THE EFFECTS OF SEROTONIN AND ITS ANTAGONISTS ON SLOWLY ADAPTING TYPE I MECHANORECEPTIVE FIBERS IN FROG SKIN

by

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ABSTRACT

THE EFFECTS OF SEROTONIN AND ITS ANTAGONISTS ON SLOWLY ADAPTING TYPE I MECHANORECEPTIVE FIBERS IN FROG SKIN

It was intended to investigate serotonin as the neurotransmitter between Merkel cell and its nerve ending, through changes in SA-I response to exogenically applied serotonin and its antagonists. Single-unit electrophysiological responses were recorded from the dorsal cutaneous nerves of the common water frog's (Rana ridibunda) skin perfused in a tissue bath. Maintained mechanical stimulation was applied at suprathreshold levels by von Frey hairs calibrated at 0.16, 0.4, 1, 1.4, and 2 g-bending forces. The effect of serotonin was tested at concentrations: 10 μM (n=8), 100 μM (n=7), 1000 μM (n=6). The responses were analyzed as spike rates. Paired-t test was used to test the significance of the results.

The spike rate increased as a linear function of the stimulus level at baseline and all tested concentrations. Additionally spike rate increased significantly at 10 μM ($p<0.05$) and at 100 μM ($p<0.05$), but decreased at 1000 μM ($p<0.05$) compared to the baseline. In order to find out which serotonin receptors are involved in the process, selective 5-HT_3 (n=7) and 5-HT_2 (n=6) receptor antagonists were applied at 100 μM concentrations. Both receptor antagonists decreased SA-I responses($p<0.05$). Recovery was obtained at each case other than 1000 μM serotonin application.

Our results confirm the role of serotonin in the mechanoelectric transduction in Merkel cell-nerve ending complex and that at least two serotonin receptors are involved in the process in frog skin.

Keywords: Somatosensory, Tactile fiber, Cutaneous afferent, Mechanoreceptor.

ÖZET

KURBAĞADA YAVAŞ ALIŞAN 1. TİP MEKANİK DUYARLI SİNİRLER ÜZERİNDE SEROTONİN VE ANTAGONİSTLERİNİN ETKİSİ

Yavaş-alışan 1.tip mekanik duyarlı sinirler periferide Merkel hücreleriyle ilişkilidirler ve Merkel hücre-sinir kompleksini oluştururlar. Bu algılayıcı organda mekanik uyarının elektriksel işarete nasıl dönüştüğü henüz tam bilinmemektedir. Merkel hücresinden yakındaki sinir lifine serotonin aracılığıyla kimyasal iletim olduğu düşünülmektedir.

Doku banyosunda perfüze edilen Ova kurbağası (Rana ridibunda) derisinin sırttaki dokunma sinirlerinden tekil aksiyon potansiyelleri kaydedilmiştir. Eşik üstü mekanik uyarılar kalibre edilmis 0.16, 0.4, 1, 1.4 ve 2 g-kuvvet seviyelerinde olan von Frey tüycükleri ile uygulanmıştır. Üç farklı serotonin derişimi test edilmiştir: 10 μM (n=8), 100 μM (n=8), 1000 μM (n=6). Uyarıya cevap olarak verilen aksiyon potansiyellerinin birim zamandaki sayıları ölçülmüştür. Tüm kontrol durumları ve derişimler için aksiyon potansiyeli sayısı uyarı şiddetinin doğrusal bir fonksiyonu olarak artmıştır. Ayrıca kontrol durumu ile kıyaslandığında serotonin aksiyon potansiyeli sayısı 10 μM ($p<0.05$) ve 100 μM ($p<0.05$) uygulamaları için artarken, 1000 μM derişimi için azalmıştır ($p<0.05$). Her derişimde ise doku banyosu ile tekrar yıkandığında serotonin etkisi giderilmiştir.

Ayrıca 100 μM 5-HT_3 ve 5-HT_2 reseptör antagonistleri uygulanmış ve mekanik uyarıya cevapta azalma görülmüştür ($p<0.05$). İki antagonist uygulamasında da spontane aktivite dönüşümdüz olarak azalmıştır($p<0.05$). Sonuçlar literatürdeki verilerle genel olarak tutarlıdır ve serotoninin yavaş alışan tip 1 mekanik duyarlı sinirler üzerindeki etkisini desteklemektedir.

Anahtar Sözcükler: Dokunma, Taktil lifler, Deri afferentleri, Mekanoreseptör.

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LIST OF ABBREVIATIONS

SA-I	Slowly Adapting Type I
SA-II	Slowly Adapting Type II
RA-I	Rapidly Adapting Type I
RA-II	Rapidly Adapting Type II
Ft-I	Frog Type I
Ft-II	Frog Type II
5-HT	5-hydroxytryptamine
5-HT_1	5-hydroxytryptamine receptor 1
5-HT_2	5-hydroxytryptamine receptor 2
5-HT_3	5-hydroxytryptamine receptor 3
5-HT_4	5-hydroxytryptamine receptor 4
5-HT_7	5-hydroxytryptamine receptor 7
MDL 72222	3-Tropanyl-3,5-dichlorobenzoate
MC	Merkel cell
VGLT1	Vesicular glutamate transporter 1
VGLT2	Vesicular glutamate transporter 2
Na^+	Sodium ion
Ca^{+2}	Calcium ion
Gd^{+3}	Gadolinium ion
PSTH	Peri-stimulus Time Histogram
ISTH	Inter-spike Time Histogram
TPH	Tryptophan hydroxilase

1. INTRODUCTION

1.1 Motivation and Objectives

Merkel cells are being investigated since 1875 [3]. Even though more than 100 years passed there is no clear agreement on their origin and function. Experimental evidence mostly support that its origin is epidermal and it functions as SA-I mechanoreceptor, but there are also experimental results suggesting that it is not a mechanoreceptor but a support cell or endocrine cell and its origin is not epidermal but neural[3]. Also Merkel cells may develop into carcinomas, which can metastasize very quickly and may affect the whole body including even the brain. Merkel cell carcinoma is very aggressive and when it spreads to lymph nodes survival rate is about 50% [5]. Thus understanding physiology and anatomy of Merkel cells is important for understanding carcinomas better.

In addition physiological and immunohistochemical findings suggest that serotonin is being secreted by both the Merkel cell and its nerve ending [6]. Thus serotonin is a candidate as neurotransmitter in the complex. The objective of the thesis is to test the serotonin hypothesis in the frog. Understanding Merkel cells' function is important, for developing alternative treatments for peripheral neuropathies. Increase or decrease SA-I mechanoreceptor activity, and therefore the sense of touch.

1.2 Outline

The thesis is presented as follows: In chapter 1 background information about morphology and function of skin, peripheral nerve types and theories on Merkel cell-nerve ending complex are given. In chapter 2 experimental procedures are explained. In chapter 3 results are presented. In chapter 4 discussion of results and possible mechanisms responsible for generating these results are interpreted.

2. SOMATOSENSORY FUNCTION OF SKIN

Somatosensory function of the skin is provided by afferent neurons innervating the skin in different layers. Anatomical studies have shown that the first sensory innervations in the skin of amphibian embryos is by neurons with cell bodies lying in a row along the dorsal spinal cord. These cells are called Rohon Beard cells which were first described in fish [7]. They innervate the skin, run into extracellular spaces between the skin cells and makes varicose terminations on superficial layers of the skin. Rohon Beard cells are present only in embryonic and young larval stages and later displaced by sensory neurons from dorsal root ganglia [7]. They were defined as primary sensory neurons whose axons leave the spinal cord at various orientations and then innervate both skin and muscles providing exteroceptive and proprioceptive information.

2.1 Peripheral Fiber Types

Even though there are no generally accepted clear-cut separation, different functions are attributed to sensory neurons with different axon sizes. For mammalian peripheral groups of the nerves are;

- Thick myelinated fibers with conduction velocity in range 70-120 m/s, Aα (type Ia, Ib) fibers which generally innervates only muscles and they may function as both afferent and efferent fibers.
- Myelinated, Aβ (type II) fibers are sensory fibers for touch, pressure and also motor fibers with 30-70 m/s conduction velocity.
- Thin myelinated, Aδ (type III) fibers are mechanical nociceptors and mechanoheat nociceptors, they may be polymodal and bring pain, touch and heat information with conduction velocity 12-30 m/s.
- B-fibers function as preganglionic autonomic fibers. They are myelinated with

conduction velocity in the range 3-15 m/s and absolute refractory period of about 1.2 ms.

- C-fibers are unmyelinated, have 0.5-2 m/s conduction velocity and about 2 ms absolute refractory period. They function as pain, temperature receptors and also mediate some mechanoreception [8]. There are also postganglionic sympathetic C-fibers that release adrenaline and noradrenaline from their nerve endings in special conditions which makes nerve endings more sensitive to bradykinin, serotonin, histamine and capsaicin [9].

Aα, Aβ and Aδ fibers have absolute refractory period between 0.4-1 ms[8], [10]. Exteroception and proprioception are achieved by mechanoreceptors in both skin (Pacinian corpuscle, Meissiner corpuscle, Ruffini ending, Merkel cell-nerve ending and polimodal nerve endings) and muscles (Golgi tendon organ, muscle spindle). According to anatomical studies, mechanoreceptors are generally concidered to be differentiated sensory nerve endings, except the Merkel cell-neurite complex [8]. Merkel cell and its nerve ending function together as a mechanoreceptor by chemical transmission from Merkel cell to nerve ending [3]. Sensory fibers of those four mechanoreceptors are Aβ fibers.

2.2 Morphology of the Skin

2.2.1 General Structure of the Mammalian Epidermis

Keratinocytes are the main cell type of the epidermis. Epidermis is divided into four or five layers depending on keratinocyte morphology. These layers from the contact with basement membrane to more superficial layers are; stratum basale, stratum spinosum, stratum granulosum, stratum lucidum, stratum corneum [11]. Mammalian epidermis is continuously renewed by the mitotic activity of the basal and spinous cells, which form the two innermost layers of epidermis. The cells produced in this level are gradually differentiated and filled with keratohyalin (protein structure found in granules in the stratum granulosum of the epidermis).

The stratum basale is the layer where keratinocytes are produced continuously. This layer contains just one row of undifferentiated columnar stem cells that divide very frequently. Half of the cells differentiate and move to the upper layer to begin the maturation process. The other half stays in the basal layer and divide to produce new cells. As the cells that move into the spinosum they start to change their alignment from horizontal to more vertical position. In spinosum layer the cells start to synthesize keratin. In the stratum granulosum they lose their nuclei and are characterized by dark clusters of cytoplasmic material. In this layer keratin proteins and water-proofing lipids are being produced and organized. The stratum lucidum layer is only present in thick skin where it helps to reduce friction and shear forces between the stratum corneum and stratum granulosum. The outer surface is the dead layer, stratum corneum [12].

2.2.2 General Structure of the Amphibian Epidermis

Amphibian epidermis is not continuously renewed like mammalian epidermis but replaced at regular intervals[13]. The names of the layers are the same but amphibian skin does not include any stratum lucidum and the horny layer is very thin. Structurally germantive cells appear comparable to these of mammalian epidermis but ascending cells are very different. Frog epidermis contains no keratohyalin granules and no granular layers and the horny layer is only one or two cells thick [14].

The epidermis of (Rana Pipiens) is about 5-7 layers thick [1]. Mucus is secreted in middle layers of the epidermis. The basal cells, which are attached to basement membrane by half desmosomes penetrate deeply into the dermis by long cytoplasmic extensions. Both basal and spinous cells contain large nuclei, numerous mitochondria and occasionally seen well developed Golgi vesicles. There is little intercellular space between basal cells. Intercellular space between the cells just above the basal layer is clean, secreted material is not evident yet. The mid portion cells are in higher stage of differentiation and they have larger intercellular space and they include mucus granules in large amounts [14]. In the horny layer, the intercellular space also contains numerous, small dense droplets of mucus. Only a few nuclear remnants, small amount of mito-

chondrial debris and occasional mucous granules are visible, Endoplasmic reticulum, Golgi vesicles can not be seen. These structures are eliminated during transformation of mucus-producing cells into horny cells [15], [13].

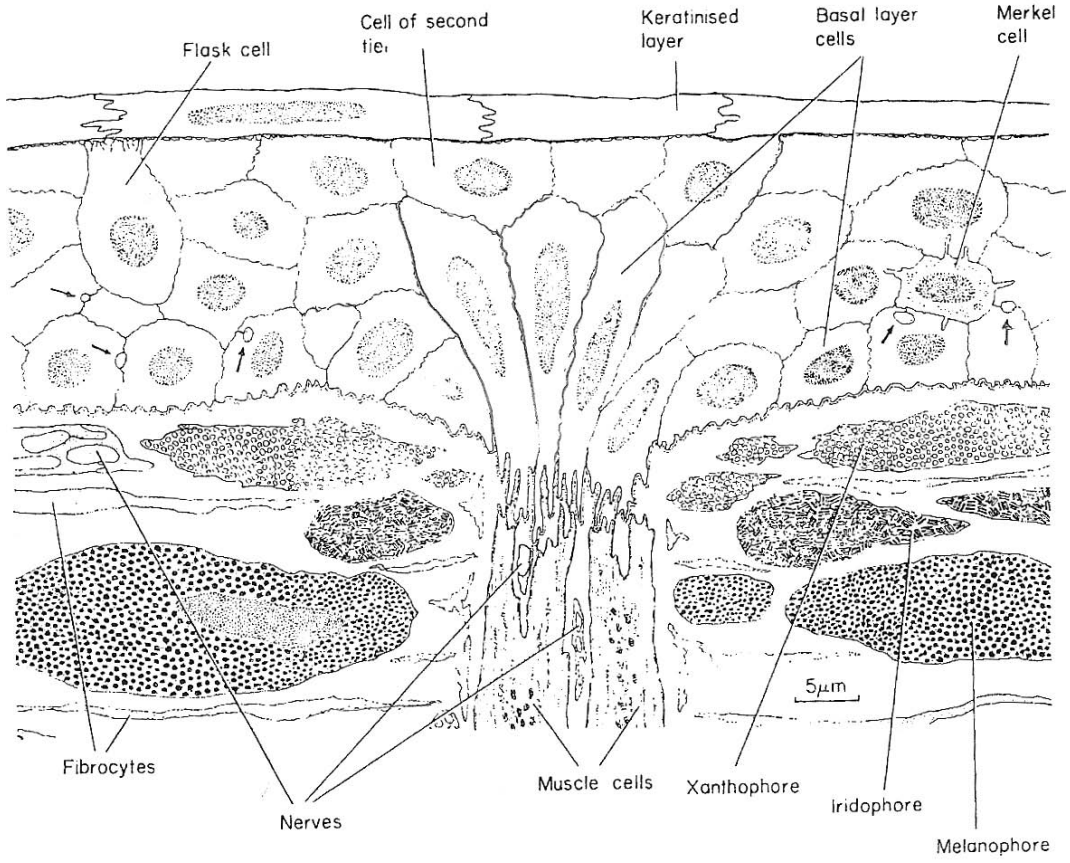

Figure 2.1 Drawing of frog skin (Rana Pipiens) observed with light microscopy by Mary Whitear in 1974 [1]. Drawing includes both dermis and epidermis. Arrows shows the nerves.

There are also other non-epidermal cells in the frog skin. Melanocytes are also non-epidermal cells found in the epidermis of frog. Merkel cells and melanocytes are clear cells by ordinary light microscopy. However, melanocytes do not have innervations in contrast to Merkel cells[16].

Mast cells which are frequently found in peripheral nerve trunks contain a unique granule and are observed in epidermis probably not in normal conditions. Mast cell granules are much larger than Merkel cell granules and different in morphology. Also no Mekel cell granules are observed in kerationcytes [16].

Flask cells are set in middle layers. In contrast to Merkel cells they are elongated in vertical direction and located in higher layers than Merkel cells. They can be recognized by their flask-like shape and numerous mitochondria. Dense core vesicles are not observed in flask cells. There are nerve innervations around flask cells but they are not found to be functional [1].

Histiocytes are part of the immune system with two distinct functions: phagocytosis and antigen presentation. A subset of cells differentiates into Langerhans cells (in mammalian skin); this maturation occurs in the squamous epithelium [1].

Schwann cells have close relationship to myelinated or unmyelinated nerve fibers, have no specific cytoplasmic granules and possess a basement membrane about themselves [16].

Merkel cells most closely resembles cells of the sympathetic ganglion with large granules containing catecholamines, but since there is no evidence for existence of catecholamines in Merkel cell granules these cells can not be equated [16].

2.2.3 Comparison of the Mammalian and Amphibian epidermis

In both mammalian and amphibian epidermis nuclei, mitochondria and other constituents disintegrate and are eliminated at corresponding stages as keratinization proceeds to the horny stage. Since mamalian epidermal cells are not included in any secretory activity they contain little amount of endoplasmic reticulum and Golgi vesicles. The most striking contrast between mammalian and amphibian epidermis is in middle layers, where the differentiation takes place. As mucous granules characteristically develop in the amphibian skin, keratohyalin granules are observed in the epidermis of land-dweeling mammalians. Additionally in contrast to mammalian skin amphibian skin is highly permeable to aqueous solutions [15].

