CHAPTER II

BACKGOUND INFORMATION

The Organization of the Vestibular Complex

The vestibular complex which lie in the pons and the dorsorostral part of medulla is subdivided into four principal nuclei based on detailed studies on cats by Brodal and his colleagues (Brodal and Pompeiano , 1957; Brodal , 1974; Ito ,1984).

1. The Superior Vestibular Nucleus (Nucleus of Bechterew)

The superior vestibular nucleus (SVN) is situated most rostrally among vestibular nuclei in the angle of the floor and lateral wall of the fourth ventricle and composed of rather loosely scattered medium-sized and small cells. This nucleus receives primary vestibular afferents and floccular Purkinje cell axons predominantly in its central part, while the peripheral parts are impinged upon predominantly by fibers from the fastigial nucleus, the uvula and the nodulus. Cells of the superior vestibular nucleus project mainly rostrally through the medial longitudinal fasiculi (MLF) but there are some cells projecting to the flocculonodular lobe, the contralateral vestibular nuclei and reticular formation.

2. The Lateral vestibular Nucleus (Nucleus of Deiters)

The lateral vestibular nucleus (LVN) is next to the inferior cerebellar peduncle at the level of the vestibular nerve entrance and characterized by the presence of the giant cells of Deiters. The afferent and efferent connection appears to be characterized as a particular unit. Deiter's nucleus receives axons from the macula of the utricle and from the cerebellum and spinal cord. Its neurons send their axons into the lateral vestibulospinal tract which terminates ipsilaterally in the ventral horn of the spinal cord, along its length.

3. The Medial Vestibular Nucleus (Nucleus of Schwalbe)

The medial vestibular nucleus (MVN) is composed of occupies parts of the rhomboid fossa and contains relatively few fibers from the cristae of the semicircular canal and utricular macula. This nucleus contains cells of different sizes, the majority being medium sized, triangular, multiform cells. Outputs running in the medial vestibular tract terminate bilaterally in the ventral horn of the cervical region of the cord, making monosynaptic connections with neck-muscle motor neurones. These provide for the reflex control of neck movement to maintain the position of the head and provide a stable base for eye movements. This nucleus and SVN participate in vestibulo-oculomotor reflexes having outputs which run in MLF to the ocular motor nuclei and give rise to rotatory nystagmus movements.

4. The Inferior Vestibular Nucleus

The inferior vestibular nucleus (IVN) extends rostrally at the level where the caudal vestibular root fibers enter the vestibular complex. This nucleus contains a certain number of large cells and its anatomical structure is characterized by the presence of bundles of longitudinal fibers. The nucleus receives excitatory inputs from the semicircular canals and from both the utricle and the saccule, and inhibitory inputs from the cerebellum.

Physiological Pathways through the Vestibular Nuclei

The peripheral branches of the bipolar cells in the vestibular (Scarpa's) ganglion cause from the specialized neuroepithelium in ampullae and from the maculae of the utricle and the saccule. The central branches enter the brain stem and terminate almost exclusively in the vestibular nuclear complex (Brodal and Pompeiano, 1957; Walberg, Bowsher and Brodal, 1958; Mugnaini, Walberg and Brodal, 1967). From here there are three main projection systems, as indicated in Fig. 1.

1. Vestibulospinal System

The vestibulospinal system has a complex organization both in aspects of its afferent connection and of its spinal projection. Neurons of origin of the vestibulospinal tract (VST) are fed by at least 3 major afferents ; the primary vestibular nerve, the cerebellum and the spinal cord. Each of these afferents makes synaptic connections with the VST neurons in specific portions of

