Long-Term Plasticity of Ipsilesional Medial Vestibular Nucleus Neurons After Unilateral Labyrinthectomy

Mathieu Beraneck,1 Mohammed Hachemaoui,1 Erwin Idoux,1 Laurence Ris,2 Atsuhiko Uno, Emile Godaux,2 Pierre-Paul Vidal,1 Lee E. Moore,1 and Nicolas Vibert1

1Laboratoire de Neurobiologie des Réseaux Sensorimoteurs, Centre National de la Recherche Scientifique, Unité Mixte de Recherche 7060, Université Paris 5, Centre Universitaire des Saints-Pères, 75270 Paris Cédex 06, France; and 2Laboratoire de Neurosciences, Université de Mons-Hainaut, 7000 Mons, Belgium

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INTRODUCTION

Destruction of one labyrinth results in major oculomotor and postural disturbances that spontaneously regress after unilateral labyrinthectomy (for reviews, see Curthoys 2000; Dieringer 1995; Smith and Curthoys 1989; Vibert et al. 1997). The static deficits, which include postural distortions and a spontaneous ocular nystagmus, disappear over 3–4 days in the guinea pig (Darlington et al. 2002; Vibert et al. 1997). In the guinea pig, we and others have shown that this recovery is limited to low acceleration and to the low and middle frequency range (0.1 to ~10 Hz) of head movement (Brousard et al. 1999; Gilchrist et al. 1998; Hamann et al. 1998; Laker et al. 2000; Vibert et al. 1993).

After unilateral labyrinthectomy (UL), the ipsilesional vestibular nucleus neurons (VNn) lose the excitatory drive provided by labyrinth afferents and become silent. In contrast, the spontaneous discharge of the contralateral VNn increases. This imbalance is responsible for the deficits triggered by the lesion. During the first week after UL, the recovery of a normal resting discharge by the ipsilesional medial VNn (MVNn) plays a key role in the disappearance of the static syndrome by restoring the balance between the activity of neurons in both vestibular nuclei (Ris et al. 1995, 1997). Because the ipsilesional labyrinth afferents stay silent (Jensen 1979; Sirkin et al. 1984), this recovery is a model of plasticity in the CNS.

Vestibular compensation involves modification of both the intrinsic properties of the ipsilesional MVNn and the vestibular-related networks in which they are embedded (Cameron and Dutia 1997; Darlington and Smith 1996; Darlington et al. 2002; Vibert et al. 1999a, 2000; Yamanaka et al. 2000). We recently suggested that in rodents, the spontaneous discharge recovered in vivo by the ipsilesional MVNn might be more and more sustained by changes in the intrinsic properties of MVNn themselves as the time of compensation increases (Vibert et al. 1999b). Extracellular recordings on slices taken from previously labyrinthectomized animals show an increase of the spontaneous discharge of the ipsilesional MVNn compared with control slices, and/or to contralesional neurons during the first week of compensation (Cameron and Dutia 1997; Ris et al. 2001a; Vibert et al. 1999b; for review, see Darlington et al. 2002). In particular, a significant increase in the firing rate of MVN neurons can be detected as early as 4 h after the lesion in the rostral third of the nucleus in rats (Cameron and Dutia 1997). In the guinea pig, we and others have shown that this increase of the spontaneous firing rate of ipsilesional neurons becomes stronger when the slices are taken after 1 or 2 mo
instead of 1 wk (Darlington et al. 1989; Vibert et al. 1999b). This late in vitro change is not directly involved in the initial recovery of a normal discharge rate by the ipsilesional MVNn observed in vivo, which is achieved by the end of the first week after UL. However, it is concomitant to the recovery of the dynamic synergies triggered by low-acceleration stimuli that has been described a few weeks after the lesion (Gilchrist et al. 1998; Vibert et al. 1993) (see preceding text).

**In vitro intracellular recordings** have led to the identification of two main types of MVNn, the type A and type B neurons, according to their membrane properties (Gallagher et al. 1985; Him and Dutia 2001; Johnston et al. 1994; Serafin et al. 1991a,b). It is generally admitted that MVNn represent a continuum of cells whose properties are distributed between those of two canonical types of neurons, the type A and B MVNn (Du Lac and Lisberger 1995a). Studies using intracellular or whole cell patch-clamp recordings have confirmed that some membrane and response properties of the ipsilesional MVNn were modified 7–10 days after UL (see also discussion). Godaux and Ris (2001) and Him and Dutia (2001) have reported an increase in the proportion of type B MVNn displaying low-threshold calcium spikes. Him and Dutia (2001) demonstrated that the average resting membrane potential of type B MVNn was depolarized by 3 mV compared with control cells, while their input resistance was increased by 10–15%. Ris et al. (2001c, 2002) reported that the nonlinear "overshoot" induced by ramp-like currents was increased in type B but not in type A ipsilesional MVN neurons. In contrast, the sensitivity of MVNn to steady-state current injection was not modified.

To determine how the properties of the deafferented MVNn were modified after longer times of compensation, we performed intracellular recordings of MVNn on slices taken from animals 1 mo after a UL and determined their static and dynamic membrane properties. We measured the responses of the ipsilesional MVNn to steps, ramps, and sinusoidal currents of various amplitudes and frequencies. The results were compared with data obtained using the same stimuli for MVNn recorded on control slices.

On all previous publications, MVN neurons have been categorized into type A and B neurons using only qualitative criteria. To assess reliably the long-term effects of UL on this heterogeneous population of cells and remove possible biases in the classification of MVNn, quantitative, objective criteria were developed to characterize the intracellularly recorded MVNn. These criteria were set from the sample of 89 cells recorded in control slices, and then the neurons recorded on the ipsilesional side of slices taken from labyrinthectomized animals were classified in exactly the same way.

We assume that a large majority (>80%) of the MVNn we record on slices are second-order vestibular neurons, which, in previously labyrinthectomized animals, have lost labyrinthine input at the time of the lesion. Indeed, 80–85% of the central vestibular neurons recorded in the MVN area of the isolated whole brain of guinea pig, using similar electrodes to those used on slices, could be identified as second-order vestibular neurons (Babalian et al. 1997). This high proportion of second-order cells is in agreement with previous anatomical (Carleton and Carpenter 1983; Sato and Sasaki 1993) and physiological studies (Chen-Huang et al. 1997; Goldberg et al. 1987).

**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 following the procedures issued by the French Ministère de l’Agriculture. The guinea pigs used to obtain control slices (i.e., intact animals, n = 60) had a mean age of ~5 wk (range: 4–8 wk) and a mean weight of ~250 g (range: 150–400 g). Because of the compensation time, the slices obtained 1 mo after UL were from slightly older animals aged 7–9 wk (n = 26), whose mean weight was ~320 g (range: 250–450 g). Compared with the rat, guinea pig is a precocious species where the CNS is almost mature at birth (after a 9-wk-long gestation period) and postnatal maturation of the CNS is minimal (Dobbing and Sands 1970; Nacher et al. 2000). Besides, there was no difference in the membrane properties of the intracellularly-recorded MVNn obtained from the smallest (150–200 g) versus the largest (300–400 g) intact guinea pigs, which proves that the properties of MVNn recorded in control slices are not significantly modified within the age range of the animals that we used.

ULs were performed under halothane anesthesia with the help of an operating microscope as described in Vibert et al. (1999a,b). The semicircular canals, utricle, and saccule were exposed via a retroauricular approach. The bony labyrinth was drilled, and the ampullae of all three canals and the otolithic maculae were removed using suction. The guinea pigs were allowed to compensate in a normal visual environment until their brain was removed to prepare the slices.

**Intracellular electrophysiological recordings**

Thick (500 μm) coronal brain stem slices were cut and maintained using standard techniques (Gallagher et al. 1985; Serafin et al. 1991a; Vibert et al. 1999b). Intracellular electrophysiological recordings were obtained with sharp, 3 M potassium acetate-containing glass microelectrodes from neurons within the medial vestibular nucleus (MVN), taking the border of the IVth ventricle as a landmark.

Given that MVN neurons constitute a heterogeneous population, it was critical to be sure that we sampled the same populations of neurons before and after labyrinthectomy. Otherwise, the differences reported in the following text could be an artifact of having recorded from different populations of cells. In particular, we have observed that the proportions of type A and B MVNn were varying along the rostrocaudal extent of the nucleus (N. Vibert, M. Serafin, M. Mühlthaler, unpublished data). Other authors have demonstrated that the cellular changes associated with vestibular compensation can be different in the caudal and rostral parts of the MVN (Cameron and Dutia 1997; Yamamaka et al. 2000). To control for this variability, we decided to restrict as much as possible our recordings to the two 500-μm coronal slices corresponding to the middle third of the guinea pig MVN, at the level of the cerebellar peduncles. Only a few cells (~10%) were recorded in more caudal slices corresponding to the last third of the MVN, both in normal and previously labyrinthectomized animals. The same investigator, who always used the same experimental setup and similar electrodes, obtained all data from previously labyrinthectomized animals as well as the majority of the control data. The other part of the control data were from a previously published study (Ris et al. 2001b) and was obtained by a different investigator (see results). There was no significant difference between the two data sets.

