Chapter 6
The Efferent Vestibular System

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6.1 Introduction

As is the case with most hair-cell organs, the vestibular labyrinth receives a dual innervation. Afferent nerve fibers arise from bipolar cells in the vestibular (Scarpa's) ganglion. The peripheral process of each ganglion cell gets synaptic inputs from hair cells in one of several discrete organs, and its central process conveys the resulting information, encoded in the spacing of action potentials, to the vestibular nuclei and the cerebellum. In addition, hair cells and afferent nerve terminals are innervated by efferent fibers originating in the brain stem and reaching the periphery by way of the vestibular nerve. This chapter reviews our understanding of the efferent vestibular system (EVS), including its neuroanatomical organization, candidate neurotransmitters, peripheral actions on afferent discharge as revealed by electrical stimulation of EVS pathways, and the underlying cellular (synaptic) and neurotransmitter mechanisms. To consider possible functions of the EVS, the chapter then describes the vestibular and nonvestibular signals carried by efferent neurons and how these signals might modify the information carried by afferents. Though our emphasis is on the mammalian EVS, results in nonmammalian species are also considered, as these provide insights into efferent function.

Efferent actions are related to the discharge properties of afferents, particularly to their regularity of discharge and their branching patterns and locations in the neuroepithelia of the various vestibular organs. Although these topics need to be reviewed, of necessity the treatment is brief. For readers wanting more detailed information, the following references can be consulted: anatomy of the peripheral vestibular organs (Lindeman 1969; Wersäll and Bagger-Sjöbäck 1974; Lysakowski and Goldberg 1997); functions of the semicircular canals and otolith organs (Wilson and Melvill Jones 1979; Lysakowski and Goldberg 2004); and relation of

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discharge properties to peripheral terminations of afferents (Lysakowski and Goldberg 2004). Previous reviews of the EVS can also be recommended (Meredith 1988; Highstein 1991; Guth et al. 1998; Goldberg et al. 2000).

6.2 Afferents and Hair Cells

6.2.1 Afferent Discharge Properties

Vestibular-nerve afferents have a resting discharge in the absence of stimulation. This discharge can be quite high; for example, it averages ~100 spikes/s in monkeys (Fig. 6.1) (Goldberg and Fernández 1971; Hacque et al. 2004; Sadeghi et al. 2007). Rotations in the plane of a semicircular canal can, depending on their direction, result in an increase (excitation) or decrease (inhibition) in afferent discharge. Similarly, appropriately directed linear forces can excite or inhibit discharge in the utricular or saccular maculae.

Some vestibular afferents have a regular spacing of action potentials, whereas in others the spacing is irregular (Fig. 6.1) (Goldberg 2000). Discharge regularity is measured by a coefficient of variation (*cv*), the ratio of the standard deviation of intervals (*s*) to the mean interval ($\overline{x}$), or $cv = s / \overline{x}$. Because *cv* varies with $\overline{x}$, a normalized statistic (*cv**), the *cv* at a standard mean interval, is used. In mammals, *cv** at $\overline{x}$ = 15 ms varies more than 20-fold, from <0.03 in the most regular units to >0.6 in the most irregular units. Discharge regularity has proved useful, as units first classified as regular or irregular differ in many of their other discharge characteristics (Table 6.1) (Goldberg 2000). A stochastic model of repetitive activity illustrates how discharge regularity impacts other neuronal properties (Smith and Goldberg 1986; Goldberg 2000).

As is generally the case (Connor 1978; Stocker 2004; Bean 2007), repetitive activity in the model reflects the interaction of an afterhyperpolarization (AHP) following each spike with synaptic and other depolarizing currents. In the model, regular units have slow, deep AHPs, whereas in irregular units, AHPs are fast and shallow. Of somewhat lesser importance in determining discharge regularity, miniature excitatory postsynaptic potentials (mEPSPs), which are the result of neurotransmission from hair cells to the afferent terminal, are smaller in regular units. Reflecting AHP differences, irregular units are much more sensitive to synaptic or externally applied currents than are regular units. Circumstantial evidence supporting the model comes from the responses of mammalian vestibular afferents to external currents (Goldberg et al. 1984). More direct evidence has been obtained from intracellular recordings of AHPs and mEPSPs in crista afferents from the red-eared turtle, *Trachemys scripta elegans* (Goldberg and Chatlani 2009).

The conclusion is that when the spike encoder, the mechanism that converts postsynaptic depolarizations into spike trains, has a much higher gain, the more irregular is the discharge. That is, a given depolarization results in a considerably larger increase in the discharge rate of irregular afferents. Sensitivity to externally

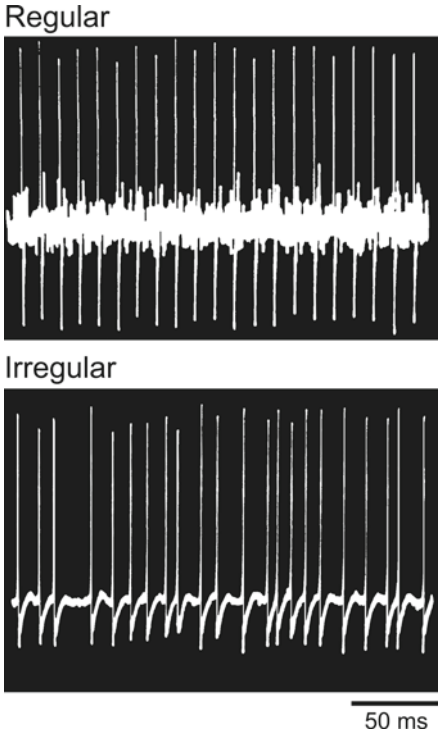

Fig. 6.1 Discharge regularity in vestibular-nerve afferents. Spike trains are shown during the resting discharge of two afferents, each innervating the superior semicircular canal in a squirrel monkey. Although both afferents have similar discharge rates of just under 100 spikes/s, they differ in the spacing of their action potentials, which is regular in the top afferent and irregular in the bottom afferent (Modified with permission from Goldberg and Fernández 1971. Copyright 1971, The American Physiological Society.)

applied galvanic currents provides a measure of encoder gain (Fig. 6.8c) (Goldberg et al. 1984). An inspection of Table 6.1 indicates that several of the differences between regular and irregular units, including the fact that efferent responses are much smaller in regular units, can be explained by differences in encoder gain (Goldberg 2000). In fact, the only item that cannot be so explained is the difference in response dynamics between the two afferent groups (Goldberg et al. 1982; Ezure et al. 1983).

6.2.2 Hair Cells and Their Innervation

In the vestibular organs of amniotes (reptiles, birds, and mammals), there are two kinds of hair cells (Fig. 6.2) (Wersäll and Bagger-Sjöbäck 1974; Lysakowski and

Table 6.1 Comparing regularly and irregularly discharging mammalian vestibular afferents

Regular	Irregular
Thin to medium-sized axons ending as bouton and dimorphic terminals in the peripheral (peripheral extrastriolar) zone (Goldberg and Fernández 1977; Yagi et al. 1977; Baird et al. 1988; Goldberg et al. 1990b; Lysakowski et al. 1995)	Medium to thick axons ending as calyx and dimorphic terminals in the central (striolar) zone
Tonic response dynamics, resembling those expected of end organ macromechanics (Goldberg and Fernández 1971; Fernández and Goldberg 1976; Schneider and Anderson 1976; Tomko et al. 1981; Curthoys 1982; Baird et al. 1988; Goldberg et al. 1990a; Lysakowski et al. 1995)	Phasic-tonic response dynamics, with sensitivity to the velocity of cupular (otolith) displacement
Low sensitivity to angular or linear forces (Goldberg and Fernández 1971; Fernández and Goldberg 1976; Schneider and Anderson 1976; Tomko et al. 1981; Curthoys 1982; Baird et al. 1988; Goldberg et al. 1990a; Lysakowski et al. 1995)	High sensitivity to angular or linear forces. Calyx afferents in cristae have unusually low sensitivity
Small responses to electrical stimulation of efferent fibers (Goldberg and Fernández 1980; McCue and Guinan 1994; Marlinski et al. 2004)	Large responses to electrical stimulation of efferent fibers
High thresholds and small responses to galvanic currents delivered to the perilymphatic space (Ezure et al. 1983; Goldberg et al. 1984; Brontë-Stewart and Lisberger 1994)	Low thresholds and large responses to galvanic currents delivered to the perilymphatic space

Goldberg 2004). Type I hair cells are distinctive in being amphora-shaped and having almost their entire basolateral surface contacted by a single calyx ending. Type II hair cells, also seen in fish and amphibians, are cylindrically shaped and receive an afferent innervation from several bud-shaped or bouton terminals. The two kinds of hair cells also differ in their efferent innervation. During development, both types of hair cells are contacted by efferent boutons, but the growth of calyces displaces these from type I hair cells (Favre and Sans 1979). With few exceptions (Wackym et al. 1991; Li et al. 2007), the efferent innervation in the adult terminates on calyx endings not on type I hair cells. Type II hair cells receive an efferent innervation, as do the bouton-shaped afferent terminals innervating these same hair cells.

Two methods have been used to characterize the afferent innervation of hair cells. First, fibers are dye-filled and their terminal fields reconstructed (Fernández et al. 1988, 1990, 1995). Second, different populations of afferents have distinctive molecular components, which can be discerned via immunohistochemistry (Desmadryl and Dechesne 1992; Lysakowski et al. 1999; Leonard and Kevetter 2002). There are three kinds of fibers in the cristae of the mammalian semicircular canals (Fernández et al. 1988, 1995; Desai et al. 2005a, b), organs that are involved in sensing angular head rotations (Fig. 6.3). *Calyx* fibers provide calyx endings to one or a few neighboring type I hair cells. *Bouton* fibers supply bud-shaped endings to several widely spaced type II hair cells. *Dimorphic* fibers provide a mixed innervation consisting of one or more calyx endings to type I hair cells and thin collaterals giving rise to bud-shaped endings to several type II hair cells. Calyx units are

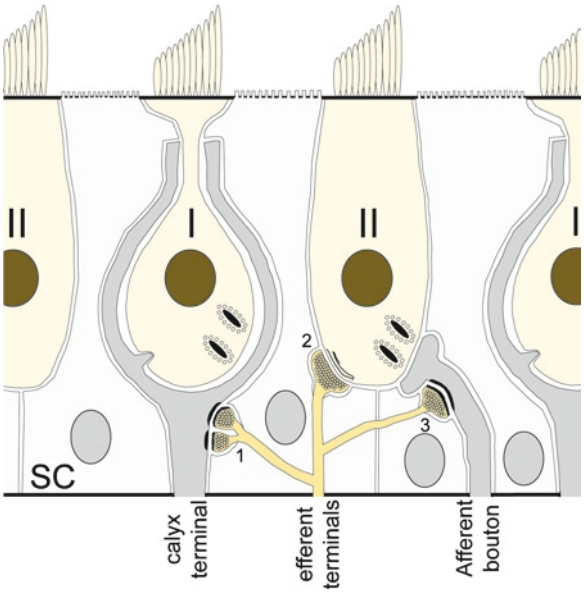

Fig. 6.2 Amphora-shaped type I hair cells are innervated by calyx endings derived from single afferent fibers, whereas cylindrically-shaped type II hair cells are innervated by bouton terminals from several afferents. In each instance, afferent synapses are marked by synaptic ribbons (a dense synaptic body surrounded by a halo of vesicles) in the hair cell. In addition to ribbon synapses, the calyx ending often invaginates into the type I hair cell. Single efferent fibers with highly vesiculated boutons terminate on the calyx ending (*1*) as well as on type II hairs (*2*) and their bouton terminals (*3*). Supporting cells (SC) span the width of the neuroepithelium and are recognized by their basally located nuclei and by microvilli on their apical surfaces

confined to a central zone (Fig. 6.3 right, CZ), whereas bouton units are confined to a peripheral zone (Fig. 6.3 right, PZ). Dimorphic units, which are the most numerous fiber type, are found throughout the neuroepithelium, as are type I and type II hair cells. Calyx fibers have the thickest axons and bouton fibers the thinnest axons. Dimorphic fibers are of intermediate caliber with those innervating the PZ being thinner and having more extensive terminal trees than those in the CZ.

The utricular and saccular maculae are sensors of linear forces acting on the head. In its afferent innervation, the utricular macula resembles the cristae (Fernández et al. 1990). There are three types of fibers, although bouton fibers are relatively infrequent. Calyx fibers are confined to the striola, a narrow stripe running throughout much of the length of the macula and separating the rest of the neuroepithelium into a medial and a lateral extrastriola. Dimorphic units are found in both the striola and the extrastriola. The relatively few bouton units are found only in the extrastriola at some distance from the striola (Fernández et al. 1990; Leonard and Kevetter 2002). Although a detailed description of the innervation patterns in the saccular macula is lacking, calyx fibers are confined to the striola (Leonard and Kevetter 2002; Desai et al. 2005a).

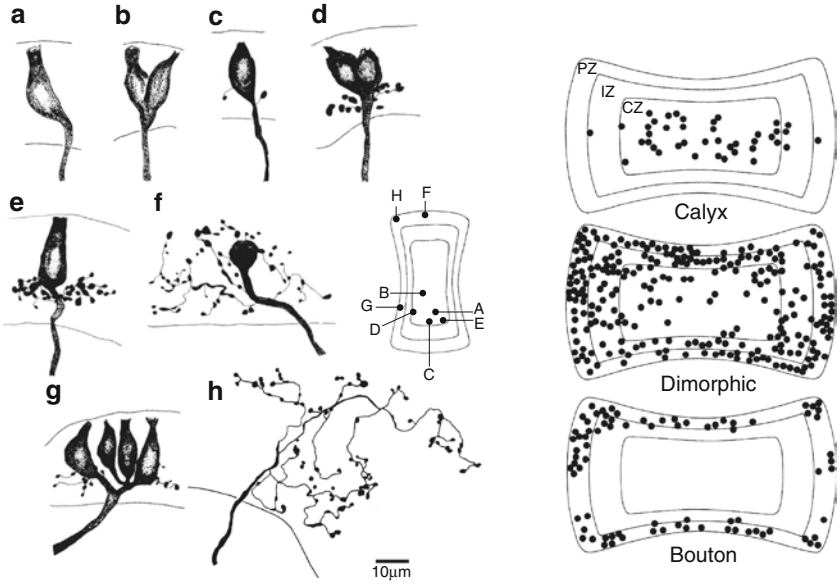

Fig. 6.3 (**a**–**h**) Branching patterns of individual semicircular canal afferents labeled by the extracellular deposit of horseradish peroxidase in the chinchilla vestibular nerve. There are three afferent classes: calyx (**a**, **b**), dimorphic (**c**–**g**), and bouton (**h**). Location of each unit is indicated on a flattened map of crista (*inset*, *middle right*). *Right column*: Distribution of calyx, dimorphic, and bouton units on a flattened reconstruction of the crista. In all maps, the crista is divided into central (CZ), intermediate (IZ), and peripheral (PZ) zones of equal areas (Modified with permission from Fernández et al. 1988. Copyright 1988, The American Physiological Society.)

