There was a significant difference between the conduction speeds of RA and SA units (t-test; $P=0.039$). There was no significant difference between the conduction speeds of SA I and SA II units (t-test; $P=0.082$). Thresholds of indentation did not differ between three groups of units. Most of the units had 0.16 g indentation threshold. Spike counts for RA units did not change as a function of indentation level, but pooled SA spike counts increase as a function of indentation amplitude. Receptive field analysis was made quantitatively and no differences were seen between groups according to their receptive field structure. Except for the receptive field information, results indicate that tactile units on mammalian and frog skin are similar.

Keywords: Mechanoreceptor, rapidly adapting units, slowly adapting units, frog skin

ÖZET

VON FREY MONOFİLAMENLERİ KULLANARAK KURBAĞA DOKUNMA BİRİMLERİNİN SINIFLANDIRILMASI

Felç edilmiş kurbağalarda elektrofizyolojik kayıt amacıyla arka ayaktaki siyatik sinirine bağlı olan 24 dokunma birimi izole edilmiştir. Onu hızlı (RA), ondördü yavaş adapte olan (SA) birimler temelde elektriksel aktivite şekillerine göre birbirlerinden ayrılmışlardır. Von Frey filamentleriyle yapılan uyarılara RA tipi birimler hızlı adapte olup peşpeşe en çok beş aksiyon potansiyeli üretmişlerdir. SA üniteleri duyar alanlarına yapılan kalıcı uyarılara uzun ve süreğen şekilde cevap vermişlerdir. İki tip SA birimi belirlenmiştir; SA Tip I rampa uyarılara düzensiz aktivite yapısı ile cevap verirken, SA Tip II daha uzun süreli ve düzenli aktivite şekliyle cevap vermiştir. SA Tip I ve SA Tip II birimleri birkaç özellikleri ile birbirlerinden farklılık göstermiş olup bunlar; anlık elektriksel aktiviteleri, dinamik cevap verme yapıları ve aksiyon potansiyelleri arası histogramlarıdır. Düzenlilik yapısı sayısal olarak belirlenmiştir. SA birimlerinin RA birimleriyle karşılaştırıldığında daha geç zamanda cevap verdiği, bir başka deyişle RA birimlerinin iletim hızlarının SA birimlerinden daha fazla olduğu belirlenmiştir. Uyarıdan sonra gelen ilk aksiyon potansiyelinin ortalama zamanı RA birimlerinde 33 ms ve SA birimlerinde 45 ms olarak ölçülmüştür. RA ve SA birimlerinin iletim hızları arasında istatistiksel bir fark elde edilmiştir (t-testi; $P=0.039$). SA tip I ve SA tip II birimlerinde iletim hızları bakımından istatistiksel bir fark gözlenmemiştir (t-test; $P=0.082$). Üç grup içerisinde uyarı eşik değerlerinde belirli bir farklılık görülmemiştir. Çoğu birimin 0.16 g eşik değerinde aktif oldukları belirlenmiştir. Aksiyon potansiyelleri sayımında RA birimleri uyarı düzeyine göre bir değişim göstermemiştir fakat SA birimleri uyarı şiddetinin artmasıyla doğrusal artan şekilde aktif olmuşlardır. Duyar alan analizi sayısal olarak yapılmış olup gruplar arasında duyar alan yapılarına göre belirli bir fark görülmemiştir. Duyar alan bilgisi haricinde diğer sonuçlar memeli ve kurbağa derisindeki dokunma birimlerinin benzer olduklarını göstermektedir.

Anahtar Sözcükler: Dokunma birimleri, hızlı adapte olan birimleri, yavaş adapte olan üniteler

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LIST OF SYMBOLS

P_{Na}	Conductance of Sodium
P_K	Conductance of Potassium
CV	Coefficient of Variation

LIST OF ABBREVIATIONS

RA	Rapidly Adapting
SA	Slowly Adapting
SA I	Slowly Adapting Type I
SA II	Slowly Adapting Type II

1. INTRODUCTION

1.1 Sense of Touch

The sense of touch is derived from information provided by mechanoreceptors innervating the skin. Mechanoreceptor is defined as the end organ that actually transduces mechanical energy into electrochemical energy, thus forming the neural signal. Often, however both of the transducing element and the associated nerve fiber is referred as a mechanoreceptor. Cutaneous mechanoreceptors are derived from functionally; those elements of the peripheral nervous system that are selectively responsive to nonnoxious mechanical stimulation of the skin [1].

Each specific nerve ending was associated with a specific sensation. Specific receptors for heat, cold, touch and pain are known receptors revealed in the previous studies [1]. For the tactile sensation, units encode the mechanical signal with their unique discharge patterns in which latency of the first spike, interspike arrival times, conduction velocities and recovery times change [2].

Touch receptor units may be classified according to their adaptation properties to a static stimulus. There are two major types of units: Rapidly adapting and slowly adapting units. Rapidly adapting class exhibits an instantaneous burst of impulse packets and for the rest of duration no significant response is given. These type of units are mainly responsible for vibration sensation and are called “phasic” units. The second major class is slowly adapting units which have continuous response during the stimulation period. Slowly adaptation class is called “tonic” units and they signal pressure information. Also there are subclasses of each unit with some intermediary properties [3].

1.2 Motivation and Objectives

This work has been undergone to provide a good insight into the classification of touch receptors innervating on the frog skin. The aim is to classify the touch units on the frog skin and to make comparison between the touch units of frogs and mammals. As will be stated later, there are many previous studies related with cutaneous receptors; but

they have not been closely examined the relationship between tactile units of different species.

The goal of this study is to find a relationship between revealed touch units of mammals (such as Merkel, Pacinian) and the units of frogs.

1.3 Thesis Outline

This thesis work is composed of six chapters. The first chapter presents an introduction of touch receptors on the skin and their adaptation properties. The second chapter presents detailed information of mammalian and frog skin, various receptor types and history of experiments related to touch units on the frog skin. The third chapter explains the methods and materials used in the performed experiments. The fourth chapter gives the results of the performed experiments and statistical computations. The fourth chapter presents the conclusions and recommended future work.

2. THEORY

2.1 Basic Properties of Mammalian Skin

Skin is an organ of the integumentary system made up of a layer of tissues that guard underlying muscles and organs. As the interface with the surroundings, it plays the most important role in protecting against pathogens. Its other main functions are insulation and temperature regulation, sensation and vitamin D and B synthesis. Mammalian skin often contains hairs, which in sufficient density is called fur. The hair mainly serves to augment the insulation the skin provides, but can also serve as a secondary sexual characteristic or as camouflage. On some animals the skin is very hard and thick, and can be processed to create leather [4].

The skin is often known as "the largest organ in the human body". This applies to exterior surface, as it covers the body, appearing to have the largest surface area of all the organs. Moreover, it applies to weight, as it weighs more than any single internal organ, accounting for about 15 percent of body weight. For the average adult human, the skin has a surface area of between 1.5-2.0 square metres, most of it is between 2-3 mm thick. The average square inch of skin holds 650 sweat glands, 20 blood vessels, 60,000 melanocytes, and more than a thousand nerve endings [4].

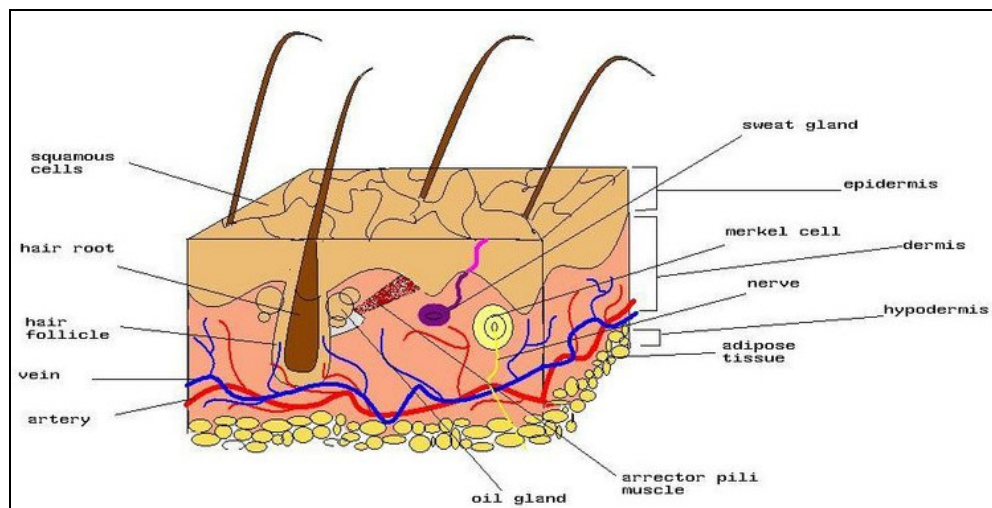


Figure 2.1.1 Diagram of the layers of human skin [4].

2.1.1 Skin Layers

Skin is composed of the epidermis and the dermis (Figure 2.1.1). Below these layers lies the hypodermis (subcutaneous adipose layer), which is not usually classified as a layer of skin. The outermost epidermis consists of stratified squamous epithelium with an underlying basement membrane. It contains no blood vessels, and is nourished by diffusion from the dermis. The main type of cells which make up the epidermis are keratinocytes, with melanocytes and Langerhans cells also present. The epidermis can be further subdivided into the following strata (beginning with the outermost layer): corneum, lucidum, granulosum, spinosum, basale. Cells are formed through mitosis at the innermost layers. They move up the strata changing shape and composition as they differentiate, inducing expression of new types of keratin genes. They eventually reach the corneum and become sloughed off (desquamation). This process is called keratinization and takes place within about 30 days. This layer of skin is responsible for keeping water in the body and keeping other harmful chemicals and pathogens out [4].

Blood capillaries are found beneath the epidermis, and are linked to an arteriole and a venule. Arterial shunt vessels may bypass the network in ears, the nose and fingertips. The dermis lies below the epidermis and contains a number of structures including blood vessels, nerves, hair follicles, smooth muscle, glands and lymphatic tissue. It consists of loose connective tissue otherwise called areolar connective tissue - collagen, elastin and reticular fibres are present. Erector muscles, attached between the hair papilla and epidermis, can contract, resulting in the hair fibre pulled upright and consequentially goose bumps. The main cell types are fibroblasts, adipocytes (fat storage) and macrophages. Sebaceous glands are exocrine glands which produce sebum, a mixture of lipids and waxy substances: lubrication, water-proofing, softening and antibactericidal actions are among the many functions of sebum. Sweat glands open up via a duct onto the skin by a pore [3].

