Efferent Neurons and Vestibular Cross Talk in the Frog

S. F. Myers, H. H. Salem, and J. A. Kaltenbach. Efferent neurons and vestibular cross talk in the frog. J. Neurophysiol. 77: 2061–2070, 1997. A galvanic stimulus (30- to 120-s, 0.3-mA constant current pulse) was used to depolarize the spike-generating region of horizontal and anterior canal afferent neurons. The galvanically induced spike activity from these neurons served as a driving input to the efferent vestibular system in the bullfrog. Efferent-mediated effects were assessed by intracellular recordings of posterior canal afferent spike activity, either ipsilateral or contralateral to the driving stimulus. Ipsilateral to the driving stimulus, efferent-mediated spike rate changes occurred in 62 (39%) of 158 posterior canal afferent neurons. Ipsilateral efferent-mediated effects were overwhelmingly excitatory (92%). Of responding units, 3% were inhibited during stimulus application and 5% showed mixed responses involving 3–20 s of inhibition followed by facilitation. Contralateral to the driving stimulus, efferent-mediated spike rate changes occurred in 18 (23%) of 77 posterior canal afferent neurons. Contralateral efferent-mediated effects were overwhelmingly inhibitory (95%). Only one unit was facilitated during stimulus application and no mixed responses to contralateral stimulation were observed. Analysis of the coefficient of variation in interspike intervals (CV) before and during stimulation showed no significant efferent-mediated effects on spike train noise. Comparisons of resting spike rates between units showing efferent-mediated effects and those that did not were in general agreement with previous studies. Responding units had a lower mean spike rate (6.8 ± 0.70 spikes/s, mean ± SE) than did nonresponding units (10.7 ± 0.42 spikes/s, mean ± SE; P < 0.001; 2-tailed t-test of log-normalized data). Comparison between groups in the regularity of their resting spike rates, as quantified by CV, showed considerable overlap. When responding and nonresponding units with similar resting spike rates were compared, responding units had more irregular resting spike rates than did nonresponding units (P < 0.004; 2-tailed, paired t-test). In most cases (77%) the temporal pattern and general shapes of efferent-mediated responses mirrored the driving input of the galvanically activated afferent neurons. The other 23% of efferent-mediated responses exhibited a marked adaptation of the response. Adapting and nonadapting units were not significantly different in their mean resting spike rates or in the regularity of their resting spike rates.

INTRODUCTION

The efferent vestibular system provides a means by which the CNS can modify sensory operations of the inner ear motion and gravity sensors. A major function of the efferent vestibular system is believed to involve adjustments of the operating range of the vestibular sensors in anticipation of a voluntary movement (Boyle and Highstein 1990). Experiments in anesthetized as well as special unanesthetized preparations have shown that efferent activation causes many vestibular afferent neurons to exhibit higher background spike rates in combination with reduced response amplitudes (Boyle and Highstein 1990; Goldberg and Fernandez 1980). These primary afferent neurons are not so easily driven into spike rate saturation or silence by a strong acceleration. This efferent control over dynamic range of the inner ear motion sensors is seen in species as diverse as fish and monkeys (Boyle and Highstein 1990; Goldberg and Fernandez 1980), and may represent the most important function of the efferent vestibular system.

A number of anatomic studies suggests that the efferent system may have other, possibly more complex or subtle functions. Vestibular efferent neurons are generally considered to be cholinergic; however, a group of vestibular efferent neurons in the gerbil appears not to be cholinergic. Also, those neurons that are cholinergic also contain calcitonin gene-related peptide and met-enkephalin (Perachio and Kvetter 1989). Studies in the rat have found that 55% of efferent neurons also contain calcitonin gene-related peptide (Ohno et al. 1991). Ultrastructural studies in the rat indicate that efferent fibers with calcitonin gene-related peptide have different peripheral innervation patterns compared with those fibers without the peptide (Wackym 1993).

Additional physiological characterization of the influence of the efferent system on vestibular afferent neurons may help in understanding the recently discovered anatomic complexities of the efferent vestibular system. Frogs are an especially useful animal model for studying the influence of the efferent vestibular system because, in addition to excitatory effects, efferent-mediated inhibitory effects are much more common in frogs than in other species. Excitatory effects and inhibitory effects probably are mediated by different efferent fibers (Valli et al. 1986). Whether both sets of fibers are activated by the CNS simultaneously or differentially, depending on the inputs to the efferent vestibular system, is not known.