6.2.3 Afferent Morphology and Physiology

Morphophysiological techniques can be used to relate terminal morphology and neuroepithelial location with the discharge properties of individual afferents. Here, an afferent fiber is impaled, its physiology characterized, after which an intracellular marker is injected into the fiber and allowed to diffuse to its peripheral termination (review: Lysakowski and Goldberg 2004). In the chinchilla cristae (Baird et al. 1988), it was found that dimorphic units in the CZ are irregularly discharging and have high rotational and galvanic gains, whereas those in the PZ are regularly discharging with low rotational and galvanic gains (Fig. 6.4). Compared to dimorphs also innervating the CZ, calyx units have a more irregular discharge, more phasic response dynamics, and larger responses to galvanic currents. This last observation implies that calyx fibers have especially sensitive spike encoders. Despite this, calyx units have considerably lower rotational gains than irregular dimorphs. In fact, a relatively low rotational gain has proved a reliable feature of calyx units in the cristae of several mammalian species (Lysakowski et al. 1995; Hullar et al. 2005; Sadeghi et al. 2007; Lasker et al. 2008). Bouton units, defined by their slow conduction velocities, resemble dimorphic afferents also localized in

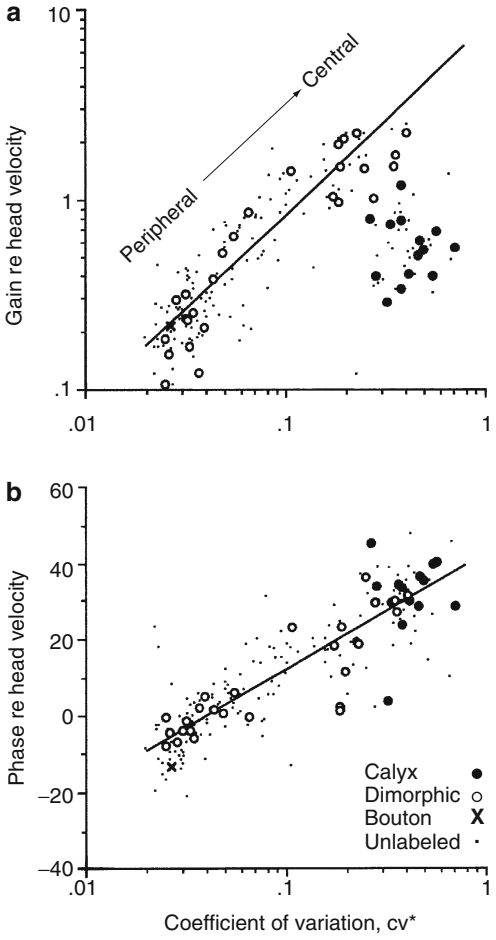

Fig. 6.4 Gains and phases vs. normalized coefficient of variation (*cv**) for labeled and unlabeled semicircular-canal afferents in the chinchilla responding to 2-Hz sinusoidal head rotations. Each point represents one unit. (**a**) Based on their gains, units fall into two groups. Straight line in (**a**) is best-fitting power law relation between gain and *cv** for one of the groups. (**b**) Sinusoidal phase re head velocity vs. *cv** for the same units shown in (**a**). Straight line in (**b**), best-fitting semi-logarithmic relationship between phase and *cv** for all units. Calyx units are the most irregularly discharging afferents, have the largest phase leads, and distinctively low gains. Gain and phase of dimorphic units increase with *cv**. Regular units are found in the peripheral zone; irregular units in the central zone. The one bouton unit was regular, had a low gain and phase, and was located in the peripheral zone (Modified with permission from Baird et al. 1988. Copyright 1988, The American Physiological Society.)

the PZ in having a regular discharge, tonic response dynamics, and low rotational and galvanic sensitivities (Lysakowski et al. 1995).

Morphophysiological experiments have also been done in the chinchilla utricular macula (Goldberg et al. 1990a). Recovered dye-filled fibers included calyx units

in the striola and dimorphic units throughout the macula. Striolar units were irregular, with calyx units being more irregular than dimorphs. Extrastriolar dimorphs located at some distance from the striola were invariably regular, whereas those in the juxtastriola, a region surrounding the striola, were intermediate in their discharge regularity. As in the case of the cristae, an irregular discharge is associated with a higher galvanic sensitivity, a more phasic response dynamics, and a greater sensitivity to natural stimulation. The one difference from the cristae is that calyx units and striolar dimorphs have similarly high gains.

6.3 Efferents: A Historical Perspective

Once Rasmussen (1946, 1953) had demonstrated the presence of olivocochlear bundles, it seemed reasonable to suppose that vestibular and other hair-cell organs also received an efferent innervation. Ultrastructural evidence for this conjecture was provided by Engström (1958), who noted that there were two kinds of nerve endings in the cochlea and vestibular organs. One group was highly vesiculated, while the other group was poorly vesiculated. Based on observations at other synapses (de Robertis and Bennett 1955; Palay and Palade 1955), it was suggested that the highly vesiculated endings were efferents and the poorly vesiculated endings were afferents. Innervation of vestibular type I hair cells was consistent with the suggestion in that only the poorly vesiculated calyx endings were in contact with hair-cell ribbon synapses and, so, could be directly affected by sensory stimulation. In contrast, highly vesiculated endings contacted the calyx and thus were in a position to modulate afferent transmission. Histochemical studies in the cochlea (Churchill et al. 1956; Schuknecht et al. 1959) and vestibular labyrinth (Dohlman et al. 1958; Ireland and Farakashidy 1961) confirmed the dual innervation, as efferents, but not afferents, were acetylcholinesterase (AChE) positive. Hilding and Wersäll (1962) connected the ultrastructural and histochemical findings by demonstrating that it was the highly vesiculated endings that were AChE positive. Further proof that these endings were efferents was obtained by showing that they degenerated following central lesions (Smith and Rasmussen 1968; Iurato et al. 1972).

Rasmussen (1946) noted that the medial group of olivocochlear efferents crossed the midline immediately below the floor of the fourth ventricle. This observation provided a convenient site at which to stimulate efferent fibers while monitoring their influence on cochlear activity (Galambos 1956; Fex 1959; Desmedt and Monaco 1961). The disposition of peripheral nerves allowed similar studies to be done in lateral-line organs (Russell 1968; Flock and Russell 1973, 1976). Using tracing methods similar to those of Rasmussen, Gacek was able to define an efferent projection to the vestibular labyrinth, but could not specify the origin of the EVS or a convenient place to stimulate its fibers (Gacek 1960; Rasmussen and Gacek 1958). AChE histochemistry also failed to ascertain the locations of EVS neurons (Rossi and Cortesina 1965). For that reason, early attempts to characterize the peripheral actions of the EVS were unsuccessful (Sala 1965; Llinás and Precht 1969). It was

only with the advent of modern retrograde tracer methods that the cells of origin of the EVS could be determined to lie bilaterally outside the confines of the vestibular nuclei (Gacek and Lyon 1974), at which point the trajectory of EVS fibers to the vestibular periphery could be traced by AChE histochemistry. This knowledge set the stage for studies of the anatomy, physiology, and pharmacology of the EVS.

6.4 Neuroanatomical Organization of the EVS

6.4.1 Location of Cell Bodies and Their Dendritic Morphology

There is an efferent innervation of the vestibular organs in every vertebrate class (Meredith 1988; Lysakowski 1996). The locations of the efferent cell bodies and their dendritic morphology in several representative species are summarized in Fig. 6.5. In mammals, separate groups of efferents innervate auditory and vestibular organs (Fig. 6.5a, cat). In all other vertebrate classes that have been examined, efferent neurons are contained in a single cell cluster, referred to in animals possessing lateral-line neuromasts as the octavolateralis efferent nucleus because cells of the nucleus innervate the lateral-line, as well as eighth-nerve derivatives. Branchiomotor facial motoneurons and octavolateralis efferents may arise embryologically from a common pool of neurons (see Simmons et al., Chap. 7). Possibly reflecting this origin, efferent neurons are found partly within the confines of the facial motor nucleus in eel and toad (Fig. 6.5a). In lizard and chicken, there is still a single efferent nucleus, but it is displaced from the facial nucleus. Retrograde tracer studies indicate that neurons destined for organs of different modalities (vestibular, vibratory, lateral line, and auditory) overlap within the efferent nucleus. In fact, some, but not all, efferent neurons can innervate the inner ear and lateral lines in fish, as well as in the African clawed frog (*Xenopus laevis*), an aquatic anuran still possessing a lateral line (Claas et al. 1981; Highstein and Baker 1986; Meredith and Roberts 1987). In reptiles (Strutz 1981, 1982a; Barbas-Henry and Lohman 1988) and birds (Whitehead and Morest 1981; Strutz and Schmidt 1982), there is a partial segregation of auditory and vestibular efferents in the efferent nucleus, with vestibular efferents being located more dorsally. Because the segregation is incomplete, it is conceivable that single efferent neurons could innervate both kinds of organs.

In mammals, vestibular efferents arise bilaterally in the brain stem from three collections of neurons (Fig. 6.6a, b). The first is a slender column of medium-sized multipolar neurons that extends rostrocaudally between the abducens and superior vestibular nuclei, just dorsal to the descending facial nerve (Gacek and Lyon 1974; Warr 1975; Goldberg and Fernández 1980; Perachio and Kevetter 1989; Marco et al. 1993). This group has been referred to as group *e* (Goldberg and Fernández 1980). A second, compact group of somewhat smaller fusiform neurons is situated dorsomedial to the facial genu (Goldberg and Fernández 1980; Marco et al. 1993). A third or ventral group of slightly larger neurons is scattered in the caudal pontine reticular formation (Strutz 1982b; Marco et al. 1993). By far, most efferent neurons are located in group *e*.

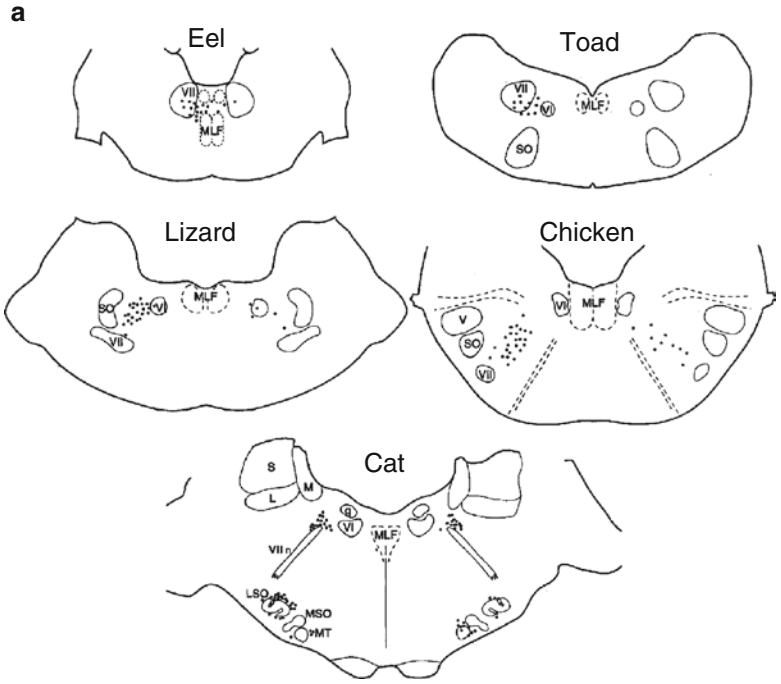

Fig. 6.5 The distribution of efferent neurons and their dendritic arborizations in the brain stem of representative vertebrates. (**a**) Distribution of efferent neurons. Locations of efferent neurons are indicated by *dots*. Examples include eel (Meredith and Roberts 1987), toad (Pellergrini et al. 1985), lizard (Barbas-Henry and Lohman 1988), chicken (Whitehead and Morest 1981), and cat (Warr 1975). (Data from eel, lizard, and cat adapted with permission from John Wiley & Sons; data from toad and chicken adapted with permission from Elsevier.)

The dendritic morphology, as well as the location of the efferent neurons, varies across the vertebrate scale. In animals ranging from the lamprey to amphibia, dendrites occupy much of the brain stem tegmentum (Fig. 6.5b, 1–4). In contrast, the dendritic arbors of the main mammalian efferent nucleus (group *e*) are quite restricted (Fig. 6.5b, 5). Another difference concerns the laterality of efferent projections. Roughly equal numbers of group *e* neurons project to the ipsilateral and contralateral labyrinths with possibly a slight contralateral preference; this is so in rats (Schwarz et al. 1986), guinea pigs (Strutz 1982b), cats (Gacek and Lyon 1974; Warr 1975; Dechesne et al. 1984), monkeys (Goldberg and Fernández 1980; Carpenter et al. 1987), chinchillas (Marco et al. 1993), and gerbils (Perachio and Kevetter 1989). There are a small number of efferent neurons projecting to both ears (Dechesne et al. 1984; Purcell and Perachio 1997). In contrast to the bilateral organization of efferent neurons in mammals, that in nonmammalian vertebrates is predominantly ipsilateral with some contralateral representation (Fig. 6.5a).

The differences in the central organization of the EVS suggest caution in extrapolating results from other vertebrate classes to mammals or vice versa.

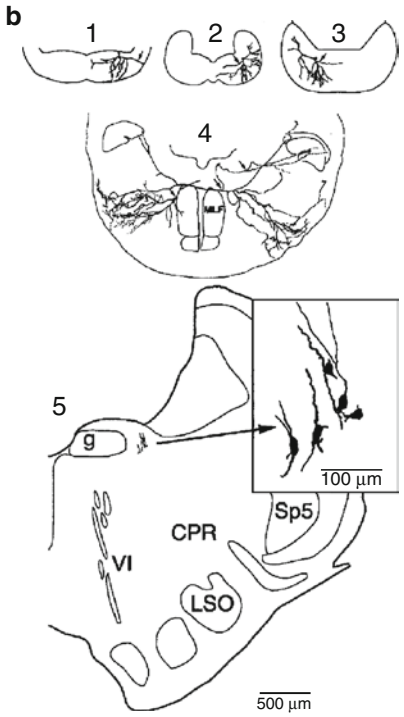

Fig. 6.5 (**b**) Efferent cell bodies and their dendritic arborizations in several vertebrates. Examples include: (1) lamprey (Fritzsch et al. 1989), (2) gymnophion (amphibian) (Fritzsch and Crapon de Caprona 1984), (3) salamander (Fritzsch 1981), (4) oyster toadfish (Highstein and Baker 1986), and (5) chinchilla (Lysakowski and Singer 2000). Scale bar found on the bottom right corner applies to all sections on the right. Efferent neurons in chinchilla shown at higher magnification in inset. (Figs. **b**1, **b**2 and **b**3 adapted with permission from Elsevier; Figs. **b**4 and **b**5 adapted with permission from John Wiley & Sons). CPR caudal pontine reticular formation; g genu of facial nerve; L, M, and S lateral, medial, and superior vestibular nuclei, respectively; LSO and MSO lateral and medial superior olive, respectively; MLF medial longitudinal fasciculus; MT medial trapezoid nucleus; SO superior olivary complex; V spinal trigeminal nucleus; VI, VII, and VIIn abducens nucleus, facial nucleus, and facial nerve, respectively.

As summarized in Sect. 6.6, peripheral efferent actions also differ, which reinforces the need for caution.