The dermis can be split into the papillary and reticular layers. The papillary layer is outermost and extends into the dermis to supply it with vessels. It is composed of loosely arranged fibres. Papillary ridges make up the lines of the hands. The reticular layer is more dense and is continuous with the hypodermis. It contains the bulk of the structures (such as sweat glands). The reticular layer is composed of irregularly arranged fibres and resists stretching [3, 4].

The hypodermis is not part of the skin, and lies below the dermis. Its purpose is to attach the skin to underlying bone and muscle as well as supplying it with blood vessels and nerves. It consists of loose connective tissue and elastin. The main cell types are fibroblasts, macrophages and adipocytes (the hypodermis contains 50% of body fat). Fat serves as padding and insulation for the body [4].

2.1.2 Types of Skin

Skin can be divided into thick and thin types. Thick skin is present on the soles of the feet and the palms of the hands. It has a larger stratum corneum with a higher keratin content. Thick skin does not grow hair; its purpose is to help grip. Thin skin is present on the bulk of the body and has a smaller stratum corneum and fewer papillae ridges. It has hair and is softer and more elastic. The characteristics of the skin, including sensory nerve density and the type of hair, vary with location on the body [4].

2.1.3 Functions of Skin

1- Protection: an anatomical barrier between the internal and external environment in bodily defense; Langerhans cells in the skin are part of the adaptive immune system.

2- Sensation: contains a variety of nerve endings that react to heat, cold, touch, pressure, vibration, and tissue injury.

3- Heat regulation: the skin contains a blood supply far greater than its requirements which allows precise control of energy loss by radiation, convection and conduction. Dilated blood vessels increase perfusion and heat loss while constricted vessels greatly reduce cutaneous blood flow and conserve heat. Erector pili muscles are significant in animals.

4- Control of evaporation: the skin provides a relatively dry and impermeable barrier to fluid loss. Loss of this function contributes to the massive fluid loss in burns.

5- Aesthetics and communication: others see our skin and can assess our mood, physical state and attractiveness.

6- Storage and synthesis: acts as a storage centre for lipids and water, as well as a means of synthesis of vitamin D and B by action of UV on certain parts of the skin. This

synthesis is linked to pigmentation, with darker skin producing more vitamin B than D, and vice versa.

7- Excretion: The concentration of urea is 1/130th that of urine. Excretion by sweating is at most a secondary function to temperature regulation.

8- Absorption: Oxygen, nitrogen and carbon dioxide can diffuse into the epidermis in small amounts. In addition, medicine can be administered through the skin, by ointments or by means of adhesive patch, such as the nicotine patch or iontophoresis. The skin is an important site of transport in many other organisms [4].

2.2 Various Types of Receptor Cells in Human Hairy Skin

One of the most important properties required to maintain the life of the living organism is the ability to react to external stimuli. Sense organs are specialized for this task. The essential element of these organs is the receptor cell, which responds to physical and chemical stimuli by sending information to the central nervous system. In general, a receptor cell may respond to several forms of energy, but each is specialized to respond primarily to one particular type. For instance, the rods and cones in the eye (photoreceptors) can respond to pressure, but they have a particularly low threshold to electromagnetic energy in the certain frequency band of electromagnetic radiation, namely visible light. In fact, they are the only receptor cells with such low thresholds to light stimulus [5].

There are at least a dozen conscious sense modalities with which we are familiar. In addition, there are other sensory receptors whose information processing goes on without our awareness. Together these may be classified as (1) extroreceptors, which sense stimuli arising external to the body; (2) introreceptors, which respond to physical or chemical qualities within the body; and (3) proprioceptors, which provide information on the body's position. Examples in each of these categories include the following :

1- Extroreceptors

- Photoreceptors in the retina for, vision
- Chemoreceptors for sensing of smell and taste

- Mechanoreceptors for sensing sound in the cochlea, or in the skin, for touch sensation
- Thermoreceptors (i.e., Krause and Ruffini cells), for sensing cold and heat

2- Interoceptors

- Mechanoreceptors in the labyrinth
- Osmoreceptors in the hypothalamus, registering the osmotic pressure of the blood

3- Proprioceptors

- Muscle spindle, responding to changes in muscle length
- Golgi tendon organ, measuring muscle tension

The sensory receptor contains membrane regions that respond to one of the various forms of incident stimuli by a depolarization (or hyperpolarization). In some cases the receptor is actually part of the afferent neuron but, in others it consists of a separate specialized cell. All receptor cells have a common feature: They are transducers that change energy from one form to another. For instance, the sense of touch in the skin arises from the conversion of mechanical and thermal energy into the electric energy (ionic currents) of the nerve impulse. In general, the receptor cells do not generate an activation impulse themselves. Instead, they generate a gradually increasing potential, which triggers activation of the afferent nerve fiber to which they are connected. The electric events in receptors may be separated into two distinct components:

1. Development of a receptor voltage, which is the graded response of the receptor to the stimulus. It is the initial electric event in the receptor.
2. Subsequent buildup of a generator voltage, which is the electric phenomenon that triggers impulse propagation in the axon. It is the final electric event before activation, which, in turn, follows the "all-or-nothing" law [3].

These voltage changes are, however, one and the same in a receptor such as the Pacinian corpuscle, in which there are no specialized receptor cells. But in cases like the retina where specialized receptor cells (i.e., the rods and cones) do exist, these voltages are separate. Because the neural output is carried in the form of all-or-nothing action pulses, it is essential to consider another form of signal than one that is amplitude modulated. In fact, the generator or receptor potentials cause repetitive firing of action pulses on the afferent

neuron, and the firing rate (and rate of change) is reflective of the sensory input. This coded signal can be characteristic of the modality being transduced. In a process of adaptation, the frequency of action potential firing decreases in time with respect to a steady stimulus. One can separate the responses into fast and slow rates of adaptation, depending on how quickly the frequency reduction takes place [5].

2.2.1 Pacinian Corpuscle

The Pacinian corpuscle is a touch receptor which, under the microscope, resembles an onion (Figure 2.2.1, Figure 2.2.2). It is 0.5-1 mm long and 0.3-0.7 mm thick and consists of several concentric layers. These corpuscles are found in mesenteries, especially the pancreas, and are often found near joints. They are found in deep subcutaneous tissue, and are considered rapidly adapting receptors. Pacinian corpuscles detect gross pressure changes and vibrations. Any deformation in the corpuscle causes action potentials to be generated. These corpuscles are especially susceptible to vibrations, which they can sense even centimeters away. Pacinian corpuscles cause action potentials when the skin is rapidly indented but not when the pressure is steady, due to the layers of connective tissue that cover the nerve ending. It is thought that they respond to high velocity changes in joint position.

Pacinian corpuscles have a large receptive field on the skin's surface with an especially sensitive center. They only sense stimuli that occur within this field.

The center of the corpuscle includes the core, where the unmyelinated terminal part of the afferent neuron is located. The first node of Ranvier is also located inside the core. Several mitochondria exist in the corpuscle, indicative of high energy production [3, 5].

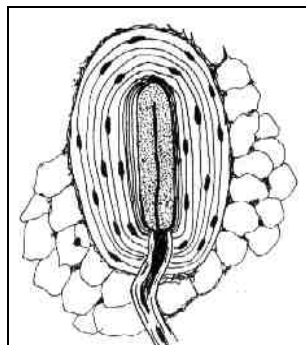


Figure 2.2.1 The Pacinian corpuscle [5].

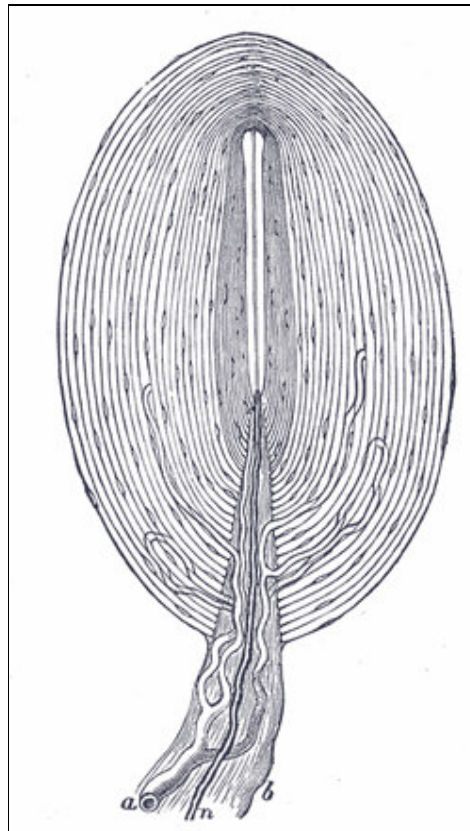


Figure 2.2.2 Pacinian corpuscle, with its system of capsules and central cavity. a) Arterial twig, ending in capillaries, which form loops in some of the intercapsular spaces, and one penetrates to the central capsule. b) The fibrous tissue of the stalk. n) Nerve tube advancing to the central capsule, there losing its myeline sheath, and stretching along the axis to the opposite end, where it ends by a tuberculated enlargement [5].

Werner R. Loewenstein (1959) stimulated the corpuscle with a piezoelectric crystal and measured the generator voltage (from the unmyelinated terminal axon) and the action potential (from the nodes of Ranvier) with an external electrode. He peeled off the layers of the corpuscle, and even after the last layer was removed, the corpuscle generated signals similar to those observed with the capsule intact [2].

The generator voltage has properties similar to these of the excitatory postsynaptic voltage. The generator voltage is a graded response whereby a weak stimulus generates a low generator voltage whereas a strong stimulus generates a large generator voltage. Even partial destruction of the corpuscle did not prevent it from producing a generator voltage. But when Loewenstein destroyed the nerve ending itself, a generator voltage could no longer be elicited. This observation formed the basis for supposing that the transducer

itself was located in the nerve ending. The generator voltage does not propagate on the nerve but, triggers the activation process in the first node of Ranvier by electrotonic (passive) conduction. If the first node is blocked, no activation is initiated in the nerve fiber.

The ionic flow mechanism underlying the generator (receptor) voltage is the same as that for the excitatory postsynaptic voltage. Thus deformation of the Pacinian corpuscle increases both the sodium and potassium conductances such that their ratio (P_{Na}/P_K) increases and depolarization of the membrane potential results. As a result, the following behavior is observed:

1. Small (electrotonic) currents flow from the depolarized unmyelinated region of the axon to the nodes of Ranvier.
2. On the unmyelinated membrane, local graded generator voltages are produced independently at separate sites.
3. The aforementioned separate receptor voltages are summed in the first node of Ranvier.
4. The summed receptor voltages, which exceed threshold at the first node of Ranvier, generate an action impulse. This is evidence of spatial summation, and is similar to the same phenomenon observed in the excitatory postsynaptic potential [5].

