Unilateral Labyrinthectomy Modifies the Membrane Properties of Contralateral Vestibular Neurons

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Beraneck, Mathieu, Erwin Idoux, Atsuhiko Uno, Pierre-Paul Vidal, Lee E. Moore, and Nicolas Vibert. Unilateral labyrinthectomy modifies the membrane properties of contralateral vestibular neurons. J Neurophysiol 92: 1668–1684, 2004. First published May 12, 2004; 10.1152/jn.00158.2004. Vestibular compensation after a unilateral labyrinthectomy leads to nearly complete disappearance of the static symptoms triggered by the lesion. However, the dynamic vestibular reflexes associated with head movements remain impaired. Because the contralateral labyrinth plays a prominent role in the generation of these dynamic responses, intracellular recordings of contralateral medial vestibular nucleus neurons (MVNn) were done after 1 mo of compensation. Their firing properties and cell type were characterized at rest, and their response dynamics investigated using step, ramp, and sinusoidal current stimulations. The sensitivity of the contralateral MVNn firing rates to applied current was increased, which, along with increased phase leads, suggests that significant changes in active conductances occurred. We found an increased proportion of the phasic type B neurons relative to the tonic type A neurons in the contralateral MVN. In addition, the remaining contralateral type A MVNn response dynamics tended to approach those of type B MVNn. Thus the contralateral MVNn in general showed more phasic response dynamics than those of control MVNn. Altogether, the firing properties of MVNn are differentially modified on the ipsilesional and contralateral sides of the brain stem 1 mo after unilateral labyrinthectomy. Ipsilesional MVNn acquire more “type A–like” tonic membrane properties, which would contribute to the stabilization of the spontaneous activity that recovers in the deafened neurons during vestibular compensation. The bilateral increase in the sensitivity of MVNn and the acquisition of more “B-like” phasic membrane properties by contralateral MVNn should promote the restoration of the vestibular reflexes generated by the remaining, contralateral labyrinth.

INTRODUCTION

Vestibular compensation is defined as the regression of the oculomotor and postural syndrome that occurs after destruction of one labyrinth (Curthoys 2000; Darlington and Smith 1996; Dieringer 1995; Smith and Curthoys 1989). This syndrome includes a spontaneous ocular nystagmus and massive postural distortions observed in the absence of movement, and abnormalities of the vestibulo-ocular and vestibulo-spinal synergies observed during head movements. Whereas the static disturbances disappear in a few days in most vertebrate species, the dynamic reflexes remain impaired indefinitely. They improve over several weeks, but this recovery is limited to low acceleration and the low to middle frequency range of head movements, both in animals (Broussard et al. 1999b; Gilchrist et al. 1998; Lasker et al. 2000; Murai et al. 2003; Vibert et al. 1993) and humans (Allum and Ledin 1999; Brandt 2000; Curthoys and Halmagyi 1999).

The vestibular system works bilaterally, using sensory information from both labyrinths simultaneously for gaze and posture stabilization. Most subdivisions of the vestibular nuclei are linked by commissural fibers that interconnect the 2 vestibular complexes located on each side of the brain stem through monosynaptic or disynaptic pathways. Commissural connections are particularly extensive at the level of the medial and superior vestibular nucleus (Epema et al. 1988; Gacek 1978; Ito et al. 1985; Newlands et al. 1989). These commissural pathways are predominantly inhibitory but also include excitatory connections (Babalian et al. 1997; Highstein et al. 1987). The role of the contralateral, intact labyrinth and of commissural pathways in the rapid disappearance of the static symptoms triggered by unilateral labyrinthectomy is controversial (Cartwright and Curthoys 1996; Graham and Dutia 2001; see Curthoys and Halmagyi 1999; Dieringer 1995; Smith and Curthoys 1989). In contrast, the contralateral labyrinth is obviously essential for the recovery of the dynamic vestibular reflexes. Indeed, it provides all the remaining sensory vestibular information related to linear and angular head movements.

Few in vivo data are available on how the contralateral vestibular neurons are modified after unilateral labyrinthectomy, but they all suggest that substantial adaptive mechanisms are triggered during vestibular compensation. Immediately after the lesion, the spontaneous discharge of contralateral MVNn recorded in awake guinea pigs increases (Ris and Godaux 1998). This is probably attributable to the loss of the commissural inhibition normally provided by the ipsilesional MVNn, which are deprived of their vestibular excitatory drive (Curthoys and Halmagyi 1995; Darlington and Smith 1996; Dieringer 1995; Smith and Curthoys 1989). The spontaneous discharge of contralateral MVNn returns to normal within 1 wk. After several months, the contralateral vestibular neurons show structural changes (Gacek et al. 1998). In patients, caloric stimulation of the intact labyrinth reveals first a reduction of the contralateral, whereby normal values in about 1 yr, which afterward show structural changes (Gacek et al. 1998). In patients, caloric stimulation of the intact labyrinth reveals first a reduction of the contralateral, whereby normal values in about 1 yr, which afterward show structural changes (Gacek et al. 1998). In patients, caloric stimulation of the intact labyrinth reveals first a reduction of the contralateral, whereby normal values in about 1 yr, which afterward show structural changes (Gacek et al. 1998).
Intracellular recordings in brain stem slices led to the identification of 2 types of MVN neurons, type A and type B neurons, based primarily on different action potential profiles (Gallagher et al. 1985; Johnston et al. 1994; Serafin et al. 1991a,b). Previous studies (Babalian et al. 1997; Ris et al. 2001) have shown that type A MVN neurons correspond to the tonic, regular vestibular neurons identified in vivo, and have a more stable spontaneous activity and more linear response dynamics. In contrast, type B MVN neurons correspond to the phasic, irregular vestibular neurons identified in vivo, and show more irregular spontaneous activity and a higher sensitivity to applied current. Recently, we showed that 1 mo after a unilateral labyrinthectomy, the intrinsic firing properties of ipsilesional MVN neurons were greatly modified (Beraneck et al. 2003a). The ipsilesional neurons were depolarized by 6–10 mV, and the static and dynamic membrane properties of type B neurons were more similar to those of type A neurons than in control slices. Altogether, the neurons on the ipsilesional side showed a more tonic behavior, which should increase the stability and regularity of the resting discharge that is restored in these MVN neurons during vestibular compensation.

In view of the prominent role of the contralateral labyrinth in the generation of vestibular synergies after unilateral labyrinthectomy, additional experiments were done on the static and dynamic membrane properties of contralateral MVN neurons after 1 mo of compensation. Statistical comparisons were made with identical measurements obtained on slices from normal animals, and on the ipsilesional side of slices taken 1 mo after unilateral labyrinthectomy (Beraneck et al. 2003a; Ris et al. 2001). Some of these data were previously published in abstract form (Beraneck et al. 2003b).

METHODS

Animals and surgical procedures

Experiments were carried out on pigmented guinea pigs of both genders (Elevage de la Garenne, Saint-Pierre d’Exideuil, France). The animals were handled in accordance with the European Communities Council Directive of November 24, 1986, and followed the procedures issued by the French Ministère de l’Agriculture.

Unilateral labyrinthectomies were performed under halothane anesthesia as described in Vibert et al. (1999a,b). Guinea pigs were allowed to compensate in a normal visual environment until their brain was removed after about 1 mo of compensation (mean of 32 days; range: 27 to 41 days) to prepare the slices. The 28 animals used in this experiment were 8–10 wk of age, and their weight ranged from 250 to 450 g (mean about 350 g). These parameters were similar to those of the animals used in our previous experiments (Beraneck et al. 2003a).