6.4.2 Axonal Pathways to the Periphery

The pathways leading from the efferent cell groups in mammals to the vestibular nerve are illustrated in Fig. 6.6c. Axons from the ipsilateral group *e* join fibers coming from the contralateral group *e*, as well as auditory (olivocochlear) efferents. All three contingents run together across the spinal trigeminal tract to join the vestibular

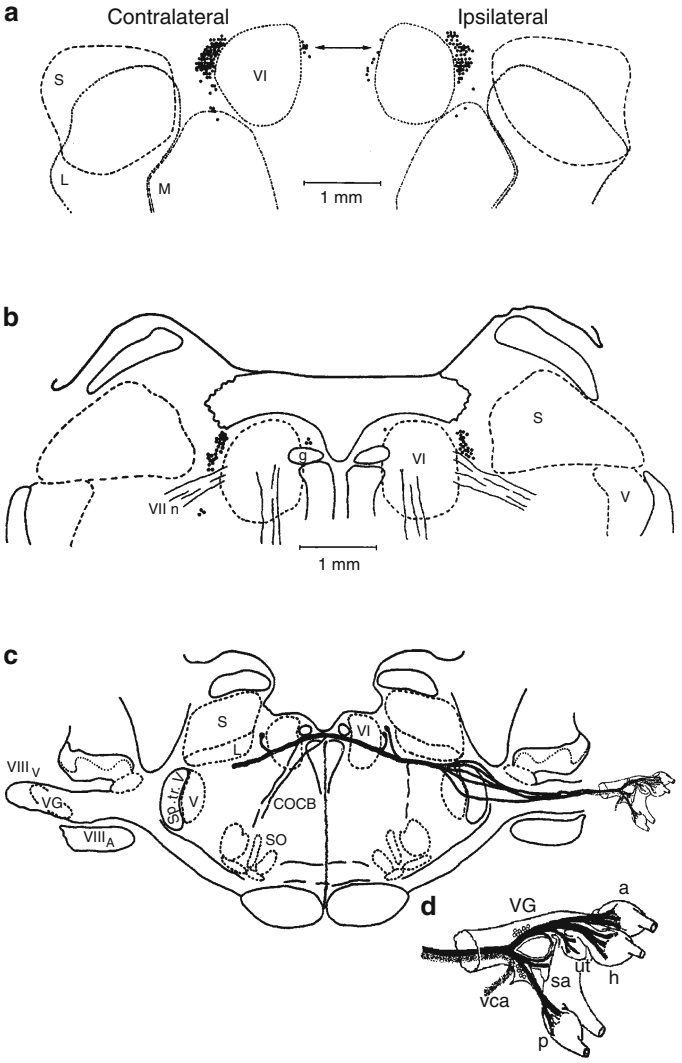

Fig. 6.6 Origin and course of efferent vestibular pathways in the squirrel monkey. (**a**) Distribution of neurons retrogradely labeled after an HRP deposit into the vestibule. Horizontal plan view. Each dot, approximately four neurons. (**b**) Same material as in (**a**). Frontal section at level indicated by *double arrow* in (**a**); each dot, one neuron. (**c**) Composite drawing of vestibular and auditory efferent pathways as revealed by AChE histochemistry. (**d**) Distribution of efferent fibers in the peripheral vestibular nerve to the different end organs. *a*, *h* and *p* anterior, horizontal, and posterior semicircular canals; *COCB* crossed olivocochlear bundle; *g* genu of facial nerve; *L*, *M*, and *S* lateral, medial, and superior vestibular nuclei, respectively; *sa* and *ut* saccular and utricular maculae; *SO* superior olivary complex; *Sp. tr. V* and *V* spinal trigeminal tract and nucleus, respectively; *vca* vestibulocochlear (Oort's) anastomosis; *VG* vestibular (Scarpa's) ganglion; *VI*, VII_n, $VIII_a$, and $VIII_v$ abducens nucleus, facial, auditory and vestibular nerves, respectively (Modified with permission from Goldberg and Fernández 1980. Copyright 1980, The American Physiological Society.)

nerve. While still in the brain, contralateral and ipsilateral vestibular efferents send collateral projections to the cerebellar flocculus, ventral paraflocculus (Shinder et al. 2001), and the interstitial nucleus of the vestibular nerve (Perachio and Kevetter 1989). In the periphery, vestibular efferents distribute to all five end organs (Fig. 6.6d). Auditory efferents pass from the inferior vestibular nerve to the cochlear nerve via the vestibulo-cochlear (Oort's) anastomosis (*vca*, Fig. 6.6d).

6.4.3 Peripheral Branching Patterns

Counting ipsilateral and contralateral projections, 300–500 efferent neurons innervate each vestibular labyrinth in mammals (Goldberg and Fernández 1980; Marco et al. 1993). In contrast, more than 10,000 afferent nerve fibers innervate one or another of the five vestibular organs (Gacek and Rasmussen 1961; Hoffman and Honrubia 2002). Despite the 20:1 discrepancy in the numbers of parent axons, afferent boutons outnumber efferent boutons by only a 3:1 ratio (Goldberg et al. 1990b; Lysakowski and Goldberg 1997). The ratio suggests that efferent fibers branch more extensively than afferents. Two kinds of branching need to be considered. Individual efferent fibers could branch within a single organ or could innervate two or more organs.

Branching within individual organs has been studied by the anterograde labeling of efferent fibers in the gerbil brain stem and tracing their trajectories in the cristae (Purcell and Perachio 1997). Branching and the resulting terminal fields are much more extensive in efferent, as compared to afferent, fibers (cf. Figs. 6.3 and 6.7a). Most branching takes place after efferent fibers enter the neuroepithelium. Despite the large size of the efferent terminal fields, many of them are restricted to the CZ or to the PZ. Remarkably, efferent neurons arising on the contralateral side of the brain stem preferentially innervate the PZ. Ipsilateral efferents show less zonal selectivity with some projecting to the CZ and others to the PZ.

Electrophysiological techniques have been used in anurans to study branching to two or more organs; in particular, the entire nerve branch supplying one organ was electrically stimulated, while recordings were made from individual afferents in other organs (Rossi et al. 1980; Prigioni et al. 1983; Sugai et al. 1991). The recorded units showed typical efferent responses. A simple interpretation is that a parent efferent axon sends branches to both the stimulated and recorded organs. Presumably, responses are the result of axon reflexes involving both branches. All five organs tested were interconnected; this included the saccular macula, which in anurans monitors substrate-borne vibrations, rather than head movements (Narins and Lewis 1984). Evidence is lacking as to whether the branching of individual efferent fibers to multiple organs occurs in mammals (Purcell and Perachio 1997).

6.4.4 Synaptic Ultrastructure of Efferent Terminals

Upon reaching the vestibular periphery, efferent fibers end as boutons and can be readily distinguished from afferent endings by their highly vesiculated appearance

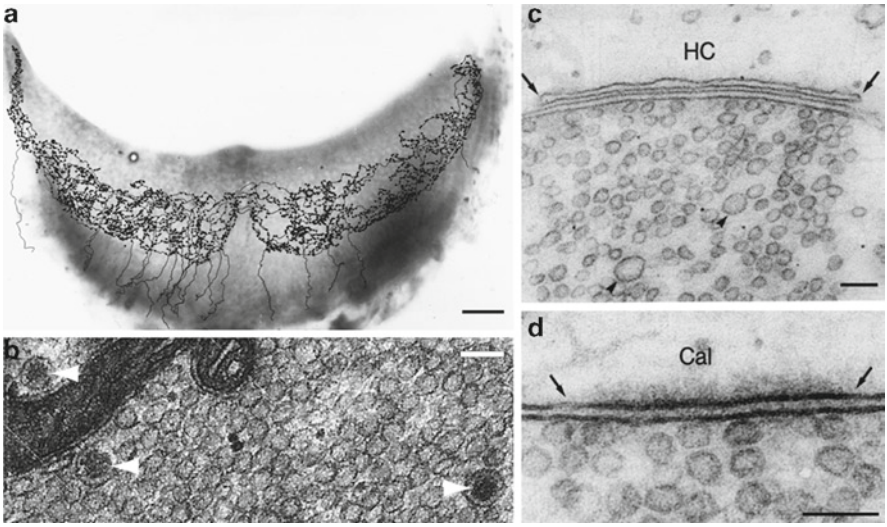

Fig. 6.7 Efferent neurons and their peripheral terminals. (**a**) Reconstructions of several efferent axons, labeled by extracellular injection of biocytin or biotinylated dextran amine in the contralateral brain stem and terminating in the posterior crista of a gerbil. These labeled efferent fibers are restricted in their innervation to the peripheral zone. Other fibers would be similarly restricted to the central zone. Each efferent fiber branches widely and gives rises to 100–300 bouton endings. (Modified with permission from Purcell and Perachio 1997. Copyright 1997, The American Physiological Society.) (**b**–**d**) Efferent terminals are recognized in electron micrographs by the large number of small, round synaptic vesicles they contain. (**b**) Electron micrograph of an efferent terminal from the red eared turtle (*Trachemys scripta elegans*) illustrating the presence of dense core vesicle (*arrowheads*) and several smaller, clear vesicles. (Unpublished micrograph, Dr. Anna Lysakowski) (**c**) An efferent synapse on a type II hair cell (HC) is marked by a subsynaptic cistern (*arrows*). *Arrowheads* point to larger vesicles that may contain neuropeptides. (**d**) Efferent ending on a calyx terminal (Cal) is marked by pre- and postsynaptic membrane thickenings delimited by *arrows*. The electron micrographs in **c** and **d** were taken from the chinchilla posterior crista. *Scale bars*: (**a**) 70 μm; (**b**–**d**) 100 μm (**c**, **d** courtesy of Dr. Anna Lysakowski, with permission)

(Engström and Wersäll 1958; Hilding and Wersäll 1962) (Fig. 6.7b–d). Efferent neurons can terminate on type II hair cells, the bouton afferents innervating type II hair cells, and the outer faces of calyx afferents (see Fig. 6.2). These terminations are characterized ultrastructurally by two types of contacts (Fig. 6.7c, d) (Iurato et al. 1972; Smith and Rasmussen 1968; Lysakowski and Goldberg 1997). Those on calyx endings and other afferent processes have asymmetric pre- and postsynaptic membrane thickenings, whereas those on type II hair cells are marked by subsynaptic cisterns, but have no obvious membrane specializations. It is thought that the cisterns are Ca^{2+} stores that might reinforce efferent synaptic actions (Sridhar et al. 1997; Lioudyno et al. 2004). Single efferent fibers can give rise to both kinds of endings (Smith and Rasmussen 1968; Lysakowski and Goldberg 1997).

6.5 Efferent Neurotransmitters and Receptors

This section considers the identity and summarizes the evidence for various efferent neurotransmitters and receptors based primarily on applying molecular biological techniques and immunohistochemistry to central neurons identified as efferents by retrograde labeling and to peripheral terminals presumed to be efferents by their being highly vesiculated. That highly vesiculated endings were AChE-positive suggested that the small clear vesicles in efferent terminals contain acetylcholine (ACh). Other substances commonly coexpressed in cholinergic neurons, such as adenosine-5′-triphosphate (ATP) and calcitonin gene-related peptide (CGRP), might also be present (Dowdall et al. 1974; Fontaine et al. 1986; New and Mudge 1986). Some context for studies of the EVS is provided by auditory efferents where there are several putative neurotransmitters other than ACh, including γ-aminobutyric acid (GABA), dopamine (DA), CGRP, and enkephalins (Eybalin 1993; see also Sewell, Chap. 4).

ACh has by far received the most attention and experimental support as the predominant efferent transmitter. Data regarding CGRP have also been reasonably compelling. The gaseous neurotransmitter nitric oxide (NO) has been implicated as a possible efferent signaling molecule. Of importance in evaluating a candidate efferent neurotransmitter are the effects of its agonists and antagonists on the responses to electrical stimulation of the EVS. Such evidence is available for ACh (Sect. 6.8), but is weak or nonexistent for most of the other candidates. For the latter, current information is based on the presence of synthesizing and degradative enzymes, of the neurotransmitter itself, and/or of various receptors. Determining the physiological effects of noncholinergic neurotransmitters remains important unfinished business. Even in the case of cholinergic mechanisms, much has to be learned about the roles of specific receptors.

6.5.1 Acetylcholine

Both the synthesizing (choline acetyltransferase [ChAT]) and the degradative enzyme (acetylcholinesterase [AChE]) are present in retrogradely labeled central EVS neurons (Schwarz et al. 1986; Perachio and Kevetter 1989; Ishiyama et al. 1994) and in highly vesiculated terminals in vestibular organs (Hilding and Wersäll 1962; Kong et al. 1994; Matsuda 1996). Clearly, ACh is a major neurotransmitter at peripheral efferent synapses. Yet, ChAT is not found in all brain stem EVS neurons, which has suggested that some of these neurons may not be cholinergic (Schwarz et al. 1986; Perachio and Kevetter 1989).

The two major classes of cholinergic receptors, nicotinic (nAChRs) and muscarinic (mAChRs), are found in vestibular neuroepithelia. nAChRs are pentameric, ligand-gated ion channels assembled from members of a currently identified family of 17 distinct subunits (α1–10, β1–4, δ, γ, and ε) (Millar and Gotti 2009;

Taly et al. 2009; Albuquerque et al. 2009). Native nAChRs expressed in the nervous system have one of the following compositions: homomeric, wherein all five subunits are identical (e.g., α7); heterodimeric, consisting of two distinct α subunits (e.g., α9α10) or of one α and one β subunit (e.g., α4β2); or multiple α and β subunits (e.g., α4α6β2β3). Differences in subunit composition and/or stoichiometry account for the diversity in the physiological and pharmacological properties of different nAChRs. mAChRs are heptahelical, G-protein–coupled receptors that are represented by five distinct subtypes (m1–5) where the odd-numbered mAChRs work predominantly through G_q and activation of phospholipase C pathways and the even-numbered mAChRs use $G_{i/o}$ to inhibit adenylyl cyclase (Caulfield and Birdsall 1998; Eglen 2005). Given their inherent differences in signaling schemes, nAChR-mediated responses should have faster kinetics than those involving mAChRs.

Molecular biological and immunohistochemical data have implicated both α9 and α10 subunits in vestibular hair cells of mammals (Elgoyhen et al. 1994, 2001; Hiel et al. 1996; Anderson et al. 1997; Luebke et al. 2005), chicken (Lustig et al. 1999), trout (Drescher et al. 2004), and frog (Holt et al. 2001). Studies in vestibular ganglia have suggested that nAChRs on afferent terminals contain the α4 and β2 nAChR subunits (Ohno et al. 1993; Wackym et al. 1995), but other subunits including α2, α3, α5–7, α9, and β3–4 have also been identified (Hiel et al. 1996; Anderson et al. 1997; Luebke et al. 2005). All five subtypes of mAChRs have been localized to the vestibular neuroepithelium in rats (Wackym et al. 1996) and pigeons (Li et al. 2007), whereas m1, m2, and m5 have been found in the human vestibular periphery (Wackym et al. 1996). Binding of the mAChR antagonist quinuclidinyl-benzilate (QNB) demonstrates that functional mAChRs are present in gerbil vestibular end organs (Drescher et al. 1999).

6.5.2 Adenosine 5′-Triphosphate

ATP is colocalized in and coreleased from synaptic vesicles in the company of a variety of classical neurotransmitters (ACh, noradrenaline, DA, GABA) (review: Abbracchio et al. 2009). ATP is likely to be a cotransmitter with ACh in vestibular efferent terminals, as it is in motor-nerve terminals (Dowdall et al. 1974; Schweitzer 1987; Silinsky 1975). ATP can activate P2X ionotropic, ligand-gated ion channels and P2Y G-protein linked receptors. Exogenous application of ATP has been shown to depolarize vestibular hair cells from several different species (Rennie and Ashmore 1993; Rossi et al. 1994; Aubert et al. 1994, 1995). Pharmacological evidence suggests that a P2Y receptor is responsible for the depolarization in *Rana* (Aubert et al. 1994, 1995). RT-PCR, Western blot, and immunohistochemical data have provided evidence for P2X receptors in the vestibular ganglia and end organs of mammals (Troyanovskaya and Wackym 1998; Syeda and Lysakowski 2001).