2.3 Mechanoreceptors in the Mammalian Skin

In mammalian skin four types of mechanoreceptors were identified according to their response characteristics to ramp and hold stimulus; Meissner(RA-I) and Pacinian corpuscles (RA-II),Merkel cell (SA-I) and Ruffini ending(SA-II). RA-I mechanoreceptors respond mostly to the ramp portion of stimuli while RA-II only responds at the on and off times of the stimulus. RA-I's are more superficial and has smaller receptive field than RA-II's. SA-I's on the other hand, responds not only to the ramp portion of the stimulus but also to the hold portion by irregular firing pattern. Finally, SA-II display a more regular firing rate and have spontaneous activity in the absence of stimulation. Spontaneous activity may also be sometimes seen in SA-I response [17].

2.4 Frog Skin Mechanoreceptors

There are different classifications of frog skin mechanoreceptors. Ogawa, Morimoto and Yamashita in 1981 identified three types of responses to steady mechanical stimulation; slowly adapting type I, rapidly adapting type I and rapidly adapting type II [18]. Calof, Jones and Roberts again in 1981 reported four response types to low frequency mechanical stimulation; slowly adapting pressure receptor, slowly adapting stroke receptors, rapidly adapting pressure receptors and rapidly adapting stroke receptors [9]. Catton in 1958 classified responses to low frequency mechanical stimulation of frog skin as type-a, type-b, type-c and type-d, where type-a is similar to rapidly adapting touch receptors, type-b is similar to slowly adapting touch, pressure and stretch receptors, type-c is similar to slowly adapting vibration receptors, type-d is similar to pain receptors [19]. There are also free nerve endings innervating the skin in different layers which are assumed to be thermoceptors and nociceptors.

Previous experiments done in our laboratory showed that there are three types of responses to mechanical stimulation; rapidly adapting, slowly adapting type I and slowly adapting type II [11]. Slowly adapting irregular responses are generally at-

tributed to Merkel cell-nerve ending complex. Additionally in our laboratory Merkel cells in frog skin were observed using fluorescence microscopy [20].

Location, structure and response type assumed to be produced by Merkel cell-neurite complex, this is to say slowly adapting type-I responses are similar in frog and mammalian skins[13]. Therefore studying on Merkel cell-neurite complex mechanisms in frog skin may give ideas for the the mechanoelectric transduction in the mammalian skin[11].

2.5 Merkel Cells

Merkel cells were first described by Friedrich Sigmund Merkel (1875) in the hairy skin of mammals, under the warts of lower vertebrates such as amphibians, reptiles and birds. They were referred to as "touch cells"[3]. So they were considered as touch receptors since their discovery. However new theories argue that they may have other functions [5]. Merkel cells are found in various parts of the skin including touch domes of hairy skin in mammals, glabrous skin, labial skin, intraoral epithelium and in the outer root sheath of sinus hair follicles. It was also shown that Merkel cells exists at various tissue without nerve terminals at normal conditions or in wound healing and that they develop into carcinomas in the skin [5]. Merkel cells are found in the skin and in those parts of the mucosa derived from the ectoderm. They are predominant in the glabrous skin and the hair follicle, in the basal epidermal layer of touch-sensitive areas of the skin, including glabrous skin, whisker follicles and touch domes. In frog skin they are observed on the walls of ducts of skin glands [18]. So it is concluded that Merkel cells may have different functions in different locations [5],[21].

2.5.1 Structure of Merkel Cells

Merkel cells are located between epidermis and dermis, mostly in the basal layer of the epidermis. They are almost spherical in shape (4-16 μm) and have a

large number of finger- or rod-like cylindrical processes (about 50 per cell, 1.5-2.5 μm) mostly on the superficial half. They make desmosomal attachments with surrounding keratinocytes. They are differentiated from surrounding epithelial cells by their larger size, pale cytoplasm and they have a single polylobullated nucleus. A well developed Golgi apparatus with a few immature granules are situated on the side of nucleus opposite the synapse [3],[16].

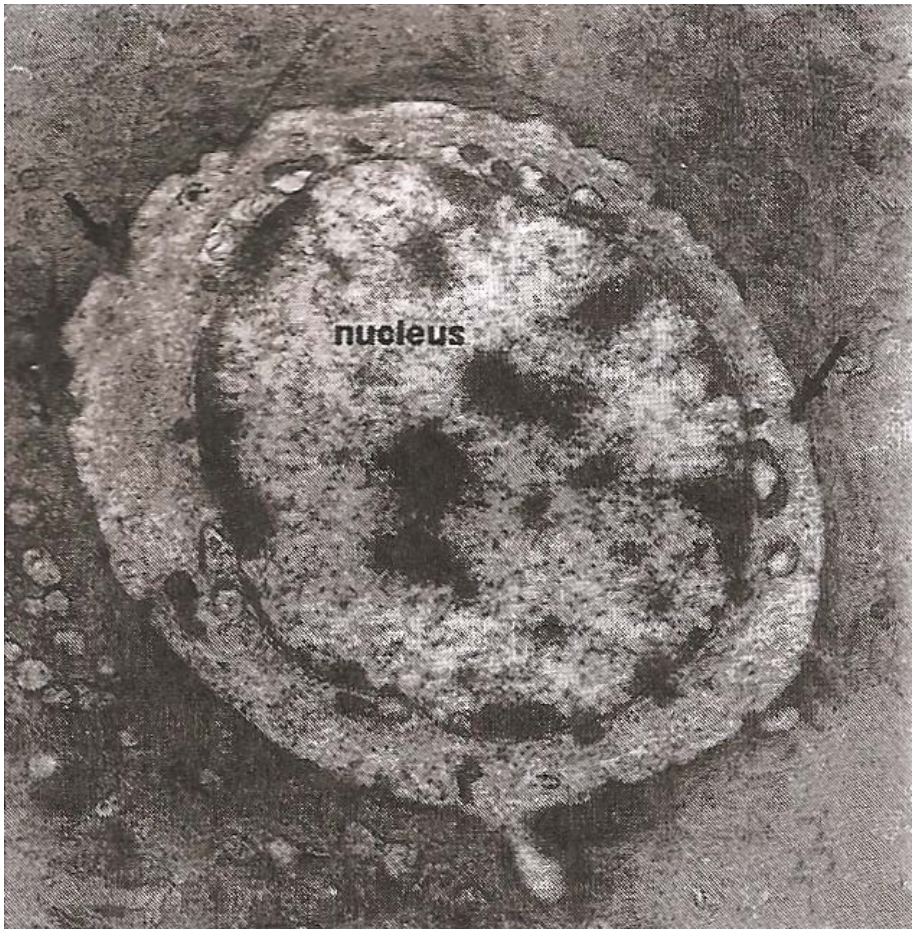

Figure 2.2 Electron micrograph of the Bufo ictericus epidermis. A Merkel-like cell with a rounded nucleus with a electron luscent cytoplasm joined to keratinocytes by desmossomes [2]

Merkel cells form reciprocal synapses with nerve endings (Aβ fiber) and the nerves make plate-, disk-like expansions (8-10 μm diameter, 1-3 μm thick) at the synapses. The terminal axon is derived from myelinated nerve fiber loses its myelin sheath close to the epidermis and continues onward as an unmyelinated axon surrounded by Schwann cell cytoplasm and basement membrane. The fiber terminates as a flat disk filled with mitochondria and both dense and clear vesicles[16]. The Merkel

cells contain numerous mitochondria at the site of the synapse which indicates that the synapse is active. Occasionally granules are observed elsewhere in the cell, but striking nerve associated polarity of these Merkel cell granules is common to the cell in all species and in all circumstances examined to date [16]. In amphibians they are also characterized by dense-cored vesicles, cylindrical processes and desmosomal attachments to neighboring keratinocytes and the reciprocal synapses with nerve making a disk-like expansion [2] .

2.5.2 Physiology of Merkel Cell-Nerve Ending Complex

There are several hypotheses on the functions of Merkel cells in the Merkel cell-neurite complex mostly based on morphological and physiological information. The most striking study showing that Merkel cell-nerve ending complex is associated with slowly adapting type I response to mechanical stimulation was performed by Ikeda (1994) applying a new approach used to kill carcinoma cells [22]. Irradiation of Merkel cells, in rat touch domes, loaded with fluorescent dye, quinacrine, using excitation light in the UV range (which is known to have propagation depth including epidermis and dermis) degenerates the the cells without affecting the nerve terminals (observed with transmission electron microscopy). As a result slowly adapting type I response was abolished, remaining responses were only phasic ones. However, this could not be repeated by other two groups; Bauman and Senok (1993) [23] , Mills and Diamond (1995) [24].

A structural observation associating Merkel cells-nerve ending complexes with SA-I responses is that the clusters of Merkel cells being close to mechanosensitive spots and being prominent at touch domes in the hairy skin of mammals [25], [26]. However developmental findings suggest that Merkel cells do not specify the characteristics of nerve fibers innervating them since slowly adapting responses are recorded from fetal, neonatal and adult rats but Merkel cells do not develop until 2 weeks postnatal [27].

In frog skin Yamashita in 1986 showed that Ca^{+2} channel blockers decreases

responsiveness of both Ft-I (SA-I) and Ft-II (SA-II) units differentially; decrease is high for Ft-I units and lower for Ft-II units[28]. This supports the idea that mechanotransduction process for Ft-I units include synaptic exosynthesis.

In summary there are five general theories on the possible functions of Merkel cells reviewed by Ogawa (1996) [21]:

- In agreement with Merkel, Merkel cell is a secondary sensory cell responding to mechanical stimulation by secretion of chemical messenger(s).
- It is a solid intermediate structure making mechanical stimuli efficient in deforming the mechanoreceptive nerve ending.
- It is a neuromodulatory cell that affects the excitability of adjacent mechanoreceptive nerve endings by secreting modulating substances.
- It is a paracrine cell regulating the surrounding epidermal and non-epidermal cells.
- It is a target for nerve extension in development or regeneration.

Although, these are not mutually exclusive, this thesis is based on the first theory. Ogawa proposed a possible model for mechanotransduction mechanism in Merkel cell-nerve ending complex [21].

2.5.3 Mechanosensitivity of Merkel cells

Until Takeda observed a decrease in Ft-I response triggered by gadolinium in 2003, no stretch-sensitive channel on Merkel cell- nerve ending complex was reported. Takeda suggested that there could be Gd^{+3} sensitive stretch activated Na^{+}, Ca^{+2} channels on both Merkel cell and neurite innervating them [4]. Activation of stretch sensitive channels causes Na^{+} and Ca^{+2} influxes for both cells of the complex which cause neurotransmitter release from both structures. Additionally non-specific cation channels,

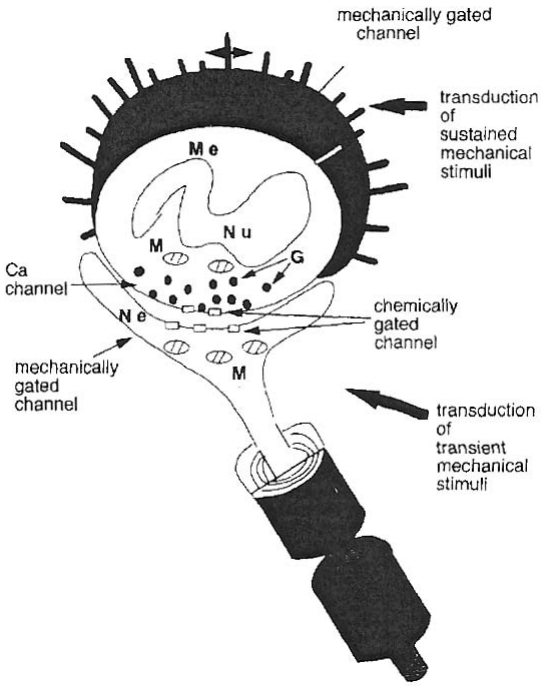

Figure 2.3 Illustration of Merkel cell- nerve ending complex summarizing the ion channels, speculated locations of mechanically gated or chemically gated ionic channels and the cites of transient and sustained mechanical stimulation [3].

delayed and transient K^+ channels and L-type Ca^{+2} channels are reported to exist on Merkel cells [21].

2.5.4 Transmitters and/or Modulators in Merkel Cell-Nerve ending Complex

Several chemicals have been proposed as transmitters in Merkel cell- nerve ending complex mostly based on immunohistochemical studies; acetylcholine (ACh), adenosine triphosphate (ATP), serotonin, met-enkephalin and vasointestinal peptide (VIP)[6].

Tachibana showed that sensory axon terminals of Merkel cell-nerve ending in sinus hair follicles of adult rat expresses positive immunoreactions for 5-HT_{1A} and 5-HT_{1B} but not for 2A, 2B and 3 receptors which is inconsistent with physiological evidence obtained by He, English and Tuckket [29]. He, English and Tuckket also

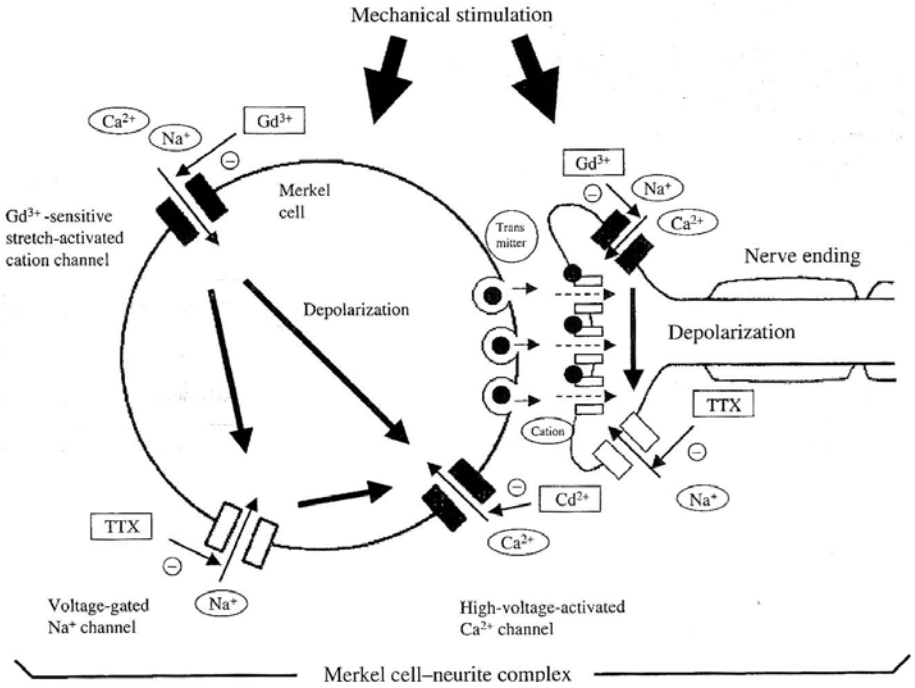

Figure 2.4 Possible model of Merkel cell- neurite complex, proposed by Takeda in 2003 [4]. Mechanical stimulation causes cation influx through a gadolinium sensitive stretch-activated cation channel in both Merkel cell and nerve terminal.

showed that both nerve ending and Merkel cell express immunoreactions for 5-HT transporter. Therefore they concluded that serotonin is released from Merkel cells towards the sensory neuron [30].

English also showed 5-HT immunoreactivity in both Merkel cells and type I endings in rat touch domes [25] . Another interesting finding is that serotonin produces hyperalgesia in rat by a direct action on the primary afferent neuron by 5-HT_{1A} serotonin receptors only not by 3 or 2 receptors [31].

Glutamate vesicular transporters VGLT1 and VGLT2 were also found to be localized in Merkel cell facing the nerve terminal. It was also reported that cutaneous axons express NMDA receptors and release glutamate [32].

According to physiological findings; ACh sensitizes mechanoreceptive sensory fibers but no specification on receptor was made [33]. Histamine, histamine and ACh, serotonin had excitatory action on slowly adapting pressure receptors in cats, but the

effect was not specific to myelinated fibers; mechanoreceptors and thermoreceptors with non-myelinated afferent fibers had diminished responses to neutral stimuli [34]. Nicotine excites SA-I fibers but not SA-II fibers [35]. Another study on cats argue that serotonin inhibited but ATP facilitated SA-I responses in rat [36]. He, English and Tuckett found serotonin increased SA-I response in rat skin in 1999[37] while 5-HT_3 and 5-HT_2 antagonist suppresses in 2003[29]. Fagan and Cahusac in 2000 found that caffeine increases SA-I responses while decreasing SA-II responses while ryanodine also increases SA-I but has no effect on SA-II of rat vibrissae. They also found that non-selective glutamate antagonist kynurante decreases SA-I responses [38].

In brief it is unclear wheter serotonin is the main neurotransmitter in the Merkel cell- neurite complex. This thesis addresses that question.

2.6 Serotonin and Its Receptors

Serotonin is a biogenic monoamine in regulation of many physiological functions. Serotonin is present in significant concentrations in neurons of central nervous system and enteric neurons. In peripheral nervous system, enterochromaffin cells of the gut and blood platelets are also important sources of serotonin. In both systems it is synthesized from L-tryptophan by the enzyme tryptophan hydroxilase (TPH) which has two isoforms by two different genes; non neural- TPH1 (both in Cnetral Newrvous System and Peripheral Nervous System) and neural- TPH2 (only in CNS)[39].

Serotonin synthesized peripherally by enterochromaffin cells is released into the circulation, and then taken up by platelets by the activity of serotonin transporter. Therefore platelets are most important reservoir for serotonin in the periphery. Serotonin is released from platelets mainly upon platelet activation and plays a role in haemostatic function. In addition, peripheral serotonin modulates a number of cardiovascular functions like vasoconstriction, vasodilatation. Altered peripheral serotoninergic function was suggested to play a role in genesis of arrhythmias. Excess serotonin circulation stimulates atrial 5-HT_4 receptors, triggers atrial fibrillation [39].

