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# Responses of Inferior Olive Neurons to Stimulation of Semicircular Canals

**Sah-Hoon Park, Jong Seong Park, Min Su Lee, and Jung Woo Shin**

*Department of Physiology, Chonnam National University Medical School, Gwangju 501–746, Korea*

**In spite of abundant anatomical evidences for the fiber connection between vestibular nuclei and inferior olivary (IO) complex, the transmission of vestibular information through the vestibulo-olivo-cerebellar climbing fiber pathway has not been physiologically established. The aims of the present study were to investigate whether there are IO neurons specifically responding to horizontal rotation and also in which subregions of IO complex these vestibularly-activated neurons are located. The extracellular recording was made in 68 IO neurons and responses of 46 vestibularly-activated cells were analyzed. Most of the vestibularly-activated IO neurons responded to signals of vertical rotation (roll), while a small number (13/46) of recorded cells were activated by horizontal canal signal (yaw). Regardless of yaw-sensitive or roll-sensitive, vestibular IO neurons were excited, when the animal was rotated to the side contralateral to the recording side. The gain and excitation phase were very similar to otolithic or vertical-canal responses. Histologic identification of recording sites showed that most of vestibular IO neurons were located in β subnucleus. Electrical stimulation of a HSC evoked an inhibitory effect on the excitability of the ipsilateral IO neurons. These results suggest that IO neurons mainly in the β subnucleus receive vestibular signals from semicircular canals and otolithic organs, encode them, and transmit vestibular information to the cerebellum.**

**Key Words: Vestibular nucleus, Inferior olive, Climbing fiber**

## INTRODUCTION

Many anatomic studies have indicated that vestibular information is transmitted to cells in several subregions of inferior olive (IO). The medial and descending vestibular nuclei (MVN, DVN) send a heavy ipsilateral projection to β subnucleus (IO β) and dorsomedial cell column (dmcc) of IO (Saint-Cyr & Courville, 1979; Carleton & Carpenter, 1983; Gerritis et al, 1985; Carpenter, 1988). In addition, the nucleus prepositus hypoglossi which receives heavy bilateral input from the vestibular nuclei projects contralaterally to the dorsal cap of Kooy (dc) and ipsilaterally to the regions of medial accessory olive (MAO) just lateral to IO β (McCrea & Baker, 1985; Balaban & Beryozkin, 1994).

Vestibular activation of IO neurons has also been demonstrated by histologic studies using deoxyglucose or Fos as a marker for neuronal activity. Practically all such studies show that neuronal activity in IO β, dc and dmcc, is specifically altered following sinusoidal rotation (D'ascanio et al, 1981), centrepetal acceleration (Kaufmann et al, 1991; Kaufman et al, 1992) and unilateral labyrinthectomy (Kitahara et al, 1995; Cirelli et al, 1996).

Physiological recording studies also revealed that IO neurons responded to vestibular signals. The vestibularly evoked climbing fiber (CF) responses of Purkinje cells could be recorded in the uvula and nodulus of the cerebellum (Precht et al, 1976; Barmack & Shojaku, 1992). More direct evidences that IO neurons responded to vestibularly-evoked signals was obtained in single-cell recording studies made upon IO β neurons (Robinson et al, 1988; Barmack et al, 1993). These studies show that the activity of IO β neurons is phasically modulated by natural (sinusoidal) vestibular stimulation.

Although the vestibular inputs are known to reach to specific IO neurons, it remains still unclear which parts of peripheral vestibular apparatus evoke these signals. At the level of cerebellar nodulus, CF activity is evoked by horizontally sinusoidal stimulation (Precht et al, 1976), and at the level of IO β neurons, neuronal activity is modulated by horizontal rotation (Robinson et al, 1988), only vertical (roll) rotation (Barmack et al, 1993), and static roll tilt (D'ascanio et al, 1981).

The aims of the present were to investigate 1) whether there are IO neurons specifically responding to horizontal rotation, 2) what their response-profiles are like, and 3) in which subregions of IO complex these vestibularly-activated neurons are located.

Corresponding to: Sah-Hoon Park, Department of Physiology, Chonnam National University Medical School, Hak-1-dong, Gwangju 501-746, Korea. (Tel) 82-62-220-4263, (Fax) 82-62-232-1242, (E-mail) hoonpark@jnu.ac.kr

**ABBREVIATIONS:** CF, climbing fiber; DVN, descending vestibular nuclei; HSC, horizontal semicircular canal; IO, inferior olive; IO β, β subnucleus of inferior olive; MAO, medial accessory olive; MVN, medial vestibular nuclei.