6.5.3 Calcitonin Gene-Related Peptide

In addition to the large numbers of small clear vesicles found in efferent terminals, the presence of occasional larger dense core vesicles (Fig. 6.7b) suggests that peptide neurotransmitters are also present. Among the various neuropeptides considered, CGRP is a 37-amino-acid peptide alternately spliced with calcitonin from the same gene transcript (Rosenfeld et al. 1983).

Cell bodies of mammalian group *e* neurons and their peripheral bouton terminals are immunoreactive (IR) for CGRP (Tanaka et al. 1989; Perachio and Kevetter 1989; Wackym et al. 1991). Consistent with the two kinds of vesicles found in individual efferent terminals, most CGRP-positive neurons also label for ChAT, suggesting that ACh and CGRP are colocalized in the same endings (Ohno et al. 1991). In the vestibular periphery, these CGRP-positive fibers terminate predominantly on calyx and bouton terminals (Tanaka et al. 1989; Perachio and Kevetter 1989; Wackym et al. 1991). Several studies have identified a possible role for CGRP and CGRP1 receptors in the lateral line (Sewell and Starr 1991; Bailey and Sewell 2000a, b), but comparable physiological studies are lacking in the peripheral vestibular system.

6.5.4 Opioid Peptides

Other efferent neuropeptide candidates likely to be present in vestibular efferents are those mediating opioid actions. Specifically, the majority of brain stem EVS neurons express preproenkephalin mRNA (Ryan et al. 1991) and show met-enkephalin-like IR (Perachio and Kevetter 1989). Endomorphin I, endomorphin II, and β-endorphin IR have been reported for ChAT-positive efferent terminals in the rat crista (Popper and Wackym 2001). Both μ and κ opioid receptors have been localized to vestibular-nerve afferents (Popper et al. 2004). Recordings in amphibian vestibular organs indicate that μ opioid receptors provide an excitatory postsynaptic modulatory input to afferent neurons (Andrianov and Ryzhova 1999; Vega and Soto 2003) and κ receptors mediate an inhibitory, presynaptic input to hair cells (Vega and Soto 2003). Both endomorphin I and dynorphin B, μ and κ receptor agonists, respectively, have also been shown to directly interact with and block nAChRs in the inner ear (Lioudyno et al. 2002).

6.5.5 γ-Aminobutyric Acid

This section considers the possibility that GABA is an afferent or an efferent neurotransmitter in vestibular organs. GABA is synthesized by glutamic acid decarboxylase (GAD) and inactivated by conversion to succinic semialdehyde by

GABA-transaminase (GABA-T). Receptors include ionotropic $GABA_A$ and $GABA_C$, as well as metabotropic $GABA_B$ varieties (Chebib and Johnston 1999; Bowery et al. 2002).

The first suggestion that GABA was an afferent neurotransmitter was provided by Flock and Lam (1974), who found that GABA was synthesized by hair-cell organs, even those lacking an efferent innervation; in addition, both spontaneous and evoked discharge were blocked by picrotoxin, a $GABA_A$ antagonist. These last results were not confirmed by Annoni et al. (1984), who found that neither GABA agonists nor antagonists had consistent effects on afferent transmission in the frog posterior canal. Despite these negative findings, there is evidence that GABA is an afferent neurotransmitter. GAD-IR has been localized to type I and type II hair cells, whereas GABA-T-IR is found postsynaptically (López et al. 1992; Usami et al. 1989). Recent studies have suggested that GABA is colocalized with glutamate in a distinctive set of hair cells in the horizontal canal of the oyster toadfish and may serve to modulate glutamatergic transmission (Holstein et al. 2004b, c).

There is, at best, mixed evidence that GABA is an EVS neurotransmitter. GAD-IR, which is an obligatory marker in GABAergic neurons, is apparently not present in brain stem EVS neurons (Perachio and Kevetter 1989) or in fibers innervating the vestibular organs (López et al. 1992; Usami et al. 1989). Attempts to localize GABA have used antibodies to its conjugation by way of aldehyde fixatives with serum albumin. Results have been variable. GABA-like-IR in vestibular organs has been described as being present only in efferent terminals (Usami et al. 1987; Kong et al. 1998); being present only in calyx endings (Didier et al. 1990); being present in calyx endings, fibers, and hair cells (López et al. 1990); or not being present (Matsubara et al. 1995).

6.5.6 Nitric Oxide

Nitric oxide (NO) is a gaseous neurotransmitter that acts by way of soluble guanylate cyclase (sGC) to activate cGMP-dependent protein kinase (reviews: Lincoln et al. 1997; Moncada et al. 1991; Garthwaite 2008). Synthesis of NO from arginine and O_2 is controlled by three isoforms of nitric oxide synthase, two of which (neuronal or nNOS, endothelial or eNOS) are constitutive, Ca^{2+}/calmodulin-dependent, and lead to a brief increase in NO in response to a transient rise in intracellular Ca^{2+}. A third or inducible form (iNOS) leads to a larger and more prolonged rise in NO, which is induced by cytokines rather than Ca^{2+}/calmodulin and is cytotoxic to invading microorganisms and tumor cells. Because NO is lipid-soluble and can pass though membranes, it can act as a paracrine agent, influencing neighboring cells, as well as the cells in which it is synthesized.

nNOS has been localized to brain stem EVS neurons and peripheral efferent boutons, hair cells, and afferent terminals (Lysakowski and Singer 2000; Takumida and Anniko 2002; Desai et al. 2004). Physiological studies have concentrated on hair cells. There is evidence that NO, likely through cGMP, inhibits $I_{K,L}$, the

distinctive K^+ current in type I hair cells (Behrend et al. 1997; Chen and Eatock 2000; Rennie 2002). This inhibition should facilitate neurotransmitter release. At the same time, NO inhibits voltage-gated Ca^{2+} channels in hair cells, which should serve to reduce afferent neurotransmission (Almanza et al. 2007). The balance between these opposing effects can potentially be assessed at the level of afferent discharge. Afferent transmission in the cristae of the axolotl, *Ambystoma tigrinum*, was facilitated by NO (Flores et al. 2001). Using criteria described later (Sect. 6.7), the effects were likely targeted to hair cells. It is unclear whether the results can be generalized to mammals as axolotls do not posses type I hair cells or an $I_{K,L}$ current. The presence of NOS in EVS neurons and its effects on hair-cell ion channels suggest a role for NO in efferent neurotransmission, but definitive evidence will require a pharmacological analysis of efferent-mediated responses.

6.6 Afferent Responses to Electrical Activation of the EVS

Given their terminations on afferent processes and type II hair cells, efferent neurons are in a position to modulate the activity of vestibular afferents. Efferent peripheral actions have been characterized by recording single-unit afferent responses to electrical stimulation of EVS pathways. The following sections consider results in mammals and then summarize findings in other vertebrates.

6.6.1 Mammals

Electrical stimulation of the mammalian EVS centrally invariably results in excitation as reflected by an increase in afferent discharge (Goldberg and Fernández 1980; McCue and Guinan 1994; Marlinski et al. 2004). This excitation, which is best seen in response to high-frequency shock trains, is similar whether efferents on the ipsilateral or contralateral sides of the brain stem are stimulated separately or simultaneously (Goldberg and Fernández 1980; Marlinski et al. 2004). This similarity is difficult to reconcile with the reported zonal projections of contralateral efferents (Purcell and Perachio 1997). Specifically, the neuroanatomical results imply that only regular units should be affected by contralateral stimulation. To the contrary, such stimulation influences both regular and irregular units.

Excitation is much larger in irregular, than in regular afferents (Fig. 6.8a). This difference is best seen by plotting efferent response magnitude vs. *cv** (Fig. 6.8b), which results in a relationship paralleling that between galvanic sensitivity and discharge regularity (Fig. 6.8c). The similarity in slopes for the two relationships suggests that much of the variation in efferent responses with discharge regularity reflects the sensitivity of the postsynaptic spike encoder. Note that calyx afferents, recognized by their irregular discharge and relatively low rotational gains

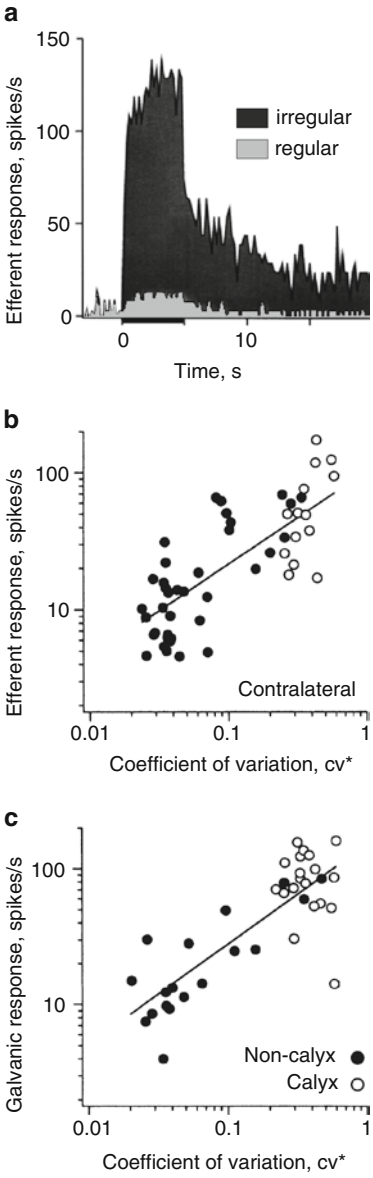

Fig. 6.8 Responses of vestibular-nerve afferents to electrical stimulation of the ipsilateral efferent nucleus in the chinchilla. (**a**) Responses of an irregular and a regular unit to 5-s shock trains, 333 shocks/s. (**b**) Relationship between efferent response magnitude and discharge regularity (*cv**). Stimulus parameters are the same as in (**a**). (**c**) Relationship between afferent response to perilymphatic galvanic polarization and discharge regularity (*cv**) (Adapted from Marlinski et al. 2004, with permission from the Association for Research in Otolaryngology.)

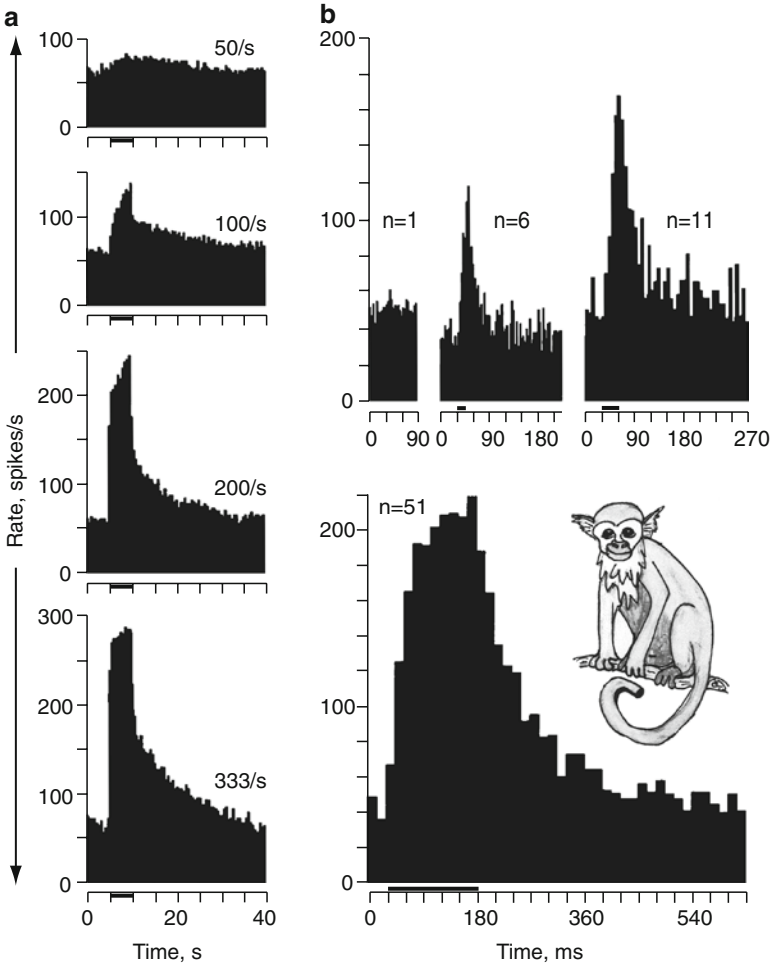

Fig. 6.9 Effects of shock-train parameters on the response of vestibular afferents in squirrel monkey to electrical stimulation of the ipsilateral efferent cell group. (**a**) Response of an irregular afferent to a 5-s efferent shock train (black bar) varying in shock frequency as stated on the *right* of each panel. (**b**) Response of another irregular afferent to efferent shock trains (*black bar*, 333 shocks/s) varying in the total number of shocks ($n = 1$–51) (Modified with permission from Goldberg and Fernández 1980. Copyright 1980, The American Physiological Society.)

(Fig. 6.4a), have efferent responses that are unexceptional in this regard (Fig. 6.8b). Consistent with their being the most irregular units in mammals, calyx units have the largest efferent responses.

For irregular units, the excitatory response can be decomposed into a fast component with kinetics of 10–100 ms and a slow response that builds up and declines with a time course of several seconds (Fig. 6.9a). The fast component is responsible for the abrupt transitions in discharge at the beginning and end of the shock train,

with the difference in the two transitions reflecting a time-dependent adaptation of the fast component. The process is time dependent in that the discrepancy between the two transitions grows with train duration. A gradual buildup of the per-train response and its persistence in the post-train period reflect the slow component. In contrast to the responses of irregular units, those of regular units are predominantly small and slow (Fig. 6.8a).

Two other features of efferent responses seen in irregular units of mammals are of potential functional significance. First, large, fast responses require high shock rates. In Fig. 6.9a, for example, only small, slow responses are seen at a shock rate of 50/s. As shock rate is increased to 100/s and beyond, the ensuing response grows disproportionately as does the fast response component. Second, even at high shock rates, large responses require multiple shocks. For the unit illustrated in Fig. 6.9b, there is only a small response to single shocks. In this case, responses grow disproportionately as the number of shocks increases. Fast responses dominate at low shock numbers (Fig. 6.9b, $n = 1–6$), but a slow response becomes evident at $n = 11$ and continues to increase as shock number increases (Fig. 6.9b, $n = 51$).

6.6.2 *Oyster Toadfish (Opsanus tau)*

Efferent-mediated responses in *Opsanus tau* are similar to those found in mammals (Fig. 6.10a). Electrical activation of the EVS almost always results in an increase in afferent discharge coupled with a small decrease in the afferent's response to head rotations or canal indentations (Boyle and Highstein 1990b; Boyle et al. 1991, 2009). Both fast and slow components are evident, with the largest responses appearing in so-called acceleration afferents, particularly those with low background activity. Based on morphophysiological studies (Boyle et al. 1991), the afferents showing large and small efferent responses are located, respectively, near the transverse center and edge of the crista. As compared to the latter units, the rotational responses of the former units have much higher gains and more phasic response dynamics.

6.6.3 *Anurans (Frogs and Toads, Rana and Bufo Species)*

Heterogeneous afferent responses to efferent stimulation are obtained in anurans (Rossi et al. 1980; Bernard et al. 1985; Sugai et al. 1991). Some irregular afferents are excited, whereas others are inhibited (Fig. 6.10b). As discussed in Sect. 6.7, both excitation and inhibition in anurans are the result of efferent actions on hair cells. Other discharge properties of excited and inhibited units appear similar. Efferent responses in regular afferents are small or nonexistent (Sugai et al. 1991).

