BEERENS / STIMULUS CODING IN THE UTRICULAR NERVE OF THE CAT

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A QUANTITATIVE STUDY OF THE RESPONSE
TO STATIC STIMULATION OF THE UTRICLE AS RECORDED
FROM PRIMARY AFFERENT FIBRES

A. J. J. BEERENS

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ACADEMISCH PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE GENEESKUNDE AAN DE UNIVERSITEIT VAN AMSTERDAM, OP GEZAG VAN DE RECTOR MAGNIFICUS MR. A.D. BELINFANTE, HOOGLERAAR IN DE FACULTEIT DER RECHTSGELEERDHEID, IN HET OPENBAAR TE VERDEDIGEN IN DE AULA VAN DE UNIVERSITEIT (TIJDELIJK IN DE LUTHERSE KERK, INGANG SINGEL 411, HOEK SPUI) OP DONDERDAG 19 JUNI 1969 DES NAMIDDAGS TE 4 UUR PRECIES

door

ADRIAAN JAN JACOBUS BEERENS

geboren te Amsterdam

1969

DRUKKERIJ CLOECK EN MOEDIGH N.V.
AMSTERDAM

PROMOTOR : PROF. DR. P.E. VOORHOEVE

COPROMOTOR : PROF. DR. L.B.W. JONGKEES

Dit proefschrift werd bewerkt in het laboratorium voor Neurofysiologie van de Universiteit van Amsterdam; en in het fysiologisch en fysisch laboratorium van de Universiteitskliniek voor Keel-, Neus- en Oorheelkunde, Wilhelmina Gasthuis, Amsterdam.

Aan de nagedachtenis van mijn moeder
Aan mijn vader
Aan Henny, Aad Jan en Robert

VOORWOORD

Hooggeleerde Voorhoeve, hooggeachte promotor, het is mij een voorrecht geweest gedurende bijna vier jaren bij u te mogen werken. De steun en medewerking welke u steeds aan het experimentele werk gegeven hebt, maakten de realisatie van dit proefschrift mogelijk. Uw waardevolle adviezen en kritiek bepaalden de juiste richting van het onderzoek.

Hooggeleerde Jongkees, hooggeachte copromotor, het is mij een grote eer bij u mijn opleiding tot keel-, neus- en oorarts te mogen ontvangen. Uw grote experimentele en literaire ervaring vormen de basis voor dit proefschrift. Door uw voortdurende belangstelling, daadwerkelijke hulp en kritische opmerkingen werd ik steeds in mijn werk gestimuleerd.

Zeergeleerde De Boer, ik ben u zeer erkentelijk voor uw vele adviezen en voor de kritische beoordeling van de verkregen gegevens.

Hooggeleerde Duyff, u dank ik voor uw nuttige kritische opmerkingen.

Zeergeleerde Hammelburg, onder uw bekwame leiding heb ik mijn eerste klinische ervaring in de keel-, neus- en oorheelkunde opgedaan. Uw veelzijdige adviezen zijn voor mij van fundamentele betekenis geweest.

Zeer geachte Van der Laarse, voor uw vele constructieve bijdragen en de grote toewijding waarmede u de illustraties verzorgde ben ik u zeer dankbaar.

Zeergeleerde James, uw belangstelling en medewerking waren voor mij van grote waarde.

Geleerde Zwaagstra, de goede samenwerking met u heb ik bijzonder op prijs gesteld.

Dr. Yokota, your help and advise have been of great importance.

Zeer geachte Bol en Schreurs, u dank ik voor uw belangrijke technische bijdragen.

Zeer geachte Varenhorst en Alkema, voor uw assistentie bij het vestibulaire onderzoek en de behandeling van de proefdieren dank ik u zeer.

Zeer geachte Anthonijsz, Faber en Groeneveld, u dank ik voor de zorg besteed aan de benodigde electronische apparatuur.

Zeer geachte Bakker en De Ruyter, u dank ik voor de illustratieve bijdragen aan dit proefschrift.

Voorts betuig ik mijn dank aan allen die bij de bewerking van dit proefschrift op enigerlei wijze behulpzaam zijn geweest, met name Mevrouw Jonge Poerink - Adams, die de Engelse tekst corrigeerde.

De prettige wijze waarop staf en medeassistenten mij altijd hebben bijgestaan, vooral toen het tijdsprobleem nijpend werd, is een uiting van de goede sfeer waarin ik met u mag samenwerken.

Lieve Henny, voor al hetgeen jij voor mij gedaan hebt, kan ik je niet dankbaar genoeg zijn.

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CHAPTER I

INTRODUCTION

1. PRELIMINARY NOTES

Labyrinthine equilibrium is based on two sensory mechanisms: perception of linear acceleration e.g. gravitational force by the otolith organs and perception of angular acceleration by the semicircular canals.

This study concerns peripheral-stimulus coding in the otolith organs of mammals as recorded from primary afferents in the utricular nerve.

Breuer (1874) and Mach (1875) were the first to describe the above mentioned differentiation in labyrinthine function. Thereafter, however, a period started (e.g. Magnus and De Kleijn 1921), during which the labyrinthologists divided labyrinthine stimuli in "static" and "dynamic". This even led to the hypothesis that one and the same organ could perceive both angular and linear accelerations.

Jongkees (1944) and Jongkees and Groen (1946) could confirm the Mach-Breuer hypothesis in their description of the physiological stimulus and the corresponding responses of the labyrinth. They stressed that there is no real qualitative difference between the action of linear acceleration whether presented as gravity, linear accelerated movements or centrifugal force. The result being a reaction of inertia by which the position of the otolith in relation to its macula is changed.

The time of indication (Jongkees and Groen 1946) for angular accelerations is approximately 40 sec (v. Egmond et al. 1949) and for linear accelerations some 100 msec (v. Békésy 1935). This difference of a factor 400 again stresses the necessity of postulating two different perceptive organs.

Linear accelerations provoke a sensation of position, given by the perception of the direction of the resultant force in its representation of the vertical (Jongkees and Groen 1946; Graybiel 1956; Brandt and Fluur 1966).

Furthermore they are involved in reflexes of the eyes (compensatory movements consisting of counter rotation and up-and-down movements), neck, trunk and extremities, accompanied by responses of the autonomic nervous system. There are indications of otolithic influence on semicircular canal function (Owada et al. 1960; Jongkees and Philipszoon 1962; Janeke 1968).

On the functioning of the semicircular canals, a generally accepted conception seems to exist. As far as the otolith organs are concerned, however, there still are some controversies.

Breuer (1891) considered the shearing force of the otolith on the macula to be most probably the appropriate stimulus for the otolith organs. Besides this conception, three other theories have been presented:

Magnus (1924), considering his studies on postural reflexes, described the pulling force of the otolith, while Quix (1924) on clinical and theoretical evidence regarded otolithic pressure as the active stimulus. Finally Werner (1928) and Mygind (1948) indicated changes in hydrostatic pressure on the macula as the stimulating factor.

Only recently von Békésy (1966) explained clearly that the shearing force alongside the macula determines the effective stimulus, although a pressure gradient will be necessary for the production of reaction.

The approach to the otolith organs is difficult on account of their anatomical location. With the introduction of electrophysiological techniques, however, a better analysis of otolith function became possible. Until then, vestibular investigation consisted mainly of observing changes in labyrinthine reflexes after partial or total labyrinthectomy.

2. ANATOMICAL CONSIDERATIONS

The vertebrate labyrinth contains three otolith organs, lying in utriculus, sacculus and lagena. In mammals the lagena is lacking. The utriculus is part of the superior part of the membranous labyrinth and the sacculus of the inferior part (de Burlet 1934). The macula contains the sensory epithelium and in the utriculus it forms a shallow bowl occupying the floor of the recessus utriculi (Flock 1964). In the normal orientation of the head the utricular macula is approximately horizontal, the saccular macula about vertical (Quix 1923). In a detailed reconstruction of the human macular planes by Corvera et al. (1958) the utricular macula measures 4.2 mm^2 and the saccular one 2.2 mm^2. The length and breadth axes are respectively 2.8/2.1 and 2.2/1.1 mm. The anterior portion of the utricular macula consists of a narrow zone demarcated from the large chief portion by a sharp upward inclination (fig. 1). The saccular macula shows a marked dorsal extension, without any sharp inclination.

The utricular "plane" exhibits a backward slope of approximately 30° to Reid's base line of the skull, with a lateral inclination of some 10°.

The macula composed of the sensory hair cells and supporting cells is covered by a gelatinous mass containing mucopolysaccharides. In this mass the otoliths are embedded. In mammals the so called otoconia are composed of $CaCO_3$ crystals. These constitute a greater specific weight (2.7) as compared to the surrounding structures and the endolymph (1.4).

The influence of gravity, or any other form of linear acceleration, is transmitted to the sensory cells by way of deviation of the protruding hairs. In mammals the hair cells can be devided into two types (Wersäll et al. 1954). The first type is bottle shaped, innervated by a nerve calix enclosing the cell body. The second type is cylindrical, innervated by a number of small nerve endings in contact with the cell base, and resembles the primitive hair cells found in fish. Each hair cell carries approximately 60 stereocilia, in a regular pattern and one kinocilium situated at the border of the hair bundle. Within each bundle the length of the stereocilia increases towards the kinocilium ($0.5-5\mu$). The kinocilium is the longest of all. The sensory cells are morphologically polarized by the arrangement of the kinocilia (fig. 2).

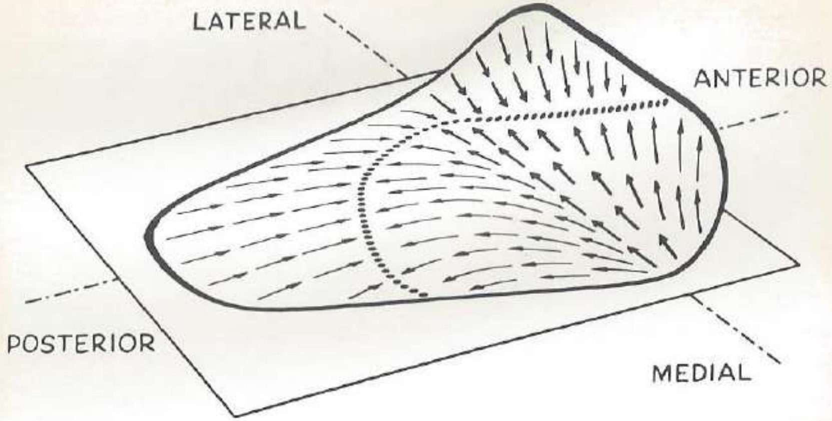


Fig. 1.

Pattern of morphological polarization of the sensory cells in the macula utriculi (cat). The arrows indicate the orientation of the kinocilia. From Spoendlin (1966).

This polarization pattern (Lowenstein et al. 1964; Flock 1964; Spoendlin 1966) appears to be of great functional significance, in so far that the position of the kinocilium within each hair bundle is related to the direction of excitatory and inhibitory displacement in the course of mechanical stimulation of the sensory cell. Deviation of the stereocilia towards the kinocilium is regarded as being excitatory.

In the mammalian utricular macula the "direction of polarization spreads fanlike from the medial and anterior part of the macula up to a curved boundary line beyond which the polarization of the sensory hairs is reversed. The kinocilia on either side of this dividing line are facing each other", thus described by Spoendlin (1966), fig. 1.

A similar pattern is described by Flock (1964) in the burbot (Lota vulgaris), in which each utricular macula covers a semicircle with its centre in the medial part of the sensory epithelium. The semicircle of directional sensitivity is divided into two halves by a line of cells, laterally oriented in a direction perpendicular to the midline of the animal. In a much smaller peripheral zone the polarization pattern is reversed. Responses from the central parts in the two maculae will oppose each other, but will coöperate with the responses from the peripheral

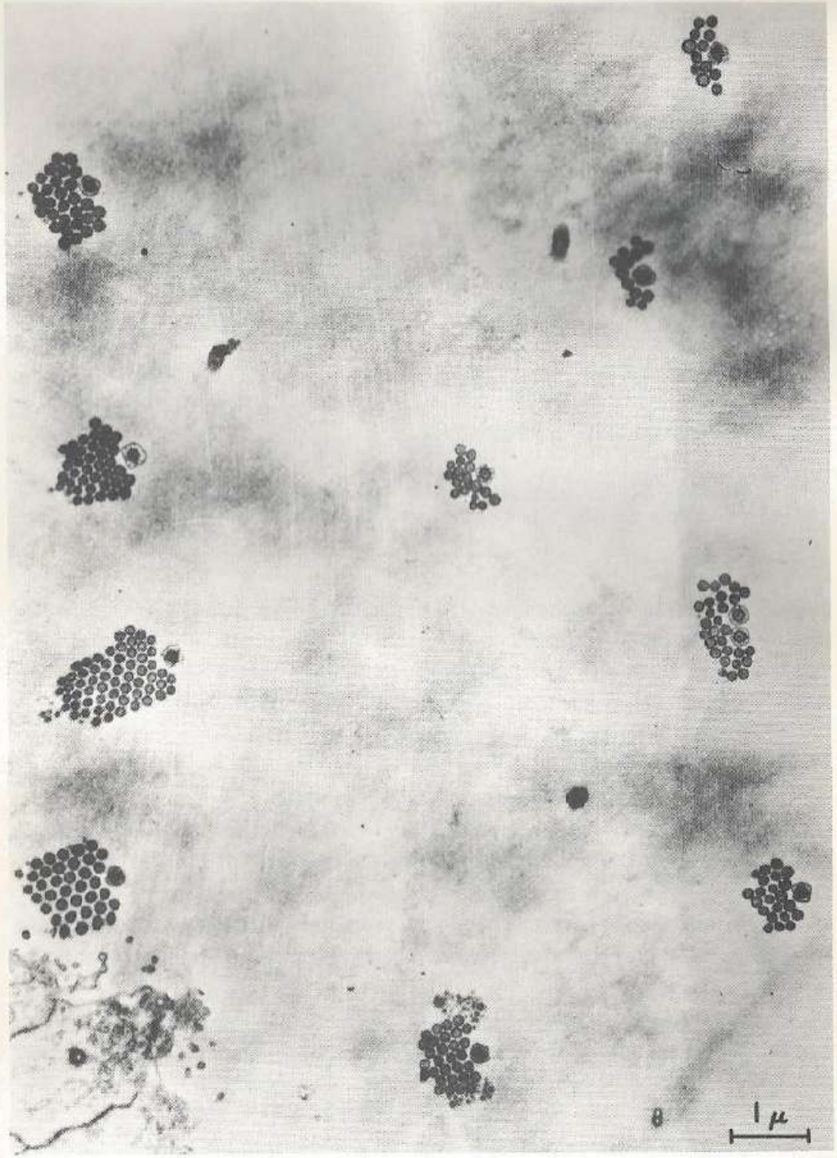


Fig. 2.

The position of the kinocilium in the hair bundle. A cross section through the sensory hair bundles reveals the consistent direction of orientation of neighbouring receptor cells. x 10,000. From Flock (1964).

zone of the opposite side. The utricular nerve fibres spread fanlike before entering the macula. Each fibre innervates many sensory cells (divergence being approximately 1 : 10).

The nerve endings are of two different types: granulated and non-granulated. The former contains an abundance of vesicles. Inside the hair cell at the area of contact with a non-granulated nerve ending, a synaptic bar surrounded by vesicles is found. This would imply an afferent function of these endings (Smith and Sjöstrand 1961).

The granulated nerve endings were suggested and later demonstrated to be of efferent nature (Engström and Wersäll 1958; Kimura and Wersäll 1962). These endings contact the non-granulated nerve calix, while the cylindrical cells at their base show connections to both types of nerve endings.

3. SPATIAL ORGANIZATION OF THE VESTIBULAR NERVE

In this series of experiments the response of primary afferent fibres in the utricular nerve to tilting in the transversal plane is studied. In order to explain the position of the microelectrode, a survey is presented of the related neuroanatomy, based principally on experimental studies in the cat, monkey, and guinea pig by Rasmussen (1946, 1953, 1960); Rasmussen and Gacek (1958); Walberg et al. (1958); Carpenter (1960, 1967); Gacek (1960, 1966); Brodal et al. (1962); Stein and Carpenter (1967) and Gernandt (1967).

The primary vestibular neurons are found in the vestibular ganglion (Scarpa) which shows a division into a superior part, with fibres both towards the utricular macula and the cristae of the lateral and anterior canals, and an inferior part, which innervates the saccular macula and the crista of the posterior canal. The vestibular ganglia are situated at the level of the internal auditory meatus. The central fibres pass to the vestibular nuclei and to some parts of the cerebellum. The peripheral and central extensions respectively form the superior and inferior divisions of the vestibular nerve. The glia-Schwann sheath junction is formed at the entrance to the internal auditory meatus. The vestibular nerve root enters the brain stem at the cerebello-pontine angle, where the root passes between fibres of the inferior cerebellar peduncle and the spinal trigeminal tract. Upon entering the brain stem the fibres bifurcate into ascending and descending branches, and are distributed to certain parts of the vestibular nuclei, in such a way that some parts of each nucleus are devoid of primary afferent fibres (Walberg et al. 1958).

The largest area devoid of primary afferent fibres is the dorsal region of the lateral vestibular nucleus. Only recently Stein and Carpenter (1967) presented data on the central projections of cells in separate divisions in the vestibular ganglia (Monkey).

Discrete lesions of cells in the superior vestibular ganglion innervating the cristae of the anterior and lateral canals revealed degeneration in the rostral and lateral parts of the vestibular nerve. These cells mainly project towards the superior vestibular nucleus and the rostral pole of the medial vestibular nucleus.

Cells of the superior vestibular ganglion innervating the macula of the utricle give rise to centrally directed fibres located in the nerve immediately caudal to those of the anterior and lateral canals, and projecting mainly into the dorsomedial part of the inferior vestibular nucleus. Collaterals pass medially into dorsolateral parts of the medial vestibular nucleus, and rostrally into ventral parts of the lateral vestibular nucleus.

Fibres to the crista of the posterior canal are situated in the caudal third of the vestibular nerve, while those to the saccular macula occupy the most caudal and medial parts of the vestibular root.

Fig. 5 (page 37) illustrates these localisations as projected upon the operating field at the level of the internal auditory meatus.

A relatively small number of primary vestibular fibres enter the cerebellum via the juxtarestiform body, or project towards the reticular formation (Gacek, 1960; Carpenter, 1960; Brodal et al. 1962).

It is beyond the scope of this paper to give a further detailed description of central projections. An important feature, however, is that all central projections of the primary afferent fibres proved to be ipsilateral (Walberg et al., 1958). Secondary fibres from the inferior and medial vestibular nuclei to the cerebellum are distributed bilaterally with ipsilateral preponderance (Brodal and Torvik, 1957). Descending secondary fibres from the lateral and medial nucleus are uncrossed, while the ascending fibres (in the medial longitudinal fascicle) are predominantly crossed.

The ascending fibres from the superior nucleus are uncrossed. According to Gacek and Rasmussen (1961) fibres in the vestibular nerve are distributed in strands with irregular fibrous septa. The number of myelinated fibres in the cat is approximately 12.000, with an average diameter of $2-5\mu$. This number comprises the whole nerve root, including fibres of the "pars intermedia" usually regarded as a division of the facial nerve which leaves the vestibular nerve at the level of the superior vestibular ganglion; also included are the efferent fibres to the vestibular epithelia and the cochlea.

The efferent fibres to the vestibular receptors (Rasmussen and Gacek, 1958) join the crossed and uncrossed efferent olivo-cochlear bundle (Rasmussen, 1946, 1960) in the vestibular root and the vestibular nerve. This compact efferent bundle separates the afferent superior and inferior part of the vestibular nerve.

At the level of the saccular part of the inferior vestibular ganglion the olivo-cochlear bundle constitutes the vestibulo-cochlear anastomosis (Oort, 1919) which enters the cochlea.

In the same region part of the efferent vestibular fibres take their course towards the superior vestibular ganglion. The few fascicles diverge as scattered fibres to the end-organs at the level of the utricular nerve, including Voit's anastomosis to the saccular macula. Efferent fibres in the inferior vestibular ganglion also reach their peripheral aims as diffusely scattered fibres.

Gacek (1966) estimates the total number of vestibular efferent fibres at 400, which means a ratio of approximately one efferent to thirty afferent fibres. The former are of smaller calibre. The total number of efferent cochlear fibres is about 600 (Rasmussen, 1960).

In the utricular nerve (frog) Robbins et al. (1967) roughly estimated the number of efferent fibres at about 2–12.

The origin of the vestibular efferents has only partly been determined. Rasmussen and Gacek (1958) and Gacek (1960) found indication of efferent involvement in the ipsilateral lateral vestibular nucleus. Carpenter (1960) considered the possibility of efferent origin in the medial and superior nuclei predominantly uncrossed but bilateral.

Rossi and Cortesina (1963) indicated several regions of vestibular efferent origin in guinea pig, respectively the ipsilateral "interposed" and lateral vestibular nuclei and the reticular formation. Finally, in contrast to the conception of Petroff (1955), Gacek (1966) indicated that the vestibular efferent complex is uncrossed.

4. BRIEF REVIEW OF ELECTROPHYSIOLOGICAL INVESTIGATIONS

a. Data on lower vertebrates and crustaceans.

The great majority of recordings from primary afferents of labyrinthine sense endings published so far, is restricted to lower vertebrates.

Ashcroft and Hallpike (1934) were the first to record discharges in the saccular nerve of the frog. There was only response to low frequency vibration up to 500 c/sec.

Mowrer (1935) described a distinctive change of impulse discharges in the vestibular nerve of the terrapin on angular acceleration. Ross (1936) recorded responses from filaments of the anterior and posterior main branch of the eighth nerve in the frog. There was no identification possible as to which type of receptor was held. Each branch contained fibres that responded to gravitational stimuli, vibration or angular acceleration respectively.

The gravity receptors were classified: type I, reacting to tilt "out-of-level" and linear acceleration in the horizontal plane, while type II responded only when tilted "into-level". This second type was found only in the posterior branch.

Lowenstein and Sand (1940) in single-fibre recordings from the lateral ampulla in the elasmobranch labyrinth observed a spontaneous (resting) discharge which increased on ipsilateral and decreased on contralateral rotation. Zotterman (1943) recorded vibration responses from the saccular nerve in the pike. Ledoux (1949) recorded in the frog from the ampullary nerves, the utricular, and the saccular nerve. Each branch showed a resting discharge. The utricule was stimulated by tilting of the head and linear acceleration. Lowenstein and Roberts (1950, 1951) from the labyrinth of the thornback ray, recorded discharges from single functional units in separate nerve branches to the otolith organs. A resting discharge of approximately 10 impulses/sec was observed. Stimulation was achieved mainly by tilting about the longitudinal or the transverse axes, both placed horizontally.

Two main types of gravity receptors in the utricular and saccular branches were distinguished, with maximum impulse discharges (ranging from 45/sec up to 65/sec) in "side-up/nose-up" position or in "side-up/nose-down" position respectively.

Receptors in the lagena showed a maximum (up till 100 imp/sec) in or near normal position. These are described as "into-level" receptors. When the tilt was stopped in any given position, the receptor would continue firing at a rate corresponding to the extent of deviation from the normal position. Although the discharge frequency reverted somewhat to the initial level, it would remain characteristically different from that in the normal position.

Apart from "static" position receptors, the maculae in the thornback ray contained receptors responding to any change in position in one and the same manner, after which the discharge frequency returned to the basic level. These were called "out-of-position" receptors, suited to signal positional change as such.

In the anterior two thirds of the saccular macula vibration responses dominated over gravity responses, in the posterior part the reverse was found. In the utricular macula the pars lacinia specifically reacted on vibration.

Spontaneous discharge activity is a characteristic of labyrinthine sense endings (Lowenstein and Sand 1940; Lowenstein and Roberts 1950, 1951; Ledoux 1949). The impulse discharge is extremely regular with deviations from the average frequency as small as 4% (Groen et al. 1952). Lowenstein (1956) concludes that, by virtue of these spontaneous active units, a peripheral threshold hardly exists. In the otolith bearing maculae the term spontaneous could have a restricted significance on account of the continuous influence of gravity. However, in the elasmobranch labyrinth the pars lacinia of the utricular macula – devoid of otolith covering – also exhibits spontaneous activity.

In the cristae of the semicircular canals of elasmobranchs Groen et al. (1952) indicated recruitment of spontaneously silent units on ipsilateral angular acceleration. This considerably improves the total increase of impulse discharges. Lowenstein (1955) supported these findings in his experiments on the effect of galvanic stimulation in the crista of the lateral canal. Either ipsilateral angular acceleration or ascending polarizing current (from the central part of the nerve to the peripheral sensory epithelium) activated a significant number of spontaneously silent units.

In the otolith organs no clear indication of such recruitment has been presented. Only once a two-fibre preparation of the utricular nerve has been described with the activity of one fibre preponderant, in which the unit with the smaller amplitude was inactive over a considerable part of the full-circle tilt (Lowenstein and Roberts 1950).

In the lobster, Cohen (1955, 1958) could distinguish two types of position receptors, and in addition to these a receptor responding to angular acceleration and a vibration receptor. Removal of the otolith abolished all position responses but did not affect spontaneous activity or vibration activity, and in some instances reactions on angular accelerations were preserved.

