COMPARATIVE PHYSIOLOGICAL STUDIES OF STATO-ACOUSTIC ORGAN IN CHICKEN AND RAT

A

THESIS SUBMITTED

TO

DEVI AHILYA VISHWAVIDYALAYA, INDORE (M.P.)

FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN

ZOOLOGY (LIFE SCIENCE)

BY

MANGILAL MAIDA

UNDER THE SUPERVISION OF

DR. G. D. SHARMA

PROFESSOR & HEAD DEPARTMENT OF ZOOLOGY
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P.M.B. GUJARATI SCIENCE COLLEGE,

INDORE (M.P.) INDIA

2018

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Ph.D. Course Work

Subject: *Zoology* Faculty: *Life Sciences*

This is certified that ~~Ms~~ / Mr. Mangilal Maida

son/~~daughter~~/~~wife~~ of Mr. K. Maida has successfully completed the Ph. D.course work conducted at *School of Life Science* from *January 2013 to May 2013*. The Ph.D. course work is as per UGC Regulation 2009 and Ph.D. Ordinance of the University. The credits and the grades earned by the candidate are as follows:

S.No.	Course Title	Credits	Grade	Credits X Grade
1	Research Methodology	5	C	25
2	Computer Applications	3	C+	18
3	Review of Literature	3	B+	24
4	Comprehensive Viva-Voice	4	C+	24
	Total	15		91

Semester Grade Point Average (SGPA) : 91/15 = 6.06 Percentage: 59%
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This is to certify that Pre Ph.D. Presentation for the thesis submission of Mr. Mangilal Maida on Topic:" Comparative physiological studies of stato-acoustic organ in chicken and Rat", in zoology (Life Science) held on August 25th Saturday 2018. MR. Mangilal Maida has Presented his research findings at Department of Zoology P.M.B.Gujarati Science College, Indore in the form of LCD power point Projection many Professors, Research Scholars were present and asked questions related his research and suggest him for improvement his presentation was satisfactory for submission of Ph.D. thesis.

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3. Faculty: Life Science
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Place: Indore

APPENDIX-IV

CERTIFICATE OF THE SUPERVISOR (Para 26 c)

CERTIFICATE

This is to certify that the work entitled **"Comparative Physiological Studies of Stato-Acoustic Organ in Chicken and Rat"** is a piece of research work done by **Mangilal Maida** under my guidance and supervision for the degree of Doctor of Philosophy of Devi Ahilya Vishwavidyalaya, Indore (M.P.) India. That the candidate has put in an attendance of more than 200 days with me.

To the best of my knowledge and belief the thesis:

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02. Has duly been completed;

03. Fulfills the requirements of the ordinance relating to the Ph.D. degree of the University, and

04. Is upto the standard both in respect of contents and language for being referred to the examiner?

Signature of the Co-Supervisor

Signature of the Supervisor

Head, Zoology Deptt.
P.M.B. Gu. Sc. College, Indore (M.P.)

Forwarded

Signature of Head UTD/Principal

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P.M.B.Gujarati Science College, INDORE

v

APPENDIX-IV

DECLARATION BY THE CANDIDATE (Para 26 b)

I declare that the thesis entitled "**Comparative Physiological Studies of Stato-Acoustic Organ in Chicken and Rat"** is my own work conducted under the supervision of **Dr. G. D. Sharma** at centre P. M. B. Gujarati Science College, Indore, approved by Research Degree Committee. I have put in more than 200 days of attendance with the supervisor at the centre.

I further declare that to the best of my knowledge the thesis does not contain any part of any work which has been submitted for the award of any degree either in this University or in any other University/Deemed University without proper citation.

Besides this-

1. I have successfully completed the Course work of one semester as per UGC-Regulation 2009 norms.

2. I have also given a pre-Ph.D. presentation and successfully incorporated the changes suggested on the basis of feedback and comments received.

3. I have also published one research paper in a ISSN/referred journal from the research work of the thesis and has produced an evidence of the same in the form of acceptance letter/or the reprint.

Signature of the Candidate

Signature of the Supervisor
Head, Zoology Deptt.
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Signature of Head UTD/Principal

Principal
P.M.B.Gujarati Science
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I express respectful regards and heartfelt sincere thanks to my guide **Dr. G. D. Sharma, Professor & Head Department of Zoology, P.M.B. Gujarati Science College, Devi Ahilya Vishwavidhyalaya, Indore** for his inspiring guidance, illuminating insights and regular encouragement throughout this work.

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Finally, my thanks goes to all the people who have supported me to complete the research work directly or indirectly.

Candidate

Mangilal maida

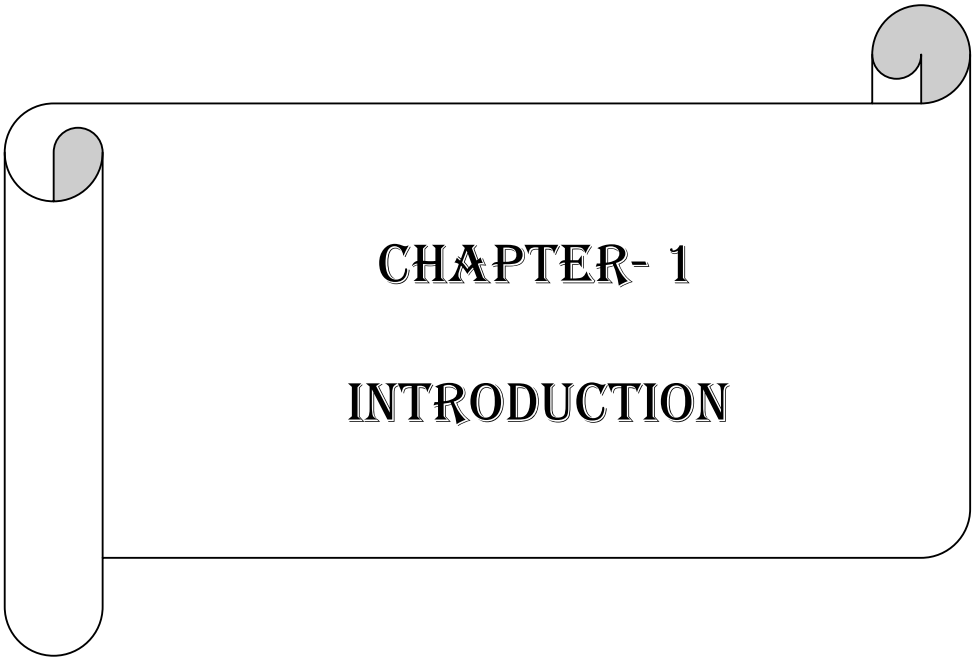
List of cross sections of SAO

List of Tables

List of Figures

K^+	Potassium Ions
AS	Acoustic System
ACF	Auto- correlation Function
AI	Adaptation Index
AVCN	Anterio-Ventral Cochlear Nerves
BHC	Basilar Hair Cells
BI	Bursting Index
BM	Basilar Membrane
BMV	Basilar Membrane Vibration
CF	Characteristics Frequency
CH1	Channel 1
CH2	Channel 2
CO_2	Carbon Dioxide
CP	Cochlear Potential
CVs	Coefficients Variations
CVP	Cochlear and Vestibular Potential
dB SPL	Disable Sound Pressure Level
EAM	External Auditory Meatus
FFT	Fast furious Transformer
FR	Frequency Range
FS	Frequency Selectivity
FTC	Frequency Tuning Curves
HF	Hazard Function
Hz	Hertz
Hz/s	Hertz Per Second
ISI	Inter Spikes Intervals
kHz	Kilo Hertz

MBPP	Minimum Burst Per Periods
MISI	Mean Inter Spikes Intervals
MS	Mean Stimulus
MS	Mechanical Stimulus
μV	Micro Watt
N_2	Nitrogen
O_2	Oxygen
OTPS	Onset Time of Preceding Spikes Discharge
OTSD	Onset Time of Spikes Discharge
RMS	Root Mean Square
RT	Response Threshold
SAEB	Sound Attenuation Experimental Booth
SAN	Spontaneous Active Neurons
SAO	Stato-Acoustic Organ
SCC	Semicircular Canals
SD	Spikes Discharge
SDISI	Standard Deviation Inter spikes Intervals
SDR	Spikes Discharge Rate
SHC	Sensory Hair Cells
Sp/s	Spikes Per Second
STI	Spikes Time Intervals
TIH	Time Intervals Histogram
TM	Tympanic Membrane
TNS	Total Number of Spikes
TTR	Total Time of Recording
VS	Vestibular System



CHAPTER- 1

INTRODUCTION

The cochlear and vestibular sensory neurons of both animals show some differences and similarities. The similarities in tonotopy of the cochleo-vestibular sensory neurons are much more impressive in all vertebrates. The sensory neurons in mammal's acoustic system is very simple than the avian, but share some fundamental characteristics, including the developmental principle, distribution of sensory hair cells and neurophysiological properties. Moreover, the provided evidences by many researchers suggest that the cochleo-vestibular sensory neurons of the human being have an affinity with a developmental plan of other vertebrate. In many species, the adaptations for detecting a particular frequency have shaped many questions in the mind of investigators. As a result, the neurophysiology has very benefitted by the use of different techniques to understand the structural and functional correlation of the SAO (stato-acoustic organ) in all animals. The marvelous advantage is that the chickens and rats offered a useful experimental model for comparative physiological studies.

Both chickens and rats are endothermic, revealed sophisticate capability to detect the mechanical sound wave and magnifying a low range of frequency by dramatic oscillation in the SHCs (sensory hair cells) and stereocilia. Although, more than 200 million years ago the aves and mammals were diverged from the reptilia (Benton, M. J. 1997). It has often been informative that how determined the dissimilarities and similarity in response properties of sensory neurons in both modern ideal model of the chickens and rats. This research planning is begin by examined that how a specific features of the neural sensitivity is intact

to their tonotopy and developmental history and then seek to know that how tonotopy of the stato-acoustic organ serve as low and high frequency detector.

The sensory neurons convey information about the external and internal environment to the brain and peripheral components by receiving from SHCs (sensory hair cells). Many mammals and birds are altricial, they spend the first several days to weeks as neonate before the onset of prominent frequency selectivity. During this period, projections rate of the sensory neurons very high in the cochlea and vestibular system. Ultimately, the information about a particular frequency conveys through these projections. This frequency magnifies by the cochlear receptive hair cells and their intact components. Thus it provides a significant basis for casting the auditory and vestibular system. The projection of sensory neurons in both systems are preserved and discriminates during ontogeny of SAO, therefore the neural synapse provides a means to pass a stimulus wave from sensory hair cells to the brain. We have understood the phases of neural refinement in regard to the sense of particular stimulus frequencies and recording the responses from active sensory neurons of both systems over the different range of stimulus frequency.

The refinements in the activity of sensory neurons are complete before the onset of proper hearing (Cant *et al.* 1998). However, the mechanisms of particular frequency selectivity underlying the prehearing development processes are not yet evident in both animals. The development of neural web in

stato-acoustic organ has been observed in neonate mammals before the onset of vision (McLaughlin *et al.* 2005). The SAO of avian and mammals are very sensitive for frequencies selectivity in a critical situation. There are several mechanisms contribute in low and high frequency detection, first is a traveling sound wave parallel to the tympanic membrane and basilar membrane, second is propagating sound wave magnify by cochlear components. The properties of this traveling sound wave have been measured in the pigeons, rats, and chickens that reveal high magnitude spikes curves at constant stimulus intensity (Von Bekesy, 1960). One pair of the ear is situated at the back side of the eyes. Two main functions of the ear, first is to receiving particular sound frequency and second, to maintain body balance in all conditions therefore, it is known as SAO in all vertebrates. Mainly ear consists three parts such as:

1.1. Outer Ear

1.2. Middle Ear

1.3. Inner Ear

Photograph-1. Stato-Acoustic Organ

1.1. Outer Ear

It is a visible part of the ear, well developed in mammals (Whale, seal, ornithorhynchus are an exception) than the other animals. It surrounds the opening of a small tubular passage called external auditory meatus (EAM), which leads into cranium and terminates at an elliptical shaped tympanic membrane (Ear drum).

1.1a Function of Pinna:

The pinna preferentially concentrates the mechanical sound frequency and transfer into the EAM. It also plays an important role to enhance the intensity of the sound frequency. In some animals such as rabbits, dogs and cows, it is movable. Although in the human being the muscles (auriculares) of the ear are vestigial, so it is immovable. It may be varied in all animals according to their use and environmental conditions, although it is absent in some classes of animals, for example Aves, Reptilia, Amphibia and super class Pisces.

1.2. Middle Ear

The air filled cavity situated between outer and inner ear known as middle ear, connected with the Eustachian tube, its first named after Bartolommeo Eustachian an Italian anatomist. In mammals, the middle ear contains a chain of three small cartilaginous bones extending between the TM (Tympanic Membrane) and fenestra ovalis. These bones are known as the Malleus, Incus

and Stapes orderly from outside to inner, homologically they represent the articular, quadrate and hyomendibular bones respectively.

The ear bones transmit the mechanical vibration of the ear drum (Tympanic Membrane) into the internal ear through oval shaped fenestra ovalis passage. It is believed that only about 0.1 % of the sound wave striking semi fluid surface, rest 99.9% wave is reflected back. If the entire mechanical waves have arrived into the semi solid fluid of the internal ear, most of the wave reflects back. One end of the malleus is connected to the tympanic membrane and the foot plate of the stapes connected to the oval passage as a movable piston. Thus the middle ear accomplishes the remarkable task of recovering otherwise lost sound wave by coordination of the mechanical action of peripheral components.

1.3. **Internal Ear**

The inner ear is an important intact part of the vertebrate's ear. It is a very complicated and delicate structure, comprise a sac that surrounds by the membranous wall known as the membranous labyrinth (ML). It is firmly embedded in periotic bone in a small cavity called the bony labyrinth. It is hollow, contains a fluid known as endolymph. A narrow outside space of the membranous labyrinth is filled with another semi-solid fluid known as perilymph; both fluids are separate from each other by the delicate membrane. The membranous labyrinth contains three delicate structures:

A. Cochlea

B. Semi-circular Canals

C. Small sac like Utricle and Saccule (Body Proper)

A. Cochlea

In all vertebrate, the cochlea is similar in many aspects, but it is varies on the basis of addition of long narrow coiled and tubular outgrowth from the lower portion of the saccule called cochlear duct. The cochlea is a bony canal; its size and shape are varying in all vertebrates in which mechanical waves propagate from the middle ear to the end part of the cochlea or organ of corti. The canal of cochlea is a part of the bony labyrinth of the auris interna (internal ear), length is about 30 mm. The spiral cochlea includes three chambers:

i. The vestibular duct (filled with perilymph) situated to the superior of the cochlear duct and adjacent to the oval window.
ii. The tympanic duct (filled with perilymph) lies inferior to the narrow cochlear canal terminates at the helicotrema.
iii. The cochlear duct (filled with endolymph) has high concentration of K^+ ions

Furthermore, many specialized components of the stato-acoustic organ that involve in the sense and enhance of the neural sensitivity at a particular frequency are such as:

a. Helicotrema

Helicotrema located at the merge point of the tympanic duct and vestibular duct, near this part a low frequency sound best detects by sensory hair cells.

b. Reissner's Membrane

This membrane separates the vestibular canal from the cochlear canal and located just above the tectorial membrane, it is important for function of the organ of Corti.

c. Basilar Membrane

It is a main functional and structural component which separates the cochlear duct from the tympanic canal. The properties of mechanical wave propagation determine by it.

d. Organ of Corti

The organ of corti is a sensory epithelium lies in scala media or middle chamber of the cochlea, in which several sensory hair cells are powered with the creating the potential difference between the semi solid fluid perilymph and endolymph.

e. Sensory Hair Cells

These cells are mechanical wave receptor in both the vestibular system and cochlea (auditory system) of all vertebrates. The sensory cells are

tonotopically arranged in four rows within the organ of corti along the entire length of the cochlear duct. These rows are situated just above a thin basilar membrane. Many fine hair like structure called stereocilia present on the sensory hair cells.

A.1. Function

The tympanic membrane vibrates when the mechanical wave transmitted via the outer ear. This membrane is connected with the malleus by which the stepes bone of middle ear transmits mechanical vibration into the oval window on the apex of the cochlea. This mechanical vibration generates the oscillatory motion in semi solid perilymph of the vestibular canal. The ear ossicles play an important role in coupling of mechanical waves into cochlea. The cochlear inner part is a delicate membrane system; it takes more pressure for transmitting sound waves via fluids. The increased pressure is achieved by the area ratio of the ear drum; thereby the pressure maintained itself. This pressure is an impedance, it equal to the mechanical waves traveling through the fluid membrane system.

At the apex of the cochlea, each duct terminates at a membranous portal that faces the middle ear cavity. The vestibular duct terminates at the fenestra ovalis, where the stapes footplate is connected. The stapes footplate vibrates when mechanical waves is transmitted via the chain of cartilage bones. The waves in vestibular perilymph move from stapes foot plate to the helicotrema.

The cochlear and vestibular duct acts mechanically as a single duct being kept apart only by a thin reissner's membrane.

The mechanical waves of the cochlear endolymph generate the vibration in the basilar membrane (BM). The organ of corti vibrates due to the movement of the outer sensory hair cells that amplify the mechanical wave. The inner sensory hair cells vibrate by the vibratory waves of endolymph, and depolarize by the inflow of potassium (K^+) ions via their apex transduction channels; after that transmits the electrical signals via neurotransmitter to the primary auditory neurons. The sensory hair cells (SHCs) of the organ of corti are tuned with a particular sound frequency in way of their location on cochlea due to the magnitude of the severity in the BM (Guenter Ehret, Dec 1978; Camhi, J. 1984).

The coiled structure of the cochlea is unique in mammals. In birds and other non-mammalian vertebrates the acoustic duct containing the sensory hair cells for receiving particular sound frequency is sometimes known as a cochlea, despite not coiled up. Instead, these animals have a blunt tube also known as cochlear duct. These structures evolve in parallel to the frequency selectivity in non-mammalian and mammalian vertebrates. The frequency range of hearing in the vertebrate is specialized due to their different mechanism of amplification of traveling sound waves by the active sensory hair cells (SHCs). The frequency resolution is not better in the mammals than the most birds and reptiles, but

several researchers believed that the upper frequency limit is somewhat higher. The frequency selectivity is very controversial in mammals and non mammalian animals.

Most species of the birds do not detect above 4-5 kHz frequency, but the current study on the barn owl have suggested that their frequency detection range extended from 6 kHz to ±11 kHz; it is high in compare to the chickens and rats. Some marine mammals focus up to 200 kHz. In the present study we have also observed the factors that alter the frequency detection such as environmental, anatomical and experimental factors in both groups of the animals that belong to different classes (Aves and Mammals). For this purpose, two groups of the animals and mechanical stimulus were included in the experiment such as:

i. Group I- Chickens (12 -14 Weeks old, Number-6)

ii. Group II- Rats (12-14 Weeks old, Number-6)

iii. Mechanical stimulus from 32 Hz to 10 kHz

The birds and mammals are an ideal model for comparison of the anatomical and physiological evolution. They have provided appreciable perception in the ontogeny of SAO. Thorough documentation of the anatomy and physiology of the ear given by Cohen *et al.* (1992), the detail descriptions of spontaneous discharge patterns recorded from the developing cochleo-

vestibular nerves and their peripheral auditory circuits of both animals. The frequency selectivity patterns (FSP) have recorded from the cochleo-vestibular sensory nerves in many neonate animals, their spontaneous burst represents to the development of the neural course in the cochlea. The neural bursting has plotted form the sensory nerves of the cochleo-vestibular system, it gives a base of the evaluation of the refinement in neural activity and sensitivity in the hair cells.

Although, further development of the sensory neurons in adult animals reveal the different distribution of neural fibers and spontaneous activity that also show the remarkable dissimilarity from pre to next developmental phase of the cochlea and cupula. This dissimilarity occurs in both animal but may be less in an individual. Therefore, several investigators have been concluded that this dissimilarity as a resultant of the many anatomical, habitual and experimental factors that influence the mechanism of the cochleo-vestibular nerves and hair cells. The experimental and theoretical observations are helpful to determine the cochleo-vestibular potential using the various mechanical stimulus on both animals and comparison between them.

In both auditory and vestibular system, stereocilia are a mechano-sensing part on the sensory hair cells which are very sensitive in response to the endolymph oscillation in many types of species. It is also play an important role in magnifying the sound frequency. Their length is varying from animal to

animals. It has some similarity with the microvilli of cupula. The sensory hair cells sense the endolymphatic force and transduce such other mechanical wave into the electrical signal via the numerous stereocilia. Stereocilia are a hair like outgrowth of the sensory hair cells and these are arranged in clusters of 30 –300 (Rzadzinska, A. K. *et al.* 2004).

The stereocilia are arranged in several rows of increasing length, as staircase. The cross linked actin fibers present at core region of the stereocilia, which can regenerate. These actin fibers face their positive and negative ends respectively at apex and base of the stereocilia, these fibers can be 120 micrometers long ((Rzadzinska, A. K. *et al.* 2004). These fibers liked with the tips of stereocilia of the contiguous rows within clusters. These cross links are made up of fine fibers that project upward from the apex region of shortest to its longest stereocilia. When the stereocilia stretched, the transduction channels open thus it permits to flow of the ions across the cells membrane into the sensory hair cells. This mechanism also plays an important role in the wave traveling across the cluster of the stereocilia and maintains the wave propagation properties of the sensory hair cells.

A. 2. Pathway of the Auditory System

The stereocilia are arranged in several rows on the organ of corti. In the hearing mechanism, the stereocilia involved as a transducer and transduce the mechanical waves into the electrical signal for the sensory hair cells that leads

to the stimulation of the auditory neurons. The cross connected actin fibers embedded in cytoplasm of the stereocilia, these actin fibers supports to the sensory neurons and apex region of the hair cells. When the mechanical wave propagates in cochlea the oscillatory movement produces in endolymph and then frequently bends the stereocilia. The bending of the stereocilia generates stretch on the apex region, whereby the opening of the apex transduction channels. The cations flow from the endolymph into the sensory hair cells that depolarized the cells and start the release of the neurotransmetters from the adjacent neurons of the cells.

In the auditory system, the movement of the tectorial and basilar membrane induced the stereocilia, whereby the stretched on the cross tip liked fibers then flow and suppress of the ions from the membrane into the sensory hair cells. The flow of the ions across the membrane into the sensory hair cells are increased with the increasing the stretched on the cross tip liked fibers. Thus, inflow of the ions induces the depolarization of the sensory hair cells; resultant in the developed of the electrical potential that ultimately leads the electrical signals to the auditory neurons. The channels of transduction related with the stereocilia; it is believed the transduction channels lie at the distal ends of the stereocilia (Hudspeth, A. J. 1982). The bending of the stereocilia toward the tall stereocilia leads to an enhanced the opening rate of the non specific ions channels. It is also induced the depolarization of receptor sensory hair cells and stimulate to the cochlear afferent neurons that are projected at the base of the

sensory hair cells. The bending of the stereocilia in the opposite direction of the small stereocilia causes transduction mechanism to suppress. In this condition, the sensory hair cells hyperpolarized and the afferent neurons are not activated (Ohmori, H. 1985; Corey *et al.* 1979; Hackney *et al*. 1995).

In the inner ear of all vertebrate animals, two types of semi solid fluid that covered the sensory hair cells. The first fluid is endolymph that covers the superior surface of the sensory hair cells; it contains the potassium ions (K^+). It is thought that the endolymph is responsible for carrying the wave in the cochlea. The second fluid is perilymph, which surrounds the bases of the sensory hair cells. The sodium (Na^+) is a major ion of the perilymph and potassium in low quantity (Bosher *et al*. 1978). The different ionic concentration of the fluid, generate the potential difference across the apex membrane of the hair cells, thus the potassium ions enter into the transduction channels. The flow of the potassium ions induced the depolarization of the sensory hair cells. It is responsible for the release of a neurotransmitter that can initiate the sensitivity of the neurons on the base of the sensory hair cells.

B. Semicircular Canals (SCCs):

A group of three SCCs connected to the utricle, these are at right angle to each other called the horizontal (lateral), the anterior (superior) and the posterior (inferior) semicircular canals. Anterior and posterior SCCs collectively are known as vertical SCCs. The movements of semi solid fluid within lateral SCCs

corresponding to the rotation of head around the vertical axis. The superior and inferior SCCs sense the rotation of the head in the sagittal and the frontal plane. Both superior and inferior canals adjusted at approximately 45° between sagittal and frontal plane. The movement of the endolymph fluid pushes on the cupula which contains several sensory hair cells that transduce the endolymphatic mechanical movement to electrical wave by the help of stereocilia. The vestibular system in all animals is physiologically very ancient and their development is an important evolutionary event because without it all animals unable to maintain equilibrium while moving in their niche.

The vestibular labyrinth in all vertebrate detects the linear and an angular movement of the head. The response of the copular sensory neurons at the different range of stimulus frequency is a matter of this review. In mammals, the horizontal SCCs are situated in the head and it aligned ordinarily with yaw axis of the head, whereas the superior (anterior SCCs) and inferior semicircular canals are positioned at right angle (orthogonal) to each other. These SCCs have an endolymph fluid filled hollow tube containing an enlarge sac like ampulla at one end. The crista ampularis is receptor organ lies inside the ampulla and anchored to a thin wall of the ampulla. This crista innervated by the delicate layer of the sensory hair cells and the stereocilia. The length of stereocilia is about 100 microns projected into a gelatinous cupula that closed in the fluid flow ampulary space.

The sensory hair cells are well innervated by the vestibular afferent neurons that nerves emerge from the base of ampula and projects toward the brain. The vestibular system is secured in the skull; therefore, the endolymph flows with the head movement. The inertial force is generated within the SCCs with oscillatory movement of semi solid endolymph. The endolymph flow is produced a drag force and a compensatory movement of the cupulary sensory hair cells that generate invigorative force, it is symmetrical to the inertial force in dynamic equilibrium (Damiano, E; Rabbitt, R. D. 1996).

B.1. Pathway of the Vestibular System:

The stereocilia are situated in the cupula of semicircular canals (SCCs) and otolith membrane of the utricles chamber in all vertebrates. These sensory hair cells in the vestibular system are somewhat vary to the cochlear hair cells. The vestibular sensory hair cells have several cilia called kinocilium. When the kinocilium bends toward the tallest kinocilium which depolarizes the sensory hair cells, results in the excitation of the responses of sensory neurons, and the stereocilia bends away from tallest kinocilium, whereby the sensory cells are hyperpolarized, results in the suppress of neural responses. In the SCCs the sensory hair cells located in the crista ampullaris. The stereocilia of the sensory hair cells projected into the cupula and their entire stereocilia bends in the same direction with the movement of the endolymph in SCCs, whereas the sensory hair cells of the otoliths membrane are located at the otoconia (calcium

carbonate crystals). Otoconia are bio-cristals which couple mechanical forces to the sensory hair cells in the utricle and saccule, a process essential for animals to sense linear acceleration and gravity for the purpose of maintaining body balance.

C. Small sac like Utricles and Saccule (Body Proper)

In the vestibular system, a large upper part is utricles and the lower small chamber is saccule, these two chambers are connected to each other by a narrow sacculo-urticulus duct. The saccule gives off a small tubular endolymphatic duct, terminating against the skull into an endolymphatic sac. Each part has a specialized cluster of the sensory cells known as macula with fine projecting sensory hair. These sensory hairs are firmly embedded in semi solid jelly also containing calcium carbonate particles ($CaCO_3$) called otoliths. These sensory hair cells are known as the macula utriculi and macula saculi respectively. Its important role in all vertebrates is to maintain body posture and position of the head. The hard bony labyrinth covered the utricle and saccule are called vestibule.

This approach has observed the common and distinct physiological features in both systems. The mechanism of frequency selectivity in the cochleo-vestibular neurons has examined and it appears that the comparative physiological approach has continued as data generative in regard to it. We have also compared the similarity and dissimilarity in response properties over the

different range of stimulus frequency, with the intent of cross-correlation in the sensitivity of the cochlear and vestibular sensory neurons in both groups of the animals.

CHAPTER- 2

AIM AND OBJECTIVES

The animal's ear anatomy firmly correlates with the neurophysiological responses in regard to the detection of particular sound frequency that also represents the specific neurophysiological and anatomical conditions. Physiological data were collected from the cochlear and vestibular neurons of the different groups of animals. Entire anatomical and physiological parameters included in the studies of the cochleo-vestibular responses. The mechanical stimulus from 32 Hz to 10 kHz generated by an audio oscillator to evaluate the cochleo-vestibular potential. The stimulus intensity from 25.5 dB to 45dB SPL was used for both animals. The potential was measured directly on the oscilloscope and expressed in μV peak to peak. The main focus of the current studies is summarized in the following contents:

i. To evaluate the established hypothesis that anatomical specialization enhanced the sensitivity of the cochlear and vestibular neurons.
ii. Compare the sound-induced cochleo-vestibular nerves responses between both animals.
iii. To determine the physiological relationship of the cochleo-vestibular system between both groups of the animals.
iv. To observe the tonotopy of the cochlea and vestibular system.
v. To study the neural innervations in the organ of corti and cupular hair cells.

vi. To observe the amplification of mechanical wave by the sensory hair cells of the cochlea in both animals.

CHAPTER- 3

REVIEW OF LITERATURE

It is generally believed that all animals tend to extend the FR (frequency range) of hearing and equilibrium of our body posture. Many researchers have been suggested that the avian's SAO (stato-acoustic organ) lies between the primitive organ of the reptilian and the anatomically complex cochlea of mammals. The oscillation in the sensory hair cells of the cochlea and cupula over a stimulus range of frequency has been observed in many species of the birds and mammals in detailed; this movement has plotted as curves. Similar studies have been completed on mechanotransducer channels in papillary hair bundle of the non mammalian cochlea (Manley and Koppl, 1998; Hudspeth *et al.* 2000; Koppl, 2011). Many researchers have been supported that this mechanism has in the birds (Cohen *et at.* 1992; Sul and Iwasa, 2009). Furthermore, this mechanism was detected in the basilar membrane of mammals (Gurmmer *et al.* 1987). According to this mechanism, we have been observed the cochleo-vestibular potentiality in both animals.

The frequency tuning curves (FTC) of the cochleo-vestibular neurons revealed spontaneous spikes discharge rate and frequency selectivity in the chickens and rats. Therefore, the dissimilarity occurred in the switching of the spikes discharge and the magnitude of the spikes on FTC. The switching was observed in the phases of the cochleo-vestibular sensory nerves at about ≤1200 Hz (spikes discharge rate 70 sp/s) and ≤1350 Hz (SDR 67 sp/s) respectively for chickens and rats. Many researchers have been observed the variation in spikes

discharge rate, that value for chickens was 86 sp/s (Salvi *et al.*1992), but this value markedly contrasted with the 1-12 days old chickens (23sp/s) (Manley *et al.*1991a). However, many researchers assumed that the variation in SDR might be attributed by anesthesia, environmental factors rather than age (Smolders *et. al.*1995). The stimulus magnitude 6 - 8º/s and 3-6 º/s respectively for chickens and rats have reported by the oscillatory movement in vestibular endolymph at about 0.06- 4.0 Hz range of frequency (Anastasio, T. J. *et al.* 1985, Baarsma, E. A. 1974, and Barmac, N. H. 1981).

The Spikes discharge patterns of the vestibular and cochlear sensory neurons have been examined in the newborn mammals (Carlier *et al.* 1975; Curthoys 1978, 1979, 1982, 1983; Desmadryl *et al.* 1986; Gummer and mark 1994; Kettner *et al.* 1985; Romand and Dauzat 1982; Walsh And McGee 1987) similar data collected from the embryo of posthatch birds (Yamaguchi and Ohmori 1990, 1993; Manley *et al.*1987, 1991a; Vaverde *et al* 1992; Jones, S.M and Jones 1995a,b; Richter *et al.* 1996**;** Sheppard *et al*. 1992), and they also measured the spikes discharge rate and bursting behavior of the cochleo-vestibular sensory neurons in the avian and mammals.