All measurements were done with an Axoclamp 2A system (Axon Instruments, Union City, CA) in either the bridge or switching discontinuous current-clamp (DCC) mode (Moore et al. 1993). 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. 2001b).
Part of the current injections and all data acquisition were performed with a PC-compatible computer using the “Acquis 1” program (version 4.0, Bio-logic S.A., Gif-sur-Yvette, France). The sampling rate used for acquisition varied between 2,000 and 5,000 Hz, depending on the length of the data-acquisition sequence. Consequently, the amplitudes of the digitized spikes were variable; however, oscilloscope traces verified that the size of the action potential was constant at any given membrane potential. The data were analyzed using program scripts with Mathematica 4.0 (Wolfram Research, Champaign, IL), or MATLAB 6.5 (The MathWorks, Natick, MA). To minimize the possibility that experimenter biases might affect the results, the same scripts were used to quantify the properties and responses of MVNn recorded on control slices and slices taken from previously labyrinthectomized animals.

**Basic membrane and firing properties of MVNn**

Because most MVNn are spontaneously active on slices, the potential was filtered with a 1-Hz low-pass filter to obtain an estimate of its average resting level that was taken as the “mean resting membrane potential” of each neuron. For each cell, this membrane potential value was corrected by measuring and subtracting the extracellular voltage offset found after removal of the electrode from the neuron. No correction was made for liquid junction potentials, but this can be assumed to be constant between slices taken from control and previously labyrinthectomized animals given that both sets of MVNn were recorded using similar electrodes.

The same criteria were used to evaluate the quality of intracellular recordings and select the neurons used for statistical analysis in control slices and in slices taken from previously labyrinthectomized animals. All cells that had resting membrane potentials more negative than –50 mV and spike amplitudes >50 mV were automatically retained. In both types of slices, we also included in the sample the MVNn whose membrane potential ranged from –50 to –40 mV if they displayed spike amplitudes >50 mV and a normal spike width. The number of such cells was 5 of 89 MVNn in control slices and 17 of 78 MVNn in slices taken from previously labyrinthectomized animals. Spike width at threshold was considered to be normal if it stayed within the range of the spike widths (0.70–2.20 ms) measured for the neurons that had resting potentials more negative than –50 mV and spike amplitude >50 mV. Recordings of the neurons at rest (i.e., with no holding current being injected through the recording electrode) were used to calculate their mean spontaneous firing rate, its coefficient of variation (CV) expressed as a percentage, and to measure the mean amplitude of the spike. For each neuron, an average of the spike shape and following inter-spike interval profile was obtained by averaging successive spontaneous spikes taken either at the resting membrane potential or while the cell was slightly depolarized (for the few neurons that were silent at rest). The spikes (mean number of ~120) were synchronized to their thresholds, taken at the point on the rising phase of the action potential where the slope of the potential trace reached an arbitrary threshold of 10 V/s (Krawitz et al. 2001). The averaged spike shape was used to determine the amplitude of the afterhyperpolarization (AHP) and the width of the spike (taken at threshold). The AHP amplitude was calculated as the membrane potential difference between spike threshold and the membrane potential minimum after the falling phase of the spike.

The cell’s firing threshold, i.e., the membrane potential for which the cells begin to fire action potentials (in mV), was assessed as the potential reached by the neuron at the threshold of the first spike triggered by a slow, depolarizing current ramp (see following text and Fig. 2D). For each cell, we determined whether long-lasting, subthreshold plateau potentials could be triggered by low-amplitude (0.1–0.2 nA), short-duration (10 ms) current pulses delivered while the neuron was maintained just below its firing threshold (Babalian et al. 1997; Serafin et al. 1991a,b) and measured their mean duration.

**Quantitative determination of the neuronal type**

In previous publications, MVN neurons have been categorized into type A and B neurons using qualitative criteria. Neurons were characterized as a type A, type B, or type B with low-threshold calcium spikes (B-LTS) MVN neurons according to their action potential profile (Serafin et al. 1991a). Type A neurons were characterized by their single, deep AHP and in general have a wider action potential than type B neurons. They display an A-like rectification when released from hyperpolarization or in response to depolarizing current pulses given from a hyperpolarized level, which is visible as an inflection point delaying the depolarization of the neuron during inter-spike intervals. Type B neurons showed a biphasic, significantly smaller AHP and narrower action potentials. Neurons that displayed intermediate properties, or did not clearly fit into any of these two categories, were grouped as type C MVN neurons.

To assess reliably the long-term effects of UL on this heterogeneous population of cells and remove possible experimenter biases in the classification of MVNn, quantitative, objective criteria were developed to characterize the intracellularly recorded neurons. For each neuron, the averaged spike profile obtained during spontaneous firing and its first derivative were used (as already done by Johnston et al. 1994) to assess the presence of the A-like rectification and double AHP, the two main criteria used previously for the qualitative classification.

The presence of an A-like rectification characterizing type A MVNn is always visible as an inflection of the voltage trace (V) within the inter-spike interval (see Fig. 1A for the following explanation). This inflection is better seen on the first derivative of the voltage trace (dV/dt) as a sudden decrease of the rate of the depolarization leading to the next spike. The A-like rectification always begins ≥2–3 ms after the end of the spike whatever the spontaneous discharge rate of the cell, and the derivative of the voltage trace remains positive. The strength of the A-like rectification was quantified as the algebraic decrease (dV/dt) in the rate of the inter-spike depolarization (dV/dt in V/s) associated with this phenomenon. In the absence of any A-like rectification, this parameter was set at zero.

The presence of an early fast AHP followed by a delayed slow one, i.e., of the double-component AHP characterizing type B MVNn, was assessed on the averaged spike profile and then confirmed using the first derivative (see Fig. 1B for the following explanation). When a double AHP is present, its second component is seen as a transient zeroing or negativity of the rate of depolarization, which always occurs within 2 ms of the end of the spike. The strength of each double AHP was quantified as the algebraic decrease (dV/dt) in the rate of the inter-spike depolarization (dV/dt in V/s) associated to the second component of the phenomenon. The strength of the double AHP was set at zero when no double AHP was present.

While most type B MVNn display a clear double-component AHP when spikes are triggered by current steps delivered from a hyperpolarized potential, the double AHP is often not visible on the averaged spontaneous spike profile obtained at rest or during a slight depolarization. A third parameter had to be used to unambiguously characterize type B MVNn recorded at their resting membrane potential, whatever their level of membrane polarization. All type A MVNn display a mostly concave voltage trace during the inter-spike interval following the peak of the AHP, because of the presence of the A-like rectification (Fig. 1C). In contrast, the averaged inter-spike profile obtained for B MVNn is always convex because the velocity of the inter-spike depolarization increases with time once the peak of the second component of the double AHP has been reached (Fig. 1D). Thus the maximum convexity of the average voltage trace obtained during the inter-spike interval was taken as a third parameter. The averaged spike profile obtained for each neuron was used to draw the line (chord) joining the peak of the AHP to the endpoint of the profile (Fig. 1, C and D). The maximum convexity of the inter-spike trace was then measured as the maximum difference (in mV) observed...
between the voltage trace and the chord in the direction of convexity (i.e., toward hyperpolarizing potentials) during the inter-spike interval. As shown on Fig. 1, typical type A MVNn have null or low convexity values, whereas type B MVNn display high convexity values (Fig. 1 D).

Altogether, a set of quantitative values describing the strength of the A-like rectification, the strength of the double AHP, and the convexity of the voltage trace during the inter-spike interval was obtained for each of the MVNn recorded in control slices. These values were used to plot a three-dimensional graph of the distribution of the neurons according to these parameters, from which quantitative criteria for classification of MVNn in type A, B, or C neurons were obtained (see RESULTS). The same criteria were then used to categorize the sample of neurons recorded on the ipsilesional side of slices taken from previously labyrinthectomized animals.

After the assessment of its basic membrane and firing properties, each neuron was submitted to the stereotyped stimulation protocol described in the following text. The instantaneous firing rate of the cell (IF in spikes/s) was estimated at any time with a Mathematica script that measured the time intervals between two successive action potentials. The time at the end of each interval between action potentials was used to indicate the time for each IF value.

