Effects of Activation of the Divergent Efferent Fibers on the Spontaneous Activity of Vestibular Afferent Fibers in the Toad

Tokio SUGAI, Michio SUGITANI, and Hiroshi OOYAMA

*Department of Physiology, Kanazawa Medical University,
Uchinada, Ishikawa, 920-02 Japan*

Abstract In anesthetized toads, spontaneous activities were recorded from single afferent fibers of three semicircular canals and three otolith organs. Since some efferent fibers ramify within the eighth nerve and innervate two or more vestibular organs, a single branchlet of the eighth nerve was disconnected from its end organ, and was electrically stimulated to activate divergent efferent collaterals leading to other vestibular organs. The stimulation elicited an inhibitory effect on spontaneous activities of about one third of the afferent population, and a facilitatory effect on those of another one third. The remaining one third was unaffected. Whether or not the inhibitory or facilitatory effect was observed in an individual unit seemed to be related to its pattern and its rate of spontaneous activity. Most of the units showing relatively high and regular spontaneous firing were insensitive to the electrical stimulation, and units with a low firing rate and an irregular pattern of activity tended to be affected by the electrical stimulation. The activation of divergent efferent fibers elicited both inhibition and facilitation on the spontaneous afferent activities in all vestibular nerve branchlets, except in the saccular branchlet, where only inhibition was elicited. Electrical stimulation of the central stump of the saccular nerve branchlet, however, could produce both inhibitory and facilitatory effects in other vestibular nerve branchlets.

Key words: frog, vestibular efferent system, divergent efferent fibers, inhibition, facilitation.

Frog vestibular organs receive efferent innervations (KLINKE and GALLEY, 1974; PRECHT, 1976). The cell bodies of the efferent neurons are clustered in the brainstem reticular formations (PRECHT *et al.*, 1974; STRUTZ *et al.*, 1980, 1982) and their axons run through the eighth nerve to reach the vestibular organs (ROBBINS

et al., 1967). Recently, a horseradish peroxidase (HRP) study using toads revealed that these efferent neurons were localized in a region of the brainstem, ipsilateral to the eighth nerve and close to the motor nucleus of the facial nerve (PELLEGRINI *et al.*, 1985). Efferent nerve terminals form axo-somatic synapses with type II hair cells, so that sensory activity might be controlled peripherally at the receptor level (HILLMAN, 1969; KLINKE and GALLEY, 1974; LLINAS and PRECHT, 1969; PRECHT *et al.*, 1974; PRECHT, 1976).

The peripheral actions exerted by efferent fibers have been studied in various kinds of hair cell systems. In the mammalian cochlea and lateral lines of lower vertebrates, electrical stimulation of efferent fibers was shown to inhibit sensory transmission (KLINKE and GALLEY, 1974; FLOCK and RUSSELL, 1976). On the other hand, electrical stimulation of vestibular efferent fibers produced both inhibition and facilitation of the afferent activity in the goldfish (HARTMANN and KLINKE, 1980) and the cat (DECHESNE and SANS, 1980). Furthermore, both inhibition and facilitation are also seen in the squirrel monkey, though fewer than 1% of the units observed were inhibited (GOLDBERG and FERNANDEZ, 1980).

ROSSI *et al.* (1980) and PRIGIONI *et al.* (1983) found the presence of efferent fiber branching in the eighth nerve of the frog using extracellular recording of compound action potentials; some of the single efferent fibers innervated several different vestibular organs; for example, the canal organs, otolith organs, and the canal and otolith organs. Furthermore, they observed that activation of efferent fibers by electrical stimulation of the central stump of any vestibular nerve branchlets elicited either inhibition or facilitation of the spontaneous activities of the afferent fibers, depending on the recorded units. Thus, there would be two types of efferents, namely inhibitory and facilitatory ones, in the vestibular organs in the frog (GRIBENSKI and CASTON, 1976), as well as in the goldfish and the cat. However, it is not known whether branches of a single efferent fiber exert the same effect on all hair cells they innervate, in different vestibular organs.

In the present study, we investigated systematically the effects of activation of the divergent efferent fibers on the spontaneous activity of afferent fibers of all vestibular organs. As a result, we present some evidence that in the toad vestibular nerve some efferent fibers exert inhibition at one of their terminals, and facilitation at another.