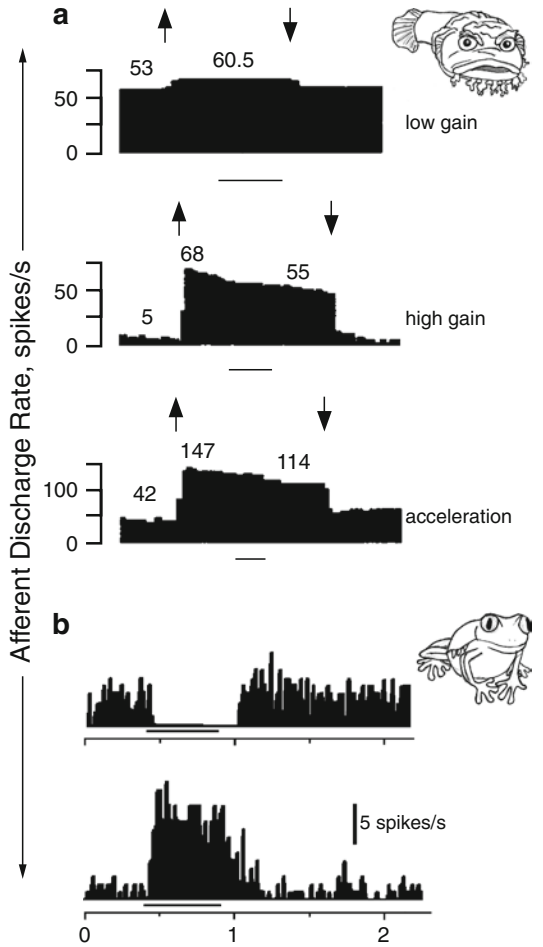

Fig. 6.10 (**a**) Firing rates of vestibular-nerve afferents during electrical stimulation of efferent pathways in the oyster toadfish, *Opsanus tau*. Three horizontal-canal afferents categorized as low-gain (*top*), high-gain (*middle*), or acceleration units (*bottom*). *Arrows* indicate the beginning and end of the efferent shock train, 100 shocks/s. *Bars*, 10 s in all cases. Numbers, firing rates at selected points. (Modified with permission from Boyle and Highstein 1990b. Copyright 1990, Society for Neuroscience.) (**b**) Responses of vestibular-nerve afferents in the Japanese toad, *Bufo vulgaris japonicus*, to electrical stimulation of efferent fibers by axon reflexes. A saccular afferent responds with inhibition to stimulation of the horizontal-canal nerve (*top*). An anterior-canal afferent is excited following stimulation of the posterior-canal nerve (*bottom*). Shock trains, 100 shocks/s, 0.5 s duration (indicated by *horizontal bar*) (Modified from Sugai et al. 1991. Copyright 1991, Physiological Society of Japan.)

6.6.4 *Red-Eared Turtles (Trachemys scripta elegans)*

Efferent-mediated excitation and inhibition are also observed in posterior crista afferents of the red-eared turtle. These efferent responses are related to the location of units in the neuroepithelium, to their afferent responses, and to the presence of efferent synapses on both hair cells and afferent processes (Brichta and Peterson 1994; Brichta and Goldberg 2000a, b; Holt et al. 2006). To understand the diversity of efferent responses in the red-eared turtle, one needs to review the organization of the posterior crista (Fig. 6.11a), which is made up of two triangularly shaped

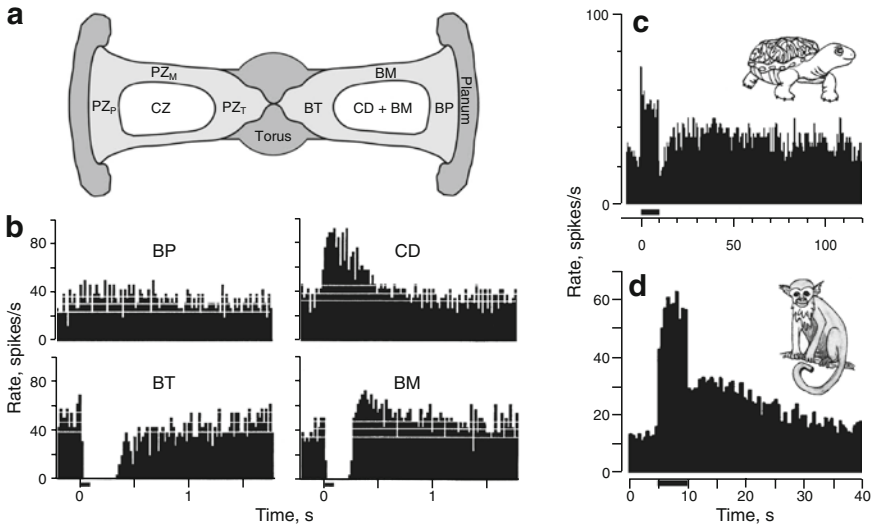

Fig. 6.11 (**a**) Regional organization of the posterior crista neuroepithelium of the red-eared turtle. The neuroepithelium consists of two hemicrista, each extending from the planum to the nonsensory torus. The hemicrista is divided into a central zone (CZ) and peripheral zone (PZ), which is further segmented as near the torus (PZ_T), near the planum (PZ_P), or between these two regions (PZ_M). Type I hair cells are located only in the CZ whereas type II hair cells are found throughout the neuroepithelium. Therefore, the PZ is innervated only by bouton afferents, further identified as those near the torus (BT), in intermediate regions (BM), or near the planum (BP). The CZ is innervated by calyx-bearing (CD) and bouton (BM) afferents. (Reproduced with permission from Holt et al. 2006. Copyright 2006, Society for Neuroscience.) (**b**) Responses of posterior-crista afferents to electrical stimulation of efferent fibers in the red-eared turtle. Shock trains, 200 shocks/s, 0.1 s duration (*bar*). Bouton afferents near the planum (BP) have a small excitatory response. Calyx-bearing (CD) units show a large excitatory efferent response. Bouton units in midportions (BM) of the hemicrista show inhibition followed by a postinhibitory excitation, whereas bouton afferents near the torus (BT) are inhibited by efferent activation without a postinhibitory excitation. (Reproduced with permission from Brichta and Goldberg 1996. Copyright 1996, John Wiley & Sons.) (**c**) Long-duration efferent stimulation (100 shocks/s, 10 s) for a CD unit results in a per-train excitation followed by a slowly developing, long-lasting (slow) excitation. (Modified with permission from Brichta and Goldberg 2000b. Copyright 2000, The American Physiological Society.) (**d**) Similar observations are seen in the squirrel monkey (333 shocks/s for 5 s) (Modified with permission from Goldberg and Fernández 1980. Copyright 1980, The American Physiological Society.)

hemicristae, each consisting of a central zone (CZ) surrounded on all sides by a peripheral zone (PZ). Calyx-bearing units are confined to the CZ, whereas bouton units are found in both the CZ and PZ. Four afferent classes need to be considered, including bouton units near the planum semilunatum (BP), at midportions (BM) of the hemicrista, and near the nonsensory torus (BT), as well as calyx-bearing (calyx and dimorphic, CD) units.

Responses of the various morphological classes to electrical stimulation of efferent fibers are illustrated in Fig. 6.11b. Resembling the situation in mammals, regularly discharging BP afferents and irregularly discharging CD units are excited by efferent stimulation with CD units having much larger responses. But unlike the situation in mammals, some afferents, including BT and BM units, are inhibited. BT/BM units are distinctive not only in their efferent responses. Their afferent responses are also unlike those seen in mammals, but are similar to units described in frogs (Blanks and Precht 1976) and the oyster toadfish (Boyle and Highstein 1990a; Boyle et al. 1991) in having distinctively high rotational gains and large phase leads re angular head velocity. Section 6.10.3 considers the possible functional implications of the high-gain (BT/BM) units being inhibited by efferent activation, while CD and BP units with more modest gains are excited.

The above responses in the red-eared turtle have relatively fast kinetics. However, slow responses, resembling those seen in mammals (Fig. 6.11d), are seen in turtle CD units in response to long-duration efferent shock trains (Fig. 6.11c) (Brichta and Goldberg 2000b). A second way to produce slow responses is to present several short efferent shock trains that are so closely spaced that the discharge does not relax to control values in the periods between trains, but continues to grow to a new asymptote (McCue and Guinan 1994; Marlinski et al. 2004; Holt and Goldberg, unpublished observations).

6.7 Sites of Efferent Actions: Hair Cells or Afferents

Resting activity is maintained by mEPSPs reflecting quantal neurotransmission to an afferent from the hair cells it innervates. Whether efferents target the hair cells or the afferent fibers can be deduced from intracellular recordings of synaptic activity in the afferent. Presynaptic actions on the hair cells should lead to a modulation of mEPSP rate, whereas postsynaptic actions on the afferent should be marked by direct efferent-mediated PSPs.

In the frog, efferent fibers contact hair cells, but not afferent terminals (Hillman 1969; Lysakowski 1996). Consistent with this innervation, efferent inhibition and excitation are associated, respectively, with decreases and increases in mEPSP rate (Rossi and Martini 1991; Bernard et al. 1985; Sugai et al. 1991). Efferent-mediated reduction in spike discharge is associated with a cessation of mEPSPs (Fig. 6.12a). When there is an increase in mEPSP traffic, these can summate to result in a depolarizing shift in membrane potential and an increased spike discharge (Fig. 6.12b).

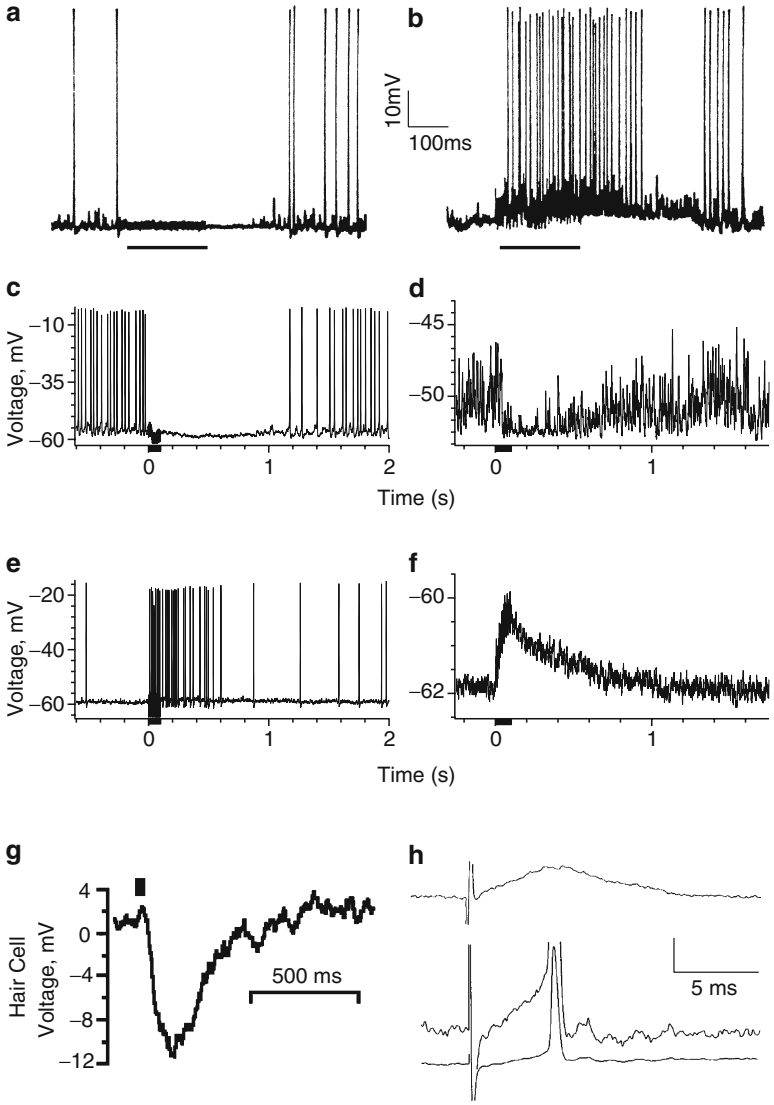

Fig. 6.12 (**a**, **b**) Efferent synaptic actions from posterior canal afferents in the frog, *Rana esculenta*. Inhibition (**a**) and excitation (**b**) of afferent discharge in two separate units are elicited by brief shock trains (200/s for 200 ms) to the anterior-horizontal canal nerves. The mEPSPs, which represent quantal transmitter release from hair cells, are seen as small depolarizations along the base of action potentials. They are eliminated in (**a**) and increased in (**b**). The increase in mEPSP traffic is associated with a depolarizing shift. (Modified with permission from Rossi et al. 1980. Copyright 1980, Elsevier Science.) (**c**, **d**) The effect of efferent actions on postsynaptic recordings from posterior crista afferents of the red-eared turtle. In bouton afferents located near the torus (BT), activation of efferent fibers results in a cessation of discharge (**c**) correlated with a reduction of quantal activity in the same unit (**d**). Efferent activation in calyx-bearing (CD, calyx or dimorphic) afferents increases discharge. (**e**) An underlying efferent-mediated EPSP from another CD

Efferent fibers in the red-eared turtle innervate both hair cells and afferent terminals, including both calyces and boutons (Lysakowski 1996; Holt et al. 2006). The efferent inhibition seen in BT and BM fibers is the result of a marked reduction in mEPSP rate and, hence, is mediated by a hair-cell action (Fig. 6.12c, d). As mEPSP rates fall, the afferent hyperpolarizes due to the loss of depolarizing quantal activity. In contrast, the excitation observed in CD units is associated with a direct efferent-mediated EPSP (Fig. 6.12e, f) consistent with efferent terminals synapsing on calyx endings. Hair cell–mediated efferent inhibition in BT/BM fibers typically masks an afferent excitation attributed to the efferent innervation of afferent boutons. This direct excitation can be revealed, however, using several pharmacological blockers of hair cell inhibition (Holt et al. 2006). Currently, intracellular recordings of efferent actions in BP units are lacking.

In the oyster toadfish, there is an efferent innervation of both hair cells (Sans and Highstein 1984; Holstein et al. 2004a) and afferents (Sans and Highstein 1984). In response to single or multiple EVS shocks, long-latency IPSPs have been recorded from hair cells (Boyle et al. 2009) (Fig. 6.12g) and monosynaptic EPSPs from afferents (Highstein and Baker 1985) (Fig. 6.12h). The hair-cell IPSPs, although delayed, lead to such large conductance changes that they might be expected to reduce spike discharge. Yet, efferent stimulation almost always increases discharge (Boyle and Highstein 1990b). How hair-cell inhibition might interact with afferent excitation to produce this result remains to be determined.

6.8 Pharmacology of Efferent Neurotransmission

The predominant efferent neurotransmitter is acetylcholine (ACh). To account for the diversity of efferent responses in vestibular organs based solely on the actions of ACh would require differences in cholinergic receptors and/or subsequent intracellular signaling. Pharmacological studies, conducted largely in frogs and turtles, have delineated the receptor and signaling mechanisms underlying the three principal effects of efferent activation: hair-cell inhibition, hair-cell excitation, and afferent excitation. These are fast efferent activations. Evidence to be considered in this section indicates that they are mediated by nAChR mechanisms.