The inner ear transduces the mechanical wave into electrochemical signals that are encoded by the sensory neurons. This mechanism initiate when the mechanically induced oscillatory wave pushed the clusters of stereocilia of cochleo-vestibular sensory hair cells. An ionic flow through the membranous

transduction channels creates receptor potentials, that modify to the neural synaptic and release neurotransmitters from the neurons of basal regions of sensory hair cells. Therefore, the sensory hair cells do not only sense the mechanical force; it is also generate the force with bending of the clusters of stereocilia that is related with the transduction channels (Howard, J *et al.* 1988; Hudspeth, A. J. 2014) and/or fast adaptation, it may also be related with the closing of the transduction channel (Ricci, A. J *et al.* 2002; Fettiplace, R. *et al.* 2014). The force generation by the cluster of stereocilia is likely to present in all type of sensory hair cells across all vertebrates and may in particular frequency selectivity (Bormuth, V *et al.* 2014).

In mammals, the force generates by outer sensory hair cells with changing their length in response to the oscillatory movement in endolymph. This mechanism is initiated with the external or internal induction in both systems. Together, these force generate by sensory hair cells with give raise otoacoustic emissions and frequency selectivity, that enhanced by the inner ear. Otoacoustic emission has been reported by many researches in all vertebrate classes (Bergevin, C *et al.* 2015). The sensory hair cells (SHCs) are main components for the cochlear amplification and particular frequency selectivity in all mammalian and avian stato-acoustic organs (Liberman *et al.* 1984; De Boer, 1991; Hubbard *et al.*1995). These features of the stato-acoustic organs (SAO) can be realized if the function of active sensory hair cells as an amplifier and

force is generated in such a way that amplified a low frequency stimulus. The frequency amplification may be vary in all animals.

Gold (1948) was a first researcher who point out the need of such amplifier in all animals. He also considered the vibration in viscous BM (basilar membrane) that was absolutely significant by which the cochlear sensitivity occurred more active, if their activity is not affected by such viscous fluid. On the ground of these studies, he has hypothesized that the cochlear sensory hair cells have a feedback of low frequency amplifier and reduce the effect of viscous fraction.

Although, it is believe that the movement of BM (basilar membrane) is not as heavily glutenized expected by Gold (1948). The presence of Feedback of the amplifier has been greatly accepted (De Boer, 1991; Zweig, 1991; Patuzzi, 1996). It has been hypothesized that the voltage dependent movement of the cylindrical body of outer hair cells referred as electromotility and makes the function of mechanosensory cells as a feedback of cochlear amplifier (Brownell *et al.*, 1985). The movement amplitude is ~5% of the cells length (Ashmore, 1987) and force generation by the cells is ~0.1 nN/ mV (Hallworth, 1995; Iwasa *et al.* 1997).

The mechanism of the outer sensory hair cells movement is similar to the piezoelectricity, it is based on direct electro-mechanical coupling (Iwasa, 1993;

Dallos *et al.* 1993; Iwasa, 1994; Gale *et al.* 1994; Kakehata *et al.* 1995; Iwasa, 2001), in which charge is conveyed across the basilar membrane (Ashmore, 1990; Santos-Sacchi, 1991; Iwasa, 1993). The movement mechanism may be fast because its motion is not controlled by diffusion. Indeed, electromotility develops a phase-locked mechanical force without reduction of 60 kHz frequency (Frank *et al.,* 1999). Moreover, prestin protein that reproduces most features of the motor neurons have been identified by Zheng and their collaborators (2000).

However, the physiological and anatomical differences in the SAO of different animals/species are inferred to the substantial differences in transduction of mechanical sound wave. For example, the sensory hair cells of the vertebrate (frogs, lizards and turtles) have been described in detailed, it has suggested that the hair cells anatomically are very similar and clustered within the complex cochlear duct. In frogs, basilar membrane is absent, on the other hand, in most reptiles (lizard and turtles) the sensory hair cells located on the basilar membrane (BM) that is not mechanically tuned (O'Neill, M. P *et al.* 1995; Peake, W. T *et al.* 1980).

Several specialized accessory cells have surrounded to the sensory hair cells which give rise to new sensory hair cells when existing cells are damaged by mechanical trauma (it mean, sensory hair cells regeneration). Physiologically hair cells of both animals have electrically resonance, in which an interaction

between voltages channels and receptor potential of all sensory hair cells for frequency selectivity (Lewis *et al.* 1983; Ashmore *et al.* 1983; Crawford *et al*. 1981). Thus, when the stereocilia on the sensory hair cells are induced over a stimulus frequency, they have more sensitivity and then it can better stimulate the sensory neurons.

In all mammals, the cochleo-vestibular sensory hair cell precisely surrounded by specialized accessory cells. Two rows of outer and inner sensory hair cells are located along the length of the cochlea in all vertebrate. Functionally, the principle characteristic of mammalian cochlea is that the passive mechanism of basilar membrane produces a spectral analysis by generating the mechanical energy that reveals as traveling waves (Von Bekesy *et al.* 1960). Stimulus energy is traveled along the length of the cochlea in a tonotopic mode so that the lowest stimulus frequency cause to vibrate maximally of the upper surface of the basilar membrane and highest stimulus frequency cause to vibrate maximally of the basal part of the basilar membrane. The active force generation properties of outer hair cells are generally referred to the locally amplify and sharpen the endemic range of BM vibration; therefore, this term is cochlear amplification, it enhance the neural sensitivity at low stimulus frequency and low frequency selectivity (Wang *et al.* 2016).

It has been observed that the active properties of the outer hair cells do not appear on the basis of cycle by cycle oscillation of the endolymph, but adjust

the magnitude of local viscidity appear within the non amplifying system (Van der Heijden *et al.* 2014). According to both theories the outer hair cells are also responsible to achieve the normal response of cochlea. Resonance has not observed within the adult mammalian cochlea. The inner sensory hair cells receive the frequency tuning of mechanical vibrations from the organ of corti and relay these signals to the afferent sensory neurons (Narayan *et al*. 1998).

The avian basilar papillae have an affinity with the mammalian cochlea and the auditory papillae of other non mammalian. Mammals have two types of sensory hair cells such as short and tall hair cells. Short hair cells (SHCs) located on vibratory basilar membrane and less innervated by the sensory nerve fibers. On the other hand, the tall hair cells located adjacent to the SHCs that are more innervated by the sensory nerve fibers. The principal role of SHCs is not confirmed, but many researchers have been hypothesized that it involves in force generation and amplify the low stimulus frequency. The inductions of stereocilia of the tall hair cells are somewhat similar to the patterns of the mammalian outer hair cells and it provides functional support to the inner hair cells (Iwasa *et al.* 2015). The sensory hair cells of the chickens have the motility that enhances bundle motility and induced the movement of the tectorial membrane in redial direction (Beurg *et al.* 2013).

It is clear that the otoacoustic emission in the bird's cochlea in a manner of the hair cells force generation. The correlation between mammalian and avian

cochlea is unclear. However, in the turtles, lizards and bird, the sensory hair cells arranged in the organ of corti are surrounded by non-specialized accessory cells, it also may regenerate and have well electrical/ mechanical tunning (Fuchs *et al.* 1988). Thus, the aves (Chickens) and mammals (Rats) offer a way to determine the active properties of sensory hair cells. The mechanism of basilar membrane is related to the evaluation of the frequency selectivity and response of auditory neurons. Furthermore, many researchers argue that the basilar papilla (have many hair cells innervated by auditory neurons) is well developed in the reptiles and mammals. This suggestion may provide a clue as to the functional roles of their mechanism in mammalian and avian hearing. Specifically, we desired to determine amplification, defined as the enhanced in cochlear response and sharpening of frequency selectivity in the auditory neurons of chickens and rats. To accomplish this, we have observed the mechanically induced response of the cochleo-vestibular sensory neuroses in both animals (invivo) and we observed traveling wave within the cochlea of both animals. However, traveling wave more amplified by the cochlea of chickens than the rats. Furthermore, the frequency of traveling wave was higher in the chicken's cochlea than the rats. It is not correspond to the cochlear potentiality have been observed in previous studies in the chicken's auditory response. These data informed that the chickens cochlea more potential to amplify the traveling wave in compare to the rat's cochlea.

The important role of the vestibular efferent fibers in the processing of the information about the body or head motion in primates has been observed in details. The vestibular receptors are innervated with the centrifugally distributed efferent nerves (Rasmussen *et al.* 1958; Gacek *et al.* 1974). The group of efferent nerves in primates is made up of ~ 400 nerves fibers that are located at the brainstem just lateral to the abducens nucleus (Goldberg *et al.* 1980).

Many animals have a different sensory neural projection on the cupular hair cells. The low and high frequency selectivity is somewhat depend on the tonotopic arrangement of the neurons on active sensory hair cells. Efferent axons distribute bilaterally on the labyrinth, it makes connection pre-synaptically with type II sensory hair cells and post-synaptically with the axons of vestibular afferent neurons (Lindeman *et al.* 1969). In frogs (Valli *et al.* 1986) and presumably in the mammals (Purcell, 1997) the efferent neural innervations are similarly diverged from central nerve fibers that terminating at basal sensory hair cells. The afferents neurons are arise from the different vestibular region.

The vestibular organs controlled by distributed efferent neurons have been explained by Golberg *et al* (2000) in many species including birds, monkeys and chinchillas (Rodent). An electrically induced the vestibular efferent neurons were revealed the increase in resting discharge rate in the toadfish and squirrel monkeys (barbiturate anesthetized) (Highstein *et al.* 1985). Further evidences

by the mechanically induced action of the efferent neurons with the afferent neural discharge rate have been provided by detailed studies in the chinchilla (plotnik *et al*. 2000). There are more different effects of mechanical inductions have been observed in many species including turtles (Brichta *et al.* 2000) and frogs (Bernard *et al.* 1985).

The several available evidences suggested that efferent neural innervations of the SCCs might be altered the rate of spike discharge and/or the sensitivity of the vestibular afferent neurons. The first experiment in toadfish has shown the modulation in afferent neurons of SCCs with passive head rotation it also decrease during electrical induction of efferent neurons. Second, the efferent nerve induction of the toadfish revealed the response but unclear phase (Boyle *et al.* 1990; Highstein *et al.*1985). Just before this unclear phase, the efferent neurons generate the active phase of spikes. The behaviorally induced of the efferent neurons lead to enhance in discharge rate and reduced the rotational sensitivity of the afferent neurons. Third, in primates, the vestibular neural sensitivity with non eye movement was related to the second order sensory neurons of vestibular nuclei, it is inference to mediate the vestibulo-ocular reflex, it preferably attenuated by the self induced head rotation on body movement (Roy *et al*. 2001; Gdowski *et al.* 1999).

The observation in several species have suggested about the important role of vestibular efferent neurons that the neurons do not contribute to modulate of the

afferent responses, it relatively have similar role of the response in barbiturate anesthetized monkeys (Fernandez *et al*. 1971; Keller, 1976; Lisberger *et al.* 1986). The premotor neurons are responsible for control the eye movement, they also do not contribute to altering the afferent activity, in head restrained animals, the afferents neurons are not modulated during the saccadic eye movement (Miles *et al.* 1980). Furthermore, there are unlike that the neck proprioceptive inputs alter to the vestibular afferents action. The second order neural response of the vestibular system in the rhesus monkey has been observed with the head movement during passive and active rotations of the head on body (Roy *et al.* 2001).

The primary function of the vestibular semicircular canals (SCCs) is to generate neural information in response to the angular rotation of the head and mechanical stimulus. This information is encoded as electrical signals by canal's primary afferents neurons. The afferent neurons of the horizontal canal are induced when the force of the angular acceleration deflect the cupula toward the utricle (Blanks *et al.* 1975; Precht *et al.* 1971). However, the sensory neuron revealed wide variation in response to the given external stimulus with increase/decrease the velocity of the endolymph within SCCs.

The accurate information is not available for this variation, but Honrubia *et al*. (1989) have been reported that the site of mechanism of an afferent dendrite in sensory epithelium might be factor. Baird *et al.* (1988) have given own view

that the distribution of individual afferent dendrites reflects a variable number of innervating sensory hair cells it may alter the mechanism of sensory neurons. Further, the dissimilarity in the degree of displacement of the different component in the cupula (Mclaren *et al.* 1979) probably also generate variation in neural responses.

Many other anatomical and physiological features could be included for the observation of different responses of afferent neurons. For example, the firmly adhering of the kinocilia in the cupula show variance on the 3-dimensional surface of the crista (Hillman, 1977), sensory hair cells may have unmatchable ratios of depolarization at a different surface, and sensory hair cells have a dissimilar size of the quantum of transmitter. Afferent neurons have different post-synaptic sensitivities in response to the transmitter released from the neurons of sensory hair cells, and the neural synaptic cleft might have variance in width, resulting in different diffusion times across it.

Many investigators attempt to determine the correlation of the semicircular canal radius and response properties of afferent neurons in mammals and avian. Ten Kate (1970) have been determined the relationship between vestibular canal's size and neural sensitivity in fish. The SCCs (semicircular canals) continue to grow with body size in adulthood, it is not relevant to the predictions of a standard model of the mechanism of SCCs; he also observed that the constant induction is required for minimum threshold of

responses and the mechanism of the vestibular organ, it did not change despite the growth of the bony labyrinth. He hypothesized that the changes in cupular mechanism, efferent frequency selectivity, the sensitivity of hair cells and neural connections compensate the large SCCs radius to keep neural sensitivity constant.

Churthoys (1982a, b) determined the relationship between SCCs size and neural sensitivity using the same developmental paradigm but he found a contradictory result. He recorded the response of vestibular afferent neurons in neonate rats as their labyrinth get mature dimension over the first week of life. He reported that the sensitivity of the afferent neurons grew with the size of the SCCs, although the neural sensitivity increased more than would be anticipated on the ground of changes in SCCs anatomy only. He hypothesized that the neural sensitivity increased beyond the described of the SCCs size, it might be due to modulation in copular mechanism or changes in the sensitivity of neuroepithelium.

Many researchers have been studied on the sensitivity of SCCs in growing animals which undergo from the disadvantage that the growing changes alter the endolymphatic movement. The tonotopy of the cupular neuroepithelium, patterns of the neural innervations themselves might generate the variation in the outcome. An alternative approach relies on the sensitivity of semicircular canals of different size for comparison among mature animals of

the same species. Different size and responses of the horizontal, anterior and posterior SCCs have been included to make such comparison, although by this observations have not been reported the relevant results.

Anderson *et al*. (1978) have reported that the posterior small semicircular canals to be more sensitive in the cat (mammal); in the squirrel monkey the large anterior SCCs have more sensitivity in compare to the both other semicircular canals (Goldberg *et al.* 1971). Similarly, in pigeons, the small horizontal and large anterior canals have similar sensitivity (Anastasio *et al.* 1985). Goldberg *et al.* (1971) observation relied on steps stimulation is a matter of the afferent neural adaptation that may affect the recording of their sensitivity (Boyle *et al.* 1990). The observation of the responses of SCCs in pigeons may be complicate because the anterior SCC is dihedral and determined the maximum response from its plane is also difficult (Dicman 1996).

CHAPTER- 4

MATERIALS AND METHODS

The experimental materials and methods used in the current studies have been described elsewhere in detail (Henson and Pollak 1972). These methods and necessary analytical procedures used here are explained in the following text. The animals used in the physiological study of SAO were approved by Institutional Animal care and use committee at IPS Academy, College of Pharmacy, Indore. We have confirmed of all guideline given by my guide, and the ethical committee. Wistar rats were procured from Aurobindo medical college, Indore and the chickens from a local poultry farm and breed in separate closed colony managed at an Institutional level.

After fully acclimatization of the animals according to the animal house and laboratory, the females of related animals were kept separately among 2 to 4 male to breeding for a period of ± 24 hours. All rats were maintained in the favorable iron cages of the animal house and chickens in the poultry farm. All animal fed on whole/fine grains such as wheat, oats barley and corn, once in 24 hrs. Chickens and rats were divided into two groups (Group I- Chickens and Group II-Rats) have 6 animals (male, females) individually, but both animals were nurtured separately in established animal house. The post-conception or total number of gestation and newly born days were included for defined the newborn age. It is thought that the animal's age closely related to the development of the organ of Corti (Sato *et al.* 1999) and vestibular sensory hair cells because of the sensitivity of both system increase with the animal's age.

4.1. Animal Preparation

Each animal was anesthetized from the effect of inhaled chloroform in anesthesia induction chamber, after some time 60 mg/kg ketamine was administered intramuscularly and the hourly supplement of ketamine (45 mg/kg) was given for maintained the anesthesia level throughout the experiment. The head stabilized with the head holder and dental cement. The prepared animals were secured in sound attenuate experimental booth (SAEB). The SAEB was perfused with O_2 humidified CO_2 mixture ($\leq$ 40 % O_2, 4% CO_2, 25% N_2 and 8% H_2O vapour) and the average respiratory rate (20-25/min) maintain in both animals with the varying of CO_2%.

The animals were maintained on the sound attenuation small animal controlled booth, and the tympanic membrane was exposed by the removing of the external auditory canal after that the cranium was exposed by microsurgery. For the recording of the cochlear and vestibular potential, the glass insulated microelectrodes were implanted in the tympanum bulla via an intracranial approach. During the stereotactic placement of microelectrodes and conveying of the controlled mechanical stimuli into the cochlea and vestibular system, the animal insensitive to the positioned in a controlling device then the animal was held at one position. The head of the animal was fixed at a sound attenuation booth by a fastener connected to a small steel rod. All experiments were carefully completed in a sound-attenuated laboratory in which temperature was

maintained at 30- 37ºC throughout the experiment.

The stereotactic implant of the microelectrodes was easily inserted in 3 to 4 months old animals than hatchlings because of the well-developed aqueduct in the adult animals. In this technique, during the plotting the FTC (frequency tuning curves) of the response of cochlear and vestibular sensory nerves was no or may be interference with any part of the auditory system, data recorded from fully recovered by the effects of anesthesia and microsurgery.

4.2. Mechanical Stimulation in Cochleo-Vestibular Endolymph [Group I-Chickens and Group II-Rats (12-14 Weeks Old, Number-6; Stimulus range from 32 Hz to 10 kHz)]

We were used audio oscillator for generating a constant stimulus (from 32 Hz to 10 kHz) that were connected to the wave analyzer (Oscilloscope Hantek 6022BE) and it has been fully described previously (Koppl, C. 2011). Acoustic stimuli were produced under computer control by a custom-built arbitrary waveform generator. The use of large on-time/ off-time ratios was prevented the induction of threshhld by repeated the presentation of sharp stimulus. The mechanical stimulus was typically given in steps. An audio oscillator provides broadband swept-source with 1, 300 nm and 16 Hz to 20 kHz sweep rate. For all experiment the power of the sample rate was 10.0 mv. The animals and stimulus frequency used for recording the sensitivity of cochleo-vestibular sensory neurons are summarized in table-1.

Table-1. Summary of the stimulus range, animal's age, stimulus intensity animals groups and time periods of response recording.

Animals Groups	Stimulus Range	Time Periods Of response Recording	Stimulus Intensity	Animal Age
Group-I (Chickens)				
1 A.S	32 Hz to 10 kHz	±60 s	25.5 to 45 dB SPL	12 to 14 Weeks old
2 V.S	32 Hz to 10 kHz	±60 s	25.5 to 45 dB SPL	12 to 14 Weeks old
Group-II (Rats)				
1 A.S	32 Hz to 10 kHz	±60s	25.5 to 45 dB SPL	12 to 14 Weeks old
2 V.S	32 Hz to 10 kHz	±60 s	25.5 to 45 dB SPL	12 to 14 Weeks old

SPL = Sound Pressure Level, S =Second, Hz = Hertz, dB= Decibel

AS = Acoustic System, VS= Vestibular Systemk, Hz= Killohurtz

The resolution of the system was maintained at 8.6 µm vertically and horizontally, defined by a visual discriminating line on the output window of the oscilloscope software. The oscillatory wave was regulated and synthesized

by software with the help of a lock loop band filter; it was connected to the oscilloscope. We have calibrated mechanical stimulus and measured cochleo-vestibular potentiality using probe-tip microelectrode inserted in the organ of corti and cupula of the SCCs, this micro probe-tip can be also induced the endolymph of the SCCs and cochlea. The motility data collected from the sensory hair cells and the curves were analyzed simultaneously.

Best MS (mechanical stimulus) range was presented between 32 and 10 kHz with frequency steps of ≥0.20 Hz at intensity between 25 and 60 dB steps. This MS range was selected so that we can collect the data from stimulated hair cells without wasting the time that was likely to be below the stimulus floor (0.5-0.8 nm under conventional experimental condition). The duration of the MS range from 30 to 100ms, this was adjusted for the vestibular system to obtain the oscillatory threshold that was 25 dB below the hair cells motility. Thus, lower stimulus intensity provides longer recording times. In addition, the data of neural response in any given bins was < 4 Spikes of the oscillatory threshold at starting intensity of stimulus. If the magnitude of stimulus frequency is underneath to the threshold set at the mean SD then the hair cells oscillation can be measured at initial stimulus frequency.

The mechanical wave was shaped by channels with 12-ms rise and fall time during stimulus frequency of 10 kHz and lower. For stimulus frequency from 60 to 580 Hz rise and fall times of spikes were determined, that generates

approximate 12 oscillatory cycles during stimulus wave onset. There are five times oscillation of 450-ms duration, each was applied with 120-ms interval between oscillations. For reconciliation of the longer and shorter rise/fall times, the oscillatory frequency was increased for both systems of the animals. The time duration of the spikes discharge and rise-fall times of the peaks were observed by the output window. The intensity of the mechanical stimulus was set in the drop-down list of CH1 and CH2 in the control panel of hantek software. The irregular response curves were plotted between 140-190 Hz and maximum bursting behavior obtained at 8-10 kHz.

The oscillator (Audio Oscillator Modal no.me-150) was attached to the band pass filter to remove low-frequency of the mechanical stimulus and to improve the burst index. To deliver the constant stimulus directly to the tympanic bulla, the microelectrode brought adjacent to the tympanic membrane and then the mechanical stimuli have driven. A constant stimulus induces the maximum responses of the cochlear and vestibular nerves throughout the experiment in both animals. Typically, the regular stimulus was presented for successive data recording from the cochleo-vestibular system after that the BI and spikes discharge rate calculated for the cochlea-vestibular sensory neurons.

Auditory Thresholds: It is determined with the reducing intensity of the mechanical stimulus (near about 32 Hz), in that cases no longer respond of the cochlear auditory nerves at about 32 Hz. After determined a primary threshold

the final threshold was determined by the applying various stimulus from 32 Hz to above 32 Hz. The recording of the sensory neurons in 12 to 14 weeks old animals also consisted below the stimulus range of 32 Hz frequency, but clear spikes were not occurred at this range. A threshold was defined as the stimulus intensity at which the responses would be determined near 32 Hz, which was usually occurred by the Interpolation of sensory neurons. The primary observation was considered completely at a particular stimulus frequency when the thresholds appeared among at least four different terms from 32 -35 Hz.

4.3. Electrophysiological Recording of Sensory Nerves Responses

A wave analyzer (Oscilloscope Hantek 6022BE) connected to the audio-oscillator (Modal no.me-150), used for recording the amplitude of the cochleo-vestibular potentials over a constant frequency and assessing the potential as spike discharge rate. The stimulus frequency was delivered into cochleo-vestibular endolymph to produce a mechanical wave, that detected by the oscilloscope with root mean square (RMS) and frequency selectivity examined at about ≤800Hz. The CP curves were plotted with the various stimulus intensities. Therefore, the curves were plotted between potentiality and frequency, these curves support to explain the auditory sensitivity and bursting patterns in the cochleo-vestibular nerves. The sensitivity of the cochlear and vestibular sensory nerves is proportional to the stimulus intensity and range of stimulus frequency. Therefore, the high and low stimulus frequency may create

some inconstancy in the curves between potentiality and frequency, in this situation the stimulus intensity should be constant at sound attenuation booth.

To calibrate the intensity of mechanical stimulus, we were measured the frequency generated from the audio oscillator as a root mean square value during the constant stimulus. Throughout the experiments, the frequency intensity level was raised in 28 dB steps from 25 to 60 dB SPL (sound pressure level), at each intensity level, 230 spikes generated from the sensory auditory nerves were sampled and averaged. The threshold of auditory nerves and spike discharge rate were defined when peak to peak value was higher than the mean stimulus (MS) level; after well pronounced by the neurons, it was assessed during first 40 ms of the repetition time. The CVP (cochleo-vestibular potential) was measured using the same microelectrodes in both animals.

We were used Fourier transform and Auto-correlation to assess the magnitude of the spikes generated from the cochleo-vestibular nerves. The spikes discharge activity of the cochleo-vestibular nerves was recorded digitally up to 1 to 3 min for each animal. In figure-1, 2, 3, 4, 5, 8 and 9 (in chapter-5) have shown a typical response recorded over a given stimulus frequency for auditory and vestibular neurons in both animals. Analysis of spikes discharge rate and patterns was accomplished offline using the responses data recorded from the neurons. Throughout this detail, the mean value would be denoted as the mean SD. Comparable data recorded from neurons having: (1) spikes

stimulus ratio (spikes amplitude /stimulus) that allows determining the ambiguous spikes and (2) the recording term that was reasonable to collect the comparable data from sensory neurons.

We used the number of spikes multiply by the time between first and last spikes generated from cochleo-vestibular nerves as an objective metric for active sensory cells. When we were analyzed the spikes rate, we did not include the poor or unclear spikes generated from the cochleo-vestibular neurons. Therefore, we were applied different stimulus frequency for recording the regular spikes patterns and rate of spike discharge.

An audio oscillator like as mechanical stimulus connected to the medium/high-frequency filter has used for the determination of CP threshold that value for chickens and rats respectively were12.23 μv and 11.34 μv. There are also used the range of ± 20 kHz stimulus frequency to oscillate the cochlear and vestibular hair cells. Therefore, we observed the high magnitude of the cochleo-vestibular response. Analytical data for each active neuron included:

i. Total recording time (between first to last spikes)
ii. Total number of spikes
iii. Spikes discharge rate (total number of spikes/total recording time)
iv. Spike time interval
v. Coefficient variation (CVs= SDI/mean)
vi. Stimulus intensity

vii. Inter-spike intervals

The spike time intervals in any selected bins were determined as follows:

$$STI = X - Y \qquad (1)$$

Where X = Onset time of spike discharge (OTSD)

Y = Onset time of the preceding spike (OTPS)

STI = Spike time intervals

Table. 2. The onset time of spike discharge and onset time of the preceding spike discharge were recorded from the auditory system and vestibular system of both animals.

Subject	OTSD	OTPS	STI
Chicken			
AS	0.2 µs	0.4 µs	0.2 µs
VS	0.4 µs	0.5 µs	0.1µs
Rat			
AS	0.4 µs	0.5 µs	0.8 µs
VS	0.3 µs	0.5 µs	0.2 µs

The CVs (coefficient of variation) is a metric used to identify and characterized the spontaneous discharge patterns of auditory neurons in terms of spike time intervals (Jones, T.A and Jones, S.M. 2000). The CVs for spontaneous

discharge was recorded from cochleo-vestibular neurons were defined with the help of spike time interval generated at a given stimulus frequency (32 Hz to 10 kHz and intensity 25.5 dB to 45 dB). These variations can be minimized to some extent of the stimulus intensity but not completely, because it has a different developmental degree in different animals. Therefore, the CVs are an important parameter for discriminating the physiological and anatomical development in both animals; CVs between 0.6 and 2.0 reflect stochastic phases of the sensory neurons, these phases often have explained as a refinement process at a particular ranges of mechanical stimulus (MS). The CVs were recorded for sensory neurons in which the recording time was sufficient to resolve the bursting. The numbers of burst spikes have included for calculation of the bursting index (BI).

Bursting rate of the sensory neurons was calculated as a general counting of the total number of burst divided by the total time of recording. The burst was defined in the concern of an initial time period of obscure sensitivity of neurons, and this was identified by the long spikes intervals on curves. There are very important to define a minimum burst per period (MBPP) for the purpose of the counting of neural bursts.

There was used a threshold to determine the onset of neural bursts and bursting patterns. Here MBPP was set equal to four times of the mean spikes interval for entire spikes discharge, similar criteria was used by Gummer and

Mark (1994) for this purpose in mammals. The invasion of prospective bursts was revealed by spikes those having a spikes interval equivalent to or greater than the MBPP. A neural burst was counted in TIH (time interval histogram) on the invasion of spikes were followed to the group of two or more spike having prominent spike intervals equivalent or more than MBPP. The obvious inter-spike intervals were included for differentiation and evaluate the responses of sensory neurons.

4.4. Determination of Endolymph Movement

We have observed the endolymph flow in the cochlea and vestibular canal in both animals over a frequency ranges from 34 to 36 Hz. According to this, here presented data were collected from hair cells movement that transduces the mechanical movement/stimulus into an electrical waveform that encodes by the sensory neurons and after that it conveys to the brain. There have used the standard approach to the recording of endolymph flow for later off-line analysis and comparison with the bursting patterns of the neurons. Measurement of the SCCs dimensions, recording SCCs sensitivity and mechanism of cupular hair cells has been explained in the rats (Curthoys *et al*. 1986).

The movement of fluid within semicircular canal is corresponding to the rotation of head around a vertical axis in response to the mechanical stimulus was driven into the vestibular system. The semicircular canals have a swelling ends called the ampulla. The ampulla has covered the cupula which comprises

several fine structures called sensory hair cells. The movement of the fluid pushes on the hair cells consequent the motion of stereocilia and that transduce the mechanical stimulus into the electrical wave. The electrical signals produced by the cupular hair cells were recorded by the oscilloscope and weak positive stimulus was driven through the oscillatory microelectrode. The microelectrode was advanced at ~ 8 µm steps adjacent to the sensory neurons. Stimulus offsets were calibrated before the implant of the microelectrode. Stimulus intensity was amplified, filtered and sampled at 8 kHz, and then data were collected in a computer using the software of oscilloscope (response analyzer).

In data analysis, very low spikes were avoided so that the inter spikes intervals (ISI) absolutely discriminated in both animals. The stimulus frequency from 32 to 80 Hz also was avoided for calculating the SDR throughout the experiment. Spontaneous stimulus rate was determined during endolymph stimulation (duration at least ± 20 s), similarly, the spikes generation was analyzed by measuring the stimulus rate in the given time period for at least four repetitions of each stimulus intensity. To determine the maximum spikes discharge rate (SDR), the stimulus intensity was adjusted at ± 45 dB SPL throughout the experiment and SDR was determined by the following formula:

$$\text{SDR} = \frac{x}{y} \qquad (2)$$

SDR= Spike Discharge Rate

X= Total Number of Spikes

Y= Total Time of Recording

Table 3. Spikes discharge rate of the stimulated cochlear and vestibular endolymph in chickens and rats.

Subject	TNS (Minimum)	TTR (Minimum)	SDR
Chicken			
AS	±4000 Spike	20 Second	200 Sp/s
VS	±2400 Spikes	20 Second	120 Sp/s
Rat			
AS	±3240 Spikes	20 Second	162 Sp/s
VS	±3200 Spikes	20 Second	160 Sp/s

TNS = Total Number of Spikes; TTR = Total Time of Recording; SDR = Spikes discharge Rate; AS = Acoustic System; VS = Vestibular System; Sp/s = Spikes Per Second.

Each cupula were stimulated over 25 s steps of the stimulus that have increased magnitude of the spikes discharge. At all level of the stimulus (in both animals) the maximum and minimum constant spikes discharge rate have defined as a mean discharge rate that was evoked in response to four repetition

of the input stimulus by which spikes discharge could be constant throughout the experiment. The Control experiments in which stimulus frequency was delivered in random order, and then the regular spikes were not observed individually in both animals. The spikes discharge more altered by the randomness of the stimulus frequency.