MATERIALS AND METHODS

Twenty-four toads (*Bufo vulgaris japonicus*) weighing 90–150 g were used in the present study. The toads were anesthetized by intramuscular injection of sodium pentobarbital (Nembutal; 70 mg/kg), placed on their back on an animal holder, and were kept moist with wet gauze throughout the experiment to aid cutaneous respiration. The eighth nerve and the otic capsule were exposed through the roof of the mouth. A large portion of the bony wall of the otic capsule was carefully removed. This preparation permitted direct view of the ampullae,

utricle, saccule, and lagena and their nerve branchlets.

A single branchlet of the eighth nerve was cut near its target organ, and the central stump of the cut nerve branchlet was sucked into a suction glass pipette for electrical stimulation. A series of square wave voltage pulses (0.3 ms duration, 1.0–4.0 V amplitude, 50–400 pulses/s) for a period of 100–800 ms were applied every 5–7 s for 10–15 times.

Single unit recordings from the other branchlets, which had been left connected to their respective organs, were made by 3 M KCl-filled glass pipette microelectrodes of 40–60 M Ω resistance. A schematic drawing of the organs within the toad labyrinth and the experimental arrangement is shown in Fig. 1.

Unit activities, stimulations and control signals were continuously recorded by an FM data recorder, and analyzed later with a signal processor and a personal computer system.

As a measure of the pattern of the interspike intervals, a coefficient of variation (CV) was computed from a record of 1,000 spontaneous spike intervals for each unit. The CV is defined as the ratio of the standard deviation (S.D.) against the mean interval (WALSH *et al.*, 1972). A large CV value indicates a large dispersion in the distribution of interspike intervals, and a small CV value relatively regular discharge activity. A CV value of zero indicates a perfectly regular discharge within the resolution of the used time bin.

Fig. 1. A schematic drawing of the experimental arrangement and the peripheral innervation by the right eighth nerve of the toad. Viewed from the ventral side. Abbreviations: RE, recording microelectrode; STE, stimulating electrode; VIII G., the eighth nerve Scarpa's ganglion; A, anterior canal; H, horizontal canal; P, posterior canal; S, saccule; U, utricle; L, lagena; BP, basilar papilla; AP, amphibian papilla.

In four preparations, the eighth nerve was acutely cut between the Scarpa's ganglion and the brainstem, to exclude the secondary effect which might be produced by stimulation through the central nervous system. Results were similar to those in uncut preparations.

RESULTS

The existence of divergent efferent fibers

In order to confirm the existence of divergent efferent fibers, a microelectrode was advanced into the nerve branchlet near the cytoneural region, while another nerve branchlet was electrically stimulated, in the preparation where the eighth nerve was acutely cut at the brainstem. Under these conditions two classes of units were observed. Many units showed spontaneous discharges, and if the electrode tip was sufficiently deep near the cytoneural junction, excitatory postsynaptic potentials (EPSPs) or the like could be recorded usually even if spontaneous spikes were absent (see Fig. 3 or Fig. 4; ROSSI *et al.*, 1977). These spontaneously active fibers never showed one-to-one response to each pulse of a stimulus given to the other branchlet. They also showed spike discharges to some mechanical stimulations. They could be afferent fibers.

In the other hand, some fibers responded with almost one-to-one to each pulse of the electrical stimulation. Figure 2A and B are records from the posterior semicircular and the utricular nerves, respectively, where the saccular (A) and the anterior canal (B) nerves were electrically stimulated. These fibers did not show any spontaneous activities, spikes or EPSPs or the like, and also did not respond to mechanical stimulation. Thus these fibers were most likely collaterals of efferent fibers which were activated by axon reflex. This observation might be good

Fig. 2. Recordings from two different divergent efferent units (A and B). Both units show 1:1 response to each pulse of electrical stimulation (50 and 100 Hz). Stimulation periods indicated by horizontal bars. The unit A was recorded in the posterior canal nerve. Electrical stimulation was applied to the saccular nerve. The unit B was recorded in the utricular nerve. Electrical stimulation was applied to the anterior canal nerve.

evidence for the existence of divergent efferent fibers bifurcating in the eighth nerve.