By discrete manipulation of the sensory hairs a displacement towards the horizontal plane decreased activity, while displacement towards the vertical showed increase up to 55°; continued bending, however, provoked a decrease of impulse-discharge frequency.

b. Data on mammals.

During the last decade electrophysiological investigation on the vestibular nuclei has shown great improvement. Gradually the gap with detailed anatomical data is being bridged.

Only papers relevant to our subject will be mentioned here. However, on recording from the peripheral vestibular nerve in mammals only a few experiments were published.

Adrian (1943) was the first to describe discharges from vestibular nuclei in a mammal (cat). Gravity receptors were located in the rostral and caudal border of the striae acusticae. A tilt in the medial or transversal plane of the head was stimulating when directed out of the normal position, i.e. for lateral tilt on ipsilateral side down. A very slow adaptation was mentioned.

Gernandt (1949) described in the cat the first recording of single functional units in the vestibular nerve connected to the lateral semicircular canal. A spontaneous activity (10–20 imp/sec) increased on ipsilateral horizontal angular acceleration and decreased on contralateral rotation. These receptors were called Type I. Two other types of receptor were encountered. They showed a spontaneous activity which increased or decreased respectively in response to horizontal angular acceleration in whatever direction. As to these last units, it was assumed that they possibly are of second order origin.

Tasaki (1954), while recording single-fibre responses from the cochlear nerve in the guinea pig, occasionally observed spontaneous discharges which could not be altered by sound. In this instance "it seemed likely that the micro-electrode was pushed in the vestibular branch of the eighth nerve."

Wing (1963), on recording action potentials and their changes in response to tilt from units in the vestibular ganglion "presumably supplying the otolith organs," concluded: "The action potentials in all units were infrequent and irregular after position was maintained for some time. The majority of the units showed no response to any change of position. In most cases, of those which exhibited a response, the responses were delayed an average of 40 sec. The evidence presented supports the view that the utricle and saccule may be vestigial organs, or at least do not function meaningfully in the orientation of the cat with respect to the gravitational field."

It must be stressed, however, that his technique for selection of otolith supplying units was not quite appropriate.

Rupert et al. (1962) recorded in the cat from medullar regions and from the vestibular nerve in the internal acoustic meatus. Two types of response on tilting of the head were found. Type I reacted to static position by characteristic frequency changes, Type II displayed a very constant impulse discharge which only showed a short change at any movement independent of the position. Although these responses were recorded at one electrode track in the medulla as well as in the vestibular nerve, the authors assumed these to be derived from primary afferent neurons. No attempt was made to relate activity in a given unit to stimulation of a particular vestibular end-organ.

In the cat, Cramer (1962) studied discharges from the descending vestibular nucleus on sustained tilt. A rather vigorous initial response on nose-down or side-down tilt diminished considerably over 15–30 seconds to a steady-state signal which only feebly represented the tilted position.

In the cat, Andersson and Gernandt (1954) investigated the cortical projection of the vestibular nerve. The peripheral branches to the utricle and cristae of the lateral and anterior vertical canals were cut close to the end-organs. The central parts were electrically stimulated by single shocks.

The utricular macula projects to the anterior portion of the ectosylvian gyrus and the posterior part of the anterior suprasylvian gyrus, mainly at the contralateral side.

Electrophysiological research on the vestibular nuclei has so far been greatly dedicated to the semicircular-canal system. Because more recent detailed descriptions of vestibular nuclear activity on position responses are closely related to these findings, several papers are mentioned somewhat more in detail.

Single-unit activity in vestibular nuclei was recorded on physiological stimulation of the lateral semicircular canals by Eckel (1954) and Duensing and Schaefer (1958). In the rabbit, these last authors described four types of receptors. Three of these had been mentioned by Gernandt (1949), the fourth type of unit showed a decrease on ipsilateral and increase on contralateral angular acceleration.

Combined rotatory and electrical stimulation of the lateral canal system, comprising introduction into the vestibule of an electrode, which was gently placed on the lateral ampullary nerve, was described by Precht and Shimazu (1965) and Shimazu and Precht (1965, 1966) in the cat's vestibular nuclei. Field potentials were recorded in the superior and medial vestibular nuclei, related to activity in the primary fibres. The field potential complex consisted of an initial positive-negative deflection (P-wave) mainly composed of spike potentials of the primary afferent fibres, followed by a large sharp negative wave (N_1) which consisted of monosynaptically evoked spikes of the vestibular neurons, and a delayed small negative wave (N_2) attributed to polysynaptically evoked spikes. Vestibular unit spikes, evoked by single shocks to the vestibular nerve, were superimposed on the field potentials, but never elicited before N_1.

The experiments on angular acceleration showed the same shape of responses in the vestibular nuclei. The response of single neurons to stimulation of primary vestibular afferents showed the four different unit-type responses. Units type I (Gernandt 1949) with increased impulse discharge on ipsilateral acceleration and decrease on contralateral acceleration were studied. These units were classified as kinetic and tonic neurons characterised by their frequency responses.

The kinetic neurons showed a high threshold, a lack of spontaneous discharge, and a rapid time course of frequency changes with a steep gradient of acceleration-frequency relations. These neurons were mainly activated monosynaptically by the primary afferents, and the evoked spikes were superimposed on the N_1 field potential. Tonic neurons showed evoked spikes during the N_2 wave, exhibited spontaneous activity, and in all respects opposite characteristics to those of the kinetic neurons. Their discharge frequency during application of long-lasting constant angular acceleration (30 sec) did not show any sign of adaptation.

While recording from the contralateral labyrinth on stimulation of the ipsilateral vestibular nerve, the authors observed decrease of activity of type-I units and excitation of half of type-II units (Duensing and Schaefer 1958). It was suggested that some of these type-II units were intercalated inhibitory neurons, activated by contralateral lateral-canal stimulation through commissural fibres that would inhibit homolateral type-I neurons.

In Deiters' nucleus of the cat Wilson et al. (1967), by antidromic stimulation of the vestibulospinal tract, located cells projecting to the spinal cord. Afferent stimulation of the nerves from hind- or foreleg revealed facilitation of Deiters' cells.

Fibres projecting to cervical levels were located in the rostral half of the nucleus, while those to lower levels were mainly represented in the caudal dorsal part.

In the same experimental set-up electrical stimulation of the ipsilateral labyrinth was achieved by implanted electrodes. To place this electrode the bulla was opened and the anterior wall of the promontory removed, exposing the scala vestibuli. The electrode was inserted into the scala vestibuli "in the direction of the vestibule" and cemented in place. Many cells were fired monosynaptically by stimulation of the ipsilateral labyrinth. These were located in the ventral half of Deiters' nucleus.

Peterson (1967) recorded the effect of tilting on the activity of neurons in the vestibular nuclei in the cat. A special analysis of Deiters' nucleus revealed that the ventral part receives the majority of primary afferents. The discharge rate increased when the ipsilateral side was tilted down and decreased if this side was tilted up.

The dorsal part of Deiters' nucleus contains cells which receive polysynaptic effects from the labyrinth. As to the vestibular nuclei in general, the greatest sensitivity to tilt was located in the descending nucleus, although position receptors could also be found in the medial and superior nuclei.

An important finding was a considerable adaptation of responses to change in position. The discharge rate decreased exponentially with attainment of a steady-state value within 10–20 sec. This same phenomenon, but with a sudden tilt, was described by Jones (1966), while recording responses from vestibular nuclear units in the cat.

On sinusoidal vertical acceleration a maximum firing frequency (70 imp/sec) was reached at the point of greatest upward acceleration. The frequency of discharges followed a sinusoidal waveform throughout the cycle. A sudden tilt of the platform to ipsilateral side down showed an instant increase of discharges from the spontaneous level. However, the response progressively declined, but quite slowly, until in 25 sec the spontaneous discharge level was reached again.

Wilson et al. (1968) studied synaptic input to cells in the medial vestibular nucleus (cat). Electrical stimulation of the labyrinth was achieved by way of an implanted electrode in the scala vestibuli in the direction of the vestibule, as described before. It should be noted that in this way the receptors on which these neurons impinge could not be identified.

Monosynaptically activated cells were mainly situated in the rostral 60% of the medial nucleus. Very little spatial summation was needed to fire these cells. Polysynaptically driven cells, on the other hand, were scattered all over the medial nucleus.

In general the electrophysiological data corresponded with the anatomical distribution of primary afferents to the vestibular nuclei (Walberg et al. 1958; Brodal et al. 1962; Stein and Carpenter 1967). It should be stressed, however, that in the majority of experiments an essential condition is not fulfilled, i.e. the stimulation is not physiological.

The more recent experiments on the effect of tilting on vestibular nuclear discharges must be considered more reliable as a comparison with primary afferent coding in the utricular nerve as described in this study.

5. THE APPROPRIATE STIMULUS FOR THE OTOLITH ORGANS

The experiments of von Holst (1950 a,b) in which freely moving fish being placed in a centrifuge, were observed under increased-gravity action, supported Breuer's theory that the shearing force of the otolith on its macula is the appropriate stimulus.

The fish will assume a position of equilibrium which is determined by three factors: the flow of the water, the "centre of illumination" and the resultant force of gravity and centrifugal force.

By keeping the first two factors constant, the influence of change in the last one was determined. This resulted in a change of the angle of tilt in such a way that the resultant shearing force of the otolith was kept constant by the fish, while the pressure gradient increased enormously.

Hence von Holst concluded that only the shearing force could be the effective stimulus for the otolith organ, while the other theories were rejected. On basis of other trials he concluded to a linear proportionality between the effect of macula stimulation and the shearing force. In these experiments no sign of adaptation was observed. Schöne (1954, 1959, 1964) confirmed these findings amongst others in crustaceans and human subjects.

The experiments of Lowenstein and Roberts (1950) also indicated a shearing force as the effective stimulus, and the discharge frequency curves looked directly proportional to this force.

In fish, de Vries (1950) demonstrated the displacement of the otoliths in various positions by making X-rays. During tilting the big saccular otolith in this species showed a displacement of 100μ. Centrifugal forces up to 11 g (g = gravitational acceleration) were applied to determine the small displacements, only 5μ for utricular and lagenar otoliths.

Only movements parallel to the macula were observed. There was a time lag (5 msec) between an attained position and the moment the utricular otolith reached its final position. This was confirmed by Vilstrup and Vilstrup (1952) with the same techniques.

Jongkees (1950) in his experiments with a steel ball brought inside the inferior part of the vestibule, however, observed that increased pressure on the saccular macula provoked by magnetic attraction of the ball, resulted in movements of the rabbit which tended to decrease this pressure. It must be added that in these experiments shearing forces cannot be excluded.

The experiments of Schoen (1957) on recording from medullar and cerebellar levels in fish, also gave support to the shearing theory. A linear relation of the shearing force to the discharge frequencies was described. Although Sasaki (1960) and Sasaki et al. (1963) regarded pressure of the otolith on its macula as the effective stimulus, the vast majority of authors supported the shearing principle. Finally von Békésy (1966) explained that the shearing force alongside the macula determines the effective stimulus as a pressure gradient is necessary for the genesis of stimulation.

However, as to the direction of this effective shearing force, some controversial findings were published. Maxwell (1923) applied direct mechanical stimulation to the utricular otolith in the dogfish. Pressure at the lateral side, resulting in medial displacement of the otolith seemed to be stimulating. However, the method of stimulation must be regarded as somewhat rude.

After Steinhausen (1935) had determined the force exerted by the otolith in the pike, Ulrich (1935) could apply adequate direct stimulation to the utricular otolith with a human hair exerting a pressure of 1.7 mg. Only pressure which effected an outward sliding of the otolith was stimulating. In the thornback ray, Lowenstein and Roberts (1950) observed a maximal impulse discharge in ipsilateral side-up position. This indicates that a shearing force which provokes a medially directed deflection of the sensory hairs is the appropriate stimulus. The authors assumed their recordings to be originating from receptors in the lateral parts of the utricular macula.

In general all experiments on recordings from the vestibular nuclei in mammals confirmed the observations of Adrian (1943) that in the transversal plane tilt to the ipsilateral side down is stimulating. The observed changes in labyrinthine reflexes after unilateral labyrinthectomy could be explained on the basis of the shearing theory, e.g. in rabbits Bos et al. (1963) recorded larger eye movements on a parallel swing when the animals were lying on the normal side as compared to lying on the operated side, and the same phenomenon was observed in supine position. Earlier, Walsh (1960) had observed a comparable effect on sensation of position in human subjects. These findings imply that a deviation of the utricular otolith to the lateral side is the more stimulating position, whereas the saccular otolith hanging freely under its macula is subjected to greater shearing forces because of diminished friction. Colenbrander (1964) in his experiments with a "human centrifuge" (a term introduced by Graybiel - Pensacola) concluded at an outward directed shearing force of the otolith on its macula as the appropriate stimulus in the transversal plane. The signal generated by the utricular macula is directly proportional to to this force.

Correia et al. (1968), however, indicated a tangent expression for the subjective visual perception of orientation in gravitational- and supragravitational-force fields. This would imply a simultaneous influence of shearing and pressure or pulling forces on otolith stimulation.

From the approximately identical polarization pattern in the utricular macula in fish and mammals, and the functional significance, we learned that the position of the kinocilium in the hair bundle (see fig. 2 page 14) is of paramount importance (Lowenstein et al. 1964; Flock 1964; Spoendlin 1966). Deviation of the stereocilia towards the kinocilium is assumed to produce depolarization of the sensory cell which effects increased nervous activity, while deviation in the opposite direction results in a hyperpolarization and a decrease of nervous activity. Basis for this assumption are the experiments on the potential shift in the guinea-pig labyrinth (Trincker 1957, 1959 a,b, 1961). The discrepancy between the findings of Lowenstein and Roberts (1950) and most other experiments concerning directional sensitivity was explained by Flock (1964) on the basis of the polarization pattern.

The suggestion of the former authors that "side-up/nose-up" receptors were located in the antero-lateral part of the macula, and "side-up/nose-down" receptors in the postero-lateral part was confirmed by Flock. As the majority of the sensory cells in the macula, however, are polarized in lateral direction, the shearing force in that direction is generally bound to be stimulating.

6. ADAPTATION

Labyrinthine sense endings are usually described as non-adapting or slowly adapting. In the functioning of "static" position receptors this is of paramount importance.

In the otolith organs, for any position in space, the influence of the otolith on its macula is a constant one, as long as that position is maintained. In this instance, changes of impulse discharge frequency could be exclusively based on adaptation.

Lowenstein (1955), however, indicated the possibility of plastic deformation of viscous portions of the otolith membrane, resulting in a time-lag between an attained position and the arrest of viscous flow of the otolith membrane. The findings of de Vries (1950), Vilstrup and Vilstrup (1952) gave support to this supposition.

Another point of interest is the possibility of inhibition by the vestibular efferent fibres as described by Sala (1965 a,b).

Lowenstein and Roberts (1950) and Lowenstein (1956) described that a tilt, arrested in whatever position in space, furnished a discharge rate corresponding to the extent of the deviation from the normal position.

The increased or decreased discharge frequency might revert somewhat to the initial level during the first 30 sec remaining, however, characteristically above or below it for periods of some 20 minutes.

Von Holst (1950 a,b) did not observe adaptation of the otolith organs in his experiments on freely moving fish. It must be noted that central nervous influences are included here.

Schoen (1957) recorded from vestibular nuclear regions in fish. Lateral tilt showed a characteristic impulse discharge for every position, which remained constant for several seconds. A maximal discharge diminished at a steady-state some 30–60% of the initial value in 30 sec or more. There was no adaptation towards the spontaneous level and a discharge-frequency/tilt relation was maintained at a lower level.

Adrian (1943) found a very slow adaptation to lateral tilt in the vestibular nuclei of the cat. Duensing and Schaefer (1959), Cramer (1962), Jones (1966) and Peterson (1967), confirmed adaptation in the vestibular nuclei (rabbit and cat), varying in extent from a considerably diminished discharge frequency towards a complete attainment of spontaneous level within 10–30 sec. Colenbrander (1964) did not observe adaptation in ocular counterrolling during his centrifuge experiments with human subjects.

As to the otolith organs we may conclude that the macular receptors concerned with indication of position show no or only a very small adaptation. In the second order neurons in the vestibular nuclei most authors describe some sort of adaptation. On higher levels of the central nervous system the observations are rather controversial. Von Holst (1950 a,b) and Colenbrander (1964) did not observe adaptation.

Groen (1957) introduced the term "pattern" adaptation in connection with the movements of a ship in cases of sea sickness as this is mainly attributed to otolith function. Lindsley (1960) described "habituation", which involves more intricate activities in the central nervous system.

In the semicircular canals, presentation of a constant angular acceleration for attainment and maintenance of a maximal cupular deviation is a major problem in studying adaptation of these end-organs.

In human subjects Hallpike and Hood (1953) could apply a constant angular acceleration for longer periods (150–15 sec depending on the magnitude of the stimulus) in the revolving chair, and concluded from data on ocular movements that the end-organs of the semicircular canals show considerable adaptation. Ek et al. (1959) showed that on continuous angular acceleration after a period of 37–40 seconds, sensation of rotation and nystagmus gradually disappear completely after another 40 sec, while the cupula deviation was still increasing. Therefore the former experiments can give no reliable information on adaptation, and certainly not on peripheral behaviour in this respect.

Guedry and Collins (1968) for periods up to 30 sec of continuous acceleration, found no evident decline of nystagmus. A difference was observed in the secondary reactions between cat and man.

Lowenstein (1955) in his experiments on the effect of galvanic polarization found indications of adaptation in the end-organs of the lateral canal. Shimazu and Precht (1965) described no significant adaptation in the vestibular nuclei on constant horizontal angular acceleration for periods of 30 seconds. They admit, that if the cupula could be deflected very rapidly and maintained at that position for longer periods, considerable adaptation might be effected. But a long lasting constant angular acceleration is seldom part of normal general behavioural patterns.

CHAPTER II

PURPOSE OF THE EXPERIMENT

The theory concerning otolith function, founded by Breuer (1891) who suggested the shearing force exerted by the otolith on the macula as the appropriate stimulus for the otolith organ, is accepted in view of most publications during this last decade.

As to the direction of the stimulating shearing force only one principal point of controversy is left.

In the transversal plane in general outward displacement of the utricular otolith over its macula is considered to be the effective direction, but there are a few indications that support a mechanism of inward sliding. The morphological polarization pattern of the sensory cells as revealed by electron microscopy and the introduction of electrophysiological recording techniques contributed to a great extent to a more complete picture of otolithic function. The information available by recording from the utricular nerve must be regarded as representative for peripheral processes in its macula. For many years labyrinthectomy has been performed in lower as well as in higher vertebrates. A wide variety of publications is available on the functional elimination of separate vestibular receptors in lower vertebrates. In mammals the otolith organs are difficult to approach on account of their anatomical position in dense bone. Functional elimination of selected parts of the vestibular labyrinth without impairment of other structures is a delicate procedure. In mammals, Versteegh (1927) was the first to perform a localized destruction of the saccular macula and section of the utricular nerve in the rabbit. Jongkees (1950) in the rabbit and Szentagothai (1952) in the cat and the dog, respectively could apply artificial and direct stimulation of the otoliths and observed the effect on labyrinthine reflexes.

The present knowledge of the electrophysiological characteristics of the various nerves of the vestibular receptors is based largely on experiments on frogs, fish, and pigeons.

The most detailed description of discharge patterns in primary afferents connected to the otolith organs was presented by Lowenstein and Roberts (1950) in the thornback ray. Although many data are available concerning discharge-frequency modulations in the vestibular nuclei in mammals, only a few papers on recording from primary vestibular afferents have been published. Gernandt (1949) described recordings from the lateral ampullary nerve in the cat. Wing (1963) on recording from units in the vestibular ganglion "presumably supplying the otolith organs" in the cat, concluded that "the utricle and saccule may be vestigial organs, or at least do not function meaningfully in the orientation of the cat with respect to the gravitational field".

Since the anatomical evidence of vestibular efferent innervation is revealed by many authors, another point of interest is the functional meaning of efferent influence on the macular receptors.

In consequence of the present situation it is of great significance to obtain exact information on the primary afferent discharges in the utricular nerve of the cat, and to compare this to the findings in lower vertebrates and to the discharge patterns in the mammalian vestibular nuclei. The object is pursued as follows:

After section of the ampullary nerves to the lateral and anterior semicircular canals on the left side, a total labyrinthectomy was performed on the right side. Pre- and postoperative electronystagmographical recordings on torsion and parallel swing confirmed an undisturbed function of the left utricle, combined with functional elimination of the lateral and anterior semicircular canals.

After some four weeks of survival, the posterior fossa was approached by an extensive craniotomy, and a microelectrode was introduced into the utricular part of the left superior vestibular nerve just inside the internal acoustic meatus. Stimulation was effected by a tilting device. The discharge patterns were recorded on tape and visualized on an oscilloscope under continuous auditory monitoring.

CHAPTER III

METHODS

1. SELECTION OF EXPERIMENTAL ANIMALS

The possibility to compare results with other experiments is of paramount interest. Our choice of animal, the cat, was based on the extensive anatomical and physiological data available.

Most electrophysiological experiments on the vestibular nuclei have been performed on the cat. The bulla tympanica is relatively large and quite superficially located. This involves a less complicated approach to the vestibule.

The frontal position of the eyes is of special interest because it procures a better comparison with electronystagmographical data in man. A significant discomfort in electronystagmographic investigation is the lively nature of the cat, which makes it react to the smallest external stimulus by voluntary eye movements (this in contrast to the rabbit).

Under special precautions and after a great number of preliminary experiments, we succeeded in obtaining reliable and constant recordings. Finally the cat presents negligible difficulties as regards anaesthesia.

The animals, young adult cats, weighing between 2,5 and 3,2 kg, were only chosen if their behaviour indicated good health, without otologic dysfunction. They had to be domestic in nature, in order that they could be submitted to the vestibular test situation. Before admittance to the experiment a gross impression of vestibular function was achieved by cold water irrigation of the external ear canals on both sides. By this procedure we ruled out some cats with suppurative otitis which we detected by either abnormal nystagmus or complete lack of nystagmus. If the animal showed a normal nystagmus, it was tested on torsion and parallel swing. In case of good semicircular-canal function, a clear nystagmus on the torsion swing (de Boer et al. 1963) is recorded by way of electronystagmography. With the same technique on the parallel swing (Jongkees and Groen 1946; Jongkees 1966) compensatory eye movements are recorded as a sign of response to otolithic stimulation (fig. 4 page 35). It must be noted that, in case of swinging sideways on the parallel swing, with the cat in prone position and the plane of the hard palate turned downward over about 30°, the stituation is optimal for utricular stimulation.

Any pathological changes found during the preparation of the animal were reason for its elimination from the experiment. The present series of experiments comprises 11 normal cats showing good vestibular function; two of these were used for checking purposes as no preliminary operations were performed.

2. PRELIMINARY SURGICAL PROCEDURES

a. Anatomical and functional considerations.

Fernandez et al. (1959) described the effect of localized lesions in the vestibular receptors of the cat. By way of fenestration in the niche of the tensor tympani just behind the oval window and below the facial nerve they could section the utricular nerve. For the exposition of the lateral and anterior ampullary nerves the fenestra was enlarged upwards.

For our purpose this approach must be considered as being less advantageous, because the bony capsule of the superior part of the labyrinth is directly opened which means a greater risk of disturbing adnex structures when the nerves are sectioned.

Money (1961) described a technique for plugging individual semicircular canals in the cat by an external approach. Otolithic function would be saved this way. However, no clear evidence on this point was presented. Furthermore, in succesful plugging operations there is still a normal ampulla left in connection with the ampullary nerve. Thus the possibility of a resting discharge remains, and, in consequence of this, the neurophysiological situation is necessarily different from a total interruption of the ampullary nerve. Moreover, the changes occurring in the endolymphatic part of the "pars superior labyrinthi" could provoke change in otolithic function.

A new technique for functional elimination of the lateral and anterior semicircular canals with preservation of utricular function had to be developed. Before description of this technique a recapitulation of the anatomical aspects is necessary.

The vestibular part of the eighth cranial nerve shows a division into an anterior and a posterior branch.

At the level of the internal auditory meatus the anterior branch contains the superior vestibular ganglion with the bipolar cells of the primary afferent fibres, whose peripheral extensions pass to the macula of the utricle and the ampullae of the lateral and anterior semicircular canals (Lorente de Nò 1933; de Burlet 1934). The central fibres pass to the vestibular nuclei and some parts of the cerebellum. The posterior branch with the inferior vestibular ganglion contains fibres from the saccular macula and the posterior semicircular canal.

Three minor branches accomplish interconnections between the vestibular parts and the cochlear part of the eighth cranial nerve. The ramus Voit leaves the utricular nerve at the point of entrance in the vestibule and communicates with the macula of the saccule. The ramus Oort (1919) connects the saccular nerve with the cochlea. The ramus Hardy achieves a contact between the cochlear (spiral) ganglion and the saccular macula.

The preservation of normal function of the superior part of the membranous labyrinth is of special interest. The main parts of the membranous labyrinth are the cochlear duct, the saccule, the utricle, the semicircular canals, and the endolymphatic duct and sac. Smaller connecting channels are the utricular duct, the saccular duct, and the ductus reuniens.