An adaptation index was calculated while the spikes discharge rate between 20 and 60 sp/s because this rate of the spikes generation absolutely corresponds to the stimulus rate. We have analyzed the time course of adaptation during spikes generation in 20 seconds while the mechanical stimulus was conveyed to the vestibular sensory hair cells and their spontaneous spikes rate corresponds to the stimulus rate in the chickens and rats. Spikes discharge rate was evoked during the initial 25 ms, the invasion of steps stimulus did not favorable to the exponential spikes discharge so it was excluded from its. Spikes generation was defined as the curves of mean spikes discharge rate and stimulus intensity. The correlation coefficients for the mean spikes discharge rate and stimulus intensity were determined from the linearity of the spikes generated from the sensory neurons. The movements of the endolymph fluid in SCCs were quantified by the adaptation index (AI) as the spikes generate on histograms.

Analysis of the action potential of neural was observed on the average range of stimulus during at least 15sp/s. As has been explained by Johnston *et*

al. (1994), in the neurons of the medial vestibular nucleus, the action potential of membrane depolarized gradually from preceding each action potential, such action potentials do not have a specific threshold. In the present studies, action potentials threshold of the sensory neurons were determined as an intersection between gradually depolarizing phases leading the action potential and the quickly rising phases of the action potential. Action potential height was examined as the variation in neural potential between threshold and the maximum spikes discharge. The action potential width was examined as the time of response threshold during the depolarization of the sensory hair cells. After hyper-polarization of the cells, the amplitude was determined as the difference between action potential threshold and the most negative neural potential was gained during the hyper-polarization.

4.5. Evaluation of the Magnitude of Spikes Discharge

To quantify the magnitude of periodic response in time interval histograms of the spontaneously active sensory hair cells and neurons, we have identified the spikes sequence with the help of the autocorrelation and Fourier transforms. To the evaluation of the magnitude of SAO (spontaneously active neurons), total 150 bins were used for correlation between both animals and the size of each selected bins was 0.2 mm. Firstly, we calculated the autocorrelation function as spikes discharge in the bins of time interval histogram and then Fourier analysis (sampling stimulus 32 to 65 Hz/s).

Probability density of spikes discharge (autocorrelation function of active hair cells) was produced using the strategies outlined by Moller (1970) for quantifying the frequency selectivity of the sensory neurons in mammals. Data were taken from digital FTC (frequency tuning curves) files saved in oscilloscope for off-line analysis. For the evaluation of the variations in spikes generation and inter-spike intervals, we have calculated the CVs by the following formula:

$$CVs = \frac{\text{Standard Deviation of the Inter-spikes Intervals}}{\text{Mean Inter Spikes Intervals}} \qquad (3)$$

Table- 4. The CVs in the response of acoustic and vestibular sensory neurons of the chickens and rats.

Animals	SDISI	MISI	CVs
Chicken			
AS	±0.56 ms	±0.9 ms	0.62 ms
VS	±0.48 ms	±0.7 ms	0.06 ms
Rat			
AS	±0.78 ms	±0.9 ms	0.86 ms
VS	±0.67 ms	±0.8 ms	0.83 ms

SDSI= Standard Deviation of the Inter- spike Intervals

MISI= Mean Inter-spikes Intervals, CVs= Coefficient Variations

Stimulus rate (total number of spikes in bins/ total time period in second), coefficient variation (CVs= standard deviation of the inter-spike intervals divided by mean inter-spike interval) and inter-spike intervals in time histograms were also determined for this purpose. Spike intervals data were collected from histogram at a time resolution between 40 and 45 μs per point. Time interval histograms were also used to evaluate the patterns and spikes time intervals for comparison of the neural sensitivity.

4.6. Determining and Characterizing of Bursting Patterns

To identify and characterizing of the regular bursting patterns, there are firstly measured the period between uninterrupted burst and spikes discharge rate during bursting in cochleo-vestibular neurons. Here we have employed two neural bursting factors A [(total time of five longest intervals)/ (sum of sample time)] and B [(mean interval length of the longest interval)/ (total number of intervals)] to readily identify the neural burst. These two factors were included as the numerical indicator of burst for stato-acoustic neural activity.

Regular bursting patterns have easily quantified than the irregular burst in 12-14 week old chickens and rats with the stimulus range between 1200-1800 Hz. Irregular bursting patterns, bursts of neural activities was occurred at regular and irregular spikes intervals detached from each other by relatively a long silence period of neural activity. It was easily interpreted using the bursting factors (A and B above maintain) and this numerical factors helps in the ranking

of neural bursts. It was considered that such neural burst factors should be proportional to the respective amount of time at which the sensory neuron spent a long silent periods during the mechanical stimulus convey in endolymph, this factors known as A. It was calculated as sum of the longest four silence periods expressed as a millisecond of the total sample (recording) time. The burst components were taken into account of the relative magnitude of neural spikes discharge occurred during the active periods. There are a greater disproportion between silence and active periods of sensory neurons. The next component of the burst known as B, it was calculated as a proportion of mean spikes intervals of five longest silent periods divided by the mean spikes intervals over the total time of recording. These components are revealed to the average long silent periods spent by sensory neurons. These two factors A and B known as burst factor and included in present studies as the numerical indicator of the busting level for neural activity. This observation reveals that the magnitude of the BF played an important role for comparison of the regular and irregular bursting of neurons like the correlogram was used to identify the bursting of retinal ganglion cells by Sernagor and Grzywacz (1999). BF also provides a mean to quantify the magnitude of regular and irregular bursting.

Hazard Functions: To identify and compared the unclear discharge activity of the active cochleo-vascular neurons in animals, the hazard function (relative probability) and TIH (time interval histogram) were calculated (Harris and flock, 1967; Harris and Milne, 1965; Li and Young, 1993; Gray, 1967). The

overall co-relation of bursting has observed by the following equation:

$$H(k) = \frac{y_k}{\sum_{n=k}^{\infty} y_n} \quad (4)$$

Where H (k) expresses to the hazard function, k is the bin's number in time histogram, and y_k is the total number of times intervals in the bins that appeared in the time interval histogram (TIH). The H (k) shows the possibility of the number of spike has occurred in selected bins (k), it is given that spike intervals in the time interval histogram increased from first to last spikes. The selected bins size or time units used in the current studies for calculating the H (k). The magnitude of the H (k) as a function of the average spike discharge rate and is a constant for all longest spike intervals. The factors such as neural refractory periods tend to bias in spikes discharge pattern. Therefore, it may reduce the probability of spike discharge at a very short interval. The hazard function of the neural TIH therefore rises and somewhat stable for the probabilities of spikes discharge as the stimulus intensity increases. By comparing of the HF and the range of stimulus frequency, we can evaluate the maturational biases imposed on the spikes discharge patterns and rate.

Overlapping of the neural bursting patterns was determined by (1) inspection of the time records, (2) cross-correlation of spikes discharge and probability density function as a bursting pattern. Overlapping neural bursts

were included in the evaluation of the bursting rate and excluding the shortest inter-spikes interval. Bursting pattern was evaluated using two prevailing computational methods: (1) probability density function of the sensory neurons and (2) BI (bursting index). These multidimensional measurements were useful for characterizing and ranking of the bursting patterns.

Ocular Inspection of Time Records: The regular and irregular spikes patterns have confirmed by the neural sensitivity curves. The times of spikes onset were averaged and calculated for evidence of overlapping spikes of the sensory nerves (cochleo-vestibular sensory nerves), these features of the neural sensitivity indicates to the sensory neurons that encode a particular frequency of sound for relay the information to the brain. These characters would be plotted on the curves and have been examined by many researchers in mammals.

Spikes discharge rate and the onset time of oscillation in endolymph have plotted. The oscillatory or movement cycles were included in time intervals histograms. These combined plots and time interval histograms were observed offline or visually in detail for confirmation of the particular frequency selectivity by the sensory neurons. The spikes recorded from sensory neurons were found in a manner that it linked to other adjacent peaks and troughs as an entrained (illustrated in fig-4).

Cross-Correlation: Cross-correlation of produced spikes time intervals and invasion time of oscillatory cycles were included in creating four separate time

interval histograms for spikes envasion times (Perkel *et al.*1967). One cycle of time interval histograms was based on oscillation onset times and onset time of spikes discharge. The time interval histogram term was used herein to express the final results of the cross- correlation of cochleo-vestibular potentiality in both animals. The duration of time for cross-correlation of sensory neurons and resultant oscillation cycle plotted in histograms was equivalent to the mean onset time of spikes discharge.

The bins (8 mm long) were used for characterizing the correlation of the cochleo-vestibular potentiality. Theoretically, the exhibited activity from sensory neurons is randomly generated and independent to the external factors. The distribution of spikes amplitudes in time interval histogram have plotted for comparison with the time histogram amplitudes have been explained by Perkel (1967) in mammals.

Probability Density Functions: There are spikes time histogram was plotted to represent spikes discharge probability as a function of frequency selectivity of the sensory neurons at a given range of frequency. The number of regular spikes has occurred in each selected time bins that taken as an amplitude of spikes discharge. This was plotted as s function of the frequency selectivity at a particular range of frequency and time period, where each selected time bins corresponded to the one cycle period of oscillation. The time represented by the total number of selected bins was corresponding to the total time of the

recording of the neural frequency selectivity. If spikes discharge strictly linked to the oscillation cycles then each selected bins have comparable numbers of spikes (regular and irregular spikes). The spikes discharge amplitudes was distributed evenly across all selected bin throughout the time periods of recording. Likewise, if the spikes of neural sensitivity (regular and irregular) are randomly generated and free from external factors then the amplitude of the spikes discharge has to be randomly distributed across all selected bins of the time histogram. However, if spike discharges from sensory neurons were stochastic and have appeared in recurrent bursts with silent periods then the distribution of the spikes in selected bins would be similar and was not correspond to the randomly mechanical stimulus.

BI (Bursting Index):

The bursting is an extremely diverse phenomenon of the acoustic and vestibular sensory neurons, where periods of rapid action potential spiking are followed by quiescent periods. The bursting is a thought to be very important in the operation of robust central patterns generation. BI was calculated only for those bins contained 12 or more spikes. The interval of each spike was ranked from longest to shortest in the selected bins. The longest interval of the spikes were used for BI calculate. There are five longest intervals of the spikes were used from bins containing 15-60 spikes. The burst index was calculated as follows:

$$BI = A \times B \quad (5)$$

$$A = \frac{TTLI}{TST} \quad (5a)$$

$$B = \frac{MILLI}{TST} \quad (5b)$$

TTLI= Total Time of Longest Intervals (ms)

TST= Total Sample Time or Number of Intervals

MILLI= Mean Interval Length of Longest Intervals (ms)

TST= Total Sample Time or Number of Intervals

The first component (A) in the equation reflects the proportion of the period in which the sensory neurons spent a long silent period during the propagation of stimulus frequency along the cochleo-vestibular pathway. The second component (B) refers to an adjustment of the BI for the relative amount of spikes appeared during the stimulus frequency given to the cochleo-vestibular endolymph, that was greater contrasted between the null and more active phase of the sensory neurons of both systems, at this condition the BI was greater for active neurons at high stimulus frequency (BIs= >2). The bursting index of the sensory neurons of both system (cochlear and vestibular system) have summarized in table-7.

Table 5. The A values for the sensory neurons of AS (acoustic system) and vestibular system (VS) of the chickens and rats.

Animals	Total Time of Longest Intervals (ms)	Total Sample Time (s)	A (ms)
Chicken			
AS	±0.55	12	0.045
VS	±0.48	12	0.04
Rat			
AS	±0.52	12	0.043
VS	±0.46	12	0.038

Table 6. The B values for the sensory neurons of the acoustic and vestibular system of the chickens and rats.

Animals	Mean Interval Length Longest Intervals (ms)	Total Sample Time/ Number of Intervals (s)	B (ms)
Chicken			
AS	±0.35	12	0.029
VS	±0.29	12	0.024
Rat			
AS	±0.31	12	0.025
VS	±0.27	12	0.022

Table 7. The bursting index (BI) for sensory neurons of the acoustic and vestibular system of the chickens and rats.

Animals	A values (ms)	B values (ms)	Bursting Index (ms)
Chicken			
AS	0.045	0.029	0.0013
VS	0.04	0.024	0.00096
Rat			
AS	0.043	0.025	0.001075
VS	0.038	0.022	0.000836

4.7. Measurement of the Ear Anatomy

To measurement of the ear anatomy we have included 12 specimens of the animals (12 to 14 week old 6 rats and 6 chickens). The tympanic membrane and middle ear bones were measured with the help of a 15× dissecting microscope, it was fitted on the ocular micrometer with 12 mm scale divided into 0.2 mm units, and the ocular micrometer had calibrated against a stage micrometer subdivided into 0.002 mm units; Here following measurements were taken (1) Tympanic membrane (2) Malleus, 3) Incus, 4) Stapes.

The diameter of the tympanic membrane is ± 9 mm in both animals.

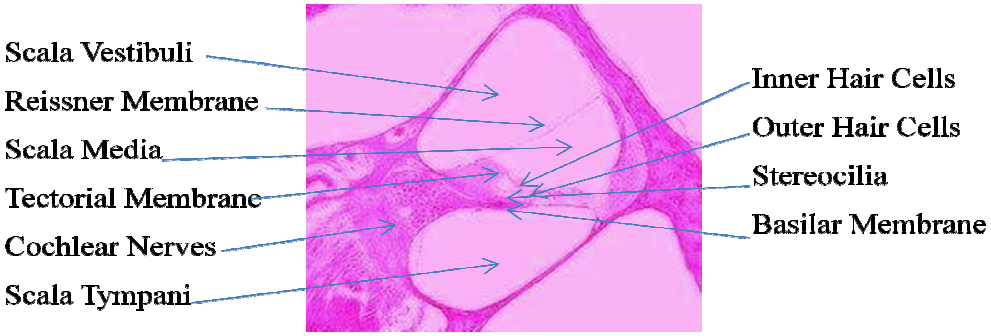
Cochlear and SCCs measurements were done by the midmodiolar axis, but the diameter is somewhat varied due to slight variations in orientation in both animals. The point to point comparative physiological data those closely correlated to the anatomical specialization are summarized in table-8 (Oaks, E. C. 1967; Pritchard, U. 1881). Measurements of the semicircular canals were made at 300 × using the Nikon microscope which calibrated with the ocular microscope and a rotating stage so that the spiral form of the cochlea and SCCs can be measured readily.

The casting technique was employed to examine the volume of the middle ear cavity. The temporal cartilage bones were clearly removed from 12 (6 specimens of chickens and 6 rats) frozen specimens. A dry cavity is well visible than the moist cavity of the middle ear. Cavities have casted with wax; it is easily pourable in liquid form in the cavity. After the casting of the cavity, the cartilage bone surrounds the cavity was broken away from the cast with a pair of fine pointed forceps under the dissecting microscope, after that the vacuities examined. The volume of each cast was examined from its weight and then the casts were weighted on a sartiorius electronic scale (weight in Gram).

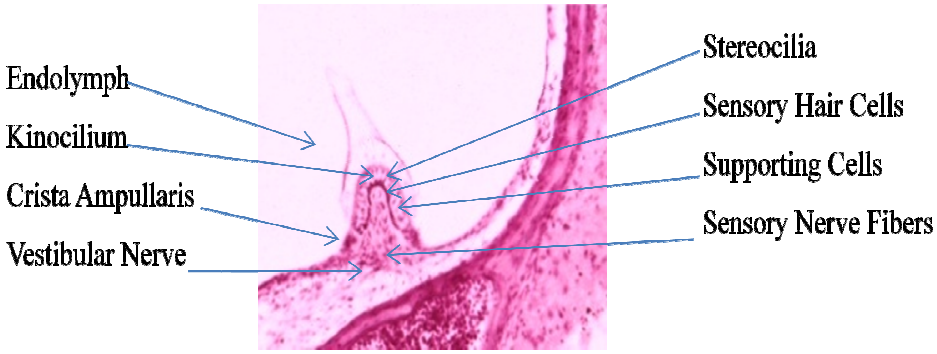
4.8. Histology of Cupula and Cochlea

The animal was sacrificed after the conclusion of the cochleo-vestibular test by overdoes of the chloroform. Before to fixation of each animal were flushed with the saline water. The cupula and cochlea were removed and fixed in

fixative (formalin 4-10 %). Before microtomy, the specimens were washed in tap water. Subsequently, several sections (15 -20 μ) was cut with microtomy, and then each section was passed in increasing percentage of the alcohol for at least 3 minutes for dehydration. After that, the section was stained and mounting. The organ of corti (part of the cochlea) and cupula (part of semicircular canals) are a very important part for the sense of endolymphatic movement. Under the organ of corti is the scala tympani and above it, the scala vestibule; both structures exist in a low potassium fluid called perilimph. Because those stereocilia are in the midst of a high concentration of potassium, once their cation channels are pulled open, potassium ions and calcium ions flow into the top of the sensory hair cells. The cross sections of organ of corti and cupula have illustrated in photograph- 2 and 3.



Photograph-2. A cross section of the auditory system (organ of corti) reveals the various components that involve in the mechanism of hearing and amplifying the low frequency.



Photograph-3. A cross section of the cupula and their peripheral components.

CHAPTER- 5

OBSERVATION AND RESULTS

COCHLEA

The cochlea is a part of the inner ear that receives sound wave in vibration form through the external and middle ear. The main component of the cochlea is the organ of corti a cellular layer on the BM (basilar membrane) and firmly covered by the bony labyrinth in the skull. The organ of corti has several sensory hair cells arranged in different types of rows and fine hair like structures called stereocilia located on the cells. These cilia are very sensitive in response to the oscillatory movement in endolymph of semicircular canals (SCCs) because these stereocilia are embedded in endolymph of the cochlear canals.

5.1. Response Properties of Stimulated Cochlea

It is mostly determined by the evaluation of the frequency selectivity (FS), spikes discharge rate (SDR), response threshold (RT), BI, CVs and ISI. There are SDR for chickens and rats respectively ≤200 sp/s and ≤162 sp/s, whereas the FS also highest of the chicken's auditory neurons than the rats. These data reflect the better response properties in regard to the particular range of stimulus frequency.

The frequency selectivity (FS) have attributed by the tendency of the auditory neurons to be most frequency selectivity with the increasing of anatomical specialization, it has explained by the plotted curves for individual animals. The highest peaks of the curve depict that the frequency at which the

active auditory neurons are most sensitive and better capability to generate regular spike. It is denoted by the characteristics frequency (CF). The cochlear response at the lower level stimulus was not similar to the stimulus waveform. At higher stimulus frequency, the responses offsets were shorter than the onsets and traces exhibit that persists well after the end of the stimulus.

We have observed the regular and irregular responses of the auditory neurons those innervate to the groups of sensory hair cells on the basilar membrane. In the present studies, it revealed that the overlying membrane imposes an offset on the sensory hair cells and the endolymph oscillation bends the stereocilia on the hair cells (Fredrickson-Hemsing, L *et al*. 2012).

The constant spike discharge was revealed by free oscillation of the sensory hair cells. The periods of regular oscillations (at ±10 kHz) was detached by the inter-spike intervals and height of spikes (maximum and minimum) when the stimulus of orderly increased amplitudes was applied (from 1 to 10 kHz). At all level the base and top of the curves were shifted by 20nm, these features for both animals were different but equivalent to the increased intensity of the stimulus applied to the endolymph. In this section, we have also observed the responses of the cochlear sensory neurons with the movement of the endolymph, that pushed on the sensory hair cells (it posses stereocilia on the apical end of the cells) and basilar membrane. Here we have also compared the responses properties of the cochlear sensory neurons between both animals (table-8).

Table 8. Comparative response properties of the cochlear sensory neurons in both animals.

Characters	Chicken	Rat
First Harmonic CP- spike	±2 kHz	±6 kHz
CP Null	appeared at ± 125 Hz	Appeared at ± 145 Hz
Switching in Phase	±1200 Hz	±945 Hz
BF (Busting Factors)	±0.06	±0.06 (similar to chicken)
Environmental and Anesthesia Effect	Marked effect on response	Marked effect on response
Induced Cochlear Response	Clear detected	Less than Chicken
Resonance Properties	Not detected	Not detected
Maximum Response	±6 kHz	±8 kHz
SDR	High than rat	Low
Characteristic Frequency	±8 kHz	±10 kHz
CVs	±0.62 ms	±0.86 ms
Auditory Neurons Susceptibility at Higher Stimulation	More Susceptible	Less than Chicken
Bursting Index	±0.0013	±0.001075

CP = Cochlear Potential, kHz = Kilohertz, Hz = Hertz, CVs = Coefficient Variations, ms = Millisecond, SDR = (Spike Discharge Rate)

At low level of the external stimulus (below 125 Hz) the sensory neurons were shown the low level bursts as the oscillation of the sensory hair cells and basilar membrane. At higher level of the stimulus (±8 kHz), the irregular phases between 8 to 10 kHz have replaced by the more stable regular phase in chicken's auditory neuron but these harmonic phases were shown by the auditory neurons of the rats at about ±10 kHz. This feature shows the variances in the potentiality of the acoustic system in both animals, but this potentiality is different in both animals. It may vary from animals to animals or species to species on the ground of environment and development of the acoustic system. As the ES (external stimulus) further increased, the response of the auditory neurons was continued, but they show the more spikes burst and little spikes time intervals in the chickens and rats auditory neurons, but not appeared similarity in both animals.

Although the mean spikes intervals decrease with the increasing of the shifting of the response phases, but their magnitude of the spikes discharge was constant. Finally, the stimulation in order of 8-10 kHz, overlapping and bistabilities in the spike discharge patterns subsequently were disappeared and prominent spikes were observed. When increases in the stimulus frequencies then the regular bursting patterns become more stable than the irregular patterns. In this condition, bistabilities in the spikes discharge phases were removed and the control parameter (μ) orderly decreased with the increase in the spikes discharge rate. The effects of slow and irregular phases offset were

observed as a spikes discharge on histogram individually for both animals (in fig- 1 and 2), in figure -1 has shown typical traces of different stimulus offset applied to the endolymph and cochlear response.

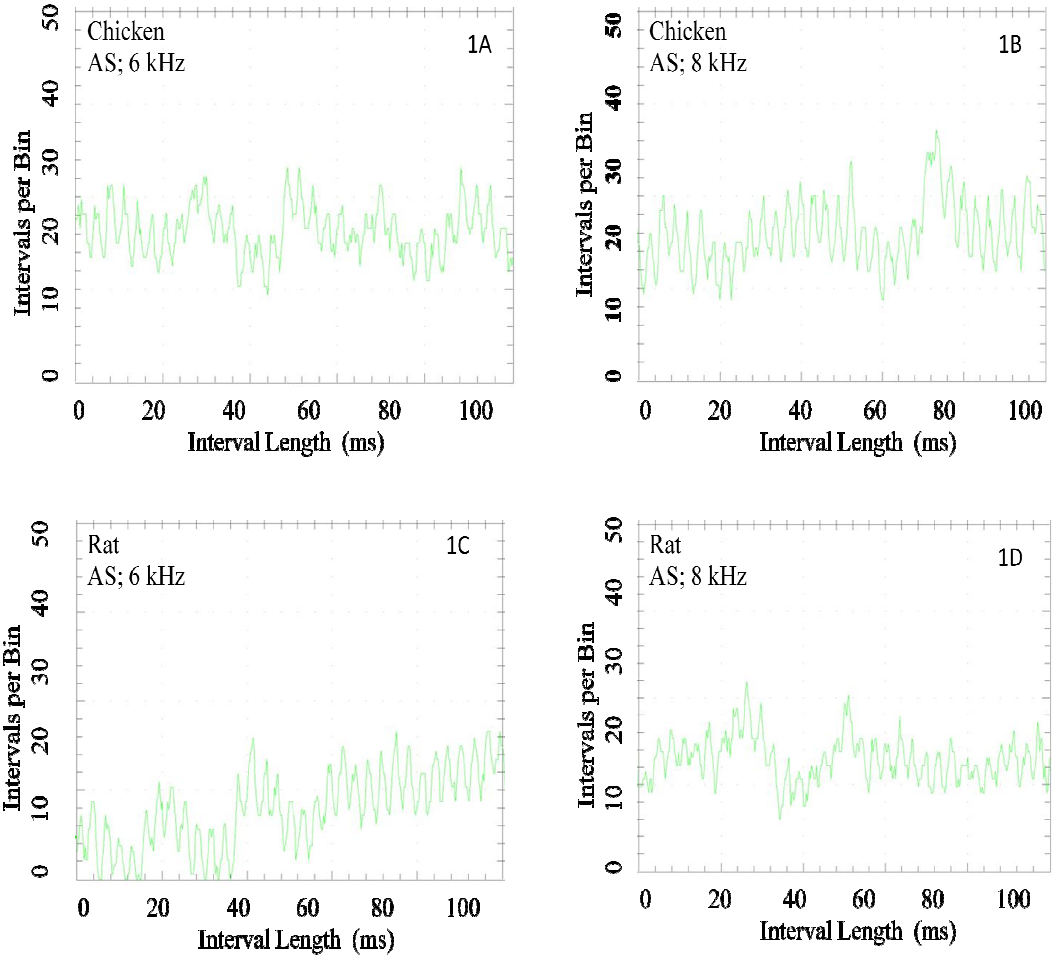


Fig-1. Intervals spike per bin vs. interval length curves 1A and 1B depict the response properties of cochlear acoustic neurons in chickens, as curves 1C and 1D depict to the cochlear neurons of the rats.

An important feature of our hypothesis is that the amplification of the stimulus wave due to the movement of the sensory hair cells of basilar membrane and stereocilia. According to the spike discharge rate (162 Sp/s) of the acoustic neurons of the rat's cochlea have less amplification properties. Although the results of the observation informed that the response of the auditory neurons is also correlated with the tonotopy and movement of the sensory hair cells in response to the endolymph oscillation.

Figure-1 reveals the blueprint of the neural sensitivity at a particular stimulus range and also illustrating their tonotopic arrangement in the cochlea. The instinct bursting mode has observed here for the chicken's auditory neurons was not similar to the bursting mode of the rat's auditory neurons. This different mode depicts the development level of the auditory neurons of chickens and rats. Despite much variation in sensitivity of the auditory neurons in both animals, the similarity and dissimilarity in the mode of spike discharge may reflect the refinement process of the active auditory neurons. Ultimately, such refinement process is not correlated only to the sensory hair cells but it also closely correlated with the peripheral components of the cochlea in both animals. This refinement process may generate the variation in bursting mode, SDR, CVs and BI at all level of the stimulus range and intensity. These variations in both animals are different and reflect the adaptation according to the stimulus range. The variation of neural response as a function of stimulus frequency and intensity may be viewed comprehensively by recasting the data.

5.2. Traveling Sound Wave Amplify by Sensory Hair Cells

Amplification of the propagating mechanical wave within the cochlea of the both animals remains unresolved, to confirm we were used various mechanical stimulus (300 Hz 350 Hz, 400 Hz, and 450 Hz) to induce the endolymph of the cochlea, consequently, we could be determined the oscillation along the length of the BHC (basilar hair cells).

It is believed that the cochlear sensory hair cells are an important component in the acoustic process in all vertebrate. These sensory cells produces electrical forces by their oscillatory movement that enhance the response of the cochlea and vibration of the basilar membrane, by which the traveling wave amplify and more sensitivity has occurred in the cochlea of both animals. When we stimulated the cochlear endolymph at 300 Hz range of frequency, the resulting frequency of the cochlea is ~4 kHz, this frequency much higher than the stimulating frequency; it is also higher than the amplitude of the stimulating frequency. It means that the SHCs have a capability to amplify the propagating wave in response to the endolymph oscillatory movement in the cochlear duct, because the SHSc firmly embedded in the endolymph, and their movements push on basilar membrane and sensory hair cell. The same experiment was performed on the rats, their resulting frequency was also high ~2 kHz but this frequency is less in compared to the chickens (Brownell, *et al.*1985). There are also determined the time taken for generating the 1-20 spikes by the cochlear auditory nerves. There have recorded the several

microsecond differences in propagation and amplification properties of the basilar papilla in the chickens and rats (in fig-2 and 3). This characteristics of the chicken's cochlea were high than the rat's cochlea.

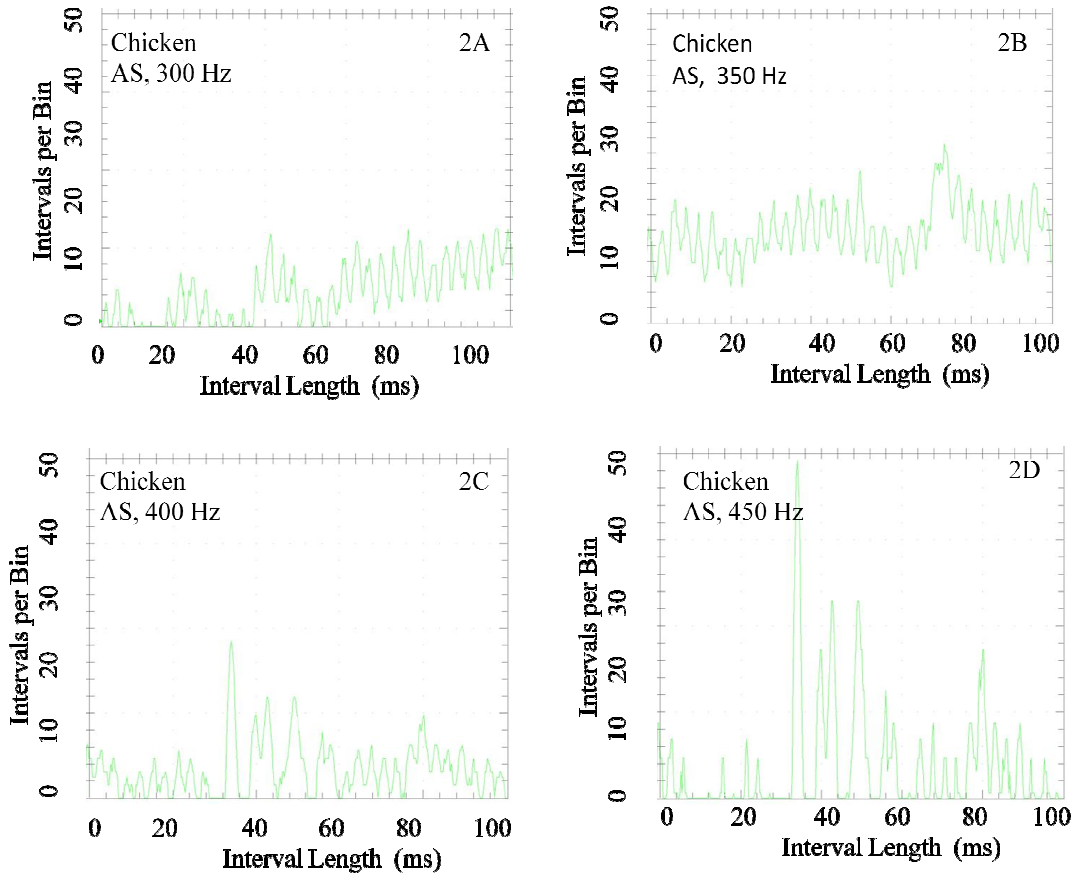


Fig 2. The variation in the time intervals histograms (TIH) plotted for the response of chicken's cochlear sensory nerves at 300 Hz, 350 Hz, 400 Hz, and 450 Hz stimulus frequency, but output frequency was observed little high from cochlear sensory neurons. The spikes discharge suppression is longer in 2A TIH than other TIH.