Peripheral serotonin mediates its effect through five types of receptors: 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4 and 5-HT_7 whereas central serotonin acts through 5-HT_{1A}, 5-HT_2 and 5-HT_3 receptors. Serotonin in CNS acts as neurotransmitter and modulates a variety of behavioral functions like appetite, mood, sexual behavior, sleep and weakness. Abnormality in serotonin secretion or uptake have been reported in psychiatric diseases, including depression, anxiety, eating disorders, and compulsive disorder[39]. Even though peripherally synthesized serotonin can not pass blood brain barrier central and peripheral serotonin effects can interact. Serotoninergic neurons in raphe nuclei innervate autonomic areas of brainstem and spinal cord and in this way modulate sympatho-parasympathetic balance. By this process central serotonin is also involved in cardiovascular regulation[39].

It is also known that higher centers modulate sensation of pain; activation of mechanoreceptors, Aβ fibers stimulate inhibitory interneurons in the spinal cord and reduces pain signal transmitted by Aδ and C-fibers (Gate control theory of pain). These findings indicate the descending modulation of autonomous nervous system on sensory systems [40].

3. METHODOLOGY

Frog skin has been studied frequently in terms of mechanoreceptors [28],[41], [4],[42]. Due to its high permeability to aquaeous solutions [43] frog dorsal skin was chosen for study.

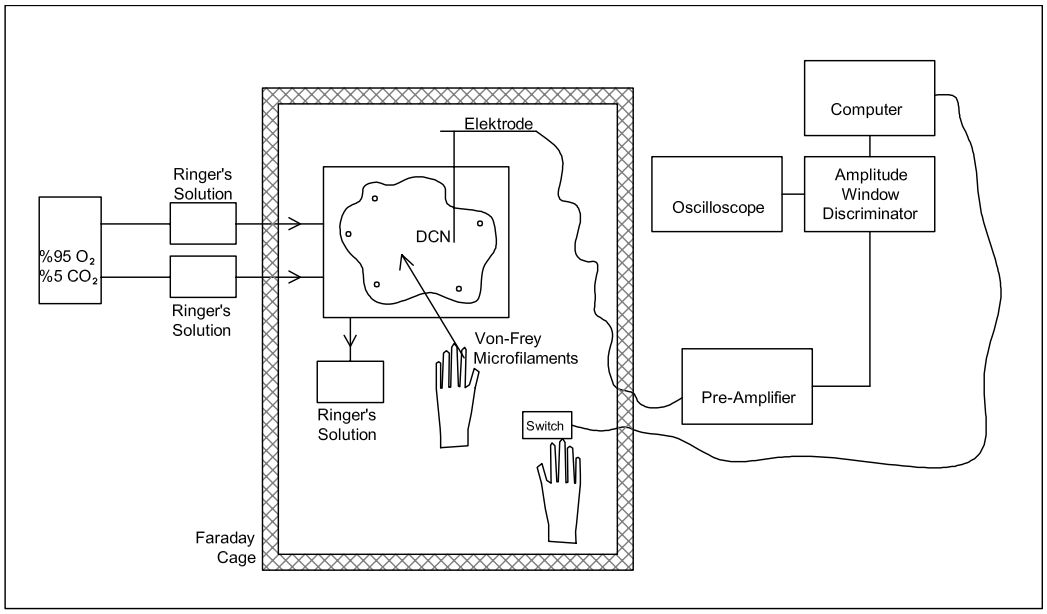

Figure 3.1 Experimental set up for recording SA-I responses to mechanical stimulation. Carbogen was applied to Ringer's solution which is used to perfuse the skin. Mechanical stimulation was applied with von- Frey microfilaments. Platinum electrode was used for recording. Single action potentials was amplified and window discriminator was used to discriminate single amplitude range of action potentials. Spike times were recorded as wave files with MATLAB.

3.1 Animals

25 common water frogs (Rana Ridibunda) of either sex, about 250-350 g in weight were used in the study. Frogs were held in a pool, with 12h light/dark cycles and fed with live grasshoppers.

3.2 Procedure

Single unit extracellular recordings were obtained from dorsal cutaneous nerves of common water frogs. The effects of exogenically applied 5-HT and 5-HT_2 and 5-HT_3 receptor antagonists and glutamate on the response to mechanical stimulation were analyzed in terms of firing rate.

3.2.1 Equipment

The diagram of the experimental set up was shown in Figure 3.1. Carbogen was applied to the Ringer's solution. Skin was perfused with Ringer's solution (flow rate 100ml/h). Mechanical stimulation was applied with von-Frey microfilaments. Amplified single action potentials were observed on the oscilloscope. Amplitude window discriminator was used to select the amplitudes of the action potentials, therefore to disriminate single units. Action potentials of selected amplitude were recorded as sound (wav) files and online analysis was performed. Faraday cage (50x90 cm) provided an electromagnetic shield around the set up. pH meter was also used to observe pH and temperature continuously. Switch was used to specify stimulus on and off times when there was high rate spontaneous activity. Platinum electrode was used for recording.

3.2.2 Dissection

Frogs were anesthetized in -4 °C for about 5 min. Cold anesthetized frogs were double pithed. Then their whole dorsal skin was removed by using conventional surgical tools. Dorsal cutaneous nerves were cut from the proximal end. The skin was mounted in the perfusion chamber without streching. Stretching the skin was observed to cause spontaneous activity. In some of the units spontaneous activity was observed and could not be abolished by reducing the skin stretch more.

3.2.3 Perfusion

The skin in perfusion chamber was perfused with frog's Ringer's solution (112 mM NaCl, 1.9 mM KCl, 1.1 mM CaCl2, 1.1 mM glucose, 2.4 mM NaHCO3, and 1.0 mM NaH2PO4 [44] pH about 7.8 and temperature about 20-25°C). After application of carbogen (95% O_2 and 5% CO_2) pH decreased to about 6.8. pH and temperature were continuously observed and were constant during the experiments.

5-HT hydrochloride (Sigma Adrich) was used at three concentrations: 10μM,100 μM and 1000 μM. It was dissolved in Ringer's solution and carbogen was applied. By carbogen application the pH was adjusted to the baseline value. In pilot experiments the effect of serotonin was observed to stabilize in 15-20 min after application. Therefore recordings were made 20 min after the application of serotonin. 5-HT was applied with another perfusion box and another perfusion canal with the same perfusion rate 100ml/h.

100 μM 5-HT3 receptor antagonist MDL 72222 (Sigma Aldrich) is not soluble in water so it was first dissolved in dimethyl sulfoxide (DMSO, 0.6ml/100ml, Sigma Aldrich) and then in Ringer's solution. Again pH was adjusted to the specific baseline value after carbogen application.

100 μM 5-HT2 receptor antagonist Ketanserin is also not soluble in water. It was also dissolved in DMSO (0.2ml/100ml, Sigma Aldrich) and then added to Ringer's solution. Carbogen was applied and baseline pH value was adjusted.

L-glutamic acid is hardly soluble in water. 100 μM L-glutamic acid was dissolved in Ringer's solution and it took about 1 hour for L-glutamic acid to completely dissolve in Ringer's solution. Then pH was adjusted to the specific baseline value with carbogen application.

3.2.4 Mechanical Stimulation

Specific point on the skin having specific SA-I response patterns discussed below, was specified. Mechanical stimulation as in Figure 3.2 was applied with calibrated, suprathreshold von-Frey microfilaments with bending forces 0.16, 0.4, 1,1.4 and 2 g for 5 seconds. 0.16-g stimulations produced response in all SA-I units. Forces stronger than 2-g could be damaging the skin, therefore they were not used. Measurements were obtained 5 times and their average was used.

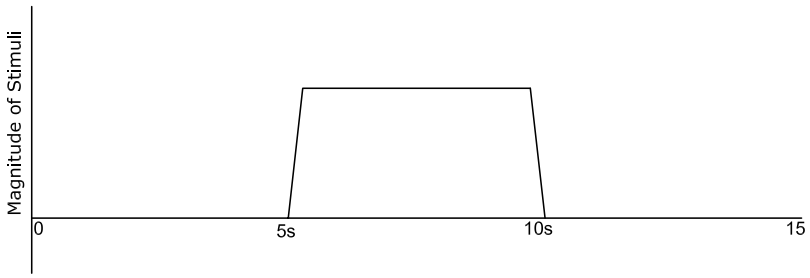

Figure 3.2 Ramp-and-hold stimulation was applied to the specific points on the skin for about 5 seconds. The diagram may be thought as the inverse of the displacement of the skin. A beep sound was produced after 5 sec recording of spontaneous activity. Then the mechanical stimulation was applied between 5 and 10 seconds of the recording. At the 10th sec of recording the second beep was produced and the mechanical stimulation was withdrawn.

3.2.5 Recording

Experimental set up is observed in Figure3.1. One of the dorsal cutaneous nerves was put on the electrode without stretching the nerve. Amplified action potentials were observed with oscilloscope. After mechanical stimulation was applied, if single amplitude action potentials were observed no dissection was applied. If action potentials with different amplitudes were observed, nerve was dissected to thinner strands and/or

amplitude window discriminator [28] was used to select specific amplitude intervals. Only one amplitude was selected by arranging the windows of discriminator. Action potentials of one amplitude is accepted to be recorded from a single axon.

An external sound device was used as an analog to digital converter. Total recording time was 15 seconds; 5s-prestimulus, 5s-stimulus, 5s-poststimulus. It was reported that slowly adapting compression receptors show considerable fatigue to mechanical stimulations in 2 sec [45]. Only spike times were analyzed on the computer. Specifically, the output pulses from the window discriminator were saved as wave files and the spike times were calculated in MATLAB.

For baseline, pharmaceutical application and for recovery data the same procedure was used, only perfusion solution was different. For baseline forces 0.16, 0.4, 1,1.4 and 2 g were applied when the skin was perfused with Ringer's solution only. Each force was applied 5 times and the average of these five recordings were used. After each force application 2 min was waited before the next stimulation. Forces were not applied by increasing the magnitude of stimulation, but they were applied randomly. After baseline recordings were done 25(five recordings for five applied forces) recordings was obtained. Then the skin was perfused with specific chemical including solution and after 20 min of application forces were applied in the same sequence explained and again 25 recordings was obtained, but this time for pharmaceutical applied conditions. After that skin was again perfused with Ringer's solution. Ringer's solution was applied for 30 min to let the pharmaceutical to be washed out. Stimulation procedure was repeated again and 25 more recordings was obtained for recovery condition.

For recording, tungsten, Ag-Cl and platinum, electrodes were tested and platinum electrode was used due to its superior signal to noise characteristic. Switch was used to synchronize the simulation times and spike times. Switch was opened when stimulus was applied and closed when it was withdrawn. Output of the switch was recorded as the second channel of wave file as in Figure 3.3. Therefore second channel of recording gave the on- and off-times of stimulation.

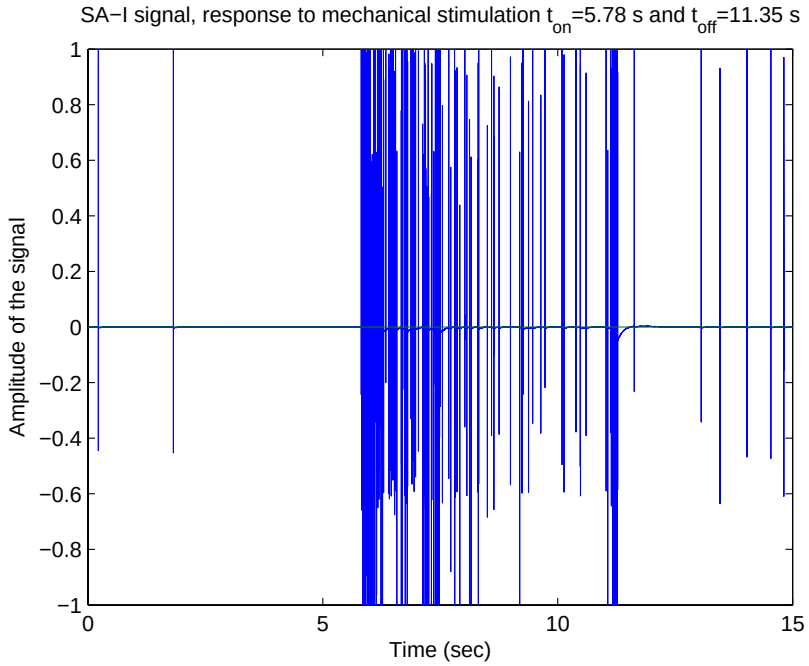

Figure 3.3 Signal recorded from the discriminator, produced in response to mechanical stimulation of slowly adapting mechanoreceptive fibers with F=0.4-g. Steady mechanical stimulus was applied between t_{on}=5.78 s and t_{off}=11.35 s

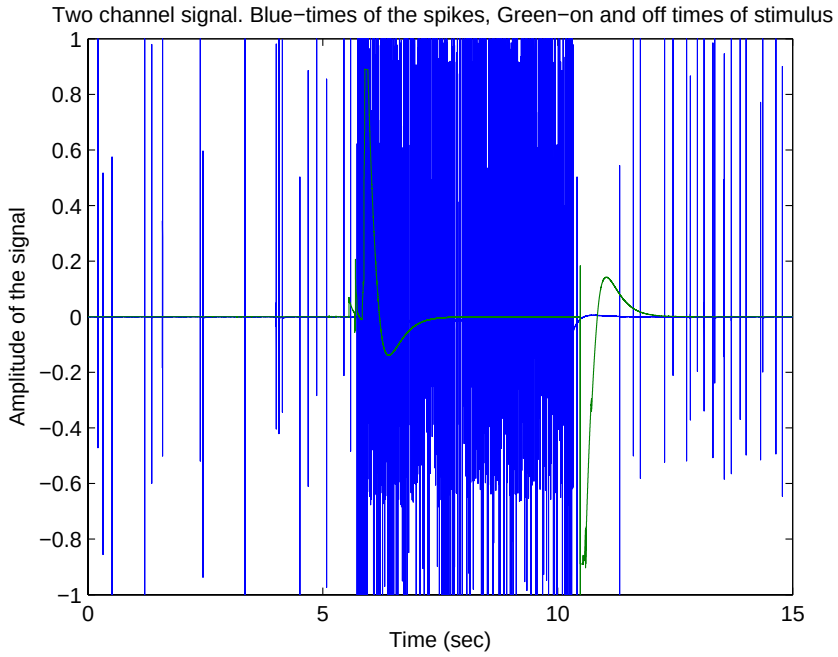

Figure 3.4 Two channel signal recorded from a SA-I mechanosensitive fiber, produced in response to mechanical stimulation F=1-g. Blue is the first channel of the recording and it shows the times of the spikes. Green is the second channel and it shows the opening (stimulus on time) and closing (stimulus off time) of the switch.

A specific peak detection algorithm was written with MATLAB to extract spike times. Therefore while performing the recordings, peristimulus time and interspike time histograms were plotted online so SA-I characteristics could be observed and to watch out for experimental error.

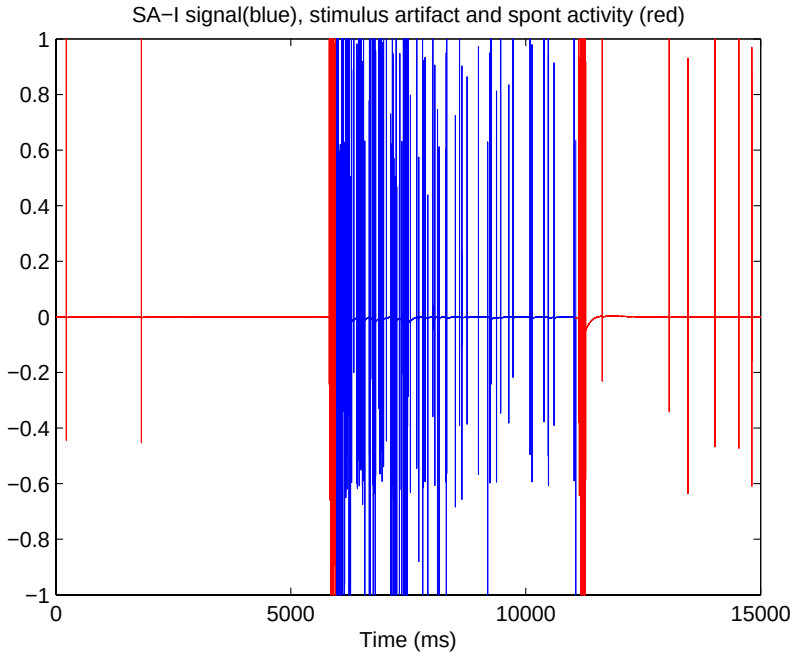

Figure 3.5 Signal recorded from the discriminator, produced in response to mechanical stimulation of slowly adapting mechanoreceptive fibers with F=0.4-g. Mechanical stimulus was applied between t_{on}=5.78 s and t_{off}=11.35 s. Spontaneous activity, stimulus artifacts and SA-I response was discriminated.

