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Commissural Inputs to Secondary Vestibular Neurons in Alert Cats After Canal Plugs

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Farrow, Karl and Dianne M. Broussard. Commissural inputs to secondary vestibular neurons in alert cats after canal plugs. *J Neurophysiol* 89: 3351–3353, 2003; 10.1152/jn.01060.2002. Gaze is stabilized during head movements primarily by the vestibuloocular reflex (VOR). After a unilateral canal plug, the VOR's response is reduced. Recovery of the VOR may be brought about by changes in the efficacy of brain stem synapses or by other mechanisms. We measured the responses of horizontal secondary vestibular neurons (HSNs) to stimulation of the contralateral labyrinth. HSN responses in normal alert cats were compared with those in cats that had recovered from unilateral horizontal semicircular canal (HSCC) plugs. After recovery, excitatory commissural inputs to HSNs on the plugged side elicited significantly smaller responses than in normal cats with no change in mean discharge rates. However, mean discharge rates tended to be higher after recovery for cells receiving inhibitory commissural inputs. The change in resting rate invalidates any direct comparison of inhibitory inputs. These results are interpreted in terms of possible mechanisms for recovery from unilateral vestibular loss by the VOR neural network. We conclude that after unilateral HSCC plugs, changes in brain stem excitatory synapses and/or excitability of secondary vestibular neurons may participate in the restoration of normal vestibular reflexes.

INTRODUCTION

The vestibuloocular reflex (VOR) keeps ocular fixation stable during head movements by rotating the eyes to counteract rotation of the head. The ratio (called VOR gain) of the eye velocity generated by the reflex to the head velocity that drives it is reduced after a unilateral canal plug. The VOR gain increases toward normal over a period of days or weeks after unilateral plug or labyrinthectomy (Courjon et al. 1977; Fetter and Zee 1989; Paige 1983). In the case of an HSCC plug, other changes such as postlesional nystagmus are small or absent (Broussard et al. 1999). The mechanism by which recovery occurs is not known, although it may involve changes in brain stem synapses (Broussard and Hong 2003; Precht et al. 1966). Previous studies have focused on either behavior or electrophysiology in reduced or anesthetized preparations. Our goal was to measure synaptic inputs to behaviorally characterized neurons in alert animals.

For rotation at frequencies <4 Hz, the intact horizontal canal provides the input to the horizontal VOR after recovery from a unilateral plug (Rabbitt et al. 1999; Yakushin et al. 1998). Ipsilesional HSNs recover some sensitivity to horizontal rotation after labyrinthectomy (Hamman and Lannou 1988; Newlands and Perachio 1990), suggesting that their firing rates can be modulated by the contralateral labyrinth. A disynaptic inhibitory pathway links HSNs on the two sides of the brain stem (Shimazu and Precht 1966), and some HSNs that receive inhibitory input from the flocculus and/or ventral paraflocculus also receive excitatory input via a polysynaptic pathway from the contralateral labyrinth (Broussard and Lisberger 1992). Changes in the commissural pathways could contribute to recovery from canal plugs. To determine whether neuronal responses to electrical stimulation of the contralateral labyrinth were modified after recovery of the VOR from a unilateral HSCC plug, we compared the responses in recovered and normal cats.

METHODS

Seven alert, young adult cats (12–24 mo old) were used in this study. Methods for training cats, monitoring eye position, implanting head holders and search coils, and plugging one HSCC have been described in detail (Broussard et al. 1999). Silver wire stimulating electrodes were implanted bilaterally in the perilymph between the oval and round windows.

HSNs were recorded in normal animals and between 80 days and 8 mo postplug in the recovered animals. A cylinder was implanted stereotaxically and a microelectrode advanced into the vestibular nuclei, while the cat was rotated around a vertical axis with its head pitched 22° nose down from the stereotaxic position. When a cell responding to rotation was isolated, rotation was continued in total darkness and spike times were recorded at 22° nose-down and 5° nose-up. Neurons whose response to rotation increased, or changed polarity, in the nose-up position were also invariably type II in the nose-up position, suggesting that they were driven by the ipsilateral vertical canals. These cells were excluded from our sample.