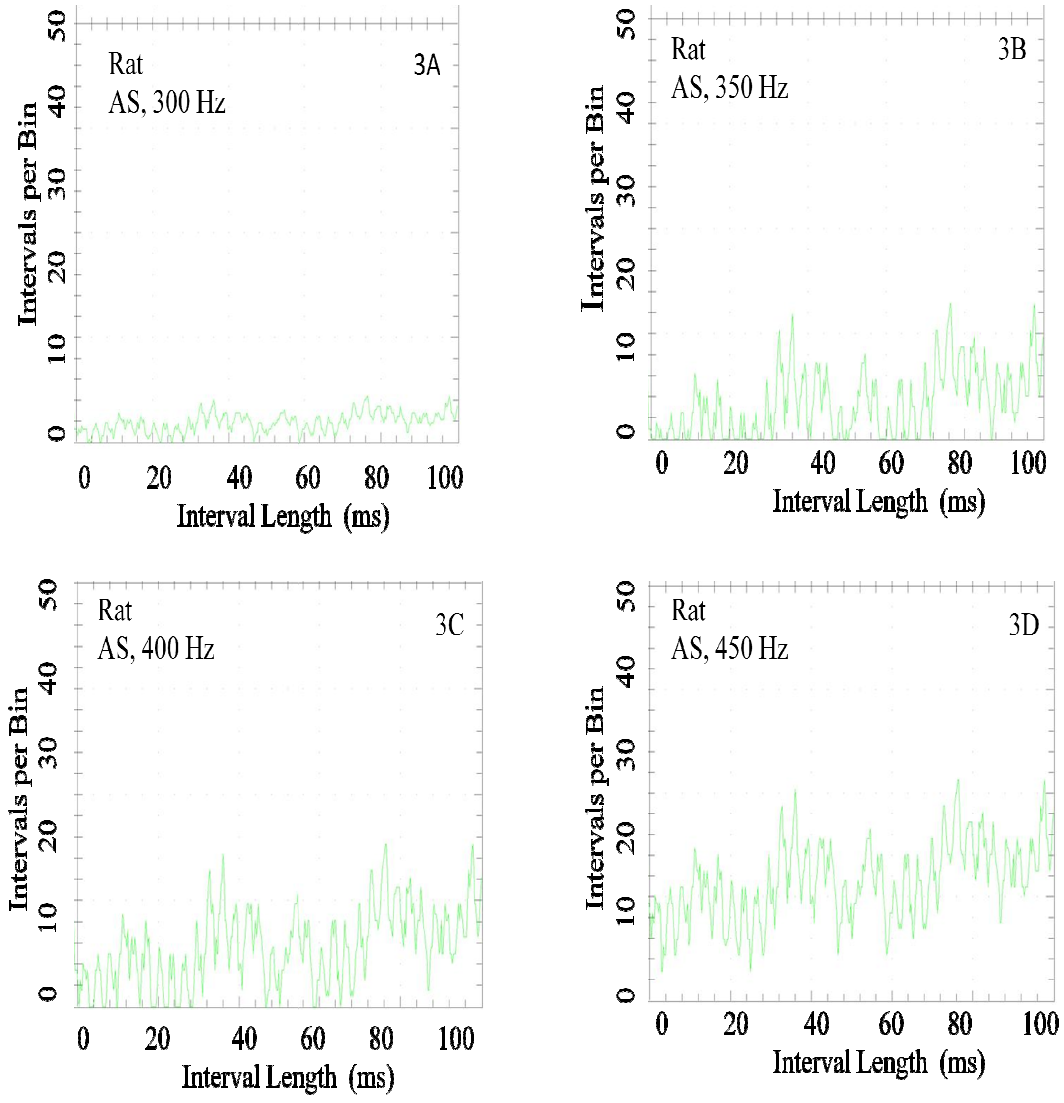


Fig 3. The variation in time intervals histograms (TIH) plotted for the response of rat's cochlear sensory neurons at a different range of stimulus frequency and intensity; we have also found the amplification of the propagating wave in rat's cochlear but less than the chickens. The spikes discharge suppression is longer in 3A TIH than the chicken's auditory neurons.

Both animals represent to the different habitat and classes so frequency selectivity is not relevant when the sensory hair cells intact into the organ of corti. The conduction of electrical signal by the auditory neurons may be dominated by the motility of the sensory hair cells. The capacitance factors are very important to the hair cells motility along the basilar membrane in that cases the characteristics frequency of the hair cells may vary. The existence of traveling waves within the endolymph of the cochlea was powered by the sensory hair cells arranged on the organ of corti in both animals. On the other hand, the vibration in basilar membrane was measured in avian and mammals revealed the response of frequency dependent phase lags (Smolders, J.W. *et al.* 1986).

The recorded of neural response have shown in the curves of bursting phases that is consistent with the traveling stimulus waves in the cochlea of both animals. On the other hand, the observation of the sensitivity of auditory neurons in barn owl does not match with the tonotopic frequency represented by the basilar papilla (it have many sensory hair cells as cluster) (Fontaine, B. *et al.* 2015).

The critical features of a traveling sound wave is that it taken time to propagate from the tympanic membrane to the organ of corti, because the frequency selectivity by the cochlear components are mainly depends on the nature and intensity of stimulus frequency. Therefore, we have used the data of endoplymph oscillation and response of acoustic neurons from both animals to

determine the amplification and propagation of the traveling mechanical wave. This was done by the observing of spontaneous response curves of the interval spikes per bin versus frequency. We were found longer delays of the spikes generation from the auditory neurons of the rats than the chickens at about 300 Hz, higher frequency stimulus had longer delayed that consistent with traveling sound wave in the cochlea.

To confirm, we have repeated the experiment for twice, where 300 Hz stimulus frequency was presented and the spikes discharge was measured in both animals. We were measured the time delayed between first spike and last spike of the bursting patterns of auditory neurons (in fig-3). Together, these data reveal that the sensory hair cells and basilar membrane in both animals support in the traveling sound wave amplification, which was consistent with the concept that the amplification of sensory hair cells in chicken's cochlea is like a series of regular spikes discharge as does in the rat's cochlea at ±400 Hz stimulus.

5.3. Spontaneous Discharge Rate in Cochlea (SDR)

The cochlear nerves of the avian and mammalian have a comparatively prominent SDR in developing animals, this rate was different from newborn to mature animals, where 25 sp/s and 65 sp/s respectively for $\leq$ 12 weeks old rats and chickens, whereas same SDR rate observed from $\geq$ 12- weeks old rats and chickens auditory nerves. This dissimilarity between 12 and 14 weeks old animals could suggest to the cochleo-vestibular tonotopic or neural refinement

in both animals.

The relationships between spike discharge rate and characteristics frequency for both animals (12 to 14 week old) are clearly different. In same ages and groups of the animals, there was a significant relationship between spikes discharge rate and characteristics frequency in both animals at a higher range of stimulus frequency. The relationship in CVs has not occurred in different ages and groups of the animals and reduced the level of spikes discharge rate has also appeared at low stimulus frequency in both groups of the animals. The changes in spikes generation occurred at high and lower stimulus level have functional implication for the ability of mature sensory neurons to convey information about the oscillation of endolymph and other components of the cochlea. The limit oscillation range and tendency of nonlinear responses were observed in the TIH, it implies that mature cochlear neurons can respond with proportion changes in stimulus rate than their immature counterparts.

Low spontaneous spikes discharge rate has been recorded from the auditory sensory neurons in developing avian and mammals. The uniformly distribution of the spikes discharge and inter-spike intervals was prominent across all responsive neurons of each group of the chickens and rats. However, the distribution of spikes in histogram was relatively varied in different ages of animals, thus the neurons of 12 weeks old have a lower spikes discharge rate. Numerical data of the SDR and silent period have summarized in table-9 for auditory and vestibular neurons of both animals.

Table-9. The spikes discharge rate and silent periods revealed by the sensory neurons of the acoustic and vestibular system in chickens and rats which representative of the different habitat and classes.

Measure	SDR	Silent Periods (ms)
Chicken		
AS	±200 Sp/s	±60 ms
VS	±120 Sp/s	±58 ms
Rat		
AS	±162 Sp/s	±90 ms
VS	±160 Sp/s	±74 ms

AS= Acoustic System; VS= Vestibular System; SDR= Spike Discharge Rate;

ms = Millisecond; Sp/s= Spikes Per Second

In the different group of animals, the spike discharge rate as a function of all intact components of the organ of corti at CF; these animals have highest discharge rate on a particular range of stimulus frequency. Generally, the relation between SDR and CF did not obtain in the different groups of the animals because the tonotopic arrangement of the auditory neurons and sensory hair cells may be different. There are very unusual that the acoustic neurons of post-hatched animal had unclear tonotopy and the spikes discharge rate, it is comparable with the higher rate examined in the chickens. Indeed the SDR of the auditory neurons in 14-weeks old animals was very peculiar as recorded in

the chickens by Manley *et al.* (1991a). In that cases, there are sufficient numbers of spike to quantify the discharge patterns. The CVs and BF (burst factors) were observed for irregular spikes discharge (fig-4).

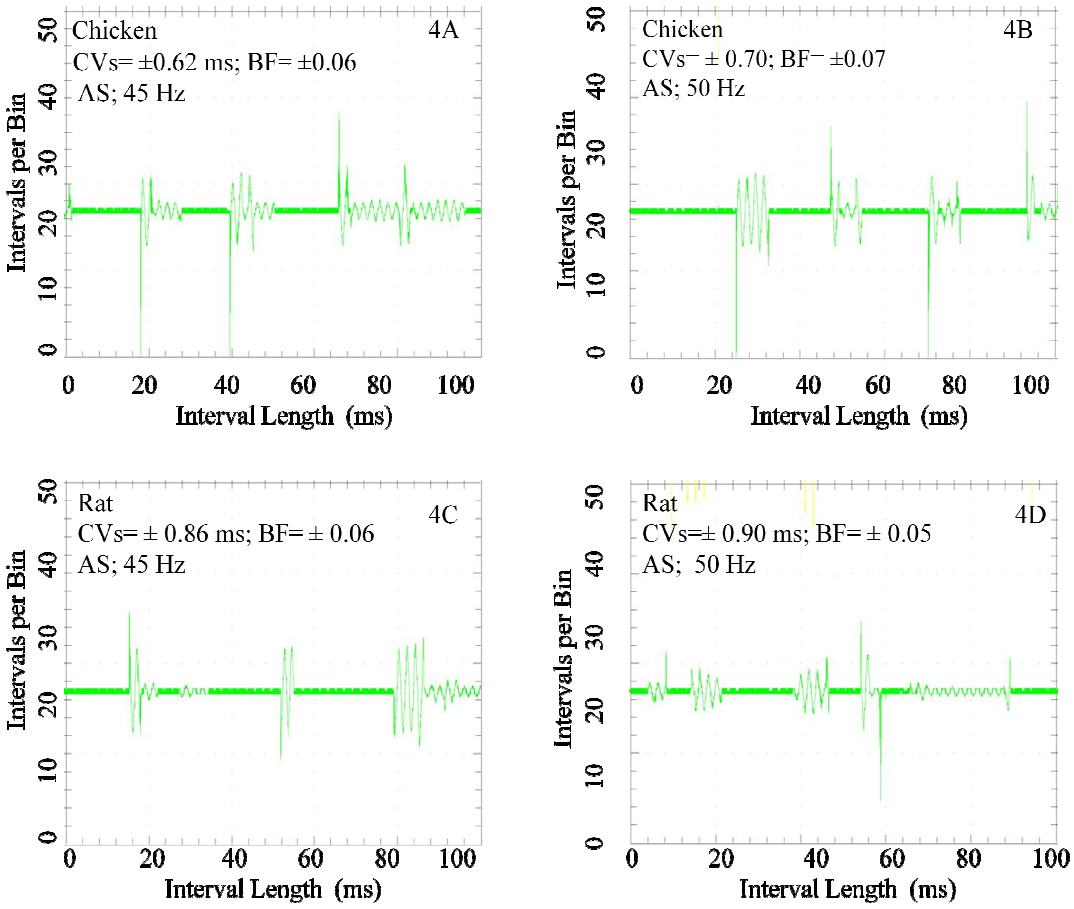


Fig 4. Irregular spikes discharge plotted form the cochlear sensory neurons in chickens and rats at 45 Hz and 50 Hz stimulus. In Curve 4A and 4B have shown the CVs and BF of the sensory neurons of the chickens, there are more different in CVs but BF is similar at 45 Hz in both animals. Curves 4C and 4D representing the irregular sensitivity of the cochlear neurons in rats.

There are not possible to obtain a well TIH (time interval histogram) for auditory neurons having minimum inter spikes intervals (≤ 12 ms), because SDR were too low. In those cases, where are minimum inter spikes interval then the data were not included in calculating the mean spikes rate and the silent period of the discharge. There are mean spikes interval in the chickens was high than the rats.

The spikes time intervals in regular spike patterns of auditory neurons were significantly longer in 14-weeks old chickens in compare with the 14-weeks old rat have shown in table-2. For auditory neurons, the spikes time intervals were independent to the mean of spikes discharge rate. In contrast, the spikes time intervals of regular and irregular spikes were varied as a function of the neural spikes discharge rate. The miner spikes intervals often indicate to the dead time, that was high for rats (±90) than the chickens (±60) (in table-9). The dead time of the sensory neurons was different in both groups and ages of the animals. However, when there were considered the discharge rate of auditory neuron individually in both groups of the animals, we found that the chicken's cochlea was shown the high discharge rate than the rat's cochlea (in table-3).

Coefficient Variation (CVs) in Inter-spikes Interval: In figure-4 have shown the sensitivity of the auditory neurons in both groups of the animals. The neurons represent the variations in the inter-spike intervals in both animals, it can be explained as regular and irregular bursting. Coefficient variations (CVs) for the cochlea has varied as a function of regular and irregular discharge and

inters spike intervals (in table-9).

The spikes generation was relatively irregular in the mode of long inter spikes interval and CVs of regular discharge was very low, whereas the irregular patterns have a CVs of ±0.86 ms, these values are contrasted to the CVs of regular and irregular spikes generated from the sensory neurons of the rats. Nevertheless, the CVs for both animals have generally varied and ranged from 0.02 to 2.4 ms from lowest to highest stimulus frequency. The highest CVs were observed for auditory neurons of the rats, whereas the lowest CVs were found in the auditory neurons of the chickens. The CVs of different patterns were obtained from auditory neurons is shown in figure- 4 for chickens and rats.

5.4. Cochlear Responses between 150 Hz and 1800 Hz

The overall curves obtained from the cochlea of both animals; thus the maximum response peaks occurred at ≥1750 Hz and ≥1800 Hz respectively for chickens and rats. Similarly, the minimum sensitivity was determined near about 140 Hz and 230 Hz respectively for chickens and rats. Observation of the figures 2 and 3 show a clear similarity in disposition of phases of the response of cochlear auditory neurons in the chickens and rats was recorded by electrophysiological. In the chickens, the spikes of the minimum responses were observed at low stimulus frequency than the rats. The second large spikes have less magnitude appeared between 1100 Hz and 1300 Hz. These two spikes have separated from each other by the downfall in cochlear sensitivity at either 1200

Hz or 1400 Hz. The clear spike between 1100 Hz and 1300 Hz does not appear for cochlea of the rats, it shows minor spikes near 1400 Hz and spikes was dropped near 1600 Hz, but auditory neurons of the rats were shown gradually rise in minor spikes generation from ± 1650 Hz to 1800 Hz. At least four major spikes have occurred at 1800 Hz and unusual spikes were examined at 700 Hz from the auditory neurons of the rats, but these unusual spikes were observed at 500 Hz in the chicken's auditory neurons. The clear discrimination in response was obtained by the plotting of the cochlear sensitivity curves for both groups of the animals; these curves reflect the increasing anatomical specialization of the cochlea, therefore, it enhance of cochlear potential or sensitivity over the range of stimulus frequency in both animals.

5.5. Spikes Discharge Pattern (SDP)

The patterns of spikes discharge were remarkably contrasted between both animals from neonates to mature. The spikes discharge in mature animals reflected continuous, irregular that contained no appreciable long period of inactivity, whereas the discharge patterns in the neonate occurred in a series of burst interrupted by long silent periods. The SDP is proportional to BI, spikes discharge rate and CVs (coefficient variation). The value of the BI, CVs, and spikes discharge rate was calculated for chickens respectively are as 0.16, 0.79 ms and 98 sp/s and the value of the BI, CV, and Spikes discharge rate for rats are as 0.14, 0.74 and 81 sp/s (Jones and Jones 2000; Jones, T.A. *et al.* 2001), This discharge rate in 14 weeks old animals reflects harmonic and regular

patterns (in fig-5).

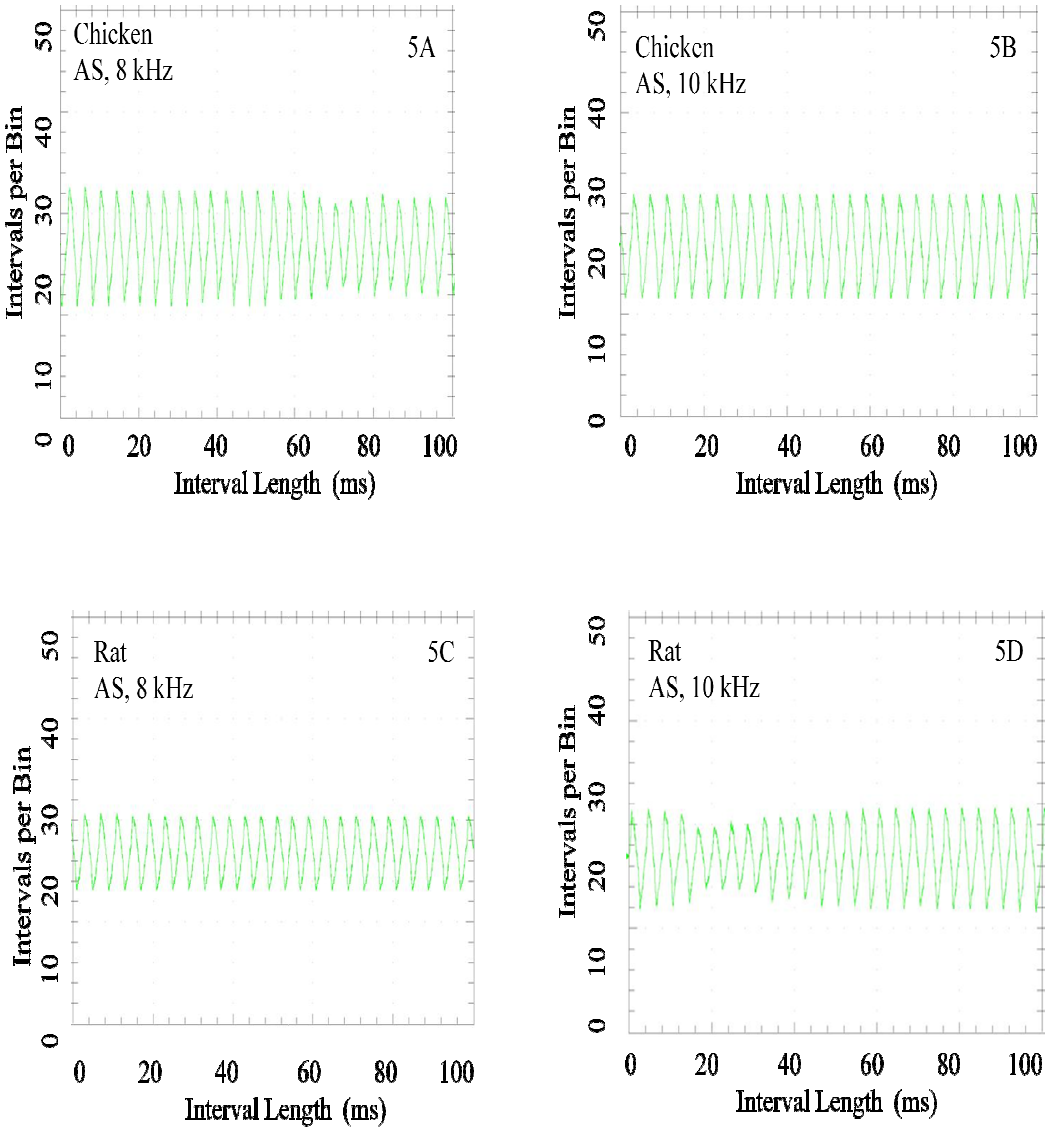


Fig 5. Regular spikes discharge patterns recorded from the cochlear sensory neurons at 8 - 10 kHz stimulus rage. At this range, the CVs and ISI were less and the neurons burst have more similarity in both animals; the dead time (0.2 ms) and switching in the patterns also very less.

The sensitivity of auditory neurons in both groups of the animals are evidence of the regular spikes discharge having a clear distribution of spikes as shown in TIH (time interval histogram) of figure-5. This spike patterns also found in both groups of the animals as widely has been reported for posthatch birds and mammals by Sachs *et al.* (1974, 1980). However, in chickens, the longest spikes interval has occurred with less probability of CVs in compared to the rat's TIH.

In curve 5D has shown the parallel appearance of the shortest spikes intervals to the longest spike in TIH of the rats, this features of chicken's auditory neuron was very less than the rats. It was contrasted with the position and clear appearance of spikes in TIH of both animals at 8 and 10 kHz range of stimulus frequency. As the time of inter-spikes interval has increased beyond the dead time (miner inter spike intervals) in the rats then sudden raise in CVs of spikes and exponential declined in the number of spikes in selected bins. In the chicken, it has also gradually raised and declined of the spikes in overall bins but comparatively the spikes interval, height and length was different in both animals. The minimum inter spikes intervals are just double in rat's sensory neurons in compared to the chickens.

The spike patterns corresponding to prefer intervals are plotted against characteristic frequency (CF) in curve 5B revealed the PIs frequency; it was a function of CFs for both animals in the current study. The preferred intervals (PI) tends to distribute near the lines of corresponding CFs (characteristics

frequency). The spikes discharge patterns were quite varied as has been recorded by Temchin (1988) in the birds. In figure-5 has illustrated four discharge patterns generated by the auditory neurons.

Figure-5D is a record of the auditory neurons that have some different regular bursting patterns in the rats and periodically appears as an instinct burst of neural activity. Discharge of spikes and silent periods was occurred at intermittently, this pattern was found only rats (14-weeks old) with miner inter spikes intervals. The regular spikes patterns were shown in the figure-5 for the rats were not corresponding to the chickens at the same range of the stimulus frequency (8 and 10 kHz).

To provide the quantitative measure of regular spikes burst, the BF (burst factors) was determined for both animals individually. In figure-4 has shown the burst factor for spikes train recorded from the auditory neurons in both animals. Generally, the magnitude of the bursting factors was correlated with the bursting of regular spikes. Fig- 4 shows the contrasts BF for auditory neurons in rats and chickens and it is a example of considerable amounts of the irregular bursting of spikes was found in both animals at lower stimulus frequency (45-50 Hz), but this feature was not obvious in rats than the chickens.

Hazard function can be used to determine the alteration of probability in the inter-spikes intervals and unclear distribution of spikes in the time intervals histograms. Figure-6 show the hazard functions for TIH of both groups of the

animals.

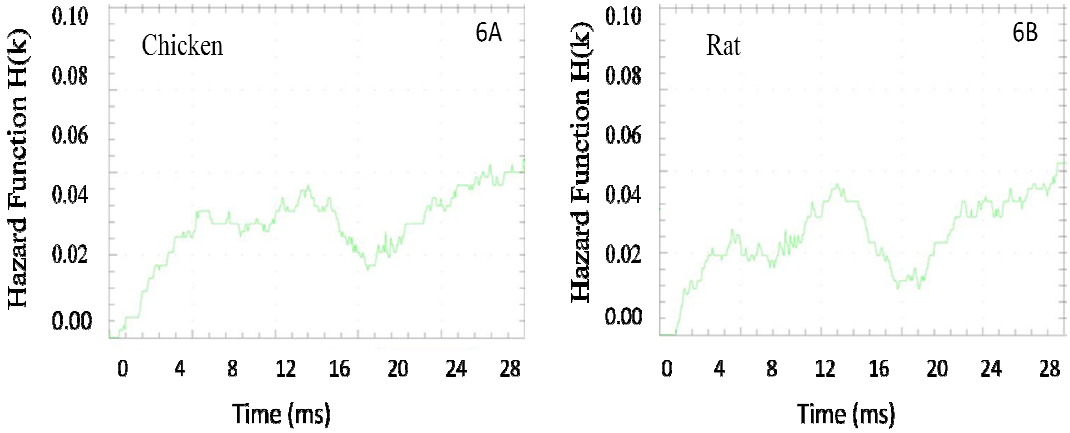


Fig 6. The hazard function for sensory neurons of both chicken and rat, both animals produced quasi Poisson distribution of the spikes and intervals between spikes at 10 kHz stimulus frequency, and it observed by the H (k) that rise quickly with spikes intervals of 4 ms.

In both animals, the large spikes intervals lead to variation in a constant mean value of spikes distributed in time interval histograms and also refer to the probability of regular inter spikes intervals. However, there are not required the significantly longer interval period to achieve the probability plateau from the acoustic neurons. There have considerable bias against the spike intervals it was less than ~3 ms, whereas the neural potential was occurred at much shorter spike intervals in rats. This result of both animals is relevant to each other and both animals provide a clear graphical illustration of the substantially longer spike time intervals. The Auditory neurons in both animals also revealed

a regular spike discharge patterns of preferred intervals (PI).

Preferred intervals in regular spike discharge patterns are obviously recorded from acoustic neurons of the chickens and rats; thus, we found only three examples of clear PI (preferred intervals) between 4 and 12 ms in the standard time interval histogram (TIH) of the rats. Autocorrelation analysis manifests only two instances of unclear periodic spike discharge patterns in rats; these optimal examples of the preferred intervals in spike discharge are illustrated in figure 6. In figure 5D has shown the differences in recorded TIH, autocorrelation function and fast Fourier transformer for the auditory neurons of rats and chickens. There are no clear IP in the time interval histogram of the 14-weeks old animals. Nevertheless, a high amplitude spikes discharge was apparently present in the autocorrelation function of the same time interval histogram at high stimulus range. The amplitude of fast Fourier transformer reveals an explanatory base near 8 kHz for the chicken's ACF. According to the ACF (autocorrelation function) chickens are higher sensitive at a low range of the stimulus frequency.

5.6. Bursting Index (BI)

BI calculated only for those curves which have more than 11 spikes, the intervals in recorded spikes were defined by the longest and shortest intervals as shown in figure 2. Therefore, the BI calculated by following formula (detail see in method):

$$BI = A \times B$$

A= Reveals the proportion of time that the hair cells spent a non oscillatory period.

B= Reflecting the relative amount of motility present during discharge burst

The spikes length and inter-spikes interval over time were also remarkably different for both groups of the animals as shown in figure-2 and 3. In figure- 2 and 3 illustrated the clear sensitivity of the auditory neurons of the chickens and rats respectively. The length and inter-spikes interval of spikes discharged from auditory neurons of the chickens are prominent than the rats. The spikes discharge have been recorded relatively irregular at 32 -45 Hz range of stimulus frequency in both group of the animals, that not contained a remarkably long period of the neural sensitivity, whereas the appreciable spike discharge has appeared between 4 and 10 kHz stimulus frequency. These spikes were used to calculating the BI. The BI value of chicken's auditory neurons is higher than the rats as shown in table-7. The variances in inter-spikes intervals over a long period of the time have plotted in time interval histograms as illustrated in figure-2. Each point in the curves of figure-5 shows the correlation between invasion time and spikes time intervals. The long time interval gives a comparable spike discharge rate in spikes per second (sp/s) for intervals per bins, which is referred to ongoing "spontaneous spike rate".

Figure-5 prominently shows the regular spontaneous spikes discharge and inter-spikes intervals from auditory neurons of both animals. The spontaneous inter-spikes intervals shown in table-10 for acoustic neurons separately and these values are varying in both animals. In table-5 has shown the BI value for the sensory neurons of acoustic neurons of the chickens and rats. These values are consistent with an exponential distribution of the inter spike intervals, which is a characteristics of the random spike discharge (Kiang, 1965; Walsh *et al.*1972).

5.7. Cochlear Potentiality (CP)

A small potential is generated due to a difference in potential between the endolymph and perilymph. The CP data were recorded with the help of microelectrode positioned near the basilar membrane. The stimulus 2.50 kHz was delivered with intensity range from 30 to 95 dB SPL. The erratic value of cochlear potential revealed as switching in the spikes and shape of the curves. Similar electrode and oscillatory frequency were used for recording of the cochlear potential and identify the potential threshold in both groups of the animals. With the help of audio oscillator and wave analyzer oscilloscope are possible to record the cochlear potential over a broad range of frequency.

The amplitude of the cochlear potential at a given range of frequency was depend on the SPL (sound pressure level) of the external stimulus and when the intensity of the external stimulus was changed gradually; there was a

corresponding change in the CP (cochlear potential). Thus, in both groups of the animals, the cochlear potential vs. frequency curves was determined with the different levels of stimulus intensity. The different length and patterns of the spikes (in fig-7 for Rats and fig- 8 for Chickens) in the curves of the neural response were not overloaded with the stimulus intensity.

The curves plotted for both groups of the animals were compared at the similar stimulus intensity. Therefore the audio oscillatory output was not constant throughout the observation of spikes patterns with 1 to 10 kHz frequency sweeps. The curves in figures- 7 and 8 are illustrating the stimulus and response values that depict the attenuation of the stimulus in regard to the maximum output of sensory neurons, as have revealed by the maximum audio oscillatory output curves. The SPL was relatively constant throughout the most frequency (1 to 10 kHz) band have studied in both animals.

The spikes generated by the cochlear auditory neurons of the rats were relatively low in amplitude and cochlear potential vs. frequency curve could only be recorded when the range of the stimulus frequency was relatively high (5 to 10 kHz). Figure-7 illustrates the cochlear potentiality of the rat's cochlea. The characteristics of these curves were constant in six rat's ears, which were: (a) The highest CP amplitudes were observed at $\geq$ 6 kHz range of the frequency, (b) the CP amplitude was declined between 32-45 Hz (d) CP amplitude null (minimum cochlear responses) recorded around 40 Hz, (e) Miner CP amplitude spikes have shown near 200 Hz.

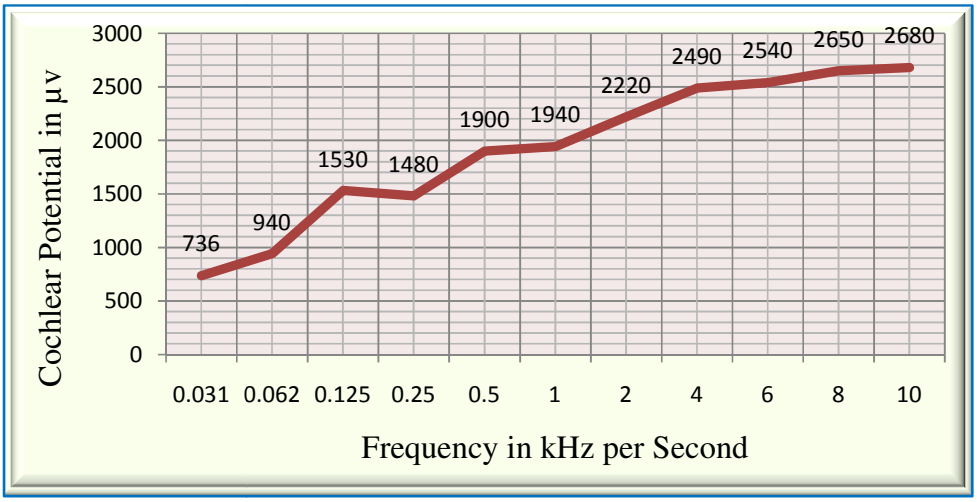


Fig-7. In Cochlear potential vs. frequency curve has shown the cochlear potentiality of the rats and this curve also reveals the less cochlear response of rats than the chicken's cochlea with the increasing range of stimulus frequency.