The endolymphatic duct communicates with the utricle through a slit-like orifice, guarded by a fold of the utricular wall. This structure was first described by Bast (1928, 1934) who presented evidence that the utriculo-endolymphatic valve may serve to maintain fluid pressure in the utricle, when due to trauma the saccule and cochlear duct collapse. Bast and Anson (1949) presented a scala of arguments to support this conception of the function of the utriculo-endolymphatic valve.

Secretory areas in the vestibular labyrinth were described by Saxen (1951). These are situated around the sensory epithelium. Dohlman (1965) and many others confirmed these observations. Kawamoto and Altmann (1967) presented the possibility of another source concerned with the metabolism of the fluids in the vestibular labyrinth, the so-called atypical epithelial formations in the inferior sinus of the utricle.

In consequence of the above-mentioned anatomical and functional considerations, interruption of the ampullary nerves to the lateral and anterior semicircular canals in the vestibule, without disturbance of other structures, will result in maintenance of utricular function.

Activity recorded from the utricular part of the superior vestibular nerve must then have its origin in the utricular macula. The possibility of recording efferent activity will be considered in chapter IV.

Since the posterior semicircular canal and the saccular macula project to the inferior vestibular nerve, elimination of their function is not necessary in order to record proper utricular discharge patterns from the superior vestibular nerve.

b. Technique for functional elimination of the lateral and anterior semicircular canals with preservation of utricular function.

Initial development of this procedure involved practicing on 26 anaesthetized cats with intact sculls, used previously for other experiments.

The cats in the present series were anaesthetized with pentobarbital (Nembutal 30 mg/kg body weight) injected intraperitoneally. Premedication consisted of 1/8 mg atropine intramuscularly. The operation was performed under antiseptic precautions and penicillin 100.000 E was given intramuscularly. This antibiotic "shield" was maintained during the next five postoperative days. The cat in supine position is fastened to a (heated) operating table, the head fixed in a specially designed clamp which permits a wide ventral approach to the tympanic bulla.

After localization of the tympanic bulla at the left side, a longitudinal incision of the overlying skin is made. Incision of the superficial nuchal fascia reveals the superior-muscle layers. With dissecting scissors all underlying structures are separated bluntly. The superficial lymphnodes (Reighard and Jennings

1963) are dissected free on the lateral side and luxated medialward. A blunt forceps pushes the neck muscles towards the lateral side and the lymphnodes with the middle-muscle groups towards the medial side. After luxation of the hyoid bone to caudal, another blunt forceps is placed transversally onto the bulla. At this moment the whole tympanic bulla becomes visible, and the periost can be removed. The bulla is opened with a chisel and fine bone tongs, rendering the middle ear accessible.

From this moment on the dissection is performed with the aid of a Zeiss operating microscope. The ossicular chain and its muscles are clearly visible with only the body of the stapes hidden under the basal cochlear turn. The chorda tympani spreads like a white band across the long leg of the incus. The round window is situated at the medial anterior side.

In order to obtain a free approach to the vestibule a part of the "annulus tympanicus" together with the tympanic membrane, the malleus, the tensor tympani muscle, and the incus are removed. The stapedius muscle is cut. With a fine curet (model House) the mucosa on the surface of the basal cochlear turn is removed. This results in a few minor haemorrhages. The middle-ear cavity is cleaned until good haemostatis is accomplished.

With a fine drill a shallow niche is made in the basal cochlear turn on the lateral side of the round window, without opening the cochlea. The bone dust is carefully removed by irrigation with warm cat's Locke solution. With a curet, placed in this niche, the basal cochlear turn is opened. Very carefully this opening is then enlarged in the direction of the vestibule. The stapes footplate is removed from the inside towards the middle ear cavity. It is of paramount importance that no particles enter the vestibule. For this reason we could not use the drill for this part of the operation. In the above-mentioned procedure there is practically no risk of contamination of the vestibule.

In the depth of the vestibule the utricle with its nerve branches is clearly visible as a white oval body occupying a large part of the cavity. At the lateral side of the utricle the ampullary nerves from the lateral and anterior semicircular canals are visible on their way to the cribrose macula (Anson et al. 1967) of the petrous bone at the medial part of the vestibule. With a very fine tabb knife ("Richards" no. 1212EE), midway between the ampullae and the point of disappearance into the bone, the nerves from the above-mentioned ampullae are cut (fig. 3).

This is by far the most delicate part of the operation. It is performed under the highest magnification (40 x), while special care is taken not to disturb the utricular partition or other structures. After sectioning the nerves a solution of chlooramphenicol is brought into the vestibule and middle-ear region. Muscles, fascia, and skin are closed in layers. At the moment the inner ear is opened there is a flow of peri- and endolymph, but this gradually slows down. By careful suction with fine pipettes at the bony edges, there is no impairment of view. Only when the basal cochlear turn is removed, there is a slight bleeding from damaged cochlear vessels. This bleeding stops rather easily and adequate suction prevents contamination of the vestibule. Further exploration is done in a blood-free field.

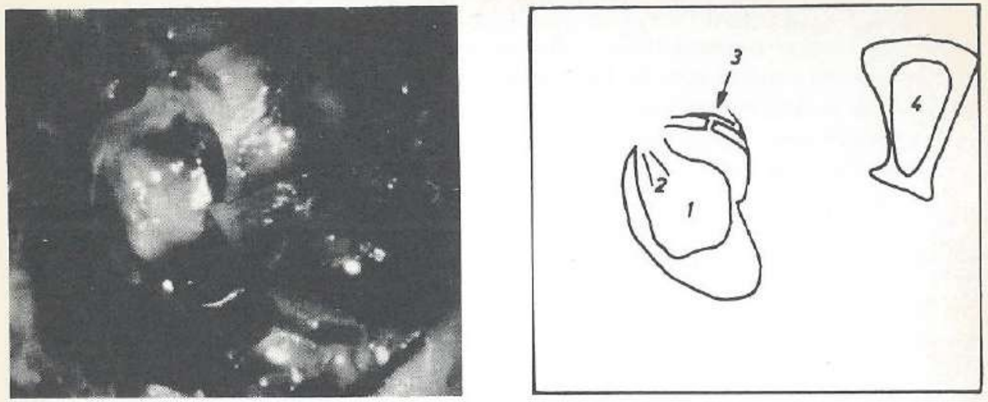


Fig. 3.

Operational view of the inner ear at the left side. The cat occupies a supine position.

1. Utricular macula.
2. Utricular nerve.
3. Point of transection of the ampullary nerves from the lateral and anterior semicircular canals.
4. Stapes.

Vascularization of the utricular part of the labyrinth is not interfered with since the utricular artery, supplying the utricular macula, is a branch from the anterior vestibular artery. The vestibulo-cochlear artery supplies the saccular macula, the posterior semicircular canal, and, with a separate branch, the basal turn of the cochlea (Kolmer, 1927; Fisher, 1967).

As to the endolymphatic system, the utriculo-endolymphatic valve preserves a normal endolymphatic condition in the superior part of the labyrinth in case of lesion to the cochlear duct (see above).

Two weeks after the functional elimination of the lateral and anterior semicircular canals on the left side a total labyrinthectomy is performed on the right side with the same antiseptic precautions and again under a "shield" of penicilline.

c. Postoperative behaviour and electronystagmographic check.

In order to be sure that the surgical procedures accomplished their objectives (and nothing more) postoperative check is needed.

After sectioning the utricular nerve in the cat, Fernandez et al. (1959) described a syndrome of disequilibrium with spontaneous and postural nystagmus towards the unoperated ear. Disturbance of equilibrium consisted of tilting of the neck towards the operated ear, swaying of the body or the head and standing with the limbs abducted.

While walking the cat frequently fell towards the operated side. As time passed, disequilibrium diminished and in about two weeks compensation was observed, but tilting of the neck remained present for a longer time. The spontaneous nystagmus towards the unoperated ear lasted 24 hours, the postural nystagmus disappeared after one week.

Section of the lateral ampullary nerve produced a syndrome very similar to that after section of the utricular nerve. The main difference is that in section of the lateral ampullary nerve the head is maintained erect; no tilting towards the operated side is observed.

The authors concluded that lesions of the utricular nerve consistently produce tilting of the head towards the operated ear.

In our experiments, after the first operation including section of the ampullary nerves to the lateral and anterior canals on the left side, the cat showed a spontaneous nystagmus towards the normal ear for one day or less, and a postural nystagmus until the fifth postoperative day. Tilting of the head was never observed after this first operation. Total labyrinthectomy two weeks later at the right side also caused the nystagmogenic features, this time directed towards the left ear. The head was tilted to the *right.*

Although our postoperative findings correspond in general with the above-mentioned features, an electronystagmographic confirmation of preserved utricular function on the left side must be regarded more conclusive.

Six weeks postoperatively a second test situation on both torsion and parallel swing took place. On the torsion swing nystagmus in the horizontal plane could no longer be recorded by electronystagmography (fig. 4). A check in the vertical plane presented major difficulties, first because this plane is not routinely investigated on our swing, and secondly there still is a functioning posterior semicircular canal. In a few instances we examined the results of stimulation in the vertical planes both pre- and postoperatively. This revealed a diminished number of nystagmic beats in the latter test situation. These data cannot be considered reliable.

However, since transection of the ampullary nerves was performed in the same way and at the same moment, we consider it justifiable to regard the outcome of the torsion-swing tests in the horizontal plane to be conclusive for functional elimination of both lateral and anterior canals.

Electronystagmographical recording on the parallel swing still shows good compensatory eye movements (fig. 4). In general duration has somewhat diminished, which can be explained by the total elimination of labyrinthine function including the utricule on the right side, and possibly also by the destruction of the saccule on the left side (Benjamins and Huizinga 1927).

If, after these two operations, the above-mentioned results were obtained, it

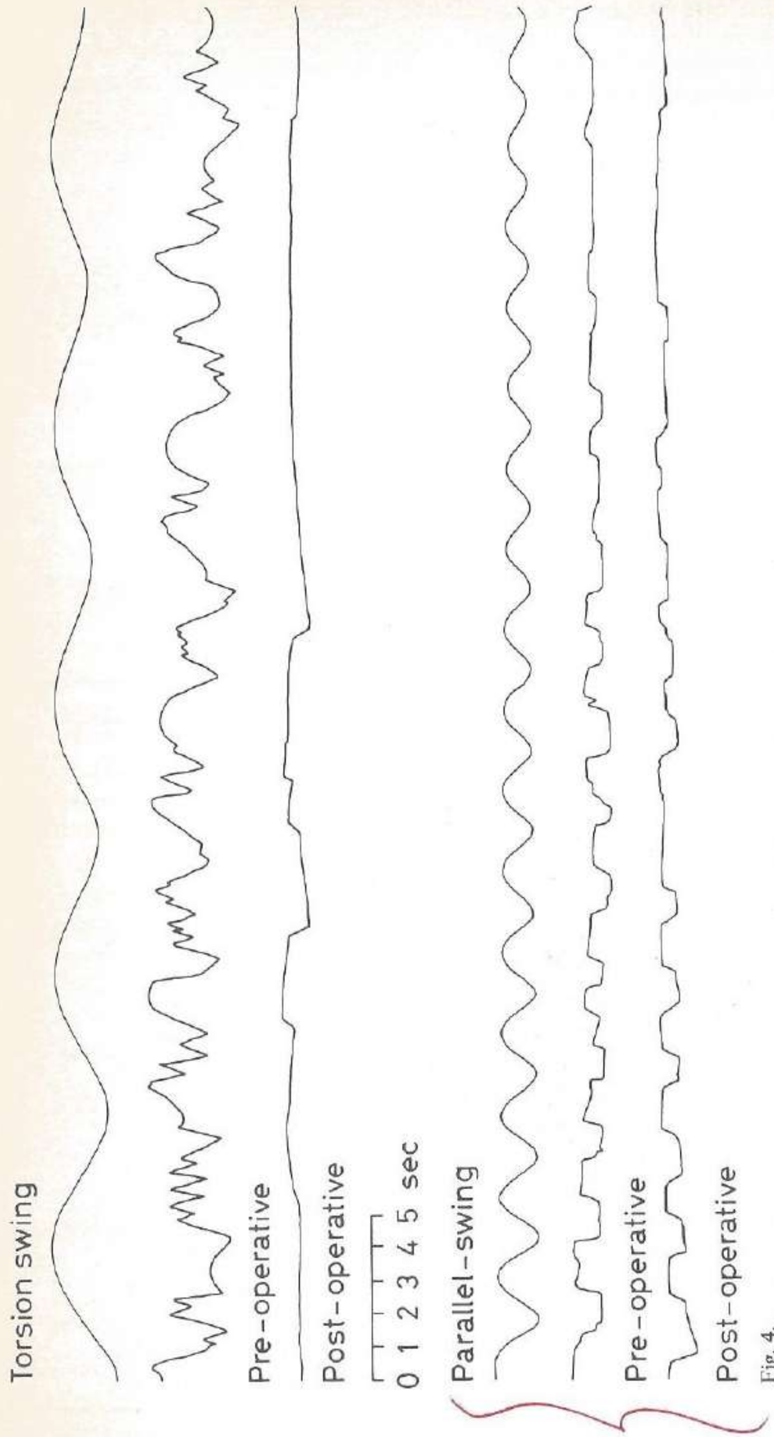


Fig. 4.
Electronystagmographic recording of eye movements of the cat on torsion and parallel swing. Upper curve before, and lower curve after operation (functional elimination of the lateral and anterior semicircular canals by sectioning of the corresponding ampullary nerves at the left side, and total labyrinthectomy at the right side). Trace above each set of curves indicates the movements of the swing.

was assumed that the preliminary operations for functional elimination of the lateral and anterior semicircular canals on the left side with preservation of utricular function, had been succesful.

3. THE ACUTE EXPERIMENTAL ARRANGEMENT

a. Surgical approach.

Most cats were anaesthetized with intraperitoneally injections of pentobarbital (Nembutal 30 mg/kg body weight). Atropin 1/8 mg was given intramuscularly as premedication. The trachea and a cubital vein were cannulated. The indifferent electrode, an Ag-AgCl wire wrapped in gauze soaked with cat's Locke solution, was placed in the neck muscles.

In some cats anaesthesia was started with the short-acting barbiturate Thiogenal (10 mg/kg body weight) injected into a femoral vein. After cannulation of the cubital vein supplementary Nembutal was given only during the initial surgical approach.

While recording from the utricular nerve, all the cats were immobilized with Flaxedil (6-8 mg/kg body weight), and respiration was artificially maintained. In general, as the surgical procedures included partial decerebration, administration of supplementary Nembutal was only rarely necessary.

Temperature was monitored by a rectal thermometer (at 37° - 39° C). In prone position the cat's head was then attached to the head holder (model Horsley-Clarke). A sagittal incision was made over the skull, and the skin and muscles at the left side over the parietal and occipital bones were removed. With a drill a parasagittal hole was made in the parietal bone; when possible bone tongs were introduced, and the caudal half of the left parietal bone was removed. After the dura was cut away, the caudal part of the left hemispere was removed by suction under constant irrigation with warm Locke solution, until the left part of the bony tentorium cerebelli became visible. The lateral cavity was packed with cotton-wool, and to the cut cerebral surface haemostatic Spongostan was applied. Concomitant sinus bleeding was coped with by application of bone wax and Spongostan.

Another hole was made caudal to the lambdoideal ridge and half of the left occipital bone was removed. This two-stage approach involved limited bleeding. After removal of the dura, hemicerebellectomy was performed by suction; during the final stages a Zeiss operating microscope was used.

The eighth cranial nerve became visible between the internal auditory meatus and the lateral cochlear nucleus. Special care was taken not to injure the vessels in this region.

The bony tentorium was removed and small pieces of cotton-wool were placed rostrally and caudally to the internal auditory meatus, in order to give support to the brain stem. Where needed Spongostan was applied to the cut cerebellar surface.

With a fine drill part of the medial edge of the internal acoustic meatus, overlying the utricular part of the vestibular nerve, was removed under intermittent irrigation with warm cat's Locke solution in order to keep the nerve free from bone dust (fig. 5).

Thereafter the animal was moved into an electrically shielded room and placed in a tilting device.

b. Stimulus device and response-recording technique.

Utricular stimulation was achieved by tilting the animal in the transversal plane about a rostro-caudal axis.

The tilting device was calibrated for steps of 15 degrees lateral tilt in both directions up to 45 degrees from the horizontal plane. The tilt was performed manually.

The cat was placed in the tilting device with the head in the Horsley-Clarke instrument and the body fastened against movements in lateral directions.

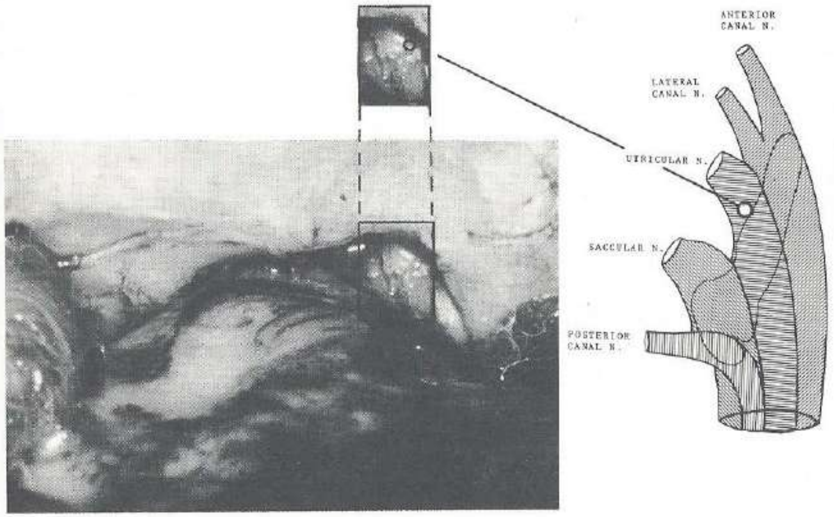


Fig. 5.

Operational view of the internal acoustic meatus (left side) after removal of the medial edge overlying the utricular part of the vestibular nerve.
Inset: indication of the position of the microelectrode into the utricular nerve peripherally to the superior vestibular ganglion.
In the anatomical scheme of the vestibular nerve as presented by Stein and Carpenter (1967) this area is also indicated as projected upon the utricular nerve.

The operating microscope and a micromanipulator were mounted on the cat board, permitting control of the microelectrode during every stage of the tilting. The microelectrodes used for recording were 4 M NaCl filled micropipettes with resistances of 5-15 megohms as measured in saline solution or in the preparation. For the marking of the electrode position, several electrodes were filled with 2 M NaCl saturated with fast green. The microelectrode mounted in the micromanipulator was connected by a cathode follower to an AC-coupled pre-amplifier.

The indifferent Ag-AgCl electrode had already been placed in the neck musculature. The pre-amplifier was connected to a Tektronix R.M. 565-dual beam cathode-ray oscilloscope, a loudspeaker, and one channel of a tape recorder ("Sony", Model T.C. 530).

Neuronal discharges from the preparation were monitored visually on the oscilloscope and acoustically with the loudspeaker. The degree of tilt of the cat was indicated on a second channel of the oscilloscope by means of a potentiometer device.

The oscilloscope was triggered at 250 msec by a digital pulse generator ("Devices", digitimer) and the screen was occasionally photographed on a Grass Kymograph camera. Protocols of the experiment were recorded on the second channel of the tape recorder.

The recording area was electrically insulated by means of liquid paraffine at body temperature. Under visual control, the microelectrode was lowered vertically in order to make contact with the utricular part of the superior vestibular nerve as far peripherally as possible. At the moment of contact the noise level diminished sharply and the micromanipulator setting was noted. The first discharges were recorded already near the surface. Very stable conditions are required for recording from single units. By application of small pieces of cotton-wool in the posterior fossa and rostrally to the internal acoustic meatus, shift of the brain stem on tilting was in general sufficiently prevented. In a few instances a firm gel of agar-agar was instilled, but this did not improve the stability of the preparation.

c. Check of topography and histology of the vestibular division of the eighth nerve as to the position of the microelectrode tip.

In the beginning of this series of experiments the position of the microelectrode tip was checked by leaving the electrode in the utricular nerve after the experiment was finished. The micromanipulator settings were noted. The animal was perfused with normal saline solution followed by formalin 10% for fixation. The electrode was removed and the temporal bone was excised for further fixation in formalin 10%. After decalcification the electrode track was traced by serial sections in the longitudinal plane of the vestibular nerve. Because of shrinkage, results were not very reliable, but on several occasions it was suspected that the tip of the electrode had been localized in the utricular nerve. This procedure was considered unsatisfactory.

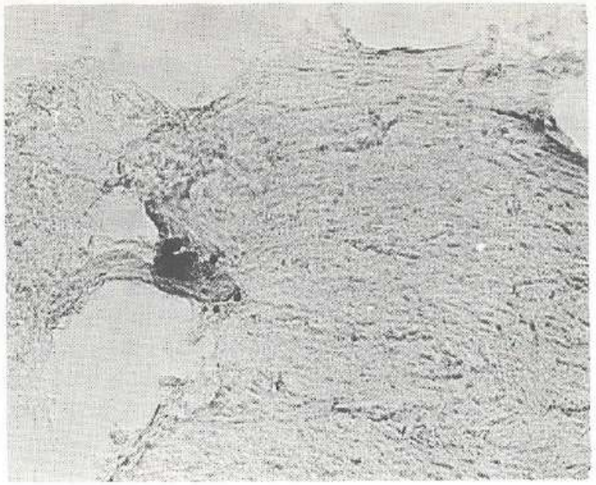


Fig. 6A.

Frozen section of the utricular nerve, photographed with an orange-red filter to show the position of the dye spot from the microelectrode, 120 x.

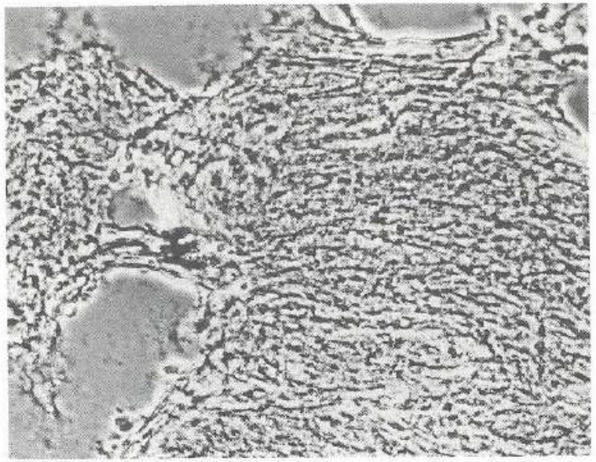


Fig. 6B.

Same section, photographed with phase-contrast and ordinary light to demonstrate the fibre pattern.

In later experiments we therefore switched to the fast-green method for electrode localisation as described by Thomas and Wilson (1965). In these experiments microelectrodes filled with 2 M NaCl saturated with fast green were used for recording.

On account of the very limited area recorded from, marking was performed only at the end of one electrode track and the micromanipulator settings were noted. After perfusion-fixation of the animal with formalin 10%, the eighth cranial nerve was excised centrally at the cerebello-pontine angle, while the peripheral branches were taken out after removal of the roof of the internal auditory meatus. Frozen serial sections of 50μ, parallel to the vestibular nerve root, were studied and a camera lucida drawing was made of the marked area. The section containing the green mark was stained by the cresyl-violet method for Nissl substance. The preceding and following sections were stained according to the Weil method for myelin sheath in order to check the fibre structure in this area. With this procedure the position of the electrode tip in the utricular nerve could be confirmed.

Another preferable method for the determination of the position of the microelectrode tip, comprises a combination of observation with red light and phase-contrast microscopy. As the dye fast green shows an absorption maximum at 628 nm, the dye spot in the utricular nerve is shown clearly with a red-orange filter (fig. 6A); subsequent photography with low-power phase-contrast with ordinary light demonstrates the fibre pattern without any staining (fig. 6B). The dye spot is seen less clearly in the latter case.

d. Data-Processing.

After the experiment was finished, representative parts of the tape recordings were photographed.

The waveform of the spikes could be observed with faster sweeps as far as the frequency characteristic of the tape recorder admitted. Two values of the amplifier's time constant, 2 msec and 100 msec respectively, were chosen so as to facilitate observation of either spikes per se or the over-all waveform. At a second playback data were processed by a special-purpose computer ("Nuclear Chicago", Type 7100). In every fixed position during tilt the discharges were recorded over periods of 10 seconds or multiples. The time clock of an electronic counter was used for gating these 10-second periods to the computing system (fig. 7a). The data were to be displayed in the form of an interval histogram (Gerstein and Kiang 1960). This is a bar graph representing the distribution of the interspike-intervals as a function of interval. The time interval is plotted on the abscissa and the number of intervals that occur in each particular range (bin) is plotted on the ordinate. In our computations a bin width of 0.625 msec was used.