Assessment of the passive input resistance of MVNn using current steps

The passive input resistance of each neuron was assessed using series of hyperpolarizing current steps of decreasing amplitudes. The cell was maintained by steady-state hyperpolarization at a few millivolts (0–10) below its threshold for discharge, to suppress spikes. The whole cell resistance for each MVNn (input resistance = voltage
Injection of depolarizing ramp-like currents

Increasing ramp currents of 0.3-nA amplitude were applied at five different slopes up to a final steady-state value, as described in detail in Ris et al. (2001b); while the cell was maintained at \(-10\) mV below its firing threshold (Fig. 2). In other words, for both control and ipsilesional MVNn, the membrane potential at which the ramps were delivered was set relative to the firing threshold of each cell. The five slopes corresponded to times to reach the plateau of current of 5,000 ms (0.06 nA/s), 3,400 ms (0.09 nA/s), 1,800 ms (0.17 nA/s), 600 ms (0.5 nA/s), and 200 ms (1.5 nA/s), respectively. Because the whole stimulus was 5,000 ms long, there was no plateau after the slowest ramp, which was used, as stated in the preceding text, to assess the cell’s firing threshold (Fig. 2D). We computed for each ramp the rate of increase of the instantaneous firing rate of the cell \((k_{IF} \text{ in spikes s}^{-1} \text{ nA}^{-1})\) during the depolarizing, ramp-like portion of the current injection, i.e., over the time taken to reach the plateau of current. This gives an indication of the sensitivity of the cell to current injections. We also measured in each case the difference between the firing rate reached at the end of the depolarizing current injection and the final, stable discharge rate reached at the end of the plateau (overshoot in spikes/s). This parameter gives an indication of the nonlinear, dynamic properties of neurons. To assess how the level

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**FIG. 2.** Measurement of the firing rate response of MVNn to ramp-like currents. A: profiles of the increasing ramp currents of 0.3-nA amplitude that were applied at 5 different slopes up to a final steady-state value. The profile of the 600-ms ramp is outlined in bold, the profile of the 5,000-ms ramp is dashed. B: example of a response triggered by a 600-ms ramp (outlined in A) delivered during steady-state hyperpolarization in a type B MVNn. C: plot of the instantaneous firing rate response obtained for the neuron displayed above, showing how the overshoot was measured. D: example of a response triggered by the 5,000-ms ramp in a type B MVNn. This ramp was used to measure the firing threshold indicated by the arrow. All spikes are shown digitally clipped at \(-10\) mV.

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of polarization of MVNn influenced their responses, the whole sequence of ramp stimulations was repeated while the neuron was at its resting membrane potential.

**Injection of sinusoidal currents**

A third series of stimuli consisted of current sine waves applied for 5,000 ms at various frequencies ranging from 0.2 to 50 Hz (Du Lac and Lisberger 1995b; see for details Ris et al. 2001b). The amplitude of the stimulus was adjusted at the 0.2-Hz frequency to keep the membrane potential variation ~10 mV peak to peak. Typically, the first series of sinusoidal currents was delivered while the cell was at its resting membrane potential and spontaneously fired action potentials (Fig. 3). For each frequency of stimulation inferior or equal to about one-third of the neuron’s resting discharge, the modulation of the instantaneous firing rate of MVNn was fitted with a sine wave that was then used to calculate the amplitude and phase of the IF modulation (ΔIF, Fig. 3). Ris et al. (2001b) have shown that in this condition, the IF modulation of MVNn was linear. When the frequency of stimulation passed a third of the neuron’s firing rate, the amplitude of the IF modulation of MVNn was calculated in an empirical way as the difference between the minimum and maximum IF reached by the neuron during the stimulation. No phase measurements were obtained in this situation. Using this method, we could evaluate ΔIF from 0.2 Hz to a maximum stimulus frequency that varied from cell to cell according to its resting discharge and the sensitivity of its discharge to current injection but could reach 50 Hz in some cases. The underlying mean membrane potential excursion (ΔV) was computed for each frequency using a Mathematica script, which performed a Fourier analysis of the total membrane potential response. The magnitude of the Fourier component corresponding to the stimulus frequency was taken as the potential response. This procedure was only valid when the components due to the shape and frequency of the action potentials were not overlapping those of the stimulation frequency. This requirement was true for frequencies <1 Hz (Fig. 3). ΔIF and ΔV were used to evaluate at 0.4 Hz the cell sensitivity to current injection by dividing ΔIF by the amplitude of the injected current (ΔIF/ΔV in spikes s⁻¹ nA⁻¹). The sensitivity of the firing rate of the cell to variations of the mean membrane potential (ΔIF/ΔV) was quantified in spikes s⁻¹ nA⁻¹. We calculated the “active” impedance Z of the cell as the amplitude of the membrane potential change obtained for the 0.4-Hz stimulus divided by the amplitude of the injected current (ΔV/ΔIF in MΩ).

When possible, a similar series of sinusoidal stimuli was given while the cell was maintained at a depolarized membrane potential by a steady-state current injection of 0.15–0.25 nA to assess how the level of discharge of MVNn modified their responses.

Some of the cells were also submitted to the same series of sinusoidal current injections while they were maintained at 10–20 mV below their threshold for discharge, so that no spike was evoked by the stimulation. The amplitude of the membrane potential change (ΔV) was computed for each frequency using a Mathematica script, and the response to the 0.4-Hz stimulus was used to evaluate the impedance Z of the cell maintained under a steady-state hyperpolarization (Z = ΔV/ΔIF in MΩ).

As reported by Ris et al. (2001b), the amplitude of the modulation of the membrane potential or instantaneous firing rate of MVNn by sinusoidal currents displayed resonant properties. For each MVNn, the response increased with increasing stimulation frequency to reach a maximum at what was defined as the peak frequency of resonance. Then the modulation progressively dropped to lower levels. The “amplitude” of the resonance was defined as the ratio between the maximum amplitude of the firing rate modulation at the peak frequency of resonance and the amplitude obtained at the lowest fre-

![FIG. 3. Measurement of the spike discharge modulation of MVNn induced by sinusoidal currents. A: profile of a 1-Hz sinusoidal current injected into the neuron. ΔI: amplitude of the current modulation. B: membrane potential response recorded in response to the sinusoidal current injection. ΔV_m is the amplitude of modulation of the mean membrane potential underlying the firing rate response. C: the instantaneous firing rate modulation (ΔIF) was calculated and fitted with a sine wave to obtain the magnitude and phase shifts of the firing rate response at different stimulating frequencies. All spikes are shown digitally clipped at ~5 mV.](http://jn.physiology.org/content/90/1/189/F3)
quency we used, namely 0.2 Hz. The amplitude of the resonance was measured in the same way for the membrane potential when the neurons were hyperpolarized to suppress action potentials.

**Statistical analysis**

Calculations of means SD and further processing of all results were carried out using the Systat 8.0 software (SPSS, Chicago, IL) on a PC-compatible computer. For each parameter, normality of the distributions was assessed using one sample Kolmogorov-Smirnov tests, with significance set at P < 0.05. Statistical comparisons between numerical values were achieved through either parametric (if the distribution of the parameter was normal for all the samples involved and each sample included ≥15 values) 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 performed to search for significant differences between the mean values obtained for type A and B neurons in control slices and in slices taken from labyrinthectomized animals (which defined 4 categories of neurons). Two-by-two comparisons among the four cell groups were then performed using Student’s t-test or the nonparametric Mann-Whitney U tests. Type C neurons were excluded from the analysis except for comparisons performed using t-test or Mann-Whitney U tests between the whole sample of neurons obtained on control slices and the whole sample of neurons recorded on slices taken from labyrinthectomized animals. Paired parametric (ANOVA followed by paired t-test) or nonparametric tests (Friedman ANOVA followed by Wilcoxon signed-rank tests) were used to compare for each cell type the responses evoked by ramps of different slopes. They were also used to determine how the responses to ramps and sinusoidal currents were modified according to the level of steady-state polarization of the cell (2 levels for the ramps and 3 levels for the sinusoidal currents).

**RESULTS**

The results presented in this study were obtained from a database of 89 MVNn recorded on slices prepared from normal, intact animals and 78 MVNn recorded on the ipsilesional side of slices taken from animals labyrinthectomized ~1 mo before (compensation times ranged from 23 to 43 days). All mean values are presented with their SD.

**Categorization of the MVNn using quantitative criteria**

The quantitative criteria used for the classification of MVNn into type A and B neurons were set from the sample of 89 control MVNn, which included 32 MVNn that were recorded by Ris et al. (2001b) and 57 MVNn recorded afterward. Because there was no significant difference between the two sets of result, data from the two samples were pooled together.