2.2.2 Merkel Corpuscle

Merkel nerve endings are mechanoreceptors found in the skin and mucosa of vertebrates that provide touch information to the brain. Each ending consists of a Merkel cell in close apposition with an enlarged nerve terminal. This is sometimes referred to as a Merkel cell-neurite complex, or a Merkel disk receptor. A single afferent nerve fibre branches to innervate up to 90 such endings. They are classified as slowly adapting type I mechanoreceptors (Figure 2.2.3) [3, 6].

In mammals, Merkel nerve endings have a wide distribution. Merkel nerve endings are found in the basal layer of glabrous and hairy skin, in hair follicles, and in oral and anal mucosa. In humans, Merkel cells (along with Meissner's corpuscles) occur in the superficial skin layers, and are found clustered beneath the ridges of the fingertips that make up fingerprints (Some other types of mechanoreceptors, such as Pacinian corpuscles

and Ruffini endings, are found primarily in subcutaneous tissue.) In birds, Merkel receptors are located in the dermis. Wherever they are found, the epithelium is arranged to optimize the transfer of pressure to the ending [6].

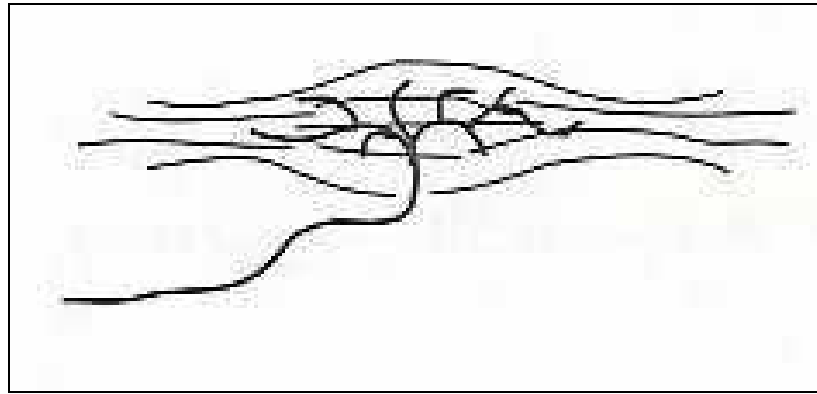


Figure 2.2.3 Merkel cells within the skin, innervated by mechanosensory axons . The axon terminals contain the force transduction machinery, which remains poorly understood. The Merkel cells convey the mechanical stimulus from the surface of the skin to the axon terminals [6].

Their somewhat rigid structure, and the fact that they are not encapsulated, causes them to have a sustained response (in the form of action potentials or spikes) to mechanical deflection of the tissue. They are the most sensitive of the four main types of mechanoreceptors to vibrations at low frequencies, around 5 to 15 Hz [6].

Because of their sustained response to pressure, Merkel nerve endings are classified as slowly adapting. This is in contrast to rapidly adapting receptors which respond only to the onset and offset of mechanical deflection, and to higher frequency vibrations [6].

In mammals, electrical recordings from single afferent nerve fibres have shown that the responses Merkel nerve endings are characterized by a vigorous response to the onset of a mechanical ramp stimulus (dynamic), and then continued firing during the plateau phase (static). Firing during the static phase can continue for more than 30 minutes. The inter-spike intervals during sustained firing are irregular, in contrast to the highly regular pattern of inter-spike intervals obtained from Slowly Adapting Type II mechanoreceptors [6].

Merkel cells fire fastest when small points indent the skin and fire at a low rate on slow curves or flat surfaces. Convexities reduce their rate of firing further still [6].

Merkel nerve endings are extremely sensitive to tissue displacement, and may respond to displacements of less than 1 μm . Type I afferent fibres have smaller receptive fields than type II fibres. Several studies indicate that type I fibres mediate high resolution tactile discrimination, and are responsible for the ability of our finger tips to feel fine detailed surface patterns [6].

A mechanoreceptor's receptive field is the area within which a stimulus can excite the cell. If the skin is touched in two separate points within a single receptive field, the person will be unable to feel the two separate points. If the two points touched span more than a single receptive field then both will be felt. The size of mechanoreceptors' receptive fields in a given area determines the degree to which detailed stimuli can be resolved: the smaller and more densely clustered the receptive fields, the higher the resolution. For this reason, Merkel nerve endings and Meissner's corpuscles are most densely clustered in the highly sensitive finger tips, and less so in the palms [3].

2.2.3 Meissner Corpuscle

Meissner's corpuscles are a type of mechanoreceptor and more specifically, a tactile corpuscle. They are distributed throughout the skin, but concentrated in areas especially sensitive to light touch, such as the fingertips, palms, soles, lips, tongue, face, nipples and the external skin of the male and female genitals. They are primarily located just beneath the epidermis within the dermal papillae [3].

Meissner's corpuscles are encapsulated unmyelinated nerve endings, which consist of flattened supportive cells arranged as horizontal lamellae surrounded by a connective tissue capsule. A single nerve fiber meanders between the lamellae and throughout the corpuscle. Any physical deformation in the corpuscle will cause an action potential in the nerve. Since they are rapidly adapting or phasic, the action potentials generated quickly decrease and eventually cease. If the stimulus is removed, the corpuscle regains its shape and while doing so (physically reforming) causes another volley of action potentials to be generated. Because of their superficial location in the dermis, these corpuscles are particularly sensitive to touch and vibrations, but for the same reasons, they are limited in their detection because they can only signal that something is touching the skin [3].

2.3 General Properties of Frog Skin

The skin is an organ of unusual importance in the life of the frog, because in addition to the functions which it commonly performs among other animals, it has a number of special functions which are peculiar to the Amphibia, and which, in most cases, reach their fullest development among the Anura. As in most of the Amphibia, the skin of the frog is smooth and moist; it is very loosely attached to the underlying musculature by thin bands of connective tissue, which separate the large subcutaneous lymph spaces. It is everywhere very tough, but it is considerably thicker on the dorsal side of the body than it is below [7].

The skin is composed of two principal layers (Figure 2.3.1): the epidermis, and the dermis or corium. A third layer of subcutaneous connective tissue, not belonging to the skin proper, lies underneath the corium and forms the septa uniting the skin to the body wall [7].

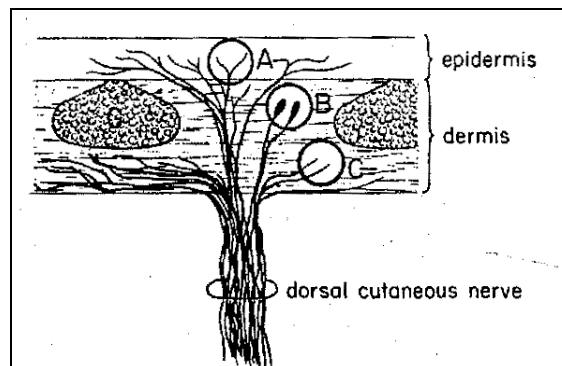


Figure 2.3.1 Semi diagrammatic illustration of the distribution of sensory endings from the dorsal cutaneous nerve in frog skin. A) endings in the epidermis; B) expanded tip endings in the upper dermis; C) free nerve endings in the lower dermis; G) mucus gland in frog skin [9]

The epidermis, or outer portion of the skin, is composed of several layers of cells. The cells of the innermost layer are columnar; but in passing toward the outer surface the cells become more and more flattened, until those of the outermost or horny layer (stratum corneum) become very broad and thin. It is the stratum corneum that is shed during the molting process. The gradual change in shape between the cells of the inner and-outer surfaces of the epidermis is due to the fact that there is a continual production of new cells in the inner layer which are gradually pushed outward, becoming more and more flattened the farther they are pressed away from their point of origin [7].

The epidermis, especially on the dorsal side of the body, usually contains more or less dark brown or black pigment. This pigment is partly within special cells, the chromatophores, and partly in and between the typical cells of the epidermis. In certain regions all of the cells of the epidermis may contain small pigment granules. Ehrmann (1891) found that in the same region of epidermis pigment would appear and disappear in the course of a few months. The chromatophores of the epidermis resemble the dark pigment cells of the corium. Whether they are derived from cells of the corium which have wandered into the epidermis, or whether they arise through the transformation of cells of the epidermis itself, is a matter of controversy. Scientists have come to the conclusion that the chromatophores that appear in the regenerated epithelium of the frog are derived from epithelial cells, and not from cells that have wandered in from the cutis. Chromatophores in the epidermis are not usually abundant. The main source of the color of the skin is in the pigment cells of the corium [7].

The inner layer of the epidermis contains several stellate cells, which, according to Mayer, arise from the modification of cells of the typical form, and, by acquiring pigment, become later transformed into chromatophores. In the outer portion of the epidermis occur scattered oval or flask-shaped cells, the upper portion or neck of which lies just beneath the stratum corneum. According to F. E. Schultze they produce a secretion which passes between the stratum corneum and the subsequent layer of cells and aids in shedding the skin. Pfitzner, on the other hand, regards them as degenerate epithelial cells which retain the mechanical function of holding the stratum corneum in contact with the underlying layer. Modifications of the outer layer or stratum corneum are found in the small stoma cells, which are situated over the necks of the cutaneous glands. The necks of these glands open to the surface through a small triradiate aperture which is raised slightly above the general level [9].

The subcutaneous connective tissue forms a loose layer beneath the stratum compactum and a second very thin layer next to the muscles, the two layers being separated by large lymph spaces except in the septa, where they become continuous. The outer of the two layers is very vascular and contains numerous stellate cells, within which are numerous grayish white pigment granules. These cells are especially abundant on the ventral side of the body, where they produce the white coloration characteristic of that region [9].

2.3.1 Types of Receptors on the Frog Skin

Touch Receptors

Unlike the mesh-like tactile strategy of the tadpole, the nerves of frogs and toads run deep under the skin. Only the ends of the nerve fibers rise towards the surface to form receptive fields. With the exception of the pain sensing nerves, the nerve fibers are myelinated meaning that they have a fatty covering for the purpose of increasing signal velocity. As shown in Figure 2.3.1 these free nerve endings near the skin show the beginnings of sensory specialization with the termination of the fibers in different regions of the skin [9].