The computer screen was photographed and the display was copied on an x-y recorder (fig. 7b). Because most interspike intervals were recorded on the

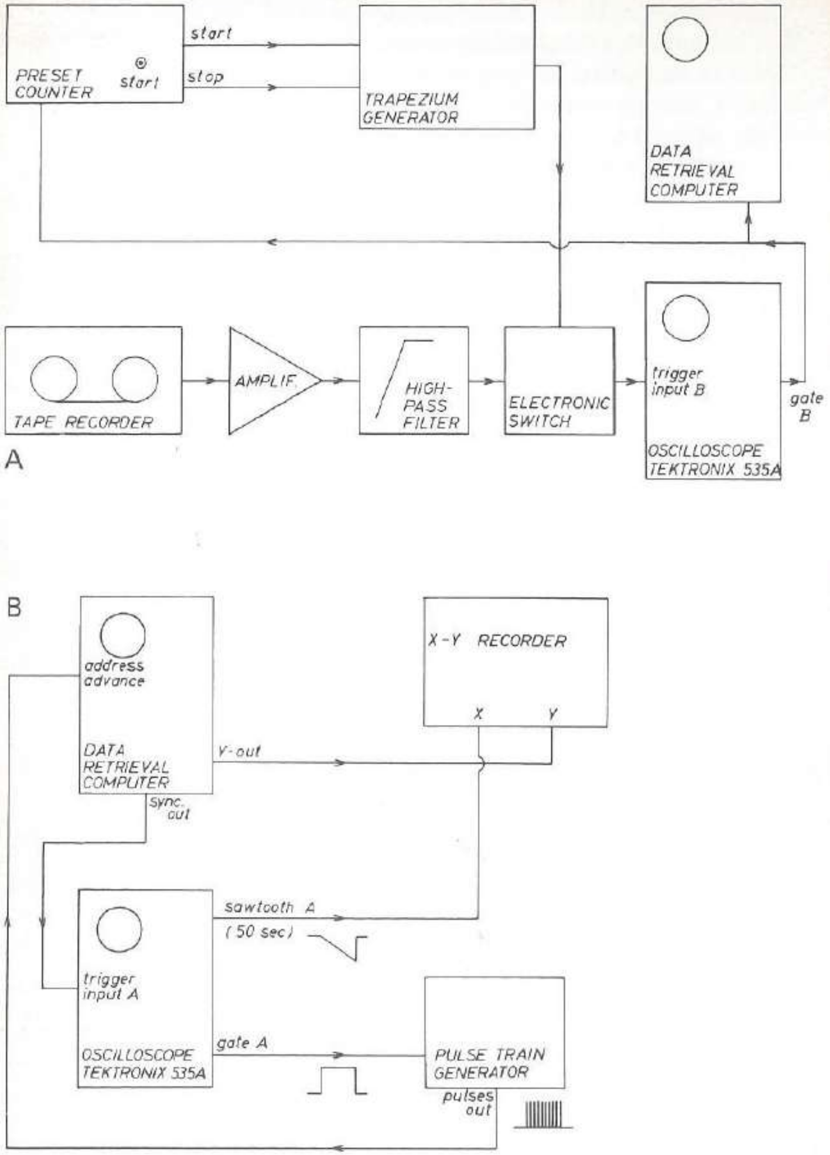


Fig. 7.

Block diagram of electronic equipment for data-processing.

A. Set-up for gating 10-second periods to the computing system. Each sweep of the oscilloscope produces a pulse which is fed both into the computer and into the electronic counter.

B. Display of data on the x-y recorder.

left side of the computer screen, the full horizontal scale was enlarged when copied out for better detail discrimination.

In order to manipulate the triggering of the computer, the discharges were fed into a separate oscilloscope. The triggering level of the oscilloscope was carefully adjusted to a level clearly distinct from the noise level. Each sweep of the oscilloscope produces a pulse which is fed both into the computer and into the electronic counter.

Excessive baseline fluctuations were prevented by the use of a high-pass electronic filter.

CHAPTER IV

RESULTS

1. INTRODUCTION

The results presented comprise experiments on 11 cats. In 9 cats, by preliminary surgical procedures, functional elimination of the ampullary nerves to the lateral and anterior vertical canals on the left side, and total labyrinthectomy on the right side was performed. Two normal cats were used for comparison.

A total of 34 units recorded from, yielded quantitatively analysable results. Of these, 7 units were recorded from in normal cats.

a. Criteria for primary afferent units.

Since no electrophysiological data were available on primary afferent units in the utricular nerve in mammals, our criteria are largely based on the anatomical situation and comparison with electrophysiological findings in lower vertebrates.

Centrally to the vestibular ganglion the vestibular nerve is composed of primary afferent fibres, of vestibular, and of cochlear efferent fibres separating the superior from the inferior part of the vestibular nerve.

At the lateral surface the nerve is joined by fibres of the "pars intermedia" of the facial nerve. Up till the level of the Schwann sheath-glia junction the peripheral fibres are covered by Schwann cells. In this context we have to consider the auditory part of the eighth cranial nerve.

Galambos and Davis (1948) presented evidence of the presence of cell bodies, probably belonging to the interstitial nucleus of the cochlear nucleus, centrally to the Schwann sheath-glia junction. Gernandt (1949) who recorded from the lateral ampullary nerve just outside the internal auditory meatus stated that probably some of his recordings could be from these second-order neurons scattered in the vestibular nerve.

The neuro-anatomical situation of the superior part of the vestibular nerve is less complicated in the sections running from the superior vestibular ganglion towards the peripheral end-organs. Here we meet primary afferent fibres with their cell body in the ganglion. These greatly outnumber the scattered vestibular efferent fibres at about 30 to 1 (see chapter I page 17).

The primary afferent utricular fibres are found in the nerve immediately caudal to those of the anterior and lateral canals (fig. 5 page 37). By removing a part of the medial edge of the internal auditory meatus overlying the utricular part of the vestibular nerve, we can introduce the microelectrode into the utricular nerve peripherally to, or at the lateral part of the superior vestibular ganglion.

The possibility of recording from the small number of vestibular efferent fibres, however, must be considered.

Electrophysiological investigation on efferent fibres is largely confined to the olivo-cochlear bundle (Galambos 1956; Desmedt and Monaco 1961; Fex 1962). These efferent fibres exert an inhibitory influence on auditory activity. A special feature of the efferents, in general, is lack of spontaneous activity. Furthermore the long latency of responses, the very regular firing pattern, and the low firing rate on stimulation mean a marked difference with the primary and secondary auditory fibres (Fex 1962).

Schmidt (1963) described efferent activity in various branches to the vestibular epithelia in the frog, but could not record spontaneous activity. Whether these vestibular efferents were inhibitory in nature was not determined.

Gleisner and Henriksson (1963) recorded efferent activity from the proximal end of the cut lateral ampullary nerve in the frog. For utriculo-fugal accelerations the threshold of efferent activity was found at a much higher level than that of afferent activity.

Bertrand and Veenhof (1964) demonstrated efferent activity in the rabbit and could show selective efferent influence on stimulation of the otoliths. Sala (1965 a,b) in the cat, found inhibitory influence on afferent vestibular activity, when the efferents were stimulated.

Goetmakers (1968) observed considerable adaptation of afferent discharges in the lateral ampullary nerve of the frog. This was attributed to efferent activity.

In primary afferent labyrinthine fibres, a spontaneous activity is an important feature (Lowenstein and Sand 1940; Lowenstein and Roberts 1950; Ledoux 1949; Groen et al. 1952). In contrast to the efferent fibres described above, all units in our series of experiments showed a distinct spontaneous discharge. When the microelectrode is inserted as far peripherally as possible into the utricular part of the superior vestibular nerve, and while other afferent input from the anterior and lateral ampullae is blocked, this spontaneous discharge must have its origin in primary afferents from the utricular macula.

b. Identification of discharges.

The microelectrode tracks were confined to a very limited area. The first neuronal discharges were encountered already near the surface. Along one electrode track, the field of recordings never exceeded a distance of 400μ from the surface. The tip of the microelectrode had a diameter of approximately 0.5–2μ. In relation to the fibre-diameter range from 1–10μ, average 2–4μ, responses from more than one unit were only occasionally found at one electrode position. Two types of unit discharges could be distinguished.

Units of the first type showed a high frequency discharge up to some 300 impulses/sec with positive-negative spikes in the 400μ V range.

The discharge frequency diminished gradually within several seconds, and then suddenly the discharge ended in complete silence. These recordings were considered to be injury discharges.

The second type of unit discharge showed a rather regular time pattern, composed predominantly of positive spikes in the order of 100–250μ V. After some incidental change as the microelectrode was introduced, spike amplitude did not change markedly in time. This spontaneous resting discharge although occasionally occurring in bursts, could be recorded over long periods of time without significant change in frequency.

During stimulation approximately the same amplitude level was maintained. This is a significant fact, because mechanical pressure of the microelectrode could involve a change in spike amplitude. On the other hand, however, in high frequency discharges on stimulation, two successive spikes could give the false impression of summation on account of capacity over the wall of the glass microelectrode which lengthens the descending phase of the recorded action potential (Tasaki 1954).

Observation of the shape of the action potentials in most instances revealed a positive spike of 1.5 msec duration with amplitude varying from 100–250 μ V (fig. 8b). Several units showed a biphasic positive-negative spike.

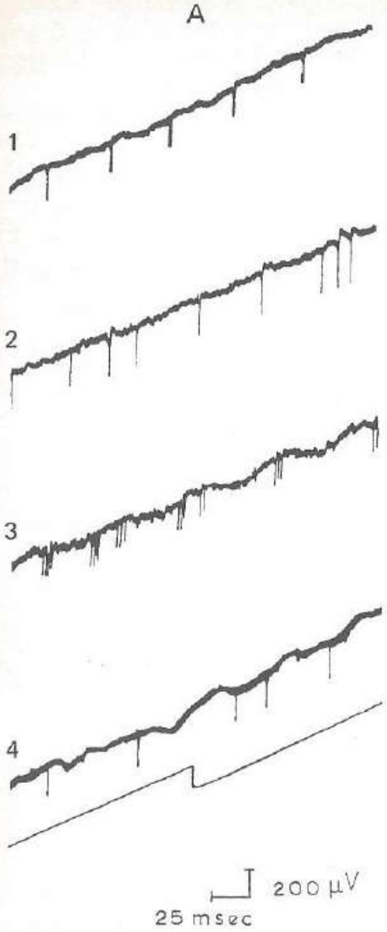
2. SPONTANEOUS DISCHARGE

All units showed a spontaneous discharge in the normal position of the cat. With the present experimental arrangement, stability conditions were such that in most preparations recordings of several units could be obtained over a period of 5–10 minutes, in one instance even of 35 minutes. Many other units, however, were held for shorter periods, sometimes only a few seconds. These of course were discarded.

In the units used for processing a rather regular spontaneous impulse-discharge frequency was generally observed.

This frequency is characteristic for each unit; and between distinct units variations of some 10 up to 40 impulses/sec are recorded. In a few instances this spontaneous activity exhibited a different pattern. The impulse discharge was composed of gross irregular "bursts" which consisted of a sequence of three or four successive spikes, followed by a rather varying period of silence before a new burst occurred. Although this firing pattern could continue for several minutes, we learned that on tilting these units were always lost, so there must have been a "graze".

Fig. 8a illustrates some different examples of the spontaneous discharge. Although careful inspection of the spike train can give valuable information, an improvement is provided by the analysis of time patterns in combination



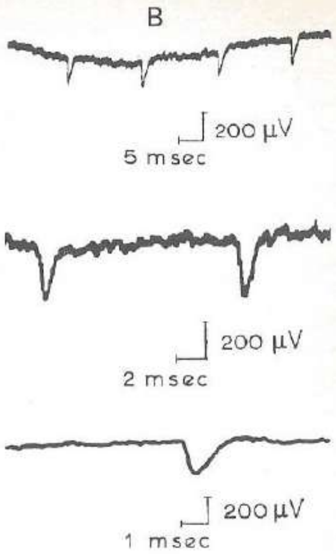


Fig. 8A.

Different examples of spontaneous discharges as photographed on moving film from the oscilloscope screen during the experiment.
Activity in samples 1, 2 and 4 respectively 22, 38 and 21 impulses per second. Sample 3 illustrates impulse discharges composed of irregular bursts. In these instances the recordings were considered a "graze" because on tilting these units were always lost. Trace below sample 4 indicates the normal position of the cat for all samples. On tilting to the left the vertical line moves to the left and "vice versa". Calibration indicated below for all figures.

Fig. 8B.

Some observations of the action potentials with different sweep speeds. The recordings were obtained during tilting and originate from different experiments. In these and in all other recordings, upward deflection indicates negativity at the microelectrode. Calibration is indicated below each figure.

with the interval histogram (Gerstein and Kiang 1960; Rodieck et al. 1962). Introducing the interval histogram involves averaging the data. In order to get an impression of the over-all activity, other aspects of the spike pattern must be discarded. For the interval histogram in our computations 400 time bins were chosen at the full horizontal scale of 250 msec, bin width being 0.625 msec.

On the ordinate the number of interspike intervals that occur in each time bin is expressed as counts. Other indications are: N = total number of spikes obtained during a gated period of 10 seconds; the cat with its registration number; the unit; and the position of the cat during the recording.

As the bin width determines the time resolution of the analysis, for comparison of the different positions of stimulation all interval histograms were plotted on the same horizontal scale.

Because most interspike intervals were recorded at the left side of the computer screen, the horizontal scale was enlarged for better detail discrimination when copied out on the x-y recorder.

A certain number of counts are missed this way, but these are represented in the overflow bin, a white bar at the right hand side of the graph.

Unit 2 cat IX is representative for the general pattern of spontaneous discharge. This unit showed an impulse discharge at 25/sec in the normal position. In fig. 9a a sample is shown of the spike discharges. Fig. 10a represents the interval histogram of the same unit in the normal position. This illustrates a slightly more irregular firing pattern in the normal position than would be observed in the pictures of the spike trains.

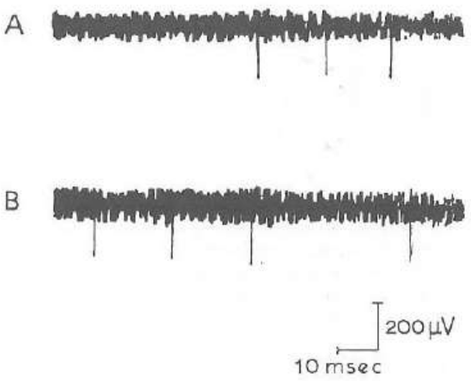
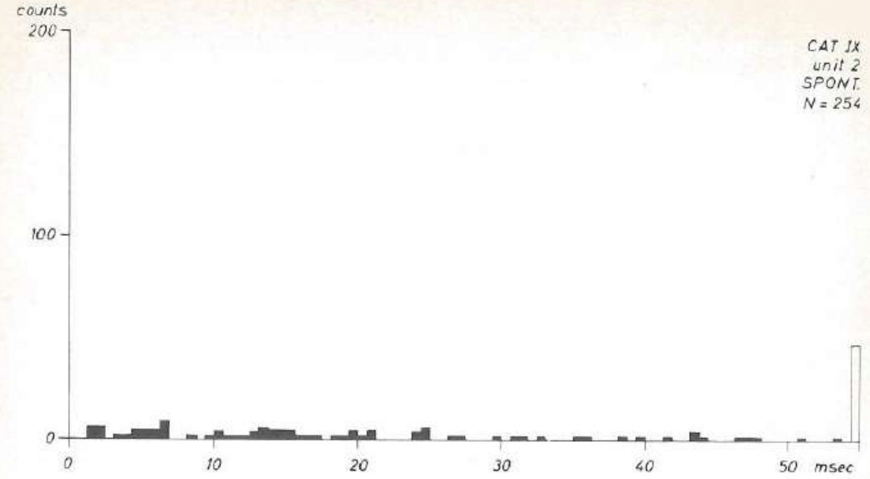


Fig. 9.

Samples of spontaneous discharges as photographed at playback of the tape.
A) unit 2 cat IX (25 imp/sec). B) unit 3 cat VIII (30 imp/sec).
At first sight these units show a rather similar discharge pattern, but comparison with the interval histograms (fig. 10a and b) reveals the more irregular firing pattern of unit 2 cat IX.



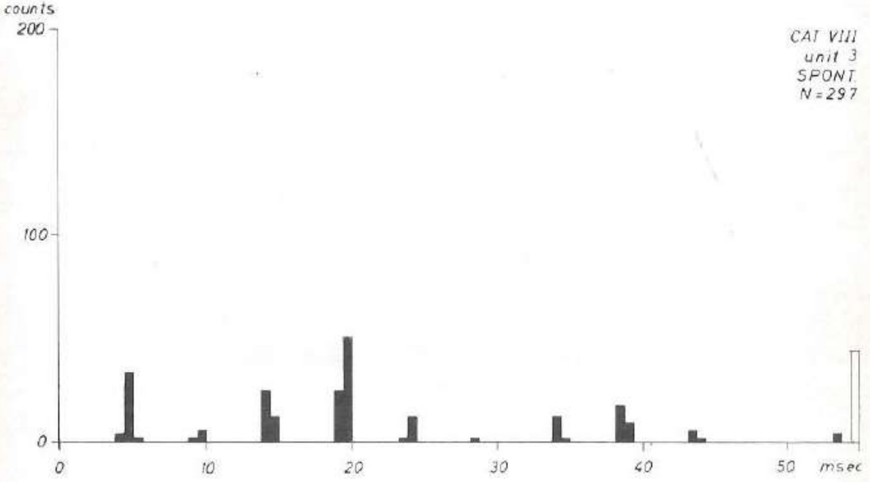


Fig. 10A.

Interval histogram of spontaneous discharges in the normal position (spont.) of unit 2 cat IX. N: total number of spikes obtained during a period of 10 seconds.

On the ordinate the number of interspike intervals that occur in each time bin is expressed as counts. As the bin width determines the time resolution of the analysis, for comparison of the different positions – as shown in other figures – all interval histograms presented in this study were plotted on the same horizontal scale (bin width 0,625 msec).

Because most interspike intervals were recorded at the left side of the computer screen, the horizontal scale was enlarged for better detail discrimination when copied out on the x-y recorder. A certain number of counts are missed this way, but these are represented in the overflow bin, a white bar at the right hand side of the graph.

Fig. 10B.

Interval histogram of spontaneous discharges in the normal position of unit 3 cat VIII. As the position of a peak on the abscissa indicates a preferred interval, in this instance several sharply defined intervals are recognised. This unit was not representative for the general pattern of spontaneous discharges.

Another example of the spike train is given in fig. 9b (unit 3 cat VIII). The corresponding interval histogram in fig. 10b shows some special characteristics of the spontaneous discharge in this unit.

As the position of a peak on the abscissa indicates a preferred interval, in this instance several sharply defined intervals are recognised. As such, this latter unit was not representative for the general pattern of spontaneous discharge. At first sight the spike trains in figs. 9a and b did not reveal this difference in behaviour.

3. RESPONSE TO TILT IN THE TRANSVERSAL PLANE

With the stimulus device described on page 37, the cat was tilted in the transversal plane – about a rostro-caudal axis – out of the normal position first towards 45° left side down and thereafter towards 45° right side down.

The tilt was performed slowly in steps of 15°. At every position the impulse discharge was observed during a period of at least 20 seconds. This arbitrary time span was found to be reliably representative. Because of technical difficulties, such as the leakage of liquid paraffine used for insulation of the area recorded from, or the greater chance of shift of the brain stem, greater angles of tilt were not tested.

This induces a restriction of the experimental results as compared to those of other authors obtained in lower vertebrates. Lowenstein and Roberts (1950) e.g. performed full-circle tilts in opposite directions.

Duensing and Schaefer (1959) and Peterson (1967), however, on recording from the vestibular nuclei in mammals, performed tilts over only about 30°. Another point stressed by Trincker (1965) is the possibility of unphysiological stimulation during full-circle tilts in opposite directions.

In the present series of experiments, before a tilt was started, the spontaneous discharge of each unit was observed over a period of at least one minute. Only on this condition an impression of quantitative discrimination in different positions was considered justified. A "scouting tilt" was performed to both sides in order to trace the behaviour of the unit. If there were a continuously variable reaction the normal position would be regained.

After this exploratory test the cat was carefully tilted towards the left and maintained in a stationary position at 15°, 30° and 45°. A distinct increase of impulse discharge was observed. On return to the normal position a quick decrease of the firing frequency was noted. In the same way a tilt towards the right, again with interruptions at 15°, 30° and 45° was performed. In all instances on tilting to the right a very clear decrease of discharge frequency was seen. Only one unit exhibited a somewhat different behaviour as will be discussed later.

While returning from the right side to the normal position, activity increased towards the spontaneous level.

During this whole performance auditory and visual monitoring of the discharges was used. In case of good responses the oscilloscope screen was occasionally photographed on moving film. The whole experiment was recorded on tape.

As described before (page 40) the discharges counted at each standard position were averaged over a period of 10 seconds. From these data the number of impulses per second was calculated. This included a maximum deviation from the original data, found on 45° tilt to the right, of about 1–8% with the only exception for unit 6 cat IV being 14%. On tilting to the left the deviation was negligible.

Careful examination of the discharges revealed that most recordings were attributable to one fibre. In several instances, however, the microelectrode recorded from two fibres simultaneously.

Only in one microelectrode location (unit 6 cat IV) these two fibres discharged approximately at the same amplitude, and as such were computed together. In the cats not operated upon before, and used as a check on our experiments, two such microelectrode positions were found in one experiment (units 1 and 4 cat 1). In other instances discharges from the second fibre did not trigger the computing system.

All units processed are presented in table I page 62. Units consisting of "two-fibre" preparations and computed as such are indicated with an asterisk (*).

In the first two experiments only the extreme positions were recorded during a period longer than 10 seconds. The first aim was to compare the effect of tilt to the left and tilt to the right while recording – as in all experiments – from the left utricular nerve.

Therefore a quantitative analysis of the intermediate positions as performed in the other trials was not possible.

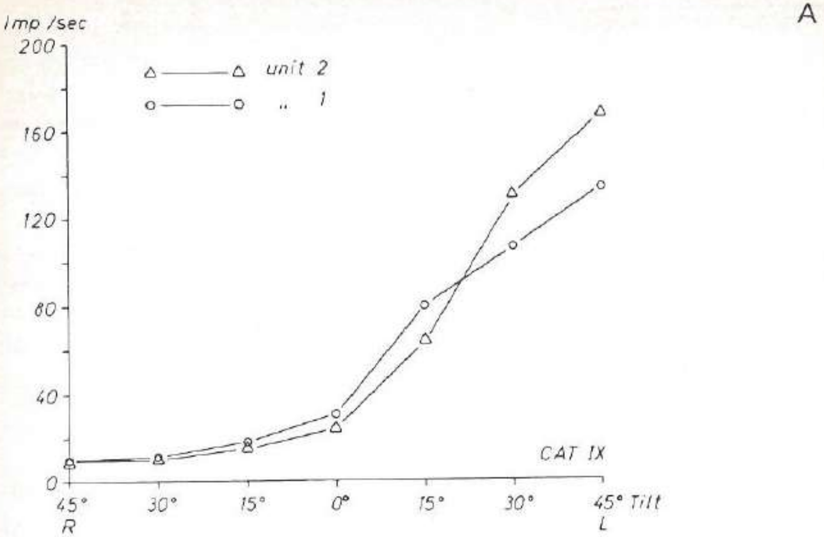
Several units will be discussed more in detail.

Units 1 and 2 cat IX are representative for the most frequently observed discharge patterns. Fig. 11a illustrates the impulse discharges/sec plotted against the angle of tilt. For the sake of convenience we will indicate a 15° tilt to the left as 15° L etc.

Unit 1 cat IX shows a spontaneous discharge at 31 imp/sec in the normal (0°) position. Tilt towards the left shows a rather steep rise to 81 imp/sec in 15° L, which becomes less steep and approximately linear to 108 imp/sec in 30° L and the maximum of 138 imp/sec in 45° L.

On tilt to the right the spontaneous discharge frequency decreased to 18 imp/sec in 15° R, after which a kind of "residue" level is reached in 30° R (12 imp/sec) with the minimum in 45° R (9 imp/sec).

Another discharge pattern often encountered is shown by unit 2 cat IX (fig. 11a). Here the spontaneous discharge at 25 imp/sec, on tilt to the left, rises distinctly to 65 imp/sec in 15° L, but a steeper increase up to 132



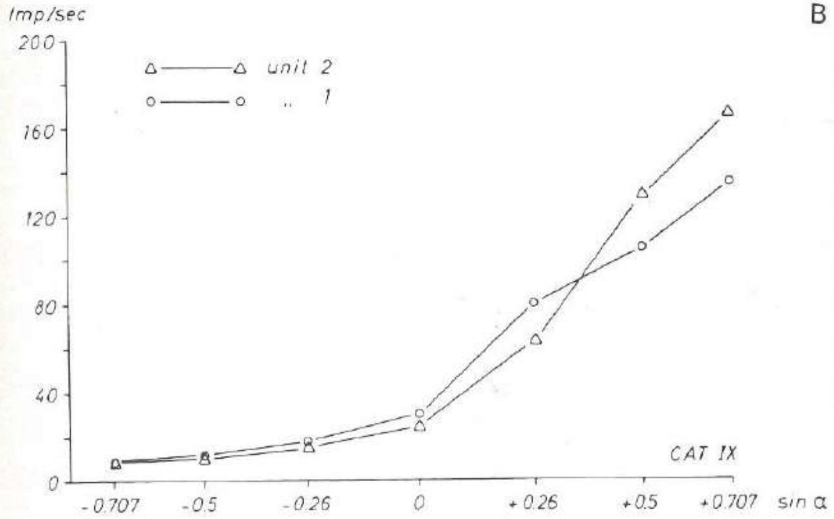


Fig. 11A.