We have defined in the methods section the three parameters we viewed as the most pertinent to categorize MVNn into type A and B neurons. They were the presence and strength of an A-like rectification during the inter-spike interval, the presence and strength of a double AHP, and the convexity of the voltage trace during the inter-spike interval. The qualitative classification of MVNn into type A and B MVNn previously used has shown that these two categories of neurons had also significantly different spike widths and AHP amplitudes. Because of that, we checked whether one or both of these parameters could be unambiguously used to categorize MVNn. When considering the whole sample of MVNn, the width of the spike ranged from 0.70 to 2.20 ms around a mean of 1.15 ± 0.23 ms, while the size of the AHP ranged from 6.3 to 29.2 mV around a mean of 16.77 ± 4.50 mV (Table 1). Both distributions were normal, and no sign for the existence of two distinct groups of neurons was obtained when considering only these parameters. Hence, neither the width of the spike nor the size of the AHP could be used to unambiguously categorize control MVNn.

Thirty-four of the 89 control MVNn (38%) displayed no measurable A-like rectification on the average spike profile obtained during spontaneous firing. For the 55 other cells, the

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**Table 1. Basic membrane and firing properties of control and ipsilesional MVNn**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control MVNn</th>
<th>Ipsilesional MVNn</th>
<th>Control vs. Ipsilesional</th>
<th>Type A</th>
<th>Ipsilesional</th>
<th>Type B</th>
<th>Ipsilesional</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of neurons</td>
<td>89</td>
<td>78</td>
<td></td>
<td>42</td>
<td>42</td>
<td>44</td>
<td>35</td>
</tr>
<tr>
<td>VM, mV</td>
<td>−58.7 ± 8.5</td>
<td>−51.4 ± 4.8</td>
<td>P = 0.001</td>
<td>−56.8 ± 7.3</td>
<td>−51.8 ± 5.5**</td>
<td>−60.8 ± 9.4</td>
<td>−51.0 ± 3.8**</td>
</tr>
<tr>
<td>FR, spikes/s</td>
<td>26.2 ± 17.2</td>
<td>31.0 ± 17.4</td>
<td>P = 0.10</td>
<td>29.1 ± 20.1</td>
<td>29.7 ± 20.3</td>
<td>24.3 ± 13.8</td>
<td>32.4 ± 14.2*</td>
</tr>
<tr>
<td>CV, %</td>
<td>8.26 ± 9.48</td>
<td>5.52 ± 4.99</td>
<td>P = 0.49</td>
<td>7.06 ± 9.61</td>
<td>6.88 ± 6.3</td>
<td>9.19 ± 9.59</td>
<td>4.21 ± 2.84**</td>
</tr>
<tr>
<td>AHP, mV</td>
<td>16.8 ± 4.5</td>
<td>18.2 ± 4.6</td>
<td>P = 0.07</td>
<td>19.6 ± 3.8</td>
<td>20.4 ± 5.1</td>
<td>14.0 ± 3.4</td>
<td>16.1 ± 2.6**</td>
</tr>
<tr>
<td>Spike amplitude, mV</td>
<td>55.2 ± 7.5</td>
<td>55.6 ± 7.2</td>
<td>P = 0.73</td>
<td>54.6 ± 7.4</td>
<td>55.4 ± 7.0</td>
<td>55.1 ± 7.3</td>
<td>55.6 ± 7.7</td>
</tr>
<tr>
<td>Firing threshold, mV</td>
<td>−67.9 ± 10.5 (39)</td>
<td>−61.3 ± 7.3 (35)</td>
<td>P = 0.003</td>
<td>−66.9 ± 10.7 (19)</td>
<td>−59.9 ± 5.4* (14)</td>
<td>−69.8 ± 10.7 (18)</td>
<td>−62.2 ± 8.4* (21)</td>
</tr>
<tr>
<td>Spike width, ms</td>
<td>1.15 ± 0.23</td>
<td>1.18 ± 0.25</td>
<td>P = 0.44</td>
<td>1.25 ± 0.21</td>
<td>1.25 ± 0.26</td>
<td>1.06 ± 0.22</td>
<td>1.10 ± 0.21</td>
</tr>
<tr>
<td>Membrane resistance, MΩ</td>
<td>94.6 ± 45.3</td>
<td>116.6 ± 46.0 (29)</td>
<td>P = 0.018</td>
<td>93.8 ± 39.9 (26)</td>
<td>112.5 ± 29.5* (11)</td>
<td>96.2 ± 54.9 (21)</td>
<td>119.1 ± 54.4 (18)</td>
</tr>
<tr>
<td>PNa⁺, mS</td>
<td>33.5 ± 40.1</td>
<td>32.3 ± 48.3</td>
<td>P = 0.54</td>
<td>32.5 ± 32.9</td>
<td>16.0 ± 34.6</td>
<td>56.9 ± 38.5</td>
<td>49.3 ± 55.0</td>
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<tr>
<td>A-like rect.</td>
<td>0.36 ± 0.51</td>
<td>0.42 ± 0.58</td>
<td>P = 0.93</td>
<td>0.72 ± 0.54</td>
<td>0.76 ± 0.60</td>
<td>0.02 ± 0.04</td>
<td>0.02 ± 0.04</td>
</tr>
<tr>
<td>Double AHP</td>
<td>0.38 ± 0.83</td>
<td>0.14 ± 0.40</td>
<td>P = 0.017</td>
<td>0.0 ± 0</td>
<td>0 ± 0</td>
<td>0.73 ± 1.07</td>
<td>0.30 ± 0.56*</td>
</tr>
<tr>
<td>Convexity, mV</td>
<td>0.80 ± 0.63</td>
<td>0.68 ± 0.67</td>
<td>P = 0.09</td>
<td>0.56 ± 0.48</td>
<td>0.34 ± 0.50**</td>
<td>1.07 ± 0.66</td>
<td>1.10 ± 0.62</td>
</tr>
</tbody>
</table>

Values are means ± SD. n values are in parentheses. This table gives the values of the parameters characterizing the membrane and firing properties of all groups of medial vestibular nucleus neurons (MVNn) considered in this study. The third column gives for each parameter the probability of significance of the differences obtained between control and ipsilesional MVNn. * indicate the values that are significantly different between the ipsilesional 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; PNa⁺, duration of the subthreshold plateau potentials; AHP, afterhyperpolarization.
strength of the A-like rectification ranged from 0.04 to 2.56 V/s around a mean of 0.59 ± 0.53 V/s. Double AHP was present during spontaneous firing in 32 of the control MVNn (36%), and its strength ranged from 0.1 to 5.4 V/s around a mean value of 1.06 ± 1.11 V/s. The 57 other MVNn had no double AHP visible during spontaneous discharge. The maximum convexity of the voltage trace during the inter-spike interval, which was added as a pertinent parameter for the reasons described in METHODS, ranged from 0 to 2.50 mV around a mean value of 0.80 ± 0.63 mV (n = 89).

These three parameters were used to plot the three-dimensional graph shown on Fig. 4A. On this plot, most of the MVNn are clearly distributed along two separate, perpendicular planes, one defined by the presence of a double AHP (double AHP vs. convexity plane), the other one by the presence of an A-like rectification (A-like rectification vs. convexity plane). In other words, the presence of a large A-like rectification and the presence of a double AHP appeared mutually exclusive, with the exception of three MVNn that clearly displayed both (\(\downarrow\) on Fig. 4A). This means that these two parameters can be used to define two distinct groups of MVNn as shown on the two-dimensional plot of Fig. 4B. On this plot, the neurons that display a double AHP (corresponding to type B MVNn) are aligned along or close to the vertical axis because most of them display no or a small A-like rectification. In contrast, the MVNn that display a large A-like rectification (corresponding to type A MVNn) are aligned along the horizontal axis because most of them (except the 3 cells marked as \(\downarrow\) on Fig. 4B) display no double AHP.

While MVNn can clearly be categorized into two groups by this method, there is still a sizeable proportion of MVNn that display “intermediate” properties (see Fig. 4A, graph). This is in accordance with the idea that there is a continuum of neurons with intermediate properties between type A and B MVNn, put forward by Du Lac and Lisberger (1995a). The “intermediate” MVNn, which have both no double AHP and no large A-like rectification, are grouped at or near the origin of the two-dimensional graph or are aligned along the “no double AHP – no A-like rectification” axis on the three-dimensional graph. According to the graphs of Fig. 4, the intermediate MVNn do not form a distinct group of neurons; this would correspond to the former type C neurons. There was therefore no objective reason to keep these MVNn as a separate category. Therefore as already suggested by Johnston et al. (1994), MVNn are best categorized by defining only two groups of neurons corresponding to the type A and B MVNn. Because there is a continuum of neurons between these two cell types, the limit between the two groups has to be set somewhat arbitrarily. As shown on Fig. 4B, we decided to fixate the threshold of what could be considered as a “large” A-like rectification at 0.15 V/s. Indeed, several MVNn displaying a clear double AHP during spontaneous discharge were also endowed with a small A-like rectification, whose strength was inferior to 0.15 V/s. Furthermore, most of the intermediate MVNn, which had both no double AHP and no or a small A-like rectification, displayed the large convexity typical of type B MVNn (Fig. 4A).