Effects of divergent efferent fiber activation

A total of 708 afferent fibers were studied in nerve branchlets innervating three canals and three otolith organs. In a considerable number of the units, the activation of the divergent efferent fibers by electrical stimulation modified their spontaneous activities. Actually 247 (34.9%) units were inhibited, while 222 (31.4%) were facilitated. The remaining 239 (33.8%) were unaffected. Thus, from the mode of responses to efferent stimulations, afferent units may be classified into three groups, each comprising roughly one third of the spontaneously firing afferent population, under our experimental conditions.

Typical inhibitory and facilitatory responses of afferent fibers to electrical stimulation are shown in Fig. 3. In most cases, inhibition was so intense that the spontaneous activity was completely suppressed during the stimulation period. The inhibitory effect persisted for 100–200 ms after the end of the stimulus. This inhibition was followed by an increase in the spontaneous activity for a few hundred milliseconds (Fig. 3A). On the other hand, facilitation (Fig. 3B) was reflected by a substantial increase in the rate of discharges above the spontaneous level, which continued during the stimulation period. Upon termination of the stimulus a gradually decreasing afterdischarge followed. Latencies of both inhibitory and facilitatory responses did not differ significantly, and ranged between 20 and 40 ms. The change in spontaneous afferent activity resulting from electrical stimulation, either inhibitory or facilitatory, will hereafter be termed efferent response.

Fig. 3. Typical inhibitory (A) and facilitatory (B) responses elicited in afferent units of the saccular and the anterior canal nerves, respectively. Electrical stimulation (100 Hz, 500 ms) was applied to the horizontal (A) and the posterior canal (B) nerves. Upper traces show single sweep recordings. Stimulation pulses appear as artifacts. The PST histograms below each recording are based on 10 trials (bin width: 20 ms). Periods of electrical stimulation are indicated by horizontal bars. Vertical bars: 5 impulses/bin.

Next, we examined the effects of changes in stimulus parameters on efferent responses. Neither inhibition nor facilitation was ever converted to the other by any change in stimulus intensity or pulse frequency. With regard to the pulse intensity, the efferent response occurred almost in all-or-none manner. It seems that branches of a single fiber or of only a few fibers with similar thresholds, innervate a single hair cell.

When pulse frequencies were varied from 50 to 400 Hz while stimulus intensity was held constant, efferent responses were seen at any pulse frequency studied, and the degree of efferent responses did not differ widely among those frequencies. Thus the stimuli we routinely used could usually be supramaximal for the efferent responses. When pulse frequency was kept constant at 100 Hz, while stimulus duration was varied from 200 to 800 ms, efferent responses were maintained at the same level as long as the stimulation was applied. There was no adaptation such as efferent responses declining gradually during the stimulus period.

Figure 4A is a record from the saccular nerve. Electrical stimulation of the anterior canal nerve reduced the occurrence and amplitude of EPSPs with a time course similar to that of inhibition of spontaneous discharges. After interruption of the electrical stimulus, a recovery of the amplitude and occurrence of the EPSPs followed with a long latency.

Figure 4B is an example of facilitatory responses of EPSPs recorded from the posterior and anterior canal nerves by electrical stimulation of the anterior and the posterior canal nerves, respectively. The responses at threshold and above threshold intensity are shown in upper and lower traces, respectively. They show that there was an increase in the occurrence and amplitude of EPSPs during and after the stimulus period. The time course was similar to that of facilitation of spontaneous discharges.

Patterns of spontaneous interspike interval histograms and types of efferent responses of afferent units

The pattern of the steady state spontaneous activity in the absence of efferent stimulation was studied in fifty units. The mean interspike intervals and S.D.s of the individual units had ranges of 30–250 and 6–250 ms, respectively. Efferent stimulation elicited inhibition, facilitation, and no effect in 19, 13, and 18 of the above units, respectively. Means and S.D.s of the interspike intervals of the individual units are shown in Fig. 5, along with the three categories of efferent responses.

Interspike intervals and their CVs are summarized in Table 1. As to the interspike interval, the F -test shows no significant difference in the variance between the inhibited and facilitated groups ($F=1.086$). Further, no significant difference was present between the mean interspike intervals of the inhibited and facilitated groups by the t -test ($t=0.445$). That is, the rates of spontaneous discharges do not differ significantly between the two groups.