Discharge frequencies in units 1 and 2 cat IX plotted against the angle of lateral tilt. 0°: normal position. L: tilt towards the left. R: tilt towards the right. These and all other recordings were obtained from the utricular nerve at the left side.

Fig. 11B.

Discharge frequencies in units 1 and 2 cat IX plotted against sinus α (α being the angle between the dorso-ventral axis of the cat and the direction of the gravitational force). + sin α: tilt towards the left; – sin α: tilt towards the right.

imp/sec occurs in 30° L. The maximum is reached in a somewhat less steeper rise at 170 imp/sec in 45° L. Tilt to the right shows the same events as described for unit 1 in this cat. In 15° R a decrease to 15 imp/sec, continues to 10 imp/sec in 30° R and to the minimum of 8 imp/sec in 45° R.
Fig. 12a and b respectively represent samples of the unit discharges as photographed from data on the tape and the interval histogram of unit 2 cat IX in all positions recorded from. The spike trains clearly show the increased discharge level on tilt to the left and the decrease on tilt to the right. The interval histogram illustrates the rather scattered distribution of interspike intervals of the spontaneous discharge. On tilt to the left these intervals gradually move to a peak at about 4 msec at 45° L. Tilt to the right shows a clear decrease of interspike intervals occurring during the first 50 msec. The relatively high overflow bin expresses the missed counts at longer intervals.

As such these units showed a distinct frequency-modulation of the spontaneous discharge on tilt in the transversal plane.
In chapter I it was already stated that the shearing force is suggested to be the most appropriate stimulus for the otoliths.
From this results the conclusion that the animal being tilted over an angle α (between the dorso-ventral axis – s.s. the axis perpendicular to the plane of the utricular macula – of the cat and the direction of the gravitational force : g), the effective force is represented by g sin α.
To check our findings in this respect, several unit-data were also plotted as discharge-frequency against sin α (resp. 15° : 0.26; 30° : 0.5; 45° : 0.707). Tilt towards the left is then expressed as + sin α, and tilt to the right as - sin α. Fig. 11b illustrates these plottings for units 1 and 2 cat IX. It will be seen that the discharge frequency shows some indication of a linear relation to sinus α on tilting to the left. If standard deviations are included conformation with linearity to sinus α improves. The standard deviations were calculated for all units. In the graphs, for the sake of surveyability, these are only indicated in figs. 13a and b and for one unit in fig. 16a.

Unit 1 cat III exhibited a discharge pattern as illustrated in fig. 13a. The spontaneous discharge was 28 imp/sec.
On tilt to the left the rise in discharge frequency was steepest at 15° L and at 30° L, becoming distinctly less steep in 45° L. Tilt to the right showed a clear decrease at 15° R, where the "residue" level is reached.
If we consider fig. 13b we see that the discharge frequency in this unit plotted against sinus α shows a rather linear relation on tilt to the left. It must be added that only a few units shared this clear picture.

Unit 3 cat VIII showed a somewhat exceptional type of reaction (fig. 14). The spontaneous discharge at 30 imp/sec, on tilt to the left, increased to a maximum at 139 imp/sec in 45° L. The steepest rise, however, occurred

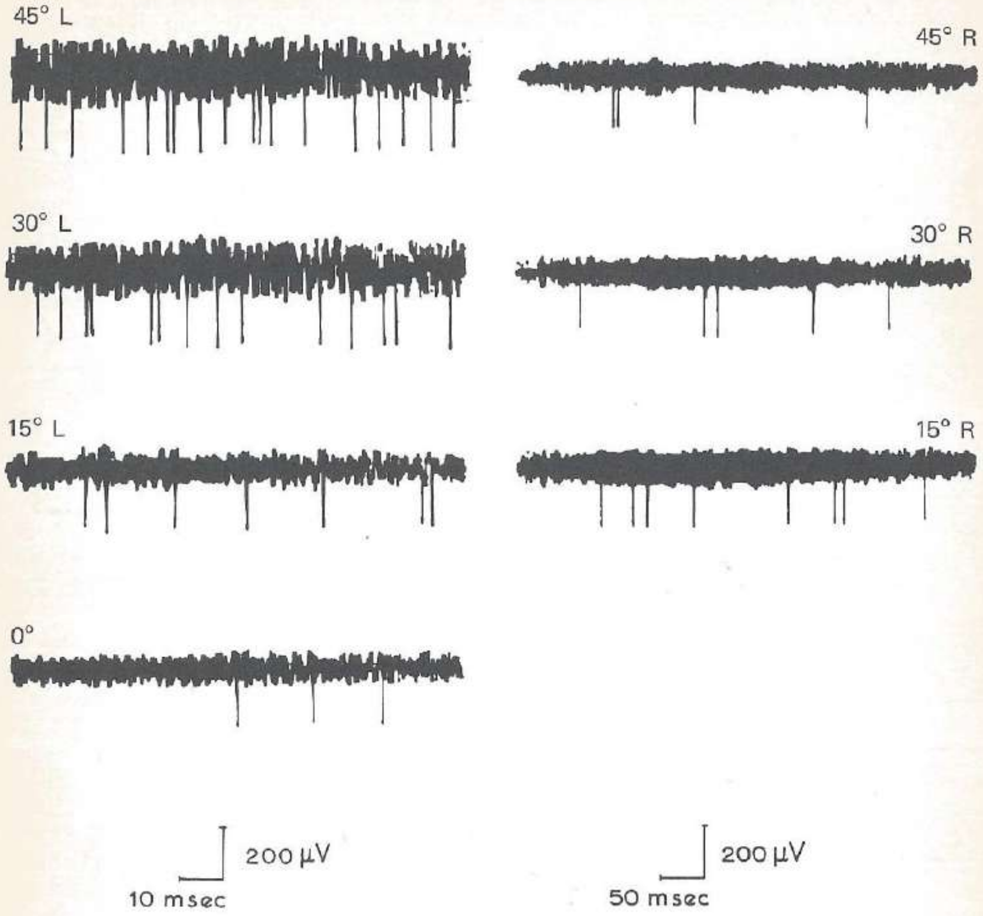


Fig. 12A.

Samples of spike trains recorded from unit 2 cat IX in all positions of the stimulus range investigated, as photographed from data on the tape.
0°: normal position; 15° L: 15° tilt to the left etc. Calibration for samples shown above. Note different sweep speeds.
The spike trains clearly show the increased discharge frequencies on tilt to the left. The total number of impulses per second in each position is given in table I page 62.

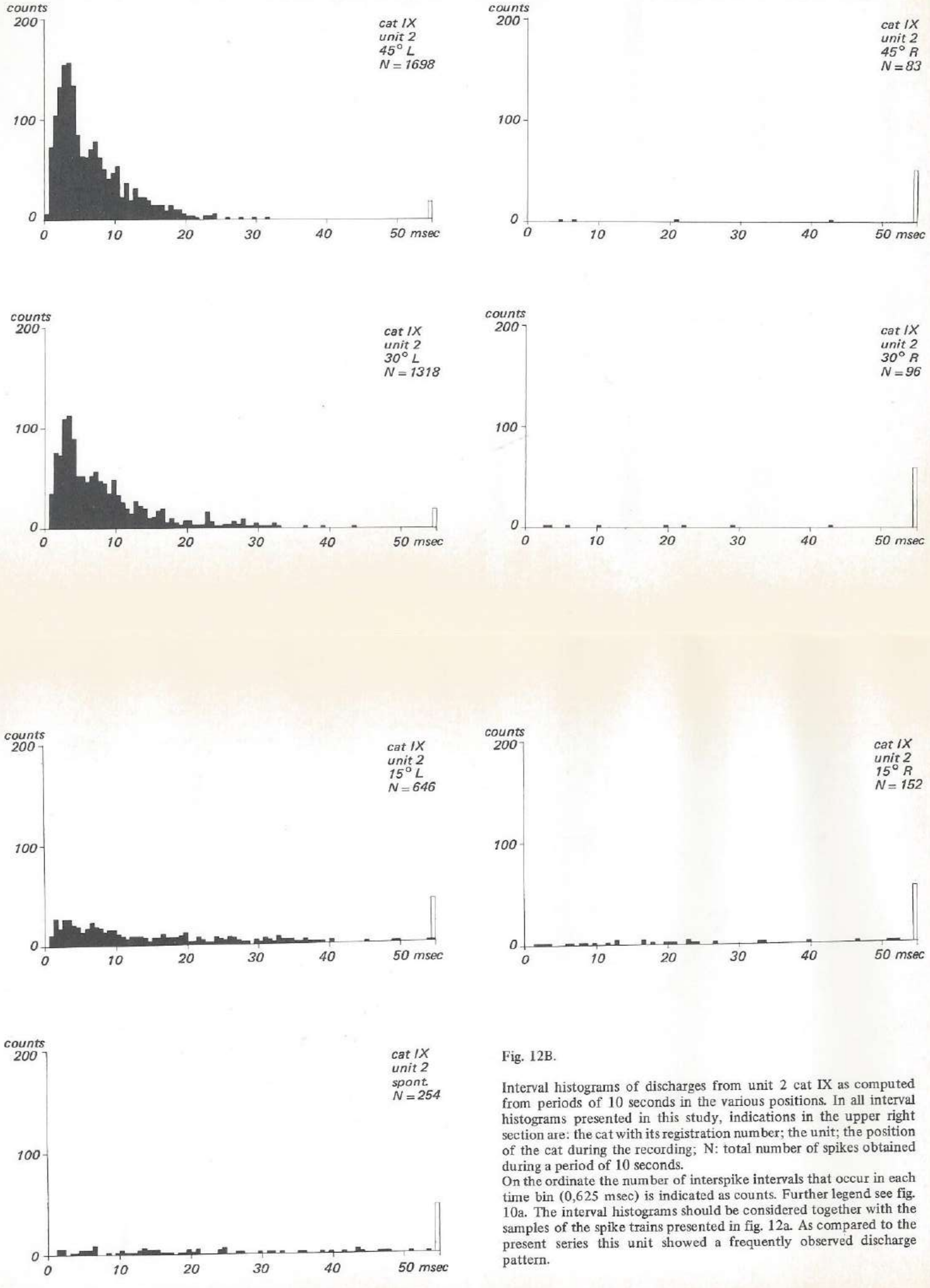
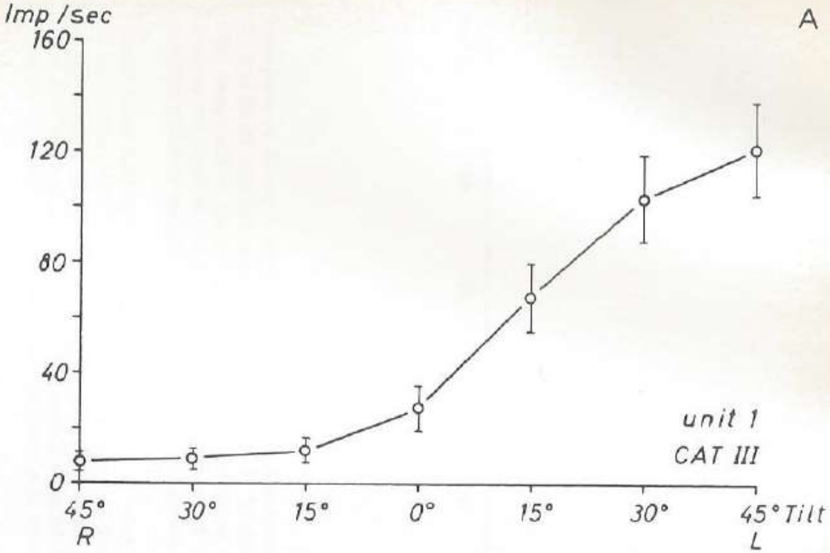


Fig. 12B.

Interval histograms of discharges from unit 2 cat IX as computed from periods of 10 seconds in the various positions. In all interval histograms presented in this study, indications in the upper right section are: the cat with its registration number; the unit; the position of the cat during the recording; N: total number of spikes obtained during a period of 10 seconds.
On the ordinate the number of interspike intervals that occur in each time bin (0,625 msec) is indicated as counts. Further legend see fig. 10a. The interval histograms should be considered together with the samples of the spike trains presented in fig. 12a. As compared to the present series this unit showed a frequently observed discharge pattern.



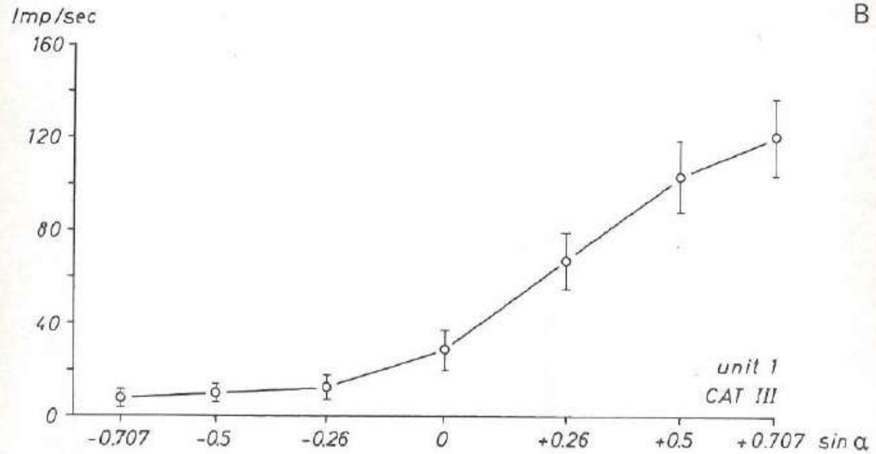


Fig. 13A.

Discharge frequencies in unit 1 cat III plotted against the angle of lateral tilt. Standard deviations are indicated.

Fig. 13B.

Discharge frequencies in unit 1 cat III plotted against sinus α (see fig. 11b). Standard deviations are indicated.

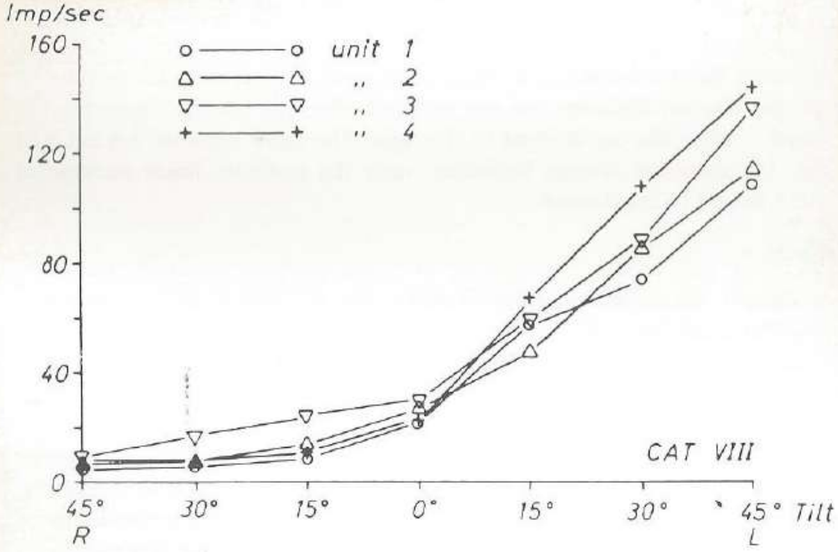


Fig. 14.

Cat VIII: discharge frequencies in all units plotted against the angle of lateral tilt. Note the special behaviour in unit 3 on tilt to the right. This unit also shows the steepest rise of discharge rate on tilt to the left from 30° L to 45° L. Furthermore the perfectly linear increase in unit 4 on tilt to the left should be mentioned.

between 30° L and 45° L. This type of discharge was occasionally observed in different units.

Tilt to the right, however, showed a remarkable sequence of impulse discharges. This unit in 15° R still offers a distinct activity (23 imp/sec) as compared to the spontaneous level. Even at 30° R 18 imp/sec are recorded, while at last in 45° R the minimum discharge frequency is reached at the more usual level of 10 imp/sec.

Thus, this unit showed a special behaviour on tilt to the right. Figs. 15a and b respectively illustrate samples of the spike trains (note different sweep speeds) and the interval histograms in the different positions. The special character of the spontaneous discharge in this unit has already been mentioned (page 49). The interval histogram shows the sharply defined preferred interspike intervals. indicating a rather "regular" firing pattern. On tilt to the left this pattern becomes even clearer, although there is some change towards the shorter intervals. Especially in 30° L the peak at 5 msec is very distinct. In 45° L this picture changes and at about 4 msec interspike-interval a peak is reached. Tilt to the right reveals in 15° R about the same pattern as the spontaneous discharge, however, there is a shift towards the longer intervals. Even at 30° R

and 45° R remnants of this pattern can be observed. As such this unit was unique in our series.
Although there is a distinct increase of impulse discharges on tilt to the left, the spontaneous discharge did not fall markedly – as is most frequently observed – when the cat is tilted to the right. The other units of this cat VIII (fig. 14) show the average behaviour, only the perfectly linear increase of unit 4 should be mentioned.

As already mentioned on page 50 unit 6 cat IV, is the only example of a two-fibre recording in the series of previously operated cats.
These two fibres showed a discharge of approximately the same spike amplitude. The discrimination level of the computing system could not be set in such a way as to abandon one of them.
In fig. 16 (page 64) this unit shows distinct characteristics. In the spontaneous discharge at 35 imp/sec there is no clear indication of the two fibres separately. On tilt to the left there is an exceptionally steep rise of the impulse-discharge frequency to 203 imp/sec in 15° L, which continues somewhat less steeply on further tilt to 30° L (320 imp/sec) and to the maximum at 423 imp/sec in 45° L. This was by far the highest maximum impulse frequency ever met in the present series.
Because the first impression on recording from this unit was that of an injury discharge obscuring the picture, this unit was observed for periods of about 30 sec in each position. There was no change in the discharge pattern and repeated tilts gave the same picture.
Another remarkable fact is the steep fall of discharge frequency on tilt to the right. In fig. 16 standard deviations for this unit are indicated on tilt to the left. Figs. 17a and b (pp. 65-67) respectively show samples of the spike trains and the related interval histograms in the different positions. In 15° L occasional bursts were seen in the spike train, but for longer periods a continuous discharge was also observed.
The interval histogram of the spontaneous discharge shows the average scatter, although there might be some greater tendency to preference for the shorter intervals.
At 15° L a distinct peak is seen about the 3 msec interspike-interval. The second hump at about 35 msec represents the interburst intervals. In 30° L and 45° L the peak about the 2 and 3 msec interval becomes still more pronounced, and comparison with the spike trains indicates a quite regular firing pattern. Special note should be made of the different vertical scale counts in these positions.
On tilt to the right most interspike intervals are missed in our graphs, these are indicated in the high overflow bin. For comparison the histograms were plotted on the same scale as used for the spontaneous level, although in this unit the number of spikes on tilt to the right was too small for appropriate

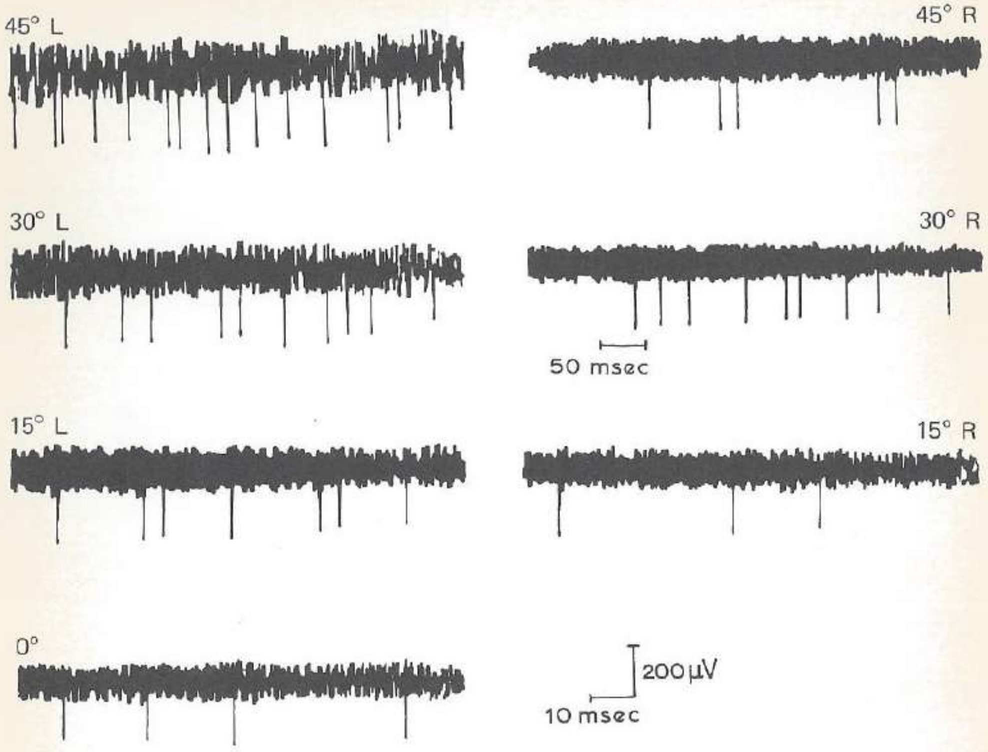


Fig. 15A.

Samples of spike trains recorded from unit 3 cat VIII in the various positions on lateral tilt. This unit in 15° R still shows a distinct activity as compared to the spontaneous level. Only samples in 30° R and 45° R were photographed at a different sweep speed (50 msec). The total number of impulses per second in each position is given in table I page 62.

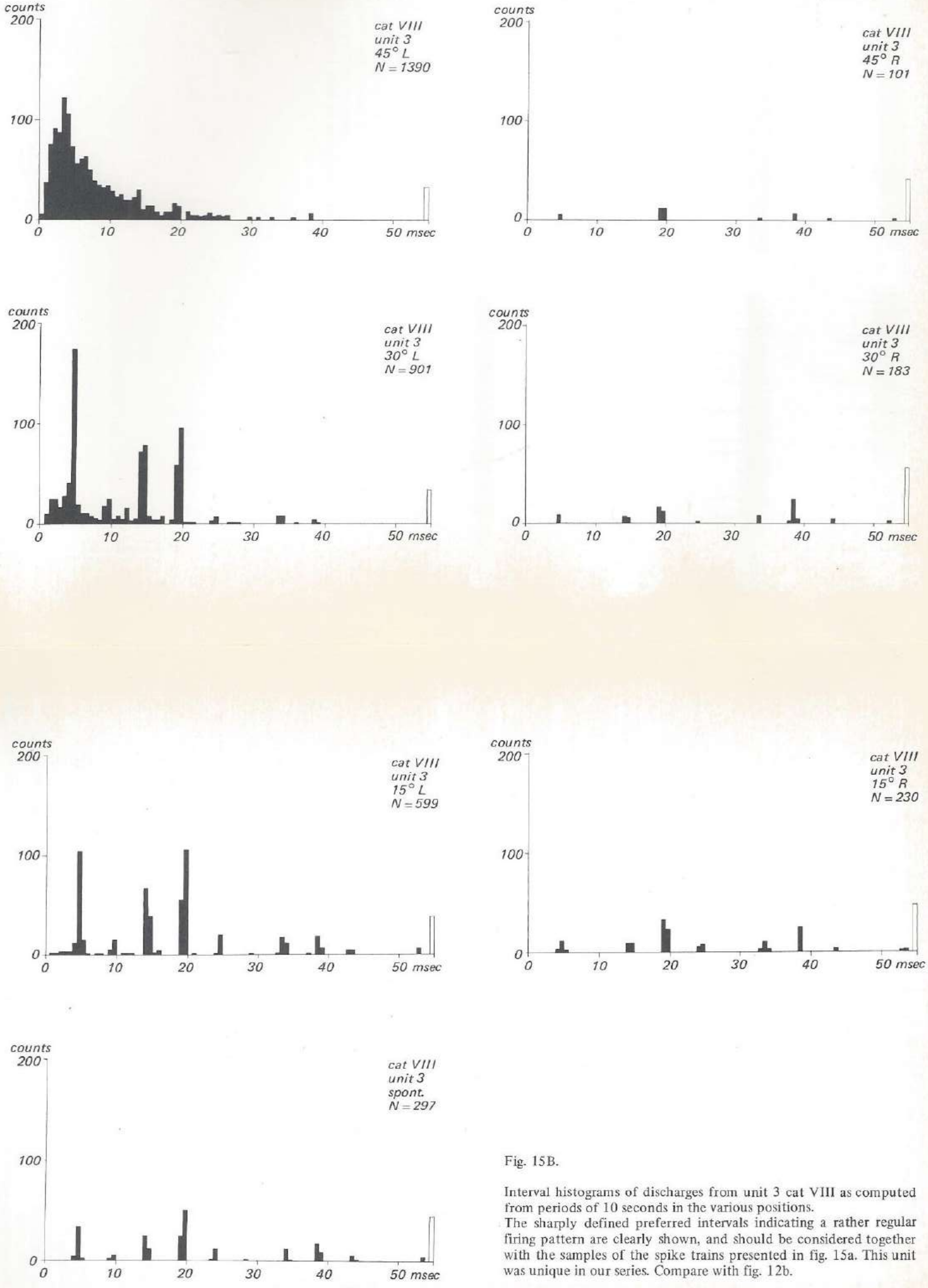


Fig. 15B.