## METHODS

### *Animal preparation*

Eighteen adult male cats, weighing 2.5~3.7 kg were used. Cats were first anesthetized with ketamine hydrochloride (40 mg/kg, i.m.), and the left saphenous vein was cannulated for infusing fluid and drugs. A tracheostomy was performed to allow artificial respiration. Anesthesia was subsequently maintained with urethane (1 g/kg, i.p.). During recording sessions, cats were paralyzed with periodic injections of pancuronium bromide (0.5 mg/hr) and artificial ventilation was employed to keep the end-tidal $CO_2$ concentration between 3.5 and 4.5%. Rectal temperature was monitored and maintained by an electrical homeothermic blanket in a range of 36.5~37.5°C.

In each animal, a midline incision was made between the vertex and the second vertebra to remove the overlying scalp and muscles. To expose the foramen magnum wider, the lower part of the occipital bone was removed and adjacent cervical muscles were deflected. Animals were then placed in a streotaxic apparatus mounted on a sinusoidal rotator. To minimize its movement during rotation, the body of the animal was fixed with soft pads and elastic straps in a case aligned with the longitudinal axis. The posterior part of animal's head was lifted upward, so that it formed an angle of about 25 degree from the horizontal. Following a fixative placing in a stereotaxic apparatus, dural flap was opened over the foramen magnum and the dorsal surface of the brain stem was exposed.

In four animals, in which electrical stimulation of semicircular canal nerves were made, retroauricular incision was made and supramastoid crest was exposed. Drilling in the small area just above the crest could make a hole on lateral semicircular canal, through which a small-diameter wire electrode was inserted toward the ampulla. This stimulating electrode, teflon-coated except its tip, was fixed onto temporal bone with dental cement and was positioned to be easily connected to stimulator.

### *Stimulation and recording*

The rotating table was sinusoidally oscillated (0.01~0.5 Hz) about the vertical axis or about the longitudinal axis. During vestibular stimulation the vision of the cat was always occluded by covering its eyes with black blindfold. In three animals, the right (ipsilateral) horizontal semicircular canal (HSC) nerves were electrically stimulated by pulse trains (4 V) repeated at the frequency of 0.2/sec. Trains were of 50 msec in duration and composed of 0.2 msec square waves at intra-train frequency of 300/sec.

Extracellular single-unit recordings were made with tungsten microelectrode (1~3 MΩ). Recording electrodes were tilted 30° posterodorsal to anteroventral and advanced with an electrically-driven hydraulic micromanipulator towards IO just rostral to the obex. The signal from the electrode were amplified (bandwidth 10~10 KHz) and were displaced on an oscilloscope. A window discriminator was used to discriminate the action potential of a single neuron. The output of the window discriminator was stored on a personal computer through A/D converting interface.

### *Identification of recording sites*

At the completion of the recording of each unit, an electrolytic lesion was made by passing a DC current (10~20 μA) for 15 sec. Animals were sacrificed with anesthetic overdose. Following transcardiac perfusion with 10% formalin solution, the brain stem was removed from the skull and was submersed in the same solution for 24 hours, and neutral red staining was performed on frozen sections (50 μm) in a frontal plane. Reconstruction of marking lesions was based on the stereotaxic atlas of the cat (Berman, 1968).

## RESULTS

We recorded from a total of 68 neurons located in the caudal half of the inferior olive. Among them, 46 neurons were activated by sinusoidal rotation. Most of IO cells showed irregular spontaneous activity less than 1 spike/sec, and characteristic waveforms that one or more wavelets follow the action potential.

### *Responses to sinusoidal rotation*

Among 46 vestibularly activated neurons, 26 cells responded specifically to the vertical (roll) stimulation, 4 cells to both vertical and horizontal (yaw) stimulation, and 3 cells to horizontal rotation only. The remaining 13 cells were activated by either roll (7 cells) or yaw (6 cells) test, although their responsiveness to the other mode or rotatory stimulation were not evaluated. Typical vestibularly-evoked responses of IO neurons are shown in Fig. 1. Most of IO neurons, regardless of yaw-sensitive or roll-sensitive, were excited, when the animal was rotated to the side contralateral to the recording side, and were inhibited, when the animal was rotated to the ipsilateral side. However, the response magnitude of yaw-sensitive neurons were usually smaller than that of roll-sensitive neurons (Fig. 2).