Those fibers ending in the epidermis are activated by light touch and have the fastest signal velocities. If the epidermis is removed by scraping with a scalpel these touch responses are abolished from the dorsal cutaneous nerve leaving only a "continued discharge of slow impulses indistinguishable from those produced by acid on the skin". These slow impulses are from the fibers ending in the dermis and they are responsible for sensing pain and temperature yet these fibers were not actually seen and identified until 1955 by Whitear. The function of those with expanded tip endings are unknown. These different tactile sensors were initially discerned only because each type had a different signal velocity which in turn meant that each type had a different sized axon [10].

Cold Receptors

The frog and toad are cold blooded (ectothermic) meaning that they cannot generate their own body heat. Consequently they change their posture or move in order to maintain their body temperature within the desired range. Different species also have different desired optimum temperatures (Lillywhite - 1970). This behavior should also be of a released motivation type since it involves a whole body strategy [9].

As shown in Figure 2.3.2 the cold sensors in the frog *Rana pipiens* are continuously active within a certain temperature range with this activity peaking at the desired body temperature. Notice that the activity level of these tonically active cold sensors is not as large as the more transient touch sensors. This would seem to be a strategy to conserve energy. The peak occurs at the desired temperature when no action needs to take place.

This is in order to convert the signal value so that the maximum occurs when some correcting action is most needed [9].

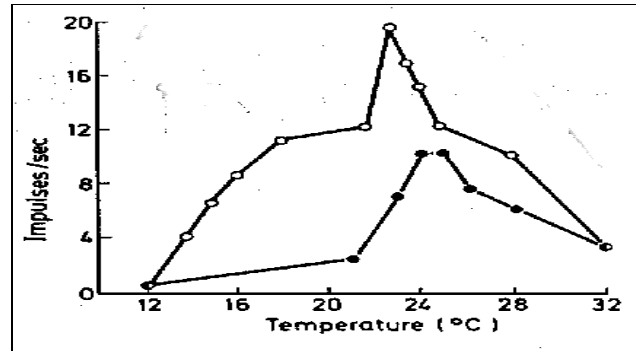


Figure 2.3.2 Responses of the Cold Sensors in Frogs Acclimated Different Temperatures (Scanned from Spray - 1976 (Originally from Spray - 1974). White dots give the response in cold acclimated animals (9.5 degrees C) while the black dots give the response in warm acclimated animals (23 degrees C). All tested after 1 hour warming to room temperature [9].

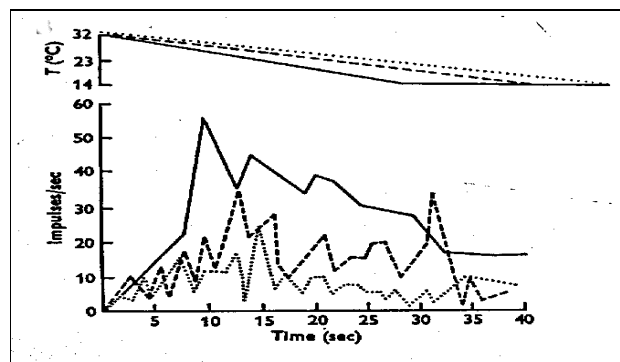


Figure 2.3.3 Dynamic Responses of Cold Sensors Showing Greater Sensitivity to Rapid Cooling. Scanned from Spray - 1976 (Originally from Spray - 1974). Dark line slope is 0.67 degrees C per second, dashed line slope is 0.5 degrees C per second, stippled line slope is 0.25 degrees C per second [9].

The fact that the cold sensor peak occurs at the desired temperature indicates that these cold sensors are used for some other purpose besides body temperature regulation. They may be used to regulate reproductive behavior by signalling the optimum situation for that to occur. This may be the reason why the responses of the cold sensor decrease in frogs living in below normal temperatures also shown in Figure 2.3.2 (the effect should be even worse for chemical reactions slow in colder temperatures meaning that the actual responses would be even lower than shown if the frog had been tested at 9.5 degrees °C) [9].

Not only do the cold sensors have the static response rate described above but they also have a dynamic response component as shown in Figure 2.3.3. This means that rapid

temperature changes, even if the body temperature is near normal, are able to trigger the same posture response as a situation in which the body is far from the desired temperature. This is a good example of a predicting strategy since large changes in temperature will eventually produce a change in body temperature sometime in the future [9].

The cold sensors in frogs (*Rana pipiens*) do not have symmetric responses to changes in temperature. Their dynamic (phasic) response is more sensitive to coldness. An increase in coldness increases the sensor's dynamic response rate while an increase in hotness (decrease in coldness) decreases the sensor's dynamic response rate. This is why they are called cold sensors instead of just temperature sensors [9].

Pain Sensors

The slowest nerve fibers are located in the dermis of the skin and are responsible for pain sensation. Unlike the other skin nerves they are not myelinated. In the frog they have signal velocities of between 0.5 and 4 meters/sec with most being in the range of 1.5 to 3 meters/sec (Spray - 1976). These signals can be triggered by weak acid, intense mechanical stimuli, and strong heating. The significant characteristic of these fibers is the long initial latency of 500 to 700 milliseconds before the maximal response in the action potential pulse (Hogg - 1935). The maximal response occurs near the beginning of the pulse and is followed by a slow decline in action potential frequency lasting up to a second for severe pain stimulus [9].

Besides these slow responding pain sensors in the frog skin another type of pain receptor has been found in the skin overlaying the gastrocnemius muscle of toads. These pain fibers had faster signal speeds being myelinated and having diameters between 6 to 9 micrometers. They produced brief responses to pinch and pin prick and were excited by a weak acid solution. Smaller fibers between 3 to 5 micrometers produced a more tonic response and these were occasionally responsive to thermal stimulation. So what one has here is a strategy similar to that used by the touch receptors and one would expect this system to trigger a different set of behaviors from the frog [9].

Since the pain signal velocities in the frog are so slow and have such a long initial latency they are not responsible for triggering any kind of withdrawal action (the fast pain system in the toad being the exception). This separation of fast withdrawal from pain

continues throughout vertebrate evolution. Since these pain signals do not trigger an action they must be responsible for the reinforcement signal in operant conditioning learning and should thus project to the reticular formation (no studies have been done to track the pain fibers in frogs or toads) and connect to the neurons differently and perhaps with different neurotransmitters than the behavior triggering neurons. They also most likely project to the forebrain region, especially to the amygdala to help characterize the environment of the animal [9].

2.4 History of Touch Experiments

In the years of 1940s, the researchers had already classified the cutaneous mechanoreceptors on the skin of the frog according to their adaptation features: fast and slow fibers [2, 12, 15]. In 1943 Fessard Rogers described a third group, called them slow fibers, which have properties intermediate between the other two. He proposed that when a weight is dropped on to the skin, the resulting nervous discharge consists of three parts: a short rapid burst of impulses in fast and slow fibers, followed by a continued discharge of slow fiber impulses, which may last as long as the weight remains on the skin. If the weight is sufficiently heavy, there is a slowly rising and falling discharge of slow impulses, suggesting that this behaviour of the slow fibers pointed to the release of some substance from the injured tissues, which could act either as a direct or additional excitant to the sensory receptors. This view was supported by Echlin&Propper (1937), who showed that scraping the skin would cause 'sensitization' of the slow fiber endings; they defined sensitization as a condition of the fiber in which a standard weight, when lowered on to a particular spot, caused decreased slow fiber discharge. Scraping had been shown by Feng (1963) to cause a gradual inhibition of the fast fiber response by release of some substance, probably potassium from the damaged cells. Sensitization was assigned to a similar cause, though it was not found possible to demonstrate it convincingly with potassium [12].

In 1950 J. S. Habgood attempted to investigate the phenomena of sensitization further, or at least to confirm or exclude the part caused by potassium. Habgood also wanted compare it with the hyperalgesia described by Lewis aster injury to human skin. He tested the discharge rates with some biochemical substances applied to skin preparation. Potassium effect was not found to be related with sensitization with controlled ringer solutions applied on to the skin. Another chemical, histamine, when dropped on the skin

sometimes caused enormous spontaneous discharge of fast and slow impulses, though Grant and Jones found no action with it on the tongue, nor on the blood pressure after intravenous injection. Habgood concluded in his study that:

- In the majority of experimental trials, spontaneous discharge was observed and rate had been seem to increase when a stimulation was applied to the skin.
- The function of slow fibers is unknown but they correspond to pressure and Fessard & Segers (1943) described them as the only responding to stretch [12].

The most striking conclusion to be drawn from these experiments is the close parallel between human and frog's skin. As Walshe (1942) has pointed out, however, the refinement of sensory function in the higher animals is not so much the result of better sensory apparatus, but because better use is made of the information received. But Foester (1925) used something analogous to the double nerve preparation, when he stimulated dorsal roots cut centrally in some of his patients; they complained of a burning pain in the skin, which was abolished the section of the nerve that, overlapping, supplied the same area. Lewis described the same effect on stimulating cutaneous nerves blocked centrally by local anesthetic.

In 1960's threshold, recovery and fatigue of the tactile receptors in frog skin were studied extensively. W.T. Catton (1961) compared properties of different receptors by localized stimulation rather than by single fibre dissection, thereby permitting a large number of observations on the same preparation, by which means the properties of different receptors could be compared. Catton found an alternative method is to excite individual endings by sharply localized stimulation, and to record from the intact skin nerve. This has the advantages of saving much time in dissection, and of ensuring that measurements, such as velocity of conduction of afferent spikes, are made on the fibre in its normal situation in the nerve trunk, and not exposed to air. Thus using a fine shielded metal tip both for mechanical and electrical stimulation (Figure 2.4.1), it was possible to elicit single unit responses by either means of stimulation. Owing to the simple nature of the method many observations could be made on one preparation [2, 13].

Catton stated that mechanoreceptors can also respond to stimulations other than mechanic indentations (e.g. to thermal) as Hunt & McIntyre (1960) declared that founding

before. It was found that weak stimuli generally produced a single spike response, strong stimuli gave rise to discharges from several receptors, characterized by differences in spike size and latency. A given spike was considered to originate from a single receptor ending and be propagated in a single axon when it showed the following properties: abrupt appearance, all or nothing at threshold strength, constant amplitude and constant latency. The stronger the stimulus the less latency on refractory periods were observed in the Catton's experiments [2].