Interval histograms of discharges from unit 3 cat VIII as computed from periods of 10 seconds in the various positions.
The sharply defined preferred intervals indicating a rather regular firing pattern are clearly shown, and should be considered together with the samples of the spike trains presented in fig. 15a. This unit was unique in our series. Compare with fig. 12b.

TABLE I

CAT	IMPULSES PER SECOND								REMARKS
	UNIT	45° L	30° L	15° L	0°	15° R	30° R	45° R	
I	1	168	–	–	25	–	–	5	
	2	151	–	–	22	–	–	9	
II	1	147	–	–	26	–	–	6	
III	1	121	104	69	28	11	9	7	
IV	1	164	101	61	29	13	10	10	
	2	134	112	87	21	15	10	9	
	3	157	103	80	28	15	8	8	
	4	123	90	67	38	17	9	8	
	5	210	149	82	31	22	11	10	
	6*)	423	320	203	35	5	5	4	fig. 17a
	7	148	110	60	22	12	10	9	
	8	185	158	82	26	16	9	9	
V	1	120	81	67	33	15	11	6	
		135	85	64	–	–	–	–	30" in each tilted pos. towards the left
		132	85	66	–	–	–	–	
	2	113	84	59	36	23	18	4	tilt as usual
		114	83	65	36	21	19	5	30" in each tilted pos.
		123	87	68	–	21	19	5	
		112	88	67	–	21	18	5	
	3	139	96	69	29	14	10	9	tilt as usual
		157	94	68	29	19	9	8	30" in each tilted pos.
		144	97	70	–	17	10	7	
		138	98	64	–	17	9	7	
	4	134	93	70	37	15	8	7	tilt as usual
		133	87	63	37	11	4	4	30" in each tilted pos.
		138	85	65	–	11	3	3	
		139	87	66	–	10	5	3	
VI	1	112	83	56	18	8	7	5	
VII	1	142	105	59	23	12	9	6	
	2	151	122	74	29	12	8	6	
	3	121	96	68	21	14	–	–	
	4	116	72	49	26	14	10	8	
VIII	1	111	76	58	22	8	7	4	
	2	117	87	48	25	13	8	5	
	3	139	90	60	30	23	18	10	fig. 15a
	4	148	110	69	24	11	8	6	
IX	1	138	108	81	31	18	12	9	
	2	170	132	65	25	15	10	8	fig. 12a
1	1*)	411	335	238	29	5	5	4	
	2	144	108	76	20	11	7	6	
	3	173	–	–	17	–	–	5	
		160	–	–	17	–	–	4	after 35′ in 45° L
	4*)	351	288	216	34	14	10	7	
2	1	122	95	54	26	16	12	6	
	2	154	121	83	36	15	10	10	
	3	162	93	64	31	11	7	6	

Table I:

Discharge frequencies in all 34 units at standard positions on lateral tilt.
0°: normal position. L: tilt towards the left. R: tilt towards the right.
The number of impulses per second calculated from the total number of discharges during a period of 10 seconds.
Cats previously operated upon are indicated with roman numerals, the normal cats with arabic numerals. Two-fibre recordings are indicated with an asterisk.
In cat V a tilt in the usual way was followed by a tilt with stops of 30 seconds in each position.

processing on this scale. In fig. 16 also several other units of cat IV are shown for comparison.

4. SOME EXPERIMENTS ON ADAPTATION

In chapter I it was concluded from literature data that the otolith organs show no or only a very small adaptation of the macular receptors concerned with indication of position.

To investigate adaptation in our experiments, cat V was tilted – as usual very slowly – in such a way that each position was occupied during a period of 30 seconds or more (see table I).

After a first tilt in the usual way, the cat was brought into the normal position again. Then a tilt to 15° L was performed and the cat kept stationary in that position for 30 seconds. The cat was returned to the normal position and a second tilt to 30° L followed etc.

This procedure was done while recording units 2, 3 and 4. Unit 1 was only examined this way on tilt to the left.

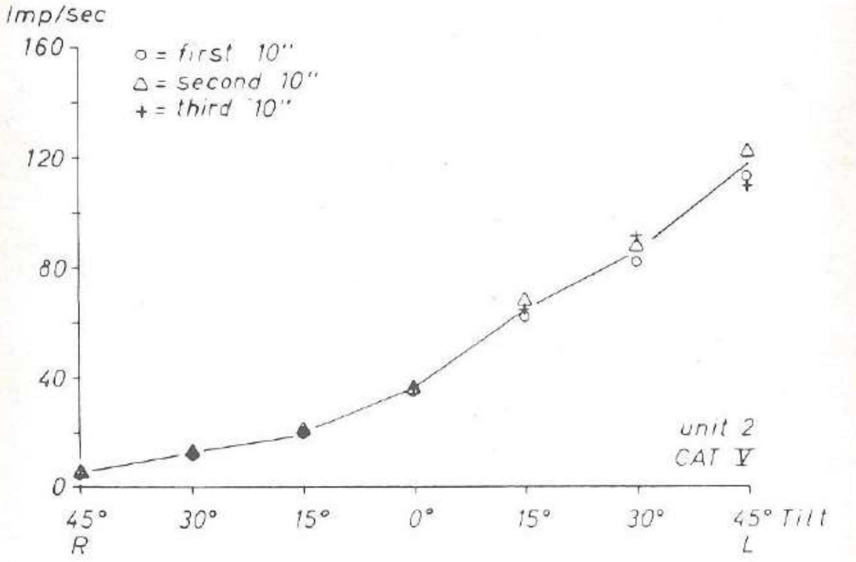


Fig. 18.

Discharge frequencies in unit 2 cat V plotted against the angle of lateral tilt. Each position was occupied during a period of 30 seconds. This period was divided in three 10-second periods, which were processed separately. These periods are indicated by different symbols. The line connects the average values in each position. In this unit the maximum spread of 9% between consecutive stages of recording was observed in 45° L.

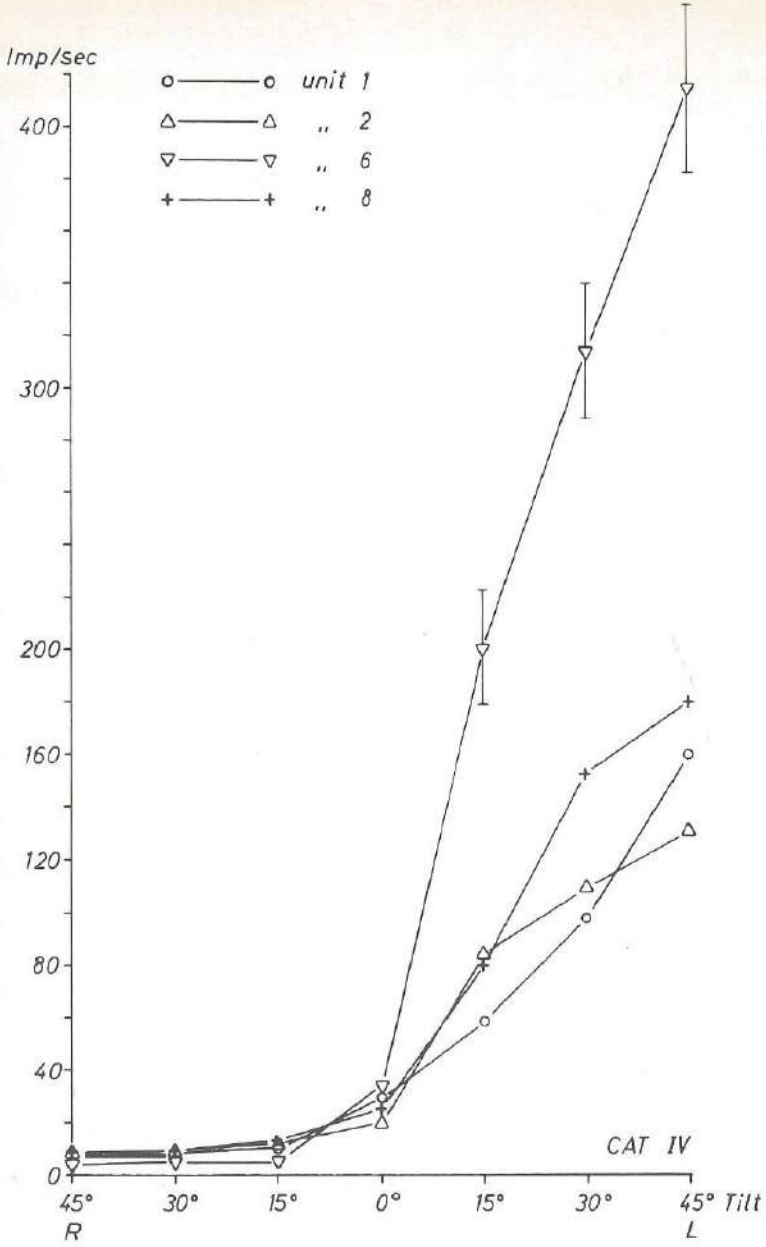


Fig. 16.

Discharge frequencies in units 1, 2, 6 and 8 cat IV plotted against the angle of lateral tilt. Unit 6 is the only example of a two-fibre recording in the series of previously operated cats. In the exceptionally steep rise on tilt to the left, standard deviations are indicated in unit 6.

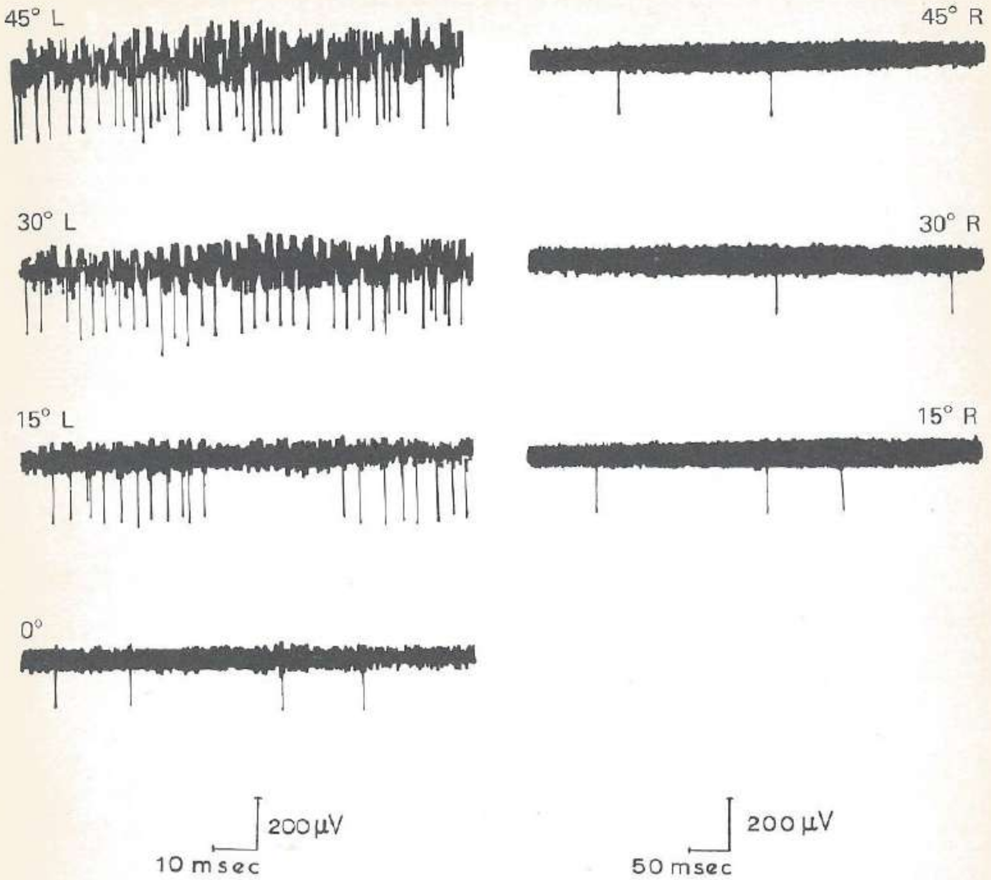


Fig. 17A.

Samples of spike trains recorded from unit 6 cat IV in the various positions on lateral tilt. In the normal position (0°) this two-fibre preparation showed the picture of one unit. On tilt to the left the very steep rise of impulse-discharge frequency is clearly demonstrated. Calibration for samples shown above. The total number of impulses per second in each position is given in table I page 62.

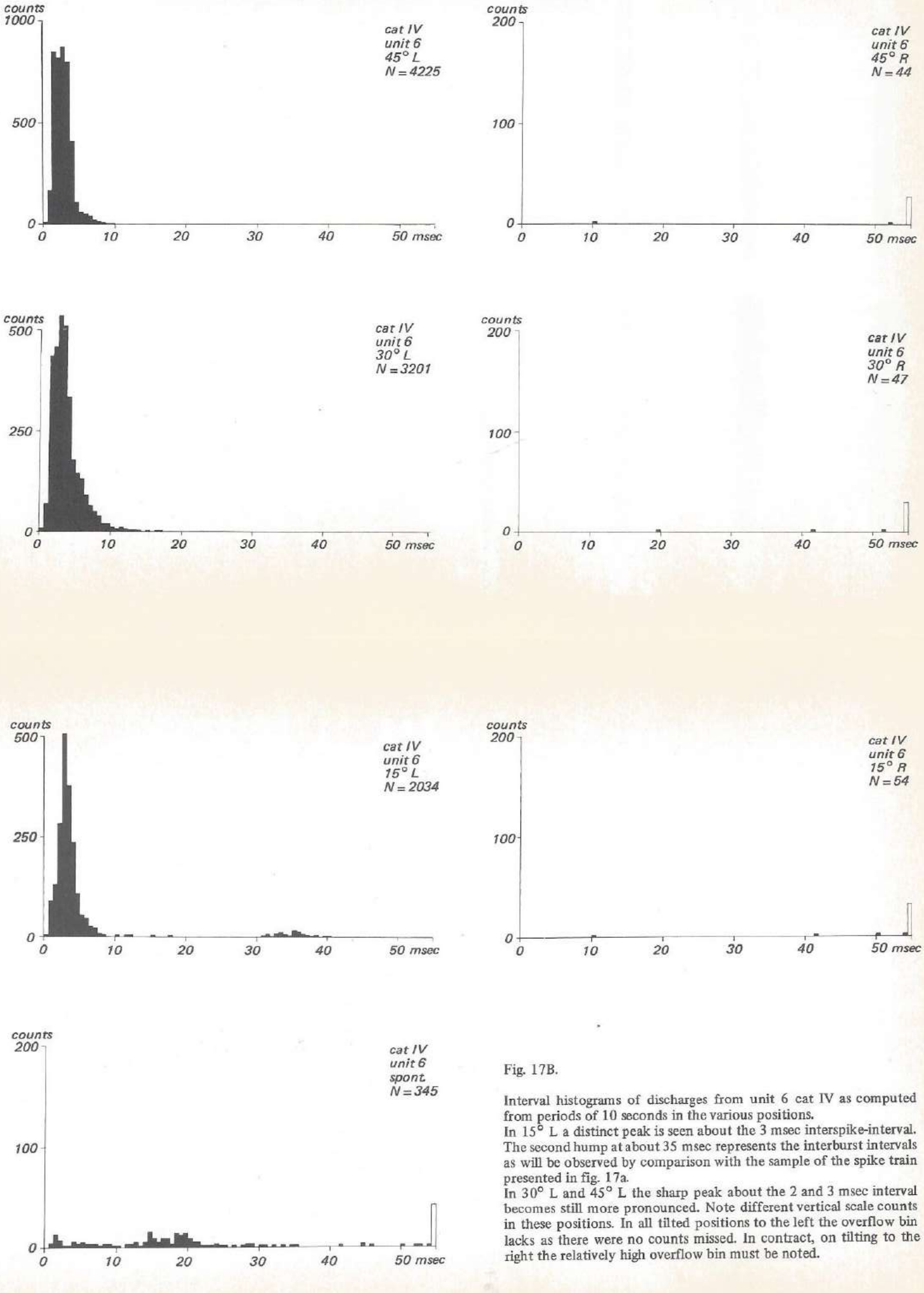


Fig. 17B.

Interval histograms of discharges from unit 6 cat IV as computed from periods of 10 seconds in the various positions.
In 15° L a distinct peak is seen about the 3 msec interspike-interval. The second hump at about 35 msec represents the interburst intervals as will be observed by comparison with the sample of the spike train presented in fig. 17a.
In 30° L and 45° L the sharp peak about the 2 and 3 msec interval becomes still more pronounced. Note different vertical scale counts in these positions. In all tilted positions to the left the overflow bin lacks as there were no counts missed. In contract, on tilting to the right the relatively high overflow bin must be noted.

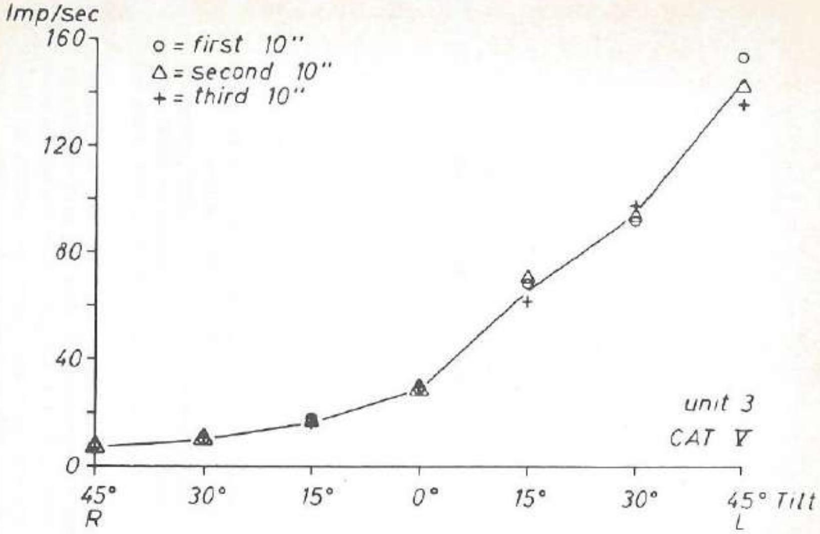


Fig. 19.

Discharge frequencies in unit 3 cat V plotted against the angle of lateral tilt. For legend see fig. 18. This unit showed the maximum spread of 12% in 45° L.

Fig. 18 illustrates the discharge frequencies of unit 2 cat V. Each 30 sec period was divided in three 10-sec periods, which were processed separately by the computer. These periods are indicated by different symbols. The line connects the average values in each position.
It is obvious that there is no marked difference in discharge frequency in whatever position between the succeeding stages of recording. In 45° L a maximum difference of 9% was observed.
Fig. 19, unit 3 cat V shows the maximum spread of 12% at 45° L. This was the greatest difference observed in this series. Unit 4 cat V (table I) only shows a difference of 4% at most. Another point has to be stressed: there is no difference of impulse-discharge frequency whether the tilted position is reached from the normal position or from a tilted position (see table I).
Concerning the subject of adaptation we mention one of the cats, not operated upon before. Unit 3 cat 1 was succesfully recorded from during a period of 35 minutes.

TABLE II (unit 3 cat 1)

Impulses per second

45° L	normal	45° R
173	17	5
160 (after 35′)	17	4

As will be seen in table II, this unit was examined only in the extreme positions. Tilt to the left was maintained for 35 minutes, the second sample was taken at the end of this period. On tilt to the left a decrease of discharge frequency is observed in the second sample of merely 7,5%.
From these data we may conclude that in these experiments no adaptation was observed over a period of 30 seconds. In one instance even over 35 minutes. Concerning the efferent influence: this will be discussed in chapter V.

5. DATA ON NORMAL CATS

In the present series of experiments two cats were used for checking purposes. No preliminary operations as described in chapter III were performed.
The microelectrode was introduced into the utricular nerve in the same area as recorded from in the other animals.
In these cats there could be confusion with spontaneous discharge in the ampullary nerves. After spontaneous discharge had been observed in every microelectrode position, a tilt was performed in the transversal plane.
In any case of observed frequency modulation we assumed the recordings to originate from a primary utricular fibre, as the slow way of tilting was far below the threshold for angular accelerations stimulating the ampullary nerves. Besides, Lowenstein and Roberts (1950) observed no significant variation of the response on full-circle tilts at various speeds within the range 1.5°/sec to 5.2°/sec.
In some instances, however, a spontaneous discharge not influenced by tilt, was observed. These latter recordings most probably were from ampullary nerve fibres.
Fig. 20 illustrates discharge frequency modulations on tilt in the transversal plane for the distinct units in cat 1 and cat 2. These units showed the normal distribution of spontaneous discharge frequency.
In the case of units 1 and 4 cat 1, two-fibre preparations as described before, were recorded from. These units exhibited the same picture of discharges as unit 6 cat IV. There was a very steep rise from the spontaneous discharge level up to 15° L, whereafter the rise diminished somewhat at 30° L and 45° L. For comparison unit 2 cat 1 (single fibre) shows the usual picture of discharge frequencies in the various positions. In the previous section unit 3 cat 1 was already mentioned. Cat 2 (fig. 20) units 1 and 2 show the more frequently observed discharge pattern, while in unit 3 there is a rather steep rise in discharge frequency from 30° L up to 45° L. This was less common, but unit 3 cat VIII for example showed the same picture.
From these experiments we may conclude that on tilt in the transversal plane there is no difference in reaction whether the cats were previously operated upon or not. The implications will be discussed in chapter V.

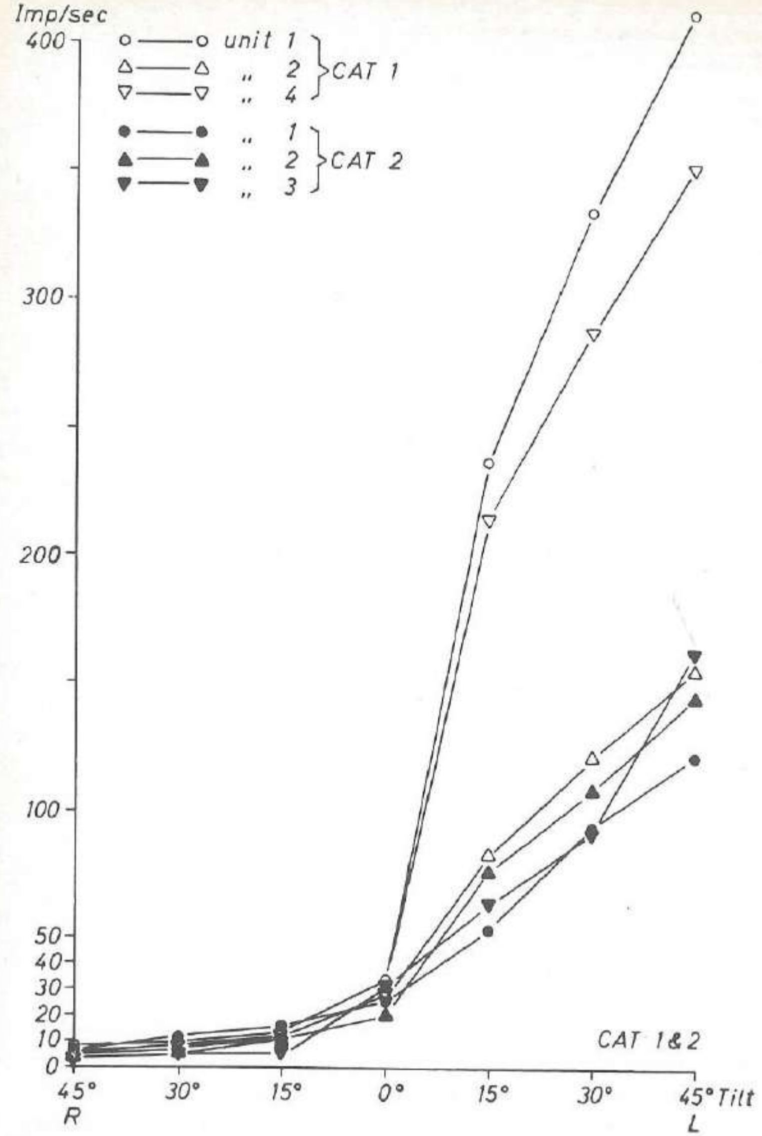


Fig. 20.

Discharge frequencies in all units recorded from in cats 1 and 2 plotted against the angle of lateral tilt. These normal cats show the same spread of responses as observed in the previously operated cats.

In units 1 and 4 cat 1, two-fibre preparations were obtained. These exhibited similar discharge-frequency modulations as compared to unit 6 cat IV (the two-fibre recording in the series of previously operated cats, fig. 16). In the normal position these former units – in contrast with the latter unit – also showed a clear indication of the two fibres separately on observation of the spike trains. Unit 3 cat 1 is not indicated as this unit was examined only in the extreme positions (see table II page 68).

6. FURTHER OBSERVATIONS ON THE MAXIMUM IMPULSE DISCHARGES

a. Distribution of rates of maximum discharge as compared to the spontaneous level.

Fig. 21 represents the distribution of the maximum discharge frequency at 45° L, expressed as multiples of the spontaneous discharge level for all 34 units processed.

The number of units in previously operated cats is indicated as black bars, those in normal cats as white bars.

It is shown that the preferential rate of increase is 4 to 6 times the spontaneous rate. All rates above 10 times spontaneous frequency were recorded in two-fibre preparations.