To measure synaptic inputs, biphasic current pulses 0.2 ms in duration were delivered to each labyrinth. The stimulus current that evoked the maximum eye movement response (1–2.4 mA) was used to identify secondary neurons. The cell's response to contralateral stimulation was measured as described in an earlier paper (Broussard et al. 1995). Briefly, contralateral stimulation was applied at half the maximum current (500–1,200 μA). Latencies to each action potential that occurred in 100–150 sweeps were used to construct a cumulative probability plot. The evoked change in probability of firing was evaluated by subtracting the extrapolated value of the baseline from the cumulative probability at the end of the response.

For rotation and fixation, spike densities were calculated. 10–30 cycles of rotation were averaged and fit with a 1-Hz sinusoid. Mean firing rates were measured during the steady state of rotation. For

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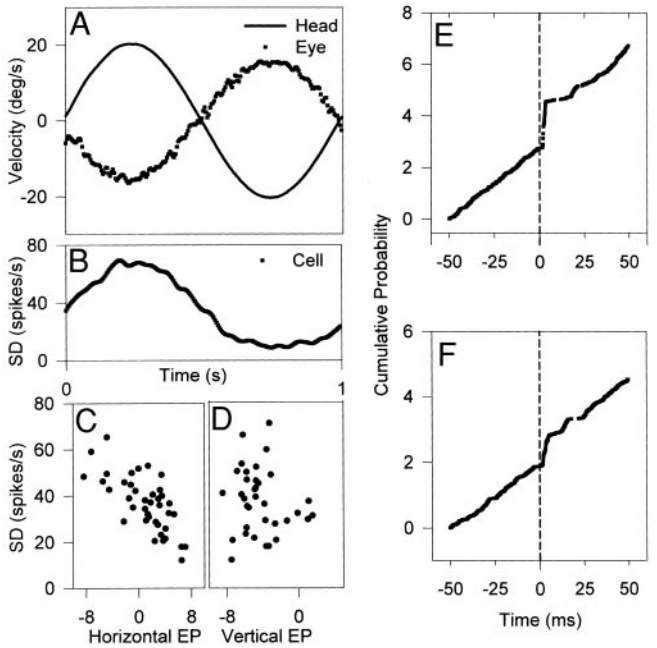

FIG. 1. Responses of a horizontal secondary vestibular neuron (HSN) in the left medial vestibular nucleus of *cat J*. SD, spike density. *A*: vestibuloocular refles (VOR) in darkness. *B*: mean spike density during the VOR. *C* and *D*: spike density during steady fixation at different horizontal and vertical eye positions. *E*: cumulative probability of firing during stimulation of the ipsilateral labyrinth. Current pulses were applied at $t = 0$. *F*: response to stimulation of the contralateral labyrinth.

eye-position sensitivity, spike density was averaged over periods (≥200 ms) of steady gaze in ambient light. For some cells, spike density was sampled at head velocity zero-crossings during rotation and correlated with eye position.

Histology was performed on five of our cats, and recording sites were located using marking lesions for 18 pre- and all postplug HSNs. Most HSNs (≥13 preplug and 11 postplug) were in the lateral part of the medial vestibular nucleus (MVN), 1.0–2.0 mm caudal to the abducens nucleus. The remaining recording sites were found in the rostral MVN, ventrolateral vestibular nucleus (VLVN), or superior vestibular nucleus (SVN).