Significant difference ($p<0.01$), however, were present between the variances

Fig. 4. Effects of electrical stimulation on spontaneous EPSPs or EPSP-like potentials (▼) of vestibular afferent units. A: Inhibition of EPSPs in the saccular nerve unit. Upper, middle and lower traces are continuous recordings. Electrical stimulation (50 Hz, 500 ms) of the anterior canal nerve is applied in the middle and stopped in the lower trace. B: Facilitation of EPSPs in two different canal units. Upper and lower traces are recordings at and above threshold, respectively. Periods of electrical stimulation (100 Hz, 500 ms) are indicated by horizontal bars. Action potentials (▼) are cut off in both A and B.

Fig. 5. The relationship between the mean interspike interval and the standard deviations of the mean. Both variables are plotted on logarithmic scales. Abbreviations in the inset: F, facilitation; I, inhibition; NE, no effect.

Table 1. Interspike intervals and coefficients of variation in inhibited, facilitated, and unaffected groups.

Type of efferent response	<i>n</i>	Interspike interval* (ms)	Coefficient of variation*
Inhibition	19	112.1±58.9	0.78±0.17
Facilitation	13	102.8±56.5	0.82±0.41
No effect	18	66.9±20.7	0.25±0.13

* Mean±S.D.

of the unaffected group and either of the inhibited ($F=8.009$) or facilitated groups ($F=7.459$). Significant difference was also present between the interspike intervals of the unaffected group and those of either inhibited ($t=3.141$, $p<0.01$) or facilitated ($t=2.182$, $p<0.05$) group, by the t -test for two populations with different variances (KENDALL and STUART, 1979).

It is clear from Fig. 5 that the S.D.s are reduced with a decrease in the interspike intervals, or with an increase in the firing rates, as previously reported (HONRUBIA *et al.*, 1981). Indeed, the present distribution seems to correspond to a subpopulation of their sample population, lacking in very long interval units.

Figure 5 shows that the S.D.s of mean interspike intervals of unaffected units are very small. This means that unaffected units seem to have a more regular firing pattern than the facilitated or inhibited units. To confirm this finding a population analysis was carried out for CV values. The CV values against the types of the efferent responses are shown in Fig. 6A, and data are summarized in Table 1.

The *F*-test showed that the variance of CV values of the facilitated group was significantly larger ($p < 0.01$) than that of the inhibited ($F = 5.538$) or unaffected ($F = 9.517$) group. Appropriate *t*-tests showed that significant differences ($p < 0.01$) were present between the CV values of the unaffected and that of the inhibited ($t = 10.516$) or facilitated ($t = 4.893$) group. Figure 6B shows representative examples of interspike interval histograms of irregular (CV = 0.56) and regular units (CV = 0.10) which are indicated by a and b, respectively, in Fig. 6A. The two units (a and b) have similar mean interspike intervals, but show a different response (facilitatory and no response, respectively) to the electrical stimulation of efferent fibers.

These results indicate that the presence or absence of the efferent response is related to the pattern of spontaneous activity of individual units. There are

Fig. 6. A: Coefficients of variation (CVs) of the spontaneous activity of vestibular afferent units ($n = 50$) are plotted separately according to efferent responses. Abbreviations are the same as in the Fig. 5. B: Interspike interval histograms of the spontaneous activity of two utricular nerve units (a, b indicated in Fig. 6A). The two units have similar mean intervals but differ greatly in CV values, exhibiting different responses to electrical stimulation.

tendencies that unaffected units are found in rapidly firing units, and that their firing patterns are more regular than those affected by efferent stimulation, in general. In contrast, units with a low firing rate and a broad interval histogram tend to be under efferent control, either inhibition or facilitation.

Proportions of types of efferent responses in different vestibular organs

Figure 7 shows the occurrence of the different efferent responses among afferent fibers innervating different vestibular organs. More detailed data are given in Table 2. Utricular branchlet stimulation has not been tried. Since the nerve branchlet invades the utricle immediately after it separated from the eighth nerve, it was difficult to detach it from the end organ for electrical stimulation.

As shown in Fig. 7, an approximately equal proportion of inhibited, facilitated, and unaffected fibers were observed in the three canals, and in the lagenar and utricular nerves. In the saccular nerve, however, only the inhibitory effect could be elicited (88%), but facilitation was never observed.