Intracellular electrophysiological recordings

After nembutal (pentobarbital) anesthesia, thick (500 μm), coronal brain stem slices were cut from previously labyrinthectomized animals and maintained using standard techniques (Gallagher et al. 1985; Serafin et al. 1991a; Vibert et al. 1999b). In most experiments, a cold (4°C) sucrose-containing artificial cerebrospinal fluid (sucrose ACSF) was used during the dissection and preparation of the slices (Devor et al. 2001; for details see Uno et al. 2003). During the recordings, however, the slices were superfused with normal ACSF maintained at 31–32°C (Serafin et al. 1991a; Vibert et al. 1999b). Use of the sucrose solution during the preparation of the slices increased the number of viable neurons recorded with microelectrodes. Because we obtained a few contralateral neurons (9 out of 74) without using the sucrose ACSF, we verified that neither their static nor their dynamic membrane properties were different from those of the contralateral MVN neurons obtained using the sucrose ACSF (see Uno et al. 2003).

Intracellular electrophysiological recordings were obtained with sharp, 3 M potassium acetate-containing glass microelectrodes from neurons within the contralateral medial vestibular nucleus (MVN). As in Beraneck et al. (2003a), recordings were restricted to the two 500-μm coronal slices corresponding to the middle third of the guinea pig MVN, at the level of the cerebellar peduncles. A few cells (<10%) were recorded in more caudal slices taken from the last third of the MVN. About 20% of the experiments were performed blind to the recording side to check for any experimenter-linked bias. It is likely that a large majority (>80%) of the MVN neurons recorded in slices were second-order vestibular neurons, given that 80–85% of the central vestibular neurons recorded in the MVN area of the isolated whole brain of guinea pig with similar electrodes were identified as second-order neurons (Babalian et al. 1997).

All measurements were done with an Axoclamp 2A system (Axon Instruments, Union City, CA) in the bridge mode, current-clamp configuration. The electrode resistance varied from 80 to 150 MΩ. Both series resistance (bridge balance) and capacitance compensation were checked throughout the recording of each individual neuron (Ris et al. 2001). The current stimulation and data acquisition were done with a PC-compatible computer using the Acquis 1 program (version 4.0, Bio-logic S.A., Gif-sur-Yvette, France) or MATLAB 6.5 (The MathWorks, Natick, MA). The data were analyzed using program scripts with Mathematica 4.0 (Wolfram Research, Champaign, IL) or MATLAB 6.5 (The MathWorks).

Basic membrane and firing properties of MVN neurons

Because most MVN neurons are spontaneously active on slices, the membrane potential was filtered with a 1-Hz low-pass filter to obtain an estimate of a “mean resting membrane potential” for each neuron. As in Beraneck et al. (2003a), all cells that had a resting potential more negative than −50 mV and a spike amplitude >50 mV were analyzed. We also analyzed the MVN neurons whose membrane potential ranged from −50 to −40 mV if their spike amplitude was 50 mV or more, with a normal spike width ranging from 0.7 to 2.2 ms (see Beraneck et al. 2003a).

Recordings of the neurons at rest were used to determine their mean spontaneous firing rate, the coefficient of variation (CV), and the amplitude of the spike. For each neuron, an average of the spike shape was used to determine the amplitude of the afterhyperpolarization (AHP) and the width of the spike at threshold (see Beraneck et al. 2003a).

The cell’s firing threshold (i.e., the membrane potential for which the cell begins to fire action potentials) was measured as the potential reached by the neuron at the threshold of the first spike triggered by a slow, depolarizing current ramp (0.06 nA/s). In each cell, the presence and duration of long-lasting, subthreshold plateau potentials were determined. These plateau potentials were elicited by low-amplitude (0.1–0.2 nA), short-duration (10 ms) current pulses, while the neurons were hyperpolarized just below their firing threshold (Babalian et al. 1997; Serafin et al. 1991a,b).

Quantitative determination of the neuronal type

In previous publications, MVN neurons were classified as type A, B, B+LTS, and C neurons using only qualitative criteria. To assess reliably the long-term effects of unilateral labyrinthectomy on ipsi...
and contralosional MVNn, quantitative, objective criteria were developed to classify intracellularly recorded MVN neurons as briefly explained below (for a complete description see Beraneck et al. 2003a).

The first derivative of the averaged spike profile of each neuron was used to assess (in V/s) the A-like rectification and double AHP, the 2 main criteria previously used for the qualitative classification. All MVNn showing an A-like rectification with an amplitude <0.15 V/s were classified as type B neurons. The type B + LTS MVNn formed a subtype of these type B neurons showing low-threshold calcium spikes when released from a strong hyperpolarization. The MVNn that had an A-like rectification stronger than 0.15 V/s and no double AHP were classified as type A neurons. The few MVNn that showed both a double AHP and an A-like rectification >0.15 V/s were considered as type C neurons.

These criteria were previously used by Beraneck et al. (2003a) to classify and compare the neurons recorded on slices taken from normal animals (control slices), and on the ipsilesional side of slices taken from animals labyrinthectomized 1 mo before. The MVNn recorded in control slices included 47% type A neurons, 50% type B neurons (of which 9% were type B + LTS neurons), and 3% type C neurons.

Measurement of the input resistance of MVNn using current steps

The passive input resistance of each neuron was assessed using a series of hyperpolarizing current steps (1-s duration) of decreasing amplitudes. The cell was maintained under a steady-state hyperpolarization at a few mV (0–10) below its threshold for discharge. The whole cell resistance for each MVNn (input resistance = voltage deflection/current input) was estimated from the final steady-state amplitude of the hyperpolarizing steps.

Stimulation with depolarizing ramp currents

Increasing ramp currents of 0.3 nA amplitude were applied at 5 different slopes up to a final steady-state value, leading to a proportionate increase in the firing rate above the resting spontaneous activity (for details see Beraneck et al. 2003a). The slope of increase of the instantaneous firing rate of the cell (kF, in spikes/s · s−1 · nA−1) was estimated during the depolarizing, ramplike portion of the applied current (Fig. 1A). The difference between the firing rate reached at the end of the applied depolarizing current and the final, stable discharge rate reached at the end of the plateau was measured as an overshoot in spikes/s (Fig. 1A). To assess how the level of polarization influenced the responses, the whole sequence of ramp stimulations was repeated from a hyperpolarized level of about 10 mV below the firing threshold. Of the 5 ramps applied to each cell, the 600-ms ramps (slope of 1.5 nA/s) gave the most significant results and were taken as the main indices of the response of MVNn to ramplike currents.

Sinusoidal current injections

A third series of stimuli consisted of constant sine waves applied for 5,000 ms at frequencies ranging from 0.2 to 50 Hz (du Lac and Lisberger 1995; for details see Ris et al. 2001). The amplitude of the stimulus (∆I, Fig. 1B) was adjusted to keep the membrane potential variation around 10 mV peak to peak. The sinusoidal currents applied in the presence of resting spontaneous activity led to a significant modulation of the firing rate. For each frequency of stimulation below or equal to about one third of the neuron’s resting discharge, the modulation of the instantaneous firing rate (IF) of MVNn was fitted with a sine wave that was used to calculate the amplitude and phase of the IF modulation (∆IF, Fig. 1B). When the frequency of stimulation passed a third of the neuron’s firing rate, the amplitude of the IF modulation was calculated empirically as the difference between the minimum and maximum IF reached by the neuron during the stimulation. No phase measurements were made in this situation. The underlying membrane potential excursion (∆V) was computed by a Fourier analysis of the total membrane potential response (Fig. 1B). The magnitude of the Fourier component corresponding to the stimulation frequency was taken as the potential response: ∆IF and ∆I were used to evaluate at 0.4 Hz the cell sensitivity to current injection by dividing ∆IF/IF by the amplitude of the injected current (IF/IF in spikes/s · s−1 · nA−1). The sensitivity of the firing rate of the cell to variations of the mean membrane potential ∆IF/∆V was also measured, in spikes · s−1 · mV−1. The “active” impedance Z of the cell was calculated as the amplitude of the membrane potential change obtained for the 0.4-Hz stimulus divided by the amplitude of the injected current (ΔV/ΔI in MΩ). In general, a spike rate transfer function can be defined for each neuron as the ratio ∆IF/∆I versus the stimulation frequency. In most experiments, a similar series of sinusoidal stimuli was given while the cell was maintained at a depolarized membrane potential by a steady-state current stimulation of 0.15–0.25 nA.