Altogether, as shown on Fig. 4B, most MVNn were categorized as either type A or B MVNn according to the following criteria (see also Table 1): 1) the 44 MVNn displaying either no

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**FIG. 4.** Classification of the control MVNn. A: 3-dimensional plot of control MVNn according to the 3 criteria used for their classification. All neurons are included either in the xy plane (A-like rectification vs. convexity plane), or in the yz plane (double AHP vs. convexity plane). Only 3 cells were obviously out of these planes, as indicated (\(\downarrow\)). Dashed line shows the value of the A-like rectification used to separate type A and B neurons (see text for details). B: 2-dimensional plot of control MVNn according to the strength of their A-like rectification and the strength of their double AHP. This plot shows how the neurons were finally categorized, depending on whether the strength of their A-like rectification was lower or higher than 0.15 V/s.
A-like rectification \((n = 32)\) or an A-like rectification with an amplitude \(<0.15\) V/s \((n = 12)\) were classified as type B MVNn. Twenty-nine of them \((66\%)\) had a clear double AHP visible during spontaneous firing (Fig. 5A). Altogether, the mean convexity of type B MVNn reached \(1.07 \pm 0.66\) mV, the mean strength of their double AHP was \(0.73 \pm 1.07\) V/s, and the mean strength of their A-like rectification was \(0.02 \pm 0.04\) V/s. 2) The 42 MVNn displaying an A-like rectification stronger than \(0.15\) V/s, and no double AHP were classified as type A MVNn. Their mean convexity was \(0.56 \pm 0.48\) mV and was lower than for type B MVNn \((P < 0.001)\). The mean strength of the A-like rectification reached \(0.72 \pm 0.54\) V/s. And 3) the three MVNn that stood out of the two main axes on the two-dimensional graph displayed both a double AHP and an A-like rectification \(>0.15\) V/s. They could not be unambiguously categorized as either type A or type B MVNn and were therefore considered as the only true type C MVNn.

According to this classification scheme, there were 42 type A neurons \((47.1\%)\), 44 type B neurons \((49.5\%)\) including 4 B+LTS neurons, and 3 type C neurons \((3.4\%)\) among the MVNn recorded in control slices.

Altogether, the five main parametric differences that characterize type A versus type B MVNn can be summarized as follows.

First, typical type A MVNn display a strong A-like rectification, a high-amplitude AHP and broad spikes; in contrast, they have low convexity values and no double-component AHP. Even when the analysis is restricted to the 42 type A MVNn defined in the preceding text, there are significant correlations between the AHP amplitude, strength of the A-like rectification, and convexity values. Type A MVNn with the strongest A-like rectification are those that display the smallest convexity values \((r = -0.43, P = 0.004)\) and the largest AHPs \((r = 0.33, P = 0.046)\), which results in a significant, negative relationship between the amplitude of the AHP and the convexity \((r = -0.39, P = 0.017)\). But none of these parameters is significantly correlated with the width of spikes when only the type A MVNn were considered.

Second, typical type B MVNn display no or only a small A-like rectification, a small amplitude AHP and thin spikes; in contrast, they have high convexity values and often display a double component AHP. When the analysis is restricted to the 44 type B MVNn defined in the preceding text, only two significant correlations persist between these five parameters. As for type A MVNn, the strength of the A-like rectification is negatively correlated with the convexity value \((r = -0.52, P < 0.001)\). Besides, the neurons displaying strong double component AHPs are those with the thinner spikes \((r = -0.49, P = 0.001)\). Within type B neurons, there was no correlation between the amplitude of the AHP, the strength of the double AHP and the convexity of the voltage trace during the interspike interval.

![FIG. 5](http://jn.physiology.org/doi/10.220.33.2) Changes in the membrane and firing properties of type B MVNn after 1 mo of vestibular compensation. A: example of the spontaneous discharge of a control type B neuron. B: example of the spontaneous discharge of an ipsilesional type B neuron. C: superimposition at spike threshold of the averaged spike profiles obtained from the neurons of A and B. Note the difference in size and shape of the AHP. D: graph showing that for both control and ipsilesional MVNn the amplitude of the AHP tended to decrease, and not increase, when their mean resting potential was more depolarized.
Membrane and response properties of the MVNn recorded in control slices

The control data that confirm what has already been reported (Ris et al. 2001b) will be only briefly summarized. Only new results will be presented in detail.

**Basic membrane and firing properties of MVNn.** As when they were classified using qualitative criteria, the type A neurons displayed a single deep AHP (19.6 ± 3.8 mV) and a wider action potential than type B neurons ($P < 0.001$). The type B neurons had narrower action potentials and were endowed with a significantly smaller AHP (14.0 ± 3.4 mV, $P < 0.001$). Whereas 91% of type B neurons displayed subthreshold plateau potentials (Serafin et al. 1991a), only plateau potentials of much shorter duration ($P = 0.004$) could be triggered in 38% of type A neurons (Table 1). There was no difference between the spontaneous firing rate of type A and B MVNn recorded at their resting membrane potential, but the regularity of the discharge of type A MVNn (assessed by the CV) was significantly greater than for type B MVNn ($P = 0.01$). The membrane resistance of hyperpolarized MVNn was similar for both types of MVNn (Table 1).

**Responses to ramp-like currents.** Of the five ramps applied to each cell, the 600-ms (slope of 0.5 nA/s) and 200-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 ramp-like currents (Figs. 6 and 7). As described by Ris et al. (2001b), type B MVNn were more responsive to ramps than type A MVNn. The mean overshoot (see METHODS) was larger for type B than for type A MVNn for both the 600-ms (5.1 ± 4.7 vs. 1.8 ± 2.1 spikes/s, $P = 0.036$) and 200-ms ramps (8.4 ± 5.3 vs. 2.4 ± 2.2 spikes/s, $P = 0.002$) delivered from the resting membrane potential. When ramps were delivered from a hyperpolarized level (Fig. 6, A and B), this difference persisted for the 200-ms ramp (13.3 ± 9.1 vs. 6.8 ± 5.6 spikes/s, $P = 0.006$) but appeared only as a trend for the 600-ms one (5.8 ± 4.5 vs. 3.5 ± 3.1 spikes/s, $P = 0.08$). In contrast, the rate of increase of the instantaneous firing rate $k_{IF}$ over the ramp-like portion of the current injection (see METHODS) was not significantly different between type A and B MVNn for any of the

![Fig. 6. Examples of responses of MVNn to 600-ms-duration ramp-like currents before and after long-term deafferentation.](http://jn.physiology.org/.../10.1152/jn.00047.2003)

A: typical response of a control type A MVNn. Note the absence of overshoot. B: typical response of a control type B MVNn. Note the clear overshoot, which contrasts with what was obtained for the type A MVNn. C: typical response displayed by a type A MVNn recorded on the ipsilesional side of a slice taken from a compensated animal. Note the presence of an overshoot. D: typical response displayed by a type B MVNn recorded on the ipsilesional side of a slice taken from a compensated animal. Note the increased overshoot compared with the control type B MVNn.
overshoot and the sensitivity of MVNn to current injection 
increase of the relationship appeared between the overshoot and the rate of 

were 123.2 

ramps delivered from the resting membrane potential (R600 and R200). *: significant differences between control and ipsilesional MVNn (P < 0.05). B: mean rates of increase kIF (slope) of the instantaneous firing rate vs. current obtained for control MVNn and ipsilesional MVNn in response to 600- and 200-ms ramps delivered during steady-state hyperpolarization (H600 and H200) or at the resting membrane potential (R600 and R200). *: significant differences between control and ipsilesional MVNn (P < 0.05).

ramps we tested. The respective kIF obtained for the 600- and 200-ms ramps delivered from the resting membrane potential were 123.2 ± 38.0 and 126.2 ± 32.9 spikes · s⁻¹ · nA⁻¹ for type B MVNn versus 112.2 ± 33.8 and 115.3 ± 34.5 spikes · s⁻¹ · nA⁻¹ for type A MVNn (P > 0.05 in both cases).

Within each cell type, there was no relationship between the overshoot and the sensitivity of MVNn to current injection given by kIF for the 600-ms ramps. A significant positive relationship appeared between the overshoot and the rate of increase of the firing rate kIF only when all MVNn were pooled together. The coefficients of correlation reached 0.33 (P = 0.048) for the 600-ms ramps delivered from a hyperpolarized level and 0.51 (P = 0.012) for those delivered from rest.

Whatever the level of polarization of the cells, the mean kIF and overshoot of both types of neurons significantly increased when the slope of the ramps went from 0.06 to 1.5 nA/s. Compared with the ramps delivered from a hyperpolarized level, the mean overshoots and kIF of the ramps delivered from the resting membrane potential tended to be smaller for all slopes.