In contrast, electrical stimulation of the saccular nerve branchlet produced facilitation as well as inhibition in all other nerve branchlets. For example, by electrical stimulation of the saccular branchlet, 10 of 74 posterior canal nerve units were inhibited, 36 were facilitated, and 28 were unaffected (Table 2). Because results similar to the above were obtained also in the preparations where the eighth nerve was acutely cut at the level of its entrance into the brainstem, it is unlikely that the inhibition in the sacculus was exerted through quite independent efferent fibers via the activation of the central nervous system.

If one collateral or branch of an efferent axon exerts inhibition on its target, it seems natural to expect that other branches of the same axon would exert inhibitory

Fig. 7. Histograms showing the effects of electrical stimulation on afferent units in six vestibular organs. The actual numbers of units are given in Table 2. Abbreviations: I, inhibition; F, facilitation; NE, no effect; anterior, anterior canal; horizontal, horizontal canal; posterior, posterior canal.

Table 2. The number of afferent units affected by electrical stimulation of different nerve branchlets.

	Nerve branchlet recorded																	
	Anterior			Horizontal			Posterior			Saccular			Lagenar			Utricular		
	I	F	NE	I	F	NE	I	F	NE	I	F	NE	I	F	NE	I	F	NE
Nerve branchlet stimulated																		
Anterior	—			5	10	8	9	13	19	19	0	3	9	6	11	3	10	11
Horizontal	9	13	3	—			6	11	4	21	0	1	6	10	6	6	5	12
Posterior	8	9	20	5	5	15	—			33	0	8	9	6	7	12	16	12
Saccular	9	14	4	7	7	12	10	36	28	—			7	6	7	6	10	7
Lagenar	6	7	11	5	12	10	8	10	6	23	0	1	—			6	6	13
Total (N = 708)	113			101			160			109			90			135		

effect on their targets. Actually it was not the case. All the efferent activities inhibited the spontaneous afferent activities in the sacculus, while antidromic stimulations of the saccular branch exerted either inhibitory or facilitatory effect on some afferent units in other vestibular organs. These results seem to demonstrate that some divergent efferent fibers could have a facilitatory effect on afferent units from the vestibular organs except the sacculus, while all their saccular branches are inhibitory to saccular afferent units.

DISCUSSION

In the present study, it was demonstrated that activation of divergent efferent fibers by electrical stimulation elicited either inhibition or facilitation on both spontaneous EPSPs and spike discharges of afferent fibers, depending on the units recorded, in all of the vestibular organs in the anesthetized toad, conforming to results on the isolated frog labyrinth (ROSSI *et al.*, 1980; PRIGIONI *et al.*, 1983).

It has been shown that in preparations where the eighth nerve had been cut between the ganglion and brainstem 2 weeks prior to the recording, the fibers with spontaneous activities were recorded as usual but were unresponsive to the stimulation of the vestibular nerve branchlet (PRIGIONI *et al.*, 1983). Therefore, they should have their soma peripheral to the cut. In other words, they are afferents, and the changes in the spontaneous activities brought about by stimulation of the nerve branchlet seen in the normal labyrinth are not the result of the antidromic spike invasion into the afferent fibers themselves. The bifurcating fibers of which we confirmed the presence in the eighth nerve are possibly efferent.

Our observation that the electrical stimulation affected not only the amplitude but also the occurrences of EPSPs (or EPSP-like potentials) shows that the effect was presynaptic, or on the hair cells. The presence of a few efferent synapses on a hair cell in the frog inner ear has been confirmed (HILLMAN, 1969; KLINKE and GALLEY, 1974; PRECHT, 1976).

The inference that only a few bifurcating efferent fibers innervate a single hair cell does not necessarily deny the presence of multiple efferent innervations on a hair cell. Together with the observation, however, that the relatively low pulse frequency (50 Hz) was enough to induce a maximal efferent response, it suggests that the effects were quite strong and there might be few efferent convergences on a hair cell.

We examined efferent responses in all the vestibular organs systematically and obtained some new findings. First, we found that the presence or absence of the efferent response was closely related to the pattern of the afferent spontaneous activity in both canal and otolith organs; units firing irregularly were more often affected by efferent activation than those firing regularly. Similar results were also shown previously in the toadfish (HIGHSTEIN and BAKER, 1985), monkey (GOLDBERG and FERNANDEZ, 1980), and cat (KASHII *et al.*, 1987).