Figure3.4 is observed on the screen after each mechanical stimulus was applied and wav file was recorded. This plot stayed on the screen until stimulus on and off time coordinates are chosen manually by clicking on the specific points of the Figure3.4. First 150 ms and last 150 ms of that SA-I signal were ignored since they were assumed to be stimulus artifacts and interferance from strong units [28]. Furthermore the remaining signal was purely the tonic response. Spikes between t_{on} and t_{off} were characterized according to two histogram types; peri-stimulus time histogram and inter-spike time histogram. Time plot of the signal and these two histograms appeared on the screen each time, after t_{on} and t_{off} were chosen, as in Figure3.6. This allowed us to observe the characteristics of the signal just after its recording and reduced the number of experiments by preventing any possible experimental error.

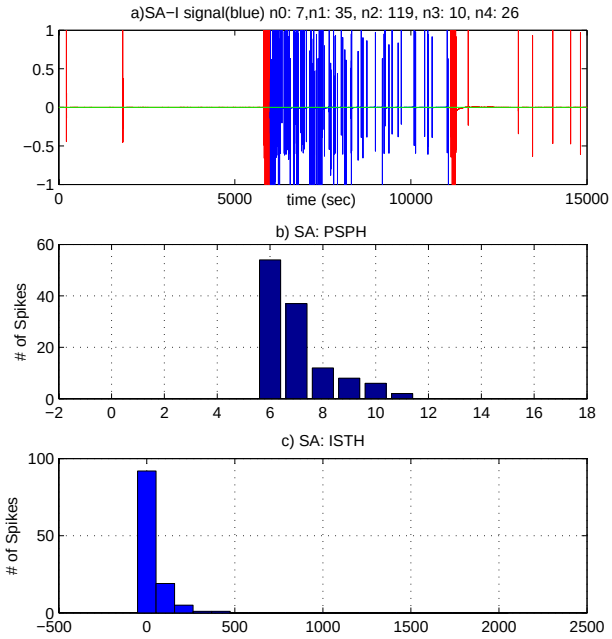

Figure 3.6 Plot that appears on the screen after each recording for online analysis. Histograms of signal recorded from a SA-I mechanosensitive fiber, produced in response to mechanical stimulation F=0.4-g. Mechanical stimulus was applied between t_{on}=5.78 s and t_{off}=11.35s

Peri-stimulus time histogram, as in Figure 3.6, with 1s bin sizes showed slow adaptation in the tonic response [29]. Interspike time interval histograms with 100 ms bins showed irregular spikes as in Figure 3.7. Each point indicated different unit. Recordings from one or two points/units was ontained from one skin preperation. Average spike rates were calculated from spike data and statistical analysis were performed with MATLAB and MS Excell.

3.3 Statistical Analysis

Paired t test was used to analyse the significance of the results. Differences in spike rates of baseline and pharmaceutical applied condition, pharmaceutical applied condition and recovery, baseline and recovery were analyzed seperately. In addition n-way anova was used to test the differences in changes percentages of spike rates with changing force and concentration of serotonin applied.

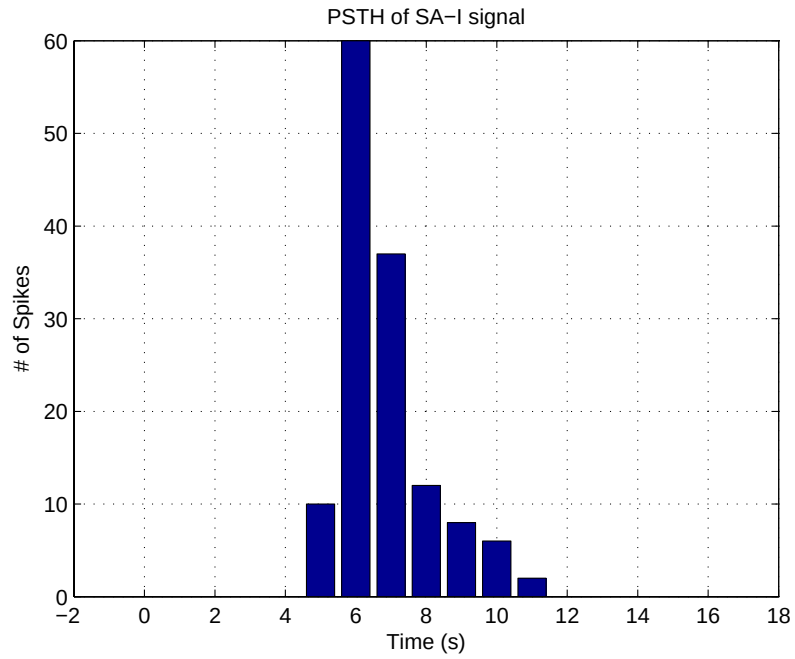

Figure 3.7 Peri-stimulus time histogram of signal recorded from a SA-I mechanosensitive fiber, produced in response to steady mechanical stimulation with F=0.4-g. Mechanical stimulus is applied between t_{on}=5.78s and t_{off}=11.35s

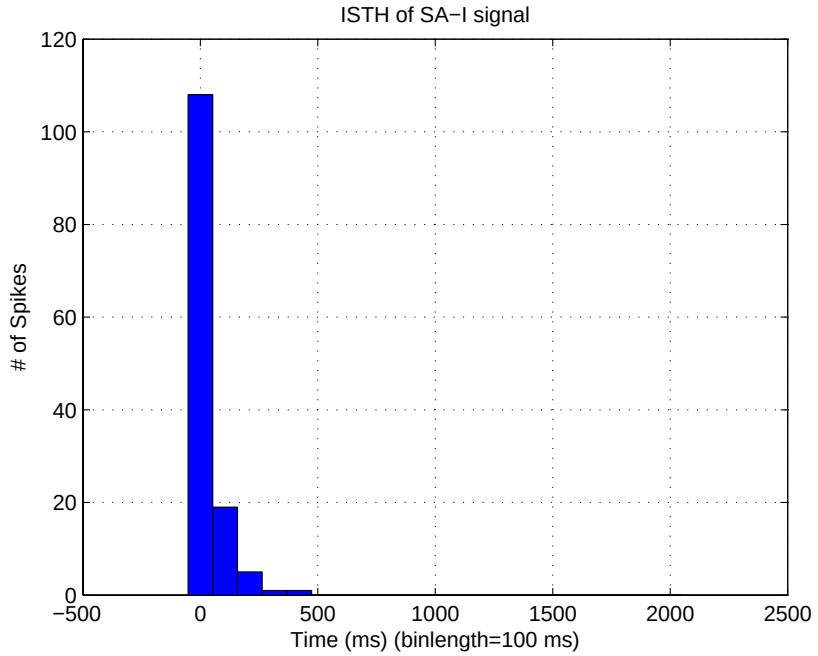

Figure 3.8 Inter-spike time histogram of signal recorded from a SA-I mechanosensitive fiber, produced in response to steady mechanical stimulation F=0.4-g. Mechanical stimulus was applied between t_{on}=5.78 s and t_{off}=11.35s

4. RESULTS

4.1 Relationship Between Stimulus Force and Spike Rate

For all conditions; baseline (n=36), 10 μM serotonin(n=8), 100 μM serotonin (n=7), 1000 μM serotonin (n=6), 100 μM MDL 72222(n=7), 100 μM Ketanserin (n=7), 100 μM L-glutamic acid (n=5) applied and for all recovery conditions (n=36) spike rate of SA-I response increased as a linear function of stimulus magnitude. Consistent with the theory that sensory neurons encode magnitude of stimulation as spike rate [10], SA-I unit encodes magnitude of mechanical stimulation as spike rate.

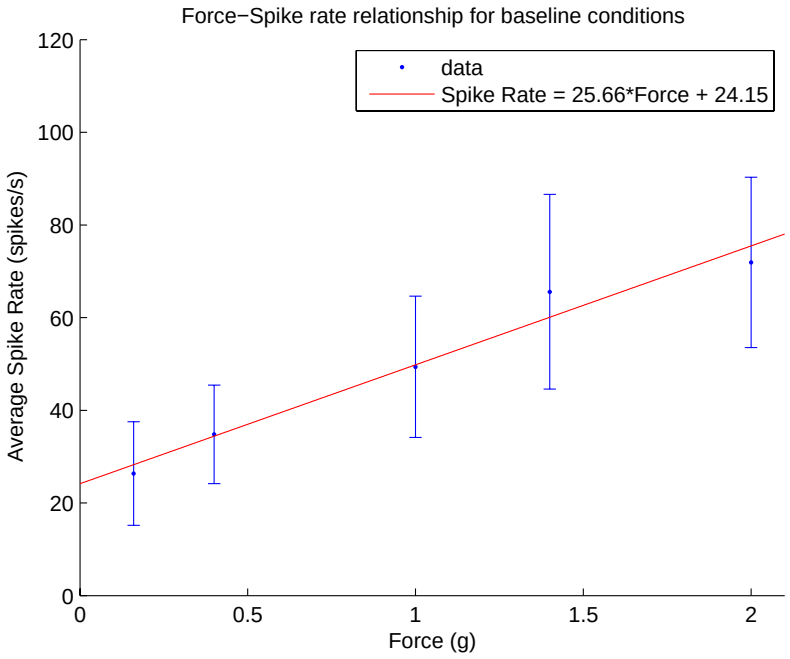

Figure 4.1 Change of SA-I spike rate with changing force for all baseline conditions (n=36), R^2=0.975.

As observed in figures 4.1- 4.8 spike rate increased as a linear function of stimulating force for all cases. Bigger slope, which is high increase in spike rate with increasing stimulus forse indicate that SA-I units are sensitive to changes in stimulus magnitude. For 10 μM serotonin applied conditions slope was almost the same as the slope of the baseline. For 100 μM serotonin application responses for all forces

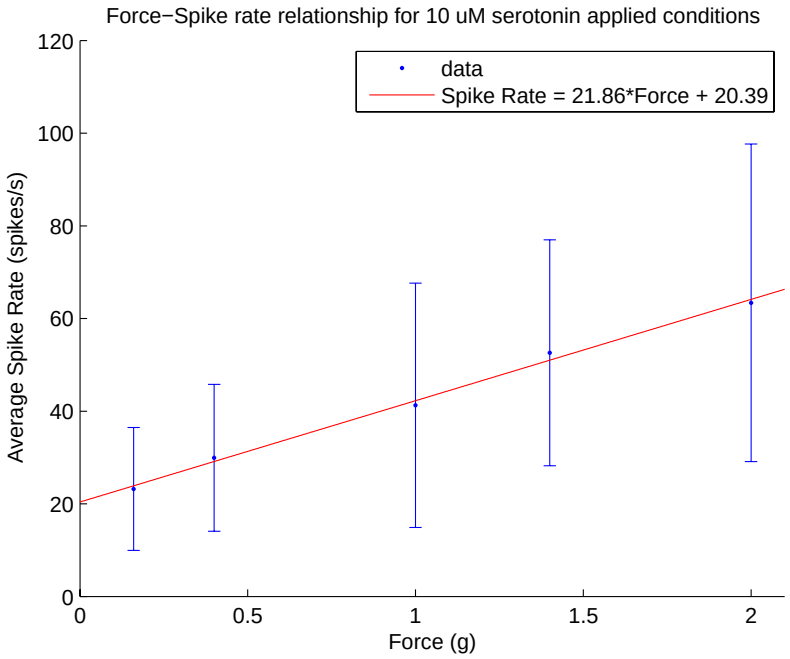

Figure 4.2 Change of SA-I spike rate with changing stimulating force in 10 μM serotonin application (n=8), R^2=0.995.

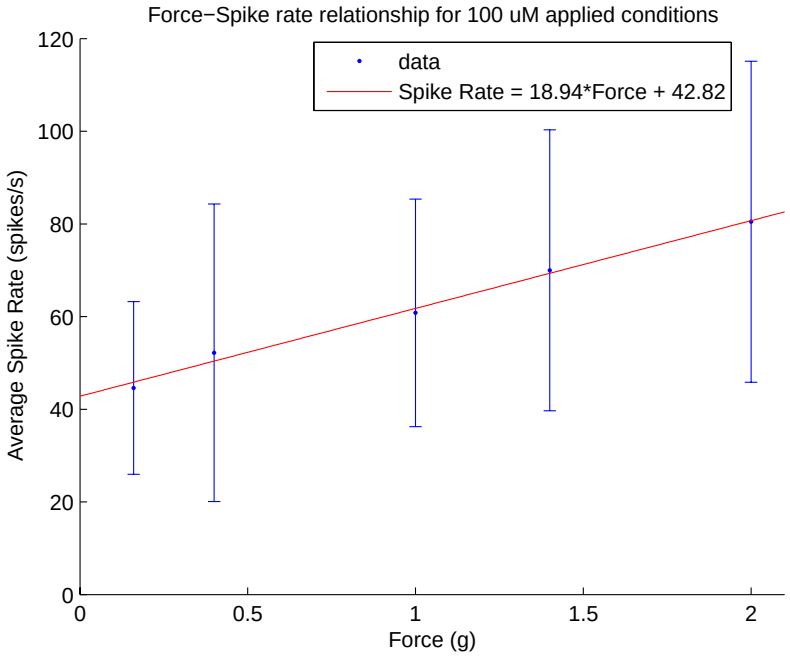

Figure 4.3 Change of SA-I spike rate with changing stimulating force in 100 μM serotonin application (n=7), R^2=0.993.

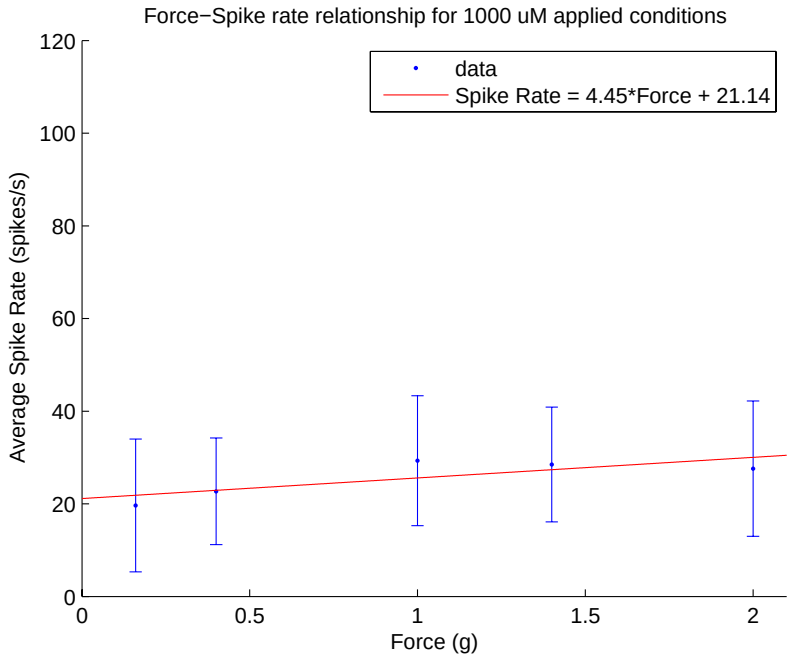

Figure 4.4 Change of spike rate with changing stimulating force in 1000 μM serotonin application (n=6), R^2=0.629.

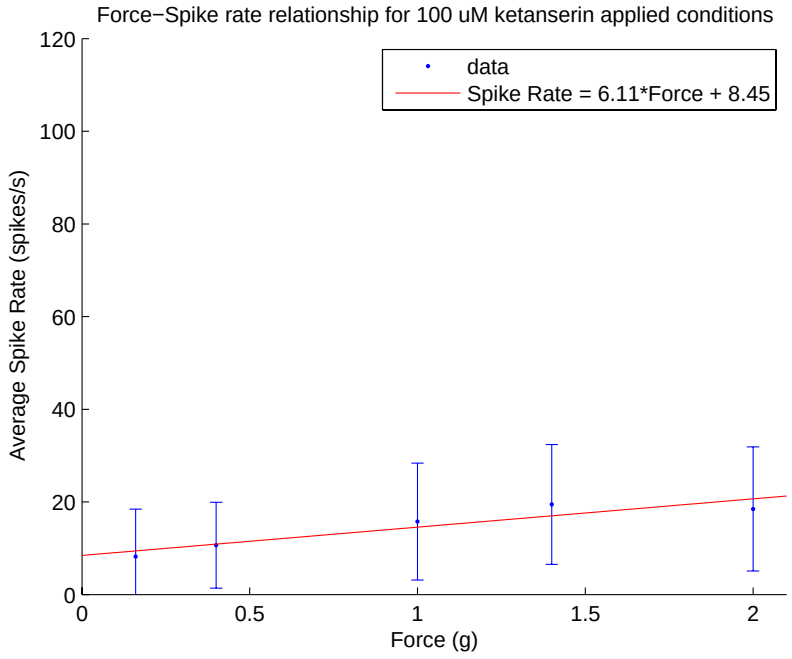

Figure 4.5 Change of spike rate with changing stimulating force in 100 μM ketanserin application (n=7), R^2=0.856.