MEMBRANE POTENTIAL RESPONSES TO SINUSOIDAL CURRENTS DELIVERED DURING STEADY-STATE HYPERPOLARIZATION IN THE ABSENCE OF ACTION POTENTIALS. Both types of MVNn recorded on control slices responded to sinusoidal current injections in a similar way. The membrane potential modulation δVh displayed a sizeable resonance at a median peak frequency of 1 Hz for type A MVNn and 0.7 Hz for type B MVNn (Table 2, Fig. 8A1). In accordance with this slight resonance, the membrane potential response of both types of MVNn displayed a small phase lead re the injected current at the lowest frequencies of stimulation, which decreased to zero and became a phase lag at higher frequencies (Fig. 8A2). These results demonstrate that the membrane does not behave in a purely passive way at these moderately hyperpolarized levels.

RESPONSES TO SINUSOIDAL CURRENTS DELIVERED AT THE RESTING MEMBRANE POTENTIAL. In terms of instantaneous firing rate, type B MVNn were more sensitive to sinusoidal current injection at low frequency than type A MVNn (Fig. 9, A1 and B1). The greater sensitivity of type B MVNn was associated with a trend for the sensitivity of their discharge to membrane potential variations (δIF/δV) to be higher than for type A MVNn (P = 0.10 at 0.4 Hz, Table 3). Interestingly, there was a strong trend for the type A MVNn to have a higher frequency of resonance than type B MVNn. Indeed, the peak of the resonance was reached at a median frequency of 8 Hz for type A MVNn versus 4 Hz for type B MVNn (P = 0.069, Fig. 9, A1 and B1). No significant difference was found between the active impedance of type A and B MVNn (Table 3), which suggests that the difference between type A and B MVNn obtained by Ris et al. (2001b) on a smaller sample of neurons might have been linked to a sampling bias.

The active impedance Z (δV/δI) at 0.4 Hz of MVNn recorded in control slices was for both cell types much lower than the impedance Z0 obtained in the absence of action potentials (P < 0.001 when pooling all MVNn together, using the paired Wilcoxon signed-rank test). The relative amplitude of the resonance of the firing rate modulation (Fig. 8B1) was not significantly different from the relative amplitude of the resonance of the membrane potential modulation induced by sinusoidal currents in the absence of action potentials (during hyperpolarization) because of the large dispersion of the values. However, the peak frequency of the resonance (Fig. 8B1) was strongly increased compared with what was observed in the absence of spikes (P = 0.004 when pooling all MVNn together, Table 3).

Consistent with the increased peak frequency of the resonance, both type A and B MVNn displayed a slightly greater phase lead at low frequency compared with the phase values obtained during steady-state hyperpolarization (Fig. 8, A2 and B2). On the other hand, the phase lag obtained at high frequency was greater than the one displayed by the potential modulation during steady-state hyperpolarization.

RESPONSES TO SINUSOIDAL CURRENTS DELIVERED DURING STEADY-STATE DEPOLARIZATION. The difference between the sensitivity of type A and B MVNn observed at rest disappeared when the neurons were maintained under a steady-state depolarization
The peak of the resonance was reached at a median frequency of 12 Hz for type A versus 8 Hz for type B MVNn (Fig. 9, A2 and B2), but the statistical trend for type A MVNn to have a higher frequency of resonance disappeared (P = 0.29) because of the large dispersion of the values (Table 3).

Compared with the values obtained at rest, the active impedance of type A and B MVNn maintained under steady-state depolarization was lower. The mean Z value at 0.4 Hz was 47.9 ± 36.4 MΩ versus 58.5 ± 33.9 for currents delivered at rest (P = 0.001 pooling all MVNn together, Wilcoxon signed-rank test). In accordance with this decrease, the sensitivity of the instantaneous firing rate of both types of MVNn to sinusoidal current injection decreased with depolarization (Table 3, Fig. 8C1). This decrease was significant when considering the

<table>
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<tr>
<th>Parameters</th>
<th>Control MVNn</th>
<th>Ipsilesional MVNn</th>
<th>Control vs. Ipsilesional</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of neurons</td>
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<td>21</td>
<td>13</td>
</tr>
<tr>
<td>Z₀, MΩ</td>
<td>131.2 ± 58.7</td>
<td>115.4 ± 45.0</td>
<td>127.9 ± 49.6</td>
</tr>
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<td>Amplitude of resonance</td>
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<td>1.19 ± 0.11</td>
</tr>
<tr>
<td>Median peak f of resonance, Hz</td>
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<td>2</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Values are means ± SD. This table gives the values of the parameters characterizing the responses of MVNn to sinusoidal currents delivered during steady-state hyperpolarization for all groups of MVNn considered in this study. The third column gives for each parameter the probability of significance of the differences obtained between control and ipsilesional MVNn. * indicate the values that are significantly different between the ipsilesional 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.

FIG. 8. Summary of the mean magnitude and phase of the membrane potential or firing rate modulations induced in MVNn by sinusoidal current injections. A: mean magnitude (A1) and phase (A2) of the membrane potential modulation displayed by control and ipsilesional MVNn recorded under 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 Z₀ of the cell (V/I) as a function of frequency. B: mean magnitude (B1) and phase (B2) of the firing rate modulation (dIF/dI) displayed by control and ipsilesional MVNn recorded at their resting membrane potential as a function of the stimulation frequency of the sinusoidal current injection. C: mean magnitude (C1) and phase (C2) of the firing rate modulation (dIF/dI) displayed by control and ipsilesional MVNn recorded during steady-state depolarization as a function of the stimulation frequency. In all cases, SDs have been omitted for sake of clarity. * values obtained on ipsilesional MVNn that were significantly different from those obtained in control neurons (P < 0.05).
23 MVNn that were submitted to sinusoidal currents both at rest and during depolarization (P < 0.001 at 0.4 Hz, Wilcoxon signed-rank test). The amplitude of the resonance increased compared with rest for type A MVNn (Fig. 9A, P < 0.047) but was not modified for type B MVNn (Table 3). The peak frequency of the resonance was increased compared with rest for both cell types. Indeed, the median peak frequency of modulation of the firing rate reached 12 versus 8 Hz at rest for type A MVNn (P = 0.005) and 8 versus 4 Hz at rest for type A MVNn (P = 0.005, Fig. 9). In accordance with this increase of the peak frequency of resonance, the mean phase function of the depolarized MVNn was shifted by a few degrees toward smaller phase lags at intermediate and high frequencies compared with rest (Fig. 8, B2 and C2).

Membrane and response properties of MVNn recorded in slices taken from guinea pigs 1 mo after UL

**CLASSIFICATION OF THE MVNn RECORDED IN SLICES TAKEN FROM LABYRINTHECTOMIZED GUINEA PIGS.** The 78 MVNn recorded on the ipsilesional side of slices taken from lesioned animals were characterized as type A, B, or C neurons according to the quantitative criteria developed in control slices. The graph showing the distribution of ipsilesional MVNn obtained when using the three parameters used for the classification is shown on Fig. 10.

First, 35 of the 78 ipsilesional MVNn displayed either no A-like rectification (n = 26) or an A-like rectification <0.15 V/s (n = 9) and were classified as type B MVNn. Only 16 of them (46%), instead of 66% in control slices, had a clear double AHP during spontaneous firing, which ranged from 0.05 to 2.20 V/s around a mean of 0.64 ± 0.68 volts/s (see Fig. 5, A and B). Altogether, the mean strength of the double AHP of the type B MVNn recorded on the deafferented side was 0.30 ± 0.56 V/s and was significantly decreased compared with the type B MVNn recorded on control slices (P = 0.013). In contrast, long-term deafferentation did not modify the convexity, or strength of the small A-like rectification, displayed by type B MVNn (Table 1).

Second, the 42 MVNn displaying an A-like rectification >0.15 V/s and no double AHP were classified as type A MVNn. Their mean convexity was 0.34 ± 0.50 mV and was...
significantly reduced compared with control slices (P = 0.004).
The mean strength of the A-like rectification of ipsilesional type A MVNn was not significantly modified compared with control slices (Table 1).

Third, only 1 of the 78 ipsilesional MVNn (1.3%) displayed both a double AHP and an A-like rectification >0.15 V/s, and was therefore categorized as a type C MVNn.

The proportion of the different types of neurons found in the MVN was slightly modified compared with control slices, but this difference was not significant (compare Figs. 4 and 10).

The proportion of type A neurons increased to 53.8% instead of 47.1% in intact animals, whereas the proportion of type B neurons decreased from 49.5 to 44.9%. Among the type B cells, the proportion of B + LTS MVNn tended to increase (6 of 35, i.e., 17 instead of 9%). As in control slices, the spikes of type A MVN were significantly wider than those of B neurons (P < 0.001), and the AHP of type A neurons significantly increased by 5 to 10 mV (Fig. 5).