Most studies of the influence of the efferent vestibular system on vestibular afferent neurons have used trains of electrical pulses to excite vestibular efferent neurons. Efferent vestibular neurons also can be activated indirectly through a variety of sensory pathways (Caston and Bricout-Berthout 1984; Precht et al. 1974; Schmidt 1963). Dickman and Correia (1993) have demonstrated the effectiveness of vestibular afferent stimulation as a driving input for efferent-mediated effects on the opposite inner ear. Given the anatomic complexities of efferent vestibular system, it is possible that ‘‘how’’ the efferent vestibular system is activated may be important in determining the character of the efferent-mediated effects on vestibular afferent activity.

The purpose of the present study was to explore how vestibular afferent activity from one set of endorgans can alter vestibular afferent activity of other ipsilateral or contralateral...
endorgans. Long-duration galvanic electrical pulses (20–120 s) were chosen as a method to galvanically depolarize the spike generator regions of anterior and horizontal canal afferent dendrites. Efferent-mediated responses of posterior canal afferent neurons were monitored via intracellular electrodes. Specific questions to be answered were as follows. 1) Does this method of inducing efferent-mediated effects result in the same variety of vestibular afferent responses as seen in studies in which direct activation of efferent neurons was employed (i.e., excitation, inhibition, mixed and no response of afferent neurons)? 2) Does the efferent-mediated afferent response have temporal characteristics that mirror the input afferent drive to the efferent system or do afferent responses adapt or extend beyond the driving stimulus? 3) Do efferent-mediated afferent responses have spike train noise characteristics significantly different from periods of resting spike activity?

METHODS

Surgical procedure

Bullfrogs (Rana catesbiana) weighing 50–180 g were anesthetized with pentobarbital sodium and ketamine hydrochloride (35 µg/g body wt of each anesthetic via intramuscular injections), with supplemental injections as necessary. During surgery and throughout the physiological experiment, the frog was covered with damp gauze to facilitate cutaneous respiration. The VIIIth nerve was approached through the roof of the mouth by removal of a small patch of mucosa and drilling through the underlying bone and cartilage with a dental drill bit to expose the dura directly over the VIIIth nerve in the cranial cavity. The otic capsule was opened postero-medially to expose the course of the posterior division of the VIIIth nerve to the branch innervating the posterior semicircular canal. Care was used when excising dura or perichondrium to avoid damage to branches of the labyrinthine artery. At the end of experimentation, the animal was killed by decapitation under deep pentobarbital anesthesia.

Experimental procedure

With the frog positioned ventral side up, the influence of efferent vestibular system on posterior canal afferent neuronal activity was investigated by galvanic activation of anterior and horizontal canal afferent neurons. Stimulating electrodes comprised paired electrodes (either tungsten or silver wire, insulated to within 1 mm of the tip with parylene or Teflon, respectively). Two sets of paired electrodes were used; one pair for each ear, placed in the otic capsule. The standard positioning was to place the cathodal electrode just anterior to the anterior canal ampulla. The anodal electrode was then placed ~8 mm posterior to the anodal electrode either within the cartilage of the otic capsule or submucosally within the middle ear. The intent of the electrode placement was to place the anterior and horizontal canal ampullae in the current path of the electrodes but in closer proximity to the cathodal electrode. Stimulation comprised long-duration (20–120 s) constant current pulses (0.1–0.45 mA) generated by an isolated pulse stimulator (AM systems model 2100).

The activity of single vestibular nerve fibers was recorded intraxonally with single-barreled glass micropipettes (World Precision Instruments, TW 150F-4). Microelectrodes were pulled with a Campden moving coil microelectrode puller (model 753) and filled with 0.5 M KCl. The electrodes were inserted into a plastic holder containing 0.5 M KCl and a Ag/AgCl pellet attached to a silver wire lead. The indifferent electrode comprised a plastic petri dish filled with an agar gel made from 0.5 M KCl, with a second Ag/AgCl pellet embedded in the agar. The moist surface of the agar was kept in contact with the animal’s skin. Electrode impedances were 10–15 MΩ.

Microelectrodes were visually positioned under low magnification and remotely advanced via a hydraulic microdrive (Soma Scientific) into the portion of posterior division of the VIIIth nerve containing fibers to the posterior canal ampulla, amphibian papilla, and basilar papilla. Nerve fibers responding to sound were excluded from this study. A commercial intracellular electrometer (Wagner Scientific, model IE-201) was used for initial signal amplification, followed by additional amplification and filtering with a Tektronix AM-502 differential amplifier (total amplifier gain of 1,000–10,000; typical filter settings: 3 kHz low-pass and 10 Hz high-pass). The electrode trace was monitored on a Tektronix digital oscilloscope, as well as on a Macintosh 8100 computer using LabView data acquisition and analysis software with lab-designed algorithms for on-line data analysis. The electrode trace also was recorded on a digital tape recorder (Vetter, model 400 PCM Recorder) for archival storage and additional data analysis off-line. A sampling rate of 4,000 Hz was used to acquire the electrode voltage signal into the computer. Algorithms were then applied to detect action potentials and determine spike rate and coefficient of variation in interspike intervals (CV = SD of interspike intervals/mean interval).