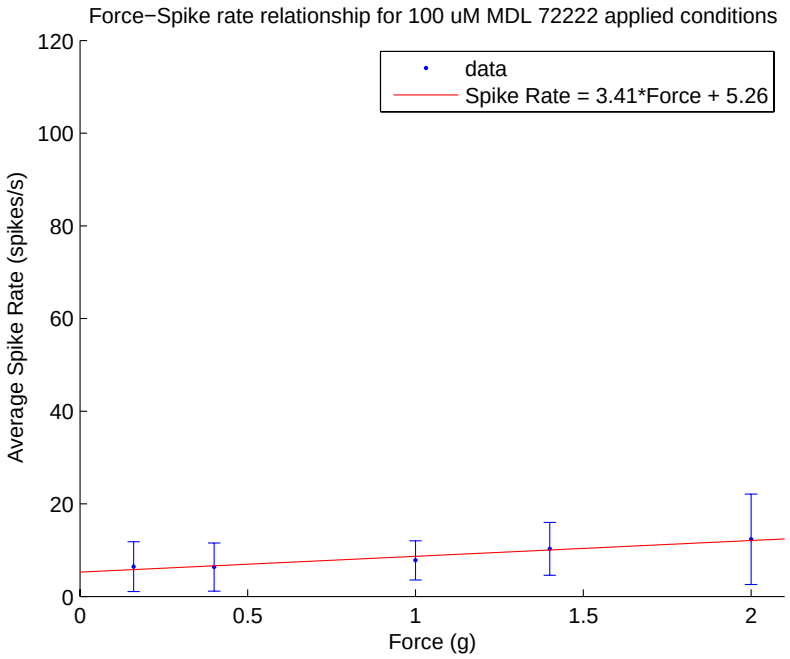

Figure 4.6 Change of spike rate with changing stimulating force for 100 μM MDL 72222 application (n=7), R^2=0.948.

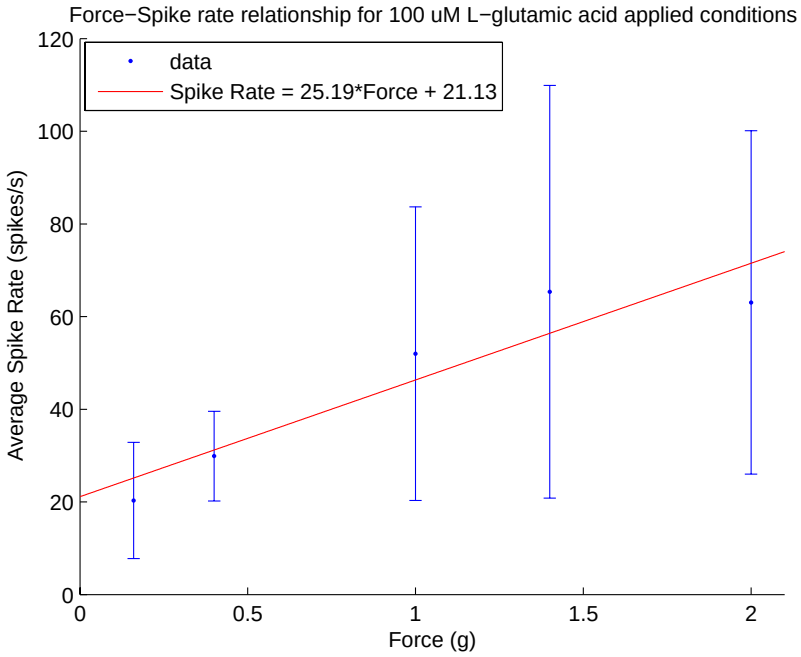

Figure 4.7 Change of spike rate with changing stimulating force for 100 μM L-glutamic acid application (n=5), R^2=0.871.

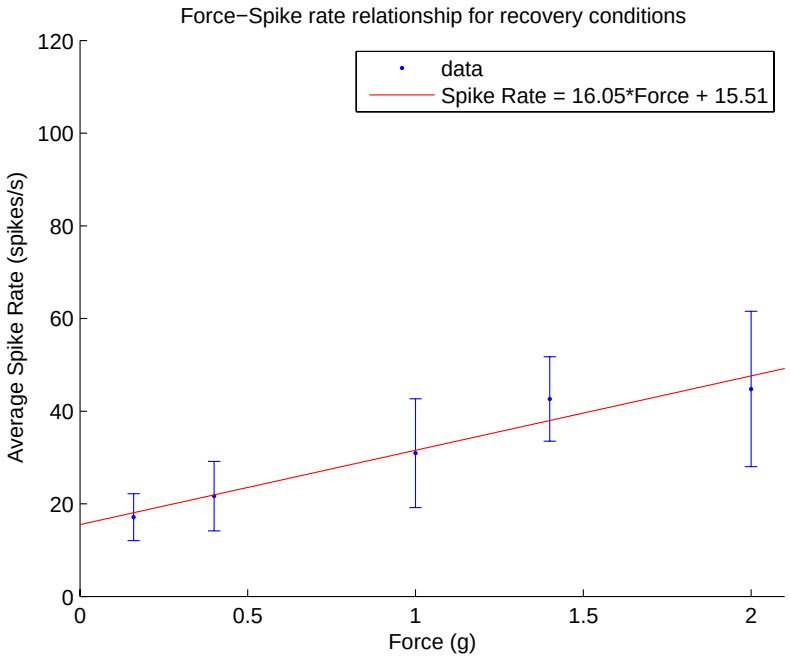

Figure 4.8 Change of spike rate with changing stimulating force for all recovery conditions, R^2=0.948.

increased but the slope decreased. This shows that increase in spike rate was high for small forces and as the force increased the the change in spike rate decreased. For 1000 μM serotonin application and for both antagonists MDL 72222 and ketanserin slope was so small, which shows that under these circumstances SA-I unit could not discriminate well between applied forces. For L-glutamic acid application the slope was almost the same as the baseline slope. Even though the slopes changed with changing chemical agents, they were always positive. Therefore the property of SA-I mechanoreceptive fibers to encode magnitude of stimulation as spike rate was not changed.

4.2 Effect of Serotonin

4.2.1 Effect of 10 μM serotonin on SA-I response

10 μM serotonin was applied to 8 SA-I units and change in response to ramp-and-hold stimulation was observed. Average spike rates of 8 units for baseline, 10 μM

serotonin and recovery conditions was given in Figure 4.9 as bars.

When all data were pooled together spike rate increased from 34.70 ± 26.39 spikes/s to 42.13 ± 28.48 spikes/s (21.41%) and after washing with Ringer's solution it decreased again to 26.24 ± 15.22 spikes/s. In this pooled data, there was a significant increase in spike rate from baseline condition to 10 μM serotonin applied condition (paired t-test, $p < 0.05$). From serotonin applied case to recovery conditions spike rate has also significantly decreased (paired t-test, $p < 0.05$), but to lower rate than baseline condition (paired t-test, $p < 0.05$). Large standard deviations indicate that there is a conciderable variation in responses of different units.

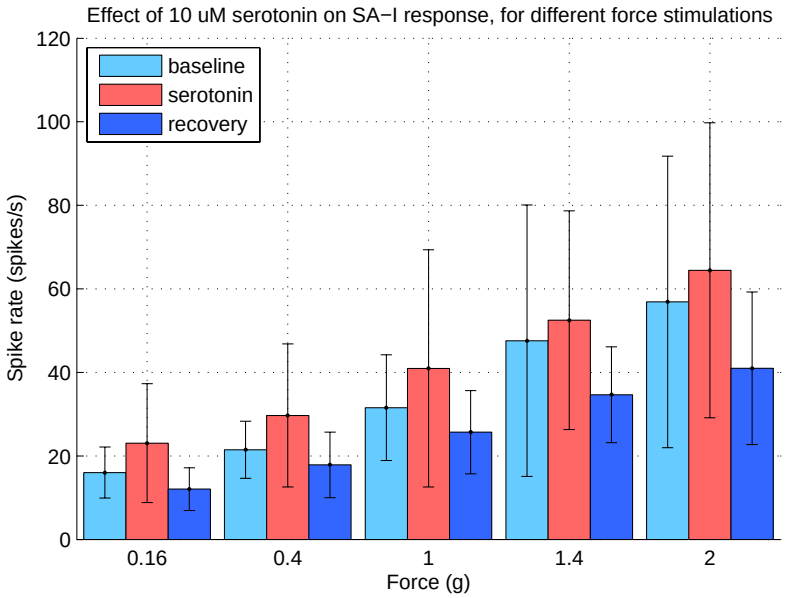

Figure 4.9 Effect of 10 μM serotonin application on SA-I responses obtained by different force stimulations (n=8).

For each force application, serotonin increased the spike rate at almost the same amount. Difference between average baseline spike rate and average 10 μM serotonin applied condition's spike rate are; 7.18 spikes/s (44.91%, paired t-test, $p < 0.05$) for 0.16 g, 8.48 spikes/s (39.48%, paired t-test, $p = 0.05$) for 0.4 g, 9.70 spikes/s (30.74%, paired t-test, $p = 0.09$) for 1 g, 5.02 spikes/s (10.54%, paired t-test, $p = 0.3$) for 1.4 g and 6.51 spikes/s (11.44%, paired t-test, $p = 0.16$) for 2 g. Recovery was obtained in each trial.

4.2.2 Effect of 10 μM serotonin on spontaneous activity

Table 4.1: Effect of 10μM serotonin on spontaneous activity

	Average spontaneous activity for 10μM serotonin (spikes/s)								
Unit	**Ave. spont. activity**			**Pre-stimulus**			**Post-stimulus**		
	B.line	**Ser.**	**Rec**	**B.line**	**Ser.**	**Rec**	**B.line**	**Ser.**	**Rec**
1	0.84	7.20	1.90	0.71	8.45	1.63	0.97	5.96	2.16
	0.38	1.40	0.65	0.27	1.54	0.59	0.58	1.62	0.85
2	2.75	3.05	2.81	2.93	3.71	3.63	2.57	2.39	1.99
	1.47	1.06	1.72	1.56	1.09	2.31	1.41	1.31	1.15
3	6.38	15.16	0.30	7.24	14.81	0.19	5.51	15.52	0.41
	7.82	3.15	0.23	9.15	2.79	0.11	6.49	3.69	0.38
4	3.54	2.94	2.13	3.08	2.99	2.28	4.01	2.88	1.98
	4.79	2.34	1.81	4.20	2.46	2.00	5.39	2.23	1.62
5	5.60	6.72	0.36	6.32	7.38	0.46	4.88	6.07	0.26
	3.38	2.09	0.08	4.57	2.08	0.19	2.87	2.23	0.03
6	1.45	0.94	0.50	1.67	0.85	0.54	1.24	1.03	0.46
	1.36	0.52	0.08	1.70	0.50	0.30	1.07	0.57	0.32
7	3.83	2.31	0.38	4.64	2.17	0.14	3.02	2.45	0.62
	2.57	0.52	0.53	2.91	0.36	0.06	2.32	0.85	1.07
8	0.59	0.54	4.16	0.42	0.51	5.26	0.76	0.57	3.06
	0.48	0.40	2.99	0.44	0.42	4.07	0.54	0.42	1.91
Ave	**2.95**	**3.15**	**1.29**	**3.24**	**3.26**	**1.49**	**2.73**	**3.11**	**1.14**
Std	**2.27**	**3.80**	**1.25**	**2.63**	**3.88**	**1.64**	**1.98**	**3.73**	**0.88**

10 μM exogenous serotonin application resulted in an increase in spontaneous activity of 3 units but decrease in spontaneous activity of 5 units. Average spontaneous activity was calculated by averaging pre- and post-stimulus activities of each recording. Generally it was concluded that average spontaneous spike rate increases from 3.12 ± 3.87 spikes/s to 4.86 ± 4.82 spikes/s and when washed again with Ringer's solution it decreases to 1.57 ± 1.86 spikes/s. Changes between all 3 conditions are significant (paired t-test, $p < 0.05$).

Therefore 10 μM exogenous serotonin application caused a reversible increase in spike rate of both spontaneous activity and the SA-I response.

4.2.3 Effect of 100 μM serotonin on SA-I response

100 μM serotonin was applied to 7 units. As a result of this, increase in spike rate of SA-I responses was observed in all units. Therefore it was concluded that 100 μM serotonin increased the response to mechanical stimulation (paired t-test, $p < 0.05$).

In general, when all data were pooled SA-I spike rate increased from 43.71±25.38 spikes/s to 61.62 ± 29.50 spikes/s (40.97%) after 100 μM serotonin application and then decreased again to 40.19 ± 19.13 spikes/s (8.05%) after washout. There were significant differences between serotonin applied condition and both baseline (paired t-test, $p < 0.05$) and recovery (paired t-test, $p < 0.05$) conditions. Average recovery spike rate is less than the average baseline spike rate, but this change was not significant ($p = 0.13$).

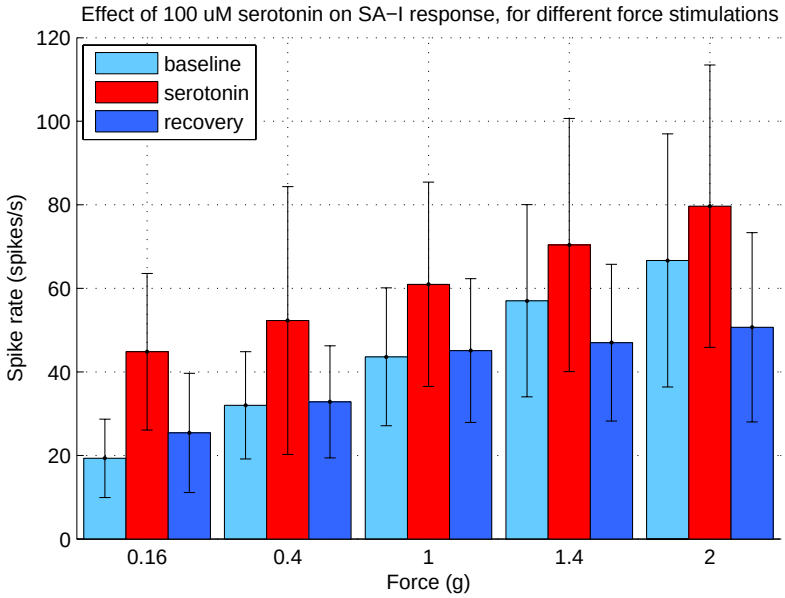

Figure 4.10 Effect of 100 μM serotonin application on SA-I responses obtained by different force stimulations (n=7).

The effect of 100 μM serotonin application was different for different forces. The

increase in spike rate is 20.60 spikes/s (164.31%, paired t-test, $p < 0.05$) for 0.16 g, 14.54 spikes/s (66.90%, paired t-test, $p < 0.05$) for 0.4 g, 10.66 spikes/s (37.33%, paired t-test, $p < 0.05$) for 1 g, 6.56 spikes/s (27.54%, paired t-test, $p < 0.05$) for 1.4 g and 11.16 spikes/s (20.84%, paired t-test, $p = 0.09$) for 2 g. There was a saturation in increase of spike rate as force was increased. Increases were so much higher in 100 μM serotonin application than in 10 μM application.

4.2.4 Effect of 100 μM serotonin on spontaneous activity

Table 4.2: Effect of 10μM serotonin on spontaneous activity

	Average spontaneous activity for 10μM serotonin (spikes/s)								
Unit	**Ave. spont. activity**			**Pre-stimulus**			**Post-stimulus**		
	B.line	**Ser.**	**Rec**	**B.line**	**Ser.**	**Rec**	**B.line**	**Ser.**	**Rec**
1	4.36	3.96	4.06	6.78	3.81	2.48	1.95	4.10	5.65
	1.97	0.85	1.08	3.66	1.62	0.43	0.50	1.98	2.21
2	1.33	1.89	0.79	1.49	2.14	1.00	1.16	1.65	0.58
	0.92	0.51	0.35	0.96	0.83	0.47	0.88	0.81	0.27
3	12.13	7.37	7.76	13.61	7.51	8.84	10.66	7.24	6.68
	3.54	5.04	1.70	3.66	5.02	1.53	3.58	5.06	2.17
4	0.43	2.09	0.29	0.24	2.35	0.20	0.63	1.83	0.38
	0.29	0.83	0.20	0.17	0.93	0.14	0.50	0.77	0.33
5	20.84	40.48	18.20	22.43	43.08	19.19	19.26	37.87	17.22
	17.27	16.29	10.10	18.51	17.35	9.44	16.18	15.48	11.00
6	0.49	1.84	1.33	0.41	1.79	1.63	0.58	1.89	1.02
	0.68	0.71	0.87	0.52	0.43	1.21	0.85	1.27	0.56
7	4.56	4.59	1.96	4.41	3.95	1.94	4.71	5.22	1.98
	1.72	2.89	1.30	1.74	2.70	1.19	1.71	3.13	1.44
Ave	**5.04**	**6.38**	**3.57**	**5.61**	**6.68**	**3.55**	**4.51**	**6.31**	**3.68**
Std	**6.72**	**10.64**	**5.15**	**7.28**	**11.33**	**5.40**	**6.25**	**9.87**	**4.98**

Spontaneous activity of 7 units was observed in Table 4.4. For the 100 μM

serotonin applied condition the average spontaneous spike rate increased from 6.39 ± 9.40 spikes/s to 8.89 ± 14.50 spikes/s (paired t-test, $p = 0.1$) and it decreased again to 4.91 ± 6.99 spikes/s (paired t-test, $p < 0.05$) in recovery. The difference between baseline spontaneous spike rate and recovery spontaneous spike rate was not significant (paired t-test,$p = 0.11$), which means there was a complete recovery.