The AHP displayed by type B neurons, which reached a mean amplitude of the AHP of ipsilesional type A neurons following depolarization of 1.3 ± 0.5 mV, was therefore categorized as a type C MVNn.

As already mentioned in the preceding text, this was a consequence of the depolarization of their mean resting membrane potential of all types of ipsilesional MVNn was increased by 5 to 10 mV (Fig. 5B, Table 1). The mean potential of type B neurons increased from –56.8 ± 7.3 to –51.8 ± 5.5 mV (P = 0.002), whereas the mean potential of type B neurons increased from –60.8 ± 9.4 to –51.0 ± 3.8 mV (P < 0.001). This depolarization of the mean resting membrane potential was accompanied by a similar increase in the firing threshold of the cells (Table 1). The other main change compared with control neurons was a significant increase of the amplitude of the AHP displayed by type B neurons, which reached a mean value of 16.1 ± 2.6 versus 14.0 ± 3.4 mV (P = 0.007, Fig. 5C).

As already mentioned in the preceding text, this was a consequence of the decrease in the proportion of type B neurons displaying a double AHP (Fig. 5B). The increase in the amplitude of the AHP was associated with a significant increase of the regularity of the spontaneous discharge of type B MVNn assessed by their CV (P = 0.008, Table 1). As a consequence, the difference in the regularity of the spontaneous discharge observed between type A and B MVNn in control slices disappeared.

The increase in the amplitude of the AHP of the ipsilesional type B MVNn was not a consequence of the depolarization of their mean resting membrane potential or of the increase of their spontaneous discharge rate. Indeed, for control as well as ipsilesional B MVNn, the AHP was significantly smaller when the neurons had more depolarized resting potentials and higher spontaneous firing rates. In other words, there was a negative correlation between the amplitude of the AHP and the level of depolarization of type B MVN (Fig. 5D). Furthermore, the amplitude of the AHP of ipsilesional type A neurons following the deafferentation was not significantly modified (P = 0.46, Table 1), despite the fact they were also depolarized.

Surprisingly, there was only a trend for the spontaneous

FIG. 10. Classification of the ipsilesional MVNn. Three-dimensional plot of ipsilesional MVNn according to the 3 criteria used for the classification of MVNn. As was the case for control MVNn, most neurons are included either in the xy plane (A-like rectification vs. convexity plane), or in the yz plane (double AHP vs. convexity plane). The dotted line shows the value of the A-like rectification used to separate type A and B neurons (see text for details).

Note that the ipsilesional MVNn form a more homogenous population than control MVNn (Fig. 4) and that the strength of the double AHP of type B MVN is strongly reduced 1 mo after unilateral labyrinthectomy.

<table>
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<th>Parameters</th>
<th>Control MVNn</th>
<th>Ipsilesional MVNn</th>
<th>Control vs. Ipsilesional</th>
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<tr>
<td>No. of neurons</td>
<td>24</td>
<td>18</td>
<td>12</td>
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<td>$Z_{rest}$, MVΩ</td>
<td>47.9 ± 36.4</td>
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<td>$\Delta F/\Delta f$ rest, spikes/s · nA$^{-1}$</td>
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<tr>
<td>$\Delta F/\Delta f$ rest, spikes/s · nA$^{-1}$</td>
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<td>Amplitude of resonance at rest</td>
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<tr>
<td>Median peak f of resonance, Hz</td>
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</tbody>
</table>

Values are means ± SD. This table gives the values of the parameters characterizing the responses of MVNn to sinusoidal currents delivered in the presence of action potentials for all groups of MVNn considered in this study. The third column gives for each parameter the probability of significance of the differences obtained between control and ipsilesional MVNn. * indicate the values that are significantly different between the ipsilesional and control neurons. ** P < 0.01, *** P < 0.001. In addition, the values that correspond to significant differences are shown in bold.

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firing rate of the whole sample of ipsilesional MVNn taken at their resting membrane potential to increase compared with control slices (Table 1, \( P = 0.10 \)). The discharge rate of type B MVNn increased by \( \sim 30\% \) (\( P = 0.015 \)), but there was no significant modification of the firing rate of type A MVNn. Neither the proportion of type A (33%) and type B MVNn (79%) exhibiting subthreshold plateau potentials nor the duration of these plateau potentials was different from control (Table 1).

Because of the general depolarization and increase of firing threshold of the ipsilesional MVNn, current steps were generally delivered at less negative membrane potentials than in control slices. Indeed, the level of steady-state hyperpolarization used was set relative to the firing threshold of each cell. Despite this, the deafferented type A MVNn displayed a higher than normal input resistance \( (R_m) \) value (\( P = 0.046 \)). The same trend was visible for type B neurons (Table 1), so that the membrane resistance of the MVNn recorded on the deafferented side increased by 23% compared with the MVNn recorded on control slices (\( P = 0.018 \), Table 1).

Responses to ramp-like currents

The main effect of previous deafferentation on the response of MVNn to 600- and 200-ms ramps was a strong increase of the overshoot displayed by both types of neurons (Figs. 6 and 7A). For the ramps delivered from a hyperpolarized level, the mean overshoot of MVNn was almost multiplied by two for the 600-ms ramps (\( P = 0.007 \)) and increased by \( 43\% \) for the 200-ms ramps (\( P = 0.037 \), Fig. 7A). The overshoot of type B MVNn stayed significantly bigger than the one of type A MVNn (\( P = 0.03 \) for the 200-ms ramps). Similar results were obtained for the overshoot of the ramps delivered from rest except that the significant difference between type A and B MVNn observed in control slices did not persist following long-term deafferentation. Indeed, the increase of the overshoot was almost restricted to type A MVNn.

For the ramps delivered from a hyperpolarized level, there was no significant variation of the rate of increase of the firing rate of MVNn during the ramps \( k_{if} \) after long-term deafferentation for either type A or B MVNn (Figs. 6 and 7B). However, a significant increase of \( k_{if} \) was observed for the ramps delivered from the resting membrane potential, while MVNn were generally spontaneously active. When considering the whole sample of MVNn, \( k_{if} \) was increased by \( \sim 20\% \) for both the 600- and 200-ms ramps (\( P = 0.05 \) in both cases, Fig. 7B). This increase tended to be stronger for type B than for type A MVNn.

As stated in Methods, ramps applied from a hyperpolarized level were always delivered while the cell was maintained at \( \sim 10 \, \text{mV} \) below its firing threshold (Fig. 2). This means that the ramps were delivered on the ipsilesional MVNn at a more depolarized level than on control cells. However, the increases of the overshoot and \( k_{if} \) displayed by ipsilesional MVNn were not linked to the fact that their mean resting membrane potential was depolarized compared with control slices. Indeed, as stated above, the overshoot and \( k_{if} \) of control MVNn always tended to decrease when the ramps were delivered from rest compared with more hyperpolarized levels.

Whatever the level of polarization of the cells, the rate of increase and overshoot of the firing rate of MVNn followed the same trends according to the slope of the ramp as for the neurons recorded in control slices (Fig. 7).

MEMBRANE POTENTIAL RESPONSES TO SINUSOIDAL CURRENTS DELIVERED DURING STADY-STATE HYPERPOLARIZATION IN THE ABSENCE OF ACTION POTENTIALS. After UL, the impedance of the deafferented MVNn tended to decrease for both cell types, but this decrease did not reach significance (Table 2). The Bode plot relating the amplitude of the response to the stimulation frequency was rather similar to the one obtained in control slices (Fig. 8A1). However, the peak frequency of resonance was significantly increased (\( P = 0.015 \)) with a median value of 2 versus 1 Hz when considering all MVNn together (Table 2). The amplitude of the resonance of the deafferented MVNn was not modified.

In accordance with this higher peak frequency of resonance, the phase lead displayed by both types of ipsilesional MVNn (Fig. 8A2) became greater at low frequency (0.4–4 Hz) and thus reached zero for a higher frequency of stimulation (median of 1 instead of 0.4 Hz, \( P = 0.023 \)). The phase lag observed at high-frequency (\( \geq 30 \, \text{Hz} \)) was significantly greater for the deafferented MVNn than for the neurons recorded in control slices.

RESPONSES TO SINUSOIDAL CURRENTS DELIVERED IN THE PRESENCE OF ACTION POTENTIALS. Whether the sinusoidal currents were delivered at rest or during steady-state depolarization, the responses of MVNn recorded on the ipsilesional side of slices taken from compensated animals were modified in the same way compared with control MVNn. Results obtained in these two situations are therefore presented together.