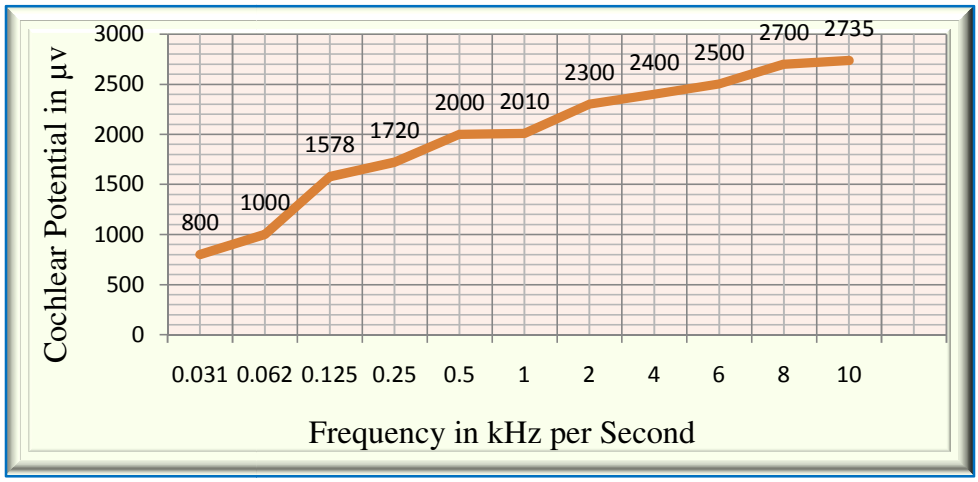


Fig-8. In curve shown the cochlear potential of the chickens, on the basis of the curve, the cochlear potentiality of the chickens is more than the rats with the gradually increasing of stimulus frequency.

In all aspects, the cochlea of chickens has shown clear response properties than the rats. Cochlear potential has plotted between 1-10 kHz range of stimulus frequency but there are 0.56 ms difference to obtain same CP on the characteristics frequency between the rats and chickens. The features that were common to the cochlea of the six chickens were: (a) A broad CP amplitude `spikes near 7 kHz range (b) Regular spike discharge at about 10 kHz, (c) A prominent CP amplitude spike at about 10 kHz, and (d) A unclear pronounced at > 10 kHz. In one chicken of six, we have examined a long amplitude spike near 11 kHz but the CP spike at >11 kHz was variable in appearance.

In chicken, the Cochlear potential vs. frequency curves revealed a broad amplitude spike in the center of the curve at about 10 kHz. It is very interesting to note that the neural potentiality and thresholds were recorded by Pollak *et.al* (1979) in rodents. This frequency is a most effective stimulus for recording the sensitivity of the cochlear auditory neurons in both chickens and rats. In all chickens, the maximum CP amplitude was examined at ±10 kHz stimulus range and the CP amplitude at this range was consistently 0.89 ms higher than that generated on the low stimulus frequency. The null of neural response was observed at about 32 Hz was very sharp and sometimes disappeared. The CP amplitude change among the spikes and nulls points was often on the order of 5 Hz stimulus. The cochlear potential is higher in the basal turn and decreases in the magnitude towards the apex part. The CP is mostly dependent on the oscillation of the cochlear components and ionic flow.

5.8. Tonotopy of Cochlea

There have been used direct and indirect techniques for determining the cochlear tonotopic arrangement. It has determined by the defining of the hair cells sensitivity and the staining techniques. It also noted that the 75% labeled auditory neurons innervates throughout the basal region of the basilar membrane only 25% to the apical part. These innervations underlie into the tonotopically arranged of the basilar membrane in both animals. These data revealed that the basal part of the basilar membrane has more innervated by the auditory neurons devoted to high-frequency selectivity rather than the apical part of the basilar membrane in both groups of animals. These features of the cochlea in the chickens are prominent in outer hair cells than the inner hair cells of the basilar membrane. On the other hand, the BM (basilar membrane) of rats has more branches of the auditory nerves that more innervate to the basal part of the basilar membrane than the upper surface.

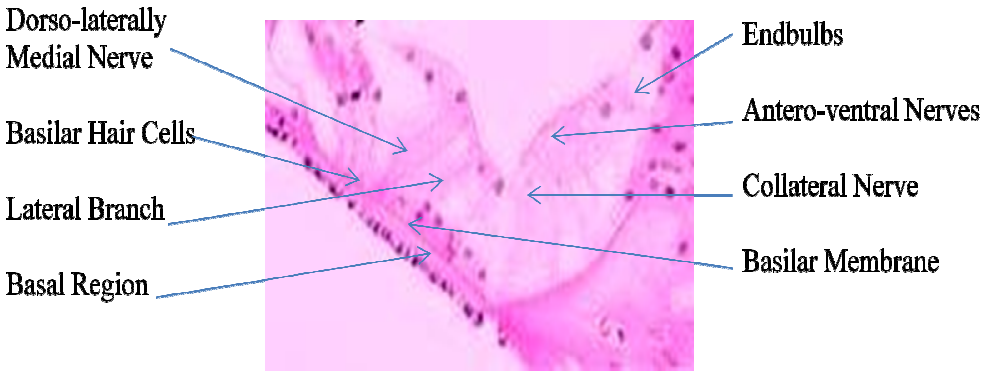
Auditory neurons innervate the outer and inner sensory hair cells of the organ of corti in the cochlea and distribute their branches from ventral to the dorso-lateral part of the organ of the corti. It terminates at the dorsal part of the SHCs (sensory hair cells) in both animals. Whereas, these neurons have innervated regularly to the basal part of hair cells and project to regularly basal parts of the basilar membrane (Photograph-2). It has been noted that 85% of the labeled nerves project throughout the ventral region of the cochlea (Fekete, D.M *et al*. 1984). By the staining of the organ of corti, we found that the individual

auditory neurons penetrate into hair cells in the different distance.

5.9. Neural Innervations in Sensory Hair Cells

We have studied of 12 to 14-week-old animal's ear invivo and exvivo. At this stage, frequency selectivity of the acoustic nerves and sensitivity of the cochlear hair cells was matured. The lateral branches of the basilar papilla continue to the basal part after bifurcation, it innervates the basilar hair cells. The basilar papilla are ≤ 4.8 mm long that was magnified by the stereo zoom microscope. There are different characteristics of bifurcated neurons at high CFs (characteristics frequencies) in chicken's basilar papilla than the rats.

The main nerve continues to dorso-laterally after rising from ventral main branch that penetrates into basilar membrane and arches across the BM (basilar membrane) to innervate the outer sensory hair cells (Photograph- 4). It is relatively a broad region through which auditory nerves fibers distribute, it does not have a counterpart to the sensory hair cells in rat's cochlea. As the acoustic nerves fibers courses ventral to dorsal region of the organ of corti then it give off more branches in chickens than the rats. The type-1 primary auditory neurons innervate inner sensory hair cells that emerge from the dorsal part of the basilar membrane. The nerve fibers of inner hair cells are also very heavily myelinated, which is in contrasted to the unmyelinated outer hair cells nerve fibers. The region of the basilar membrane supplying the inputs to a particular afferent nerve fibers can be considered to be its receptive field.



Photograph- 4. Cross section of the cochlear sensory hair cells and the neurons that innervated to the sensory hair cells; there are basal regions of the cells more innervated than the upper surface of the sensory hair cells.

Each collateral nerve fiber ascends dorsally to innervate the apical region of the sensory hair cells and the projection of the branches corresponding to the tonotopic arrangement of the basilar membrane (Ryugo, D. K. 1992). The auditory nerves form the calyx like end bulbs, it is a common character of the all animal's cochlea, including birds and mammals. Anatomical features are suggested that each auditory neuron on average contained two endbulbs with several branches of the sensory nerves.

There are very difference in the tonotopy of cochlear auditory nerves in regards to the low (32-40 Hz) and high (5-10 kHz) frequency selectivity at characteristics frequency in both animals. The nerves ending in the ventro-lateral region of the rat's basilar membrane do not form the endbulbs but these

branches are terminated as a lobulated ending (Koppl, C. 1994). This feature is contrasted with the endbulbs situation in the rats in which the bushy fibers of lower characteristics frequency in antero-ventral cochlear nerves (AVCN) contains endbulbs. The neurons of highest characteristics frequencies in the chicken's cochlea do not have bifurcate fibers but it terminates as endbulbs on the basilar membrane. It is clear that the low frequency receiving part (bifurcated fibers) of the basilar membrane does not receive inputs from the endolymph wave. It is very interesting to consider that the functional and anatomical correlations are not appeared in the neural ending. Sensory neurons are well specialized in the chicken's auditory organ than the rats, because this feature might be needed for receiving a low frequency in the birds.

VESTIBULAR SYSTEM

The vestibular system is a part of the inner ear in most animals which involved in the sense of balance. The vestibular system comprise three SCC (semicircular canal) is filled with the endolymph which oscillates in response to the mechanical stimulus transmits from the middle ear. With the movement of the fluid, the hair cells sense the mechanical movement through their stereocilia, subsequently that mechanical wave converts into electrical signals.

5.10. Responses of Vestibular Sensory Nerve

A constant range of the stimulus (130 Hz) at which vestibular neurons of the chickens have responded 120 sp /s. The spikes discharge was not obvious of

the vestibular neurons in the rats at this stimulus range (130 Hz). The stimulus range increases (150 Hz) then the spikes discharge was corresponding to the spikes discharge of the chicken's vestibular neurons. There we could explain the dissimilarity in response properties of the vestibular sensory neurons. The variation in the sensitivity was much more when the stimulus level gradually increased up to 10 kHz. The silent periods of the vestibular sensory neuron in chickens were less than the rats**.**

The responses of individual afferent fiber are divers in both animals, it receive input from afferent neurons. It does not encode high oscillatory movement in the endolymph randomly mechanical stimulus delivered into endolymph. The vestibular sensitivity of low oscillatory movement in endolymph are related to the second order neurons of vestibular nuclei, which are thought to mediate the vestbulo-ocolar reflex; it was selectively attenuated for self generated variation in time interval histogram. The vestibule neurons in both animals are very sensitive to constant stimulus, but not to random stimulus and intensity.

Three main patterns of the vestibular neural sensitivity have been explained in avian (chickens) and mammals (rats): (1) regular patterns (2) irregular patterns and (3) regular bistability patterns, present results revealed the low gain irregular and regular responses of vestibular sensory nerves. An irregular spikes discharge patterns have appeared near ± 350 Hz, this sensitivity continues increased when the stimulus range was gradually increased but the

mode of the response were different in chickens at different stimulus (in fig-9).

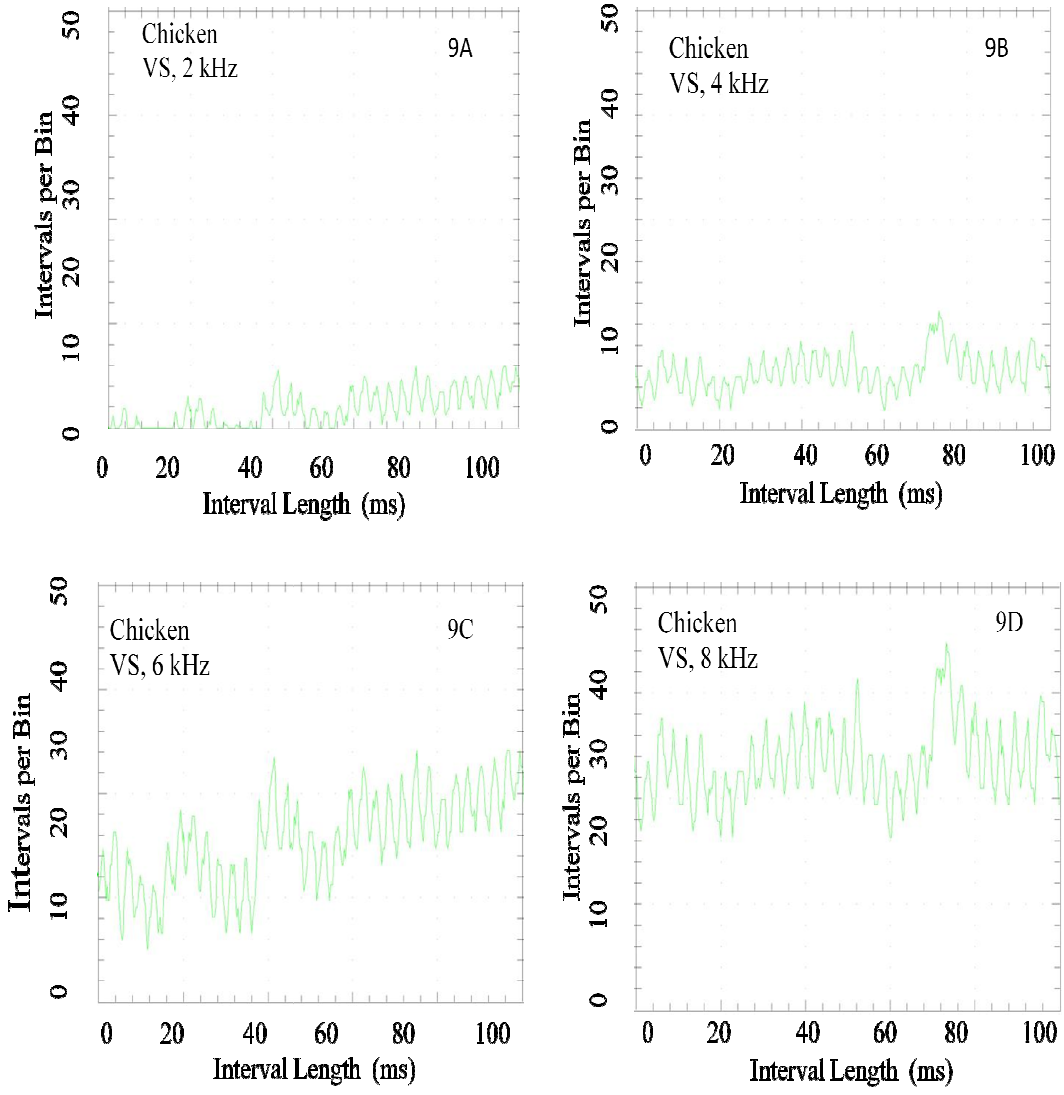


Fig-9. Response mode of the vestibular sensory neurons of the chickens plotted between interval length and intervals per bin at different stimulus frequency. There are more variance occurred with the increasing level of the stimulus from 2 – 8 kHz.

The sensitivity of vestibular neurons was recorded in order to the high to low sensitive neurons and maintains a consecutive phase lead on all stimulus frequency, it was very accurately for sensory nerves (Hullar *et al.* 2005). We have categorized the patterns of spikes generated from vestibular sensory neurons on the basis of their normalized coefficient variation (Goldberg, 1984) in figure-9. The neural response of the chickens and rats over the range of stimulus frequencies are not consistent with the previous observation in other avian species but the sensitivity of vestibular system increased when the stimulus intensity was constant between 2-8 kHz frequencies. These responses were continued from 2 to 10 kHz stimulus frequency in both animals nevertheless the responses are contrasted in both animals.

The correlation between coefficient variation (in table-4) and spikes discharge rate (in table-3) was calculated by measuring the inter spikes intervals and CVs in spikes patterns was plotted from 6 chickens and 6 rats. The coefficient variations of each spikes patterns were explained as its CVs when stimulus level was high and inter spike interval was ± 0.14ms. In figure-9 has shown the sensitivity of the vestibular system at different rage of stimulus. The overall sensitivity of sensory neurons in the chicken (fig-9) and rat (fig-10) to oscillation of endolymph, assuming that the neurons have unequal sensitivity to one. The coefficient variations for each patterns were explained on the basis of the relationship formula of CVs and the ISI (inter spikes intervals, in Millisecond). The values of coefficients a and b related to the ISI are shown in

table-10.

Table-10. The inter spikes intervals (ISI) for both groups of the animals. These data have collected from cupular sensory neurons during endolymphatic tilts.

Animals	a (ms)	b (ms)	Inter Spikes Intervals (ms)
Chicken VS	±0.035 ms	±0. 2 ms	±0.17 ms
Rat VS	±0.053 ms	±0.4 ms	±0.13 ms

The mean value and coefficients of ISI during spontaneous spikes discharge of the cupular sensory neurons is not similar in both animals. The superimposed spikes discharge curves was discriminated using the coefficients values of the ISI (in table-10) and CVs (in table-4) and then separate the magnitude of spikes into regular spikes (regular sensitive neurons) with CVs of < 0.3 and irregular spikes (irregular sensitive neurons) with CVs of > 0.3. This level for discriminating a regular from irregular spikes discharge is like to that used for studies of the chinchillas (Baird *et al.* 1988), but slightly lower than the rats with CVs of < 0.6 for regular and CVs of > 0.6 for irregular spikes discharge. The correlation between magnitudes of ISI and CVs of the spontaneously spikes discharge was not obvious in both animals, but on the

basis of CVs and ISI here we have observed the response properties of the vestibular sensory neurons in both animals are very different.

The sensitivity of the cupular sensory neurons at 6 kHz has shown as a function of cupular hair cells movement in figure-9 (for Chicken) and figure-10 (for Rat). Both the regular and irregular spikes discharge has revealed the functional correlation of the SCCs (semicircular canal) in both animals, although the correlation between regular and irregular spike discharges patterns is much more substantial in both animals. The response of the cupular sensory neurons in chickens and rats recorded at 6 kHz is illustrated respectively in figures-9 and 10; there is a little difference between mean responses of the cupular sensory neurons of the chickens and rats. The difference in the spike rate of the vestibular neural responses at 8 kHz range of stimulus frequency is 40sp/s.

Neural Responses in Frequency Domain: Categorization of response patterns of the vestibular sensory neurons for individual animals revealed the different sinusoidal movement of the endolymph in SCCs. Their variances could provide a way for categorization of the discharge patterns into comparative groups. All patterns of the sensory neurons were not correlated to each other in chickens and rats. The curves 9A (in fig-9) and 10A (in fig-10) have an average phases and responses at 2 kHz range of stimulus frequency for the chickens and rats respectively.

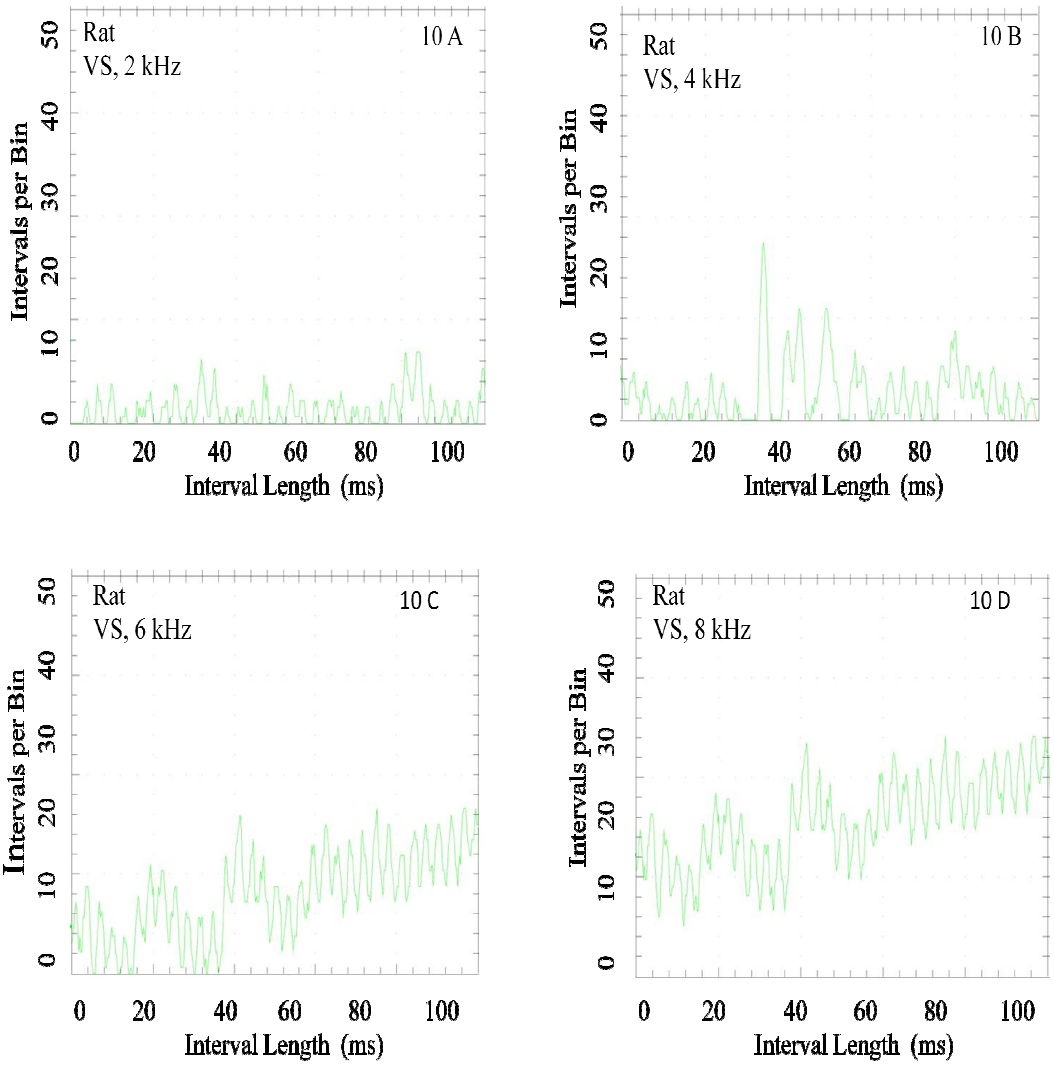


Fig-10. The variations in sensitivity of the neurons of rat's vestibular system at different stimulus frequency, it also revealed that the sensory neurons of the vestibular system are less sensitive in compare to the chicken's vestibular sensory neurons at all level of stimulus frequency.

The response of sensory neurons in the chickens is not similar to the responses curves (in fig-10) of rats, but the sensitivity of cupular neurons had

increased from low to high stimulus frequency. There are also increased in phases switching at all stimulus ranges of the frequencies. The SDR, CVs, and sensitivity of the cupular neurons in both animals are shown in table-11.

Table 11. Characteristics of the vestibular sensory neurons in chicken and rat.

Characters	Chicken	Rat
First Harmonic VP- spike	±4 kHz	±6 kHz
VP (Vestibular Potential) Null	Appeared at Low (±35 Hz) Stimulus	Appeared at High (±47 Hz) Stimulus
Relationship of CVs at	±0.11	±0.12 (Vary to Chicken)
BF (Busting Factors)	±0.05	±0.07
Environmental and Anesthesia Effect	Marked Effect on Response	More Effect on Response
Induced Cochlear Response	Clear Detected	Less than Chicken
Resonance Properties	Not Detected	Not Detected
Maximum Response	±3 kHz	± 4kHz
Spike Discharge Rate	120 sp /s	160 sp/s High than Chickens
Burst Index	±0.00096	±0.000836
Spike Time Interval	±0.1µs	±0.2µs
CVs	±0.06 ms	±0.83 ms

5.11. Vestibular Potentiality (VP)

In all view, the vestibular system of the chickens and rats have shown more sensitive respectively at ≥1800 Hz and 2000 Hz. Potentiality in both groups of the animals could be examined throughout the experiment over the stimulus range from 32 Hz to 10 kHz. The potentiality may be influenced by the various factors such as anatomical, experimental, and habitual. Sensory nerves potential have been recorded by Landolt Jack, P. *et al.* (1980), there are clear evidence of the potential recorded from the vestibular sensory neuron, and it was used as a functional test of the neurons of chickens and rats. The neuro-physiological substrate for such neural potential is that the sensory neural stimulation of the vestibular system gives rise to post-synaptic potentials over all range of stimulus frequencies.

The most remarkable variations have observed in the response of the chickens and rats vestibular neurons at 8 kHz range of the stimulus frequency (in fig-11 and12). Spike discharge detection theory suggests that low responsive neurons would require more regular and constant stimulus frequency to carry the information as the higher responsive neurons; because all low responsive neurons have very high responsive threshold and silent time periods at low stimulus range. The lower response of the rat's vestibular neurons is likely to reduce their capability to encode the low level stimulus frequency. The negative polarization of the sensory hair cells and the action potentials of the vestibular neurons are obvious in the chickens but have distinct features at all stimulus

frequencies (in fig-11).

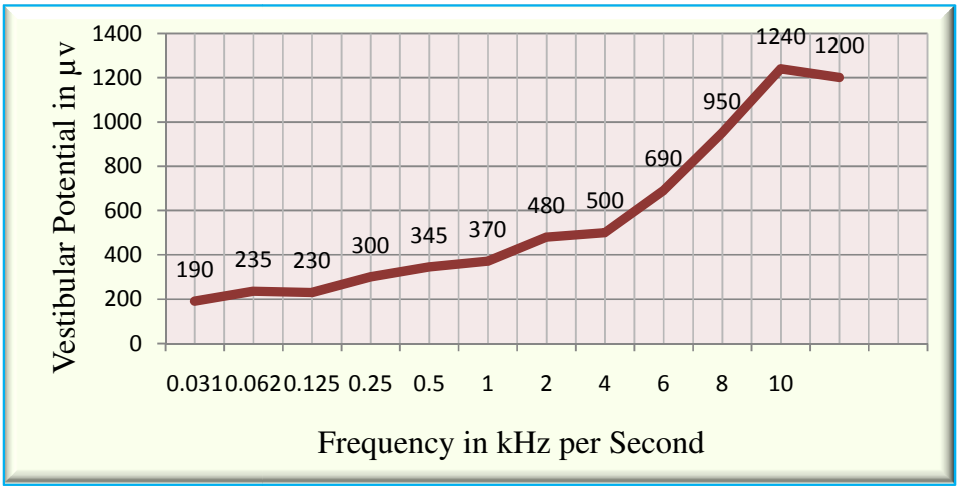


Fig-11. In vestibular potential vs. frequency curves have shown the vestibular potentiality of the chickens. There are increased the potentiality with increasing the stimulus range of frequency.

The absolute magnitudes of the nerves potentials and endolymphatic movement are almost different in rat's semicircular canal. If we have stimulated with the high stimulus frequency (10 kHz), then we were found the high action potentiality of the vestibular sensory neuron in both animals. Actually, the potential difference between both animals is ± 30 mv (Millivolts) at 10 kHz stimulus frequency. Eventually, there are obvious potential differences were observed [in fig -11 (for Chicken) and fig-12 (for Rat)] in both animals might be attributed by the potassium and sodium concentration in the endolymph of SCCs.

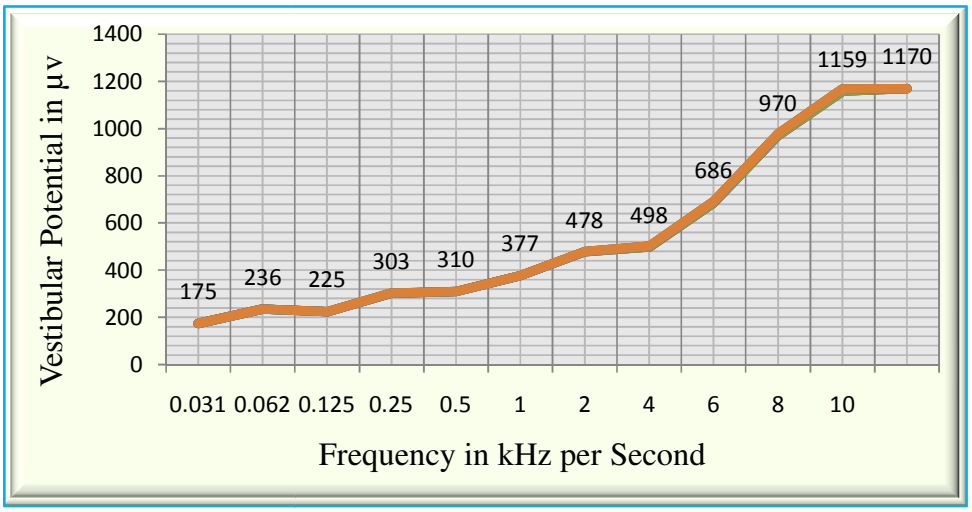


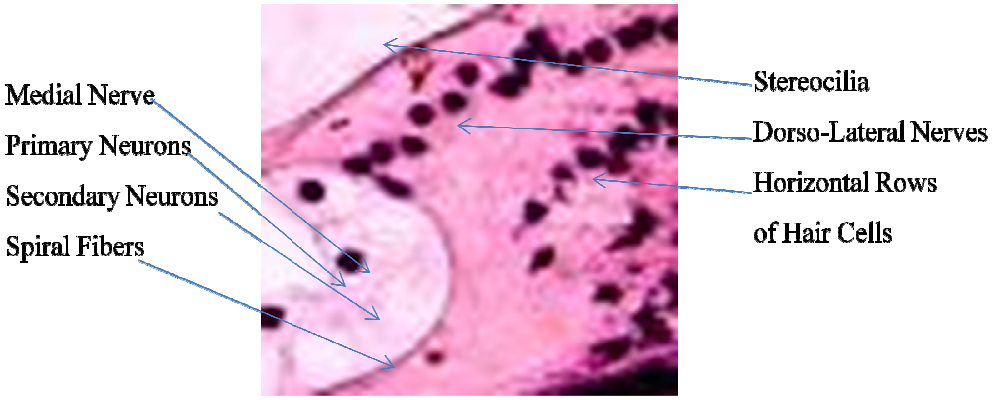
Fig-12. In vestibular potential vs. frequency (kHz) curves illustrated the comparative sensitivity of rat's vestibular neurons at different stimulus range; there are increased the potentiality with increasing the stimulus frequency but the potentiality is varying in compared to the chicken.

5.12. Neural Innervation in Cupular Hair Cells

The medial nerve of the cupular sensory hair cells continues to the dorso-laterally after bifurcation, that innervates the horizontal rows of the hair cells in the chickens. The patterns of the neural innervations are different in the cupula of both groups of the animals. In chickens, the vestibular nerves enter into the lateral region of cupular hair cells and terminate at the junction of hair cells and stereocilia.

There are redial neural fibers run to the base of the hair cells and their each collateral fiber innervates only one hair cells. These radial fibers are achief

supply to the cupular hair cells. The basal part of the hair cells are richly innervated by spiral fibers. There are several branches that project lengthwise of the cupular hair cells neurons in distance of several micrometers in both chickens and rats (in photograph-5).



Photograph. 5. Cross section of the cupular sensory hair cells and its innervated by the various types of neural fibers involve in the relay of information.

5.13. Comparison of Vestibular Potentiality

The comparison of vestibular potential between both animals by plotting vestibular potential vs. frequency curves (in fig- 11 and 12), some features of these curves were constant which were: (1) Determined the highest amplitudes peaks at 8-10 kHz stimulus range; (2) As the oscillatory frequency swept downward, the magnitude reduced mostly between 5 - 7 kHz; (3) The frequency selectivity was null around >10 kHz, and (4) Maximum peaks amplitude

occurred between 9 - 10 kHz.

In all consideration, more sensitivity has revealed by the sensory neurons of the chickens, those reared in open environment of the poultry farm, on the other hand, the rats were breed in the cage of the animal house (not open environment). Sensitivity could be measured at ≥ 220Hz frequency with the stimuli intensity was around 25 dB SPL for both animals. The amplitude peak near 10 kHz range was remarkably constant for chickens. The vestibular potential vs. frequency curves represents a broad oscillation peaks at about 10 kHz range, while in the rats, this broad oscillation peaks was examined near 8 kHz range of frequency, thus on the basis of these curves, we can assume that the vestibular potentiality of the chickens are much more than the rats. Rats were reared in cages, on the other hand, the chickens those breeds in open environment. Hence, we can say that the auditory specialization somewhat correlated with anatomy and the environment of the animal's habitat.