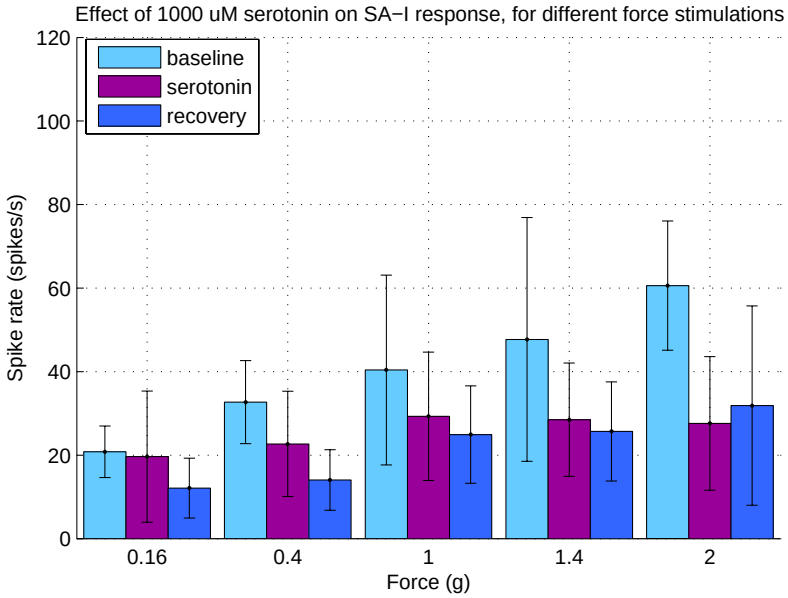

Figure 4.11 Effect of 1000 μM serotonin application on SA-I responses obtained by different force stimulations (n=6).

In conclusion 100 μM exogenous serotonin application increased the SA-I response but did not affect the spontaneous activity as much as it affected SA-I response.

4.2.5 Effect of 1000 μM serotonin on SA-I response

1000 μM serotonin was applied to 6 units. SA-I responses were consistent for 5 units but for unit 1, in Table 4.5, SA-I spike rate increased reversibly. For other five units SA-I spike rate decreased irreversibly in response to 1000 μM serotonin application. In other words recovery was not obtained all units.

When all the data were pooled the average spike rate decreased from 40.44 ± 15.03 spikes/s to 25.54 ± 4.18 spikes/s (36.84%), and in recovery condition it decreased

further to 21.73 ± 8.37 spikes/s (46.26%). Decrease in spike rate from baseline to 1000 μM serotonin applied condition was significant (paired t-test, $p < 0.05$) similar to the decrease from serotonin to recovery conditions (paired t-test, $p < 0.05$).

Decrease in spike rate was smaller for smaller forces and as the stimulus force increased, the decrease increased. Decrease in spike rate obtained with 1000 μM serotonin application was 1.16 spikes/s (5.57%, paired t-test, p=0.4) for 0.16 g, 10.00 spikes/s (30.55%, paired t-test, $p < 0.05$) for 0.4 g , 11.09 spikes/s (27.45%, paired t-test,p=0.06) for 1 g, 19.22 spikes/s (40.27%, paired t-test, $p < 0.05$) for 1.4 g and 33.00 spikes/s (54.44%,paired t-test, $p < 0.05$) for 2 g stimulations.

4.2.6 Effect of 1000 μM serotonin on spontaneous activity

Table 4.3: Effect of 1000μM serotonin on spontaneous activity

	Average spontaneous activity for 1000μM serotonin (spikes/s)								
Unit	**Ave. spont. activity**			**Pre-stimulus**			**Post-stimulus**		
	B.line	**Ser.**	**Rec**	**B.line**	**Ser.**	**Rec**	**B.line**	**Ser.**	**Rec**
1	1.66	2.50	0.80	1.39	3.24	0.92	1.93	1.76	0.67
	0.85	0.44	0.22	0.62	0.70	0.41	1.09	1.05	0.47
2	1.73	0.45	0.60	2.26	0.63	0.99	1.22	0.30	0.21
	0.81	0.20	0.32	1.19	0.28	0.41	0.47	0.11	0.27
3	1.61	0.69	0.75	1.44	0.50	0.71	1.77	0.87	0.79
	0.32	0.20	0.62	0.33	0.30	0.61	0.36	0.20	0.71
4	1.90	1.15	3.44	1.55	1.12	3.15	2.24	1.18	3.73
	0.77	0.65	2.68	0.93	0.80	2.95	0.72	0.66	2.45
5	4.26	16.67	0.57	4.49	17.52	0.21	4.02	15.82	0.92
	4.48	9.30	0.61	5.60	7.85	0.21	3.45	10.94	1.16
6	4.76	0.73	0.08	4.87	0.48	0.13	4.65	0.99	0.02
	2.27	0.72	0.10	1.63	0.61	0.18	3.03	0.91	0.04
Ave	**2.12**	**2.81**	**0.90**	**2.19**	**2.84**	**0.91**	**2.08**	**2.90**	**0.95**
Std	**1.54**	**5.04**	**1.05**	**1.77**	**5.10**	**1.04**	**1.43**	**5.02**	**1.09**

Irreversible decrease in SA-I response was seen consistently in spontaneous activity of 5 of the units. However, for one unit, spontaneous activity showed a sharp increase in response to 1000 μM serotonin application. When data were pooled average spontaneous activity increased form 2.62 ± 2.39 spikes/s to 3.71 ± 6.88 spikes/s and then in recovery it decreased to 1.01 ± 1.55 spikes/s. Unit 5 which showed a considerable increase in spontaneous activity changed the overall average. When the unit that showed a high increase in spontaneous activity was considered separately, a consistent irreversible decrease in spontaneous activity was seen for 5 units. For those units, spontaneous spike rate decreased from 2.29 ± 1.76 spikes/s to 1.12 ± 0.99 spikes/s (paired t-test, $p < 0.05$) and decreased further to 1.10 ± 1.16 spikes/s (paired t-test, $p < 0.05$) in recovery conditions. For these 5 units decrease from baseline to 1000 μM serotonin applied conditions (paired t-test, $p < 0.05$) and decrease from baseline to recovery condition (paired t-test, $p < 0.05$) were significant. However, decrease from 1000 μM serotonin applied conditions to recovery (paired t-test, $p = 0.4$) was not.

4.2.7 Effect of 10, 100 and 1000 μM serotonin on SA-I response

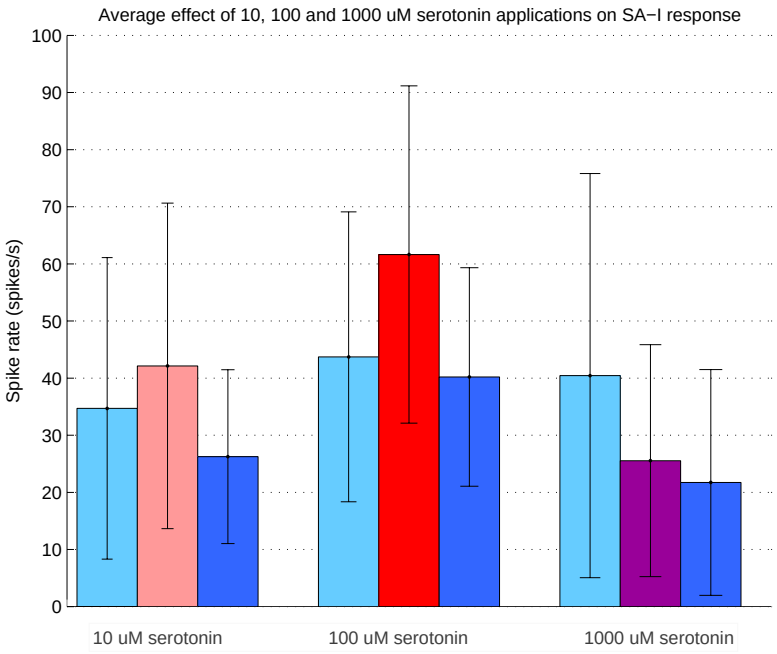

Figure 4.12 Effect of 10, 100 and 1000 μM serotonin application on SA-I responses obtained by averaging the data for all different force applications.

Analysis of Variance

Source	Sum Sq.	d.f.	Mean Sq.	F	Prob>F
X1	104864.9	4	26216.2	4.04	0.0046
X2	155504.1	2	77752	11.99	0
X1*X2	87467.6	8	10933.4	1.69	0.1124
Error	583408.2	90	6482.3		
Total	929044.3	104			

Constrained (Type III) sums of squares.

Figure 4.13 Output table of n-way anova X1=different forces, X2=different molarities of serotonin.

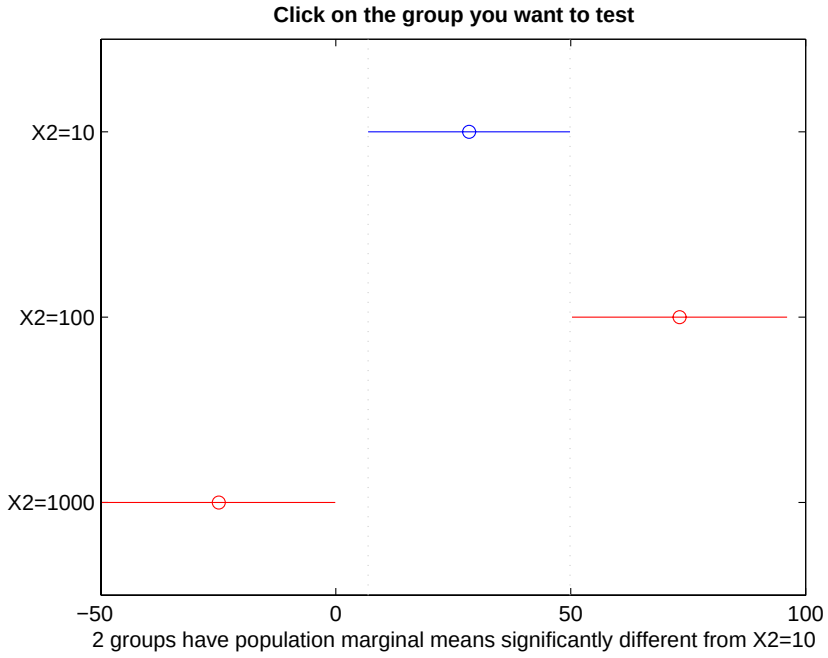

Figure 4.14 Marginal mean of percenteges of changes of 10 uM serotonin applied condition was different than means of 100 and 1000 uM serotonin applied conditions.

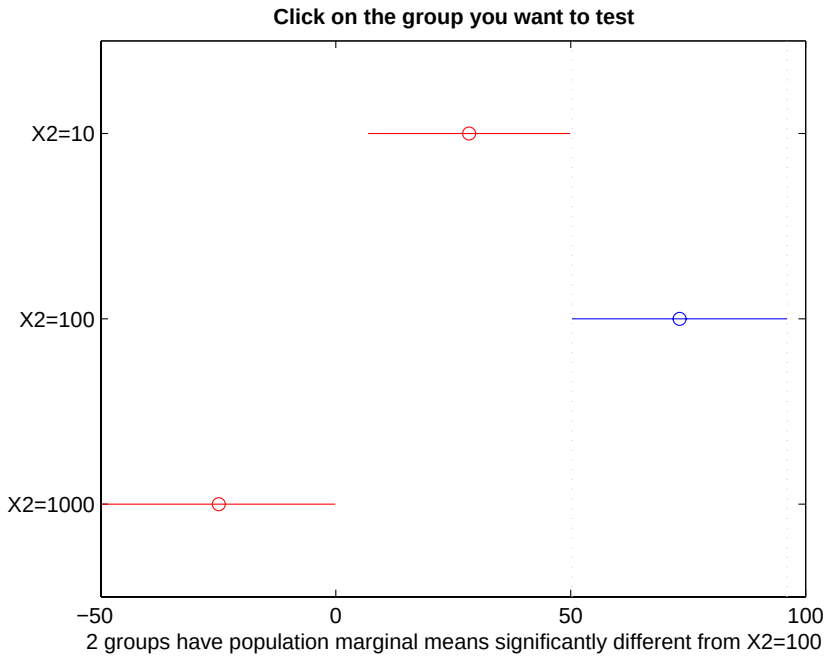

Figure 4.15 Marginal mean of percenteges of changes of 100 uM serotonin applied condition was different than means of 10 and 1000 uM serotonin applied conditions.

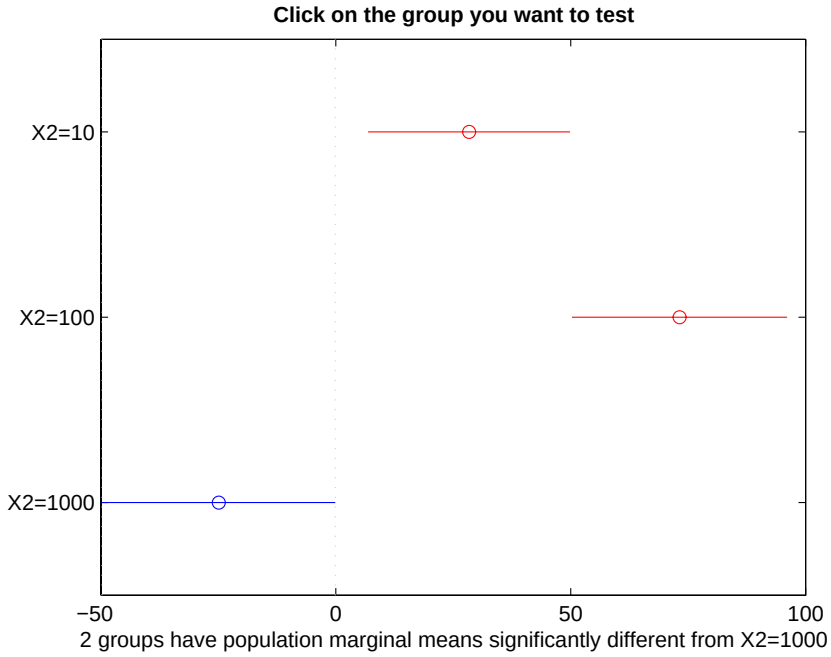

Figure 4.16 Marginal mean of percenteges of changes of 1000 uM serotonin applied condition was different than means of 10 and 100 uM serotonin applied conditions.

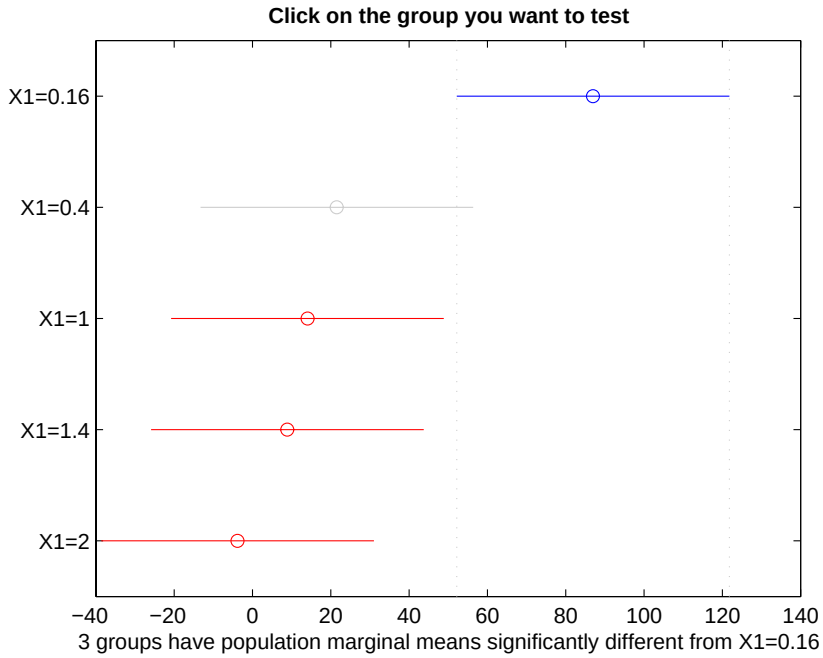

Figure 4.17 SA-I mechanoreceptive fibers responded to 0.16 g differently than they respond to 1, 1.4 and 2 g stimulations.

Figure 4.12 shows the effect of 10, 100 and 1000 μM serotonin according to the averaged data for all force applications. In pooled data, there was a significant increase in spike rate from baseline condition to 10 μM serotonin applied condition (21.41%,paired t-test, $p < 0.05$) and from baseline to 100 μM serotonin applied condition (40.97%, paired t-test, $p < 0.05$). A significant decrease from baseline to 1000 μM serotonin applied condition was observed (36.84%, paired t-test, $p < 0.05$).

As observed in Figures: 4.13-4.17 that marginal means of the percentages of increases obtained by 10 and 100 μM serotonin application and the decrease obtained by 1000 μM serotonin application were significantly different from each other (n-way anova, $p < 0.05$).

Additionally, according to the averaged data for all three concentrations of serotonin applications, SA-I mechanoreceptive fibers respond to 0.16 g stimulations with spike rates significantly different than 1, 1.4, 2g stimulations (n-way anova, $p < 0.05$), independent of the concentration of serotonin applied (n-way anova, p=0.11).

4.3 Effect of 5-HT$_3$ Receptor Antagonist MDL 72222

4.3.1 Effect of 100 μM MDL 72222 on SA-I response

MDL 72222 which is a 5-HT$_3$ receptor antagonist was exogenically applied to 7 SA-I mechanosensitive units. Spike rate in response to mechanical stimulation decreased considerably for all units.

When data were pooled the spike rate decreased from 63.43 ± 25.14 spikes/s to 8.64 ± 6.42 spikes/s (86.37%) with 100 μM MDL 72222 application, and in recovery conditions spike rate increased to 35.21 ± 21.56 spikes/s. Changes in all three conditions were significant (paired t-test, $p < 0.05$).