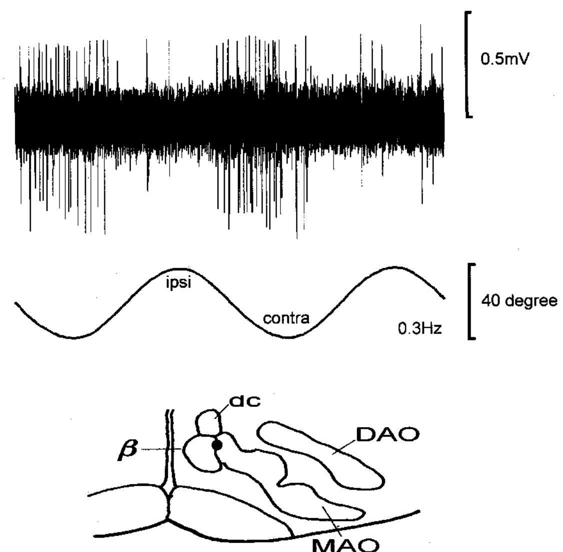


**Fig. 1.** Response of yaw-sensitive neuron to sinusoidal stimulation. This cell was recorded in the boundary area between beta subnucleus and medial accessory olive. Ipsi, ipsilateral; contra, contralateral.

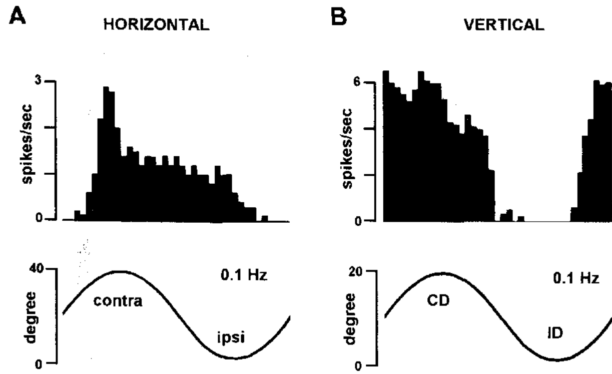


**Fig. 2.** Comparison of neuronal response between yaw-sensitive (horizontal) and roll-sensitive (vertical) IO neurons. CD, contralateral side down; ID, ipsilateral side down.

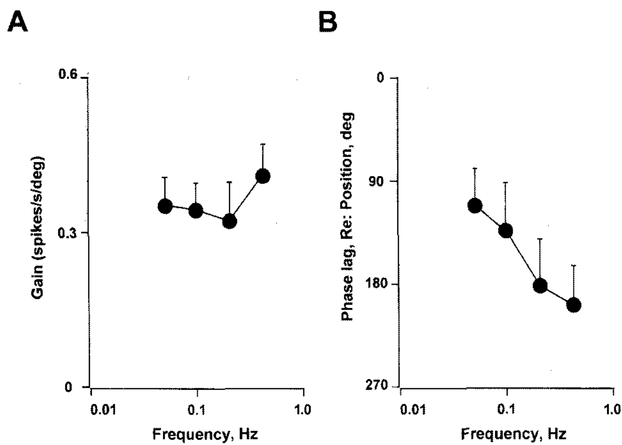


**Fig. 3.** Gain (A) and phase lag (B) of the responses in yaw-sensitive neurons.

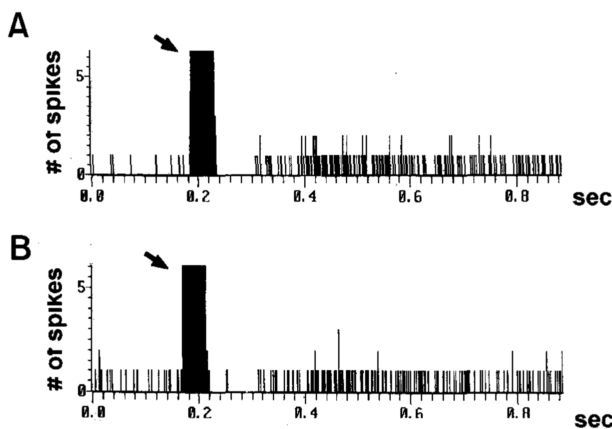


**Fig. 4.** Peristimulus histogram of the effect of stimulating a horizontal canal on the spontaneous firing rate of ipsilateral IO neurons. Arrow indicates artifact of stimulation with pulse trains.