5.14. ANATOMICAL-FUNCTIONAL CORRELATES

Many researchers believed that the anatomical components are correlated with the neural responses over the constant stimulus. Furthermore, the anatomical specialization enhanced the frequency selectivity of the sensory neurons of both cochlear and vestibular system. We have observed a very high correlation between the anatomical components and physiological data recorded from both animals. Therefore, we have summarized the anatomical-functional

correlation in the following text:

5.14.1. Correlation between SDR (Spike Discharge Rate) and Cochlear Sensory Nerves

Anatomical development of the hair cells has been observed in regard to the SDR. Low SDR (< 25) of the hair cells have closed and thin spikes, whereas high SDR (> 25) have thick and clear spikes in the curves. Furthermore, there is clear segregation in SDR of the cochlear hair cells in both animals and auditory nerves give rise to the normal spikes discharge in presence of controlled MS (mechanical stimuli). There are correlated the neural innervations with spikes discharge rate and these compared to the spikes discharge patterns those have a high and low SDR. The higher SDR neurons traverse along the nucleus and give rise more collaterals nerves fibers. There is obvious that the projection of sensory neurons with different spike discharge rate might be segregated along basilar membrane, but the studies of the neural single unit labeling do not obviously support to this suggestion (Liberman, M.C. *et al.* 1991, 1992, 1984).

There are generally one or two swelling node at the terminal end of the ascending neurons and the remaining branches being as blunt terminals. There have found that these swelling parts are points of synaptic neurons that connect to other sensory neurons in the basilar membrane. There is a clear that the SDR related variations in sensory neurons and the numbers of swelling parts are different in both animals. High SDR neurons give rise to the greater collateral

nerves of the cochlea in compared to those neurons that have low SDR. In rats, the ascending nerves of low SDR give rise to the greater collaterals nerves that are responsible for high CVs (coefficients variations).

5.14.2. Correlation of Vestibular Potential between Both Animals

The bar on the lower portion of each curve expresses the regular sound frequency selectivity by the vestibular system, bar position was compared with the potential vs. frequency graph of each animal. The curves represent the highest peaks in most cases that corresponded to the stimulus sound frequency. Consequently, 14.2- 16.4 V RMS (root mean square) were determined for the vestibular sensory neurons of chickens, and 10.3- 12.5 V RMS for the rat's vestibular neurons. There is a close correlation occurs at the peak to peak of the second harmonic patterns of spikes discharge, when the frequency band was ± 8 kHz in the chickens and ± 6 kHz in the rats.

The responses of vestibular neurons in both chickens and rats were recorded over the external stimulus range extended from 1 to 10 kHz at a constant intensity (12 dB SPL) and the values of the neural responses were observed at each range of stimulus frequency. The phase changing values of the sensory neurons were averaged at different external stimulus for comparison between both animals. Generally, the responses increased with the increase of the stimulus frequency from 1 to 10 kHz in both animals but these values for both animals are different as shown in figure-11 and 12. The difference in

neural response was ± 40 sp/s in both chickens and rats.

The spikes of the neural response in chickens and rats show a gradually increases as stimulus frequency increased up to 10 kHz there are broadly change in the response between 1 and 4 kHz, (in fig-11 and 12). The sensitivity of the vestibular system was little declined at 32 Hz stimulus frequency and ± 8 kHz the highest stimulus frequency at which vestibular neurons sensitivity more sensitive. The potentiality of the vestibular neurons of the chickens was higher than the rats, it has determined according to the values of the BI (in table-7) and CVs (in table- 4).

In chickens and rats, the vestibular potential vs. frequency curves were plotted for comparison individually. There have been observed the similar and sharp spike at **±2** kHz from the chicken's vestibular nerves and the same stimulus was used for plotting the potentiality of the rat's vestibular system. It is interesting to note that the amplitude of VP (vestibular potential) for first harmonic characteristics frequency (CF) in the chickens is prominent because the appearance of null of neural response on the low frequency side of spikes. On the other hand, in rats the amplitude of VP (vestibular potential) are not prominent because of: (1) The magnitude of spike was low at the level of preferred stimulus frequency; (2) The null of neural response at high frequency has appeared on the side of high amplitude spike but the values of bursting factor are same in both animals.

5.15. EFFECT OF ENVIRONMENT AND ANESTHESIA

The frequency selectivity was recorded from the animals those recovered by the effect of anesthesia and microsurgery. The disposition of the peaks and the variation in spikes discharge has examined on the curves that switched thousand kHz in both animals. The switching and bistability in the spikes were very high on high dose of the kitamine and low stimulus frequency in rats than the chickens.

For examlple, the phase switching appeared at about 1200 Hz among the peaks recorded on the oscilloscope. Comparable types of effects were also determined in *pteronotus* (bat, mammal), and have been documented elsewhere by Pollak *et al.* (1972). However, in all animals, these effects ordinarily have been evidenced, because considered that the apparent variable factors could not reasonably minimized or controlled, i.e., the dose of anesthesia, environmental factor, and body temperature.

It is observed that the spike rate decreased in deep anesthetized chickens and rats (12 to 14 weeks old). The high variation was determined in the phase change of bursting patterns in both groups of animals which was deeply anesthetized with the effect of ketamine (in fig-13 and 14). Both the cochleo-vestibular responses and its maximum amplitude can be altered by the administration of the anesthesia which would tend to increase the depolarization of the sensory hair cells and then cochleo-vestibular potential may be decreased according to the level of anesthesia. The action potentials of the cochlear and

vestibular neurons also gradually decreased as shown in figure- 13A and 13B respectively for cochlear and vestibular neurons of the chickens, whereas figure-14A and 14B respectively represent to the cochlear and vestibular neurons of the rats.

Generally, the cochleo-vestibular response is affected by high doses (70-75 mg/kg) and temperature; these effects belong to the sensitivity trauma. They are broadly corresponding with the principle of an auditory theory and the precise results are confirmed by the distribution of spikes on time interval histograms at all range of stimulus frequency. This evidence is very important for the question of frequency selectivity of the neurons over different stimulus frequency. The effects on potentiality are unlikely when the appropriate dose of anesthesia and other environmental factors are maintained.

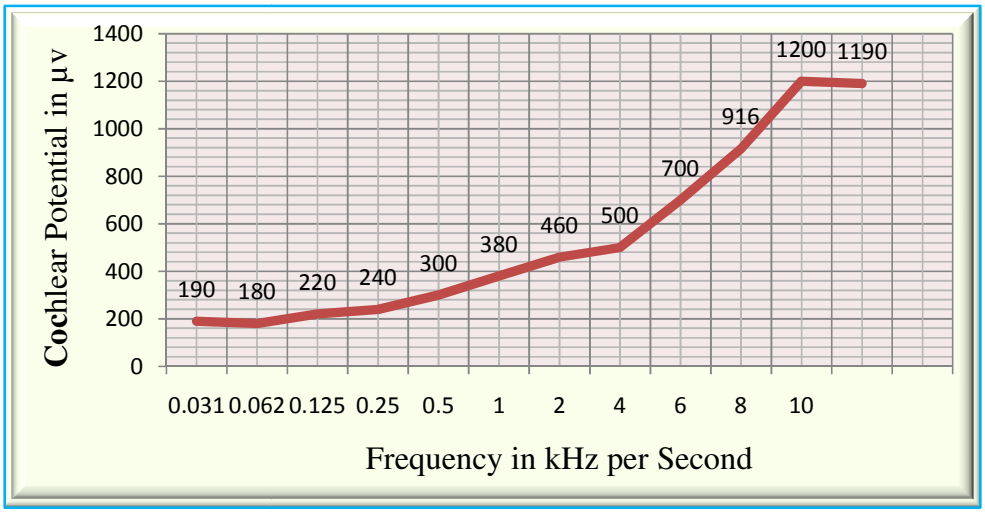


Fig-13A

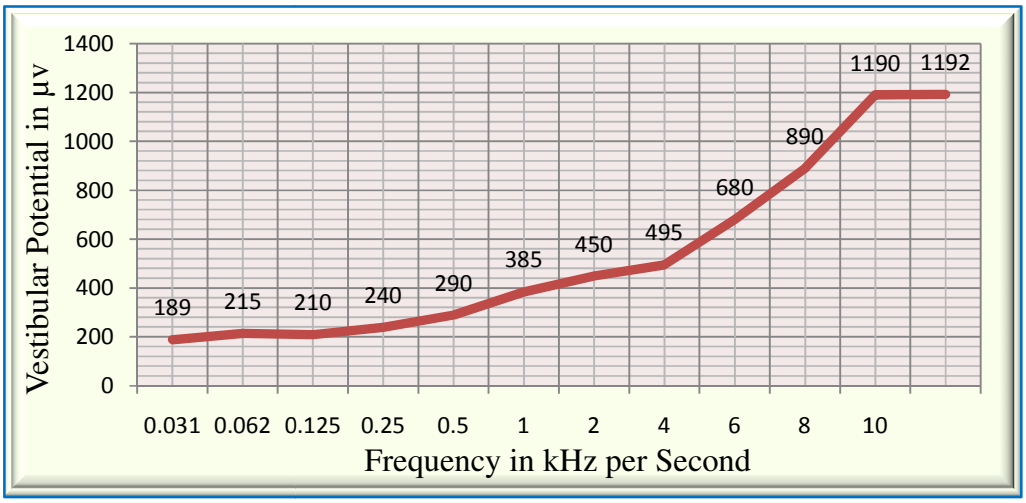


Fig-13B

Fig-13. The curves 13A and 13B respectively for acoustic and vestibular neurons of the chicken that revealed the variations in sensitivity at different stimulus frequency when deeply anesthetized from the effect of ketamine.

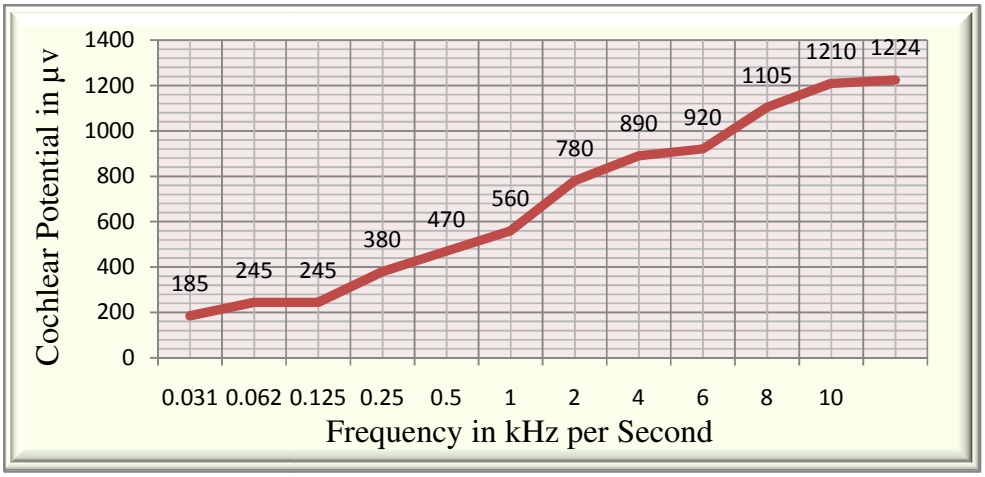


Fig-14A

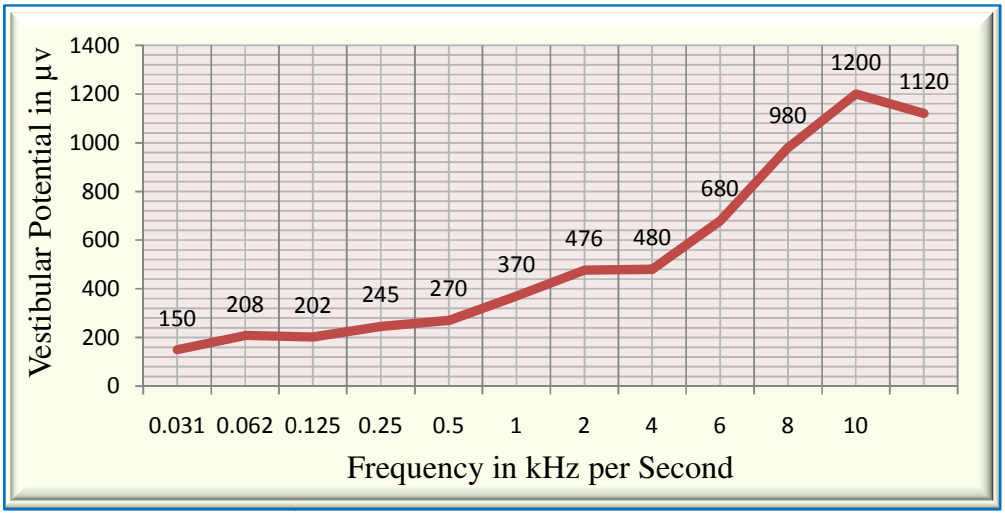


Fig- 14B

Fig-14. In curves 14A and 14B have shown the variances in response properties respectively for cochlear and vestibular sensory neurons of the rats at different stimulus frequency. Cochleo-vestibular neurons of the rats are more influenced by the effects of the ketamine at all level of the stimulus frequency.

On the basis of the potential vs. frequency curve, deeply anesthetized rats are more affected by the effect of ketamine than the chickens. This result suggests that captivity of the animals reduced the tolerancy and development of the SAO. Recording was made day time for comparison between both animals. There are shifting in the position of the peaks, changes in the cochlear and vestibular evoked potential curves (in fig-13 and 14) are illustrated for anesthetized animals. There are notable changes occurred in the apex point of the curves at ±1 kHz and the maximum spikes at 10 kHz, this effect very less in the chickens than rats.

CHAPTER- 6

DISCUSSION

The main purpose of this study was to make a direct or indirect comparison of the physiological properties of the ear in birds and mammals, those rearing entirely different environmental lines. Typically, the comparative physiological studies are accommodation with erstwhile observations have shown in the following text: (a) continuous sharp Spikes observed from the auditory nerves (Pollak *et al.* 1972), (b) closely relation of the resting frequency and second harmonic response peak of the audiogram (Henson *et al*. 1980), the changes in the response properties of the ear in regard to the change of body temperature or administration of the anesthesia; (c) a notable distinct amplitude of the cochleo-vestibular resonance in avian and mammalian; (d) dissimilarity in the cochleo-vestibular responses at10kHz stimulus.

The variations in SDR (Spike discharge rate) in chickens and rats may be common because the responses of sensory nerves with a CF (characteristics frequency) derived from many hair cells lying along the basilar membrane (BM) between the inferior and superior portions. These variations are depend on the coordination of the frequency with the numbers and compatibility of the sensory hair cells on the basilar membrane.

Many hair cells lie on the 0.95 nm part of the basilar membrane, according to this number and orientation of the cells play a significant role to determine the potentiality of the cochlea in both groups of animals. The sensitivity in the inferior and superior parts of the papillary cells in the chickens are

comparatively prominent than rats (mammals). Nerves fibers synapses are more in cochlear hair cells of the chickens that demonstrated with the threshold of CF and SDR (Spike discharge rate) by the response curves. Similar data reported from the labeled cochlear nerves units in the starling (bird) (Gleich 1989), but these data reveal dissimilarity with the current data collected from the chickens and rats.

Different instinct spike discharge rate has determined in many avian (barn owls, starling, chickens) that have a similar spike discharge rate (Koppl, C. l. (1997). One of the basic characteristics of the cochleo-vestibular neurons is more sensitivity to a specific frequency of the external stimulus. It may be elaborate and compare by the plotting of frequency tuning curves (FTC) at a given range of frequency. These curves reveal the response phases and frequency co-ordination when the neural response was harmonic at the constant external stimulus delivered into the cochlea.

The apex of the curves shows the single stimulus frequency at which the cochleo-vestibular neurons is most stimulated or sensitive, denotes its characteristic frequency. These curves tend to be regular spikes discharge and the characteristics frequency of the neurons observed by its distribution end on the basilar papilla (Chen *et al.*1994).The neurons that receiving the low frequency range are emerge from the apical end and neurons that receiving the high frequency arising from the basal end of the basilar papilla (a group of the

sensory hair cells) have comparatively more sensitive than apical sensory neurons. All chicken's acoustic nerves were appeared relatively same in their response and morphological features, but show variation in their primary characteristics frequency (CFs). The central distribution of the acoustic neurons had also revealed the stereotypic morphology (Pigeon, Boord, R. L. G. L.1963; Barn Owl, Carr *et al.* 1991)**.**

The oscillatory force generated by the cochlear bundle of hair cells which induced the cochlear sensory neurons. This oscillatory movement in the endolymph is profoundly and it has a linear FTC (frequency tuning curves) at about 8 kHz in mature chickens, whereas this feature was appeared at about 10 kHz range of frequency in the rats. Artificially amplification of the cochlear hair cells in mammals and birds has been yet identified. When such an artificial stimulus is not given to cochleo-vestibular organ, then the cochlear hair cells was not exhibited the specific CF and have less spikes discharge rate. According to this significant feature, we have comprehensively explained of the cochleo-vestibular potential and the loss of frequency selectivity in cochlear hair cells. The declines in sensitivity of both hair cells were same and that suggest to the standard mode of cochlear hair cells stimulation (Kurian *et al.* 2003).

In addition to the mechanical stimulus, the electrical wave is one of the several factors derived from the cochleo-vestibular hair cells, which influenced

the cochlear sensitivity or particular frequencies selectivity in all vertebrates, this frequency has been reported in chicken's hair cells and rats located in papilla (Fuchs *et al.* 1988). The temperature controlling of laboratory and body is must because the coordination between both parts of the papilla are very susceptible with varying of the temperature and that responsible for higher sensitivity of cochleo-vestibular nerves. We have examined the highest spike discharge rate (70- 180 sp/s) at 35 to 38ºC in both animals, but spikes discharge rate reduced when the temperature was below 35ºC and greater than 38ºC.

In avians, the tonotopic organization and coordination in cochlear papilla are very prominent than the rats, thereby the variations appear in the responses of cochleo-vestibular nerves. The motion of the bird's basilar membrane was revealed the traveling wave and broadly sensitive (Von Bekesy *et al*. 1960), Apart from this; the increasing in BMM (basilar membrane movement) and sensitivity of cochlear hair cells partially proportional to the stimulus intensity, that can be damaged with the high stimulus intensity.

In the curves (in fig- 2) clearly has illustrated that the propagating wave amplifies in the cochlea of chickens and rats. There are explained a sharp spikes discharge with distinct sensitivity on the low and high frequency, but there is more variation in frequency selectivity between both animals; therefore, it is comparable to the other species of rodents and mammals. Similar studies have been done on cochlear sensitivity in mammals and reported that the frequency

selectivity in auditory nerves and the sharpness of the BM (basilar membrane) is same in mammals on common characteristics frequency (Narayan *et al.* 1998).

Data collected from the ears of chickens and rats were antagonism with earlier studies concerning with following text: (a) the precise relationship between the CP and stimulus frequency on the 1st and 2nd harmonic cochlear responses (b) the amplitude of the peaks of the cochlear sensitivity recorded from rats when the stimulated at about 8 kHz. (c) The effects on the CP and FTC when the high frequency propagates from cochleo-vestibular hair cells. The preparation of animals, anesthesia, the technique used to recording and implantation of the microelectrodes may generate variation in the frequency selectivity. Pollak *et al.* (1979) were used Nembutal anesthesia and then drilled the micro hole by microsurgery in the cochlea for implant of the microelectrode, their studies were carried out on anesthetized *Rhinolophus ferrumequinum* (Mammal), Suga *et al.* (1975) were used ether anesthesia added with the Nembutal for the studies on the *Pteronotus*, and the microelectrode implanted near the round window via a surgical approach through the middle ear. Their experimental set up was maintained at about 35ºC by using the temperature controlling room. They have been observed more variation in response of the cochleo-vestibular sensory neurons.

In the current studied we have used the chloroform and kitamine for anesthetized of the animals. There have implanted the microelectrode in scala

tympani via an intracranial approach, and then the cochlear potentials were recorded over long periods of time in both animals. These were fully recovered from the effect of anesthesia and microsurgery. There was maintained the body temperature of the animals at about 37ºC by keeping them in the temperature controlled room and we have plotted the switching in spikes discharge phases in both animals at 2 kHz. This switching was more in the rats than chickens.

The finding of the current study reveals that the sensory neurons of stato-acoustic organ in rats (12 to 14 weeks old) are capable for frequency selectivity on low stimulus range as found in the chickens (12 to 14 weeks old). There are included both regular and irregular patterns of the neural response in concern with the interval histograms that reflect both linear model and binomial distribution of spikes. These broad patterns of the spontaneous neural responses are very consistent with the mature CF and tonotopic arrangement; it has been explained for chicken's sensory neurons innervating to the hair cells of cupula and the cochlea (CF ~100-2000 Hz) (Jones, S. M and Jones 1995a, b). However, some data of the current study shows that the responses of the sensory neurons of the rats are not corresponding to the responses of the chickens at all level of stimulus frequency.

There have comparison with chickens and we observed the sensory neurons of the SAO of rats have significantly lower spikes discharge rates, greater variance in spikes discharge rate reflected by a broad range of CVs

(coefficient variation), longer inter spikes intervals, maximum intervals (dead times) and more irregular bursting patterns. Moreover, the preferred intervals were high in the rats. There were no characteristics frequency higher than1500 Hz in rats of the current study, this finding not consistent with the previous observation by Jones, S. M and Jones (1995a, b). Here we have discriminated the variations between chickens and the rats by comparison of SA (statoacoustic) sensitivity at mature stages as discussed in subsequently sections.

Many researchers have been described the results of comparative physiology of the cochleo-vestibular sensory neurons in both mammals and avians (Carlier *et al.* 1975; Curthoys *et al.* 1978, 1979, 1982, 1983, Desmadryl *et al.* 1986; Gummer *et al.* 1994; Kettner *et al.* 1985; Romand 1984; Romand and Dauzat 1982; Walsh and Mcgee 1987) and late or early posthatch birds (Manley *et al.* 1987, 1991a; Richter *et al.* 1996; Sheppard *et al*. 1992; Valverde *et al.* 1992; Yamaguchi and Ohmori 1990, 1993). The current study provides the first evidence of insitu recording of the spontaneous sensory neural response from SAO in mature chickens. The basic frequency of periodic waveforms found in the ACF (autocorrelation function) of irregular response, which is plotted against time of phase switching. These plots revealed the irregular spikes discharge have unclear inter-spike intervals in the rats at 2 kHz stimulus frequency. These features of the cochlear and vestibular sensory neurons of the rats were not corresponding to the neurons of the chickens but these features

have similarity at different stimulus frequency.

Discharge rate: A low instinct spikes discharge rate has been recorded broadly for cochlear and vestibular sensory neurons in mature chickens and rats. The mean spontaneous rate of cochlear and vestibular sensory neurons of the rats is less clear than chickens at ± 6 kHz. Manley *et al.* (1991a) reported no variation in spontaneous spikes discharge rate for chickens at age P2 (20 sp/s) and P 21 (23 sp/s); same rate was examined in the Emu chicks aged P1-P14 and rate 26 sp/s by Manley *et al.* (1997). These values markedly are contrasted with the rate of adult chickens (86 sp/s) recorded by Salvi *et al.* (1992).

Higher spikes discharge rate has been also recorded in mature animals of other avian species including the pigeons (65 sp/s, Gummer 1991; 34 sp/s Hill *et al.* 1989; 67.4 sp/s, Klinke *et al.* 1994; 90 sp/s Sachs *et al.*1974; 78 sp/s, Temchin 1988) for starling 48 sp/s by Manley *et al.* (1985) and for barn owl 72 sp/s by Koppl (1997). The recorded rate little differences between chickens and the rats have reflected the maturational refinements in the response of cochleo-vestibular sensory neurons. However, some researchers have inferred that the difference of spikes discharge rate in several studies might be attributed by temperature or other factors than the age of the animals (Smolder *et al.* 1995).

Moreover, Manley *et al*. (1991a) have informed that the anesthesia may involve in generating the variations in spikes discharge rate in all adult animals. This latter suggestion is supported by the research of Anastasio *et at.* (1985) and

informed that the average spontaneous rate of vestibular sensory afferent neurons were decreased up to ~55% in anesthetized animals (spike rate 93sp/s) in compared with the anesthetized (spike rate168 sp/s) matured pigeons.

In anesthetized chickens of the current studies, the mean spontaneous rate was 45 sp/s; this rate is approximate to that determined for mature chickens by Salvi *et al.* (1992) as well as other avian species as explained in the preceding text. The mean spontaneous discharge rates of the cochlear and vestibular neurons of the rats have relatively lower than the chickens.

In the current study, we have observed the effects of anesthesia on the sensitivity of neurons in mature animals and the lower spontaneous rate in rats might be due to the different level of anesthesia and body temperature in both groups of the animals. The animals were anesthetized using the same agent, but the variances in spikes discharge rate are not likely to be described only on the basis of this factor. We conclude that the variances in instinctive spikes discharge rate recorded here from chickens and rats reflect developmental and adaptation changes in the cochlear and vestibular sensory hair cells and neurons. Richter *et al.* (1996) also have examined the increases in instinctive discharge rates in newly born pigeons during the first 4 weeks.

Despite being broadly reported the responses of sensory neurons across all animals, it is not cleared that why instinctive discharge rates increase during development. The stimulus of ambient noise may be a factor, it involve in the

generate of instinctive neural activity as we have explained here. In the current study, ambient noise stimulus was attenuated for both chickens and rats; As a result, reduces in spikes discharge rate of the neurons, it could appear only on reduction of ambient external or internal factors in both animals.

Although the mean threshold of neural response at characteristic frequency (CFs) have reported here for chickens are dissimilar to those explained for chickens (hatchling) by Manley *et al.* (1991a), it was significantly high than rats of the current study. The threshold of chickens was comparable with the mean threshold in mature chickens recorded by Salvi *et al.* (1992) on characteristics frequency between 500 and 1500 Hz.

Moreover, it is reported elsewhere that instinctive discharge rate increase with decreasing responsive threshold at the characteristic frequency in mature animals (Manley *et al*. 1991a; Salvi *et al.* 1992). This finding in both animals tends to support the hypothesis that the spikes discharge was low in rats due to the higher threshold of the neural response. Despite, these observations of the responses of the cochleao-vestibular neurons informed that the relative sensitivity of the SA elements may not depend only on it.

The spontaneous spike discharge rate was significantly lower in rats than chickens. The irregular sensory neurons probably do not depend on the ambient stimulus level to convey the information. Second, instinctive spikes discharge rate did not increase with decreasing thresholds at characteristics frequency in

the rats. Our finding is not corresponding with the hypothesis that the spontaneous spikes discharge rate closely related to the ambient noise stimulus level. Third, there are clear that the substantial region of instinctive sensitivity in hair cells of SAO is not related to ambient noise stimulus only, it is produces autogenously within SAO by coordination between frequency and cochleo-vestibular components. The autogenous characters of instinct response are based on several lines of observation. Many researchers have been isolated the sensory neurons (Santos Sacchi, 1993) or exposing sensory hair cells of the cochlea and vestibular organ in both animals the chickens and birds (Kiang *et al.* 1976; Li and Correia 1998; Muller *et al.* 1997; Salvi *et al.* 1994,1998) for eliminates the instinctive responses. They have suggested that instinct neural responses depend mainly on the sensory basilar membrane.

The instinctive spikes discharge may be altered by external ambient stimulus and independ endogenous activities of the basilar membrane. A unimodal distribution of instinctive spikes discharge rates has been reported broadly for cochleo-vestibular sensory neurons of both groups of the animals and this is true for both animals in current study that the mean spikes discharge rate varies systematically as a function of characteristics frequency in the chickens and rats, although the spikes discharge rate was decreased at higher CF in both animals.

Regularity of Spikes Discharge: Many researchers have evaluated the

developmental appearance in instinctive spikes discharge regularity in cochleo-vestibular sensory neurons. Regular bursting patterns in regular active neurons were reported in which to be either present or absent a small proportion of sensory neurons in mature chickens and rats. The numbers of regular spikes increase obviously with the age of animal (Curthoy 1983; Desmadryl *et al.*1986; Romand and Dauzat 1982).

The Mean spikes discharge rate was also increased with age in rats and the irregular response continued in few cases at about 1 kHz as both animals matured. The regular spikes patterns reflect a mature phase of the tonotopy of nerves of the sensory hair cells. No information is available pertaining to the appearance of regular spikes discharge patterns during the development of the sensory neurons in neonate animals. Our present study on 12 to 14 weeks old animals (chickens and rats) revealed that both regular and irregular patterns of spikes appear respectively at high and low stimulus frequency. Although the sample of irregular sensory neurons was small and the proportion of regular spikes discharge in 14-weeks old animals was generally large in compared to those reported for immature animals.

Furthermore, there are some evidences of the substantial amount of variation in mean CVs of both cochleo-vestibular sensory neurons. These results indicate that the periods of instinct discharge regularity of sensory neurons are comparatively mature in 14-weeks old animals (both animals). It is

noticeable that the CVs (0.86 ms) of rat's auditory neurons were higher than the chickens (CV is 0.62 ms). This could be described as a tendency toward more regular bursting patterns in the chickens. The lower CVs may be related to the higher spike discharge rate and developmental refinement in cochleo-vestibular sensory neurons.

There are insufficient samplings of irregular spikes discharge; this matter deserves a more detailed study on the cochlear and vestibular system of both animals. Very little is known about the changes in CVs during the development of sensory neurons in these animals. Although the sensory neurons were revealed an irregular and regular spikes discharge patterns at low stimulus frequency, it was a tendency to increase CVs as a function of neurons. The lower CVs in chicken' auditory neurons informed a deviation in the regular spikes distribution in the form of very large spikes that was corresponding to the anticipated variances in spikes discharge. This may be related with the increased regular, irregular busting patterns and overall variations of instinct discharge in both animals; it is commonly reported for developing sensory neurons in many species of avians and mammals. The coefficient variations of sensory neurons with bursting factors (BFs) > 3.5 are observed in other animals; this value indicates the more sensitive characteristics of the sensory hair cells and its innervated neurons.

Spikes interval and coefficient variations (CVs) curves have plotted as a

function of cochlear and vestibular sensory neurons in response to the particular stimulus frequency in both animals. CVs of regular sensory neurons were independent to the mean spike interval. Coefficient variation is a function of irregular sensory neurons as mean spike intervals that explained by the regression line and the equation. The inclusion of irregular neural burst produced high CVs is shown in table-4. The sensory neurons of the chickens have high sensitivity and their CVs were low than the rat's sensory neurons. The CVs of both animals may also be affected by observable biases against the occurrence of short spike intervals during instinct response of the neurons.

Neural Bursting Patterns and Refinements: The basis of variation in neural bursting in both animals is unknown. There are plotted curves of the neural bursting patterns indicates the unclear neural activity. It is altered by the intensity of stimulus and appears to be very high with high CVs of neural bursting. It shows the variation in pre-synaptic stimulatory process and modified post-synaptic responses, it compared between both animals are a reasonable question to address for the future studies.

Several finding has suggested that the instinctive response plays an impotent role in the refinement of neural connection during development (Shatz, 1996, 1990). The synchronous neural bursting have been observed in ganglion cells in the ocular system prior to invasion of sight (Galli and Maffei, 1988). The bursting patterns are provided a trace for identified the neurons

arising from the central sensory nerve as they run to primary receiving regions of the cortex. Modification of these early traces has been reported in the altered neural synaptic refinements in the ocular cortex and lateral nucleus (Mooney *et al.*1996). In chickens, Lippe (1994, 1995) has observed the patterns of instinctive spike discharge from sensory neurons in the region of auditory relays and cochlear nucleolus. Using single and multiple recording units he has reported a rhythmic synchronized discharge patterns which disappeared in E19 (embryo 19 days old). Gummer *et al.* (1994) have been recorded the response of the cochlear nucleus and also reported the action potential with rhythmic inter spike intervals in wallaby (Mammal).

In the current study, the neural bursting patterns were observed clearly in the chickens and rats, but there are no similarities in this spike patterns. We have assumed that these patterns as an indicator of neural adaptation in response to the environmental and/or anatomical development of the stato-acoustic organ. The similarity in these patterns was found at the different rage of stimulus only. The rhythmic bursting patterns were found synchronized only at the constant stimulus range in both animals individually. Although the irregular neural bursting have recorded here may be a residue of some putative rudimentary bursting patterns. We do not have any reason to confirm that it could serve as the basis of the central bursting patterns (Lippe, 1994). Moreover, Lippe (1994) recorded that the synchronous rhythmic burst was disappeared in E19, but this characteristics of the neurons are not corresponding with the neural bursting of

the cochleo-vestibular neurons of the chickens and rats.