Two basic experimental paradigms were used (see Fig. 1). In both paradigms, single posterior canal afferent units were recorded in the absence of any stimulation and then while indirectly driving the efferent vestibular system. In paradigm 1, galvanic stimuli were applied ipsilateral to the posterior canal afferent neuron being recorded. In paradigm 2, galvanic stimuli were applied to drive the efferent system from the contralateral inner ear.

Recordings of vestibular afferent responses to galvanic stimulation were made from electrodes placed in the anterior division of the VIIIth nerve root within the cranial cavity. Horizontal canal units could be identified by monitoring afferent responses to gentle motions of a horizontal rotational stage. Units not responding to gentle horizontal rotations were assumed to be either anterior canal or utricular units. Some units responded weakly or not at all to our galvanic stimulus. These units were probably utricular or saccular units at a greater distance from the cathodal electrode.

Galvanic chemical reactions between paired stimulating electrodes, as evidenced by gas bubbling around the electrode tips, sometimes occurred. Reactions between stimulating electrodes can cause voltage spike artifacts in the recording electrode trace as well as a prolonged voltage gradient between electrodes after termination of the stimulus pulse. Galvanic chemical reactions between stimulating electrodes were not observed in initial experiments in which tungsten electrodes were used; however, for an unknown reason, these reactions later became a common problem, even with new electrodes from the same supplier. Galvanic reactions were minimized by limiting stimulus currents to 0.3 mA and by the use of insulated silver wire electrodes, newly prepared at the beginning of each experiment.

The care and use of the animals for this study were approved by the University Committee on Use and Care of Animals at the University of Michigan (National Institutes of Health Grant R29 DC-00971).

RESULTS

Galvanic stimulation of anterior and horizontal canal afferent neurons

Basic experimental protocols are illustrated in Fig. 1. The anterior and horizontal canal afferent responses shown in

1 The electrode puller used allowed control of the final taper angle of the electrode tip so that sharp electrodes could be pulled with relatively low impedances.
Fig. 1. Experimental paradigms used in this study. In paradigm 1, ipsilateral effects of vestibular afferent drive to efferent vestibular system were investigated. In paradigm 2, contralateral effects were investigated. Efferent vestibular neurons project widely throughout the labyrinth. For illustrative purposes only the efferent projection to the posterior semicircular canal is shown. Efferent-mediated effects on posterior canal afferent activity, either ipsilateral or contralateral to the driving stimulus, were recorded with the use of intracellular microelectrodes.

Fig. 2. Peristimulus spike rate histograms with 1-s spike bins demonstrate the effect of direct electrical activation of the afferent dendrites (A: horizontal canal nerve; B and C: anterior canal nerve). These responses provide an indication of the “driving” input to the efferent vestibular system. Horizontal bars: duration of galvanic stimulation (0.3-mA constant current pulse). A and B show the typical pattern with an “onset” response, then a period of relatively constant elevated spike rate, followed by a poststimulation spike rate depression. Response patterns like that shown in C, with little onset response and no poststimulus depression, also were observed.

Fig. 2. provide an indication of the “driving” input to the efferent vestibular system. Long-duration, constant current pulses (30–120 s, 0.3 mA) applied to the otic capsule in the vicinity of the anterior and horizontal canal ampullae caused a two- to eightfold increase in spike rates (increase of 6–30 spikes/s over resting spike rate) of afferent nerve fibers innervating those same ampullae (Fig. 2). Spike rate changes occurred abruptly at the onset and termination of the galvanic stimulus. Partial response declines during stimulation as well as poststimulus depression of spike rates were
common but not evident in all cases (compare Fig. 2, A and B, with Fig. 2C).

Comparison of ipsilateral and contralateral stimulation effects on posterior canal afferent spike rates

In paradigm 1 (Fig. 1), galvanic activation of vestibular afferent neurons of the anterior and horizontal canal ampullae was used to provide input drive to the efferent vestibular system. The influence of the efferent vestibular system on posterior canal afferent activity was examined in the presence and absence of this input drive. The incidence of efferent-mediated responses and the response types (facilitated, inhibited, or mixed) are summarized in Fig. 3. Single-unit recordings of posterior canal afferent neurons identified 62 of 158 units (39%) that changed spike rate during 0.3-mA galvanic stimulation. Ninety-two percent (57 of 62) responded with increased spike rates (prestimulus spike rate: 7.0 ± 0.72 spikes/s, mean ± SE; during-stimulus spike rate: 11.9 ± 0.91 spikes/s, mean ± SE; increase of 4–839% or 0.8–21 spikes/s; mean = 5.0 ± 3.8 spikes/s, mean ± SD). Two units (3%) responded with decreased spike rates (24% and 88%; 3 and 2 spike/s). Three units (5%) had mixed responses involving an initial 3–20 s of reduced spike rate followed by an elevated spike rate.