Fig. 6.12 (continued) unit is shown (**f**). Shock train, 20 shocks, 200 Hz. (Adapted with permission from Holt et al. 2006. Copyright 2006, Society for Neuroscience.) (**g**, **h**) Intracellular recordings from a hair cell (**g**) and afferents (**h**) in the horizontal canal of the oyster toadfish during efferent stimulation. (**g**) Large IPSP generated in a canal hair cell during the delivery of an efferent shock train (100/s, *gray bar*). (Modified with permission from Boyle et al. 2009. Copyright 2009, The American Physiological Society.) (**h**) Depolarization of a canal afferent elicited by a single efferent shock (*top*). The single-shock, efferent-mediated EPSP can elicit action potentials (*bottom*). Voltage *scale bar* in *bottom* applies to both: *top*, 4 mV; *bottom*, 8 mV (Modified with permission from Highstein and Baker 1985. Copyright 1985, The American Physiological Society.)

In addition, there is a slow excitation, which involves mAChR-mediated and/or possibly non-cholinergic actions.

6.8.1 Hair-Cell Inhibition

When it was first reported that efferent stimulation exerts an exclusively excitatory action on mammalian vestibular afferents (Goldberg and Fernández 1980), it seemed rather surprising since efferent actions in other hair-cell organs were known to be inhibitory (Fex 1962; Russell 1968; Ashmore and Russell 1982). Even in frogs, almost half of vestibular afferents were excited by efferent stimulation (Rossi et al. 1980). At that time, it was also known that: (1) inhibitory efferent actions in the cochlea are the result of cholinergic nicotinic neurotransmission (Bobbin and Konishi 1971, 1974); and (2) activation of nicotinic receptors typically results in excitation (Cooper et al. 2002). The initial challenge, it seems, was to explain how an efferent action based on nicotinic receptors could give rise to inhibition, rather than excitation.

A possible clue was provided by hair-cell recordings in the red-eared turtle basilar papilla, which showed that efferent inhibition was mediated by a hyperpolarization that was preceded by a brief depolarization; the suggestion was made that the early depolarization was the primary synaptic event that triggered the slower hyperpolarization (Art et al. 1984). Consistent with this idea, central recordings indicated that nicotinic transmission led to inhibition by triggering a calcium-dependent increase in a potassium conductance (Wong and Gallagher 1991). Within a few years, a similar mechanism was shown to be responsible for efferent inhibition in auditory hair cells in the chick basilar papilla (Fuchs and Murrow 1992a, b). Later work showed that inhibition is the result of the efferent-mediated activation of α9/α10-nicotinic ACh receptors (α9/10nAChRs) (Elgoyhen et al. 1994, 2001), whose opening allows the entry of Ca^{2+} ions (Weisstaub et al. 2002) that activate small-conductance, calcium-dependent potassium (SK) channels (Yuhas and Fuchs 1999; Oliver et al. 2000). Outward K^+ currents through SK channels hyperpolarize the hair cell, inhibit neurotransmitter release, and reduce afferent discharge.

As noted previously, inhibition of afferent discharge during efferent stimulation is seen in recordings from the vestibular organs of anurans (Rossi et al. 1980; Bernard et al. 1985; Sugai et al. 1991) and red-eared turtles (Brichta and Goldberg 2000b). As with auditory hair cells, efferent inhibition in the turtle vestibular labyrinth also involves the linked activation of α9/10nAChRs and SK channels. The situation is illustrated by recordings from a BT afferent innervating the posterior crista (Fig. 6.13a, b). The reduction of quantal activity and the ensuing afferent hyperpolarization resulting from efferent stimulation are completely blocked by the α9/10nAChR antagonist tropisetron (ICS-205390 or ICS) (Fig. 6.13a). In line with the sequential activation of α9/10nAChRs and SK channels, single efferent shocks generate a biphasic voltage response in the afferent comprised of an initial

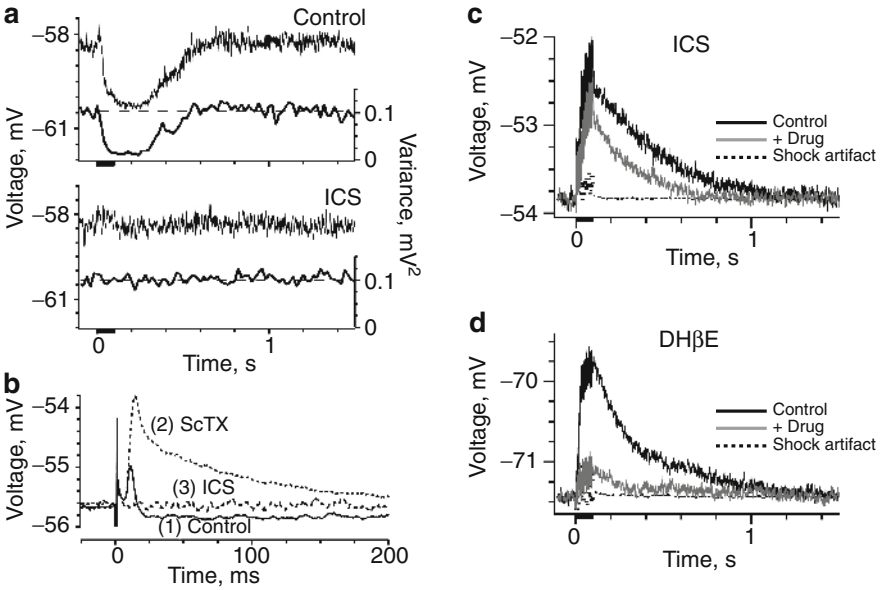

Fig. 6.13 Pharmacological dissection of efferent responses in the posterior crista of the red-eared turtle. (**a**) Tropisetron (ICS), a pharmacological blocker of α9/α10 nicotinic receptors (α9/10nAChRs), eliminates the response of a BT afferent to efferent shock trains (20 shocks at 200/s, *black bar*). Average responses are compared before and during the application of 10 μM ICS. In each panel, the ensemble mean voltage (*top trace*) and variance (*bottom trace*) are shown. Variance should be proportional to quantal rate. *Dashed line* indicates the average prestimulus variance. ICS blocks the effects of efferent stimulation on both voltage and variance. (**b**) Scyllatoxin (ScTX), an SK blocker, converts afferent responses to single efferent shocks from a biphasic excitation–inhibition to a monophasic excitation. In the red eared turtle, the biphasic voltage response (*1*, Control) to single efferent shocks, seen here in a BT unit, is a signature for the sequential activation of α9/10nAChRs and SK where a brief depolarization is followed by a prolonged hyperpolarization. The biphasic response is converted to an exclusively depolarizing response by 1 μM ScTX (*2*), which is then completely blocked by 10 μM ICS (*3*). (**c**, **d**) Pharmacology of postsynaptic depolarizing responses in CD afferents can be distinguished from presynaptic responses in BT/BM afferents. The effects of ICS and the nicotinic antagonist, dihydro-β-erythroidine (DHβE), on the response of CD afferents to efferent shocks (20 shocks, 200/s) are shown. (**c**) In a CD afferent, 10 μM ICS has only a small effect on the response. (**d**) In another CD unit, 300 nM DHβE blocks most of the response (All panels reproduced with permission from Holt et al. 2006. Copyright 2006, Society for Neuroscience.)

brief depolarization followed by a more prolonged hyperpolarization (Fig. 6.13b, control). Application of scyllatoxin (ScTX), an SK antagonist, blocks the hyperpolarization and unmasks a substantial afferent depolarization (Fig. 6.13b, ScTX). That the depolarization is mediated by α9/10nAChRs is confirmed by its subsequent blockade with ICS (Fig. 6.13b, ICS). Other blockers of α9/10nAChR (e.g., strychnine) and SK channels (e.g., apamin) confirm the linked participation of the nAChR and SK (Holt et al 2006). Similar mechanisms have been identified in vibratory and lateral line organs (Yoshida et al. 1994; Holt et al. 2001; Dawkins et al. 2005).

6.8.2 Hair-Cell Excitation

Efferent-mediated excitation, as indicated by an increase in afferent discharge, is commonly seen in a subset of irregularly discharging afferents in anurans (Rossi et al. 1980, 1994; Bernard et al. 1985; Sugai et al. 1991) and in calyx-bearing afferents in turtles (Figs. 6.10–6.12) (Brichta and Goldberg 2000b). It is the predominant action in the horizontal crista of *Opsanus tau* (Fig. 6.11a) (Boyle and Highstein 1990b; Boyle et al. 1991) and in the entire vestibular labyrinth of mammals (Figs. 6.8 and 6.9) (Goldberg and Fernández 1980). The synaptic bases of excitation in different species are heterogeneous and attributed to the actions of efferents synapsing on hair cells and/or afferents. This section discusses the mechanisms underlying efferent-mediated excitation of hair cells as seen in anurans and the red-eared turtle.

In anurans, efferent-mediated excitation is associated with an increase in quantal activity (Rossi et al. 1980; Bernard et al. 1985; Sugai et al. 1991), consistent with an efferent action on hair cells. A slow excitation of frog canal afferents, generated with cholinergic agonists, is attributed to mAChRs also on hair cells; however, such slow excitation has not been demonstrated with genuine efferent stimulation (Guth et al. 1986; Holt et al. 2003; Derbenev et al. 2005). The observation in red-eared turtles that efferent inhibition can be converted to excitation when SK channels are blocked suggests that hair cells expressing α9/10nAChRs uncoupled from SK could account for such hair-cell excitation (Holt et al. 2006). However, several pharmacological observations suggest, at least in frogs, that the receptor underlying hair-cell excitation is nicotinic but not α9/10nAChR: (1) application of the nicotinic agonist 1,1-dimethyl-4-phenylpiperazinium (DMPP) or carbachol mimics efferent stimulation in increasing the rate of both mEPSPs (Fig. 6.14a) and action potentials (Fig. 6.14b–d), and can do so in the same afferents previously excited by efferent stimulation (Bernard et al. 1985; Holt et al. 2003); (2) efferent-mediated hair-cell excitation and DMPP responses are blocked by *d*-tubocurarine (dTC) (Fig. 6.14c) but not by strychnine (Fig. 6.14d), the latter a potent α9/10nAChR antagonist; (3) finally, the nAChRs underlying hair-cell excitation are more sensitive to ACh and DMPP than are α9/10nAChRs (Fig. 6.14e, f)

Fig. 6.14 (continued) rate of mEPSPs. (**b**) Single unit spike histograms illustrating the actions of the cholinergic agonists, DMPP and carbachol (in mM), upon the firing rates of frog vestibular afferents. NS, normal saline. DMPP elicits a fast excitation (*top*) whereas carbachol produces both fast and slow excitation. (Modified with permission from Bernard et al. 1985. Copyright 1985, Elsevier Science.) (**c**, **d**) Effects of the nicotinic antagonists *d*-tubocurarine (dTC) and strychnine on the response of multiunit afferent firing to DMPP in posterior semicircular canal of *Rana pipiens*. Similar to single-unit observations in (**b**), the application of 10 μM DMPP (*small bars*) results in a rapid increase in background discharge of canal afferents (**c**, **d**, *left*). This DMPP-mediated excitation was mostly antagonized by 1 μM dTC (**c**, *right*) but not by 10 μM strychnine (**d**, *right*). (**e**, **f**) Current clamp recordings demonstrate that frog semicircular-canal hair cells are strongly depolarized by low concentrations of both ACh (1 μM) and DMPP (0.1 μM). AP, artificial perilymph (Modified with permission from Holt et al. 2003. Copyright 2003, The American Physiological Society.)

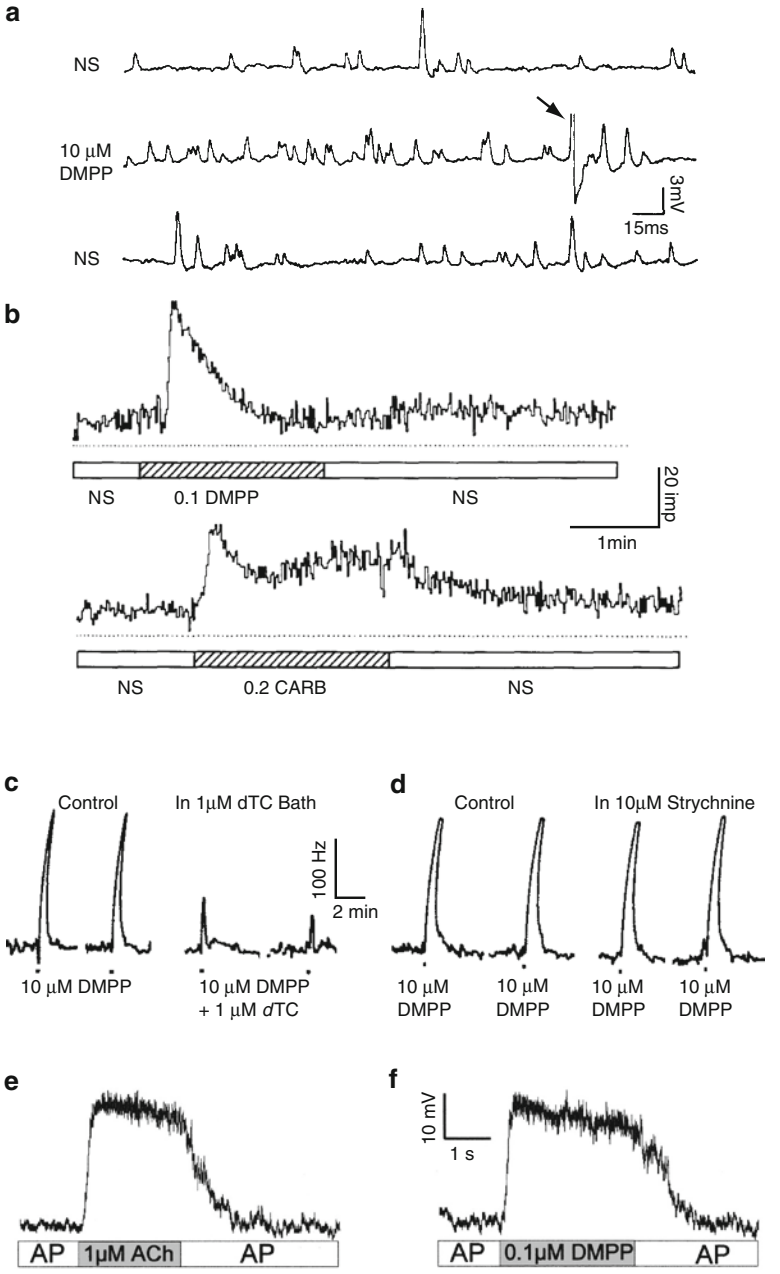

Fig. 6.14 Pharmacological dissection of excitatory efferent responses in frogs, *Rana temporaria* and *Rana pipiens*. (**a**) Effect of the nicotinic agonist 1,1-dimethyl-4-phenyl-piperazinium (DMPP) on spontaneous synaptic potentials recorded intracellularly from vestibular afferents. Spontaneous mEPSPs were recorded before (*top*), during (*middle*), and after (*bottom*) the application of 10 μM DMPP. A single action potential has been truncated (*arrow*). DMPP significantly increases the

(Elgoyhen et al. 2001; Holt et al. 2003). This DMPP-sensitive excitatory nAChR may contain α4 and/or α6 subunits but its exact composition has not yet been determined (Guth et al. 2002).