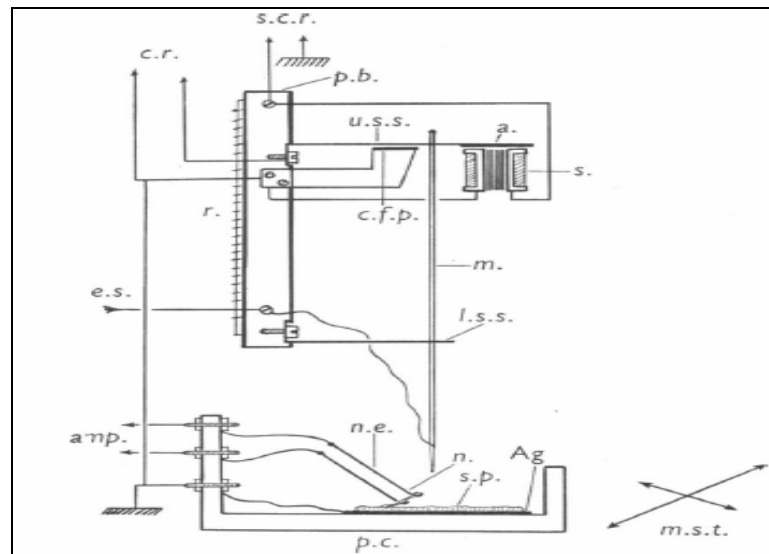


Figure 2.4.1 Diagram of apparatus for skin stimulation. c.r.) capacitance recorder; s.c.r.) solenoid current supply; p.b.) Perspex bar; U.&S.) upper support strip; a.) armature; s.) solenoid; c.f.p.) capacitor fixed plate; in.) micro-electrode; l.s.s.) lower support strip; n.) nerve; s.p.) skin preparation; Ag) silver plate; p.c.) Perspex chamber; n.e.) nerve electrodes; r.) rack: amp.) leads to amplifier; e.s.) lead from stimulator; m.s.t.) mechanical stage traverse [13].

Tactile stimulation in frogs (*Rana pipiens*) seem to produce either prey capture actions or withdrawal actions. A soft brush or the edge of a pipe cleaner stroked on a frog's skin produces prey capture actions as if some bug were crawling on it. In contrast pokes result in a limb withdrawal or a turning away from the stimulus. A reasonable hypothesis is that the fast touch receptors are responsible for triggering the prey capture actions while the slow touch receptors are responsible for triggering the withdrawal actions [9].

Supporting this hypothesis is the fact that some touch receptors (presumably the slow touch) have much higher stimulus thresholds. A good description is given by W.T. Catton :

"As one traverses a vibrating stylus over the surface of the skin of a frog, whilst recording from a cutaneous nerve, responses can be obtained from nearly every point. When responses in a single fiber are examined, it is found that there are a number of discreet points at which a response is evoked; the envelop of these points makes up the receptive field of that fiber. Receptive fields overlap extensively.

If the response at one point is evoked by a brief stimulus, it consists usually of only one or two spikes, even for markedly supraliminal (strong) stimuli. When longer pulses are used, there is still in most cases only a very brief discharge at pulse onset, although when the strength is raised another brief burst occurs at the end of the pulse. Thus the majority of receptors are fast adapting and would be termed 'touch' receptors; they give brief responses only at the beginning and end of prolonged skin deflection.

However, here and there, one encounters receptors of much higher threshold, which continue to discharge during sustained pressure of the stylus. Such slowly adapting 'pressure' receptors are probably sited deeper in the skin than the touch receptors but there is little positive evidence to confirm this. They do not appear to discharge for very long periods and maximum spike trains in the frog were found not to exceed about 16 spikes, however long the stimulus."

As Catton stated above [9], the responses of a single fiber show varying degrees of sensitivity within its receptive field. This is due to the wide branching of its free fiber endings as shown in Figure 2.4.2 so that it is more sensitive to small stimuli near its endings than to stimuli further away. If a weak (non-spike triggering) stimulus is applied to one free fiber ending it affects the other free nerve endings because its charge moves down its own branch and then up the others (called antidromic stimulation). The effect is to raise the stimuli threshold of the other ends just as it does at its own end since, for the fast touch receptors turn themselves off to a degree proportional to their stimulation so that they only produce a few action potentials per stimulus. This effect was measured in toads by Lindblom (1958) who excluded other effects such as skin deformation. A typical skin deformation was 100 microns deep and the skin pit it formed was not wide enough to affect other free fiber endings. Upon the release of the stylus stroboscopic measurements showed that the skin recovered its shape in 5 milliseconds [9].

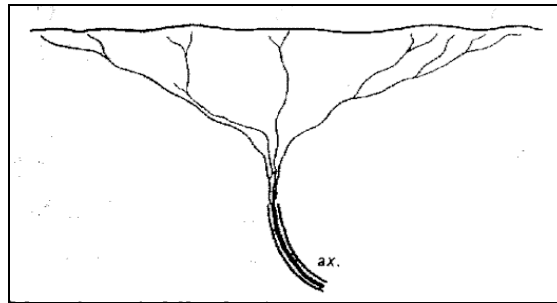


Figure 2.4.2 Close-up of a Single Touch Receptor Fiber Scanned from Catton [6].

In addition to this local antidromic effect about half the dorsal cutaneous nerves have fibers exhibiting a non-local antidromic effect showing that some fibers innervate widely different regions of the skin. These fibers were best activated by narrowly focused air blasts so most likely they are the slow touch receptors responsible for general withdrawal or escape actions and not the spatially localized prey acquisition actions. These fibers always innervate the same side of the frog at approximately the same distance from the head such that they are within their nerve's receptive field. The receptive fields of these single fibers range from 4 to 100 square mm. Typical measurements are 3 mm wide and 8-14 mm long. This is the only description of the receptive field of individual fibers in the literature [9].

Using swift pokes the depth threshold range for touch receptors in the frog is 2 to 20 microns which is 5 times smaller and sensitive than the toad's 10 to 150 microns. The reason for this difference is not known but one wonders if toads can react to bugs on their skin in a fashion similar to frogs. If the rate of the poking is measured one can get the minimum rate required to produce a response which is called the critical slope [9].

The poke response rates in the European toad *Bufo bufo* for the fast touch receptors (called very rapidly adapting by Lindblom) and slow touch receptors (called less rapidly adapting by Lindblom) are shown in Figure 2.4.3 and 2.4.4 (from Lindblom - 1962). These represent the extreme ends in a range of touch receptors types for many intermediate responses were found as well to correspond to the many intermediate fiber diameters. The significant thing to notice is that both the frequency and the number of the action potential spikes were modulated. The frequency response is given by the line graph while the number of action potential spikes was given by the histogram at the bottom with

each dot representing one spike. The fast touch receptors only produce a few action potentials while the slow touch receptors produce a longer train at low poking rates. If the stimulus is suddenly withdrawn from a slow touch receptor before its normal end of action potential production the action potential production will cease [9].

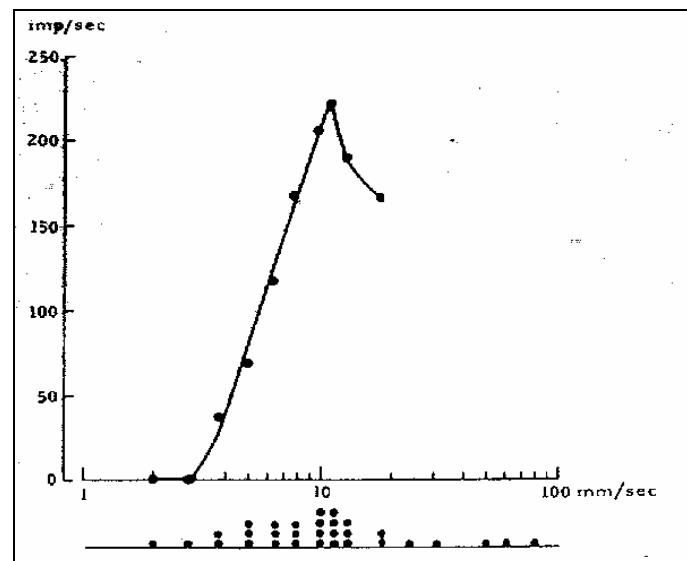


Figure 2.4.3 Response of a Fast Touch Sensor in a Toad Scanned from Lindblom - 1962. Top graph gives the impulse rate versus stylus skin penetration rate. Dots at the bottom represent the number of impulses [9].

The evolutionarily earliest tactile sensory inputs in vertebrates seem to be the Rohon-Beard cells (named after the people who first described them in fish, Rohon (1885) and Beard (1896)). These Rohon-Beard cells are found in adult lampreys which represent the evolutionarily earliest class of vertebrate - the jawless fish. In amphibians and fish they are present only in the embryonic and young larval (tadpole) stages and originate in the spinal cord. In adults they are displaced by a more specific sensory system originating from cells in the dorsal root ganglions located just outside and along the segments of the spinal cord. The Rohon-Beard cells in fish and amphibians are an example of the "ontogeny follows phylogeny" observation in which more recent evolutionary advances are grafted on top of older structures in the developing embryo [10].

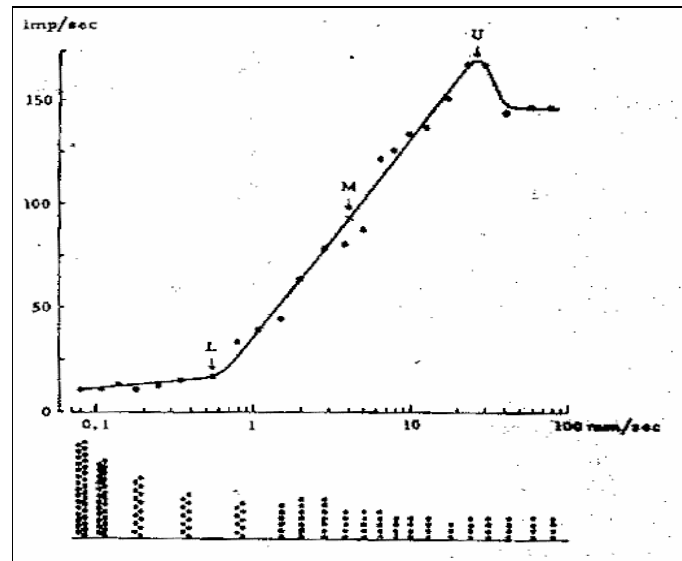


Figure 2.4.4 Response of a Slow Touch Sensor in a Toad Scanned from Lindblom - 1962. Top graph gives the impulse rate versus stylus skin penetration rate. Dots at the bottom represent the number of impulses [9]

Rohon-Beard cells run towards the spine from the underside of the fish or tadpole. They are sensitive to a light touch as produced by a light stroking with a hair. These fibers have no specialized endings so that any deformation along their length tends to produce a signal [10].