Most of the cells were also submitted to the same series of sinusoidal current injections while they were maintained at 10 to 20 mV below their discharge threshold, so that no spike was evoked by the stimulation. The amplitude and phase of the membrane potential change (∆Vh) was computed for each frequency, and the responses to the 0.4-Hz stimulus was used to evaluate the impedance Zh of the cell maintained under a steady-state hyperpolarization (Zh = ∆Vh/ΔI in MΩ). An impedance transfer function can be defined for each neuron as the ratio ∆Vh/∆I versus the stimulation frequency.

For each MVNn, the modulation of the firing rate (i.e., the amplitude of the spike rate transfer function) increased with stimulation frequency to reach a maximum at the peak frequency of resonance. Then, the modulation progressively decreased to lower levels. The “amplitude” of the resonance was defined as the ratio between the amplitude of the firing rate modulation at the peak frequency of resonance and the amplitude obtained at the lowest frequency (0.2 Hz). When the neurons were hyperpolarized to suppress action potentials, the amplitude of the resonance was measured for the membrane potential response. Both the impedance and spike rate transfer functions showed a small phase lead with respect to the injected current at the lowest frequencies of stimulation, which decreased to zero and became a phase lag at higher frequencies (Beraneck et al. 2003a). For all MVNn, we could therefore define as the “zero-crossing frequency” the lowest frequency for which a phase lag was measured.

Statistical analysis

Data presented in this study were obtained from a database of 74 MVNn recorded on the contralosional side of slices taken from animals labyrinthectomized about 1 mo before (contralosional neurons). All mean values are presented with their SD. Statistical analysis was carried out using the Systat 8.0 software (SPSS, Chicago, IL). For each parameter, normality of the distributions was assessed using one-sample Kolmogorov–Smirnov tests, with significance set at P < 0.05. Statistical comparisons were achieved through either parametric (for normal distributions including a minimum of 15 samples) or otherwise nonparametric tests, with the threshold for significance set at P < 0.05. Type B + LTS neurons were pooled together with the other B neurons for analysis. ANOVA or the nonparametric Kruskal–Wallis ANOVA was first performed to search for differences between the average values obtained for type A and B neurons recorded in control slices, and on both sides of slices taken from labyrinthectomized animals (which define different distributions from normal distribution in terms of controllability). The comparison between the 2 × 2 between the cell groups were then performed using Student’s t-test or the nonparametric Mann–Whitney U-tests. Paired parametric (ANOVA followed by paired t-test) or nonparametric tests (Friedman
ANOVA followed by Wilcoxon signed-rank tests) were used to compare the responses evoked by ramps of different slopes, and to determine how the responses to ramps and sinusoidal currents were modified by the polarization level of the neurons.

RESULTS

Classification of contralesional MVNn based on action potential profiles

As described in METHODS, quantitative criteria were developed to categorize MVNn according to the measure of the A-like rectification and/or double AHP shown by each neuron (see METHODS; Beraneck et al. 2003a). Among the 74 contralesional MVNn we recorded (Fig. 2A), there were 20 type A neurons (27.0%) and 53 type B neurons (71.6%). Four of the type B neurons (7.5%) displayed low-threshold calcium spikes and were B+LTS MVNn. Only 1 contralesional MVNn (1.4%) was a type C neuron.

Compared with control slices, the proportions of the different types of neurons found in the contralesional MVN were significantly modified (Pearson chi-square test, $P = 0.007$, Fig. 2B). Although the number of type C cells stayed very low, there was an increase in the proportion of type B neurons matched by an equivalent decrease in the proportion of type A neurons. The proportion of B+LTS MVNn was not modified compared with control conditions (7.5 vs. 9.5%). Thus the ratio of type A to type B MVNn, which was roughly 1 in control conditions, changed to about 0.4 on the contralesional side after 1 mo of compensation. This contrasts with the ipsilesional side, where the proportion of type A MVNn tended to increase (Fig. 2B).

In addition, the parameters used to categorize MVNn, that is, the A-like rectification and the double AHP (Table 1) also confirmed these opposite changes between the 2 sides after 1 mo of compensation. The average measure of the double AHP of contralesional MVNn was multiplied by more than 2 compared with control MVNn ($P = 0.01$, Fig. 2A). In contrast, the double AHP of ipsilesional MVNn was divided by more than 2 compared with control conditions ($P = 0.01$; Beraneck et al.
2003a). Whereas the double AHP of contralesional MVNn was increased, the average measure of their A-like rectification tended to decrease (Table 1) compared with control MVNn ($P < 0.08$), and was lower than that on the ipsilesional side (0.42 ± 0.58 V/s, $P < 0.02$).

In general, contralesional MVNn compared with control MVNn were characterized by an increased proportion of type B neurons, larger double AHPs and smaller A-like rectifications (Fig. 2). This is the opposite to that observed on the ipsilesional side (Beraneck et al. 2003a), that is, an increased proportion of type A neurons and a smaller double AHP.

**Membrane potential and firing rate of contralesional MVNn**

Contralesional MVNn had a mean resting membrane potential of $-56.3 \pm 5.4$ mV (Table 1) and were slightly but significantly depolarized compared with control MVNn ($-58.7 \pm 8.5$ mV, $P = 0.048$). This change was restricted to type B MVNn, whose resting membrane potential was depolarized by almost 5 mV ($P = 0.004$) compared with control type B neurons. The depolarization of contralesional type B neurons was accompanied by a significant depolarization of their firing threshold ($P = 0.003$, Table 1).

The mean resting discharge displayed by contralesional MVNn (24.8 ± 19.9 spikes/s) was similar to the one of control MVNn ($P = 0.56$, Table 1). Type A and B contralesional MVNn had the same mean resting discharge ($P = 0.68$). The increase in firing threshold that accompanied the depolarization of contralesional type B MVNn was consistent with the lack of an increase in their average firing rate. Interestingly, the distribution of the resting discharges of contralesional MVNn was modified compared with control slices (Fig. 3A). Specifically, 17.4% of the contralesional neurons were silent at rest (Fig.
TABLE 1. Basic membrane and firing properties of control and contralesional MVNn