Patterns of Regular and Irregular Sensory Neurons: The spikes patterns in time interval histogram obviously different of the regular and irregular sensitive neurons found in chickens and rats. Both regular (CVs of regular response is < 0.6) and irregular (CVs of irregular response is > 0.6) spikes discharge were found distinctly in the bin of TIH. Manley (1991b) have suggested that in the birds, numerous ganglion cells project into the cochlea and otolith organ take a position at the distal end of the cochlea. The regular instinctive spike discharge patterns are produced only by the high CFs neurons that innervate to the cochleo-vestibular sensory hair cells and peripheral components.

It is facts that the regular neural bursting was most abundant in the current study, it is clearly contrasted with the results as reported from mammals and aves in the preceding text and suggests that these neurons are somewhat well mature in post-hatch chickens; this finding support to the current study on cochleo-vestibular evoked potential has recorded in the mature animals. In the later case, the action potential was represented by the vestibular neurons in mature animals than E18 (Jones and Jones 1996). On the other hand, the characteristics of the action potential of irregular sensory neurons points to the immature response. It is comparable with the regular sensory neurons as explained above. Irregular sensory neurons have prolonged patterns of inter-spike intervals and dead times in both animals at low stimulus frequency.

Significantly, the kinetic rates of the slow channel (CH1 and CH2) could attribute to these findings as argued for regular sensory neurons. Moreover, progressively increases in both channels kinetic rate would also included in observations; the action potentials of cochleo-vestibular neurons have shown the maturity after posthatching of the animals (Jones T.A. *et al* 2000). The late developmental changes appeared in sensory neurons, it is like to accompany refinement in the movement of the basilar membrane, sensory hair cells and synaptic neurons. The extent of the mechanism of frequency selectivity may involve to detecting the higher range of stimulus frequency is remains to be explained.

The variances have been observed on TIH in regard to the response of irregular sensory neurons of the avian semicircular canals (Correia and Landolt 1973; 1977; Landolt and Correia 1978; Lifschitz 1973). The TIH of neural response, longer dead times and patterns of the irregular sensory neurons have been well explained for post-hatch chickens, (Manley *et al*. 1985, 1991a). Walsh *et al*. (1972) suggested that all regular sensory neurons have modal spikes intervals of <10 ms, whereas irregular sensory neurons had shown the longer modes of inter-spike intervals. In the chickens, it can not rely on minimum inter-spike intervals and it distinguished from regular inter-spike intervals in irregular TIH. Some regular sensory neurons have longer spike discharge patterns and dead time particularly those have lower CFs (Fig-4) (salvi *et al.* 1992).

Another difference between regular and irregular sensory neurons was a correlation between coefficient variations (CVs) and spikes discharge rate. The high CVs of irregular response is a functions as a neural spikes discharge rate, whereas it is very low for regular sensory neurons. We have explained the correlation between CVs in irregular sensory neurons and instinctive spike discharge rate using frequency tuning curves. Many researchers have recorded the correlation for sensory neurons of the semicircular canal in Pigeons (Anastasio *et al.* 1985; Dickman and Correia 1989). Although, the characteristics of these sensory neurons are very prominent and its CVs has been shown as a function of neural spikes discharge rate in rat's cochleo-vestibular sensory neurons (Fernandez and Goldberg 1976; Fernandez *et al.* 1972; Goldberg and Fernandez 1971). Indeed, irregular sensory neurons may have high values of CVs depending on the rate of spikes discharge.

It is very important to note that the coefficient variations of irregular sensory neurons decline with the gradually increasing rate of spikes discharge. In figure-2 has shown two outliers of response from the general trend of irregular sensory neurons. These irregular sensory neurons revealed relatively high spikes discharge rate (±120sp/s) with coefficient variations up to ± 1.5ms. Schermuly *et al*. (1990a, b) have been observed high spikes discharge rate and lack in the substantial rate of variation. The mechanically evoked neurons have dissimilar mean discharge rate at 10 kHz stimulus in both animals. It is apparent that the outlier sensory neurons have an obvious correlation with CVs and

spikes discharge rate, it has more inconsistency between chickens and rat's irregular sensory neurons. These sensory neurons are less sensitive at high stimulus intensity.

Immature Spikes Discharge Patterns: It has been believed that the bursting of the shortest spike intervals from the cochleo-vestibular sensory neurons is related to the refractoriness of dendrite (Gray 1967). Absolute longer and limit refractory periods are responsible for the appearance of shortest spike intervals and lowest spike discharge rate. Generally, in hatchling, it is rapidly rise in time interval histogram. These spike patterns are typically observed in developing auditory afferents in the birds and mammals (studies on the bird by Manley *et al*. 1997; Manley *et al.* 1985, 1991a, b; Sachs *et al*. 1974; Temchin 1988; studies on mammals by Kiang 1965; Walsh *et al.* 1972). The exponential decline in the time period of the spike intervals is a probability process where spike intervals are independent to the initial sensitivity of sensory neurons.

In avians, the time inter histogram was plotted that have common features in the patterns of longer spike intervals than the shortest intervals. These features have suggested that stochastic stimulation process is likely to present in most sensory neurons in the chickens. However, the declined of the longer spike intervals, shortest spike intervals and dead time also indicates to some adaptation in sensitivity of sensory neurons over high and low stimulus range, it is not obviously reflected by rat's cochleo-vestibular neurons.

In mammals (Rat), there is very short information available in regard to the overall modal spike intervals and dead time for spontaneous activity in sensory neurons. Romand (1984) reported that shorter spike intervals become more prevalent with the age of animals. Gummer and their collaborators (1994) reported a significant reduction in the modal inter spikes intervals with increasing animals age.

Many researchers have been observed the reduction in maximum spike discharge rates and the prolonged latency period of stimulated neurons. To describe well mature spike patterns, many researchers mostly emphasized on the development of the stereocilia of sensory hair cells, the properties of basilar membrane and the neural synapse (Desmadryl *et al.*1992). These descriptions are plausible for some facts of developing cochleo-vesibular potential; however, they are somewhat unclear and very difficult to recording. Prolonged patterns of interspike intervals and dead times are an indicator of low potentiality of the cochleo-vestibular sensory neurons that may be known by simple observation and more direct and indirect methods. These specific features have observed by the functional phases of ion channels in the sensory hair cells.

The appearance of longer modal spike intervals and dead times in both chickens and rats might be an indicator of altered the ionic channel kinetics; thus the biases in the probability of spike patterns could generate by pre-synaptically. For example, as a result of an immature pre-synaptic, the

stochastic stimulation process rapidly increased in both animals at about 46 Hz. The transformations in the spikes patterns from neonate sensory neurons to adult sensory neurons may involve in generating the variations at any level of action potential cycle, refinements in pre-synaptic and post-synaptic stimulation. These variation my alter the BI, CVs and spike rate in both animals.

There are no direct evidence of the pre-synaptic stimulation biases in both animals, but there are direct evidences for support the role of channels of basilar membranes and the refractory phases of post-synaptic sensory neurons. Yamaguchi *et al.* (1990) explained the kinetic channels in afferents sensory neurons of the chickens (E16 –E19) and Compared with adult mammalian channel kinetic rate (Santos Sacchi, 1993) it was substantially low. These observations support the present working hypothesis that decreased patterns of spikes intervals and the longer dead times observed in rat's sensory neurons are results of lower kinetic rate of the ion channel. Thus, these features were directly compared with the kinetic rate of an ion channel in chicken's sensory hair cells (12-14 weeks old) and we were found that the age of the animals also alters the spike intervals and kinetic rate of an ion channel.

Significance of Auditory Specialization

Both animals have shown a very high correlation for the degree of auditory specialization in regard to the habitat; thus the auditory specialization augments the auditory sensitivity over the low frequencies of sound. Both

animals show the convergence in the cochleo-vestibular anatomy. In the current studies, we have included the various anatomical and physiological parameters of the cochleo-vestibular for explained the significance of the auditory specialization. A well developed middle ear of the chickens and rats have attracted the attention of many researches since the detaile studies on the ear anatomy and physiology by Webster (1960) and Orr (1940), they have been observed the wide range of the hearing in Rabbits and Hares in the southern desert , they posses large and well developed pinna and bullae of the tympanum. He also informed that increasing in middle ear size was closely correlated with the great acoustic response. Knudsen (1931) has been suggested that the transmission of sound in the ear is extensively decreased at above 1000 Hz in the air with high temperature or humidity or in both conditions.

CHAPTER- 7

SUMMARY & CONCLUSIONS

Mechanically stimulated neural responses of the cochleo-vestibular organ are mainly depend upon the tonotopy of the cochlear and cupular sensory hair cells. Here we explained the distinctive features of the hair cells motility in cochlea-vestibular organ over nonlinear MS (mechanical stimulus). The data were collected from both cells (cochlear and cupular hair cells) graphically that implies the bistable oscillatory motion.

Furthermore, the disappearance was observed in the bursting behavior of the cochleo-vestibular sensory neurons over the range of ≤ 185 Hz frequency in the rats, whereas this feature was observed from the chicken's cochleo-vestibular sensory neurons at about ≤ 135 Hz. According to this, we concluded that the neurophysiological responses are not correlated only to the vestibular sensory hair cells but also correlated to the various factors of the SAO such as anatomical, physiological, tonotopy of the organ, experimental and habitat of the organism. Therefore, the sensitivity of SAO (stato-acoustic organ) of the chickens and rats are different.

The maximum response peak of the sensory neurons of the chickens (at ≥ 1750 Hz) and rats (at ≥ 1800 Hz) represents the great variation in the cochleo-vestibular potentiality and specialization. Thus these data provides the base for evaluating the hypothesis that the specialization of the SAO enhances the cochleo-vestibular sensitivity in all animals of different classes and habitat. The chickens and rats are an example of the different class and habitat. The neural

responses have also influenced by the over dose and administration of different anesthesia.

The results of the current observation suggest that the active sensory neurons of the chickens well potential than the rats. Furthermore, the adult cochleo-vestibular neurons fundamentally evidence for different spontaneous discharge patterns. At low range of stimulus frequency, these sensory neurons were revealed irregular spike patterns and generate phase switching in time interval histogram (TIH). At a high range of stimulus frequency, these neurons have revealed clear regular spike patterns in TIH. On the other hand, the coefficient variations (CVs) varied orderly as a function of the active sensory neurons of cells in both animals at a low range of stimulus frequencies but not at high stimulus frequency.

The regular activities of sensory neurons in the rats are remarkable but not similar to the chickens. The present results also revealed the remarkable characteristics of primary afferent neurons at lower stimulus range including: a lower spontaneous spike discharge rate at low stimulus frequency; longer interval of spike discharge (dead time); appearance of shortest inter-spike interval accompanied longer spikes discharge patterns; absence of correlation in many aspects and has shown the dissimilarity between both animals in some other features as described in observations and results. Futhermore, the longer irregular bursting patterns were commonly observed in rat's cochleo-vestibular

sensory neurons, this feature has also shown by the chicken's cochleo-vestibular sensory neurons but for a very short time. This features mostly appeared at low stimulus frequency and low stimulus intensity in both animals.

The dissimilarity in spontaneous discharge rate and some other features in both animals may be an adaptation in the sensory hair cells for a particular stimulus frequency and the peripheral components of the SAO; it may alter the mechanism of sensory hair cells. This contrasting sensitivity of the sensory neurons may helpful for identifying the potentiality of sensory neurons and discriminating the organ's tonotopy.

Regular and irregular instinctive discharge patterns are basically different in regard to the relationship between the spike discharge rate and spike interval regularity. The basis of variations is to be tonotopical arrangement of sensory hair cells, and/or neural projection. The contrasting spike discharge rate of the regular and irregular discharge patterns was very unclear at low (32 Hz) and high stimulus frequencies in both animals.

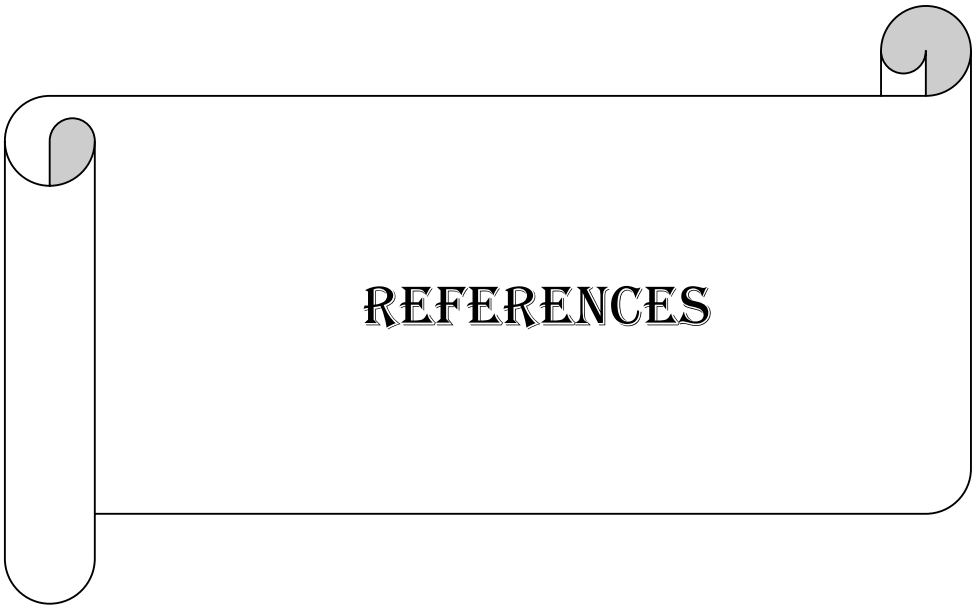
The cochleo-vestibular neurons in chickens and rats have shown somewhat affinity at different stimulus range and intensities of frequencies because these two classes (Aves and Mammals) diverged from their same ancestor. The dissimilarities in sensitivity of the cochleo-vestibular neurons are remarkable at 10 kHz. The cochleo-vestibular neural responses are much more peculiar in the chickens than the rats, but many basic principles of the tonotopic development

of the SAO and mechanism are somewhat same in both animals.

In both chickens and rats, two types of the sensory hair cells have common features as in many other mammals, on the ground of sensory hair cells; there are obvious evidences of the arrangement of the sensory hair cells of chicken's cochlea. It is not correspond to the arrangement the mammal (rats) sensory hair cells in the cochlea. The capability of mechanical wave amplification associated with instinct oscillation of the sensory hair cells bundle. This mechanism in both animals are varied at all level of stimulus frequencies (32Hz to 10 kHz), but this mechanism has increased when stimulus intensity increased and this mechanism inhibits at higher stimulus intensity.

Cochleo-vestibular studied to date demonstrates the tonotopy with the neural response that also reflect the refinement and adaptation process. Convergences of the stimulatory and inhibitory mechanism of the sensory neurons have played an important role at low stimulus frequencies, but the appearance of fast adaptation in responses have not yet observed.

There are recorded miniature endolymphatic oscillatory motion at low and higher stimulus frequencies in both systems have exhibited the constant dynamics but it is not similar to that response of sensory neurons have plotted on TIH (time interval histogram); this dissimilarity indicates the magnifying mechanism of the sensory hair cells of the cochlea and vestibular system.


REFERENCES

Allen, J. (1980). Cochlear micromechanics a physical model of transduction. J. Acoust. Soc. Am.68: 1660–1670.

Anastasio, T. J., Correia, M. J and Perachio, A. A. (1985). Spontaneous and driven responses of semicircular canal primary afferents in the unanesthetized pigeon. J. Neurophysiol. 54: 335–347.

Anderson, J. H., Blanks, R. H. I and Precht, W. (1978). Response characteristics of semicircular canal and otolith systems in cat. I. Dynamic responses of primary vestibular nerve fibers. Exp Brain Res 32: 491–507.

Ashmore, J. F. (1983). Frequency tuning in a frog vestibular organ. Nature 304: 536–538.

Ashmore, J. F. (1987). A fast motile response in guinea-pig outer hair cells: the molecular basis of the cochlear amplifier. J. Physiol. (Lond). 388: 323-347.

Ashmore, J. F. (1990). Forward and reverse transduction in guinea-pig outer hair cells: the cellular basis of the cochlear amplifier. Neurosci. Res. Suppl. 12: S39–S50.

Baarsma, E. A and Ollewijn, H. (1974). Vestibulo-ocular and optokinetic reactions to rotation and their interaction in the rabbit. J. Physiol. Land.

238: 603-625.

Baird, R. A., Desmadryl, G., Fernandez, C and Goldberg, J. M. (1988). The vestibular nerve of the chinchilla. II. Relation between afferent response properties and peripheral innervation patterns in the semicircular canals. J Neurophysiol 60: 182–203.

Baird, R.A., Desmadryl, G., Fernandez, C and Goldberg, J. M. (1988). The vestibular nerve of the chinchilla. II. Relation between afferent response properties and peripheral innervation patterns in the semicircular canals. J. Neurophysiol 60: 182-203.

Barmac, N. H. A. (1981). Comparison of the horizontal and vertical vestibuloocular reflexes of the rabbit. J. Physiol. Land. 314: 547-564.

Batchelor, G. K. (1967). An Introduction to Fluid Dynamics. Cambridge University Press, Cambridge, UK.

Bekesy, G. (1952). resting potentials inside the cochlear partition. J. Acoust. Soc. Am. 24: 72–76.

Bergevin, C., Manley, G. A and Koppl, C. (2015). Salient features of otoacoustic emissions are common across tetrapod groups and suggest shared properties of generation mechanisms. Proc. Natl Acad. Sci 112: 3362–3367.

Benton, M. J. (1997). Vertebrate Paleontology second ed.,Chapman and Hall, London.

Bernard, C., Cochran, S. L and Precht, W. (1985). Presynaptic actions of cholinergic agents upon the hair cell afferent fiber synapses in the vestibular labyrinth of the frog. Brain Res 338: 225–236.

Beurg, M., Tan, X and Fettiplace, R. (2013). A prestin motor in chicken auditory hair cells: active force generation in a nonmammalian species. Neuron 79, 69–81.

Blanks, R. H. I., Estes, M. S and Markham, C. H. (1975). Physiologic characteristics of vestibular first order canal neurons in the cat. II. Response to constant angular acceleration. J Neurophysiol 38: 1250-1268.

Boord, R. L. G. L. (1963). Rasmussen, Projection of the cochlear and lagenar nerves on the cochlear nuclei of the pigeon, J. Comp. Neurol. 120: 463–473.

Bormuth, V., Barral, J., Joanny, J. F., Julicher, F and Martin, P. (2014). Transduction channels' gating can control friction on vibrating hair-cell bundles in the ear. Proc. Natl Acad. Sci. USA 111: 7185–7190.

Boyle, R and Highstein, S. M. (1990). Efferent vestibular system in toadfish: action upon horizontal semicircular canal afferents. J Neurosci 10:

1570–1582.

Bosher, S. K and Warren, R. L. (1978). "Very low calcium content of cochlear endolymph an extracellular fluid".Nature.273 (5661): 377–378. Doi:10.1038/273377a0. PMID 661948.

Boyle, R and Highstein, S.M. (1990). Resting discharge and response dynamics of horizontal semicircular canal afferents of the toadfish, Opsanus tau. J Neurosci 10: 1557–1569.

Brichta A. M and Goldberg J. M (2000). Responses to efferent activation and excitatory response-intensity relations of turtle posterior-crista afferents. J Neurophysiol 83:1224–124.

Brownell, W. E., C. R. Bader., D. Bertrand and Y. de Ribaupierre. (1985). Evoked mechanical responses of isolated cochlear outer hair cells. Science. 227: 194–196.

Cant, *et al.* (1998). Structural development of the mammalian auditory pathways. In: Rubel E.W, Popper A.N, Fay R.R, editors. Development of the Auditory System. New York: Springer, p. 315-413.

Camhi, J. (1984). Neuroethology: nerve cells and the natural behavior of animals. Sinauer Associates.

Carlier, E., Abonnenc, M and Pujol, R. (1975). Maturation des responses

unitaires a la stimulation tonale dans le nerf cochlear du chaton. J. Physiol. (Paris) 70: 129–138,

Carr, C. E and Boudreau, R. E. (1991). The central projections of auditory nerve fibers in the barn owl, J. Comp. Neurol. 314: 306–318.

Chen, L., Salvi, R and Shero, M. (1994). Cochlear frequency-place map in adult chickens: intracellular biocytin labeling, Hear. Res. 81: 130–136

Chen, L., Sun, W and Salvi, R.J. (2001). Electrically-evoked otoacoustic emissions from the chicken ear. Hear. Res. 161: 54–64.

Cody, A. R and I. J. Russell. (1987). The response of hair cells in the basal turn of the guinea-pig cochlea to tones. J.Physiol. (Lond.).383:551–569.

Cohen, G. M and Cotanche, D. A. (1992). Development of the sensory receptors and their innervation in the chick cochlea. In: Development of Auditory and Vestibular Systems 2, edited by R. Romand. Amsterdam: Elsevier, p. 101–138.

Cole, K. S. (1968). Membranes, Ions, and Impulses. University of California Press, Berkeley, California.

Corey, D. P and Hudspeth, A. J. (1979). "Ionic basis of the receptor potential in a vertebrate hair cell". Nature. 281 (5733): 675–677. doi:10.1038 /

281675a0. PMID 45121.

Correia, M. J and Landolt, J. P. (1973) .Spontaneous and driven responses from primary neurons of the anterior semicircular canal of the pigeon. Adv. Oto-Rhino-Laryngol. 19: 134–148.

Correia, M. J and Landolt, J. P. (1977). A point process analysis of the spontaneous activity of anterior semicircular canal units in the anesthetized pigeon. Biol. Cybern. 27: 199–213.

Crawford, A. C and Fettiplace, R. (1981). An electrical tuning mechanism in turtle cochlear hair cells. J. Physiol. 312, 317–338.

Curthoys, I. S and Oman, C. M. (1986). Dimensions of horizontal semicircular duct ampulla and utricle in rat and guinea pig. Acta Otolaryngol 101: 1–10.

Curthoys, I. S. (1978). The development of the function of horizontal semicircular canal primary afferents in the rat. In: Vestibular Mechanisms in Health and Disease, edited by J. D. Hood. London: Academic, p. 3–9.

Curthoys, I. S. (1979). The development of the function of horizontal semicircular canal primary neurons in the rat. Brain Res. 167: 41–52.

Curthoys, I. S. (1982). Postnatal developmental changes in the responses of rat primary horizontal semicircular canal neurons to sinusoidal angular

accelerations. Exp. Brain Res. 47: 259–300.

Curthoys, I. S. (1982a). Postnatal development changes in the response of rat primary horizontal semicircular canal neurons to sinusoidal angular accelerations. Exp Brain Res 47: 295–300.

Curthoys, I. S. (1982b). The response of primary horizontal semicircular canal neurons in the rat and guinea pig to angular acceleration. Exp Brain Res 47: 294 –1982.

Curthoys, I. S. (1983). The development of the function of primary vestibular neurons. In: Development of Auditory and Vestibular Systems, edited by R. Romand. New York: Academic, p. 425–461.

Dahl, D and D. Kleinfeldt. (1976). Perilymph viscosity as a function of temperature and noise stress. Ann. Acad. Med. Stetin. 14:83–86.

Dallos, P., R. Hallworth and B. N. Evans. (1993). Theory of electrically driven shape changes of cochlear outer hair cells. J. Neurophysiol. 70: 299-323.

Damiano, E and Rabbitt, R. D. (1996). A singular perturbation model of fluid dynamics in the vestibular semicircular canal and ampulla. J Fluid Mech, 307:333–372.

De Boer, E. (1991). Auditory physics. Physical principles in hearing theory.

III. Phys. Rep. 203:125–231.

Desmadryl, G., Raymond, J and Sans, A. (1986). In vitro electrophysiological study of spontaneous activity in neonatal mouse vestibular ganglion neurons during development. Dev. Brain Res. 25: 133-136.

Desmadryl, G., Dechesne, C. J and Raymond, J. (1992). Recent aspects of development of the vestibular sense organs and their innervation. In: Development of Auditory and Vestibular Systems 2, edited by R. Romand. Amsterdam: Elsevier, p. 461–482.

Dickman, J. D and Correia, M. J. (1989). Responses of pigeon horizontal semicircular canal afferent fibers. I. Step, trapezoid, and low-frequency sinusoid mechanical and rotational stimulation. J. Neurophysiol. 62: 1090–1101.

Dickman, J. D. 1996). Spatial orientation of semicircular canals and afferent sensitivity vectors in pigeons. Exp Brain Res 111: 8 –20.

Dong, X. X., M. Ospeck and K. H. Iwasa. (2002). Piezoelectric reciprocal relationship of the membrane motor in the cochlear outer hair cell. Biophys. J. 82: 1254-1259.

Eguiluz, V.M., M. Ospeck., Y. Choe., A.J. Hudspeth and M. O. Magnasco.

(2000). Essential nonlinearities in hearing. Phys.Rev. Lett. 84: 5232–5235.

Fekete, D. M., Rouiller, E. M., Liberman, M. C and Ryugo, D. K. (1984). The central projections of intracellularly labeled auditory nerve fibers in cats, J. Comp. Neurol. 229: 432–450.

Fernandez, C and Goldberg, J. M. (1971). Physiology of peripheral neurons innervating semicircular canals of the squirrel monkey. II. Response to sinusoidal stimulation and dynamics of peripheral vestibular system. J Neurophysiol 34:661–675.

Fernandez, C and Goldberg, J. M. (1976). Physiology of peripheral neurons innervating otolith organs of the squirrel monkey. I. Response to static tilts and to long-duration centrifugal force. J. Neurophysiol. 39: 970–984.

Fernandez, C., Goldberg, J. M and Abend, W. K. (1972). Response to static tilts of peripheral neurons innervating otolith organs of the squirrel monkey. J. Neurophysiol. 35: 978–997.

Fettiplace, R and Kim, K. X. (2014). The physiology of mechanoelectrical transduction channels in hearing. Physiol. Rev. 94: 951–986.

Fontaine, B., Koppl, C and Pena, J. L. (2015). Reverse correlation analysis of auditory-nerve fiber responses to broadband noise in a bird, the barn owl. J. Assoc. Res. Otolaryngol. 16: 101–119.

Frank, G., W. Hemmert and A. W. Gummer. (1999). Limiting dynamics of high-frequency electromechanical transduction of outer hair cells. Proc. Natl. Acad. Sci. USA. 96: 4420–4425.

Fredrickson-Hemsing, L., S. Ji and Bozovic, D. (2012). Mode-locking dynamics of hair cells of the inner ear. Phys. Rev. E Stat. Nonlin. Soft Matter Phys. 86: 021915.

Freeman, D. M and T. F. Weiss. (1988). The role of fluid inertia in mechanical stimulation of hair cells. Hear. Res. 35:201–208.

Fuchs, P.A., Nagai, T and Evans, M.G. (1988). Electrical tuning in hair cells isolated from the chick cochlea. J. Neurosci. 8:2460-2467. (PubMed).

Gacek, R. R and Lyon, M. (1974). The localization of vestibular efferent neurons in the kitten with horseradish peroxidase. Acta Otolaryngol 77: 92–101.

Galli, L and Maffei, L. (1988). Spontaneous impulse activity of rat retinal ganglion cells in prenatal life. Science 242: 90–91.

Gale, J. E and J. F. Ashmore. (1994). Charge displacement induced by rapid stretch in the basolateral membrane of the guinea-pig outer hair cell. Proc. Roy. Soc. (Lond) B. Biol. Sci. 255: 233–249.

Gdowski, G. T and McCrea, R. A. (1999). Integration of vestibular and head movement signals in the vestibular nuclei during whole-body rotation. J

Neurophysiol 82: 436–449.

Gleich, O. (1989). Auditory afferents in starling: Correlation of function and morphology. Hear. Res. 37: 255-267 Doi: 10.1016/0378-5955(89)90026-9. (PubMed)

Gold, T. (1948). Hearing. II. The physical basis of the action of the cochlea. Proc. Roy. Soc. (Lond.) B. Biol. Sci. 135: 492–498.

Goldberg, J. M and Fernandez, C. (1971). Physiology of peripheral neurons innervating semicircular canals of the squirrel monkey. III. Variations among units in their discharge properties. J. Neurophysiol. 34: 676–684.

Goldberg, J. M and Fernandez, C. (1980). Efferent vestibular system in the squirrel monkey: anatomical location and influence on afferent activity. J Neurophysiol 43: 986–1025.

Goldberg, J. M., Smith, C. E and Fernandez, C. (1984). Relation between discharge regularity and responses to externally applied galvanic currents in vestibular nerve afferents of the squirrel monkey. J Neurophysiol 51: 1236–1256.

Goldberg, J. M., Brichta, A. M and Wackym, P. A. (2000). Efferent vestibular system: anatomy, physiology and neurochemistry. In: Neurochemistry of the vestibular system (Anderson JH, Beitz AJ, eds),

pp 61–94. Boca Raton, FL: CRC.

Gray, P. R. (1967). Conditional probability analyses of the spike activity of single neurons. Biophys. J. 7: 759–77.

Greenwood, D. D. (1990). A cochlear frequency-position function for several species–29 years later. J. Acoust. Soc. Am. 87:2592–2605.

Guenter Ehret. (Dec 1978). Stiffness gradient along the basilar membrane as a way for spatial frequency analysis within the cochlea" J. Acoust Soc Am. 64 (6): 1723–6. doi:10.1121/1.382153. PMID 739099.

Gummer, A. W and Mark, R. F. (1994). Patterned neural activity in brain stem auditory areas of a prehearing mammal, the tammar wallaby Macropus eugenii. Neuroreport. 5: 685–688. [PubMed: 8199338]

Gummer, A. W. (1991). First order temporal properties of spontaneous and tone evoked activity of auditory afferent neurones in the cochlear ganglion of the pigeon. Hear. Res. 55: 143–166.

Gummer, A. W., Smolders, J. W and Klinke, R. (1987). Basilar membrane motion in the pigeon measured with the Mossbauer technique. Hear. Res. 2: 63-92.

Hackney, C. M and Furness, D. N. (1995). "Mechanotransduction in vertebrate hair cells: Structure and function of the stereociliary bundle". The American Journal of Physiology. 268 (1 Pt 1): C1–13. PMID

7840137.

Hallworth, R. (1995). Passive compliance and active force generation in the guinea pig outer hair cell. J. Neurophysiol. 74: 2319–2328.

Harris, G. G and Flock, A. (1967). Spontaneous and evoked activity from the xenopus laevis lateral line. In: Lateral Line Detectors, edited by P. H. Cahn. Bloomington, IN: Indiana Univ. Press, p. 135–161.

Harris, G. G and Milne, D. C. (1965). Input-output characteristics of the lateral line sense organs of Xenopus laevis. J. Acoust. Soc. Am. 40: 32–42.

Heffner, H. E and Heffner, R. S. (1998). Hearing. In: Greenberg, G, Harawy, M.M (Eds.), Comparative physiology, a handbook. Garland, New York, pp. 290-303.

Henson, O.W J. R., Henson, M. M., Kobler, J.B and Pollak G. D. (1980). The constant frequency component of the biosonar signals of the bat, Pteronotus Parnellii. In: Busnel R-G, Fish L (eds) Animal Sonar system. Plenum press, New York, pp 913- 916.

Henson, O.W. J. R., Pollak, G. D. (1972). A technique for chronic implantation of electrodes in the cochleae of bats. Physiol Behav 8: 1185-1188.

Highstein, S. M and Baker. R. (1985). Action of the efferent vestibular

system on primary afferents in the toadfish, Opsanus tau. J Neurophysiol 54: 370–384.

Hill, K. G., Mo, J and Stange, G. (1989). Excitation and supression of primary auditory fibers in the pigeon. Hear. Res. 39: 37–48.