Primary vestibular afferents in the frog are characterized by a broad range of

axon diameters (from 3 μm up to 20 μm) (DUNN, 1978). In the posterior canal of the frog, HONRUBIA *et al.* (1981) reported that the largest vestibular afferents were characterized by their irregular spontaneous activities, while the smaller axons by more regular activities. Furthermore, from morphological observations with HRP they showed that these largest axons consistently terminated at or near the central region of the crista. In combination with their results, our spontaneously active afferent fibers affected by electrical stimulation could correspond to those axons mainly innervating hair cells at or near the central region of the crista. Efferent innervations might also be limited in the same region.

Second, no facilitation was seen in the saccular afferents by efferent activation, while both inhibitory and facilitatory effects were observed in the afferents of the five other otolith and canal organs. On the other hand, PRIGIONI *et al.* (1983) reported that there were no clear differences in the occurrence of the efferent response between the canal and otolith organs, although their data were not presented in detail. While it has been reported that in the frog posterior canal the efferent has both inhibitory and facilitatory effects on the afferent discharge (ROSSI *et al.*, 1980), in the frog sacculus stimulation of the efferent produced only hyperpolarizing synaptic potential in the hair cell (ASHMORE and RUSSELL, 1982; ASHMORE, 1983). Such a difference in the efferent response between the sacculus and other vestibular organs would be seen also in the toadfish. HIGHSTEIN and BAKER (1985) reported that the effects of efferent activation were facilitatory on the posterior canal afferent discharges. In intracellular recording from the saccular hair cells, acetylcholine (ACh) was found to increase the open time and opening rate of a high conductance K^+ channel (STEINACKER and ROJAS, 1988). In addition, in goldfish, both types of efferent responses have been found in semicircular canal afferents (HARTMAN and KLINKE, 1980), while FURUKAWA (1981) demonstrated that electrical stimulation of the efferents reduced or abolished the sound-induced EPSP in saccular afferents.

Thus, our results agree with those by other investigators in that the sacculus differs from the other vestibular organs in view of the efferent control; only inhibition occurs in the sacculus and both inhibition and facilitation in others. It appears that the efferent facilitation can be observed mostly in the vestibular organs such as semicircular canals, but not in the auditory organs (see introduction). It has been known that the frog sacculus has seismic sensitivity (sensitivity to substrate-borne vibration) (KOYAMA *et al.*, 1982; LEWIS *et al.*, 1982). Therefore, the functional differences may be related to the different efferent actions in the sacculus and five other vestibular organs. Nevertheless, the fact that the electrical stimulation of the saccular nerve branchlet could produce both types of efferent responses in all other vestibular afferents is quite interesting. This would indicate that divergent efferent fibers elicit different responses on the spontaneous activities of the afferent fibers of different organs.

A number of observations suggest ACh as the inhibitory efferent transmitter in the inner ear. However, no conclusive data are yet available concerning the neural

mechanism involved in the efferent facilitation. Some investigators suggest exclusion of chemical transmission for the facilitatory effect (GOLDBERG and FERNANDEZ, 1980; HARTMANN and KLINKE, 1980; ROSSI *et al.*, 1980). Others suggest involvement of ACh for the facilitatory efferent synapse (BERNARD *et al.*, 1985; GUTH *et al.*, 1986; NORRIS *et al.*, 1988). Considering Dale's law, the present result may add evidence that both the inhibitory and facilitatory effects are mediated by the same transmitter, probably ACh.

Concerning the basic mechanism of the two types of efferent responses, BERNARD *et al.* (1985) suggested that the method of preparation may be an important factor in determining whether efferent activation results in a predominant increase or decrease in afferent spontaneous activity, from the result that facilitation was elicited more frequently in the isolated semicircular canal than in the isolated, intact whole vestibular labyrinth. Their explanation was as follows. Endolymphatic K^+ would be expected to be lower in the isolated canals than in the isolated whole labyrinth. Therefore, with respect to the equilibrium potential of the efferent transmitter, the hair cells in the isolated whole labyrinth are more depolarized in the resting state, and the hair cells in the isolated canal are more hyperpolarized. As a result, activation of the receptor at the efferent synapse would result more often in a depolarization of the hair cells in the isolated canal than those in the isolated intact labyrinth.