While the Rohon-Beard fibers signal touch in these animals other skin disturbances which might be interpreted as pain are signaled by a general electrical skin response. The skin itself becomes depolarized and propagates a signal which is received elsewhere by the nervous. Further development of this property allows some fish (such as *Apteronotus albifrons*) to sense electric field disturbances and other fish such as electric eels give a shock when touched [10].

3. MATERIALS AND METHODS

3.1 Animals

20-30 frogs (*rana ridibunda*) weighing 30-65 g. were used in this study. Frogs were placed in a prone position on the table, and left leg foot will be fixed on the plate covered by solid wax. Animals were pithed with a needle. Paralysed frog's left leg were dissected so that sciatic nerve was freed from the surrounding tissue. Sciatic was further dissected to its small fascicles.

3.2 Equipment

Conventional surgical tools were used in the dissection process of the frog. In the micro-dissection procedure a light microscope was used externally illuminated by a two-armed, adjustable fiber-optic device. For the electrophysiological recording a preamplifier, an oscilloscope, an accelerometer and a notebook computer were used. For mechanical stimulation, von Frey hairs with different bending forces were used. Also to "hear" the spikes 2 amplified speakers were employed in the experiment. In receptive analysis a high resolution digital camera was used.

3.3 Methods

Some predetermined areas were mechanically stimulated by von Frey hairs. These hairs are special kinds of hairs with different bending forces of indentation. When the skin is stimulated, the action potentials could be recorded from the afferent axon in the sciatic nerve.

Receptive fields were determined for each type of units by mapping on the topographically organized anatomy depictions. After the related data was obtained experimentally, the statistical computations were performed. Standard statistical tests like t-test and Anova test were employed.

The block diagram of the data gathering and processing was depicted in the Figure 3.1. Mechanical stimulations were made hand movements of the experimenter with up and down motions by his left hand. Hand motions were transduced to electrical signals by an

accelerometer fixed on the experimenter's wrist so that physiological signal and signal coming from the accelerometer were able to be recorded in different channels of the computer's sound card slot.

The precise location of the receptive field was determined by using a suprathreshold von Frey monofilament and mapped onto a drawing of the hind limb of the frog. Next, mechanical threshold of the fiber was ascertained by applying von Frey monofilaments with increasing bending forces to the most sensitive area of the receptive field. The threshold value was determined as the minimum force (g) that evokes a response in more than 50 percent of the trials.

Action potentials were recorded extracellularly from the sciatic nerve using conventional microdissection and recording techniques. Sciatic nerve was kept at its original place but was isolated from the surrounding tissue. Fine filaments were teased from the fascicles of the sciatic nerve using a sharpened surgical freezer.

In order to calculate the conduction speeds, the instantaneous time of stimulation must be stored so that the difference between the stimulation time and the arrival time of A.P can be obtained. Conduction speed of each unit was calculated by dividing the distance in between point of stimulation and the point of recording to the time interval obtained previously.

Only single units that could be easily discriminated were studied. Action potentials from the the fiber of interest were discriminated from those of other.

Forces produced by the von Frey monofilaments were presented as grams. Responses were expressed as spikes per second and average spike counts. The number of impulses evoked during the indentation were reported as mean + SE . Comparisons of response thresholds for indentation stimuli and conduction velocities (m/s) between groups were made by t-tests. Comparisons of thresholds of indentation between different fiber types were made by one way Anova. A value of P was determined if any significantly difference occurs.

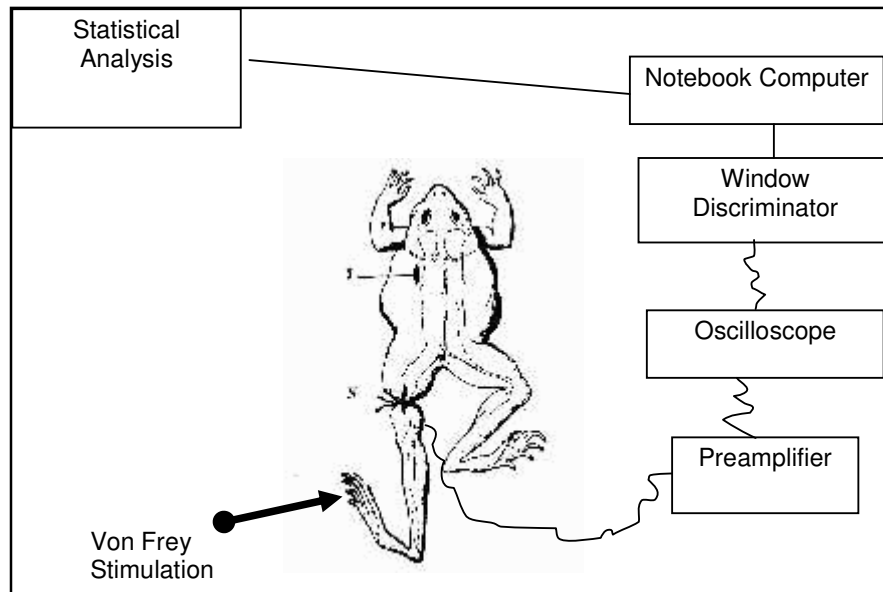


Figure 3.1 Block diagram of the experimental process.

4. RESULTS

11 multi-unit sensory fibers were studied electrophysiologically. 24 units innervating the plantar surface on the hind foot of the frog were discriminated by offline analysis. Among these units 10 rapidly adapting (RA) and 14 slowly adapting (SA) units were classified on the basis of discharge patterns. Slowly adapting units were further classified into two groups as slowly adapting Type I (SA I) and slowly adapting Type 2 (SA II) according to their interspike interval histograms. All discriminated units were listed with their response parameters in Table 4.1.

4.1 Discharge Patterns

Post stimulus time histograms of the units were plotted. In the neural response to von Frey indentation, RA units had a rapid transient discharge with the maximal 5 sequential action potentials. RA units produced on and off responses to von Frey stimulation, whereas slowly adapting units generated sustained discharges during the indentation. RA types responded immediately when applied a mechanical stimulus enough to generate neural discharge. Burst activity was not observed. Rapidly adapting units discharged typically in the first 50 ms after stimulus onset. Both on and off responses were observed for RA units, however some RA units had either on or off response.

SA units had various responses. SA units had burst activity with higher latencies than the activity of RA units. Two kinds of SA units were distinguished; one group of SA units (SA type I) generated irregular discharge pattern at a gradually decreasing rate when was applied ramp stimulus, while the other group (SA type II) generated fewer transient discharges followed by sustained regular discharges lasting longer seconds. SA I and SA II units were differentiated on the basis of several features: i.e, spontaneous firing, transient response and interspike interval histograms. The regularity was determined by interspike interval histograms with 10 ms bin size. Coefficient of variation (CV) of each histogram was determined. Low CV values imply regular discharge. The regularity of maintained discharges of SA II units was confirmed by the low coefficient of variation in ISI histograms (0.02-0.16).

Most of the SA II units had exponentially decaying ISI histograms with peaks at 10 ms intervals. However there were also some regularly discharging units with 30 ms peak intervals. The irregularity of maintained discharges in SA I units was confirmed by a large coefficient of variation (0.24-0.75). Both on and off responses were obtained for SA units.

Table 4.1
Units classified according to their adaptation properties.

UNITS	RA	SA I	SA II	Threshold (g)	Latencies (s)	Conduction Speed (m/s)	Coefficient of Variation
01050125-1			X	0.008	0.0315	34.9206	0.159
015019-1	X			0.008	0.0149	73.8255	Not Calculated
013015-1			X	0.008	0.0317	34.7003	0.034
01014-2	X			0.16	0.0068	117.647	Not Calculated
019022-2	X			0.16	0.0235	51.0638	Not Calculated
01500158-2			X	0.16	0.0315	34.9206	0.176
015801668-2		X		0.16	0.0315	34.9206	0.75
0119501255-2	X			0.16	0.0299	36.7893	Not Calculated
00640073-1			X	0.16	0.0311	35.3697	0.187
012013-1		X		0.16	0.0298	36.9127	0.303
0240255-1	X			0.16	0.0312	35.2564	Not Calculated
01250145-1		X		0.16	0.0323	34.0557	0.345
01690193-1			X	0.16	0.0378	29.1005	0.13
007008-1			X	0.008	0.0398	27.6382	0.152
0130195-1	X			0.008	0.0255	43.1372	Not Calculated
01450147-1	X			0.16	0.0502	21.9123	Not Calculated
0070076-1		X		0.16	0.0315	34.9206	0.295
00580060-1	X			0.16	0.0081	111.111	Not Calculated
00600062-1	X			0.16	0.0364	30.2197	Not Calculated
01010103-1	X			0.4	0.0559	19.6780	Not Calculated
01060108-1			X	0.4	0.0328	33.5365	0.064
01070111-1			X	0.4	0.0317	34.7003	0.156
0060065-1			X	0.16	0.0365	30.1369	0.048
00550060-1			X	0.16	0.0332	33.1325	0.016

4.2 Conduction Speeds

RA units differed from SA units by their higher conduction speeds. Latencies of the first spikes were recorded from each unit; then the latencies were divided by the distance (average distance was 0.109 m) to find the conduction speeds. The average latency of the first spike was recorded as 33 ms for RA units and 45 ms for SA units. There was a significant difference between the conduction speeds of RA and SA units (t-test; $P=0.039$) (Figure 4.2.1). There was no significant difference between the conduction speeds of SA I and SA II (t-test; $P=0.082$) (Figure 4.2.2).

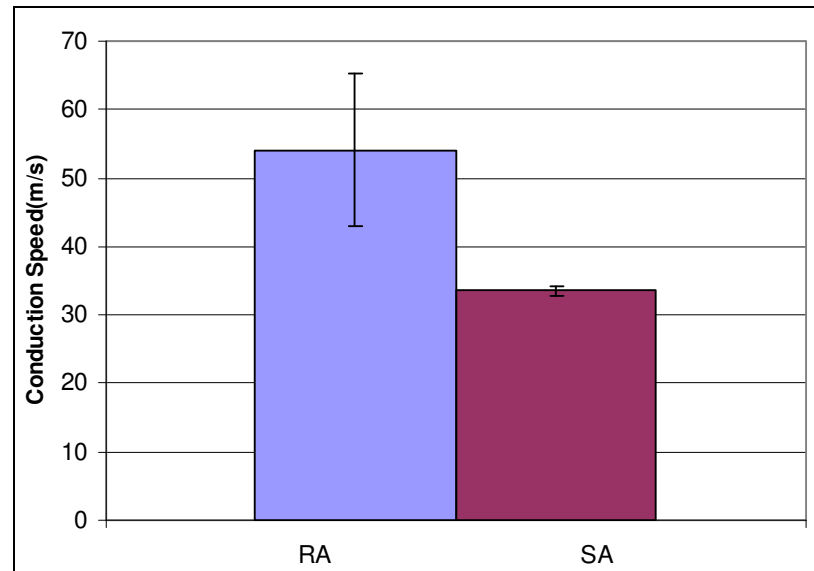


Figure 4.2.1 Conduction speed of RA units were found to be significantly larger than SA units (t-test; $P=0.039$). Error bars indicate standard error of the means.