RESULTS

We characterized the responses of 22 HSNs in five normal cats and 16 HSNs in three cats that had recovered from unilateral HSCC plugs. Recovered cats had VOR gains, measured at 1 Hz in darkness, between 0.53 and 0.66, averaging 75% of the gain in normal cats. All of the HSNs in our sample responded to stimulation of the contralateral labyrinth. The identification of secondary neurons was based on the latency of the response to ipsilateral stimulation. Neurons activated ≤1.4 ms after labyrinthine stimulation in the cat are thought to be monosynaptically activated from the ipsilateral labyrinth (Kasahara and Uchino 1974; Sato et al. 2002). This interpretation is consistent with the distribution of latencies in the current data. Neurons were included in our sample only if activated by ipsilateral stimulation at a latency of ≤1.4 ms.

Figure 1 illustrates the responses of an HSN from the medial vestibular nucleus of a normal cat. The neuron showed increased firing for contralateral rotation and was therefore classified as type II (Fig. 1. *A* and *B*). It was sensitive to eye position (Fig. 1, *C* and *D*). Stimulation of the ipsilateral labyrinth increased the probability of firing by 1.58 (Fig. 1*E*); contralateral stimulation increased the probability by 0.62 (Fig. 1*F*).

All HSNs showed some change in probability of firing after contralateral stimulation. HSNs that received inhibitory commissural inputs were likely to be type I, increasing their discharge rates for ipsilateral rotation (17 of 18 neurons). Neurons receiving excitatory commissural inputs were more evenly divided between type I (11 neurons) and type II (9). Most HSNs also carried eye position signals with either ipsi- or contralateral on directions, which presumably contributed to their responses during the VOR.

Excitatory responses to contralateral stimulation tended to be smaller in cats that had recovered from HSCC plugs. After recovery, the mean evoked increase in probability of firing for excitatory inputs was 0.099 ± 0.070 (mean ± SD, $n = 9$; Fig. 2, *E*, *F*, and *I*), compared with 0.38 ± 0.20 ($n = 9$) in normal cats (Fig. 2, *A*, *B*, and *I*). The difference was significant ($P = 0.0019$, Wilcoxon rank sum). Distributions of evoked increases and mean discharge rates are shown in Fig. 2, *B* and *F*. Mean discharge rates for these cells, averaged over all cycles of rotation, were 32 spikes/s in normal and 37 spikes/s in recovered cats (Fig. 2*I*); the difference was not significant ($P = 0.46$, *t*-test; $P = 0.28$, Wilcoxon).

The mean efficacy of the inhibitory inputs from the contralat-

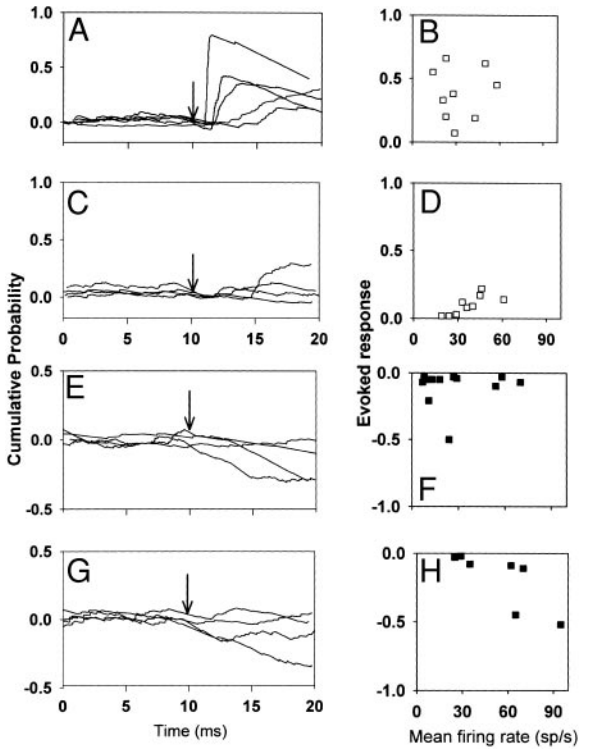

FIG. 2. Excitatory and inhibitory commissural inputs and resting rates of HSNs. *A*: examples of excitatory responses to commissural input in normal cats; the resting rate has been subtracted from each trace. Four or 5 examples are shown that span the range of recorded responses for each case. ↓, stimuli presentations. *B*: summary plot of commissural input as a function of mean firing rate for all excitatory responses in normal cats. *C*: examples of excitatory responses after recovery from horizontal semicircular canal (HSCC) plugs. *D*: summary of excitatory responses and mean firing rates after HSCC plugs. After recovery, excitatory responses were significantly smaller than in normals. *E* and *F*: examples and summary for inhibitory responses in normal cats. *G* and *H*: inhibitory responses in plugged cats. After recovery, cells with inhibitory responses had significantly higher discharge rates than in normals.