Neuronal responses evoked by horizontal rotation revealed different excitation phases of an oscillation cycle depending on the rotation frequency The gain and phase of neuronal excitation evoked by horizontal rotation were measured for 7 HSC-related IO neurons (Fig. 3). At the rotation frequency of 0.1 Hz, the mean of the response gain was about 0.34 spikes/sec/deg, and the evoked activity lagged head acceleration by 140°. As the rotation frequency increased, this phase lag became more prominent.

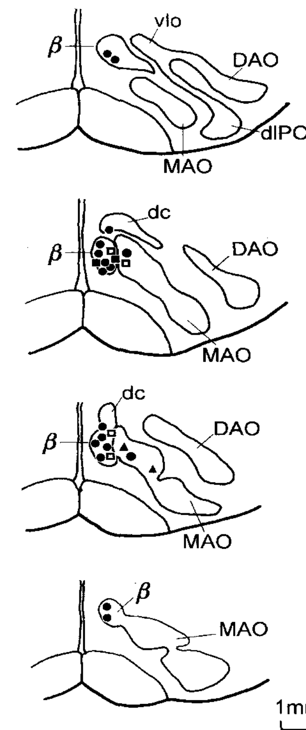


**Fig. 5.** Histological reconstructions of recording sites of vestibularly-activated neurons in the inferior olive. Solid circle, cells responding only to roll stimulation. Solid square, cells responding to both roll and yaw stimuli. Solid triangle, cells responding only to yaw stimulation. Open square, cells in which only the response to yaw stimulation was checked. DAO, dorsal accessory olive; dc, dorsal cap; dlPO, dorsolateral part of principle olive; MAO, medial accessory olive; vlo, ventrolateral out growth.

### *Responses to electrical stimulation of HSC*

With two cells whose responding sensory modalities were not identified, the electrical stimulation of the ipsilateral HSC inhibited spontaneous firing rate of IO neurons (Fig. 4).

### *Distribution of vestibularly-activated IO neurons*

The location of 25 vestibularly-activated neurons was histologically identified in the lower part of inferior olive. As shown in Fig. 5, most (20/25) of these cells were located in IO $\beta$, and recording sites of two cells responding only to horizontal rotation were found in MAO.

## DISCUSSION

Earlier studies on the vestibular inputs to cerebellar Purkinje cells (Eccles et al, 1966; Precht & Llians, 1969; Llinas & Precht, 1972; Ito, 1982) support the theory that

Purkinje cells do not receive afferent information through CF pathways. Their supports are derived from the finding that the field potential evoked by stimulation of the ipsilateral vestibular nerve are mainly of a mossy fiber (MF) type. They suggest that the CF afferents to the vestibullocerebellum may be activated either via pathways other than the vestibular nerve or by means of special patterns of afferent activity which cannot be evoked by electrical activation of the vestibular nerve. Based on the later-accumulated anatomic data, their reasoning seems to have neglected three major observations: 1) MVN and DVN send directly and indirectly projecting fibers to IO neurons (Saint-Cyr & Courville, 1979; Gerritis et al, 1985). 2) vestibular inputs to IO are mainly GABAergic inhibitory neurons (Nelson et al, 1989). 3) efferent fibers of IO (CF) reach to the contralateral vestibullocerebellum (Voogd et al, 1996). Therefore, the effect of stimulating a vestibular nerve should be reflected in a field potential recorded in the contralateral vestibulocerebellum.

In the present study, most of vestibularly-driven IO neurons were activated in a phase when the head was rotated towards the contralateral side, which is consistent with previously reported data (Robinson et al, 1988; Barmack et al, 1993). This finding implied that the IO neurons were activated while the contralateral semicircular canal was physiologically stimulated. Considering the fact that MVN and DVN projected GABAergic inhibitory fibers to the ipsilateral IO, it was quite understandable that the IO neurons showed inhibitory responses to the excitation of the ipsilateral semicircular canals.