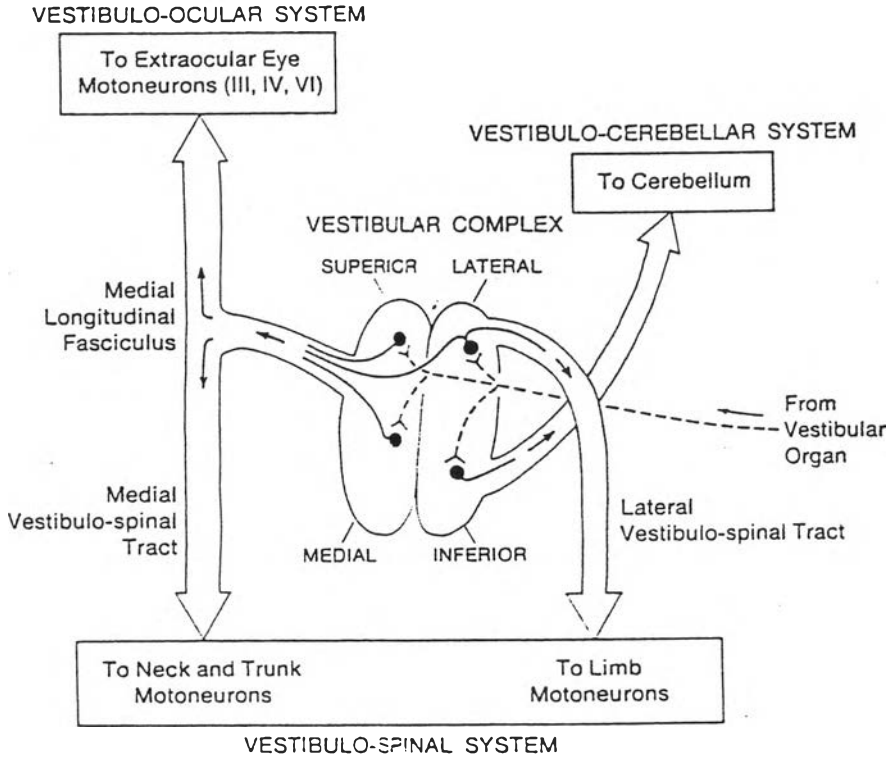

Figure 1. Divisions of the vestibular nucleus, and their output connections to different parts of the brain.

the vestibular nuclear complex. It contains two separate vestibulospinal tracts ; medial (MVST) and lateral (LVST). The medial tract consists of fibers that arise from cells in most of the vestibular nuclei, and gather in the midline to form the MLF. The descending fibers terminate in anterior segments of the spinal cord, where they connect to motoneurons that control the axial muscles of the neck and trunk. In contrast, fibers to motoneurons that control limb muscles arise from the LVN, and descend in the lateral tract.

2. Vestibulo-ocular System

Vestibular projections to the extraocular motor nuclei (EOM) arise in the SVN and in rostral portions of the MVN, the regions which receive the central projections of the semicircular canal ganglion. Fibers ascending from these two regions follow separate courses. The majority of ascending SVN fiber pass rostromedially into the ipsilateral MLF from which they enter the ipsilateral trochlear and cranial nerve nuclei. From the MVN, ascending fibers pass medially into the ipsilateral cranial nerve nuclei and a few fibers are distributed to the contralateral cranial nerve nuclei. Ascending fibers pass in the contralateral MLF to the EOM nuclei bilaterally. Small groups of extraocular muscle motor neurons receive projections from large areas in the SVN and MVN. The disynaptic pathway from vestibular afferents to extraocular motoneurons is supplemented by polysynaptic connections through the reticular formation. this pathway is

consistent with the rapid transmission necessary in controlling eye movement.

3. Vestibulocerebellar System

The vestibular system projects to the cerebellum, both as primary vestibular fibers and with secondary afferents, originating from the vestibular nuclei in the brain stem. The primary vestibular fibers terminate in the nodulus, the adjoining ventral folia of the uvula, the paraflocculus and the ventral paraflocculus. A few fibers end in the lingula and the ventral paraflocculus. Secondary vestibular fibers take origin from restricted parts of the vestibular complex. Neurons in the medial and descending nuclei and cell groups f and x project bilaterally to the entire cerebellar vermis, the flocculus, the fastigial nucleus, and the anterior and posterior interpositus nuclei. In addition, some cells in the superior and lateral vestibular nuclei project to the nodulus, flocculus, fastigial nucleus, and vermis.

Electrophysiological Studies of the Vestibular Nerve on Cells in the Vestibular Nuclei

The major inputs to vestibular nuclei are provided by the primary vestibular afferents from labyrinthine and commissural afferents from the contralateral vestibular nuclear complex (Shimazu and Precht ,1965; Wilson, 1972; Epema, Gerrits and Voogd, 1988; Cochran, Kasik and Precht, 1987). Stimulation of the vestibular nerve with single shock excites some cells in the vestibular nuclei monosynaptically, others polysynaptically; some

cells are inhibited. Shimazu and Precht (1965) made the first detailed analysis of single unit activity and field potentials produced in the vestibular nuclei by stimulation of the ipsilateral nerve.

The fields (Fig.2) consist of a positive or positive-negative P wave, indicating the arrival of afferent impulses, followed by a negative N1 potential consisting of monosynaptically-evoked activity. In some areas, there is a later negative N2 potential, due to postsynaptic activity with a latency longer than 1 msec (2.2-2.7 msec). The latencies of these components vary somewhat with recording location; that of the N1 potential usually ranges from 0.8-1.2 msec. The threshold of the N1 potential is similar to the threshold of the largest vestibular nerve fibers. Because this potential is easier to monitor than the P wave, the strength of vestibular nerve stimulation has usually been expressed as a multiple of N1 threshold (xN1T).