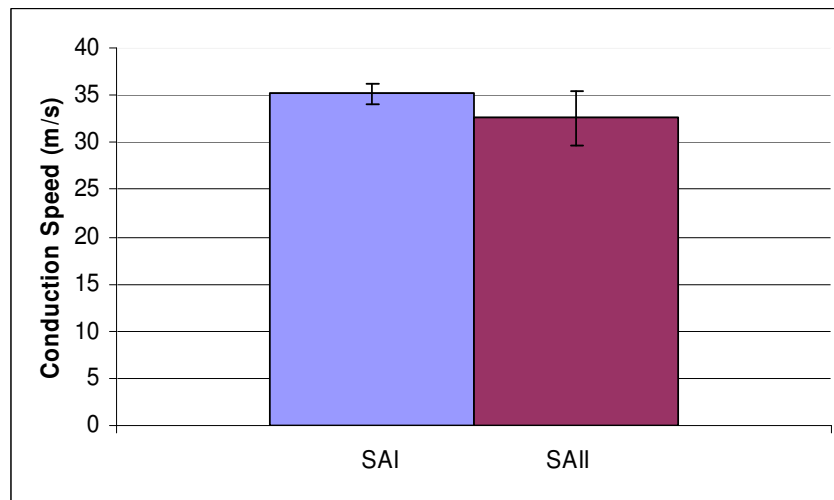


Figure 4.2.2 Conduction speeds of SA I and SA II were found to be not different from each other (t-test; $P=0.082$). Error bars indicate standard error of the means.

4.3 Indentation Threshold

The indentation thresholds for on responses were examined for the three groups of units by using von Frey hairs. The thresholds were expressed as grams. RA units had a mean threshold of 0.1688 ± 0.1667 g (mean \pm SD, $n=10$), and most of them ($n=8$) had a value of 0.16 g (Figure 4.3.1). The mean threshold of SA units was 0.1617 ± 0.1433 g (mean \pm S.D). Thresholds of SA I and SA II units were not significantly different (t-test;

$P=0.5$). No significant difference was found between the thresholds of RA and SA units (t-test; $P=0.057$) (Figure 4.3.2).

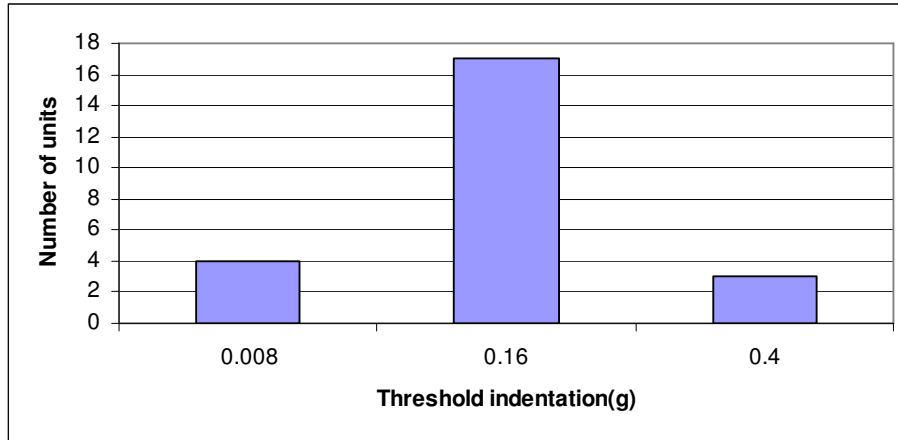


Figure 4.3.1 Most of the units (RA and SA units pooled) had 0.16 g thresholds.

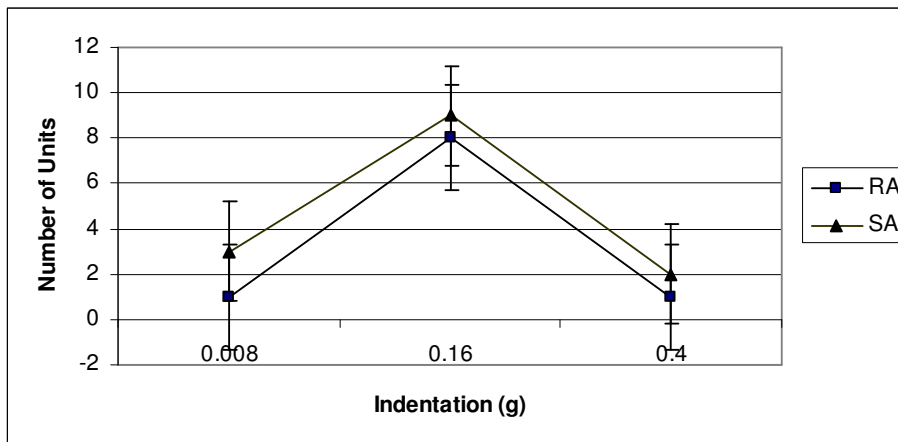


Figure 4.3.2 Units were not found to be significantly different according to their threshold levels. (t-test; $P=0.057$) Error bars indicate standard error of the mean.

4.4 Relation Between Indentation Amplitude and Firing Rate

Spike counts of RA units did not increase as a function of stimulus level. The ‘on’ and ‘off’ responses of the RA units were nearly constant. However, pooled SA spike counts increase as a function of indentation level (Figure 4.4.1). These results are consistent with the previous results in the literature, which were obtained with controlled mechanical indentations [2, 14, 15].

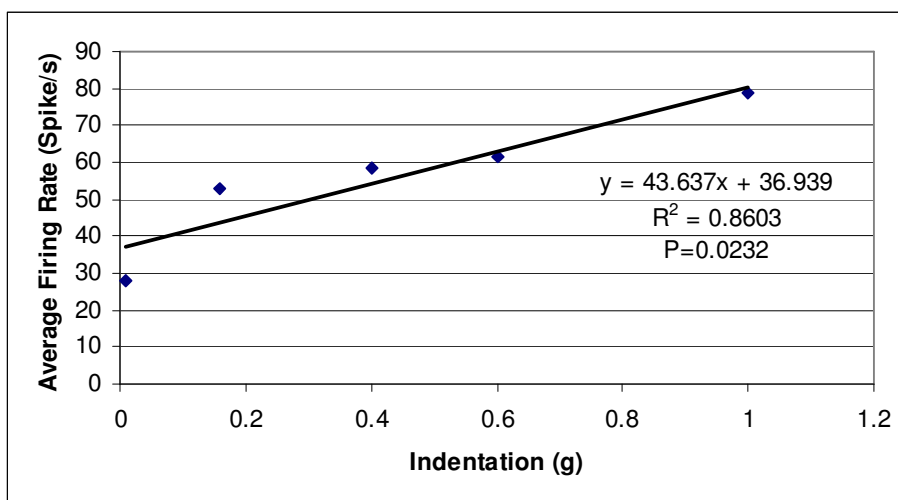


Figure 4.4.1 Indentation amplitude vs Average Firing Rate of the SA Units.

4.5 Receptive Fields

The distribution of receptive fields were shown in the depicted Figure 4.5.1 and Figure 4.5.2 for the three groups of mechanoreceptive afferent units. Of the total sample, three RA units and six SA units were analysed in detail with regard to their receptive field structure. Fields consisted of multiple spots which produced intensive firing in response to light indentation. Each group of units was found to be distributed in the various parts of the skin examined, warty, non-warty skin and web areas. As the indentation level increased, boundary of the receptive fields expanded. Some of the units intensively responded to light indentations in the junctions of web area and skin. RA and SA units did not differed between each other according to their receptive field structures. RA units had the average receptive area of $0.75 \pm 0.5 \text{ cm}^2$ (mean \pm SD, n=3) and SA units had a mean area of $0.85 \pm 0.65 \text{ cm}^2$ (mean \pm SD, n=6). Both units were uniformly sensitive, wherever the receptive field was located.

The receptive fields of both groups do not seem to have any relation to type of skin coloration or conformation, whereas the tactile endings seem to be most abundantly supplied to the small elevations on the skin.

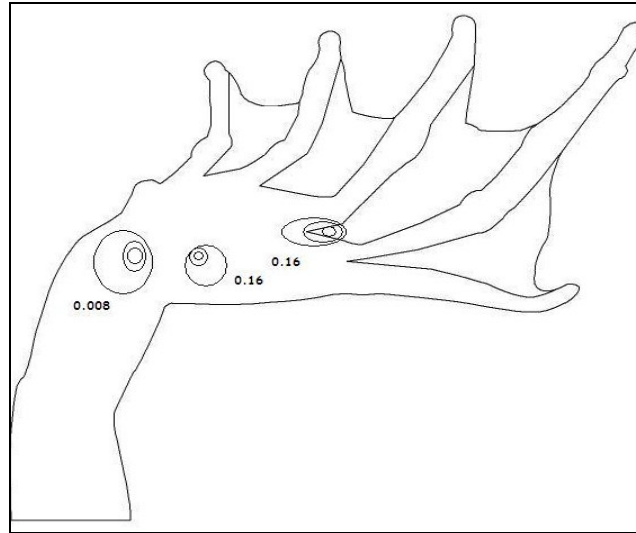


Figure 4.5.1 RA receptive field. Decimals represent the threshold level of the unit. The inner circle represents the threshold responsive area. Concentric circles refer to the boundary of the receptive field with the given stimulus level. For 0.008 g threshold: smallest circle to largest circle: 0.008, 0.16, 0.4, 1 g. For 0.16 g threshold: smallest circle to largest circle: 0.16, 0.4, 1 g.