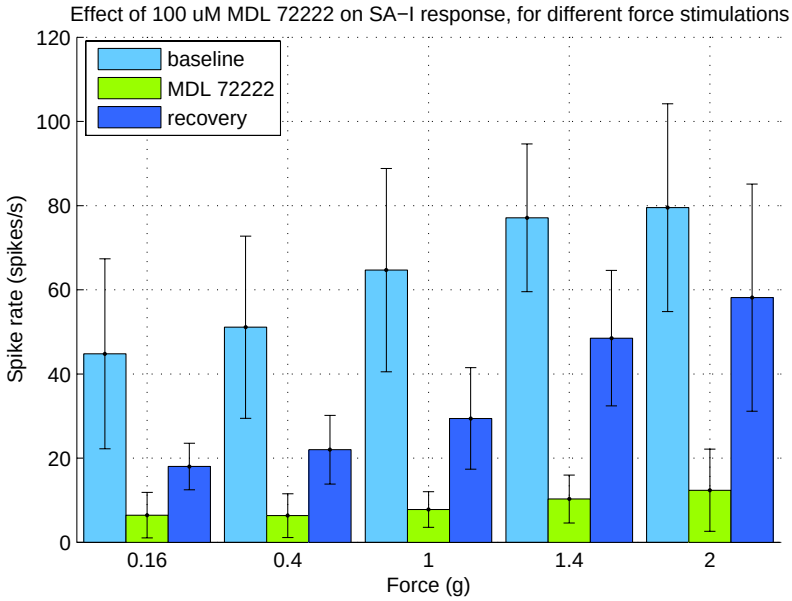

Figure 4.18 Effect of 100 μM MDL 72222 application on SA-I responses obtained by different force stimulations (n=7).

Effects of 100 μM MDL 72222 was consistent for all force applications. In MDL 72222 applied conditions, maximum spike rate is 31.03 spikes/s. 5-HT$_3$ receptor antagonist MDL 72222 considerably decreased the SA-I response to stimuli in the specified range.

SA-I response spike rate decreased to 38.34 spikes/s (85.60%,paired t-test, $p < 0.05$) for 0.16 g, 44.75 spikes/s (87.56%, paired t-test, $p < 0.05$) for 0.4 g, 56.89 spikes/s (87.95%, paired t-test, $p < 0.05$) for 1 g, 66.81 spikes/s (86.66%, paired t-test, $p < 0.05$) for 1.4 g, 67.14 spikes/s (84.44%, paired t-test, $p < 0.05$) for 2 g stimuli.

4.3.2 Effect of 100 μM MDL 72222 on the spontaneous activity

Average spontaneous activity decreased from 11.59±13.00 spikes/s to 0.41±0.43 spikes/s when 100 μM MDL 72222 was applied (paired t-test, $p < 0.05$). Since in recovery conditions spike rate did only increased to 0.52 ± 0.48 spikes/s it can be said that the decrease due to the MDL application was irreversible (paired t-test, $p = 0.14$). The decrease of baseline activity to recovery conditions was significant (paired t-test, $p < 0.05$). In summary MDL 72222 decreased the spontaneous activity irreversibly but decreased the SA-I response reversibly.

Table 4.4: Effect of 100μM MDL 72222 on spontaneous activity

	Average spontaneous activity for 100μM MDL (spikes/s)								
Unit	**Ave. spont. activity**			**Pre-stimulus**			**Post-stimulus**		
	B.line	**MDL**	**Rec**	**B.line**	**MDL**	**Rec**	**B.line**	**MDL**	**Rec**
1	17.25	0.34	0.67	21.02	0.30	0.70	13.49	0.38	0.64
2	17.62	0.40	0.65	26.38	0.21	0.81	8.87	0.58	0.48
3	34.06	0.68	0.61	38.73	0.23	0.36	29.38	1.13	0.86
4	0.54	0.17	0.20	0.54	0.14	0.20	0.55	0.19	0.20
5	5.37	0.26	0.25	5.44	0.24	0.29	5.31	0.28	0.20
6	4.74	0.14	0.24	5.77	0.17	0.19	3.71	0.11	0.28
7	1.56	0.92	1.04	0.61	0.73	0.40	2.51	1.11	1.68
Ave	**11.59**	**0.41**	**0.52**	**14.07**	**0.29**	**0.43**	**9.12**	**0.54**	**0.62**
Std	**12.12**	**0.29**	**0.31**	**14.81**	**0.20**	**0.24**	**9.92**	**0.42**	**0.53**

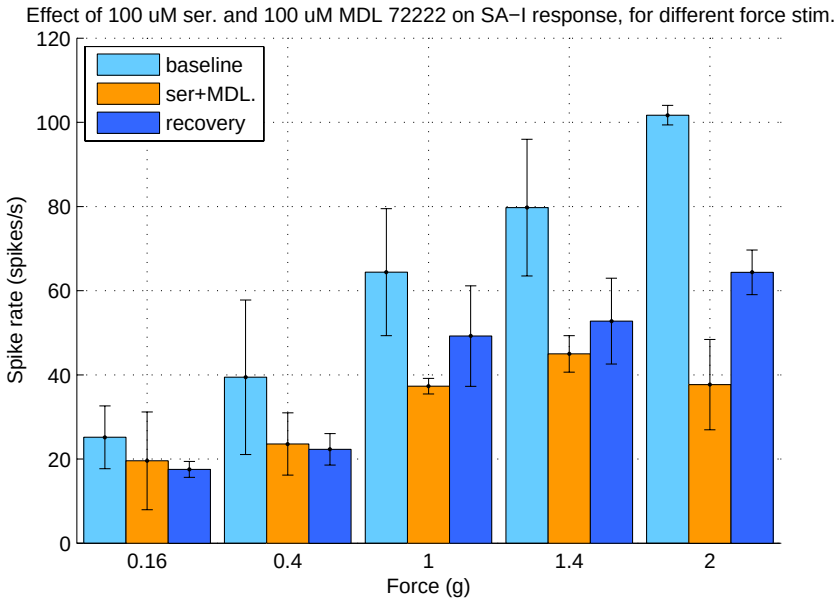

Figure 4.19 Effect of 100 μM serotonin and 100 μM MDL 72222 application on SA-I responses obtained by different force stimulations (n=2).

4.4 Effect of Serotonin and MDL 72222

4.4.1 Effect of 100 μM Serotonin and 100 μM MDL 72222 on SA-I response

Due to the strong effect of 100 μM MDL 72222 it was thought that 5-HT_3 could be the only receptor that caused an increase in SA-I activity when serotonin was applied exogenically. Two experiments were performed to test this theory.

As observed in Table 4.4, when 100 μM serotonin was applied alone it caused a significant increase in SA-I spike rate while 100 μM MDL 72222 was applied alone it causes a sharp decrease in SA-I spike rate. They were subsequently applied together and the result was a decrease in the SA-I spike rate not as much as in the case when MDL 72222 was applied alone. This suggests the presence of another serotonin receptor for the mechanotransduction of SA-I units.

When data were pooled together for all units and forces it was observed that spike rate decreased from 62.08 ± 30.57 spikes/s to 32.63 ± 11.69 spikes/s. This decrease

was statistically significant (paired t-test, $p < 0.05$). SA-I spike rate increased (paired t-test, $p < 0.05$) to 41.22 ± 19.99 spikes/s in recovery conditions. There was also a significant decrease (paired t-test, $p < 0.05$) in SA-I response from baseline condition to recovery.

The effects of serotonin and MDL 72222 mixed solution on SA-I response to different force applications was analyzed. No increase in the spike rate was observed and decreases were not as sharp as only MDL 72222 application for any given force. Therefore the responses were similar for all the forces in the specified range.

4.4.2 Effect of 100μM Serotonin and 100μM MDL 72222 on spontaneous activity

Table 4.5: Effect of 100μM serotonin and 100μM MDL 72222 on spontaneous activity

		Average spontaneous activity for 100μM MDL (spikes/s)								
Unit	**F(g)**	**Ave. spont. activity**			**Pre-stimulus**			**Post-stimulus**		
		B.line	**MDL**	**Rec**	**B.line**	**MDL**	**Rec**	**B.line**	**MDL**	**Rec**
1	**0.2**	2.39	0.28	0.59	1.62	0.09	0.43	3.15	0.46	0.75
	0.4	10.13	0.69	1.09	8.05	0.52	0.78	12.20	0.85	1.40
	1	2.20	2.84	1.02	2.61	0.76	0.34	1.78	4.92	1.70
	1.4	4.06	2.52	1.75	3.78	1.05	0.29	4.33	3.99	3.20
	2	4.00	1.38	1.74	3.15	1.56	0.57	4.85	1.20	2.90
2	**0.2**	1.02	0.31	0.10	0.66	0.14	0.04	1.38	0.48	0.15
	0.4	2.62	1.69	0.16	2.22	0.24	0.17	3.01	3.14	0.14
	1	3.16	0.35	1.82	3.19	0.12	0.92	3.12	0.58	2.72
	1.4	5.06	0.55	0.37	5.09	0.32	0.40	5.02	0.78	0.33
	2	5.23	1.55	0.32	5.19	0.81	0.19	5.26	2.29	0.45
	Av.	**3.98**	**1.22**	**0.89**	**3.56**	**0.56**	**0.41**	**4.41**	**1.87**	**1.37**
	St.Dev.	**2.53**	**0.94**	**0.69**	**2.12**	**0.48**	**0.28**	**3.04**	**1.63**	**1.20**

Spontaneous activity decreased in response to 100 μM Serotonin and 100 μM MDL 72222 application from 3.98 ± 2.52 spikes/s to 1.21 ± 0.93 spikes/s (paired t-test, $p < 0.05$). In recovery condition it decreased even further to 0.89 ± 0.68 spikes/s, but the difference between mixed solution application and the recovery was not significant (paired t-test, $p = 0.1$). SA-I spike rate decreased significantly from baseline to recovery condition (paired t-test, $p < 0.05$). Therefore spontaneous activity decreased irreversibly when 100 μM Serotonin and 100 μM MDL 72222 was exogenically applied. It may be concluded that 5-HT_3 was not the only serotonin receptor subtype active in generating SA-I response.

4.5 Effect of 5-HT_2 Receptor Antagonist Ketanserin

4.5.1 Effect of 100 μM Ketanserin on SA-I response

100 μM 5-HT_2 receptor antagonist ketanserin was exogenically applied and the changes in the responses to mechanical stimulation and in spontaneous activity of 7 SA-I units were analyzed. 100 μM ketanserin application effectively decreased the SA-I spike rate.

Considering the pooled data from all units and all force applications it was observed that spike rate decreased from 65.46 ± 34.84 spikes/s to 14.50 ± 11.94 spikes/s (77.84%) when 5-HT_2 receptors were blocked by 100 μM ketanserin (paired t-test, $p < 0.05$). In recovery conditions spike rate increased again to 37.26 ± 24.51 spikes/s (42.97%) (paired t-test, $p < 0.05$). In recovery conditions spike rate decreased (paired t-test, $p < 0.05$) compared to baseline condition.

The decrease in SA-I spike rate is consistent for all force applications. SA-I spike rate decreased to 20.03 spikes/s (71.00%, paired t-test, $p < 0.05$) for 0.16 g, 25.28 spikes/s (70.36%, paired t-test, $p < 0.05$) for 0.4 g , 50.16 spikes/s (76.09%, paired t-test, $p < 0.05$) for 1 g, 76.95 spikes/s (79.81%, paired t-test, $p < 0.05$) and 82.37 spikes/s (81.67%, paired t-test, $p < 0.05$) for 2 g stimuli.

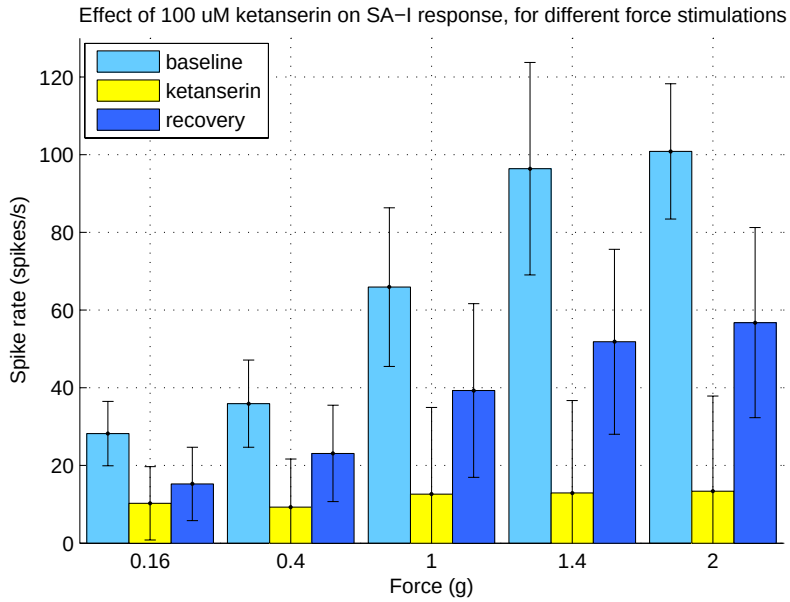

Figure 4.20 Effect of 100 μM ketanserin application on SA-I responses obtained by different force stimulations (n=7).

4.5.2 Effect of 100 μM Ketanserin on spontaneous activity

The effect of 100 μM ketanserin was analyzed with data from 7 SA-I units. Response patterns were consistent for all 7 units. Spontaneous activity decreased irreversibly from 4.22 ± 4.55 spikes/s to 1.04 ± 0.71 spikes/s and in recovery it decreased more to 0.75 ± 0.88 spikes/s.

There was a significant difference between baseline and ketanserin applied condition (paired t-test, $p < 0.05$) and between ketanserin applied condition and recovery spontaneous spike rate (paired t-test, $p < 0.05$). There was a significant difference between baseline and recovery spontaneous spike rates (paired t-test, $p < 0.05$). There was a significant difference between all three conditions.

Briefly 100 μM exogenous ketanserin application resulted in a reversible decrease in response mechanical stimuli of SA-I mechanoreceptive fibers but an irreversible decrease in spontaneous activity.

Table 4.6: Effect of 100μM ketanserin on spontaneous activity

	Average spontaneous activity for 100μM Ketan. (spikes/s)								
Unit Numb.	**Ave. spont. activity**			**Pre-stimulus**			**Post-stimulus**		
	B.line	**MDL**	**Rec**	**B.line**	**Ketan.**	**Rec**	**B.line**	**Ketan.**	**Rec**
1	2.64	0.61	0.21	1.88	0.66	0.19	3.39	0.56	0.23
2	1.83	1.84	0.32	2.27	1.96	0.41	1.40	1.72	0.22
3	1.05	0.46	0.46	0.54	0.56	0.47	1.56	0.36	0.45
4	12.64	0.76	0.40	12.60	0.79	0.39	12.68	0.72	0.41
5	4.03	1.68	1.56	5.49	1.82	1.59	2.58	1.54	1.53
6	4.12	1.21	1.44	6.03	1.83	2.18	2.21	0.59	0.71
7	3.26	0.72	0.83	3.95	0.63	0.93	2.56	0.81	0.74
Average	**4.22**	**1.04**	**0.75**	**4.68**	**1.18**	**0.88**	**3.77**	**0.90**	**0.61**
St.Dev.	**4.5527**	**0.71**	**0.88**	**4.909**	**0.8**	**1.2361**	**4.573**	**0.737**	**0.6914**

4.6 Effect of L-glutamic acid

4.6.1 Effect of 100 μM L-glutamic acid on SA-I response

100 μM L-glutamic acid was applied to 5 SA-I units. It caused an irreversible decrease in response to mechanical stimuli of 4 units, but for 1 unit, spike rate increased reversibly. When data were pooled for all units and all force applications spike rate decreased from 51.40 ± 31.38 spikes/s to 46.12 ± 28.98 spikes/s and decreased further in recovery conditions to 37.37 ± 28.98 spikes/s. Decrease in spike rate from baseline to L-glutamic acid applied condition was significant(paired t-test, $p < 0.05$). Decrease from L-glutamic acid applied condition to recovery was also significant(paired t-test, $p < 0.05$).

L-glutamic acid had almost no significant change in SA-I response for different force stimuli. For 0.16 g SA-I spike rate decreased to 20.30 spikes/s (11.80%, paired

t-test, $p = 0.17$), for 0.4 g to 29.88 spikes/s (8.00%, paired t-test, $p = 0.2$), for 1 g to 51.99 spikes/s (1.59%, paired t-test, $p = 0.46$), for 1.4 g to 65.35 spikes/s (4.68%, paired t-test, $p = 0.29$) and for 2 g to 63.05 spikes/s(21.23%, paired t-test, $p = 0.05$). For all five forces 100 μM L-glutamic acid decreased SA-I activity and, in recovery SA-I activity decreased to smaller spike rates than L-glutamic acid applied conditions. This concentration may have caused excitotoxicity in Merkel cells. In conclusion the effect of L-glutamic acid on SA-I activity should be tested in smaller concentrations to see the effect in detail.