Amino Acids as Possible Vestibular Primary Afferent Transmitters

During the last decades, data have been accumulated which have provided detailed insight in anatomy and physiology of the vestibular nuclear complex. However, there is still need for more detailed studies to elucidate the chemical substance utilized by this system as neurotransmitter(s) for complete understanding of its function and dysfunction.

Previous electrophysiological work studied effects of norepinephrine (NE), D-amphetamine and acetylcholine (ACh) on

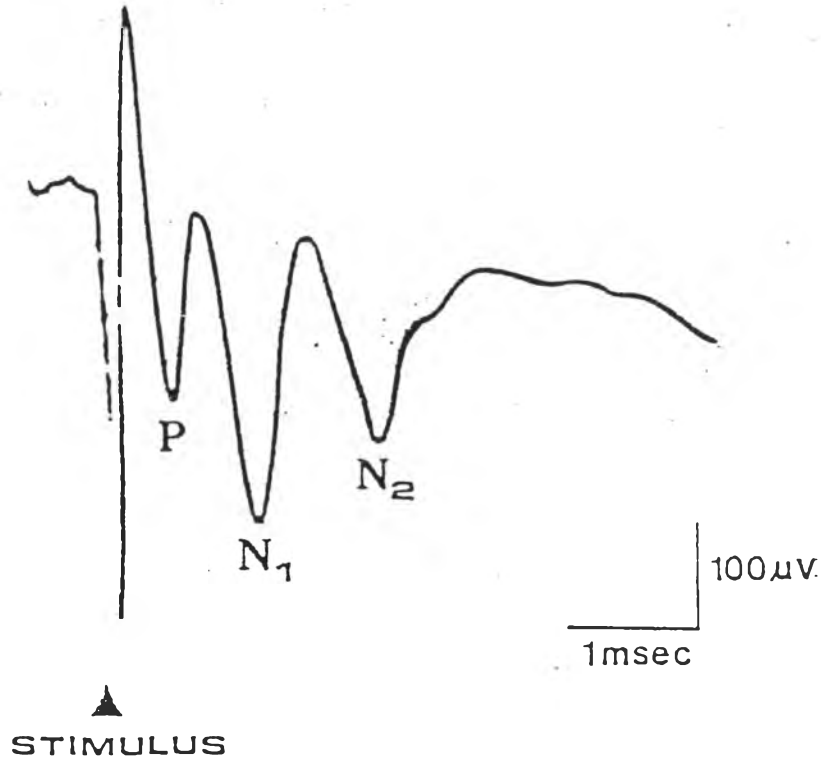

Figure 2. Field potential evoked in vestibular nurons by stimulation of ipsilateral vestibular nerve.

spontaneously firing cells which responded to a motion stimulus and others which were in the MVN or LVN. From these observations it has been suggested that NE-containing terminals are in close proximity to the vestibular neurons and further implicate both NE and acetylcholine (ACh) as neurotransmitters in afferent pathways to vestibular nuclei (Kirten and Sharma, 1976).

Ito et al. (1981) reported that monosynaptic spike generation of LVN neurons with vestibular nerve stimulation in cats was inhibited by microiontophoretic atropine and gamma-aminobutyric acid (GABA). Spontaneous firing of the LVN monosynaptic neuron increased with iontophoretic application of ACh and glutamate (Glu) and atropine inhibited ACh-induced firing without affecting Glu-induced firing, while GABA blocked spike generation produced by both ACh and Glu. These results suggested that ACh probably plays a role in transmission from the vestibular nerve to the LVN monosynaptic neurons.

According to Raymond et al. (1984), evidence that Glu acts as a neurotransmitter in the vestibular nerve fibers was sought by electron microscopic radioautographic identification of the uptake site of [^{3}H]-glutamic acid after incubation of slices of cat vestibular nuclei and measuring changes in sodium-dependent high affinity Glu uptake in nerve endings containing homogenates from normal and deafferented vestibular nuclei. It is concluded that Glu (or aspartate, Asp) is used by the vestibular nerve fibers as a neurotransmitter in the vestibular nuclei.

Dememes, Raymond and Sans (1984) used [^{3}H]D-Asp as a selective retrograde marker in the putative glutaminergic and/ or aspartatergic vestibular system. Labelling patterns resulting from retrograde axonal transport by vestibular nerve fibers were observed in the vestibular ganglion neurons and also in the nerve fibers. These observations indicated that Glu and/or Asp may be the putative neurotransmitter(s) in the afferent vestibular pathways. In addition, [^{3}H]Glu binding sites in frozen sections of rat vestibular nuclei were characterized biochemically and pharmacologically *in vitro* by quantitative autoradiography in order to investigate the possible role of Glu as a synaptic transmitter in this region. It was found that the [^{3}H]Glu binding sites were distributed unevenly in the projection areas of the vestibular nerve which has been described as a glutamate-mediated pathway (Touati, Raymond and Dememes, 1989).