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All Neurons</th>
<th>Type A</th>
<th>Type B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of neurons</td>
<td>(n = 89)</td>
<td>(n = 42)</td>
<td>(n = 44)</td>
</tr>
<tr>
<td>VM, mV</td>
<td>−58.7 ± 8.5</td>
<td>−56.8 ± 7.3</td>
<td>−60.8 ± 9.4</td>
</tr>
<tr>
<td>FR, spikes/s</td>
<td>26.2 ± 17.2</td>
<td>29.1 ± 20.1</td>
<td>24.3 ± 13.8</td>
</tr>
<tr>
<td>CV, %</td>
<td>8.26 ± 9.48</td>
<td>7.06 ± 9.61</td>
<td>9.51 ± 9.59</td>
</tr>
<tr>
<td>AHP, mV</td>
<td>16.8 ± 4.5</td>
<td>19.6 ± 3.8</td>
<td>14.0 ± 3.4</td>
</tr>
<tr>
<td>Firing threshold, mV</td>
<td>−67.9 ± 10.5</td>
<td>−66.9 ± 10.77</td>
<td>10.7</td>
</tr>
<tr>
<td>Spike width, ms</td>
<td>1.15 ± 0.23</td>
<td>1.25 ± 0.21</td>
<td>1.06 ± 0.22</td>
</tr>
<tr>
<td>Membrane resistance, MΩ</td>
<td>94.6 ± 45.3</td>
<td>93.8 ± 39.9</td>
<td>96.2 ± 54.9</td>
</tr>
<tr>
<td>PNa+, ms</td>
<td>33.5 ± 40.1</td>
<td>15.2 ± 32.9</td>
<td>56.9 ± 38.5</td>
</tr>
<tr>
<td>A-like rect., V/θ</td>
<td>0.36 ± 0.51</td>
<td>0.72 ± 0.54</td>
<td>0.02 ± 0.04</td>
</tr>
<tr>
<td>Double AHP &amp;d(V/dt), V/θ</td>
<td>0.38 ± 0.83</td>
<td>0.38 ± 0.83</td>
<td>0.38 ± 0.83</td>
</tr>
</tbody>
</table>

Values are means ± SD. This table gives the values of the parameters characterizing the membrane and firing properties of all groups of control and contralesional MVNn. Asterisks indicate the values that are significantly different between the contralesional and control neurons. *P < 0.05, **P < 0.01, ***P < 0.001. In addition, the values that correspond to significant differences are shown in bold. The numbers of neurons shown at the top of the table apply to all parameters, except the firing threshold and membrane resistance. VM, resting membrane potential; FR, spontaneous firing rate; CV, coefficient of variation of the spontaneous firing rate; AHP, amplitude of the afterhyperpolarization; PNa+, duration of the subthreshold plateau potentials.

Responses of contralesional MVNn to ramp currents

The basis of the change in the neuronal populations after a labyrinthectomy clearly lies in the intrinsic voltage-dependent conductances of vestibular neurons. Changes in these membrane properties can be demonstrated by probing the neurons with a range of ramp stimuli having different slopes providing low- and high-frequency stimulation. Contralesional type A MVNn showed significantly increased overshoots (Fig. 4A) and slopes of firing rate increase k_f (Fig. 4B) in response to ramps delivered from rest. For the 600-ms ramp, the overshoot of contralesional type A MVNn increased nearly a factor of 3 compared with control type A MVNn (P = 0.01), and the k_f increased by 33% (P = 0.03). Because the overshoots and slopes of contralesional type B MVNn did not increase significantly compared with control type B MVNn (Fig. 4), the significant difference observed between the overshoots of type A and type B MVNn in control slices disappeared. The k_f measured on the 600-ms ramp became greater in type A than in type B contralesional MVNn (P = 0.042), which is the reverse of that observed in control slices where the k_f of type A MVNn was lower than that of type B MVNn.

Interestingly, the slope of firing rate increase k_f of contralesional type A MVNn was higher for the ramps delivered from rest than for those delivered from a hyperpolarized level (Fig. 5). A similar trend was observed for the overshoots, except for the 200-ms ramp. This contrasts with all other groups of MVNn recorded in control slices or on slices taken after 1 mo of compensation, for which the mean overshoots and k_f measured on the ramps delivered from rest always tended to be...
FIG. 3. Spontaneous activity of intracellularly recorded MVNn. A: histograms showing the distributions of the spontaneous firing rates obtained for control (A$_1$) and contralesional (A$_2$) MVNn. B: proportions of silent neurons recorded in control slices, and on the contralesional and ipsilesional sides of slices after 1 mo of vestibular compensation.
smaller (Beraneck et al. 2003a). This reveals a strong and unusual dependency of the sensitivity of contralesional type A MVNn on their membrane potential as revealed by high-amplitude ramp current stimulation (Fig. 5).

As suggested above, the modifications occurring in compensated MVNn appear to modify the voltage dependency of the ion channels responsible for spike discharge. This is illustrated by the lack of significant increases in the firing rate slopes for ramps elicited from hyperpolarized contralesional MVNn (Fig. 4B) compared with normal neurons. Nevertheless, the average overshoot (see Fig. 4A) evoked in contralesional MVNn by the 600-ms ramps delivered from a hyperpolarized level reached 7.4 ± 6.3 spikes/s and was increased compared with control conditions (P = 0.015, Fig. 4A). Because as in control slices, the overshoot of contralesional type B MVNn was greater than that of type A MVNn (P = 0.04, Fig. 4A), this increase was attributed in part to the higher proportion of type B MVNn among contralesional neurons. However, there was also a strong trend for the overshoot of contralesional type B MVNn to be higher than that in control type B MVNn (P = 0.09). In contrast, no significant change was observed for the overshoot elicited by the 200-ms ramp delivered from a hyperpolarized level in either type A or type B contralesional MVNn, possibly because the adaptive changes had reached some maximum value.

The increased overshoot displayed by contralesional MVNn in response to ramps is similar to that observed on the ipsilesional side (Beraneck et al. 2003a). However, the strong and selective increase of the k_{IF} of contralesional type A MVN for the ramps delivered from rest is specific to the contralesional side.

**Impedance functions of contralesional MVNn measured with sinusoidal currents delivered during steady-state hyperpolarization and in the absence of action potentials**

When measured at 0.4 Hz, the impedance of hyperpolarized contralesional MVNn decreased by 31% compared with control MVNn (P = 0.001, Table 2). This decrease was significant for all frequencies of sinusoidal stimulation from 0.2 to 50 Hz (Fig. 6A). This reflected a selective decrease of the impedance of contralesional type B MVNn, which was significantly lower than that of control type B MVNn (Fig. 7B) as well as contralesional type A MVNn over the whole range of frequencies tested (Table 2, Fig. 7). The decreased impedance of contralesional type B MVNn was not associated with any change in the peak frequency or amplitude of the resonance (Table 2). However, there was a selective increase of their zero-crossing frequency (Table 2), which reached a median value of 0.8 Hz instead of 0.3 Hz for control type B MVNn (P = 0.02). In accordance with this increase, contralesional type B MVNn displayed significantly bigger phase leads at low frequencies (P = 0.035 at 0.6 Hz) and smaller phase lags at intermediate frequencies (P = 0.02 at 2 Hz). In contrast, neither the phase nor the amplitude function of contralesional type A MVNn was modified compared with control conditions. These findings suggest that the marked decrease in the impedance of contralesional type B MVNn could be a result of the potentiation of a current activated during hyperpolarization.

The selective decrease in the impedance of contralesional type B MVNn neurons during steady-state hyperpolarization followed the trend previously observed for ipsilesional type B MVNn, but was more pronounced on the contralesional side. The increased zero-crossing frequency and associated phase changes obtained for contralesional type B MVNn were also observed on the ipsilesional side. However, the peak frequency of resonance of contralesional type B MVNn was not modified, contrary to findings on the ipsilesional side.