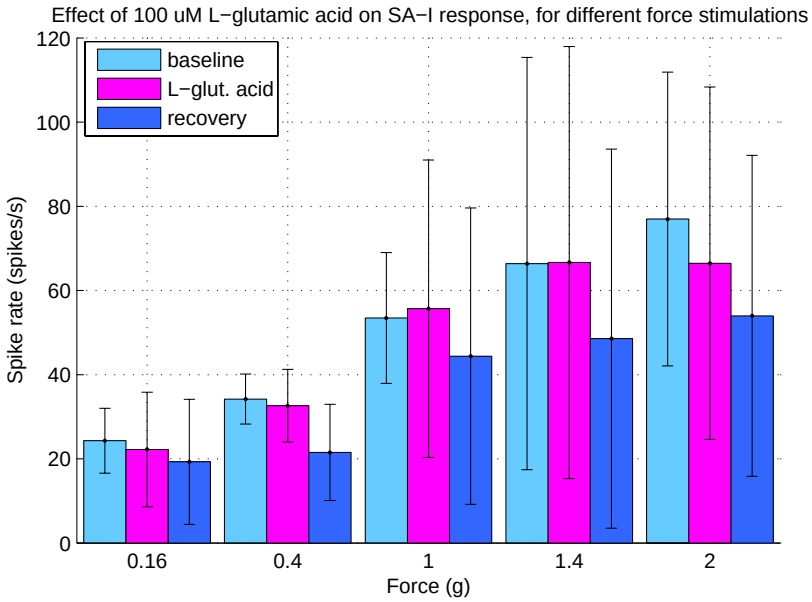

Figure 4.21 Effect of 100 μM L-glutamic acid application on SA-I responses obtained by different force stimulations (n=5).

4.6.2 Effect of 100 μM L-glutamic acid on spontaneous activity

As observed in the Table 4.7, 100 μM L-glutamic acid had no significant effect on spontaneous activity. There was only significant decrease from 100 μM L-glutamic acid applied condition to recovery condition (paired t-test, $p < 0.05$). Therefore 100 μM L-glutamic acid had a decreasing effect on SA-I activity but no effect on spontaneous activity.

Table 4.7: Effect of 100μM L-glutamic acid on spontaneous activity

		Average spontaneous activity for 100μM L-glut. (spikes/s)							
Unit	**Ave. spont. activity**			**Pre-stimulus**			**Post-stimulus**		
	B.line	**L-glut.**	**Rec**	**B.line**	**L-glut.**	**Rec**	**B.line**	**L-glut.**	**Rec**
1	0.82	0.73	0.27	0.78	0.66	0.28	0.87	0.80	0.26
2	3.79	3.77	6.76	2.31	4.16	6.98	5.27	3.37	6.53
3	0.54	0.15	0.67	0.55	0.19	0.60	0.52	0.11	0.75
4	1.55	0.81	0.24	0.86	0.77	0.18	2.25	0.85	0.30
5	1.35	1.34	2.08	1.13	1.14	1.79	1.57	1.53	2.36
Av.	**1.68**	**1.37**	**1.99**	**1.13**	**1.45**	**2.01**	**2.23**	**1.28**	**1.96**
Std	**1.47**	**1.63**	**3.19**	**0.80**	**1.83**	**3.32**	**2.16**	**1.43**	**3.06**

5. DISCUSSION

5.1 Effect of Serotonin

10 μM serotonin application was observed to cause significant increase in both SA-I response and in spontaneous activity. 100 μM serotonin increased SA-I response more than 10 μM, but spontaneous activity did not change significantly. 1000 μM serotonin application decreased both SA-I response and spontaneous activity irreversibly.

Table: 5.1 shows that serotonin affected SA-I response and spontaneous activity differently. This difference suggested that increase in SA-I activity was not due to peripheral sensitization of nerve fibers. Serotonin must be affecting SA-I activity and spontaneous activity by taking part into different reactions.

Table 5.1: Brief summary of all results obtained.

Applied chemical agent	SA-I resp.	Spont. activity
10 μM ser.	$\uparrow$ (*rev*)	$\uparrow$ (*rev*)
100 μM ser.	$\uparrow$ (*rev*)	no change
1000 μM ser.	$\downarrow$ (*irrev*)	no change
100 μM MDL.	$\downarrow$ (*rev*)	$\downarrow$ (*irrev*)
100 μM MDL+ser.	$\downarrow$ (*rev*)	$\downarrow$ (*irrev*)
100 μM ketan.	$\downarrow$ (*rev*)	$\downarrow$ (*irrev*)
100 μM L-glut.	$\downarrow$ (*irrev*)	no change

These results are consistent with the results of He, Tuckett and English published in 1999 [37]. They performed in vitro and in vivo physiological recording from rat dorsal skin, dorsal cutaneous nerves. They applied 5, 50 and 500 μM serotonin dissolved in SIF (synthetic interstitial fluid) and serotonin was intravenously applied by electronically controlled syringe pump (0.2 ml/min). They found that 5 μM serotonin application increased SA-I activity and 50 μM increases SA-I activity more than 5 μM

but 500 μM caused a decrease in SA-I response, which is exactly the same pattern that I have found for effects of 10, 100 and 1000 μM serotonin on frog dorsal skin.

I also have found that spontaneous activity only increased significantly for 10 μM serotonin application. For 1000 μM application spontaneous activity of one unit increased significantly but for other five units spontaneous activity decreased irreversibly. He, Tuckett and English reported no significant change between spontaneous activity of baseline and serotonin applied conditions.

According to our results for 10 μM serotonin application the change in SA-I response (21.40%) and the change in spontaneous activity (35.72%) were qualitatively similar, they both increased significantly. Due to the increase in spontaneous activity it may be suggested that 10 μM serotonin affects the nerve ending by changing the excitibility. This could be due to the decrease in K^+ gradient so a subtype of serotonin receptor subtypes permeable to K^+ is a candidate. 5-HT_3 and 5-HT_2 are two candidates since their antagonists decreased spontaneous activity. Existence of 5-HT_1 autoreceptors on both Merkel cells and on nerve ending was mentioned in the introduction section. So since 100 μM serotonin application caused increase in SA-I response (29.05%) more than 10 μM application but there is no significant change in spontaneous activity, event hough the average spontaneous activity increases in average (29.04%). This brings the idea that 5-HT_1 autoreceptors are activated at higher concentrations of serotonin than 10 μM, and are active at 100 μM application conditions. 1000 μM serotonin application caused irreversible decrease of both SA-I activity and spontaneous activity. This irreversible decrease is thought to occur due to induction of serotonin excitotoxicity in the Merkel cells. It is concluded that 100 μM serotonin application is close to optimal for observing the changes in SA-I activity since there is no significant change in spontaneous activity and changes were reversible.

5.2 Effect of MDL 72222 and Ketanserin

I found that ketanserin decreased SA-I activity from 65.46±34.84 spikes/s to 14.50±11.94 spikes/s and in recovery SA-I response increased to 37.26±24.51 spikes/s. At the same time, spontaneous activity decreased from 4.22±4.55 spikes/s to 1.04±0.71 spikes/s but in recovery there was further decrease to 0.75±0.88 spikes/s. And the changes between both baseline and ketanserin applied condition and baseline and recovery conditions were significant ($p<0.05$). But difference between ketanserin applied condition and recovery was almost significant ($p=0.04$).

MDL 72222 decreased SA-I response from 63.43±25.14 spikes/s to 8.64±6.42 spikes/s then in recovery SA-I response increased to 35.21±21.56 spikes/s also caused the same changes as ketanserin did on spontaneous activity. MDL 72222 decreased spontaneous activity from 11.69±13.19 spikes/s to 0.41±0.44 spikes/s and in recovery it decreased further to 0.52±0.49 spikes/s. Again changes between both baseline and MDL 72222 applied case and baseline and recovery conditions were significant ($p<0.05$). However difference between MDL 72222 applied condition and recovery was not significant ($p=0.14$).

He, Tuckett and English also performed experiments investigating the effect of 5-HT_3 and 5-HT_2 receptor antagonists on SA-I activity in 2003 [29]. They found that ketanserin decreased spontaneous activity from 1.4±0.25 spikes/s to 0.75±0.35 spikes/s and in recovery it increased again to 1.1±0.4 spikes/s, but changes were not found to be significant. For MDL 72222 again spontaneous activity decreased from 1±0.9 to 6.5±0.4 spikes/s and recovered to 0.8±0.35 spikes/s but changes were not significant.

All these differences show that 5-HT_2 and 5-HT_3 receptor antagonists cause different changes in spontaneous activity in frog skin than in rat skin, changes in SA-I responses were consistent for both antagonists in rat and frog skin. For ketanserin, He, Tuckett, English observed a decrease of about 74% - 68% and I have found a decrease of 74.13%. For MDL 72222 consistency was not that high; they found a decrease between 44% and 67% but I observed a stronger decrease at 86.36%.

5.3 Possible Mechanisms in SA-I mechanotransduction

Based upon all these findings serotonin is affecting both rat and frog SA-I mechano-response by acting at least two of the receptors 5-HT_2 and 5-HT_3.According to immunohistochemical findings it is probable that 5-HT_1 autoreceptors are also involved in mechanotransduction of SA-I unit.

Spontaneous activity seems to be affected by serotonin and by both antagonists in frogs but in rat it is reported to be unaffected by them [29]. Beck showed that exogenous serotonin alone can induce spontaneous firing of slowly adapting mechanoreceptors with thick and thin myelinated fibers even at low concentrations in cat [46].

Although our experiments were in vitro preparations, it is possible that during the catching and/or double pitthing the frogs specific amount of serotonin (and may be other sensitizing substances) may be secreted by symphatetic activation of Aδ fibers or by mechanically destroying spinal cord or/and brain. This may explain the higher baseline activity and the lower recovery.

As a conclusion I brought together all information found in this study and those reported before and propose a model of Merkel cell-nerve ending complex and its functioning as observed in Figure 5.1. According to the model, as it was mentioned in "two transduction sides hypothesis" of Yamashita and Ogawa, in 1991, that mechanical stimulation opens stretch-activated channels, which are permeable to both Na^+ and Ca^{+2}, located on both Merkel cell and nerve ending [3]. This may cause activation of voltage gated channels on nerve ending and may be the source or rapidly adating on and off response to mechanical stimulation, which was mostly observed in our experiments as well as in reported by different groups [38]. Another group showed that these stretch-activated channels are Gd^{+2} sensitive [4]. For the maintained response to occur, Merkel cell, which was excited mechanically, by Ca^{+2} influx, secretes serotonin and chemically excites the nerve fiber. There are at least three serotonin receptors involved in the process; 5-HT_3, 5-HT_2 and 5-HT_1 receptors. Eventhough previously it was reported that only 5-HT_{1A} and 5-HT_{1B} but not 5-HT_2 and 3 receptors were expressed

immunohistochemically on type I nerve ending physiologically it was repeated that 5-HT_2 and 3 antagonists decreases SA-I responses for rat and frog [29]. In addition serotonin transporters were reported in both Merkel cells and nerve ending. Therefore it may be concluded that serotonin is secreted by both Merkel cell and its nerve ending.

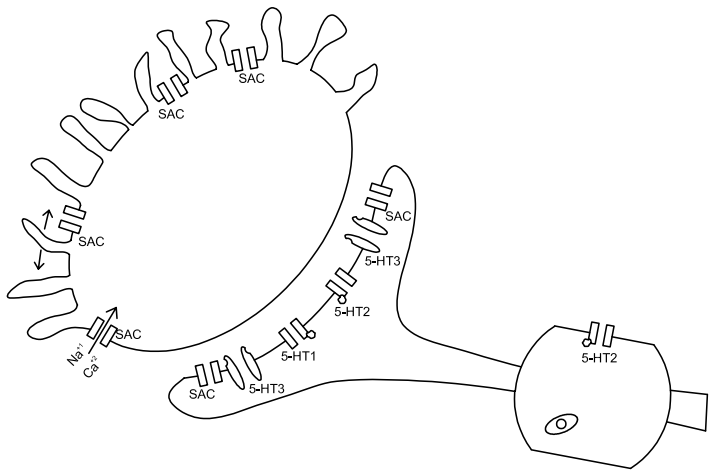

Figure 5.1 Theoretical model for Merkel cell- nerve ending complex structure and its function.SAC: Gd^{+3} sensitive stretch activated non Na^{+}/Ca^{+2} channel. 5-HT_1, 2 and 3 receptors are located at least on nerve fiber according to observations up to date including our results. There is a strong possibility that Schwann cells join the information processing of SA-I nerve ending at least by 5-HT_2 receptors.

Another possibility is Schwann cells may be modulating the signal propagating on SA-I fibers, since they were shown to be immunoreactive to ATP, acetylcholine, bradykinin and serotonin. 5-HT_{2A} receptors were reported on Schwann cells [47]. Also according to the effects of 5-HT_{2A} and 3 antagonist on spontaneous activity and SA-I activity receptors may be located at least on the SA-I nerve fiber. Serotonin may be acting on Schwann cells, so directly on nerves. Changing the responsiveness, threshold for mechanical stimulation. Since it is reported that these cells are observed at higher layers of dermis near up to epidermis [1]. It was reported that Schwann cells cultured from rat sciatic nerves exhibit responses to 5-HT that are specifically mediated by the 5-HT_{2A} receptor subtype [48]. However to find out their exact location (Merkel cells and/or nerve ending and/or Schwann cells) further immunohistochemical work needs to be done. Also since the blood vessels only passes from deep to middle layers of

dermis it is not really probable for serotonin in blood circulation from these layers to go up to Merkel cell as in the model proposed by He, English and Tuckett. But serotonin in blood can still effect, modulate the activity of peripheral nerves by serotonin (and many other substances that Schwann cells are responsive to like ATP, acetylcholine, bradykinin, but not glutamate), as it was mentioned before, by 5-HT_{2A} receptors on Schwann cells.

Even though our results showed that glutamate decreased SA-I response without affecting spontaneous activity, another theory is that glutamate may be released by Merkel cell or nerve ending to participate the mechanotransduction process. Since it was reported that non-selective glutamate antagonist kynurante decreased SA-I activity for rat sinus hair, by Fagan in 2001 glutamate is another candidate as neurotransmitter in Merkel cell-nerve ending complex [38].

Another possibility is that more than one interdependent transmitters may be active in the synapse. But all these theories needs further investigation.

The Merkel cell-nerve ending synapse could be more complex than we assume. There could be many substances that sensitizes or desensitizes the nerve ending and they may be secreted by keratinocytes, nerve itself, glands or other non-epithelial cells in skin and even substances in blood circulation could affect information processing in nerve. But all evidences show that there is at least one especially for SA-I mechanotransduction. A lot of immunohistochemical and physiological work needs to be done to explain the exact mechanotransduction precess of SA-I unit. Evidences found up to date including our results supports the serotonin hypothesis.

As future work selective glutamate receptor antagonists, 5-HT_1 receptor antagonist, Serotonin and glutamate receptor antagonist together could be used to see if they are involved in the mechanoelectric transduction in SA-I unit. In addition receptors on the Merkel cells and its nerve ending could be identified by using antibodies.

APPENDIX A. OVERVIEW OF THE SEROTONIN RECEPTORS

5-HT_{1A}: The receptor is negatively coupled to adenylyl cyclase and principally causes hyperpolarisation. 5-HT_{1A} receptors in the raphe nuclei, act as somatodendritic autoreceptors which inhibit neuronal cell firing and 5-HT release onto postsynaptic sites.

5-HT_{1B}: Studies have established that the terminal autoreceptor controlling 5-HT release in both the rat, guinea pig and man is of the 5-HT_1B type.

5-HT_{1D}: It has been proposed that neurogenic inflamation and nociceptive activity within trigeminovascular afferents may be 5-HT_{1D} receptor mediated due to the presence of 5-HT_{1D}, but not 5-HT_{1B} receptor mRNA in the guinea pig and human trigeminal ganglia24. The location of 5-HT_{1D} receptor mRNA in the raphe,1 suggests that it may function as an 5-HT autoreceptor.

5-HT_{2A}: This receptor (previously termed 5-HT_2) is located on human chromosome 13q14-q21 and is widely distributed in peripheral tissues where it mediates contractile responses of many vascular, urinary, gastrointestinal and uterine smooth muscle preparations, platelet aggregation and increased capillary permeability in both rodent and human tissue.

5-HT_3: Receptor binding site is widely distributed both centrally and peripherally and has been detected in a number of neuronally derived cells. The highest densities are found in the area postrema, nucleus tractus solitarius, substantia gelatinosa at all levels and nuclei of the lower brainstem such as the trigeminal nucleus and the dorsal vagal complex. It is also found in higher brain areas such as the cortex, hippocampus amygdala and medial habenula, but at lower densities.1 Peripherally, it is principally found on the neurones of the sensory and enteric nervous systems and

pre-and postganglionic autonomic neurones. Unlike other 5-HT receptors, 5-HT_3 receptor subunits form a pentameric cation channel that is selectively permeable to Na^+, K^+ and Ca^{+2} ions causing depolarisation. In vivo, administration of 5-HT_3 receptor ligands can either stimulate or inhibit cardiac function, induce vasodilation, affect lung and intestinal function, cause pain and sensitisation of nociceptive neurons and induce nausea and vomiting. This latter action is thought to underlie the emetic side effects of cancer chemotherapy and radiotherapy and has led to the use of selective 5-HT_3 receptor antagonists as antiemetic agents.

5-HT_7: The serotonin receptor encoded by this gene belongs to the superfamily of G protein-coupled receptors and the gene is a candidate locus for involvement in autistic disorder and other neuropsychiatric disorders.

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