Twenty-nine of the posterior canal units that responded to ipsilateral galvanic stimulation (paradigm 1) were also presented with contralateral galvanic stimulation (paradigm 2). Only 11 of these 29 units responded to a contralateral stimulation. An additional 7 posterior canal units responded to contralateral galvanic stimulation, but these units along with 39 nonresponding units were not held long enough to test their response to ipsilateral stimulation. Ninety-five percent of posterior canal units that responded to contralateral stimulation did so with a reduced spike rate (prestimulus spike rate: 7.0 ± 0.72 spikes/s, mean ± SE; during-stimulus spike rate: 4.1 ± 0.91 spikes/s, mean ± SE; reduction of 7–95% or 0.5–11 spikes/s; mean = 3.2 ± 2.7 spikes/s, mean ± SD; n = 17; 0.3-mA stimulus). The remaining unit showed a very mild excitatory response, changing from a resting spike rate of 4.6 spikes/s to 6.2 spikes/s during stimulation. No mixed responses were seen with contralateral stimulation.

Ipsilaterally evoked spike rate changes were significantly larger than contralaterally evoked spike rate changes (P < 0.05; 1-tailed t-test; log-normalized data). Of 11 units that responded to both ipsilateral and contralateral stimulations, 10 had the greater spike rate change to ipsilateral stimulation. The unit shown in Fig. 4 had the greatest asymmetry between ipsilateral and contralateral responses. The first and third stimulus applications were ipsilateral to the recorded unit, the second and fourth stimulus applications were contralateral. The first two stimulus applications were 0.3-mA constant current, the second set of stimuli consisted of 0.4-mA constant current. The stronger ipsilateral stimulus caused a 50% greater response than the weaker ipsilateral stimulus. The stronger contralateral stimulus caused a transient, initial silencing of spike activity not seen during the weaker stimulus; however, the overall spike rate for each stimulus period was the same (0.2 spikes/s) for both stimulus strengths. All inhibited posterior canal units (either contralateral or ipsilateral) still had some residual spike rate during stimulation.

Control experiments

To verify that the efferent vestibular system was responsible for the observed modulations of posterior canal afferent spike rates, three sets of control experiments were conducted (3 frogs per set). The first set involved transection of the VIIIth nerve root ipsilateral to the stimulating electrodes, but contralateral to the recording electrode. Figure 5A shows the inhibitory response of a posterior canal unit to a contralateral galvanic stimulus. After this recording, the VIIIth nerve ipsilateral to the stimulating electrodes was carefully cut without disturbing the recording electrode. Subsequent spike activity of the same posterior canal unit as well as its lack of response to additional contralateral stimuli are shown in Fig. 5B. These experiments demonstrated that for the galvanic stimulus to modify the spike rate of a posterior canal unit of the opposite side, there must be intact vestibular afferent connections to the brain stem from the stimulated inner ear. These experiments also rule out current spread from the stimulating electrodes as well as electrical activation of general sensory nerve fibers as explanations for the observed responses to contralateral galvanic stimulation.
The second set of control experiments involved recordings of posterior canal units ipsilateral to a transected VIIIth nerve (labyrinthine artery intact). Unfortunately, units isolated before the nerve was transected were lost during the cutting process. All 12 units isolated after ipsilateral VIIIth nerve transection showed no response to either ipsilateral or contralateral stimulation. If an intact VIIIth nerve was not necessary for the observed 39% ipsilateral response incidence, then the chances of no units responding in 12 trials, on the basis of a binomial probability distribution, are $<0.4\%$. Similarly, if an intact VIIIth nerve was not necessary for the observed 23% contralateral response incidence, then the chances of no units responding in 12 trials, on the basis of a binomial probability distribution, are $<0.9\%$. These results indicate that for ipsilateral galvanic stimulation to be effective, there must be an intact vestibular afferent pathway from the stimulated anterior and horizontal canal ampullae to the brain stem. For contralateral galvanic stimulation to be effective, there must be an intact efferent pathway through the VIIIth nerve root to the posterior canal ampulla.