**Impedance and spike rate transfer functions of contralesional MVNn: amplitude functions**

The active impedance, Zrest, of contralesional MVNn measured at their resting membrane potential (45.8 ± 35.8 MΩ at
0.4 Hz) was decreased by 22% compared with control MVNn (58.5 ± 33.9 MΩ, P = 0.01, Table 3). This decrease was observed at rest for both type A (−30%, P = 0.02) and type B (−24%) contralesional MVNn, in contrast to being restricted to type B neurons at hyperpolarized potentials (Table 3). During steady-state depolarization, the active impedance of contralesional MVNn decreased by 19% compared with control MVNn (but P = 0.13 only, Table 3).

In addition to this general decrease of their active impedance, the sensitivity ΔIF/ΔI of the discharge of contralesional MVNn to applied current increased compared with control MVNn, both at rest and during depolarization. This increase of con...
TABLE 2. Membrane potential responses of MVNn to sinusoidal currents delivered during steady-state hyperpolarization, in the absence of action potentials

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All Neurons</th>
<th>Type A</th>
<th>Type B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Contralesional</td>
<td>Control</td>
</tr>
<tr>
<td>Number of neurons</td>
<td>(n = 24)</td>
<td>(n = 48)**</td>
<td>(n = 13)</td>
</tr>
<tr>
<td>Zm, MΩ</td>
<td>131.2 ± 58.7</td>
<td>90.8 ± 40.5***</td>
<td>127.9 ± 49.6</td>
</tr>
<tr>
<td>Amplitude of resonance</td>
<td>1.18 ± 0.12</td>
<td>1.17 ± 0.17</td>
<td>1.19 ± 0.11</td>
</tr>
<tr>
<td>Median peak f of resonance, Hz</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Zero-crossing, Hz</td>
<td>0.4</td>
<td>0.8</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Values are means ± SD. This table gives the values of the parameters characterizing the responses of contralesional and control MVNn to sinusoidal currents delivered during steady-state hyperpolarization. Asterisks indicate the values that are significantly different between the contralesional and control neurons. *P < 0.05, **P < 0.01, ***P < 0.001. In addition, the values that correspond to significant differences are shown in bold.

the average sensitivity of MVNn to current injection was either significant (P ≤ 0.05) or visible as a strong trend (P values ranging from 0.05 to 0.1) for all frequencies of stimulation between 0.2 and 30 Hz at rest (Fig. 6B1) and between 0.2 and 40 Hz during depolarization (Fig. 6C1). As discussed in methods, this sensitivity is a spike rate transfer function of the MVNn firing rate modulation relative to the current input for the entire range of stimulation frequencies used. As a rule, the sensitivity of the discharge of contralesional MVNn to applied current increased more for type A than type B MVNn, particularly at low frequencies (Table 3, Fig. 7). Indeed, the sensitivity of type A MVNn to applied current was significantly increased for most frequencies between 0.2 and 20 Hz at rest (Fig. 7A2), and between 4 and 50 Hz during depolarization. In contrast, the sensitivity of contralesional type B MVNn was increased only for high frequencies of stimulation ranging from 12 to 20 Hz at rest, and from 12 to 40 Hz during depolarization (Fig. 7B2). Altogether, the significant difference in sensitivity to current that was observed between type A and type B control MVNn (Beraneck et al. 2003a) disappeared.

The increased sensitivity of the discharge of contralesional MVNn (ΔIF/ΔI) to current injection combined with the decrease in their active impedance (ΔV/ΔI) means that there was a strong increase in the sensitivity of their discharge relative to the membrane potential ΔIF/ΔV, both at rest and during depolarization. At 0.4 Hz, ΔIF/ΔV increased by 51% at rest (P = 0.004, Table 3), and by 57% during steady-state depolarization (P = 0.025). This increased sensitivity of contralesional neurons to membrane potential was observed for both types of MVNn (Table 3).

The amplitude of the resonance shown by contralesional MVNn was not modified compared with control values, either at rest or during steady-state depolarization (Table 3), nor was the peak frequency of resonance of contralesional type A MVNn. However, the median peak frequency of resonance of contralesional type B MVNn tended to increase (Table 3), and the significant difference between the peak frequency of resonance of type A and B MVNn observed in control slices (Beraneck et al. 2003a) was no longer present.

Interestingly, data from individual neurons demonstrated that most contralesional MVNn did not show the gradual decrease of the modulation of their firing rate by current injection usually observed for control and ipsilesional MVNn after the peak frequency of resonance (Beraneck et al. 2003a). In contrast, the modulation of spontaneous firing stopped rather abruptly, given that at higher frequencies of stimulation contralesional MVNn tended to synchronize their firing with the depolarizing phase of sinusoidal current injections. This phenomenon, which was previously described for lateral vestibular nucleus neurons (Uno et al. 2003), was observed for both types of contralesional MVNn.

Spike rate transfer functions of contralesional MVNn: phase functions

Just as active conductances can manifest themselves as a resonance in amplitude functions, phase functions show a phase lead at low frequencies, then cross zero near the peak resonant frequency, and finally show a phase lag that increases with higher frequencies. Compared with control MVNn, the phase functions of the firing rate modulation displayed by contralesional type A and type B MVNn were significantly modified. The median zero-crossing frequency of contralesional MVNn reached 3 Hz instead of 1 Hz at rest and 4 Hz instead of 1 Hz under steady-state depolarization (Table 3, P < 0.001 in both cases). Significant phase shifts toward higher phase leads and smaller phase lags were associated with this shift of the zero-crossing frequency. For the whole sample of contralesional MVNn, there was a significant change of the phase function for most frequencies of stimulation between 0.4 and 10 Hz at rest (Fig. 6B1) and for all frequencies between 4 and 20 Hz under steady-state depolarization (Fig. 6C2). The changes in phase functions were more important in type A than in type B MVNn. Indeed, the zero-crossing frequency of contralesional type A MVNn reached 6 Hz instead of 1 Hz in control slices at rest (P = 0.001) and 7 Hz instead of 2 Hz during steady-state depolarization (P = 0.009). As a result, the zero-crossing frequency of contralesional type A MVNn was higher than that of contralesional type B MVNn (Table 3), whereas the phase functions of control type A and type B MVNn were similar. Contralesional type A MVNn also displayed significantly larger phase leads and smaller lags than those of contralesional type B MVNn for frequencies of stimulation between 2 and 20 Hz at rest, and 2 and 30 Hz during depolarization.

Responses of contralesional MVNn to sinusoidal currents delivered in the presence of action potentials: comparison with the ipsilesional side

The increased sensitivity of the firing rate of contralesional MVNn to current was similar to that observed on the ipsilesional side (Beraneck et al. 2003a), but was associated with a
stronger increase of the sensitivity of the discharge of contrale-
sional MVNn to membrane potential ($/H9004 IF/H9004 V$). Although a
shift of the zero-crossing frequency was not observed on the
ipsilesional side, ipsilesional MVNn did show a slight shift of
their phase function and had larger phase leads and smaller
phase lags compared with those of control MVN neurons. In
general, the modifications of the phase functions were small for
ipsilesional MVNn compared with those of contralesional
MVNn. In addition, the most pronounced phase effects were on
type A MVNn on the contralesional side, whereas they were