Apparently for single fibres there is an unimodal distribution, without difference between the normal and the previously operated cats.

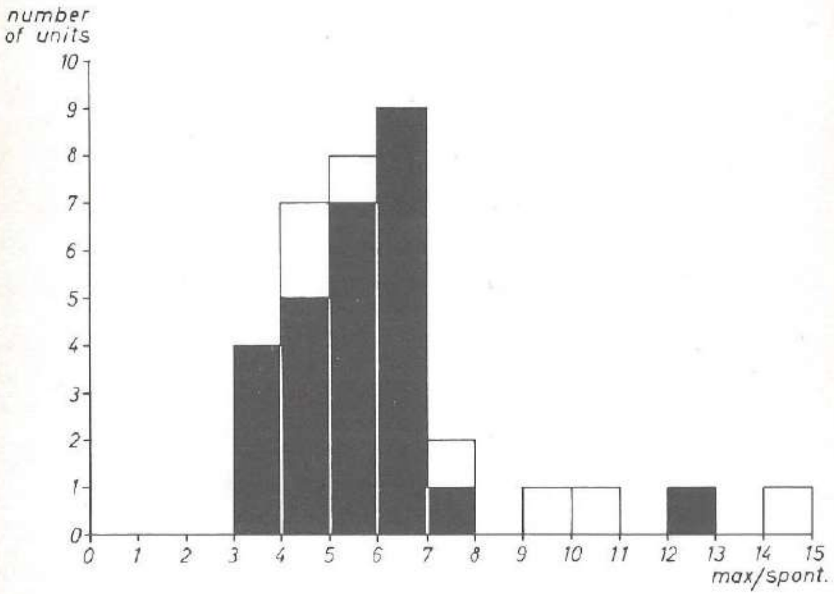


Fig. 21.

Distribution of the maximum discharge-frequency at 45° L, expressed as multiples of the spontaneous-discharge-level for all 34 units processed. The number of units in previously operated cats is indicated as black bars, those in normal cats as white bars.

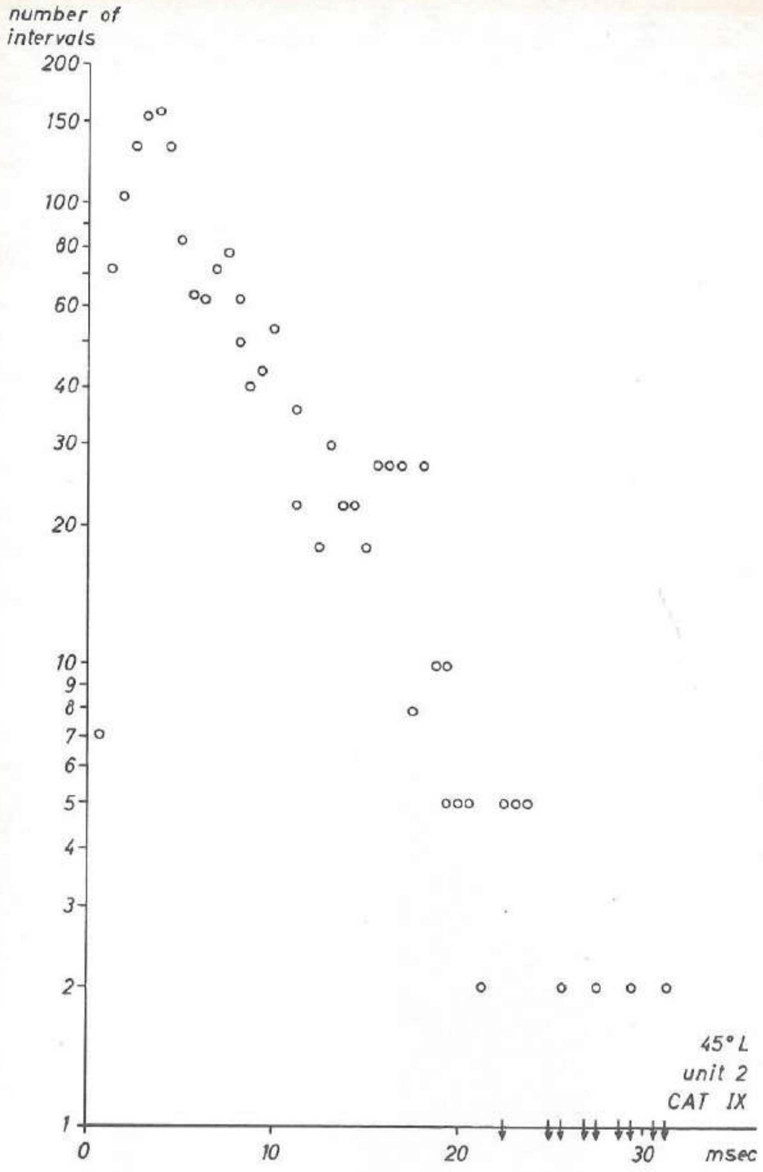


Fig. 22.

Interval histogram of unit 2 cat IX in 45° L (fig. 12b) plotted on a semilogarithmic scale. Maximum discharge rate: 170 imp/sec. The total number of spikes obtained during a period of 10 seconds was 1698. Arrows indicate those bins in which no intervals were counted.

b. Time pattern of the maximum discharge.

Further information on the time pattern of the maximum discharge is found in a semilogarithmic re-plot of the interval histograms at 45° L, that were presented in figs. 12b, 15b and 17b.

A re-plot of the interval histogram of unit 2 cat IX on a semilogarithmic scale as shown in fig. 22, reveals that, except for the shortest intervals, all points roughly fall on a straight line. This demonstrates an exponential decay of the number of interspike intervals as a function of time. Unit 3 cat VIII, fig. 23, approximately shows the same picture. Unit 6 cat IV, fig. 24, exhibits a very steep decay, also matched by a straight line.

In statistical nomenclature the exponential decay is indicative of a Poisson process. This would implicate that the probability of firing is independent of the moment of the previous discharge.

It must be stated that this hypothesis was not directly verified in this study. Kiang (1965), however, in his paper on primary auditory fibres, suggested that one would expect a greater number of short intervals if the spontaneous activity was generated by a Poisson process. This deviation was attributed to the refractory properties of the units.

7. SOME SUPPLEMENTARY EXPERIMENTS

Some supplementary experiments were performed to investigate the response of macular receptors as recorded from primary afferents in the utricular nerve to tilt in the sagittal plane and to linear accelerations in the horizontal plane. These latter experiments comprise 4 cats, two of these were previously operated as described in chapter III.

a. Response to tilt in the sagittal plane.

The tilting device was adapted in such a way, that the cat board was placed transversally to the long axis. Stability conditions this way restricted the range of for-and-aft tilting to merely 15°.

As the nature of stimulation is fundamentally equal to tilt in the transversal plane and the object being only to obtain a qualitative impression of the responses, this stimulus range was regarded sufficient. The experiments were performed on both a previously operated cat and a normal cat. A total of 6 units was recorded from.

On tilting to the nose-down position in general an increase of the spontaneous discharge was observed, while on tail-down tilting a decrease was noticed. In one unit (in the normal cat) a reversed discharge pattern was observed. With this exception there was no difference in responses between the two cats. The findings can be explained on basis of the morphological polarization pattern of the utricular macula (fig. 1, page 13).

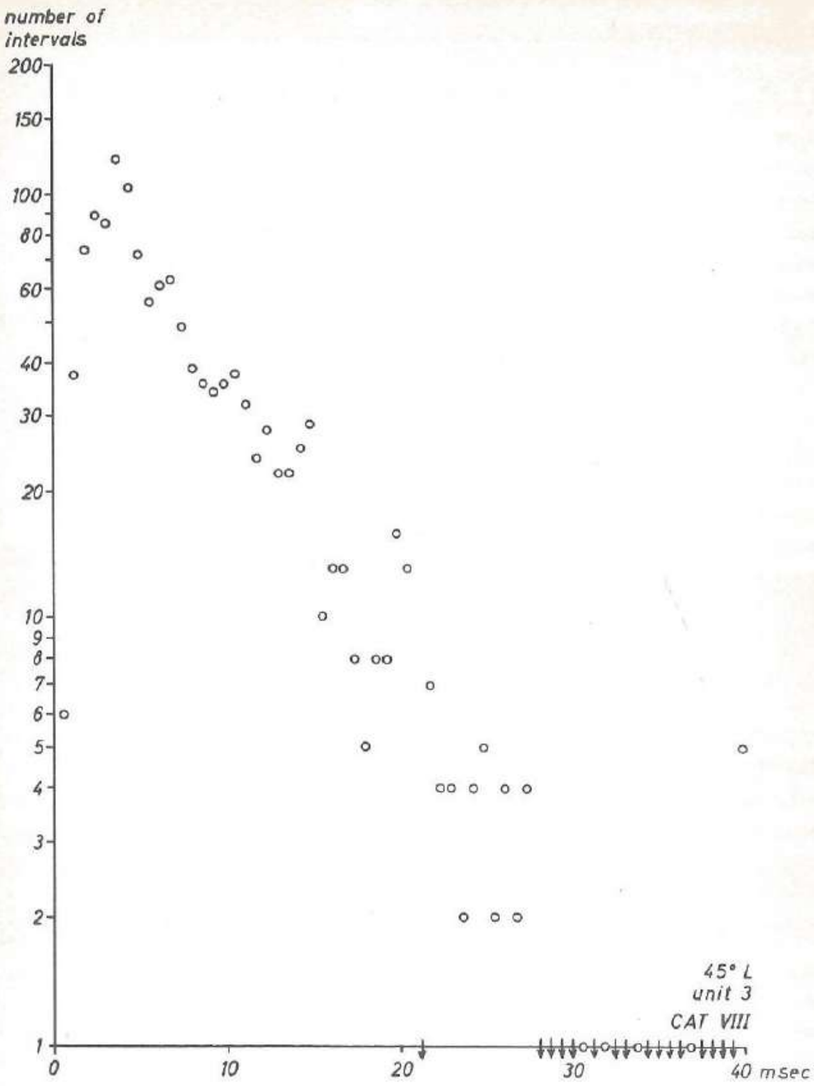


Fig. 23.

Interval histogram of unit 3 cat VIII in 45° L (fig. 15b) plotted on a semilogarithmic scale. Maximum discharge rate: 139 imp/sec. The total number of spikes obtained during a period of 10 seconds was 1390. Arrows indicate those bins in which no intervals were counted.

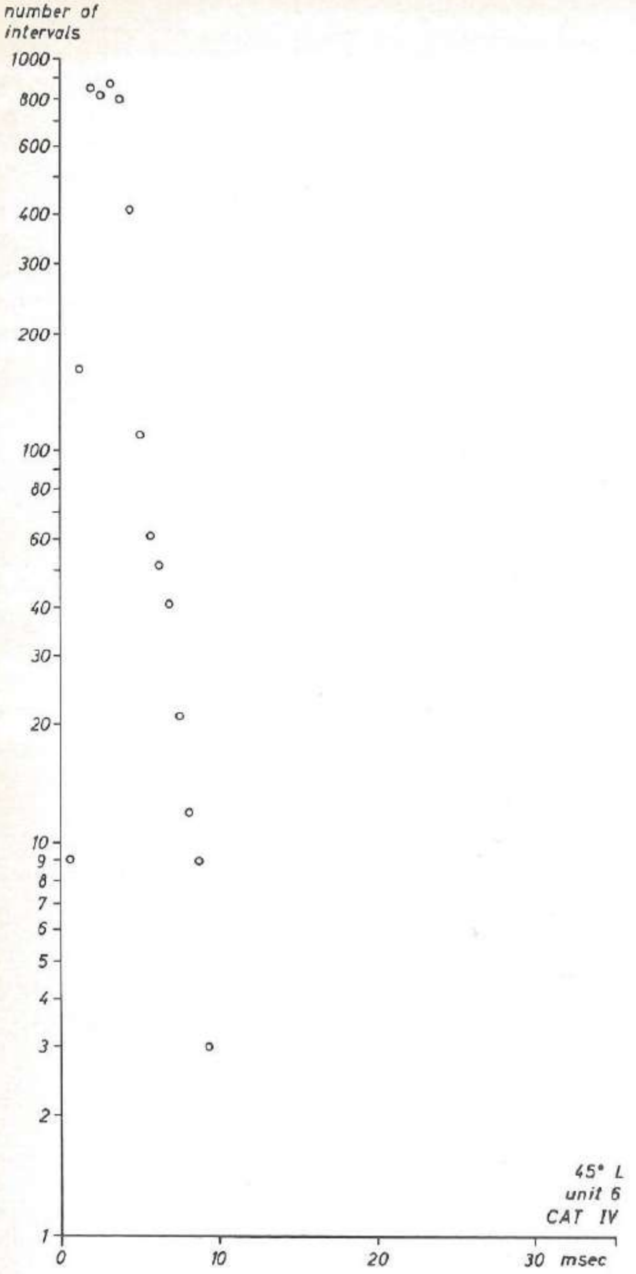


Fig. 24.
Interval histogram of unit 6 cat IV in 45° L (fig. 17b) plotted on a semilogarithmic scale. Maximum discharge rate: 423 imp/sec. The total number of spikes obtained during a period of 10 seconds was 4225.

b. Responses to linear accelerations in the horizontal plane.

For the application of linear accelerations in the horizontal plane a parallel swing was used. A normal and a previously operated cat were tested. The following observations represent a preliminary report.
The cat in the tilting device (as described in chapter III) was mounted on the parallel swing. The movements of the swing were indicated on the oscilloscope screen by means of a potentiometer device placed at one of the turning points of the steel bars.
With the swing fixed in mid-position, the localization of the microelectrode in the primary afferent utricular nerve (at the left side) was confirmed by the increase of the spontaneous discharge on tilt to the left and decrease on tilt to the right side down.
The cat was brought into the normal position again and stimulation was applied by swinging sideways. The sinusoidal movements of the swing evoked a distinct frequency modulation of the discharges. An increased discharge frequency was observed when inertia movements of the otolith were directed laterally.
At a first qualitative impression the maximum response occurred approximately at the point of maximum acceleration (to the right) or deceleration (to the left) in the extreme left position of the swing and "vice versa".
Both cats showed the same pattern of response. These preliminary observations need further quantitative analysis, amongst others as to the determination of a time-lag between stimulus and response, and will be complemented by further experiments.

CHAPTER V

DISCUSSION

1. GENERAL CONSIDERATIONS

a. Check on physiological circumstances in recording situation.

The present study deals with the analysis of stimulus coding in the utricular nerve of the cat. It is of paramount interest that utricular function is preserved in the preliminary surgical procedures for functional elimination of the lateral and anterior semicircular canals as described in Chapter III. Post-operatively the animals did not show tilting of the head to the left side. Together with the electronystagmographical recordings on the parallel swing which show good compensatory eye movements these features are indicative of normal utricular function at the left side.
It must be added that on recording from the utricular nerve just inside the internal acoustic meatus, (as in general) the acute neurophysiological approach involves interference with normal physiological circumstances.
Since exact recording from the utricular nerve, as far peripherally as possible, could not be achieved stereotactically, the surgical approach included partial decerebration. Concomitant bleeding was minimized by constant irrigation with warm Locke solution, and could be stopped adequately by the application of Spongostan or bone wax. In general, the animals were in good condition during recording circumstances.
In most experiments Nembutal was given only during the surgical procedures. Administration of supplementary Nembutal was rarely necessary. During recording Flaxedil was used to prevent voluntary movements and the cats were artificially respirated.

b. Some remarks on vestibular receptors in vertebrates.

The labyrinthine receptors are secondary sensory cells. In Chapter I a description has been given of the special anatomical features and a brief review of electrophysiological data from literature.
The macula is covered by a gelatinous mass containing mucopolysaccharides (Dohlman et al. 1959).
Vilstrup and Jensen (1961) described a "displacement potential" in vitro on movement of acid mucopolysaccharides. The hypothesis on transformation of mechanical energy into electrical activity as presented by these authors includes the fact that these acid mucopolysaccharides form complex connections with proteins.
The distance between two ciliae on the surface of the hair cell corresponds

with the length of two such macromolecular complexes. Deviation of the ciliae would provoke potential changes.

Trincker (1959 a) found that the ciliae on the apical part of the hair cells exhibit an electrostatic potential. Tangential displacement of the sensory hairs by movement of the otolith membrane (utricle) provoked depolarization in the direction towards the kinocilium, and hyperpolarization on deviation of the hairs in the reverse direction.

The effected depolarization constantly exceeded hyperpolarization (Trincker 1959 a). The bi-directional sensitivity of the sensory hair bundle coincides with the morphological polarization pattern.

Flock and Wersäll (1962) for the lateral line organ of fish, and Lowenstein et al. (1964) for the utricular macula of the guinea pig indicated that depolarization of the hair cell evokes increased impulse discharge frequency in the afferent nerve fibres. The mechanism of stimulus transmission to the afferent fibres, however, is not clear. The presynaptic structures at the basal part of the hair cells (Smith and Sjöstrand 1961) are indicative of chemical transmission. The nature of the possible transmitter substance involved with activation of the afferents is unknown. As to the efferent fibres, acetylcholine could be of importance (Dohlman 1958; Desmedt and Monaco 1961; Rossi and Cortesina 1963; Rossi et al. 1964).

The morphological polarization pattern in the utricular macula of mammals, fig. 1, page 13 (Spoendlin 1966), in general corresponds with that in teleost fish (Flock 1964).

It is obvious that a laterally directed shearing force is excitatory for the central part of the macula and inhibitory for the lateral part. Only cells with the kinocilium pointing exactly in the lateral direction will be maximally stimulated or inhibited by tilting in the transversal plane. On tilting about any horizontal axis there will be a plane of maximum stimulation for those macular regions morphologically "polarized" in the direction of that plane. Units in intermediate positions with respect to the stimulating plane will respond accordingly to the degree of deviation. Lowenstein and Roberts (1950) described preparations with only a single functional unit which showed a response both on lateral and on fore-and-aft tilting.

The utricular macula is curved, and the "plane" shows a backward slope of approximately 30° to Reid's base line with a lateral inclination of some 10°. This implies an improvement of the indication of position.

The potential changes produced by all hair cells of the utricular macula (and all other maculae) will be simultaneously perceived at central levels. Stimulus coding on central level is based upon the frequency-modulated input from the peripheral receptors. In the transversal plane position is indicated by the relative stimulus difference between the left and the right side.

In general the labyrinthine receptors show a spontaneous discharge by virtue of which a peripheral threshold hardly exists and the functional range is enlarged (Lowenstein 1956).

In most vertebrates the utricle is described as a position receptor, although sensitivity to vibration is mentioned (as all derivatives of the otic placode show vibrational sensitivity). The function of the saccule in phylogenetic development is more variable. The lagena lacks in mammals.

If we want to compare data from lower vertebrates with those in mammals these facts must be borne in mind.

As the utricle shows a rather uniform function in the vertebrate series, there may be a marked difference in central processing with the development of the central nervous system.

2. RESULTS AS COMPARED TO EARLIER OBSERVATIONS

Stimulus range in the present experiments covered a 45° tilt to both sides in the transversal plane about a rostro-caudal horizontal axis. It should be stressed that quantitative data are restricted to this plane and concern static stimulation of the otolith organ.

Only Lowenstein and Roberts (1950) thus far presented quantitative data on primary afferents in the utricular nerve. Recordings were obtained from separate utricular fibres in the isolated labyrinth of the thornback ray, during full-circle tilts in opposite directions.

These tilts were mainly performed about the transversal or the longitudinal axes. Strictly speaking only the few recordings on interrupted tilts can be used for comparison with our data. As such, an example of a "static" position receptor in a two-fibre preparation was described by these authors. The frequency modulation was slightly less, and at a somewhat higher level as compared to the continuous tilt of the same preparation. Another preparation in each position showed a return of discharge frequency to the basic level. This type, called "out-of-position" receptor, is suited to signal positional change as such. On continuous full-circle tilts the positions of maximum and minimum discharge showed variations with the direction of the tilt. Trincker (1965) indicated the possibility of unphysiological stimulation in these tilts.

a. Spontaneous activity.

The utricular primary afferents in the cat show a rather regular spontaneous discharge in the normal position as described in a preliminary note (Beerens 1969). Discharge rate was observed at a slightly higher level (20-40 imp/sec) as compared to findings in lower vertebrates. When inspecting the spike train at lower sweep speeds one finds a great resemblance to the discharges presented in the thornback ray. No previous processing of data on discharges from vestibular primary afferents or secondary neurons in the form of interval histograms has been presented in the literature.

The interval histograms as presented in this study reveal a more scattered distribution of the interspike intervals of the spontaneous discharges (figs. 9a, 10a) as would be observed by conventional methods. In our series, only in one instance a quite distinct distribution of preferred intervals was seen (unit 3 cat VIII, figs. 9b, 10b), indicating a more regular firing pattern.

The extremely regular discharge rate described by Schoen (1957) on recording from medullar levels of fish might be characteristic of vestibular second-order-neurons in this species.

About spontaneous discharges in the mammalian vestibular nuclei, only some incidental remarks but no quantitative data have been presented so far.

b. Static position and its stimulus coding in the utricular nerve.

In general the present findings in the cat show great conformity with the data on "static" position receptors in the utricular nerve of the thornback ray (Lowenstein and Roberts 1950).

Although the stimulus range is restricted in our series, it is obvious that a distinct increase of discharge rate occurs on tilting to the recorded (left) side down. On tilting to the right side a decrease is noticed at 15° R, whereafter a rather constant lower level is attained in 30° R and 45° R. The term "residue" level is introduced as neither of the units showed a decrease of discharge rate up to zero.

In the elasmobranch mostly a "basic" level was reached in the extreme inhibiting position (180° opposite to maximum stimulation), only a few preparations showed a complete absence of discharges.

In our series unit 3 cat VIII (fig. 14 page 57) showed a different discharge pattern. The "regular" discharges on tilt to the right side, up till 30° showed conformity with the spontaneous pattern. While even at 45° R remnants of this pattern could be observed in the interval histogram (fig. 15b page 60). The origin of these responses is not clear. On pure speculation these could be assumed to be asymmetrical borderline recordings at the point of reversal of the morphological polarization pattern. The distinct decrease of the discharge rate at 45° R, however, does not support this conception.

Mostly the discharge frequencies plotted against the angle of tilt in the stimulating direction of the tilting, indicate an approximately linear relation. Individual responses, however, vary in several ways. Some units show a linear increase up till 30° L, after which the agreement becomes poor, while in several units a rather steep rise on greater angles of tilting is observed.

If discharge frequencies are plotted against sinus α (see Chapter IV) the conformation with linearity becomes more obvious in several units; but in those units which show the steepest rise at the greater angles of tilting divergence of linearity is accentuated. When standard deviations are included the results point to a direct proportionality, but the latter unit-type keeps its different pattern.

The possibility of introducing minor variations in stimulus conditions by tilting the device by hand must be considered in this respect. Another fact that could influence the shape of the curve might be the spatial position of the macular area recorded from.

The data of Lowenstein and Roberts (1950) on interrupted full-circle tilts show a better resemblance to a sine curve in the stimulating direction; however, these recordings were from two-fibre or few-fibre preparations which includes the averaging of responses. The indication of position in the inhibitory range of the tilt is poor; this is in agreement with our findings.

On continuous full-circle tilts an improvement of bi-directional sensitivity is shown, but in the stimulating direction indication is still better.

In the vestibular nuclear regions of fish, Schoen (1957) found several types of unit discharges. Only about 30% showed a bi-directional sensitivity.

Although curves that show a sinusoid pattern were described, we found some deviations from linearity if the discharges were plotted against sinus α.

Other units were described which presented a distinct rise of discharge frequency on tilting in one direction, while on tilting to the opposite side a basic level is gradually reached.

These latter uni-directional units show the greatest conformity in discharge pattern both with the present data and with those on interrupted tilts in the thornback ray.

On the ground of the polarization pattern of the utricular macula we have to consider the possibility that most recordings in the present experiments were obtained from central macular regions, since invariably the discharge rate increased upon tilt to the left side down.

As Lowenstein and Roberts (1950) stated, this does not imply that they are necessarily derived from neighbouring neuromasts, on account of the fanlike spread of the end-ramifications of the afferent nerve fibre upon entering the macula.

The recordings from the lateral parts of the macula in the thornback ray showed increased activity on side-up tilting. In comparison with the present findings and on the ground of the corresponding polarization pattern of the utricular maculae in fish and cat, we might tentatively assume that the central and lateral macular regions receive a separate nerve supply. Up till now, however, anatomical evidence has not yet been presented.

As described in this study, the "static" position receptors in the cat also show resemblance to type I gravity receptors in the frog (Ross 1936), and to statocyst type I receptors in crustaceans (Cohen 1955). A representation of this type of receptor in the vestibular nuclei in the cat was described by Adrian (1943), Cramer (1962), Jones (1966) and Peterson (1967).

In the present experiments no evidence was found for the existence of "out-of-position" receptors as described in the utricular nerve of the thornback ray by Lowenstein and Roberts (1950), and in crustaceans by Cohen (1955). The type II response described in the cat by Rupert et al. (1962) displayed a very constant impulse discharge which only showed a short change at any movement independent of position. As described in Chapter I these recordings could have been from second-order neurons.