As an additional test for possible current spread from galvanic stimulation to the ipsilateral posterior canal, a third set of control experiments was conducted. These experiments involved intentional misplacement of a second pair of stimulating electrodes, such that the cathodal electrode was 6–8 mm posterior to the anterior canal ampulla. The anodal electrode was placed in the posterior wall of the middle ear. Under these conditions, both the misplaced and properly placed cathodal electrodes were approximately the same distance from the posterior canal ampulla. If current spread from the cathodal electrode were directly activating posterior canal afferent dendrites, then both the misplaced and properly placed cathodal electrodes should have been able to activate posterior canal afferent neurons. If, on the other hand, posterior canal afferent activation was due to an efferent reflex in response to galvanic activation of anterior

![FIG. 4. Peristimulus spike rate histogram (1-s bins) of a posterior canal afferent neuron that responded to both ipsilateral (excitatory responses) and contralateral (inhibitory responses) applied stimuli. Horizontal bars: duration of galvanic stimulation. The 1st 2 stimulus periods were at 0.3-mA constant current; the 2nd 2 periods were at 0.4 mA. Note residual spontaneous activity during inhibitory responses.](image-url)

![FIG. 5. Peristimulus histograms of 2 units used in control studies to verify that the efferent vestibular system was responsible for observed changes in posterior canal afferent responses. A: inhibitory response of a posterior canal unit to a 120-s current pulse applied to the contralateral labyrinth. B: lack of response of the same unit as in A to 2 60-s current pulses after the contralateral VIIIth nerve was transected at the brain stem. C and D: another unit, ipsilateral to galvanic stimulation (a portion of the record was omitted in which a connector to a stimulating electrode came loose and had to be replaced). Two sets of ipsilateral electrodes were used to test for current spread from the stimulating electrodes to the posterior canal ampullae. The cathodal electrode of each set was approximately the same distance from the ipsilateral posterior canal ampulla but only 1 was close to the anterior and horizontal canal ampullae. When current was passed through this electrode, responses were observed (stimulus in C and 2nd stimulus in D). When current was passed through the other cathodal electrode, placed 6–8 mm posteroiateral to the horizontal canal ampulla, no response occurred (1st stimulus in D). See RESULTS for further discussion.](image-url)
and horizontal canal afferent neurons, then only the properly placed cathodal electrode would be effective in altering posterior canal afferent activity. Eighteen posterior canal units were tested with both sets of electrodes. Six units responded to the properly placed electrodes, and no units responded to the misplaced electrodes. Figure 5, C and D, shows spike activity of a posterior canal unit that responded to properly placed stimulating electrodes but not to the improperly placed electrodes. These experiments offer good evidence that the observed posterior canal afferent responses were not due to current spread from ipsilateral stimulating electrodes.

Comparisons between posterior canal units that did and did not respond to ipsilateral galvanic stimulation

Posterior canal units that responded to ipsilateral galvanic stimulation had a mean spike rate of 6.8 ± 0.70 (SE) spikes/s (range: 0.6–21 spikes/s; n = 62), compared with a mean spike rate of 10.7 ± 0.42 (SE) spikes/s (range: 0.5–41 spikes/s; n = 96) for nonresponding units (Fig. 6). These differences were statistically significant (P < 0.001; 2-tailed t-test of log-normalized spike rates). Responding units also had more irregular spike rates, which can be quantified in terms of CV, with higher CV values indicating a greater level of spike train noise. The mean CV value for responding units was 0.91 ± 0.12 (SE) (range: 0.43–1.26), compared with a mean CV of 0.72 ± 0.08 (SE) (range: 0.23–1.10) for nonresponding units. Valid statistical comparisons of CV values require correction for differences in mean spike rates (Goldberg et al. 1984). This correction can be made by developing empirical formulae to normalize CV values to an arbitrary mean spike rate. This approach was attempted by the use of data from 20 afferent units in which galvanic stimuli were used to excite each unit for 20- to 30-s periods at several different levels of stable spike rates. However, individual variability between units was too great to determine formulas that could be used to reliably normalize CV values of other units.

An alternate method was devised to test the hypothesis that responding units had higher CV values than nonresponding units with comparable mean spike rates. For this purpose each responding unit with a mean spike rate >3 spikes/s was systematically paired with a nonresponding unit with an identical or nearly identical spike rate. When more than one nonresponding unit could be matched to a responding unit, the match was biased in favor of the null hypothesis by choosing the nonresponding unit with the highest CV value. Figure 7 shows spike rate and CV distributions for responding and nonresponding posterior canal units. The mean spike rates of the spike rate matched units were 9.5 ± 4.9 (SE) spikes/s for responding units and 9.5 ± 5.0 (SE) spikes/s for nonresponding units. CV distributions for the two groups showed separate peaks (Fig. 7), centered around mean values of 0.87 ± 0.17 (SE) for responding units and 0.74 ± 0.20 (SE) for nonresponding units. These differences were statistically significant (P < 0.004; 2-tailed, paired t-test).