---

**FIG. 6.** Summary of the mean magnitude and phase of the membrane potential or firing rate modulations induced in MVNn by
sinusoidal current stimulation. **A:** mean magnitude ($A_1$) and phase ($A_2$) of the membrane potential modulation shown by control and
contralesional MVNn during steady-state hyperpolarization (in the absence of action potentials) as a function of the stimulation
frequency. Because the amplitude of the injected current was constant for any given neuron, the membrane potential modulation
is given as the impedance $Z_\text{m}$ of the cell ($/H9004 V_\text{m}/H9004 I$) as a function of frequency. **B:** mean magnitude ($B_1$) and phase ($B_2$) of the firing
rate modulation ($/H9004 IF/H9004 I$) shown by control and contralesional MVNn at their resting membrane potential as a function of the
stimulation frequency of the sinusoidal current injection. **C:** mean magnitude ($C_1$) and phase ($C_2$) of the firing rate modulation
($/H9004 IF/H9004 I$) shown by control and contralesional MVNn during steady-state depolarization as a function of the stimulation frequency.
In all cases, SDs have been omitted for the sake of clarity. Asterisks indicate the values obtained on contralesional MVNn that were
significantly different from those obtained in control neurons ($P < 0.05$).
FIG. 7. Summary of the mean amplitude of the membrane potential or firing rate modulations induced in type A and type B MVNn by sinusoidal current stimulation. Top 2 panels: mean magnitude of the membrane potential modulation shown by type A ($A_1$) and type B ($B_1$) control and contralesional MVNn during steady-state hyperpolarization (in the absence of action potentials) as a function of the stimulation frequency. Because the amplitude of the injected current was constant for any given neuron, the membrane potential modulation is given as the impedance $Z_h$ of the cell ($V_h/I$) as a function of frequency. Bottom 2 panels: mean amplitude of the firing rate modulation ($IF/I$) shown by control and contralesional type A MVNn as a function of the stimulation frequency, at rest ($A_2$) and during steady-state depolarization ($B_2$). In all cases, SDs have been omitted for the sake of clarity. Asterisks indicate the values obtained on contralesional MVNn that were significantly different from those obtained in control neurons ($P < 0.05$).

**TABLE 3. Responses to sinusoidal currents delivered in the presence of action potentials**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All Neurons</th>
<th>Type A</th>
<th>Type B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Contralesional</td>
<td>Control</td>
</tr>
<tr>
<td>Number of neurons ($n$)</td>
<td>(48)</td>
<td>(43)</td>
<td>(23)</td>
</tr>
<tr>
<td>$Z_{rest}$, $M_\Omega$</td>
<td>58.5 ± 33.9</td>
<td>45.8 ± 35.7</td>
<td>57.5 ± 20.9</td>
</tr>
<tr>
<td>$\Delta F/\Delta I$ rest, $Sp/s/nA$</td>
<td>130.5 ± 48.1</td>
<td>150.1 ± 52.7</td>
<td>116.2 ± 43.8</td>
</tr>
<tr>
<td>$\Delta F/\Delta V$ rest, $Sp/mV$</td>
<td>3.08 ± 2.05</td>
<td>4.66 ± 3.12</td>
<td>2.48 ± 1.35</td>
</tr>
<tr>
<td>Amplitude of resonance at rest</td>
<td>1.31 ± 0.23</td>
<td>1.32 ± 0.30</td>
<td>1.28 ± 0.22</td>
</tr>
<tr>
<td>Median peak $f$ of resonance at rest, Hz</td>
<td>6</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Zero crossing at rest, Hz</td>
<td>1</td>
<td>3***</td>
<td>1</td>
</tr>
<tr>
<td>Number of neurons ($n$)</td>
<td>(24)</td>
<td>(35)</td>
<td>(11)</td>
</tr>
<tr>
<td>$Z_{depo}$, $M_\Omega$</td>
<td>47.9 ± 36.4</td>
<td>38.6 ± 41.1</td>
<td>46.8 ± 39.0</td>
</tr>
<tr>
<td>$\Delta F/\Delta I$ depo, $Sp/s/nA$</td>
<td>119.0 ± 38.1</td>
<td>144.2 ± 51.9</td>
<td>113.2 ± 34.2</td>
</tr>
<tr>
<td>$\Delta F/\Delta V$ depo, $Sp/mV$</td>
<td>3.96 ± 2.05</td>
<td>6.23 ± 3.95</td>
<td>4.44 ± 3.41</td>
</tr>
<tr>
<td>Amplitude of resonance at depo</td>
<td>1.38 ± 0.15</td>
<td>1.43 ± 0.3</td>
<td>1.42 ± 0.18</td>
</tr>
<tr>
<td>Median peak $f$ of resonance at depo, Hz</td>
<td>10</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Zero crossing at depo, Hz</td>
<td>1</td>
<td>4***</td>
<td>2</td>
</tr>
</tbody>
</table>

Values are means ± SD. This table gives the values of the parameters characterizing the responses of contralesional and control MVNn to sinusoidal currents delivered in the presence of action potentials. Asterisks indicate the values that are significantly different between the contralesional and control neurons. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. In addition, the values that correspond to significant differences are shown in bold.
practically restricted to type B MVNn on the ipsilesional side (Beraneck et al. 2003a).

**DISCUSSION**

**Summary of the long-term effects of unilateral labyrinthectomy on contralesional MVNn**

The plasticity of intrinsic membrane properties is strikingly demonstrated in the nearly opposite changes observed in the excitability of ipsilesional and contralesional vestibular neurons after a unilateral labyrinthectomy. These complementary modifications are even apparent in the distributions of particular populations of neurons based solely on action potential profiles, that is, type A and B neurons described previously (Beraneck et al. 2003a). After 1 mo of vestibular compensation (Fig. 8), there was a strong increase in the proportion of type B MVNn and a concomitant decrease in the proportion of type A MVNn among contralesional neurons. The AHP of contralesional MVNn decreased, in contrast to the increase observed on the ipsilesional side. The dynamic responses of contralesional MVNn (i.e., spike rate transfer functions) were also modified. Although the active impedance of contralesional MVNn was decreased, the amplitude of the spike rate modulation increased. These findings are consistent with an increase in active voltage-dependent conductances manifested by an increase in the sensitivity of the discharge of contralesional MVNn to membrane potential, ΔIF/ΔV. There was also a significant phase shift toward greater phase leads and smaller lags over the observed frequency range. In general, the dynamic responses of contralesional type A MVNn were more modified than those of type B MVNn, in contrast to that observed on the ipsilesional side. In both cases, the response dynamics of MVN neurons became more homogeneous than in the control situation (Fig. 8).

**Origin of the changes in the membrane and firing properties of contralesional MVNn induced by unilateral labyrinthectomy**

There is a wide range of examples in the literature in which unilateral peripheral lesion produces bilateral effects (for review see Koltzenburg et al. 1999). The contralesional effects are usually qualitatively similar to those that occur on the ipsilesional side but often of different magnitude. The putative neuronal mechanisms include the release of trophic signals during stressful conditions, or altered patterns of neuronal activity attributed to the compensatory behavior of the animal. Gacek et al. (1996) demonstrated in cats that contralateral labyrinthectomy was followed by significant morphological changes of the superior vestibular nucleus neurons, including a decrease in cell size. Whether these morphological changes occur in the guinea pig and are linked to the modifications of the membrane properties we observed remains an open question. Supporting the trophic factor hypothesis, other studies by the same authors using knock-out mice suggest that neurotrophin 3 might be involved (for review see Gacek et al. 1998).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CONTRALESIONAL MVNn</th>
<th>IPSILESIONAL MVNn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion type A</td>
<td>↓↓</td>
<td>↑</td>
</tr>
<tr>
<td>Proportion type B</td>
<td>↑↑</td>
<td>↓</td>
</tr>
<tr>
<td>A-like rectification</td>
<td>↓</td>
<td>=</td>
</tr>
<tr>
<td>Strength of the double AHP (type B)</td>
<td>↑</td>
<td>↓↓</td>
</tr>
<tr>
<td>AHP amplitude</td>
<td>↓</td>
<td>↑↑ type B only</td>
</tr>
<tr>
<td>Proportion of Silent neurons</td>
<td>↑</td>
<td>=</td>
</tr>
<tr>
<td>Firing rate</td>
<td>=</td>
<td>↑ type B only</td>
</tr>
<tr>
<td>Resting potential</td>
<td>↑ type B only</td>
<td>↑↑</td>
</tr>
<tr>
<td>Firing threshold</td>
<td>↑ type B only</td>
<td>=</td>
</tr>
<tr>
<td>CV</td>
<td>=</td>
<td>↓ type B only</td>
</tr>
</tbody>
</table>