The absence of "into-level" responses in the utricular nerve of the cat is understandable since these responses specifically originated in the lagena of lower vertebrates.

The recordings in the two-fibre preparation (unit 6 cat IV, fig. 16) showed a very steep rise of the discharge rate on tilting to the left side down, and a sharp decrease on tilting in the opposite direction.

In normal position the picture was that of one unit (fig. 17a). Although conclusions on data related to this particular unit must be regarded as purely speculative, it seems tempting to assume recruitment of spontaneously silent receptors. In macular receptors, however, only once has a unit which was inactive over a considerable part of the stimulus range been described (Lowenstein and Roberts 1950).

Recruitment of spontaneously silent units, however, considerably improves the total impulse discharge in the canal cristae (Groen et al. 1952; Lowenstein 1955). In Deiters' nucleus (cat), Peterson (1967) described the occurrence of spontaneously silent cells which showed large changes in firing rate when the preparation was tilted.

The average ratio between spontaneous and maximum frequencies observed in the present study as being approximately 1 to 6 is distinctly greater than the ratio found in lower vertebrates and in secondary neurons in the vestibular nuclei of the cat (Peterson 1967). A correlation of the spontaneous discharge level to the maximum frequency during tilting was not found (table I, page 62). The time pattern of maximum discharge in re-plots of the interval histograms on a semilogarithmic scale (figs. 22, 23, 24) showed a sharp rise of the number of interspike intervals to a peak before 10 msec and a decay that appears to be exponential. In a paper on primary auditory fibres Kiang (1965) described the above-mentioned features as characteristic for primary units.

c. Considerations on the influence of efferent activity.

Schmidt (1963) recorded efferent activity in all branches to the vestibular epithelia in the frog on stimulation of any ampulla or a "variety of unidentified extralabyrinthine proprioceptors". A feedback from the lateral and anterior ampullae to themselves was described. Stimulation of the otolith organs, however, was not found to provoke efferent activity in any of the branches of the vestibular nerve on the contralateral side or in the ipsilateral branches to the ampullae. The possibility of a direct feedback from an otolith organ to itself has not been investigated.

In the cat, Sala (1965 a,b) found inhibitory influence on afferent vestibular activity when the efferents were stimulated electrically. Considerable adaptation of the afferent discharges in the lateral ampullary nerve of the frog to stimulation on a torsion swing was attributed to efferent activity (Goetmakers 1968); but rather strong stimuli were used to provoke these adaptational effects.

The phenomenon actually observed by Goetmakers (1968) was a decrease of the response on a continuously changing stimulation, and the term "adaptation" in this context does not seem to be universally used. Adaptation is by definition the decrease with time in afferent discharges from a receptor that is subjected to a *maintained* constant stimulus strength. Whether a sinusoidally changing stimulus may be called continuous is a matter for discussion.

"Habituation" is not an appropriate term either since the word is already in use for a central phenomenon of decreasing responsiveness.

Since the gravitational force is acting continuously on the otolith receptors, the term adaptation will be used in the present discussion to indicate the decrease in afferent discharge frequency after attaining a new position, irrespective of its mechanism. In the present experiments no adaptation of the macular receptors was observed over periods of 30 seconds (figs. 18 and 19), in one instance even for 35 minutes (Chapter IV).

On attainment of a new position, occasionally some minor variations – either increase or decrease – of discharge frequency could be observed during the first few seconds. The data have been computed from 10-second samples of the spike train which included averaging. However, no marked difference was observed in consecutive samples in the same position.

The possibility of lateral inhibition between adjoining receptor cells cannot be excluded. Moreover, any influence of higher levels of the central nervous system upon efferent activity must be left open in our experimental set-up. The data show great conformity with the findings of Lowenstein and Roberts (1950) in the isolated labyrinth of the thornback ray. In the interrupted full-circle tilts these authors did not observe adaptation, and compared to continuous tilts of the same preparation the discharges were found to be on a slightly higher level. On incidental stationary periods, in tilted positions, they found in other experiments that during the first 30 sec the discharge frequency returned somewhat to the initial level although it remained characteristically above or below it over periods of 20 minutes.

In the present study the influence of anaesthesia upon efferent activity (Gleissner and Henriksson 1963) should also be considered. As to this, in cat V (figs. 18 and 19) anaesthesia was started with the short-acting barbiturate Thiogenal, and supplementary Nembutal was given intra-venously only during the surgical approach to the internal acoustic meatus. While recording from the utricular nerve during a period of some four to eight hours later we gave Flaxedil (i.v.). The cat was artificially respirated. In these circumstances we may assume normal vestibular efferent activity, the influence of extra-labyrinthine efferent stimulation being greatly reduced by suppression of voluntary movements.

The findings on the absence of adaptation were representative for the whole series of experiments. In a few instances supplementary Nembutal was given during the latter stages of the experiment; there was no difference in responses as compared to those initially recorded.

In normal cats (fig. 20) discharge-frequency curves revealed the same picture. In one instance the discharge rate was steady for 35 minutes in the position that provoked maximum stimulation. Since in these cats no total labyrinthectomy was performed on the contralateral side, these findings also support the anatomical description of Gacek (1966). This author indicated that there are no direct efferent connections from the contralateral side. Furthermore the findings of Schmidt (1963) who did not observe efferent activity in response to stimulation of the otoliths are in agreement with the present data. Only very recently, Giessen and Klinke (1969) reported recordings from primary afferent units of macular receptors in guinea pigs. There was no clear indication as to whether utricular or saccular units were held.

Although the exact nature of their recordings as originating from primary afferent units is open to doubt (see chapter IV, page 43), their approach on stereotactic coordinates – with neither hemicerebellectomy nor removal of the caudal part of the left hemisphere – revealed recordings that in some aspects are in good accordance with the present findings in the utricular nerve of the cat, since these authors did not observe any efferent influence either. These facts are contradictory to the findings of Bertrand and Veenhof (1964) in the rabbit. However, their sinusoidal stimulation on the parallel swing could represent a different functional property of the otolith system.

The preliminary findings in our experiments on the parallel swing need further quantitative analysis before any conclusions on the subject of efferent influence are justified.

If we assume that efferent activity would enhance adaptation in the otolith organ, as it does in the semicircular-canal system according to Goetmakers (1968), then an effect of efferent activity was not found in this series of experiments on static stimulation of the macular receptors. It has not been possible in the present experimental set-up to transect the utricular nerve centrally in order to settle this question.

d. Conclusions on the appropriate stimulus for the otolith organs.

Most authors indicate the shearing force of the otolith along the macular surface as the appropriate stimulus for the macular receptors, and thus confirm the original conception of Breuer (1891); e.g. the observations on freely moving fish placed in a centrifuge (v. Holst 1950 a,b); the changes in discharge rate in separate utricular fibres in the ray (Lowenstein and Roberts 1950); the recordings from medullar levels in fish (Schoen 1957) and the observations on the influence of gravity in human spatial orientation (Schöne 1964) all indicate a (approximately) linear relation to the shearing force.

Thus the latter authors found that the usual logarithmic relation between stimulus and effect in receptors (e.g. in the cochlea) is given by a linear relation in the vestibular apparatus.

The shearing principle for the utricular macula includes a maximum of sensitivity about the normal position, minor deviations may be based on the position of the macular plane in space.

If a pressure gradient constituted the appropriate stimulus one can only expect minor changes about the normal position, since this force is proportional to the cosine of the angle of tilting.

The present data, although founded on a limited stimulus range, may be regarded conclusive since the most effective stimulus range must be around the normal position, if shearing forces are involved.

Most units showed discharge-frequency modulations on tilting in the stimulating direction that can be regarded to be about linearly proportional to the shearing force, i.e. the sine of the angle of tilting. However, several units showed deviations from a sine curve because of a distinct rise at greater angles of tilt.

In addition to other factors mentioned before, in these latter units the possibility of concomitant influence of pull or pressure must be considered. Lowenstein and Roberts (1950) already indicated that besides shearing forces, relief of pressure could have some meaning in the functioning of "static" position receptors.

If we consider the morphological polarization pattern it is obvious that the majority of recordings in our series were not in the optimum-stimulus situation since only purely laterally "polarized" cells could be maximally stimulated. This was clearly demonstrated by Giessen and Klinke (1969) in their experiments on a cardanic table.

In contrast with the above-mentioned authors, Correia et al. (1968) formulated a predictive tangent equation for subjective judgements of orientation in gravitational- and supragravitational force fields in human subjects. At higher stimulus levels the initial linear relation was found to become nonlinear. Von Békésy (1966) indicated that where there is a pressure gradient, shearing forces will always be present. In the otolith organs we will have to consider the shearing force pattern along the macular surface and the pressure pattern perpendicular to the surface since they can always be viewed as two components in a parallelogram of forces. The shearing force determines the effective stimulus but a pressure gradient is necessary to produce sensation.

The author concluded that since the otoliths are made to detect movements and static positions as well, the question how the nervous system reacts to such a complicated pattern will not be solved by oversimplification and emphasis of one pattern.

As far as the present experiments on a limited stimulus range allow for conclusions as to the appropriate stimulus, it is assumed that the shearing force of the otolith alongside the macula is bound to be the appropriate stimulus as indicated by Breuer (1891). However, in accordance with the conception of von Békésy (1966) some deviations from a linear relation to the shearing force at greater angles of tilting could be based on concomitant increasing influence of e.g. relief of pressure. The supposition seems justified that in supragravitational circumstances the influence of a pressure gradient on the macular receptors will increase.

3. FINAL CONCLUSIONS

1. The present study has confirmed that the equilibrium function of the utricular otolith in the cat is concerned with the indication of static position. Thus the conclusion of Wing (1963) who assumed that the otolith organs in the cat are vestigial and without functional significance in spatial orientation is rejected.

   The quantitative data are restricted to the transversal plane. They are derived from static stimulation of the otolith organ. The recordings from the primary afferent utricular nerve show great conformity to the description of "static" position receptors in lower vertebrates (Lowenstein and Roberts 1950).
2. An approximately linear relation of the discharge frequencies to the shearing force in the stimulating direction of the tilt i.e. ipsilateral side down, was found. Since on tilting in the opposite (inhibitory) direction a distinct discharge-frequency indicative of a position was not observed, we may assume an uni-directional sensitivity of the macular receptors as regards static stimulation in the cat.

   In several units deviations from a linear relation to the shearing force at greater angles of tilting could be based on concomitant increasing influence of a pressure gradient.
3. It is concluded that probably most recordings in the present experiments originated from central regions of the utricular macula, since a laterally directed shearing force is found to be stimulating conforming to the morphological polarization pattern.
4. No evidence was found for the existence of "out-of-position" receptors as described in the maculae of lower vertebrates.
5. Only in one instance an indication of spontaneously silent receptors might be speculatively assumed. Only once has a unit which was inactive over a considerable part of the stimulus range been described in macular receptors (Lowenstein and Roberts 1950).

   Such recruitment of spontaneously silent receptors, however, considerably improves the total impulse discharge in the canal cristae (Groen et al. 1952, Lowenstein 1955).
6. Upon static stimulation of the macular receptors no evidence was found of adaptation. The involvement of efferent activity in stimulus coding of static position is not clear in the present experiments.
7. The recordings in normal cats as compared to those which were previously operated upon (amongst others a total labyrinthectomy on the contralateral side) did not show any differences.

   In the present series we were not able to demonstrate influences exerted by the contralateral labyrinth upon the effect of static stimulation on the ipsilateral one.
8. Supplementary experiments on tilting in the sagittal plane provided qualitative confirmation of the quantitative findings on tilting in the transversal plane.
9. Some preliminary experiments on the response to linear accelerations in the horizontal plane applied by means of a parallel swing are described. Its sinusoidal movements evoked distinct frequency-modulations of the discharges. These observations need further quantitative analysis.
10. Since vestibular efferent fibres have been shown clearly by anatomists the physiological evaluation of efferent involvement in otolith stimulation is a tempting subject for future investigation.

    In view of the interest in vestibular function in this era of space flight, the fact that in the present study on static stimulation of the otolith organs in mammals adaptation was not demonstrated, deserves special attention.

    As already indicated by Jones (1966) the very intricate patterns of movements in rotating space vehicles to re-establish a "gravitational" field will present a variety of stimuli to all parts of the vestibular system. In these circumstances the phenomena of adaptation, pattern copy building (Groen 1957), habituation etc. will be of paramount significance.

SUMMARY

For many years vestibular investigation, i.a. of the function of the otolith organs consisted mainly of observing labyrinthine reflexes and their changes with partial or total labyrinthectomy.

The morphological polarization pattern of the sensory cells as revealed by electron microscopy, and the introduction of electrophysiological recording techniques contributed to a great extent to a more complete picture of otolithic function.

Fairly recently most authors indicated the shearing force of the otolith along the macula to be the appropriate stimulus for the macular receptors and thus confirmed the original conception of Breuer (1891).

The present knowledge of electrophysiological characteristics of the various nerves of the vestibular receptors is based largely on experiments on lower vertebrates.

Quantitative data were presented by Lowenstein and Roberts (1950) on recordings from separate branches of the otolith organs in the thornback ray. Although many electrophysiological data are available concerning the vestibular nuclei in mammals, only a few papers on recording from primary afferents in the vestibular nerve have been published.

As to the otolith organs Wing (1963), basing himself on recordings from units in the vestibular ganglion, assumed that the utricle and saccule in the cat might be vestigial organs without functional significance in spatial orientation.

The present study concerns peripheral-stimulus coding in the otolith organs as recorded from primary afferents in the utricular nerve in the cat.

To obtain exact recordings from the utricular nerve a technique for functional elimination of the lateral and anterior semicircular canals with preservation of utricular function had to be developed.

After sectioning the lateral and anterior ampullary nerves at the left side, a total labyrinthectomy was performed at the right side. Pre- and postoperative electronystagmographic recordings on torsion and parallel swing confirmed an undisturbed function of the utricle at the left side and functional elimination of the above-mentioned semicircular canals.

Some weeks after these operations, the posterior fossa at the left side was approached by an extensive craniotomy. Thereafter a microelectrode was introduced into the utricular part of the superior vestibular nerve. This was performed under visual control, since exact location of primary afferent fibres just inside the internal acoustic meatus could not be achieved stereotactically. The fast-green method was used for electrode localisation, and in frozen serial sections the dye spot in the utricular nerve was confirmed by a combination of observation with red light and phase-contrast microscopy.

– Utricular stimulation was achieved by tilting the animal in the transversal plane about a rostro-caudal horizontal axis. Stimulus range covered a 45° tilt to both sides.

In the normal position a fairly regular spontaneous discharge is observed. Data processed in the form of interval histograms, however, revealed a more scattered distribution of interspike intervals as would be observed by conventional methods.

A distinct increase of discharge rate occurred on tilting to the ipsilateral side down, a tilt in the opposite direction showed a decrease of spontaneous activity. An approximately linear relation of the discharge frequencies to the shearing force was found in the stimulating direction of the tilting. In the inhibitory range a distinct discharge-frequency indicative of the position was not observed. This includes an uni-directional sensitivity of the macular receptors concerned with static stimulation.

In several units deviations from a linear relation of discharge rate to the shearing force could be attributed to concomitant influence of a pressure gradient as indicated by von Békésy (1966).

– The recordings in normal cats as compared to those which were previously operated upon (involving amongst others a total labyrinthectomy on the contralateral side) did not show any difference. As such in the present series we could not demonstrate influence of the contralateral labyrinth upon static response.

– In the present study we found no evidence of adaptation upon static stimulation of the macular receptors. If we assume that efferent activity would enhance adaptation in the otolith organ, as it does in the semicircular-canal system according to Goetmakers (1968) then an effect of efferent activity was not found.

– Supplementary experiments on tilting in the sagittal plane provided qualitative conformation of the quantitative findings on tilting in the transversal plane.

– Some preliminary experiments on the response to linear accelerations in the horizontal plane on a parallel swing are described. The sinusoidal movements evoked a distinct frequency-modulation of the discharges, in such a way that an increase was observed when inertia movements of the otolith were directed laterally. These first qualitative impressions need further analysis.

– The interval histograms presented together with samples of the spike trains provided an improvement of the analysis of the time patterns of discharges on tilting in the transversal plane. The implications of these findings and the relation to the morphological polarization pattern of the utricular macula are discussed.

The present study has confirmed that the equilibrium function of the utricular otolith in the cat is concerned with the indication of static position. The present recordings show great conformity to the description of "static" position receptors in the thornback ray.

In comparison with the findings by other authors in the vestibular nuclei of the cat, the discharge rate in the primary afferents proved to be on a slightly higher level. The variety of response patterns in the vestibular nuclei can be attributed to participation of intercalated neurons.

Upon static stimulation of the macular receptors no evidence was found of adaptation in the primary afferent fibres. This constitutes a distinct difference between primary and secondary neurons in the vestibular system.

SAMENVATTING

Vestibulair onderzoek, o.a. naar de functie van de otoliet-organen (utriculus en sacculus), berustte gedurende lange tijd voornamelijk op waarnemingen van labyrinthaire reflexen en de veranderingen die hierin ontstaan na gedeeltelijke of totale labyrinthectomie.

Met behulp van de electronenmicroscoop werd het morfologische polarisatiepatroon van de zintuigcellen in de maculae ontdekt. Tezamen met de invoering van electrofysiologische technieken, met behulp waarvan de informatie van het geïsoleerde perifere orgaan duidelijk werd, kon een vollediger beeld van de functie van de otolieten verkregen worden.

Sinds betrekkelijk korte tijd wordt door de meeste auteurs de schuifkracht van de otoliet langs de macula aangegeven als de adekwate prikkel voor de maculareceptoren, waarmee de oorspronkelijke opvatting van Breuer (1891) bevestigd werd.

De huidige kennis van de electrofysiologische eigenschappen van de verschillende zenuwen van de vestibulaire receptoren berust grotendeels op proeven bij lagere vertebraten.

Lowenstein en Roberts (1950) publiceerden kwantitatieve gegevens over afleidingen uit de afzonderlijke zenuwtakken van de otoliet-organen bij de rog. Ofschoon vele electrofysiologische gegevens beschikbaar zijn over prikkelverwerking in de vestibulaire kernen bij zoogdieren, werden slechts enkele artikelen over afleidingen van primair afferente vezels in de nervus vestibularis gepubliceerd.

Zo nam Wing (1963) aan, op grond van afleidingen uit het vestibulaire ganglion, dat de utriculus en de sacculus bij de kat waarschijnlijk rudimentaire organen zijn zonder functionele betekenis voor de ruimtelijke oriëntatie.

In dit proefschrift wordt de codering van de perifere prikkel in het otolietsysteem onderzocht door middel van afleidingen van primair afferente vezels in de nervus utricularis bij de kat.

Om exacte afleidingen uit de nervus utricularis te verkrijgen moest een techniek worden ontwikkeld voor het functioneel uitschakelen van de laterale en voorste halfcirkelvormige kanalen, met behoud van de functie van de utriculus. Na doorsnijden van de laterale en de voorste ampullaire zenuw links, werd een totale labyrinthectomie rechts verricht. Pre- en postoperatief electronystagmografisch onderzoek op torsie- en parallelschommel bevestigde een ongestoorde functie van de utriculus (links) en een functionele uitschakeling van de bovengenoemde kanalen.

Enkele weken na deze operaties werd de achterste schedelgroeve aan de linkerzijde benaderd via een uitgebreide craniotomie. Vervolgens werd een microelectrode in het utriculaire deel van de nervus vestibularis superior geplaatst. Dit geschiedde onder visuele controle, omdat exacte afleiding van primair afferente vezels net binnen de meatus acusticus internus op stereotactische wijze niet mogelijk was.

Ter verificatie van de localisatie van de micro-electrode in de nervus utricularis werd de "fast-green" methode (Thomas en Wilson 1965) gebruikt. In de vriescoupes (gesneden evenwijdig aan de nervus vestibularis) werd de gekleurde plaats in de nervus utricularis aangetoond door middel van gecombineerde observatie met rood licht en phase-contrast microscopie.

– De utriculus werd geprikkeld door de kat in het transversale vlak te kantelen om de rostro-caudale as, naar beide zijden over een hoek van 45° ten opzichte van het horizontale vlak.

De kwantitatieve gegevens hebben alleen betrekking op dit gebied. In de normale houding werd een vrij regelmatige spontane ontlading waargenomen. Indien de gegevens worden bewerkt in de vorm van een interval-histogram, zien we echter een meer gespreide verdeling van de intervallen tussen de actiepotentialen dan bij observatie met conventionele onderzoekmethoden.

Bij kantelen naar de ipsilaterale zijde ontstond een duidelijke toeneming van de ontladingsfrequentie, terwijl een kanteling in tegenovergestelde richting een afname van de spontane activiteit te zien gaf.

In de richting van de kanteling die tot verhoogde activiteit leidt, werd een bij benadering lineaire verhouding van de ontladingsfrequentie tot de schuifkracht gevonden. Kanteling in tegenovergestelde richting toont geen voor de positie karakteristieke ontladingsfrequentie. Dit houdt in dat de macula-receptoren die betrokken zijn bij statische prikkeling een een-zijdige richtingsgevoeligheid tonen.

In enkele vezels werden afwijkingen gevonden van een lineaire verhouding van de ontladingsfrequentie tot de schuifkracht. Deze zouden kunnen worden toegeschreven aan begeleidende invloed van een drukgradiënt zoals door von Békésy (1966) werd aangegeven.

– De afleidingen bij normale katten toonden geen verschil in vergelijking met de reacties van de tevoren geopereerde katten (o.a. totale labyrinthectomie aan de contralaterale zijde). Bij onze experimenten werd dus geen invloed van het contralaterale labyrinth op de reactie bij statische prikkeling aangetoond.

– Voorts werden geen tekenen van adaptatie bij statische prikkeling van de macula-receptoren gevonden. Als we aannemen dat adaptatie van de otolietorganen zou optreden ten gevolge van de efferente activiteit, zoals dit voor de halfcirkelvormige kanalen beschreven werd door Goetmakers (1968), dan moeten wij vaststellen dat in onze experimenten geen effect van efferente activiteit werd waargenomen.

– Aanvullend onderzoek waarbij in het sagittale vlak werd gekanteld gaf een kwalitatieve bevestiging van de kwantitatieve bevindingen bij kantelen in het transversale vlak.

– Enkele inleidende experimenten worden beschreven betreffende de reactie op lineaire versnellingen in het horizontale vlak, opgewekt door prikkeling op een parallelschommel. De sinusvormige bewegingen gaven een duidelijke frequentiemodulatie van de ontladingen te zien, zodanig dat een toeneming ontstond wanneer de traagheidsbewegingen van de otoliet naar lateraal gericht waren. Deze voorlopige, kwalitatieve indrukken dienen nader geanalyseerd te worden.

De interval-histogrammen, beoordeeld in samenhang met beelden van de opeenvolgende actiepotentialen, maken een betere analyse mogelijk van de tijdspatronen der ontladingen bij kantelen in het transversale vlak.

De resultaten van deze bevindingen en het verband met het morfologische polarisatie-patroon van de macula utriculi worden besproken.

Ons onderzoek heeft de evenwichtsfunctie van de utriculus voor de ruimtelijke oriëntatie bij de kat bevestigd. De reacties tonen grote overeenkomst met de beschrijving van "statische" positie-receptoren bij de rog.

In de primair afferente vezels wordt een hoger niveau van de ontladingsfrequentie waargenomen dan in het vestibulaire kerncomplex van de kat, zoals dit door anderen is beschreven.

De verscheidenheid van reactiepatronen in de vestibulaire kernen kan worden toegeschreven aan de aanwezigheid van coördinerende neuronen.

In de primair afferente vezels kon bij statische prikkeling geen adaptatie worden aangetoond. Dit vormt een duidelijk verschil tussen neuronen van de eerste en de tweede orde in het vestibulaire systeem.

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STELLINGEN

I

De functionele betekenis van het vestibulair-efferente systeem bij statische prikkeling van het otoliet-orgaan is niet duidelijk.

II

Amniocentese is een onmisbaar diagnostisch hulpmiddel bij de behandeling van actief rhesus-antagonisme.

III

Homotransplantatie van bot na verwijdering van grote kaakcysten via een intra-orale incisie betekent een verkorting van de postoperatieve behandelingsperiode.

IV

Voor het hart als geheel is versterkte werking van de orthosympathicus niet identiek aan verminderde werking van de nervus vagus.

V

Dislocatie van het neusseptum bij de pasgeborene moet op de derde dag post partum gecorrigeerd worden.

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VI

Bij de behandeling van gonorroïsche urethritis verdienen tetracyclines in het algemeen de voorkeur boven de gebruikelijke penicillines.

VII

Wanneer de diagnose basilarisinsufficiëntie wordt overwogen, verdient het aanbeveling een hypercalcemie uit te sluiten.

VIII

Vooralsnog bestaat er geen indicatie tot het verrichten van larynxtransplantaties.

IX

Prothesen in de mond moeten vervaardigd worden van materiaal dat bij röntgenologisch onderzoek de prothese in zijn geheel zichtbaar kan maken.

X

De bepaling van de activiteit van het enzym ornithine-carbamyl-transferase is van geen nut voor de vroege opsporing van levermetastasen.

XI

De wijze waarop de medische keuring ter beoordeling van de geschiktheid tot het besturen van motorrijtuigen geschiedt, beantwoordt niet aan het doel van deze keuring.