Adaptation of efferent-mediated responses

In most cases, the temporal pattern and general shapes of efferent-mediated responses of posterior canal afferent neurons qualitatively mirrored the driving input of the galvanically activated anterior and horizontal canal afferent neurons. Of posterior canal responses, 77% approximated a step response, sometimes with an “onset” enhancement of the response. The other 23% of posterior canal units exhibited a marked response adaptation. These units, like the one shown in Fig. 8, often showed an offset response that was more pronounced than the depression of spike activity generally seen after galvanic activation of afferent neurons (compare with Fig. 2). Mean spike rates and CV values of adapting posterior canal units (6.9 ± 1.52 spikes/s, mean ± SE; CV = 0.96 ± 0.05, mean ± SE; n = 14) and nonadapting units (7.0 ± 0.8 spikes/s, mean ± SE; CV = 0.9 ± 0.13, mean ± SE; n = 45) were not significantly different (P > 0.4; 2-tailed t-test; log-normalized spike rate data).

Analysis of CV

Analysis of CV values before and during efferent modulation of posterior canal spike activity was performed on non-
adapting units with ≥50 s of stable spike rate during stimulation. Although some units had changes in CV values as large as 0.23, CV values of most units changed little or not at all during stimulation. As a group, units with excitatory responses (spike rate change: 5.7 ± 3.7 spikes/s, mean ± SD) had a mean prestimulus CV value of 0.92 ± 0.23 (SD) and a mean during-stimulation CV value of 0.87 ± 0.19 (SD). Although this difference was statistically significant ($P < 0.02$; 2-tailed, paired $t$-test; $n = 24$), the difference is most likely due to differences in spike rates, not spike train noise levels. Similarly, units with inhibitory responses (spike rate change: −4.9 ± 3.2 spikes/s, mean ± SD) had statistically

FIG. 7. Histograms comparing spike rate and CV distributions of responding and nonresponding posterior canal afferent units to ipsilateral stimulation. A: high degree of overlap for spike rates <21 spike/s, in spike rate distributions between responding and nonresponding units. B: responding units tend to have higher CV values than nonresponding units. C: when the influence of mean spike rate is compensated for by selecting spike-rate-matched units between the 2 groups, CV distributions have more closely placed, yet separate peaks in their distributions (see Fig. 6B and RESULTS).

FIG. 8. Peristimulus histogram of a posterior canal unit showing adaptation of efferent-mediated responses during 2 60-s ipsilateral stimulations.
higher CV values in conjunction with lower spike rates ($P < 0.02$; 2-tailed, paired $t$-test; $n = 14$; prestimulation $CV = 0.90 \pm 0.11$, mean $\pm$ SD; during-stimulation $CV = 0.95 \pm 0.14$, mean $\pm$ SD).

**DISCUSSION**

**Afferent drive to efferent system**

In this study a galvanic stimulus was used to depolarize the spike-generating region of horizontal and anterior canal afferent neurons to serve as a driving input to the efferent vestibular system. Vestibular afferent neurons can act monosynaptically on vestibular efferent neurons, but polysynaptic pathways are likely to be invoked as well (see Hightstein 1991 for review). The galvanic stimuli applied may have caused one action potential per efferent axon in the vicinity of the stimulating electrode during each stimulus pulse (Highstein and Baker 1985). These single action potentials could be transmitted via efferent collateral axons to multiple ipsilateral endorgans. Early studies in the frog (Llinas and Precht 1969) suggested that single, brief electrical pulses could induce measurable efferent-mediated effects; however, the light level of anesthesia used in some of those experiments may have allowed for an arousal or behavioral activation of efferent neurons. Effective efferent stimulation requires many spikes per second (Rossi et al. 1980; Valli et al. 1986). Single efferent action potentials during a 20- to 120-s stimulus pulse would not be expected to induce significant efferent-mediated changes in afferent spike activity.

Galvanic activation of vestibular afferent neurons in our study resulted in spike rate increases of 6–30 spikes/s, representing two- to eightfold increases depending on the afferent unit’s resting spike rate. On the basis of previous work with rotational stimuli, these spike rate increases are comparable with those resulting from relatively modest $10^\circ$ to $120^\circ$ s accelerations.\(^3\)

**Temporal characteristics of responses**

The efferent-mediated responses in the present study had temporal characteristics that generally mirrored the driving afferent input. The galvanically induced driving afferent activity generally showed a poststimulation spike rate depression. This spike rate depression of the driving afferent activity usually was reflected in efferent-mediated responses of posterior canal afferent neurons as a postfacilitation depression or a postinhibition rebound. These extensions of the efferent response beyond the time period of the galvanic stimulus are not analogous to the poststimulation phenomena reported by Valli et al. (1986). Poststimulation phenomena, seen in studies in which isolated labyrinths and direct efferent fiber activation were used, comprised 2–5 s of elevated resting spike rate after either a facilitation or an inhibition. Any such phenomena probably were obscured in the present study because of extensions of the driving afferent input (i.e., poststimulation spike rate depression) to the efferent system beyond the end of the galvanic stimulus pulse.