Hillman, D. E. (1974). Cupular structure and its receptor relationship. Brain Behav. Evol 10352-68.

Hillman, D. E. (1977). Relationshin of the sensorv cell cilia to the cupula. Scan Elec Microscopy 2:415-420.

Honrubia, V., Hoffman, L. F., Sitko, S and Schwartz, I. R. (1989). Anatomic and physiologic correlates in bullfrog vestibular nerve. J Neurophysio161: 688-701.

Housley, G. D and J. F. Ashmore. (1992). Ionic currents of outer hair cells isolated from the guinea-pig cochlea. J. Physiol. (Lond). 448: 73–98.

Howard, J and Hudspeth, A. J. (1988). Compliance of the hair bundle associated with gating of mechanoelectrical transduction channels in the bullfrog's saccular hair cell. Neuron 1: 189–199.

Hubbard, A. E and D. C. Mountain. (1995). Analysis and syhthesis of cochlear mechanical function using models. *In* Auditory Computation. H. L. Hawkins, T. A. McMullen, A. N. Popper, and R. R. Fay, editors. Springer, New York. 62–120.

Hudspeth, A. J and R. S. Lewis. (1988). A model for electrical resonance and frequency tuning in saccular hair cells of the bull-frog, *Rana Catesbeiana*. J. Physiol.(Lond.). 400: 275–297.

Hudspeth, A. J. (1982). Extracellular current flow and the site of transduction by vertebrate hair cells. The Journal of Neuroscience. 2 (1): 1–10.

Hudspeth, A. J. (2014). Integrating the active process of hair cells with cochlear function. Nat. Rev. Neurosci. 15: 600–614.

Huxley, A. F. (1969). Is resonance possible in the cochlea after all? Nature. 221: 935–940.

Hudspeth, A. J., Choe, Y., Mehta, A. D and Martin, P. (2000). Putting ion channels to work: mechanoelectrical transduction, adaptation, and amplification by hair cells. Proc. Natl. Acad. Sci. USA 97: 11765–11772.

Hullar, T. E., Della santina., C. C, Hirvonen, T. P., Lasker, D. M., Carey, J. P and Minor, L. B. (2005). Responses of irregularly discharging chinchilla semicircular canal vestibular-nerve afferents during high-frequency head rotations. J Neurophysiol 93: 2777–2786.

Ikeda, T. (1990). Fundamentals of Piezoelectricity. Oxford University Press, Oxford, UK.

Iwasa, K. H. (1993). Effect of stress on the membrane capacitance of the auditory outer hair cell. Biophys. J. 65:492–498.

Iwasa, K. H. (2001). A two-state piezoelectric model for outer hair cell motility. Biophys. J. 81: 2495–2506.

Iwasa, K. H and M. Adachi. (1997). Force generation in the outer hair cell of the cochlea. Biophys. J.73: 546–555.

Iwasa, K. H and Ricci, A. J. (2015). In Mechanics of Hearing eds Corey D. P., Karavitak K. D. American Institute of Physics.

Iwasa, K. H. (1994). A membrane model for the fast motility of the outer hair cell. J. Acoust. Soc. Am.96: 2216–2224.

Johnston, A. R., Macleod, N. K and Dutia, M. B. (1994). Ionic conductances contributing to spike repolarization and after-potentials in rat medial vestibular nucleus neurones. J Physiol (Lond) 481: 61–77.

Johnstone, B. M., R. Patuzzi and G. K. Yates. (1986). Basilar membrane measurements and the travelling wave. Hear. Res. 22: 147–153.

Jones, S. M and Jones, T. A. (1995a). The tonotopic map in the embryonic chicken cochlea. Hear. Res. 82:149–157.

Jones, S. M and Jones, T.A. (1995b). Neural tuning characteristics of auditory primary afferents in the chicken embryo. Hear. Res. 82: 139–

148.

Jones, S. M and Jones, T. A. (1996). Short latency vestibular evoked potentials in the chicken embryo. J. Vestib. Res. 6: 71–83.

Jones, T. A and Jones, S. M. (2000). Spontaneous activity in the statoacoustic ganglion of the chicken embryo. J. Neurophysiol, 83: 1452–1468. [PubMed: 10712472]

Jones, T. A., Jones, S. M and Paggett, K.C. (2001). Primordial rhythmic bursting in embryonic cochlear ganglion cells. J. Neurosci. 21: 8129–8135. [PubMed: 11588185].

Kanis, L. J and E. de Boer. (1993). Self-suppression in a locally active nonlinear model of the cochlea. J. Acoust. Soc. Am. 64:3199–3206.

Kakehata, S and J. Santos-Sacchi. (1995). Membrane tension directly shifts voltage dependence of outer hair cell motility and associated gating charge. Biophys. J. 68: 2190–2197.

Kawase, T and Liberman, M.C. (1992). Spatial organization of the auditory nerve according to spontaneous discharge rate, J. Comp. Neurol. 319: 312–318.

Keller, E. L. (1976). Behavior of horizontal semicircular canal afferents in alert monkey during vestibular and optokinetic stimulation. Exp Brain Res 24: 459–471.

Keller, J. B and J. C. Neu. (1985). Asymptotic analysis of a viscous cochlear model. J. Acoust. Soc. Am. 77: 2107–2110.

Kettner, R., Feng, J and Brugge, J. (1985). Postnatal development of the phase locked response to low-frequency tones of auditory nerve fibers in the cat. J. Neurosci. 5: 275–283.

Kiang, N. Y. S. (1965). Discharge Patterns of Single Fibers in the Cat's Auditory Nerve. Cambridge, MA: MIT Press.

Kiang, N. Y. S., Liberman, M. C and Levine, R. A. (1976). Auditory nerve activity in cats exposed to ototoxic drugs and high intensity sounds. Ann. Otol. Rhinol. Laryngol. 85: 752–768.

Kiang, N.Y.S and Sachs, M. B. (1965). Effects of acoustic stimuli on spontaneous spike discharges in auditory nerve fibres (Abstract). Physiologist 8: 208.

Klinke, R; Muller, M., richter, C. P and Smolders, J. (1994). Preferred intervals in birds and mammals: a filter response to noise. Hear. Res. 74: 238-246.

Knudsen, V. (1931) The effect of humidity upon the absorption of sound in a room, and ;I determination of' the coefficients of absorption of sound in air. J. Acoust. Soc. America. 3: 126-138.

Koay, G., Heffner, R. S and Heffner, H. E. (1998). Hearing in

megachiropteran fruit bat Rousettus aegyptiacus. J.Comp. Psychol. 112:371-382.

Koppl, C. (1994). Auditory nerve terminals in the cochlear nucleus magnocellularis: differences between low and high frequencies, J. Comp. Neurol. 339: 438–446.

Koppl, C. (2011). Birds same thing, but different? Convergent evolution in the avian and mammalian auditory systems provides informative comparative models. Hear. Res. 273: 6 5–71.

Koppl, C. l. (1997). Frequency tuning and spontaneous activity in the auditory nerve and cochlear nucleus magnocellularis of the barn owl, Tytoalba, J. Neurophysiol. 77: 364–377.

Kossl, M and M. Vater. (1995). Cochlear structure and function of bats. *In* Hearing by Bats. A. N. Popper, and R. R. Faye, editors. Springer, New York. 191–234.

Kros, C. J A. Rusch and G. P. Richardson. (1992). Mechano-electrical transducer currents in the hair cells of the cultured mouse cochlea. Proc. R. Soc. Lond. B. Biol. Sci. 249:185–193.

Kros, C. J. (1996). Physiology of mammalian cochlear hair cells. *In* The Cochlea. P. Dallos, A. N. Popper, and R. R. Fay, editors. Springer, New York. 318–385.

Kros, C. J., J. P. Ruppersberg and A. Rusch. (1998). Expression of a potassium current in inner hair cells during development of hearing in mice. Nature. 394:281–284.

Kurian, R., Krupp, N. L and Saunders, J. C. (2003). Tip link loss and recovery of chick short hair cell following intense exposure to sound Hear.Res. 181 (1-2):40-50.Dio:10.1016/So378-5955(03) 00165. (PubMed) (CrossRef).

Kurian, R., Krupp, N. L and saunders, J. C. (2003). Tip link loss and recovery on chick short hair cells following intense exposure to sound. Hear. Res. 181(1–2): 40–50.

Landolt Jack, P and Correia Manning, J. (1980). Neurodynamic response analysis of anterior semicircular canal afferents in the pigeon. J. Neurophysiol. 43: 1746-1770,

Landolt, J. P and Correia, M. J. (1978). Neuromathematical concepts of point process theory. IEEE Trans. Biomed. Eng. 25: 1–12.

Lewis, R. S and Hudspeth, A. J. (1983). Voltage and ion-dependent conductances in solitary vertebrate hair cells. Nature 304 :538–541.

Li, J and Young, E. D. (1993). Discharge rate dependence of refractory behavior of cat auditory nerve fibers. Hear. Res. 69: 151–162.

Li, W and Correia, M. J. (1998). Recovery of semicircular canal primary afferent activity in the pigeon after streptomycin ototoxicity. J. Neurophysiol. 80: 3297–3311.

Liberman, M. C and L. W. Dodds. (1984). Single neuron labeling and chronic cochlear pathology. III. stereocilia damage and alterations of threshold tuning curves. Hear. Res. 16: 55–74.

Liberman, M. C. (1992). The cochlear frequency map for the cat: labeling auditory nerve fibers of known charactaristices frequency. J. Acoust. Soc.Am. 75: 1441-1449.

Liberman, M.C. (1991). Central projections of auditory-nerve fibers of differing spontaneous rate. I. Anteroventral cochlear nucleus, J. Comp. Neurol. 313: 240–258

Lifschitz, W. S. (1973). Responses from the first order neurons of the horizontal semicircular canal in the pigeon. Brain Res. 63: 43–57.

Lighthill, J. (1981). Energy flow in the cochlea. J. Fluid Mech. 100:149–213.

Lim, D. J. (1980). Cochlear anatomy related to cochlear micromechanics. a review. J. Acoust. Soc. Am. 67:1686–1695.

Lindeman, H. H. (1969). Studies of the morphology of the sensory regions of the vestibular apparatus. Erg Anat 42: 1–113.

Lippe, W. R. (1994). Rhythmic spontaneous activity in the developing avian auditory system. J Neurosci, 14:1486–1495. [PubMed: 8126550]

Lippe, W. R. (1995). Relationship between frequency of spontaneous bursting and tonotopic position in the developing avian auditory system. Brain Res. 703: 205–213.

Lisberger, S. G and Pavelko, T. A. (1986). Vestibular signals carried by pathways subserving plasticity of the vestibulo-ocular reflex in monkeys. J Neurosci 6: 346–354.

Mammano, F and J. F. Ashmore. (1996). Differential expression of outer hair cell potassium currents in the isolated cochlea of the guinea-pig. J. Physiol. (Lond). 496: 639–646.

Manley, g. A and Koppl, C. (1997). Activity of primary auditory neurons in the cochlear ganglion of the emu dromaius novaehollandiae: spontaneous discharge, frequency tuning, and phase locking.J. Acoust. Soc. Am. 101: 1560–1573.

Manley, G. A., Brix, J and Kaiser, A. (1987). Developmental stability of the tonotopic organization of the chick's basilar papilla.Science, 237: 655-656.

Manley, G. A., Gleich, O., Leppelsack, H. J and Oeckinghaus, H. (1985). Activity patterns of cochlear ganglion neurons in the starling. J. Comp.

Physiol. [A] 157: 161–181.

Manley, G. A., Kaiser, A., Brix, L and Gleich, O. (1991a). Activity patterns of primary auditory nerve fibers in chickens: development of fundamental properties. Hear. Res. 57: 1-15.

Manley, G. A., Haeseler, C and Brix, J. (1991b). Innervation patterns and spontaneous activity of afferent fibres to the lagenar macula and apical basilar papilla of the chick's cochlea. Hear. Res. 56: 211–226.

Manley, G. A and Koppl, C. (1998). Phylogenetic development of the cochlea and its innervation. Curr. Opin. Neurobiol. 8: 468–474.

Martin, P and A. J. Hudspeth. (1999). Active hair-bundle movements can amplify a hair cell's response to oscillatory mechanical stimuli. Proc. Natl. Acad. Sci. USA. 96:14306–14311.

McLaren, J. W and Hillman, D. E. (1979). Displacement of the semicircular canal cupula during sinusoidal rotation. Neuroscience, 4: 2001-2008.

McLaughlin, T. O., Leary and D. D. M. (2005). Molecular gradients and development of retinotopic maps. Annu Rev Neurosci, 28: 327–355. (PubMed: 16022599)

Miles, F, A and Braitman, D. J. (1980). Long-term adaptive changes in primate vestibuloocular reflex. II. Electrophysiological observations on

semicircular canal primary afferents. J Neurophysiol 43:1426–1436.

Moller, A. R. (1970). The use of correlation analysis in processing neuroelectric data. Prog. Brain Res. 33: 87–100.

Mooney, R., Penn, A. A., Gallego, R and Shatz, C. J. (1996). Thalamic relay of spontaneous retinal activity prior to vision. Neuron 17: 863–874.

Mountain, D. C and A. E. Hubbard. (1994). A piezoelectric model of outer hair cell function. J. Acoust. Soc. Am. 95:350–354.

Muller, M., Smolders, J. W. T., Ding-Pfennigdorff, D and Klinke, R. (1997). Discharge properties of pigeon single auditory nerve fibres after recovery from severe acoustic trauma. Int. J. Dev. Neurosci. 15: 401–416,

Narayan, S. S., Temchin, A. N and Ruggereo, M. A. (1998). Frequency tuning of the basilar membrane and auditory nerve fibers in the same cochlea. Science. 282: 1882- 1884. Dio: 10.1126/scienc.282.5395.1882. (PCM free article) PubMed) (CrossRef)

Neely, S. T and D. O. Kim. (1986). A model for active elements in cochlear biomechanics. J. Acoust. Soc. Am. 79:1472–1480.

Nobili, R and F. Mammano. (1993). Biophysics of the cochlea: linear approximation. J. Acoust. Soc. Am. 93:3320–3332.

O'Neill, M. P and Bearden, A. (1995). Laser-feedback measurements of turtle basilar membrane motion using direct reflection. Hear. Res. 84, 125–138.

Oaks, E. C. (1967). Structure and function of inflated middle ears of rodents. Ph.D. Thesis,Yale Univ, 411 pp, 99 figs. 13 tables.

Ohmori, H. (1985). "Mechano-electrical transduction currents in isolated vestibular hair cells of the chick".The Journal of Physiology. 359:189–217.Doi:10.1113/jphysiol.1985.sp015581.PMC 1193371.
PMID 2582113.

Orr, R. (1940). The rabbits of California. Occ. Pap. Calif. Acad. Sci., 19: 207 pp., 9 pl.

Ospeck, M. C., X. X. Dong and K. H. Iwasa. (2002). RC time constant paradox of outer hair cells. Biophys. J. 82:1215a.

Patuzzi, R and D. Robertson. (1988). Tuning in the mammalian cochlea. Physiol. Rev. 68:1009–1082.

Patuzzi, R. (1996). Chochlear micromechanics and macromechanics. *In* The Cochlea. P. Dallos, A. N. Popper, and R. R. Fay, editors. Springer, New York. 186–257.

Peake, W. T and Ling, A. (1980). Basilar-membrane motion in the alligator

lizard: its relation to tonotopic organization and frequency selectivity. J. Acoust. Soc. Am. 67: 1736–1745.

Perkel, D. H., Gernstein, G. L and Moore, G. P. (1967). Neuronal spike trains and stochastic point processes I.: The single spike train. Biophys J. 7: 391–418. [PubMed: 4292791].

Plotnik, M., Marlinski, V and Goldberg, J. (2000). Efferent-mediated type III rotational responses recorded from vestibular-nerve afferents in the chinchilla. Twenty-Third Annual Meeting of the Association for Research in Otolaryngology, St. Petersburg Beach, FL, February.

Pollak, G. D., Henson, O.W Jr and Novick, A. (1972). Cochlear microphonic audiograms in the 'pure tone' bat, Chilonycters parnellii Parnellii. Science 176: 66-68.

Pollak, G.D., Henson, O.W., Johnson, R. (1979). Multiple specializations in the peripheral auditory system of the CF-FM bat, Pteronotus parnellii. J Comp Physiol 131:255-266.

Precht, W., Llinas, R and Clarke, M. (1971). Physiological responses of frog vestibular fibres to horizontal angular rotation. Exp Brain Res 13: 378-407.

Pritchard, U. (1881). The cochlea of the Omitkorhy? crrs plntgpzrs compared with that of ordinary mammals and of birds. Trails. Roy. Soc. London,

vol. 172. Part 2: 267-282.

Purcell, I. M. (1997). Three-dimensional analysis and patterns of innervation of vestibular efferent and afferent terminal endings in the semicircular canals and otolith organs of the gerbil. PhD dissertation, University of Texas-Galveston.

Rasmussen, G. L and Gacek, R. R. (1958). Concerning the question of the efferent fiber component of the vestibular nerve of the cat. Anat Rec 130: 361–362.

Ren, T. (2000). Longitudinal pattern of basilar membrane vibration in the sensitive cochlea. Proc. Natl. Acad. Sci. USA. 99:17101–17106.

Rhode, W. S. (1971). Observations of the vibration of the basilar membrane in squirrel monkeys using the Mössbauer technique. J.Acoust. Soc. Am. 49:1218–1231.

Rhode, W. S and Smith, P.H. (1985). Characteristics of tone pip response patterns in relationship to spontaneous rate in cat auditory nerve fibers.Hear.Res. 18:159-168.Doi: 10.1016/0378-5955 (85)90008-5. (PubMed) (CrossRef)

Ricci, A. J., A. C. Crawford and R. Fettiplace. (2000). Active hair bundle motion linked to fast transducer adaptation in auditory hair cells. J.

Neurosci. 20:7131–7142.

Ricci, A. J., Crawford, A. C and Fettiplace, R. (2002). Mechanisms of active hair bundle motion in auditory hair cells. J. Neurosci. 22, 44–52.

Richter, C., Sauer, G., Hoidis, S and Klinke, R. (1996). Development of activity patterns in auditory nerve fibers of pigeons. Hear. Res. 95: 77–86.

Roberts, W. M., R. A. Jacobs and A. J. Hudspeth. (1990). Colocalization of ion channels involved in frequency selectivity and synaptic transmission at presynaptic active zones of hair cells. J. Neurosci. 10: 3664–3684.

Robles, L., W. S. Rhode and C. D. Geisler. (1976). Transient response of the basilar membrane measured in squirrel monkeys using the Mössbauer effect. J. Acoust. Soc. Am. 59:926–939.

Romand, R. (1984). Functional properties of auditory-nerve fibers during postnatal development in the kitten. Exp. Brain Res. 56: 395–402.

Romand, R. and Dauzat, M. (1982). Modification of spontaneous activity in primary vestibular neurons during development in the cat. Exp. Brain Res. 45: 265–268.

Roy, J. E and Cullen, K. E. (2001). Selective processing of vestibular reafference during self-generated head motion. J Neurosci 21:2131–

2142.

Roy, J. E and Cullen, K. E. (2002). Vestibuloocular reflex signal modulation during voluntary and passive head movements. J Neurophysiol 87:2337.

Rubio, M. E. J. R. (1997). Wenthold, Glutamate receptors are selectively targeted to postsynaptic sites in neurons, Neuron 18, 939–950.

Rubel, E. W. T. N. (1975). Parks, Organization and development of brain stem auditory nuclei of the chicken: tonotopic organization of n. magnocellularis and n. laminaris, J. Comp. Neurol. 164:411–433.

Rubio, M. E. J. M. (1998). Juiz, Chemical anatomy of excitatory endings in the dorsal cochlear nucleus of the rat: differential synaptic distribution of aspartate aminotransferase, glutamate, and vesicular zinc, J. Comp. Neurol. 399: 341 358.

Ruggero, M. A and N. C. Rich. (1991). Furosemide alters organ of corti mechanics: evidence for feedback of outer hair cells upon the basilar membrane. J. Neurosci. 11:1057–1067.

Ruggero, M. A. (1992). Responses to sound of the basilar membrane of the mammalian cochlea. Curr. Opin. Neurobiol. 2:449–456.

Russell, I. J., A. R. Cody and G. P. Richardson. (1986a). The responses of inner and outer hair cells in the basal turn of the guinea-pig cochlea and

in the mouse cochlea grown in vitro. Hear. Res. 22:199–216.

Russell, I. J., G.P. Richardson and A. R. Cody. (1986b). Mechanosensitivity of mammalian auditory hair cells *in vitro*. Nature. 321:517–519.

Ryugo, D. K. (1992). The auditory nerve: peripheral innervation, cell body morphology, and central projections, in: R.R. Fay (Ed.), The Mammalian Auditory Pathway: Neuroanatomy, Springer, New York, pp. 23–65.

Ryugo, D. K. L.W., Dodds, T.E. Benson, N. Y. S. (1991). Kiang, Unmyelinated axons of the auditory nerve in cats, J.Comp. Neurol. 308: 209–223.

Rzadzinska, A. K., Schneider, M. E., Davies, C., Riordan G. P and Kachar, B. (2004). "An actin molecular treadmill and myosins maintain stereocilia functional architecture and self- renewal". J Cell Biol.164 (6): 887-97.

Sachs, M. B., woolf, N. K and Sinnott, J. M. (1980). Response properties of neurons in the avian auditory system: comparisons with mammalian homologues and consideration of the neural encoding of complex stimuli. In: Comparative Studies of Hearing in Vertebrates, edited by A. N. Popper and R. R. Fay. New York: Springer-Verlag, p. 323–353.

Sachs, M. B., Young, E. D and Lewis, R. H. (1974). Discharge patterns of

single fibers in the pigeon auditory nerve. Brain Res. 70: 431–447.

Salt, A. N., N. Inamura., R. Thalmann and A. Vora. (1989). Calcium gradients in inner ear endolymph. Am. J. Otol. 10: 371–375.

Salvi, R. J., Chen, L., Trautwein, P; Powers, N and Shero, M. (1998). Hair cell regeneration and recovery of function in the avian auditory system. Scand. Audiol. Suppl. 48: 7–14,

Salvi, R. J., Saunders, S. S., Hashino, E and Chen, l. (1994). Discharge patterns of chicken cochlear ganglion neurons following kanamycin-induced hair cell loss and regeneration. J. Comp. Physiol. [A] 174: 351–369.

Santos-Sacchi, J. (1991). Reversible inhibition of voltage-dependent outer hair cell motility and capacitance. J. Neurophysiol. 11:3096–3110.

Salvi, R. J., Saunders, S. S., Powers, N. L and Boettcher, F. A. (1992). Discharge patterns of cochlear ganglion neurons in the chicken. J. Comp. Physiol. [A] 170: 227–241.

Santos-Sacchi, J. (1993). Voltage-dependent ionic conductances of type I spiral ganglion cells from the guinea pig inner ear. J. Neurosci. 13: 3599- 3611.

Sato, M., Leake, P. A and Hradek G. T. (1999). Postnatal development of the organ of Corti in cats: A light microscopic morphometric study.

Hear Res.127:1–13. (PubMed: 9925011).

Schermuly, L and Klinke, R. (1990a). Origin of infrasound sensitive neurones in the papilla basilaris of the pigeon: an HRP study. Hear. Res. 48: 69–77.

Schermuly, L and klinke, R. (1990b). Infrasound sensitive neurones in the pigeon cochlear ganglion. J. Comp. Physiol. [A] 166: 355–363.

Schnitzler, H. U and Suga N; Simmons, J.A. (1976). Peripheral auditory tuning for fine frequency analysis in the CF-FM bat, Rhinolophus ferrumequinum.J Comp Physiol. 106: 99-110.

Segal B. N, Outerbridge J. S (1982). Vestibular (semicircular canal) primary neurons in bullfrog: nonlinearity of individual and population response to rotation. J. Neurophysiol. 47:545-562.

Sernagor, E and Grzywacz, N. M. (1999). Spontaneous activity in developing turtle retinal ganglion cells: pharmacological studies. J. Neurosci. 19: 3874–3887.

Shatz, C. J. (1990). Impulse activity and the patterning of connections during CNS development. Neuron. 5: 745–756.

shatz, C. J. (1996). Emergence of order in visual system development. Proc. Natl. Acad. Sci. USA. 93: 602–608.

Sheppard, D. N., Valverde, M. A., represa, J and Giraldez, F. (1992). Transient outward currents in cochlear ganglion neurons of the chick embryo. Neuroscience. 51: 631–639.

Smolders, J. W., Gummer, A. W. & Klinke, R. (1986). Travelling wave motion along the pigeon basilar membrane. ORL. J. Otorhinolaryngol. Relat. Spec. 48: 93–97.

Smolders, J.W.T., Ding-pfennigdorff, D and Klinke, R. (1995). A functional map of the pigeon basilar papilla: correlation of the properties of single auditory nerve fibres and their peripheral origin. Hear. Res. 92: 151–169.

Snastasio, T. J., Correia, M. J and Perachio, A. A. (1985). Spontaneous and driven responses of semicircular canal primary afferents in the unanesthetized pigeon. J. Neurophysiol. 54: 335-347.

Suga, N and Jen, PH-S; (1977). Further on the peripheral auditory system of CF-FM bats specialized for fine frequency analysis of doppler-shifted echoes.J Exp Biol 69: 207-232.

Suga, N *et al.* (1975). Peripheral specializations in fine frequency analysis of Doppler-shifted echoes in the auditory system of the CF-FM bat, Pteronotus parnellii J Exp Biol 63:161-192.

Sul, B and Iwasa, K. H. (2009). The effectiveness of hair bundle motility as

the cochlear amplifier. Biophys. J. 97: 2653–2663.

Temchin, A. (1988). Unusual discharge pattern of single fibres in the pigeon's auditorynerve. J. Comp. Physiol. [A] 163: 99–115.

Ten Kate, J. H., van Barneveld, H. H and Kuiper, J. W. (1970). The dimensions and sensitivities of semicircular canals. J Exp Biol 53: 501–514.

Valli, P., Botta, L., Zucca, G and Casella, C. (1986). Functional organization of the peripheral efferent system in the frog. Brain Res 362: 92–97.

Valverde, M. A., Sheppard, D. N., Represa, J and Giraldez, F. (1992). Development of Na1-and K1-currents in the cochlear ganglion of the chick embryo. Neuroscience 51: 621–630.

Van der Heijden, M. (2014). Frequency selectivity without resonance in a fluid wave guide. Proc. Natl Acad. Sci. USA 111, 14548–14552.

Von Bekesy, G. (1960). Experiments in hearing. New York: McGraw-Hill.

Walsh, B. T., Miller, J. B., Gacek, R. R and Kiang, N.Y.S. (1972). Spontaneous activity in the eighth cranial nerve of the cat. Int. J. Neurosci. 3: 221–236.

Walsh, E. J and Mcgee, J. A. (1987). Postnatal development of auditory

nerve and cochlear nucleus neuronal responses in kittens. Hear. Res. 28: 97–116.

Walsh, E. J and Mcgee, J. A. (1988). Rhythmic discharge properties of caudal cochlear nucleus neurons during postnatal development in cats. Hear. Res. 36: 233–248.

Wang, Y., Steele, C. R and Puria, S. (2016). Cochlear outer hair cell power generation and viscous fluid loss. Sci. Rep. 6, 19475.

Webster, D. B. (1960). The morphology and functional significance of the ear of the kangaroo rat, Dipodomys. Ph.D. Thesis, Univ. Microfilms, Inc., Ann Arbor. 135 pp; 37 figs.

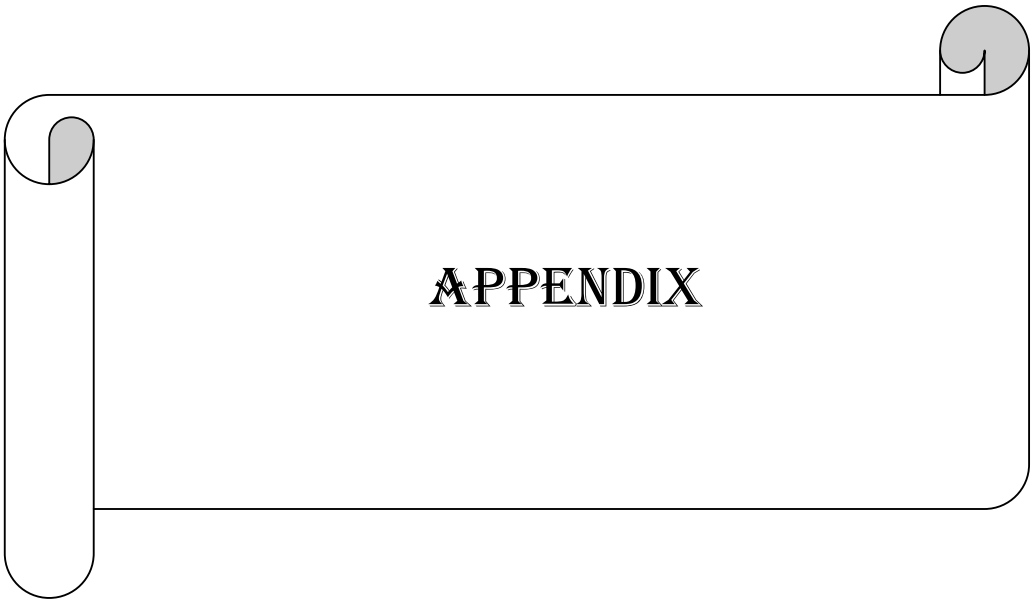
Yamaguchi, K. and Ohmori, H. (1990). Voltage-gated and chemically gated ionic channels in the cultured cochlear ganglion neuron of the chick. J. Physiol. (Lond.) 420: 185–206.

Yamaguchi, K. and Ohmori, H. (1993). Suppression of the slow K1 current by cholinergic agonists in cultured chick cochlear ganglion neurons. J. Physiol. (Lond.) 464: 213–228.

Zheng, J., W. Shen, D. Z. Z. He., K. B. Long., L. D. Madison and P. Dallos. (2000). Prestin is the motor protein of cochlear outer hair cells. Nature. 405: 149–155.

Zweig, G. (1976). Basilar membrane motion. Cold Spring Harbor Symp. Quantit. Biol. 40: 619–633.

Zweig, G. (1991). Finding the impedance of the organ of corti. J. Acoust. B. Soc. Am. 89: 1229–1254.


APPENDIX

Publications

- **Mangilal, Maida, G.D Sharma, Sandeep Charpota and Sujeeta Kanare (2016).** Comparative Study of the Effect of Different Incubation Temperature on the Spontaneous Activity of Auditory Neurons in Chicken Embryo and Posthatch Chicken, International Journal of Innovative Research and Advanced Studies 3:325-328 ISSN: 2394-4404.

- **Mangilal, M, Sandeep Charpota and G. D Sharma (2017).** Evaluation of the Physiological Specialization of the Hearing Organs of Posthatch Chicken Rearing at Different Environmental Circumstances, International Journal for Innovative Research in Multidisciplinary Field,3:207-211, ISSN-2455-0620.

Poster Presentation

- Evaluation of the Physiological Specialization of the Hearing Organs of Posthatch Chicken Rearing at Different Environmental Circumstances Presented in International Conference, 16 to 18 February 2017 at School of Life Sciences, DAVV, Indore, India.

Conferences Attained

- International Conference on Scientific Impacts of Music and Environmental on Sustainable Development; 07th to 09 January-2018; DAVV, Auditorium, Khandwa Road, Indore

- International Conference on Ecosystem Responses to Global Environmental

Changes and their Impacts; 16 to 18 February 2017 at School of Life Sciences, DAVV, Indore, India and Adarsh Institute of Management & Science, Dhamnod, (M.P.), India In Collaboration with Zoological Society of India, Gaya, India.

- International conference on Global Perspective on Multidisciplinary Approaches in Pharmaceutical Sciences; 18 to 19 November 2017 at IPS Academy College of Pharmacy, Indore.