Sangchantra (1986) tested effects of excitatory amino acid (EAA) and their antagonist. Iontophoretically applied Asp and Glu and their agonists have produced marked excitation of all spontaneously firing vestibular neurons tested, while their antagonist : glutamic acid diethylester (GDEE) and D-α amino adipic acid (DαAA), produced inhibition of spontaneous firing, and blocked the evoked excitation following stimulation of the vestibular nerves. These results support the proposal that excitant amino acid related to 'glutamate receptor' may involve as a neurotransmitter of the vestibular primary afferent.

From neurochemical study by using push-pull cannula superfusion technique to measure endogenous amino acids released in the vestibular nuclei under normal condition and neuronal stimulation it was found that both stimulation of vestibular nerve and perfusion with artificial cerebrospinal fluid (aCSF) containing high concentration of potassium ions (100 mM) produced significant increase in Glu and Asp contents recovered in the perfusate, suggested that Glu and/or Asp may be neurotransmitter of the vestibular primary afferent (Warunee, 1987).

Recent pharmacological studies have demonstrated that at least three types of EAA receptors can be distinguished according to their most sensitive agonist : N-methyl-D-aspartate (NMDA), quisqualate (QA) and kainate (KA) receptors (Cotman and Iversen, 1987; Watkins and Olverman, 1987; Collingride and Lester, 1989; Watkins, Krogsgaard-Larsen and Honore 1991).

From electrophysiological and pharmacological experiments using brain slice preparation, NMDA, QA, KA and homocysteate are able to depolarize the MVN neurons while atropine, hexametronium or diphenhydramine are not able to block synaptic transmission at this synapse, concluded that ACh is not the transmitter but rather suggested that an EAA or similar substance may be the transmitter at this central afferent vestibular synapse (Lewis, Gallagher and Shinnick-Gallagher, 1987).

Cochran, Kasik and Precht (1987) have investigated the excitatory synaptic transmission of two input : vestibular afferent via the VIIIth cranial nerve and commissural afferents from the contralateral vestibular nuclear complex. It was found that both application of Glu receptor antagonists block the postsynaptic component of the field potential in dose dependent manner without affecting the presynaptic volley. In addition, the slow orthodromic excitatory postsynaptic potentials (EPSPs) evoked from contralateral VIIIth nerve or contralateral vestibular nuclear complex stimulation are completely blocked by kynurenate (KYNA), a broad spectrum antagonist for three types of EAA. Low concentrations of KYNA and 2-amino-5-phosphonovalerate (APV), a selective antagonist for NMDA receptor, reduce depolarizations to bath-applied NMDA, while depolarizations due to KA and QA are resistant to these antagonist at these concentrations. The results indicated that the VIIIth nerve afferent released Glu and/or similar substance as its transmitter which activates second-order neuron through KA/QA synaptic receptors and suggested that the transmitter released by the commissural afferents is also Glu and/or relate compound which generated largely through NMDA synaptic receptors.

Another experiment was performed on the MVN neurons in transverse brain slices containing the root of the vestibular nerve. Electrical stimuli applied to the vestibular nerve tract evoked an EPSPs. These orthodromic EPSPs were insensitive to the following antagonists : atropine, hexametronium, diphenhydramine and caffeine, concluded that the primary

afferent excitatory transmitter is not ACh, histamine or adenosine, respectively. However, KA blocked the orthodromic EPSP while having no effect on the resting membrane potential, input resistance or action potential configuration to MVN neurons, suggesting that an EAA or amino acid-like substance, is responsible for primary afferent excitatory transmitter in rat MVN (Lewis et al.,1989).

Finally, electrophysiological study in brain stem slice preparation, Doi, Tsumoto and Matsunaga (1990) revealed that application of APV through the perfusion medium suppressed 82% of cells activated monosynaptically from vestibular commissures, while it suppressed only 9 % of cells activated monosynaptically from vestibular afferents. The application of KYNA proved much less selective, suppressing 83% of the formal groups of cells and 93% of the latter. In addition, 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX), a selective antagonist for non-NMDA type suppressed almost all the cells of both groups and the sensitivity of monosynaptic inputs to KYNA, CNQX or APV was not significantly different from that of polysynaptic inputs irrespective of sources of inputs. These results suggested that excitatory synaptic inputs to MVN neurons are mediated mainly through non-NMDA type of EAA receptors from vestibular afferents and through NMDA as well as non-NMDA types of EAA receptors from commissures.