**FIG. 8.** Summary of the main modifications of the contralesional and ipsilesional MVNn observed 1 mo after unilateral labyrinthectomy. This figure is an attempt to synthesize the main changes in the membrane properties and dynamic responses observed after 1 mo of vestibular compensation in either ipsilesional or contralesional MVNn. Upward and downward arrows indicate increases or decreases, respectively, compared with control conditions, whereas = indicates an absence of modification. Double arrows emphasize the strongest modifications.
Static membrane and firing properties of contralesional MVN

A striking result of this study is the finding of an increase in the proportion of type B neurons among contralesional MVN and a concomitant decrease in the proportion of type A neurons (Fig. 8). Because identical criteria were used to classify all contralesional, ipsilesional, or control MVN, this change could not be the result of differences in the subjective assessment of neurons. The 2 experimenters who performed the recordings obtained similar ratios of type A to type B neurons. The proportion of cells that were silent at rest was similar among type A and B neurons, which indicate that there was no specific silencing of type A MVN on the contralesional side.

Interestingly, on the ipsilesional side of slices taken after 1 mo of compensation, the proportion of type A neurons among MVN tended to increase, whereas the remaining type B MVN were modified and displayed more “type A–like” membrane properties (Beraneck et al. 2003a). In particular, the amplitude of the AHP of ipsilesional type B MVN was selectively increased. Complementary modifications between the 2 sides were observed for the 2 parameters used to categorize MVN (Fig. 8): A-like rectification (that characterizes type A MVN) and double AHP (typical of B MVN).

Serafin et al. (1991a), who used qualitative criteria to categorize MVN in slices taken from normal guinea pig, obtained about 30–35% of type A, 50% of type B, and 15–20% of type C MVN neurons. The type C MVN corresponded to those that showed “intermediate properties.” Using the quantitative method of classification, most of the neurons previously considered as type C MVN had no double AHP and an A-like rectification >0.15 V/s, and thus fell into the type A category. This led to an almost equal division of control MVN between type A (47%) and B (50%) neurons (Beraneck et al. 2003a). We suggest that the changes in the ratio of type A to B MVN observed on each side of the brain stem are linked mainly to modifications of the properties of these 20% of “intermediate” neurons that were qualitatively classified by Serafin et al. (1991a) as type C cells. On the ipsilesional side, the shift of MVN toward more A-like properties did not significantly modify the ratio of type A to type B MVN because most of the “intermediate” neurons that could have switched categories were already classified as type A MVN in control slices. On the contralesional side in contrast, the shift of MVN toward more B-like membrane properties, and in particular the decrease of the A-like rectification, led to a reclassification of the “intermediate” MVN as type B neurons.

Thus our data suggest that during long-term postlesional plasticity, the intrinsic membrane properties of guinea pig MVN can change in different ways, leading to a shift of the MVN population toward either “A-like” (on the ipsilesional side) or “B-like” (on the contralesional side) properties. This is consistent with the “continuum theory” proposed by Du Lac and Lisberger (1995), which states that the membrane properties of MVN are distributed between those of the 2 canonical A and B neuronal types. This “population shift” in the membrane properties that leads to substantial modifications of the ratio of type A to type B MVN could depend on the species and/or the exact criteria used to categorize MVN. In the rat and mouse, for instance (Dutta and Johnston 1998; Him and Dutia 2001; Johnston et al. 1994), contrary to the guinea pig, the type B MVN make up the vast majority of the population and all show a double AHP during spontaneous firing.

Although long-term vestibular compensation induced a significant depolarization of the average resting membrane potential of ipsilesional MVN, this effect was slight for contralesional MVN and was restricted to type B cells (Fig. 8). In the rat (Him and Dutia 2001), an early depolarization of the ipsilesional, deafferented type B MVN appears within the first week of compensation. This depolarization develops and extends progressively to type A neurons during the first month of compensation, consistent with findings in the guinea pig (see Discussion in Beraneck et al. 2003a). Hence, the average resting membrane potential of both ipsilesional and contralesional type B MVN appears to be more sensitive to unilateral labyrinthectomy (i.e., more dependent on the permanent activity of the vestibular sensory afferents than the resting potential of type A neurons).

The mean resting activity of contralesional MVN was not modified compared with control slices, and tended to be less than that on the ipsilesional side (Fig. 8). The absence of a strong decrease in the spontaneous firing rate of the whole sample of MVN contrasts with that found at the same stage of compensation using extracellular recordings (Vibert et al. 1999b). This discrepancy may be ascribed to the use of sharp electrode intracellular recording techniques, which may provoke an artificial increase of the neuronal firing rate (for a detailed discussion of that point see Beraneck et al. 2003a).

However, it is noteworthy that the proportion of neurons silent at rest reached 17.5% among contralesional MVN, and was thus higher than that obtained in control slices (5%) or on the ipsilesional side (3%). Accordingly, the proportion of contralesional MVN whose spontaneous firing rate ranged from 10 to 20 spikes/s was half that of normal MVN. This is consistent with both our extracellular study and that of Darlington et al. (1989), who observed in the guinea pig a marked decrease of the number of spontaneously active neurons on the contralesional side of slices 2 mo after the lesion.

After unilateral labyrinthectomy, the spontaneous discharge of contralesional MVN recorded in vivo increases, before returning to normal in 1 wk (Ris and Godaux 1998). At the cellular level, the intrinsic depolarization of neurons that develops with time on the side of the lesion might partially sustain the spontaneous discharge that has been restored in deafferented MVN (Beraneck et al. 2003a; Him and Dutia 2001). The increased proportion of silent and slowly discharging neurons in the MVN could be the contralesional mirror of this phenomenon. The long-term plasticity of firing properties on the contralesional side might contribute to the decrease of the spontaneous discharge rate of contralesional MVN observed in vivo, which should act to maintain the new balance of activity that is reached between both vestibular complexes 1 wk after the lesion (Ris and Godaux 1998).

The amplitude of the AHP of the whole population of contralesional MVN decreased compared with control MVN (Fig. 8), which should decrease the stability and regularity of their resting discharge. This and the increased sensitivity to membrane potential variations (see following text) should render contralesional MVN more responsive to synaptic inputs (Babalian et al. 1997). On the ipsilesional side in contrast, there was an increase in the amplitude of the AHP of type B MVN compared with control MVN neurons, which would
on both sides of the brain stem, in the sensitivity of those normally seen in type B neurons (Fig. 5). MVNn developed responses to ramp currents that resembled sensitivity to large-amplitude current injections. Type A ring rate increase during the ramps. Thus on both sides, the overshoot was associated with an augmentation of the slope of MVNn for the ramps delivered during hyperpolarization. For increased for both types of contralesional as well as ipsilesional MVNn for the ramps delivered during hyperpolarization. For the ramps delivered from rest, the increase of the overshoot occurred mainly in type A MVNn on the ipsilesional side, and was restricted to them on the contralesional side. The increased overshoot was associated with an augmentation of the slope of firing rate increase during the ramps. Thus on both sides, the MVNn recorded after 1 mo of compensation showed a higher sensitivity to large-amplitude current injections. Type A MVNn developed responses to ramp currents that resembled those normally seen in type B neurons (Fig. 5).