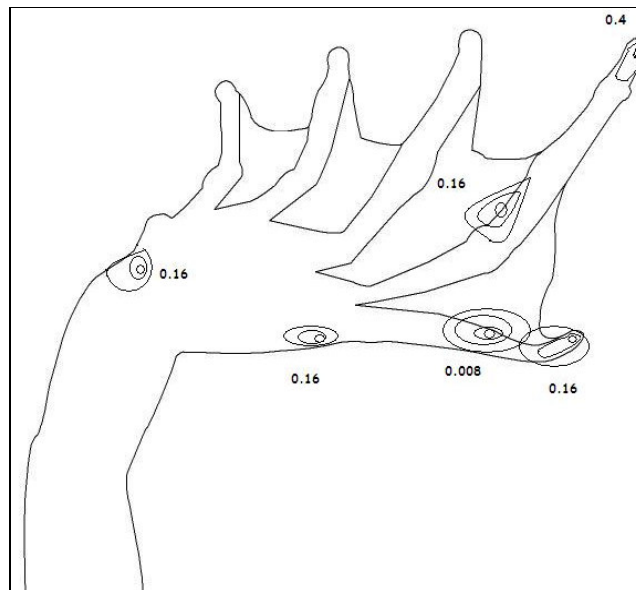


Figure 4.5.2 SA receptive field. Decimals represent the threshold level of the unit. The inner circle represents the threshold responsive area. Concentric circles refer to the boundary of the receptive field with the given stimulus level. For 0.008 g threshold: smallest circle to largest circle: 0.008, 0.16, 0.4, 1 g. For 0.16 g threshold: smallest circle to largest circle: 0.16, 0.4, 1 g. For 0.4 g threshold: smallest circle to largest circle: 0.4, 1 g.

5. DISCUSSION

In the present study mechanoreceptor afferent units with fast conducting axons could be classified into slowly adapting units and rapidly adapting units. SA units were further subdivided into two types mainly based on differences in the rate of adaptation.

5.1 Properties of Mechanoreceptors

In SA units the number of impulses per second was linearly related to the magnitude of indentation suggest that the SA units signal the magnitude of indentation.

In previous experiments of the controlled stimulations, RA units were reported as they discharged spikes phasically in response to ramp stimulation and the maximum instantaneous frequency of discharge was expressed by monotonically increasing functions of indentation velocity. These findings indicate that RA units can encode indentation velocity into spike trains. However in this study the above finding could not be proved due to the lack of velocity information during the manually controlled stimulations. The experimental results show that RA units had a rapid transient discharge with the maximal five sequential action potentials supporting Ogawa and Yamashita that RA units did not exhibit pressure information encoding [1].

RA units had axons fastest in conduction speeds among the three groups and axons of SA I and SA II units showing similar conduction speed to each other, composed the slower conduction fiber group. The present finding is compatible with previous reports that phasically responding units were faster in conduction speed than tonically responding ones [1]. In a record of the discharge produced by mechanical stimulation of the skin, there is no difficulty in distinguishing the rapid impulses which are produced by light touch from slower type due to severe pressure. The former had a shorter duration and are conducted more rapidly; although the slow impulses form a distinct group clearly separable from the fast type [2]. This differential responsiveness of RA and SA units to a ramp stimulation was quite similar to that found in phasically and tonically responding units in the glabrous skin of cats [1].

5.2 Correlation Between Mammalian Skin and Frog Skin

Electrophysiological investigations of the sensory responses of mammalian skin have indicated that in a general way it is possible to correlate specific modes of sensation with characteristic discharges in corresponding types of afferent fiber, grouped according to diameter, spike amplitude, and conduction speed. Such findings have not been widely accepted by many anatomists who have closely studied the morphology of mammalian cutaneous sensory endings, and little correlation appears to exist at present between the physiological and anatomical investigations [2, 14]. But modern anatomical studies have led to the conclusion that mammalian and frog skin have the identical physiological and anatomical characteristics [16].

The frog skin tactile receptors shows a mechanical sensitivity of the same order as that of Pacinian corpuscle or Merkel corpuscle when measured under optimal conditions. It is likely that the prolonged discharges in the irregularly type (SA I) are produced by active involvement of Merkel Cells, whereas those in SA II units are the result of a direct activation of afferent nerve terminals. RA units showed similar discharge patterns to that of Pacinian corpuscles when compared to previous reports [13]. Apart from the discharge patterns many studies supported the Pacinian and Merkel involvement by detailed receptive field analysis. Specifically dyed corpuscles were seen to locate at the very sensitive spots of the receptive field indicating a potential involvement of mechanic displacement transduction [16].

Receptive fields of tactile units innervating on human and frog skin exhibit a considerable similarity [18]. All the units seem to overlap to each other with uniform sensitivity on the sensitive spots. The fields expanded when indentation magnitude was increased. Some of previous studies show that SA receptive fields are quite larger than receptive fields of RA units [1]. In this study no significant difference was found between the receptive field sizes of the units.

6. CONCLUSION

Both type of mechanoreceptors discussed here can play crucial role in frog's environmental adaptation. Rapidly Adapting (RA) units can detect motions of small bugs and parasites leading to wiping response on the skin. Slowly Adapting (SA) units can also encode the information of bugs especially of leeches that stand on the skin without any motion.

The irregular discharges of the SA I units may be for preventing the adaptation of the information sent to the brain for the prolonged stimulations. In mammals, brain ignores some continued and monotype information (such as pain at low levels) for building information hierarchy. The same case would not be useful for the frogs that, for instance, information of a leech on the skin would be inhibited in long time. This may be the reason why SA I units discharge in an irregular pattern. SA II units, by their regular discharges can encode the stretch situation of the skin indicating if the skin is wet or dry.

Merkel Cells function either as an actual mechanoelectric transducer or as the modulator that effects the excitability of mechanosensitive nerve endings by secreting modulator substances. In either case Merkel Cells in frogs may be involved in the mechanism generating sustained responses. Merkel cells act as targets for growing nerves [15].

Since my study was based on manually guided stimulations, the stimulation intervals were not constant and units could not be investigated for their response to repeated or sinusoidal indentations. The future work would consist of computer controlled stimulations which can give a better understanding of threshold, latency times, recovery and fatigue properties of the units during the classification.

Experimental data obtained in this study can be used for future works on diseases such as peripheral paralysis, diabetic neuropathy and ischemia. It can also give useful information for the robotics science in the fields of tactile sensing, texture perception and object recognition in the computer manipulated robotic hands.

APPENDIX A MATLAB CODE FOR OFFLINE ANALYSIS

A.1 Determination of “On” - “Off” Times During Stimulation (gui)

```

function varargout = channel2(varargin)
gui_Singleton = 1;
gui_State = struct('gui_Name',    mfilename, ...
                  'gui_Singleton', gui_Singleton, ...
                  'gui_OpeningFcn', @channel2_OpeningFcn, ...
                  'gui_OutputFcn', @channel2_OutputFcn, ...
                  'gui_LayoutFcn', [], ...
                  'gui_Callback', []);
if nargin & isstr(varargin{1})
    gui_State.gui_Callback = str2func(varargin{1});
end
if nargout
    [varargout{1:nargout}] = gui_mainfcn(gui_State, varargin{:});
else
    gui_mainfcn(gui_State, varargin{:});
end
% End initialization code - DO NOT EDIT
% --- Executes just before channel2 is made visible.
function channel2_OpeningFcn(hObject, eventdata, handles, varargin)
% This function has no output args, see OutputFcn.
% hObject    handle to figure
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
% varargin   command line arguments to channel2 (see VARARGIN)
% Choose default command line output for channel2
handles.output = hObject;
handles.dataAr=[0];
% Update handles structure
guidata(hObject, handles);

```

```

% UIWAIT makes channel2 wait for user response (see UIRESUME)
% uiwait(handles.figure1);
% --- Outputs from this function are returned to the command line.
function varargout = channel2_OutputFcn(hObject, eventdata, handles)
% varargout cell array for returning output args (see VARARGOUT);
% hObject handle to figure
% eventdata reserved - to be defined in a future version of MATLAB
% handles structure with handles and user data (see GUIDATA)
% Get default command line output from handles structure
varargout{ 1 } = handles.output;
% --- Executes on button press in pushbutton1.
function pushbutton1_Callback(hObject, eventdata, handles)
% hObject handle to pushbutton1 (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles structure with handles and user data (see GUIDATA)
[y,fs]=wavread('file path');
h=size(y);
s=h(1,1);
[b,a]=butter(2,[0.0001,.001]);
u=filter(b,a,y(:,2));
t=[1/s:(s/fs)/s:s/fs];
plot(t,u);
axes(handles.axes1);
grid;
% --- Executes on mouse motion over figure - except title and menu.
function figure1_WindowButtonMotionFcn(hObject, eventdata, handles)
% hObject handle to figure1 (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles structure with handles and user data (see GUIDATA)
currPoint=get(handles.axes1,'CurrentPoint');
set(handles.text1,'String',[ 'x:' num2str(currPoint(1,1)) ' y:' num2str(currPoint(1,2))]);
% --- Executes on button press in pushbutton2.
function pushbutton2_Callback(hObject, eventdata, handles)

```

```
% hObject handle to pushbutton2 (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles structure with handles and user data (see GUIDATA)
a=ginput(1)
%disp(a)
handles.dataAr=[handles.dataAr; a(:,1)];
guidata(hObject, handles);
% --- Executes on button press in pushbutton3.
function pushbutton3_Callback(hObject, eventdata, handles)
% hObject handle to pushbutton3 (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles structure with handles and user data (see GUIDATA)
handles.dataAr=[0];
guidata(hObject, handles);
% --- Executes on button press in pushbutton4.
function pushbutton4_Callback(hObject, eventdata, handles)
% hObject handle to pushbutton4 (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles structure with handles and user data (see GUIDATA)
disp(handles.dataAr);
dlmwrite('file path',handles.dataAr);
```

A.2 Determination of Spike Times

```

[y,fs]=wavread(file path');
h=size(y);
x=h(1,1);
u=y(:,1);
t=[1/x:(x/fs)/x:x/fs];
splot=zeros([480000 1]);
stime=zeros([480000 1]);
a=1;
below=uplimit(number);
above=downlimit(number);
for i=1:480000
    if u(i)>below && u(i)<above
        if u(i) > u(i+1);
            stime(i)=t(i);
            splot(i)=1;
            spikerhorizontal(a)=stime(i);
            a=a+1;
        end
        if u(i)<u(i+1)
            stime(i+1)=t(i+1);
            splot(i+1)=1;
            spikerhorizontal(a)=stime(i+1);
            a=a+1;
        end
    else
        splot(i)=0;
        stime(i)=0;
    end
end
end
spikereporter=transpose(spikerhorizontal);
subplot(2,1,2);

```

```
plot(t,splot); axis ([0 60 0 3]);  
grid;  
subplot(2,1,1);  
plot(t,u);  
grid;  
hold on  
plot([0 60],[below below],'c')  
hold on  
plot([0 60],[above above],'r')
```

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