Although there is no pharmacological evidence for a comparable DMPP-sensitive nAChR in turtle vestibular hair cells (Holt et al. 2006), an efferent-mediated hair-cell excitation can be seen in recordings from a subset of afferents innervating the turtle posterior crista. With few exceptions, efferent-mediated inhibition in BM afferents is followed by excitation (Fig. 6.11b, BM). This post-inhibitory excitation (PIE) is associated with an increase in quantal activity that is initiated by the preceding inhibition (Brichta and Goldberg 2000b; Holt et al. 2006). Blockade of either α9/10nAChRs or SK also blocks PIE. Despite its dependence on an α9/10nAChR/SK-mediated hyperpolarization of type II hair cells, PIE is peculiar to BM, but not BT, afferents. These observations suggest that the efferent-mediated hyperpolarization activates a conductance that would depolarize the hair cell as the hyperpolarization terminates. Two likely candidates are a T-type calcium current or an I_h (HCN) current. PIE is also seen in vestibular and lateral-line afferents of anurans (Rossi and Martini 1991; Dawkins et al. 2005). Here, similar mechanisms could be involved. Alternatively, there could be a convergence of efferent-mediated inhibition (e.g. α9/10nAChR/SK) and excitation (e.g. DMPP-sensitive nAChRs) onto the same hair cells, or on different hair cells innervating the same afferent. PIE could result were the inhibition to outweigh the excitation during the evoking shock train, but to decay faster in the post-train period.

6.8.3 Fast Afferent Excitation

While efferent terminals in the frog are confined to hair cells, those in most other species also contact afferent terminals (Sect. 6.4.4). In species containing both type I and type II hair cells, calyx endings and afferent boutons receive an efferent innervation. As far as we know, this postsynaptic efferent innervation is excitatory in all species where it is present. In addition, there is circumstantial evidence that excitation may also occur in the mammalian cochlea at efferent synapses onto afferent dendrites underneath inner hair cells (Walsh et al. 1998; Zheng et al. 1999).

Pharmacological experiments in the red-eared turtle have further demonstrated that the direct efferent-mediated excitation of both kinds of afferent terminals involves nicotinic ACh receptors distinct from α9/10nAChRs (Holt et al. 2006, 2010). In particular, the receptors underlying efferent-mediated afferent EPSPs can be distinguished from α9/10nAChRs in the potency of their blockade by various agents. Strychnine and ICS are more potent blockers of α9/10nAChRs (cf. Fig. 6.13a, c), whereas dihydro-β-erythroidine (DHβE) is a qualitatively more effective blocker of the efferent-mediated EPSPs (Fig. 6.13d). Molecular biological and pharmacological data suggest that α4β2-containing nAChRs may underlie efferent-mediated excitation of vestibular afferents (Wackym et al. 1995; Anderson et al. 1997; Holt et al. 2006, 2008, 2010).

How do the efferent actions on hair cells and afferent terminals interact? Most afferents in mammals and turtles receive synaptic inputs from type II hair cells. This connectivity is obviously the case for afferents with bouton endings, but even the calyx terminals innervating type I hair cells can be contacted on their outer faces by ribbon synapses from type II hair cells (Lysakowski and Goldberg 1997, 2008; Holt et al. 2007). Type II hair cells also receive a conspicuous efferent innervation; yet inhibition is not seen in mammalian afferents, even those receiving type II inputs. This observation as well as recent work in the oyster toadfish (Boyle et al. 2009) and the red-eared turtle (Holt et al. 2006) suggests one of two possibilities: (1) there is a presynaptic efferent inhibition of hair cells that is outweighed by the postsynaptic excitation of calyx endings and other afferent processes; or (2) the presynaptic action is also excitatory. Concerning the latter possibility, a presynaptic excitatory action might be mediated by a novel receptor, as is suggested by work in the frog (Bernard et al. 1985; Rossi et al. 1994; Holt et al. 2003), or it could result from an α9/10nAChR-mediated excitation that is not completely checked by an activation of SK channels. The matter is currently unresolved.

6.8.4 Slow Afferent Excitation

The fast excitatory component seen in mammals has kinetics of 10–100 ms, while the slow excitatory component can take several seconds to increase and decrease (Goldberg and Fernández 1980; McCue and Guinan 1994; Marlinski et al. 2004). Slow responses have also been recorded in the oyster toadfish (Boyle and Highstein 1990b; Boyle et al. 1991) and in turtles (Brichta and Goldberg 2000b) by the electrical activation of the EVS as well as in frogs by the application of cholinergic agonists (Bernard et al. 1985; Holt et al. 2003). Because of the slow kinetics involved, it is natural to suspect a G-protein–coupled receptor such as a mAChR. At the same time, it should be noted that fast and slow efferent effects in the mammalian cochlea can involve one and the same nicotinic receptor, likely α9/10nAChR (Sridhar et al. 1995, 1997).

There is pharmacological evidence for the participation of mAChRs in the etiology of slow responses. Such receptors are present in vestibular neuroepithelia, nerve fibers, and ganglia (Wackym et al. 1996; Drescher et al. 1999; Li et al. 2007). Application of mAChR agonists in the crista of frogs and turtles results in a slow excitation (Bernard et al. 1985; Holt et al. 2003; Jordan et al. 2010). Furthermore, in red-eared turtles, mAChR antagonists block slow responses in CD afferents evoked by prolonged electrical stimulation of efferent fibers (Jordan et al. 2010). How might mAChR activation lead to slow responses? One possibility is the activation of the G protein (G_q) that inhibits so-called M currents through depletion of intramembranous phosphatidylinositol 4,5-bisphosphate and/or the elevation of intracellular calcium (Hernandez et al. 2008; Brown and Passmore 2009). M currents are outwardly rectifying K^+ channels whose suppression by activation of mAChRs or other metabotropic

receptors results in a slow depolarization coupled to an increase in impedance (Fukuda et al. 1988; Brown 1988; Delmas and Brown 2005). KCNQ4, one of several channels that can give rise to M currents (Selyanko et al. 2000; Brown and Passmore 2009), has been immunolocalized to type I hair cells and calyx endings (Kharkovets et al. 2000; Hurley et al. 2006; Sousa et al. 2009). Calyx endings are also immunoreactive for KCNQ5 (Hurley et al. 2006). M-currents have been recorded from chick and rat vestibular ganglion cells (Yamaguchi and Ohmori 1993; Pérez et al. 2009a, b).

It is also possible that mAChRs tap into the nitric oxide (NO) pathway by activating nNOS in the calyx or in efferent terminals to produce NO that then diffuses to block $I_{K,L}$, an M-like current in type I hair cells, and thereby to depolarize the hair cell and enhance neurotransmitter release (Chen and Eatock 2000; Lysakowski and Singer 2000; Hurley et al. 2006). Whether the slow response arises from hair cells or afferents or whether KCNQ channels and/or NO are involved is currently unresolved.

Alternatively, vestibular efferent neurons also contain CGRP (Tanaka et al. 1989; Wackym et al. 1991; Ishiyama et al. 1994) and possibly other neuroactive peptides (Sects. 6.5.3 and 6.5.4; review: Goldberg et al. 2000). In lateral lines, CGRP causes a slow excitation (Sewell and Starr 1991; Bailey and Sewell 2000a, b). In mammals, postsynaptic actions could be mediated by CGRP-containing efferent axons, which have been observed to contact calyces and other afferent processes (Tanaka et al. 1989; Wackym et al. 1991; Ishiyama et al. 1994).

6.9 Efferent Modulation of Afferent Responses to Natural Stimulation

Most studies of the physiological actions of the EVS have looked at the consequences of efferent activation on the background activity of vestibular afferents. However, it is equally important to understand how different efferent actions impact the afferent's response to natural stimulation. In posterior-canal afferents of frog and turtle, efferent inhibition can completely abolish responses to cupular deflections produced either by rotations (Rossi et al. 1980) or indentations of the canal duct (Holt 2008). More modest changes in rotational sensitivity are seen with efferent-mediated fast excitation. In the squirrel monkey (*Saimiri sciureus*), when fast efferent excitation is paired with rotation, there can be a small decrease in the rotational gain of irregularly discharging units (Goldberg and Fernández 1980). Similar gain reductions have been observed during efferent-mediated fast excitation in the oyster toadfish (Boyle and Highstein 1990b). A somewhat different result was obtained in the cat under conditions that may have favored slow responses; here there was an enhanced sensitivity of saccular afferents to intense air-borne sounds (McCue and Guinan 1994).

A conventional parallel-conductance model suggested that fast and slow efferent excitation could have opposite effects on afferent gain (Fig. 6.15a). Fast responses, by increasing conductance, should decrease gain; slow responses, were they to

decrease conductance, for example, by inhibiting an M current, should have the opposite effect. The prediction has been confirmed in the posterior canal of the red-eared turtle; pairing indentation of the canal duct with an efferent-mediated fast response is associated with a modest gain decrease (Fig. 6.15b), whereas pairing with a slow response is associated with a more substantial gain increase (Fig. 6.15c) (Holt 2008; Shah et al. 2010). In both situations, there is an increase in the background discharge and in the average discharge during sinusoidal stimulation.

6.10 Functional Studies of the EVS

The afferent responses to electrical stimulation of the EVS can provide clues as to function. But to go beyond speculation, an understanding is needed of how efferent neurons are influenced by natural stimulation and how they, in turn, can modify afferent discharge under physiological conditions. Studies of efferent discharge characteristics have almost exclusively been done in fish and anurans.

6.10.1 Response of EVS Neurons to Natural Stimulation

Efferent neurons respond to vestibular stimulation, including activity arising from semicircular canals (Schmidt 1963; Gleisner and Henriksson 1964; Precht et al. 1971; Blanks and Precht 1976; Hartmann and Klinke 1980) and otolith organs (Klinke and Schmidt 1968). These studies provide evidence that efferent neurons receive a convergent input from several vestibular organs in both ears. Possibly reflecting such a bilateral convergence, efferent neurons respond in a type III manner, increasing their discharge for angular rotations in either direction (Precht et al. 1971; Blanks and Precht 1976). A type III response may be contrasted with the invariable type I responses of afferents, where discharge is increased (excited) by rotations in one direction and reduced (inhibited) by oppositely directed rotations. Only inconsistent rotational responses were recorded from efferent neurons in the oyster toadfish (Highstein 1991), even though these neurons receive a monosynaptic excitatory input from the vestibular nerve (Highstein and Baker 1985).

Efferent neurons also respond to nonvestibular stimulation, including pressure applied to the skin (Schmidt 1963; Precht et al. 1971), passive movement of limbs (Schmidt 1963; Precht et al. 1971), and visual stimulation (Klinke and Schmidt 1970). As has also been observed in lateral-line efferents (Russell 1971; Roberts and Russell 1972), vestibular efferents also respond in anticipation of active body movements (Schmidt 1963; Gleisner and Henriksson 1964; Precht et al. 1971). In the oyster toadfish, efferents are excited by a large variety of sensory stimuli (Highstein and Baker 1985; Highstein 1991). Here, the responses have a long latency (≈150 ms) and may outlast the stimulus by 500 ms or more. Efferent activation in the oyster toadfish is most likely associated with arousal, a stereotyped

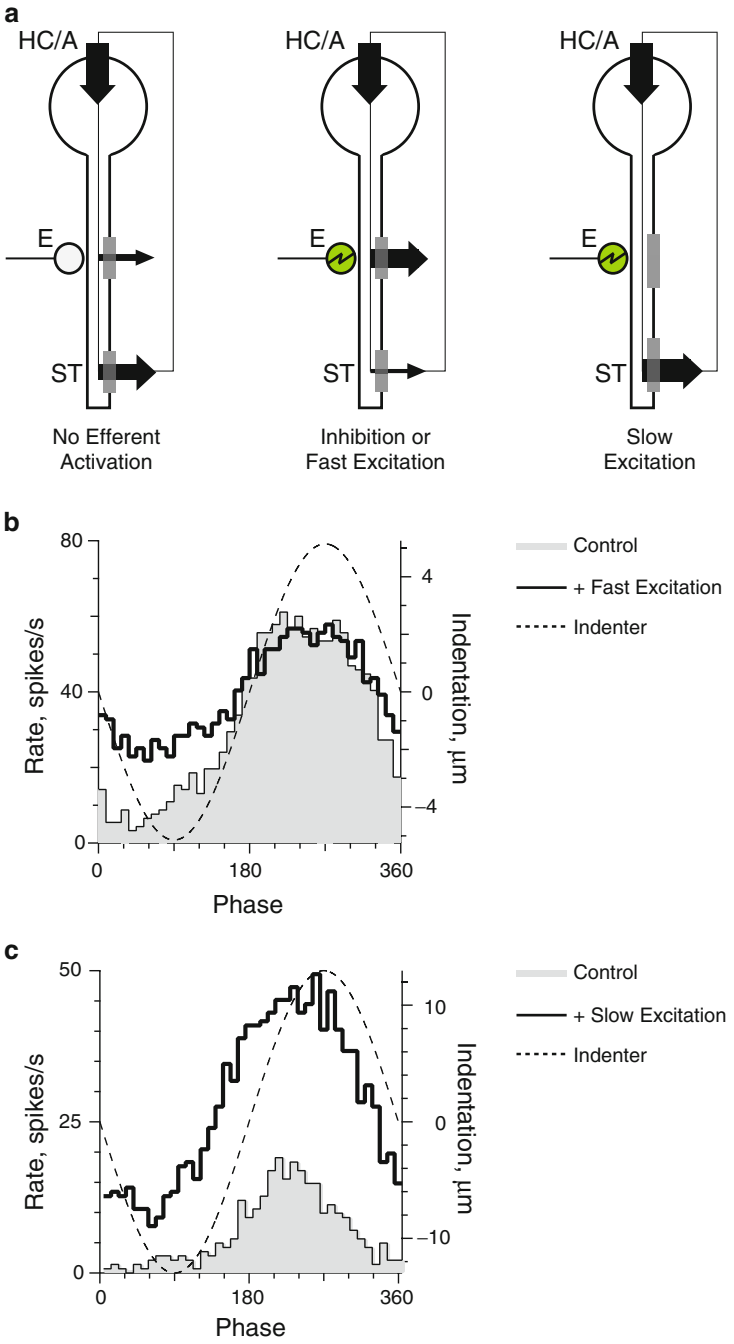

Fig. 6.15 Effects of efferent activation on the indenter response of posterior canal afferents of the red-eared turtle. (**a**) Parallel conductance model indicating how efferent activation (E) can attenuate or augment synaptic currents arising from a hair cell or afferent (HC/A) and their subsequent interaction with synaptic transmission or the spike-trigger site (ST), respectively. Upon efferent-mediated

behavior that can be evoked by sensory stimulation and can also occur spontaneously (Highstein and Baker 1985). The behavior can be a prelude to movement. With one possible exception (Marlinsky 1995), discharge properties of efferent neurons have not been studied in mammals.

6.10.2 *Efferent-Mediated Modulation of Afferent Discharge*

A potential function of efferents is to modify afferent discharge on a short time scale. An example is provided by the arousal response in the oyster toadfish, which is associated with an excitation of afferents, as well as of efferents (Highstein and Baker 1985; Boyle and Highstein 1990b). To study efferent-mediated vestibular responses, a mechanical indenter was used in the decerebrate pigeon to stimulate the horizontal canal contralateral to the afferents being recorded (Dickman and Correia 1993). Some afferents were excited by contralateral stimulation, others were inhibited, and still others showed mixed responses. The diversity of afferent responses presumably reflects a similar diversity in the peripheral actions evoked by electrical stimulation of efferent pathways in birds.