Consistent with these observations, we reported an increase, on both sides of the brain stem, in the sensitivity of the firing rate of neurons $\Delta I/F/\Delta t$ to injection of sinusoidal currents (Fig. 8). On the ipsilesional side, this increased sensitivity was associated with a slight, nonsignificant increase in the sensitivity of the discharge of MVNn $\Delta I/F/\Delta V$ to membrane potential. However, on the contralesional side, there was a marked and significant increase in the sensitivity of their discharge to membrane potential $\Delta I/F/\Delta V$, which was presumably attributable to an increase of their voltage-dependent conductances, as indicated by the decrease in the active impedance $\Delta V/\Delta I$.

On both sides, the increased $\Delta I/F/\Delta I$ was associated with a general phase shift of the responses toward greater phase leads and smaller phase lags, also suggesting a major involvement of voltage-dependent conductances (Fig. 8). On the ipsilesional side, this more active behavior might result from the fact that the resting membrane potential of MVNn was more depolarized in control slices. However, this is not likely on the contralesional side, where only type B neurons were slightly depolarized. The fact that this shift was stronger on the contralesional side than on the ipsilesional side correlates with the greater dependency of the firing rate of contralesional neurons on the membrane potential $\Delta I/F/\Delta V$.

Although the neuronal properties at rest of contralesional type A MVNn were not different from those of control type A MVNn, their evoked responses were strongly modified. Interestingly, the amplitude of these changes depended on the level of their membrane potential. During steady-state hyperpolarization, contralesional and control type A MVNn showed very similar responses to sinusoidal current injections, whereas the active impedance of contralesional type B MVNn was already lower than normal. At rest or under steady-state depolarization, in the presence of action potentials, the sensitivity of type A contralesional MVNn to applied current was strongly increased and became very similar to that of type B neurons. Thus type A contralesional MVNn displayed enhanced dynamic properties and behaved like the phasic type B MVNn as soon as they discharged spikes. Interestingly, Uno et al. (2003) previously observed that, although the lateral vestibular nucleus neurons had membrane properties similar to a composite of type A and type B MVNn, they showed very different dynamic responses. Thus it appears that subtle changes in the membrane properties of central neurons are sufficient to strongly modify their dynamic responses.

When the amplitude of the firing rate modulation of contralesional MVNn by sinusoidal currents to increasing stimulation frequency reached the modulation limit imposed by their essentially constant spontaneous discharge rate, they tended to synchronize their discharge with high-frequency inputs more than control or ipsilesional MVNn neurons. Interestingly, the control type B MVNn also show a better synchronization than that of the control type A MVNn (Ris et al. 2001).

Altogether, these data demonstrate that long-term plasticity after sensory deafferentation can lead to bilateral modifications of the membrane properties of central neurons. Most of the voltage-sensitive conductances expressed by neurons are potentially subject to persistent use-dependent modulation. In a recent review on intrinsic neuronal properties, Zhang and Linden (2003) suggested several specific $K^+$, $Ca^{2+}$, and $Na^+$ conductances as possible molecular substrates of long-term plasticity. In MVNn, recent studies by Du Lac and colleagues (Nelson et al. 2003; Smith et al. 2002) proposed that the level of intracellular calcium and the calcium-activated potassium conductances are major determinants of the excitability of MVN neurons.

**Functional implications of the long-term changes of the membrane properties of contralesional MVNn during vestibular compensation**

In vivo experiments on guinea pigs demonstrated that after unilateral labyrinthectomy, there is at first a similar decrease of the vestibulo-ocular responses triggered by head rotations directed toward either the lesioned or intact side (Gilchrist et al. 1998; Vibert et al. 1993). On the contralesional side, the decrease arises from the loss of the disinhibition normally coming from the deafferented side through commissural pathways. In guinea pigs, as in other vertebrates (Broussard et al. 1999b; Gilchrist et al. 1998; Lasker et al. 2000; Murai et al. 2003; Vibert et al. 1993), the vestibulo-ocular reflex triggered by rotations toward the contralesional side largely recovers within several weeks. In contrast with the behavior on the ipsilesional side, the quality of the recovery does not decrease with the amplitude of the movement, and high acceleration impulses directed toward the contralesional side trigger high-frequency responses similar to those obtained in intact animals. Many of the modifications of the membrane properties of contralesional MVNn reported here might be involved in this recovery. The increased sensitivity of the firing rate of neurons to membrane potential modulation and their smaller AHP should lead to more efficient responses of contralesional neurons to synaptic inputs. The increased sensitivity of the firing rate of contralesional MVNn to both ramplike and sinusoidal current stimulation was associated with a greater involvement of active conductances, which should increase both the efficacy
and frequency range of the neuronal responses. Altogether, the contralesional MVNn displayed a more phasic behavior than that of control MVNn. The proportion of the more phasic type B neurons among MVNn was increased, and the dynamic properties of the remaining tonic type A MVNn were strongly modified and resembled more those of type B neurons than in control slices. There was also an amplification of the nonlinear properties of MVNn such as the overshoot and synchronization capabilities.  

Contrary to changes on the ipsilesional side, where the type B MVNn tended to acquire the AHP and response dynamics of type A neurons (see DISCUSSION in Beraneck et al. 2003a), the ability of type B contralesional MVNn to respond to high-frequency, high-amplitude stimuli was not impaired.

In 1999 Minor et al. proposed a mathematical model of the vestibulo-ocular reflex dynamics where the system includes distinct linear and nonlinear pathways that would be mediated by different sensory vestibular afferents and central vestibular neurons. Based on their observations in the monkey and those of Broussard et al. (1999a,b) in the cat, Lasker et al. (2000) suggested that an extension of the nonlinear component of vestibular reflexes was responsible for the increase in contralesional gain after unilateral labyrinthectomy or canal plugging. This should apply to guinea pigs because the dynamics of the vestibulo-ocular reflex and the way they are affected by labyrinthectomy are similar in all mammalian species (Broussard et al. 1999b; Escudero et al. 1993; Gilchrist et al. 1998; Minor et al. 1999; Smith and Curthoys 1989). The changes observed on the contralesional side confirm at the cellular level the assumption of Lasker et al. (2000) concerning the enhancement of contralesional gain observed in vivo. It also strengthens the idea that in the intact MVN the tonic type A cells show relatively linear behavior, whereas the more phasic type B neurons have more marked nonlinear properties.

In conclusion, this study demonstrates that after 1 mo of vestibular compensation after unilateral labyrinthectomy, the properties of contralesional medial vestibular nucleus neurons (MVNn) are strongly modified. The increased proportion of silent neurons among contralesional MVNn might be involved in the permanent decrease of the spontaneous firing rate of MVNn observed during vestibular compensation in vivo. The marked improvement of vestibular related synergies observed in vivo for rotations toward the contralesional side could be sustained by the increased sensitivity of contralesional MVNn to current injection and membrane potential. The general shift of contralesional MVNn toward more B-like, phasic properties would explain the full recovery of vestibular responses triggered by high acceleration impulses directed toward the contralesional side.

Altogether, these data show that long-term vestibular compensation differentially affects ipsilesional and contralesional MVN neurons. However, the 2 sides continue to work synergistically. The 2 MVN switch from their normal “push–pull” mode of action to a system where the ipsilesional nucleus is mainly stabilizing the tonic activity responsible for normal posture, whereas the more dynamic responses of the contralesional nucleus allows more efficient modulation of this activity by the remaining sensory vestibular afferents.

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