However, despite the fact that the whole animal preparation, which has a greater integrity than the isolated whole labyrinth, was used in the present study, facilitation occurred as frequently as inhibition did. Further, under the same conditions as other vestibular organs, no facilitation was seen in the saccular afferents. Considering these results, the important factor determining whether the efferent activation results in facilitation or inhibition of afferent activities would not be the condition of the preparation as discussed by BERNARD *et al.* (1985). So far, it is not clear why electrical stimulation of the saccular nerve branchlet produces both types of efferent responses in all other vestibular afferents, while efferent effects to the saccular afferents are all inhibitory if present. One possibility may be that saccular hair cells receive both types of efferent control, but the efficiency of facilitatory action is very low compared to that of the inhibitory one, so that only inhibition was observed in the sacculus.

In conclusion, although the interpretation of results may be somewhat speculative, the data obtained in the present study show that not all the vestibular organs in the frog would receive homogenous, non-selective efferent control. They also suggest that the actions of divergent efferent fibers would be more complex than usually imagined.

We are thankful to Dr. J. Yano for valuable suggestions, H. Adachi and S. Muramoto for technical assistance and computer analysis of the data, and Mrs. K. Yamada for her help in the preparation of figures and manuscript.

REFERENCES

- ASHMORE, J. F. (1983) Frequency tuning in a vestibular organ of the frog. *Nature*, **304**: 539-541.
- ASHMORE, J. F. and RUSSELL, I. J. (1982) Effect of efferent nerve stimulation on hair cells of the frog sacculus. *J. Physiol. (Lond.)*, **329**: 25P-26P.
- BERNARD, C., COCHRAN, S. L., and PRECHT, W. (1985) Presynaptic actions of cholinergic agents upon the hair cell-afferent fiber synapse in the vestibular labyrinth of the frog. *Brain Res.*, **338**: 225-236.
- DECHESNE, C. and SANS, A. (1980) Control of vestibular nerve activity by the efferent system in the cat. *Acta Otolaryngol. (Stockholm)*, **90**: 82-85.
- DUNN, R. F. (1978) Nerve fibers of the eighth nerve and their distribution to the sensory nerves of the inner ear in the bullfrog. *J. Comp. Neurol.*, **182**: 621-636.
- FLOCK, A. and RUSSELL, I. J. (1976) Inhibition by efferent nerve fibres: Action on hair cells and afferent synaptic transmission in the lateral line canal organ of the burbot *Lota lota*. *J. Physiol. (Lond.)*, **257**: 45-62.
- FURUKAWA, T. (1981) Effects of efferent stimulation on the saccule of goldfish. *J. Physiol. (Lond.)*, **315**: 203-215.
- GOLDBERG, J. M. and FERNANDEZ, C. (1980) Efferent vestibular system in the squirrel monkey: Anatomical location and influence on afferent activity. *J. Neurophysiol.*, **43**: 986-1025.
- GRIBENSKI, A. and CASTON, J. (1976) Tonic influence of the efferent vestibular system on the spontaneous afferent activity from semicircular canals in the frog (*Rana esculenta* L.). *Exp. Brain Res.*, **26**: 275-283.
- GUTH, P. S., NORRIS, C. H., GUTH, S. L., QUINE, D. B., and WILLIAMS, W. H. (1986) Cholinomimetics mimic efferent effects on semicircular canal afferent activity in the frog. *Acta Otolaryngol. (Stockholm)*, **102**: 194-203.
- HARTMANN, R. and KLINKE, R. (1980) Efferent activity in the goldfish vestibular nerve and its influence on afferent activity. *Pflügers Arch.*, **388**: 123-128.
- HIGHSTEIN, S. M. and BAKER, R. (1985) Action of the efferent vestibular system on primary afferents in the toadfish, *Opsanus tau*. *J. Neurophysiol.*, **54**: 370-384.
- HILLMAN, D. E. (1969) Light and electron microscopical study of the relationships between the cerebellum and the vestibular organ of the frog. *Exp. Brain Res.*, **9**: 1-15.
- HONRUBIA, V., SITKO, S., KIMM, J., BETTS, W., and SCHWARTZ, I. (1981) Physiological and anatomical characteristics of primary vestibular afferent neurons in the bullfrog. *Intern. J. Neurosci.*, **15**: 197-206.
- KASHII, S., SASA, M., MATUOKA, I., ITO, J., and TAKAORI, S. (1987) Inhibition of vestibular nerve activity by efferent fibers in the cat. *Acta Otolaryngol. (Stockholm)*, **104**: 13-21.
- KENDALL, M. and STUART, A. (1979) The Advanced Theory of Statistics, Charles Griffin and Company, London, Vol. 2, 159.
- KLINKE, R. and GALLEY, N. (1974) Efferent innervation of vestibular and auditory receptors. *Physiol. Rev.*, **51**: 316-357.
- KOYAMA, H., LEWIS, E. R., LEVERENZ, E. L., and BAIRD, R. A. (1982) Acute seismic activity in the bullfrog ear. *Brain Res.*, **250**: 168-172.