\(^3\)Low range based on high-gain unit, 1.5 spikes $\cdot$ s$^{-1}$ $\cdot$ deg$^{-1}$ $\cdot$ s$^{-2}$; 8-fold response $\equiv 15$ spikes/s. High range based on low-gain unit, 0.25 spikes $\cdot$ s$^{-1}$ $\cdot$ deg$^{-1}$ $\cdot$ s$^{-2}$; 2-fold response $\equiv 30$ spikes/s.

**Comparisons of responding and nonresponding afferent neurons**

As a group, responding units had lower, more irregular spontaneous spike rates than nonresponding units; however, there was extensive overlap between groups in these parameters (see Fig. 7). Only the most regularly discharging units, those with CV values $<0.4$, were absent from the responding group. Studies in other species, such as squirrel monkey (Goldberg and Fernandez 1976), pigeon (Dickman and Correa 1993), and toadfish (Boyle and Hightstein 1990), have also found either lower occurrence of or weaker efferent effects in regularly versus irregularly discharging vestibular afferent neurons.

**Types of efferent-mediated responses**

When direct activation of efferent fibers is used in the frog, responding afferent neurons fall into three categories on the basis of changes in their spontaneous spike rates: facilitation, inhibition, or mixed (combined inhibition/facilitation) (Bernard et al. 1985; Rossi et al. 1980; Valli et al. 1986). The same categories of responses were observed in the present study; however, our use of an indirect method of efferent activation resulted in a much lower incidence of inhibitory responses. Increased vestibular afferent drive to the efferent system caused predominantly facilitation of spike rates of responding units of the ipsilateral labyrinth (92%) and predominantly inhibition of responding units of the contralateral labyrinth (95%). These findings are consistent with the study by Gribenski and Caston (1976) on the tonic influence of the efferent system on spontaneous afferent activity in the frog. The study by Dickman and Correa (1993) of bilateral communication between the vestibular labyrinths also found increased afferent activity to cause predominantly inhibition of contralateral afferent neural activity. This preponderance of inhibition was related to whether the responding afferent neuron had regular or irregular spontaneous spike activity. In the study by Dickman and Correa, a mechanical stimulus was used to excite canal afferent neurons of one ear during recording of afferent unit activity from the contralateral labyrinth. Of the units that responded to contralateral stimulation, 84% of the irregularly discharging units were inhibited. In contrast, 71% of regularly discharging units that responded to the contralateral stimulus were excited.

The few cases in which efferent-mediated responses involved an initial inhibition followed by facilitation represent another pattern of interaction between excitatory and inhibitory efferent fibers. Something akin to this pattern was reported in the study by Guth et al. (1986), in which a few single-unit responses shifted from inhibition to facilitation as the concentration of applied carbachol was increased to $>1 \mu$M. The current data, however, are too limited, without repeated stimulus applications, to make any meaningful inferences about underlying mechanisms.

**Comparisons of inhibitory and excitatory efferent effects**

That some efferent nerve fibers may mediate only inhibitory responses and others only excitatory effects is suggested by findings of Valli et al. (1986). Those investigators identi-
Efferent fibers are known to innervate vestibular hair cells in the frog (Dunn 1980). There is no evidence that efferent fibers innervate afferent dendrites in the frog. Physiological evidence from Guth et al. (1986) indicates that excitatory efferent neurons do not innervate afferent dendrites, because acetylcholine application does not cause increased spike rates when the hair cell synapse is blocked by low Ca\(^{2+}\) and high Mg\(^{2+}\) concentrations. Pharmacological studies of isolated hair cells provide evidence that both the excitatory and inhibitory actions of the vestibular efferent neurons are due to synapses on hair cells that modulate a calcium-dependent potassium current (Guth et al. 1994; Housley et al. 1990).