The first aim of our present study was to investigate whether specific IO neurons receive vestibular input mainly from HSCs, and the data obtained showed that HSC-related (yaw) activity was observed in a small number (13/46) of cells in IO complex. Considering that about half (6/13) of these yaw-sensitive neurons were evaluated only in the yaw test, the number of specifically yaw-sensitive IO cells might have been smaller, if 3-dimensional rotation had been adopted on these cells. Barmack (1993) pointed out that certain HSC-related activity of IO neurons observed in yaw test can be abolished by readjusting head position without changing the horizontal plane. This finding suggests that a certain amount of yaw-sensitive responses in yaw test are actually originated not from HSC but from vertical semicircular canals. In the present study using 3-dimensional rotator, the number of cells responding specifically to yaw stimulation (3/33) was found to be much smaller than that (26/33) of specifically roll-sensitive neurons. This finding was consistent with data that, at least in rabbits IO $\beta$ neurons, vestibular signals originated mainly from vertical semicircular canals (Barmack, 1993). The reason of why only a small number of IO cells were related with HSC activity is not clear. However, it is quite likely that vestibular signals detected by the peripheral vestibular receptors are transmitted to IO neurons through secondary or tertiary vestibular neurons following being transformed in the vestibular nuclei (McCrea et al, 2001). It is also possible that vestibular information detected by a HSC may be converged or combined in the vestibular nuclei with other types of vestibular signals originating from either vertical semicircular canals or otolithic organs.

Anatomic findings up to now confirm that vestibularly-activated IO neurons are most abundant in IO $\beta$ subregion (see review of Azizi, 1989). This was the reason of why we focused on the IO $\beta$ region to record the activity of putative HSC-related neurons. During our recording session, we encountered many numbers of roll-sensitive IO neurons, which is consistent with a previous report (Barmarck et al, 1993). Possibly, we did not select proper vestibular subregion in IO. However, any other subregions of IO complex have not been introduced as a vestibular IO so far.

The responsiveness of HSC-related IO neurons seemed to be not specific only to HSC, as mentioned above. However there were a few differences between HSC-related neurons and roll-sensitive neurons. First, the gain of yaw-sensitive neuron responses was less affected by the rotation speed, while those of roll-sensitive neurons became apparently decreased (Barmack et al, 1993). Second, the responses of HSC-related neurons was much less affected by angular acceleration vector, compared with those of roll-sensitive neuron (Robinson et al, 1988).

These response characteristics supports the assumption that vestibular signals from HSC might be handled and encoded together with other sensory modality, such as a vision or collic inputs, in IO neurons (Maekawa & Simpson 1973; Alley et al, 1975; Maekawa & Taketa, 1979; Takeda et al, 1980). And somatosensory inputs to IO have been well established (Gellman et al, 1983; Gellman et al, 1985). Histological identification of recording sites suggested a possibility of different locations of yaw-sensitive cells and roll-sensitive cells. In the present study, most of roll sensitive neurons were recorded in IO $\beta$. However, two cells which did not respond significantly to roll stimulation, and showed moderate response to yaw stimulation were found not in IO $\beta$, but in MAO. This is quite consistent with the finding obtained from rabbit IO $\beta$ (Barmack et al, 1993). In his study, neurons responding to roll test were identified in the medial portion of IO $\beta$.

In the present study, we electrically stimulated HSC ampullary nerve in the ipsilateral side to recording site in three animals. In an earlier study (Robinson et al, 1988), similar electrical stimulation of bony labyrinth was tried, however, it failed to activate any IO neurons. The failure of activating IO neurons by electrical stimulation might have partly been due to inhibitory nature of GABAergic projection from MVN and DVN to ipsilateral IO. Another possibility was that a single pulse might not be adequate for evoking excitatory response of IO neurons. In the present study, pulse trains were used in the electrical stimulation and inhibitory responses were observed in 2 cells in the ipsilateral IO. However, because the strength (voltage) of electrical stimuli was higher ($8 \sim 10$ V) than the voltage level usually used for stimulating canal nerves (less than 5 V), this electrical current might have spreaded over the ampulla of HSC and the response of IO neurons to this electrical stimulation could not be regarded as HSC-specific. Nevertheless, to the best of our knowledge, this is the first data to show that IO neurons are inhibited by electrical stimulation of peripheral vestibular receptors.

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