- LEWIS, E. R., BAIRD, R. A., LEVERENZ, E. L., and KOYAMA, H. (1982) Inner ear: Dye-injection reveals peripheral origins of specific sensitivities. *Science*, **215**: 1641-1643.
- LLINAS, R. and PRECHT, W. (1969) The inhibitory vestibular efferent system and its relation to the cerebellum in the frog. *Exp. Brain Res.*, **9**: 16-29.
- NORRIS, C. H., HOUSLEY, G. D., WILLIAMS, W. H., GUTH, S. L., and GUTH, P. S. (1988) The acetylcholine receptors of the semicircular canal in the frog (*Rana pipiens*). *Hear. Res.*, **32**: 197-206.
- PELLEGRINI, M., CECCOTTI, F., and MAGHERINI, P. (1985) The efferent vestibular neurons in the toad (*Bufo bufo* L.): Their location and morphology. A horseradish peroxidase study. *Brain Res.*, **344**: 1-8.
- PRECHT, W. (1976) Physiology of the peripheral and central vestibular systems. In: *Frog Neurobiology*, ed. by LLINAS, R. and PRECHT, W., Springer, Heidelberg, pp. 481-512.
- PRECHT, W., RICHTER, A., OZAWA, S., and SHIMAZU, H. (1974) Intracellular study of frog's vestibular neurons in relation to the labyrinth and spinal cord. *Exp. Brain Res.*, **19**: 377-393.
- PRIGIONI, I., VALLI, P., and CASELLA, C. (1983) Peripheral organization of the vestibular efferent system in the frog: An electrophysiological study. *Brain Res.*, **269**: 83-90.
- ROBBINS, R. G., BAUKNIGHT, R. S., and HONRUBIA, V. (1967) Anatomical distribution of efferent fibers in the eighth cranial nerve of the bullfrog (*Rana catesbeiana*). *Acta Otolaryngol. (Stockholm)*, **64**: 436-448.
- ROSSI, M. L., VALLI, P., and CASELLA, C. (1977) Post-synaptic potentials recorded from afferent nerve fibres of the posterior semicircular canal in the frog. *Brain Res.*, **135**: 67-75.
- ROSSI, M. L., PRIGIONI, I., VALLI, P., and CASELLA, C. (1980) Activation of the efferent system in the isolated frog labyrinth: Effects on the afferent EPSPs and spike discharge recorded from single fibers of the posterior nerve. *Brain Res.*, **185**: 125-137.
- STEINACKER, A. and ROJAS, L. (1988) Acetylcholine modulated potassium channel in the hair cell of the toadfish saccule. *Hear. Res.*, **35**: 265-270.
- STRUTZ, J., SPATZ, W. B., SCHMIDT, C. L., and STURMER, C. (1980) Origin of the centrifugal fibers to the labyrinth in the frog (*Rana esculenta*). A study with fluorescent retrograde neuronal tracer "Fast blue." *Brain Res.*, **215**: 323-328.
- STRUTZ, J., BIELENBERG, K., and SPATZ, W. B. (1982) Location of efferent neurons to the labyrinth of the green tree frog (*Hyla cinerea*), a horseradish peroxidase study. *Arch. Otorhinolaryngol.*, **234**: 245-251.
- WALSH, B. T., MILLER, J. B., GACEK, R. R., and KIANG, N. Y. S. (1972) Spontaneous activity in the eighth cranial nerve of the cat. *Intern. J. Neurosci.*, **3**: 221-236.