When vestibular efferent axons are activated directly by rapid current pulses applied to isolated frog labyrinths, the results are somewhat controversial. Some investigators have found efferent activation to have a predominantly inhibitory effect on afferent activity of the same labyrinth (75–83% of responding units) (Rossi et al. 1980; Valli et al. 1985), whereas other investigators have found a predominantly excitatory effect (~60% of responding units) (Bernard et al. 1985). Rossi et al. (1980) felt that careful dissection was necessary to obtain the consistent inhibitory effects of efferent activation. They suggested that the efferent nerve fibers mediating inhibitory effects were more susceptible to dissection damage than were nerve fibers mediating excitatory effects. Taken as a whole, studies in which direct electrical activation of efferent fibers was used indicate that at least a large proportion of vestibular afferent neurons responding do so with a decrease in spike rate. With the use of vestibular afferent activity as a driving input to the efferent vestibular system in the present study, we found only 3% of responding afferent fibers to be purely inhibited by an ipsilateral driving stimulus (5% showed a mixed, inhibited/facilitated response). This suggests that moderate vestibular afferent input to the efferent system preferentially activates excitatory efferent fibers to the ipsilateral labyrinth. If a significant resting efferent activity (tone) was present in our ketamine/pentobarbital-anesthetized frogs, an alternate explanation for predominantly excitatory responses could be that the vestibular afferent input to the efferent system preferentially inhibited the inhibitory efferent fibers to the ipsilateral labyrinth. Attempts in our laboratory to measure resting and evoked efferent activity with the use of suction electrodes have not been successful. Efferent activity in unanesthetized toadfish is low (4–5 spikes/s) when the animal is at rest (Boyle and Highstein 1990). Pentobarbital anesthesia is believed to reduce efferent vestibular activity (Schmidt 1963). Ketamine blocks N-methyl-D-aspartate glutamate receptors (Anis et al. 1983), but how this might alter vestibular efferent activity is not known. The level of ketamine/pentobarbital anesthesia in the current study was kept as low as possible but undoubtedly varied between animals. Still, resting efferent tone, if any, probably was low. Efferent-mediated responses limited to spike rate changes of a few spikes per second may have been caused by a disinhibition or disfacilitation of vestibular hair cells/afferent neurons (i.e., decreased efferent activity); however, the stronger efferent effects were more probably due to active inhibition or facilitation of vestibular hair cells/afferent neurons (i.e., increased efferent activity).

The level of anesthesia also could have differential effects on excitatory and inhibitory efferent neurons. If inhibitory efferent fibers were more sensitive to the level of anesthesia, then relatively fewer efferent-mediated inhibitory responses ipsilateral to the driving stimulus would be expected. This argument, however, would require a disfacilitation of excitatory efferent fibers to explain the predominantly inhibitory effects of a contralateral driving stimulus. Although this explanation is plausible for small spike rate changes, the stronger inhibitions of 10–22 spikes/s are better accounted for by activation of inhibitory fibers, assuming a low level of resting efferent tone as discussed in the previous paragraph.

Guth et al. (1986), in their pharmacological study, used primarily whole nerve, multunit recordings, which emphasized responses of small-diameter fibers. That study showed excitatory responses to acetylcholine application with an underlying inhibitory response when muscarinic blockers were given in combination with acetylcholine. Other studies involving direct activation of efferent fibers with rapid current pulses (50 Hz) relied on single-unit recordings, which mainly select larger-diameter, more irregularly discharging afferent fibers (Rossi et al. 1980; Valli et al. 1985). The stimuli applied in these studies were reported to produce maximal activation of efferent fibers (Valli et al. 1986). Combining the results of the single-unit and multunit studies suggests that larger-diameter afferent fibers are under a greater inhibitory influence than the more numerous small-diameter fibers (Guth et al. 1986). In the present study we used intracellular single-unit recordings and therefore mainly sampled larger-diameter afferent fibers; however, the efferent-mediated response of the ipsilateral labyrinth was predominantly facilitation and rarely inhibition. These findings suggest that how efferent fibers are activated is important in determining efferent responses, at least for large-diameter afferent fibers.

A substantial number of units (23% of ipsilaterally facilitated units) had a marked adaptation of their efferent-mediated response during the stimulus period. If we assume that the efferent neurons themselves are not adapting, then it is possible that these adapting responses represent a convergence of excitatory and inhibitory efferent neurons where the inhibitory processes are of slower onset and build in strength over time. Response adaptations often extended to or below the initial resting spike rates. Such was the case for the unit whose spike activity is shown in Fig. 8. If all of the hair cells that supplied this afferent neuron contributed equally to its spontaneous spike rate, then this adaptation could be explained by a dual excitatory and inhibitory innervation of most of those hair cells. Restricting excitatory and inhibitory efferent innervations to separate subpopulations of this afferent neuron’s hair cells limits the ability of an adapting unit to both double its spike rate and adapt back to or below the resting spike rate. If the excitatory-controlled hair cells were fewer in number and/or “silent” in the absence of efferent stimulation, then efferent activation with a slower developing inhibitory response could conceivably return a doubled afferent spike rate to approximately resting levels.
Spike train noise

On an individual basis, responses of a few units suggested that the efferent vestibular system might influence spike train noise. Analysis of CV, a measure of spike train noise, found that CV values were marginally lower (less noisy) during nonadapting, facilitated responses compared with prestimulus periods (see RESULTS). This effect, however, can be attributed simply to the increased spike rate and not to any real increased regularity in action potential generation (Goldberg et al. 1984).

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