eral labyrinth increased from −0.104 ± 0.129 (n = 13) in healthy animals (Fig. 2, *C*, *D*, and *J*) to −0.18 ± 0.21 (n = 7) in canal-plugged animals (Fig. 2, *G*, *H*, and *J*). This difference was not significant (P = 0.38, Wilcoxon). However, the mean discharge rate of neurons receiving inhibitory input was 54 spikes/s in recovered animals, which was significantly higher than 26 spikes/s measured in normals (P = 0.017, t-test; P = 0.012, Wilcoxon).

DISCUSSION

Stimulating the contralateral labyrinth in alert cats increased the probability of firing in some HSNs as has been reported in alert monkeys (Broussard and Lisberger 1992). Excitatory signals from the contralateral HSCC arriving at HSNs would be of the wrong polarity to drive the horizontal VOR. Although it is possible that the excitatory inputs arose from the other semicircular canals or otoliths, such convergence is rare among oculomotor HSNs in the cat medial vestibular nucleus (Sato et al. 2002; Zhang et al. 2001). HSNs receiving excitatory inputs either were type II after a plug or expressed eye-movement signals that could generate a type I response. We have proposed that type II HSNs oppose type I HSNs at the motoneuron level and that relative changes in their inputs can modify VOR gain adaptively (Broussard and Lisberger 1992). If so, a decrease in the excitatory response to stimulation of the contralateral labyrinth, such as we observed, would be expected to contribute to the recovery of the VOR. In fact, a decrease was predicted by a recent model of recovery from unilateral labyrinthectomy (Galiana et al. 2001). Our failure to measure any change in spontaneous discharge rates in cells receiving excitatory inputs suggests that the decrease is due to reduced synaptic efficacy rather than reduced excitability of the postsynaptic neuron. The modified synapse(s) could be located on the soma or dendrites of the recorded neuron and/or upstream, for example on a commissural neuron.

The postplug increase in mean firing rates of postsynaptic neurons in our study could be responsible for the slightly larger values we obtained for inhibitory responses after recovery. Because discharge rates cannot be negative, measurements of inhibitory synaptic inputs that use probabilities of firing depend critically on the resting discharge rate of a neuron (for example, see Broussard et al. 1995). We conclude that the mean firing rates of the type I ipsilesional neurons receiving inhibitory inputs increased after a plug, but we can draw no conclusions regarding possible changes in the inhibitory commissural inputs themselves. Changes in the inhibitory commissural pathway, including either increased or decreased inhibitory responses to commissural inputs (Dieringer and Precht 1977; Graham and Dutia 2001), as well as increased intrinsic excitability on the ipsilesional side (Cameron and Dutia 1997), have been reported after hemilabyrinthectomy. Increased excitability is presumed to aid in compensating for a bilateral imbalance in discharge rates, which would not be expected to occur after a plug. However, the change in intrinsic excitability is due in part to an increase in glucocorticoid levels (Cameron and Dutia 1999). Activation of the hypothalamic-pituitary axis may also occur after canal plugs, possibly contributing to the differences between recovery from canal plugs and motor learning in the VOR of normal animals (Broussard and Hong 2003). Dutia and co-workers have shown that the increased excitability occurs primarily in type B neurons, which they suggest are type I neurons (Him and Dutia 2001). Our data add the new observation that increases in resting rate occur in secondary neurons that receive inhibitory commissural inputs and that we have shown to be type I.

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