Efferent-mediated rotational responses were obtained in the decerebrate chinchilla (Plotnik et al. 2002) from otolith afferents, which do not otherwise respond to head rotations (Goldberg and Fernández 1975), and from canal afferents after positioning the head so that conventional rotational responses of each fiber were nulled by placing the innervated canal nearly orthogonal to the plane of motion. High-intensity (320°/s) rotations led to type III responses (Fig. 6.16a–c), which resembled those obtained by electrical stimulation of efferent pathways in several ways. The responses were always excitatory. They were considerably larger in irregular, as compared to regular, afferents (Fig. 6.16d–f). In irregular units, both fast and slow responses were seen, whereas the responses in regular units were predominantly slow. Canal-plugging and labyrinthine galvanic polarization were used to show that type III responses could be

Fig. 6.15 (continued) inhibition and/or fast excitation, opening of ion channels (e.g., nAChRs or SK) in the hair cell and/or afferent will shunt current away from ST effectively reducing the sensitivity of the afferent to the same vestibular stimulus. Closure of ion channels, as is thought to occur with efferent-mediated slow excitation, should have the opposite effect. (**b**) Phase histograms illustrating the effect of fast excitation on a CD afferent's response to sinusoidal canal-duct indentation. For this particular unit, there was no discernible efferent-mediated slow excitation. Comparing the afferent's response to the indenter alone to that when the efferent stimulus (+ Fast Excitation) and the same indenter stimulus are given simultaneously demonstrates that fast excitation reduces the peak-to-peak modulation. (**c**) Phase histograms of another CD afferent illustrating the effect of slow excitation on the indenter response. Slow excitation was generated using an efferent shock protocol similar to that shown in Fig. 6.11c. The afferent's response (control, 18 cycles, 0.3 Hz) to indentation alone is significantly enhanced when the same indenter stimulus is applied during efferent-mediated slow excitation

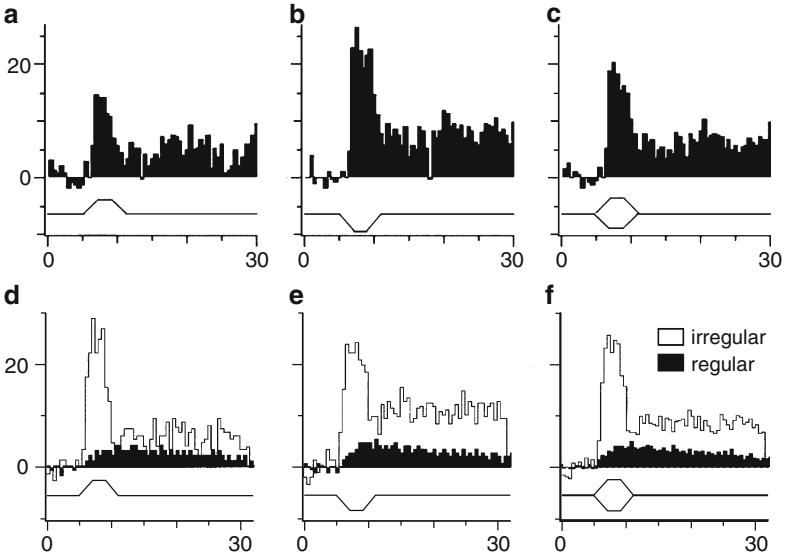

Fig. 6.16 (**a**–**c**) Responses (discharge rate minus background rate) in response to (**a**) counterclockwise (CCW) and (**b**) clockwise (CW) rotations, 320°/s in the horizontal plane, for an irregular otolith afferent ($cv^* = 0.32$) from a decerebrate preparation. Velocity profiles are below each trace and directions are as viewed from above. Both rotations gave excitatory responses, which are averaged in (**c**). (Adapted from Plotnik et al. 2005, with permission from the Association for Research in Otolaryngology.) (**d**–**f**) Comparison of responses of two otolith units, one regular and the other irregular, to horizontal angular head rotations. Other details as above in (**a**–**c**) (Modified with permission from Plotnik et al. 2002. Copyright 2002, The American Physiological Society.)

obtained from stimulation of either the ipsilateral or contralateral labyrinths. Remarkably, after unilateral canal plugging, efferent-mediated excitatory responses could be produced by rotations in either direction, including those resulting in the excitation or inhibition of afferents on the unplugged side. Some of the potential mediating pathways are depicted in Fig. 6.17a. There is evidence that group *e* receives direct inputs from the ipsilateral vestibular nerve (White 1985; Li et al. 2005) and bilaterally from the vestibular nuclei (Chi et al. 2007). Because of the bilateral projections of the ipsilateral and contralateral efferent cell groups, it is easy to see how an afferent could be excited by excitatory rotations of either ear. How inhibitory rotations are converted into excitation is less clear. In Fig. 6.17a, the conversion is accomplished by disinhibition, that is, the inhibition of crossing inhibitory fibers.

Efferent-mediated rotational responses, which have also been seen in alert monkeys (Sadeghi et al. 2009), are small, typically less than 20 spikes/s even in irregular afferents. These small responses suggest that the efferent system has only weak actions when not stimulated with high-frequency shock trains. That the EVS can exert powerful effects on afferent discharge is shown by large fluctuations in background discharge in the decerebrate chinchilla (Plotnik et al. 2005). A particularly striking example is shown in Fig. 6.17b1. Even in the absence of stimulation, there are nearly periodic fluctuations in the background discharge, which ranges from less than 50 to almost 300 spikes/s. Fluctuations are unusually large in this case. More

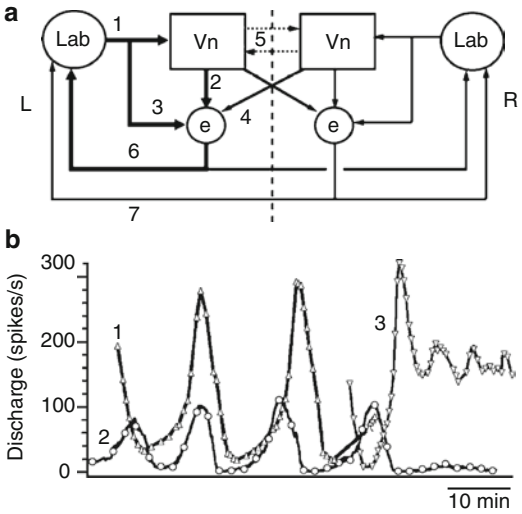

Fig. 6.17 (**a**) A bilaterally symmetric model of central efferent vestibular pathways. *Bold lines* indicate a positive-feedback loop. Labyrinth (*Lab*) sends direct projections (*3*) to ipsilateral efferent group (*e*) and indirect projections via the ipsilateral (*1*, *2*) and contralateral (*4*) vestibular nuclei (*Vn*). The Vn on the two sides are interconnected by inhibitory fibers (*5*). All other connections are excitatory. Efferent neurons to each labyrinth come from the ipsilateral (*6*) and contralateral (*7*) efferent group. (Adapted from Plotnik et al. 2005, with permission from the Association for Research in Otolaryngology.) (**b**) Large fluctuations in the background discharge of three irregular units (otolith afferents (*1* and *2*); horizontal-canal afferent (*3*) with animal stationary in the horizontal plane (Adapted from Plotnik et al. 2005, with permission from the Association for Research in Otolaryngology.)

typically, the oscillations have peak-to-peak amplitudes of 50–100 spikes/s (Fig. 6.17b2) and may be damped (Fig. 6.17b3). In all cases the fluctuations have a period lasting several minutes. Such fluctuations are confined to irregular units in decerebrates and are not seen in either regular or irregular units in anesthetized or alert preparations. Several lines of evidence suggest that the fluctuations are efferent mediated, of which the two most salient are the fact that (1) they are abolished when the vestibular nerve is cut central to the recording site and (2) the presence of a positive correlation between fluctuation amplitude and the size of type III, efferent-mediated rotation responses. A possible explanation for the fluctuations is provided by the positive feedback loops between efferents and afferents (Fig. 6.17a, thick lines): afferents and efferents are mutually excitatory. A theoretical model indicates that such a positive feedback loop could give rise to the observed periodic fluctuations (Plotnik et al. 2005). The excitation provided by efferents could also explain another finding, viz., the increase in background discharge of decerebrate, as compared to anesthetized, animals (Perachio and Correia 1983; Plotnik et al. 2005). Once again, the effect is targeted to irregular afferents, even those not showing large fluctuations in background discharge.

There can be no question that the fluctuations are an artifact of decerebration since they are not seen in alert, behaving animals (Keller 1976; Louie and Kimm

1976; Sadeghi et al. 2007, 2009). Presumably the decerebration releases the efferent cell groups from a descending tonic inhibition. Even though the fluctuations are an artifact, they show that positive feedback loops involving efferents can have a powerful impact on afferent discharge and that modification of these loops by other systems could provide a novel mode of efferent control.

6.10.3 Possible Functions of the EVS

The functions of efferent modulation have not been clearly established in any vertebrate, including mammals. One reason for a lack of progress may relate to the properties of the peripheral efferent synapse. Large responses of mammalian afferents to electrical stimulation of the EVS require several closely spaced shocks. Since single shocks are relatively ineffective, the implication is that large responses require potentiation at the peripheral efferent synapse, likely attributed to the presynaptic facilitation of neurotransmitter release and/or the amplification of postsynaptic effects. Regardless of the mechanisms involved, the efferent synapse acts as a filter, maximizing the effects of high-frequency bursts of activity in central efferent neurons and minimizing the influence of lower, tonic discharge rates. As a result of such filtering, a sensory stimulus may be quite effective in exciting efferents, yet have only a small or no influence on afferent discharge. This property suggests that recordings from efferent neurons might be more revealing than recordings from afferents. Yet, although there have been several recordings from afferent fibers in alert, behaving mammals (Keller 1976; Louie and Kimm 1976; Lisberger and Pavelko 1986; Cullen and Minor 2002; Sadeghi et al. 2009), the only study possibly recording from efferents in mammals was done in decerebrate, decerebellate animals (Marlinsky 1995).

Large efferent-mediated afferent responses require a high rate of efferent discharge, which is likely to occur in bursts associated with active head movements. As a specific hypothesis, it has been supposed that the resulting excitation would serve among other things to prevent the silencing of afferent discharge during rapid head movements in the inhibitory direction (Goldberg and Fernández 1980; Highstein 1991). Afferent recordings in monkeys free to move their heads have been used to test this hypothesis (Cullen and Minor 2002). Contrary to expectations, there were no differences between active and passive head movements. The conclusion can be related to recordings from head-restrained animals (Keller 1976; Louie and Kimm 1976). In the latter studies, it was found that vestibular-nerve discharge was insensitive to eye saccades. When an animal makes a gaze saccade, there is usually a coordinated head and eye movement (Sadeghi et al. 2007). When the head is restrained, a head torque can be measured and indicates that the motor strategy still includes a head movement. So the head-restrained studies imply that efferents are not sufficiently excited by efference-copy signals to affect afferent discharge.

These last results imply that active head movements do not lead to an efferent modulation of afferent discharge in mammals over and above the vestibular stimulation they produce. It remains possible that active head movements play a role in

other vertebrates, specifically in red-eared turtles. Recall that BT/BM units are characterized by much higher rotational gains than BP and CD units (Sect. 6.6.4) (Brichta and Goldberg 2000b). The former units are inhibited by EVS stimulation, whereas the latter units are excited. One implication of the difference in rotational gains is that BT/BM afferents have limited dynamic ranges, responding well to small head movements (<10°/s), but approaching saturation during modest head movements of 20–50°/s. In addition, responses become highly distorted for even modest head velocities. Excited (BP and CD) units, in contrast, have dynamic ranges in excess of 100°/s. Given their responses to head rotations, BT/BM units would seem suited to monitor the small head movements characterizing postural control during quiet standing. BP and CD units, on the other hand, could provide useful information during rapid, voluntary head movements, for example, during prey striking. If one assumes that efferents in turtles fire in anticipation of rapid head movements, the crista could be switched from a postural to a volitional mode. It must be emphasized that the suggestion is speculative, as there have been no observations of either afferent or efferent discharge in behaving turtles. Nor would the scheme work in the oyster toadfish because the high-gain afferents, which might function in postural control, are excited, rather than inhibited by efferents (Sect. 6.6.2) (Boyle and Highstein 1990b; Boyle et al. 1991).

Most speculations about efferent function have focused on the rapid modification of afferent discharge. One theme that emerges from studies in mammals and in other vertebrates is that any such actions are likely to be nonspecific, affecting afferent discharge in several vestibular organs similarly. This theme is exemplified in lower vertebrates by the anatomical organization of both central and peripheral efferent pathways. A similar conclusion in mammals is suggested by the fact that efferent-mediated afferent responses from all organs are similar for head rotations in either direction and in several canal planes (Plotnik et al. 2002).

In addition to rapid modifications of afferent discharge, it is quite possible that the EVS could also serve a slower, modulatory role, shaping the function of the peripheral organs during development and/or during adult life. An indication of this possibility is the presence of slow responses, which suggests that some efferent actions are mediated by metabotropic, rather than by ionotropic, receptors (see Sect. 6.8.4). Of particular interest in this regard are the efferent responses of regularly discharging afferents. These are quite slow and quite small. Were the only function of the efferents to modify spike discharge on a relatively fast time scale, it would be difficult to understand why the peripheral/extrastriolar zones of the cristae and maculae, where regular fibers reside (Baird et al. 1988; Goldberg et al. 1990a), receive as rich or richer an efferent innervation as central/striolar zones (Nomura et al. 1965; Lysakowski and Goldberg 1997; Purcell and Perachio 1997).

A possible target of efferent modulation relates to the balance between the vestibular organs on the two sides. Such a balance is important functionally. When the activity of one labyrinth is depressed, for example, by some pathological process, it is necessary to redress or compensate for the imbalance. Much of compensation involves central mechanisms that rebalance the activity of the two vestibular nuclei (Curthoys and Halmagyi 1995). But because of the bilateral organization of the efferent pathways (Figs. 6.6 and 6.17a), the activity of one labyrinth can influence

that of the other labyrinth. From this relationship, it is conceivable that some of the compensation could involve an efferent modification of afferent activity. This hypothesis has been investigated in behaving monkeys by recording from one vestibular nerve after destruction of the contralateral labyrinth. Remarkably, no obvious change in overall activity was detected (Sadeghi et al. 2007).

6.11 Summary

This chapter summarizes our current knowledge of the EVS, including its neuroanatomical organization, the responses of afferents to electrical stimulation of the EVS, the extent to which these responses are the result of actions on hair cells or afferent terminals, the neurotransmitters and neurotransmitter receptors involved, and the efferent modulation of afferent responses to natural stimulation. While studies of mammals were emphasized, nonmammalian vertebrates were also considered. When compared to other hair-cell systems, the peripheral actions of the EVS are unusually diverse and involve excitation and inhibition targeted to hair cells and afferent terminals. Both ionotropic and metabotropic receptors participate.

Several questions remain, of which two may be mentioned here. (1) ACh is a neurotransmitter at efferent synapses. Other transmitters, including CGRP, ATP, and nitric oxide, may be colocalized with ACh in the same efferent terminals. The physiological effects of these agents remain to be determined, as does the role of various cholinergic receptors. (2) To explore possible functions of the EVS, the responses of efferents and the efferent modification of afferent discharge have been studied. Despite this effort, the functions of the EVS have remained elusive. It is suggested that a promising approach to a study of function would be to record from efferent neurons under a variety of behavioral circumstances.

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