After UL, the active impedance \( Z \) of the ipsilesional MVNn was not modified, whether the sinusoidal currents were delivered at rest (Table 3) or during depolarization. Despite the lack of impedance modification, the sensitivity of the discharge of both types of MVNn to current injection \( (\delta I / \delta I) \) tended to increased (Table 3). When all MVNn were pooled together, the sensitivity of the neurons was significantly increased at most frequencies ranging from 0.2 to 12 Hz at rest and from 0.2 to 40 Hz during steady-state depolarization (Fig. 8, B1 and C1). For instance, the \( \delta I / \delta I \) at 0.4 Hz of the deafferented MVNn increased by 16% compared with normal controls at the resting membrane potential (\( P = 0.049 \), Table 3) and by 27% during steady-state depolarization (\( P = 0.036 \)). The sensitivity of the discharge to membrane potential variations, measured at 0.4 Hz (\( \delta I / \delta V \)), tended to increase after deafferentation, but this change did not reach significance because of the large dispersion of the values.

When type A and B MVNn were considered separately, there was a stronger increase of the sensitivity of the discharge to current injections \( (\delta I / \delta I) \) for type B MVNn than type A MVNn. Indeed, the sensitivity of the ipsilesional type A MVNn did not increase significantly compared with the type A MVNn recorded in control slices. In contrast, the sensitivity of the ipsilesional type B MVNn was significantly increased at all frequencies ranging from 12 to 20 Hz when the sinusoidal currents were delivered at the resting membrane potential, and from 6 to 30 Hz during steady-state depolarization (Fig. 9).

Whatever the level of polarization of the cells, the mean amplitude of the resonance of MVNn was not significantly modified by the lesion (Table 3). However, the median peak frequency of the resonance of the deafferented MVNn tended
neurons (Fig. 9B, 1) increase was actually restricted to the deafferented type B neurons (Fig. 9B, 1 and 2). Their median peak frequency reached 8 Hz instead of 4 Hz at the resting membrane potential (P = 0.033) and 14 Hz instead of 8 Hz during steady-state depolarization (P = 0.06). This increase might be linked to the fact that the ipsilesional type B MVNn were more depolarized than control type B MVNn, because in control slices, steady-state depolarization was also modified compared with control slices (Fig. 9A, 1 and 2). Altogether, the peak frequencies of resonance were now similar for type B and A MVNn, and the Bode plots of the deafferented type A and B MVNn displayed more similar profiles than on control slices (Fig. 9).

The phase response of both type A and type B MVNn was slightly modified by long-term deafferentation. The mean phase function of both types of deafferented MVNn was shifted by a few degrees toward greater phase leads and smaller phase lags over the whole range of frequencies compared with control slices (Fig. 8, B2 and C2).

**Summary of the main long-term effects of labyrinthectomy on the ipsilesional MVNn**

Long-term deafferentation of the vestibular nuclei induced a depolarization of the average resting membrane potential and firing threshold of all MVNn by ~7 mV (Fig. 5). This depolarization was associated with an increase in the resting discharge of type B but not type A MVNn. In addition, there was a selective increase of the size of the AHP and regularity of discharge of type B MVNn associated with a decrease in the proportion of type B neurons displaying a double AHP (Fig. 5). The input resistance of MVNn, obtained from current steps, increased after the lesion for both types of neurons. The ramps (Figs. 6 and 7) revealed a strong increase of the overshoot induced by the steepest slopes for both types of deafferented MVNn. The sensitivity kΩ of the discharge of MVNn to ramp-like current injection increased, but only when ramps were delivered from the resting membrane potential. The modulation of the instantaneous firing rate of MVNn by sinusoidal currents was also modified after UL (Fig. 8). The sensitivity of their discharge to sinusoidal current injection (δIF/δI) was increased over the whole range of frequency tested. This increase in sensitivity was greater for type B than for type A MVNn. Long-term deafferentation did not modify the amplitude of the resonance of type B MVNn, which shifted from 4 to 8 Hz at rest and from 8 to 14 Hz during steady-state depolarization (Fig. 9).

**Discussion**

Our data reveal that UL induces long-term changes in the neuronal properties of the ipsilesional MVN neurons. Qualitative evaluation of the modifications observed one month after the lesion suggests that they were more extensive than the changes reported 7–10 days after the lesion in the rat or guinea pig (see following text). This means that at the neuronal level, the plastic processes triggered by labyrinthectomy continue well after the first few days of compensation, long after the ipsilesional vestibular neurons have recovered their spontaneous discharge and the static deficits have disappeared.

**Categorization of MVNn**

In our previous publications, MVN neurons have been categorized into type A and B neurons using qualitative criteria only. For this study, quantitative criteria were developed to characterize the intracellularly recorded MVNn. Using a method introduced by Johnston et al. (1994), an averaged spike profile was obtained for each MVNn during spontaneous firing. This spike profile and its first derivative were used to assess the presence and quantify the strength of the A-like rectification and double AHP, which were the two main criteria used previously for the qualitative classification. The convexity of the voltage trace during the inter-spike interval was added as a third parameter for classification. While two distinct groups of MVNn could be defined using these parameters, a sizeable proportion of MVNn still displayed “intermediate” properties (in accordance with the idea of a continuum of neurons put forward by Du Lac and Lisberger 1995a). However, there was no objective reason to keep these intermediate MVNn as a separate category. In agreement with Johnston et al. (1994), our analysis shows that MVNn are best categorized by defining only two distinct groups of neurons corresponding to the qualitatively defined type A and B MVNn.

According to the quantified classification scheme, control MVNn included ~50% each of type A and B neurons in the guinea pig. Compared with the proportions previously reported using qualitative classification (in average 35% of type A MVNn, 50% of type B MVNn, and 15% of type C MVNn), the main change was an increase in the proportion of MVNn classified as type A neurons. Most of the MVNn that we previously considered as type C neurons were re-classified as type A MVNn. In previous works performed both in the guinea pig and rat (Him and Dutia 2001; Johnston et al. 1994; Serafin et al. 1991a), the qualitatively defined type B MVNn displayed significantly thinner spikes and smaller AHPs than type A MVNn. They were also characterized by the significantly different overshoots obtained in response to steep current ramps (Ris et al. 2001b). Despite the increase in the proportion of MVNn classified as type A neurons, all these significant differences between type A and B MVNn persisted when the quantified classification scheme was used (see RESULTS).

Compared with the data obtained in the rat and mouse (Dutia and Johnston 1998; Him and Dutia 2001; Johnston et al. 1994), in the guinea pig, the proportion of type A MVNn is higher (~50% instead of 20–25%) and the proportion of type B MVNn is lower. All type B MVNn display a double AHP during spontaneous firing in the rat and mouse (because this is the criterion used by these authors to define type B MVNn). In contrast, only 66% of the type B neurons and 33% of all MVNn display a double AHP at rest in the guinea pig. Altogether, rat and mice seem to have more phasic MVNn and less tonic neurons than guinea pigs (see Babalian et al. 1997 for the equivalence between the in vitro and in vivo classifications of
shown that the spontaneous firing rate of type B MVNn was associated with an increase in the spontaneous firing rate of type B MVNn but not type A MVNn. In our case, however, there was a discrepancy between the depolarization of the membrane potential and firing threshold that affected both type A and B MVNn and the fact that only the spontaneous firing rate of type B MVNn was increased. This discrepancy might be a consequence of the different membrane properties displayed by type A versus type B MVNn. Indeed, the sensitivity of the firing rate of MVN to membrane potential variations assessed at their resting membrane potential from their responses to sinusoidal stimuli tended to be higher for type B than for type A MVNn even if this trend was not significant. The overall absence of strong increase of the spontaneous firing rate of the whole sample of MVNn (Table 1) contrasts with what was found at the same long-term stage of compensation using extracellular recordings (Vibert et al. 1999b) and may be due to our use of intracellular recording techniques. Indeed, the average discharge rate of MVNn assessed using extracellular (Darlington et al. 1995; Ris et al. 2001a; Vibert et al. 1999b) or patch-clamp electrodes (Him and Dutia 2001; Murphy and Du Lac 2001) is 10–15 spikes/s in control slices. In contrast, intracellular recordings with sharp electrodes give values of 20–30 spikes/s even when performed by the same groups as in the preceding text (Du Lac and Lisberger 1995a; Gallagher et al. 1985; Johnston et al. 1994; Serafin et al. 1991a). The firing rate of the ipsilesional MVNn recorded on slices after various times of compensation using extracellular or whole cell patch electrodes reached at most 23 spikes/s despite its increase compared with control slices (Him and Dutia 2001; Vibert et al. 1999b). It stayed lower than the spontaneous discharge obtained with sharp electrodes in control slices. Thus the increase in the firing rate of control MVNn induced by sharp electrode penetration might reduce the apparent relative increase of firing rate normally associated with the compensation process. No other difference between the membrane properties of MVNn recorded with patch-clamp versus sharp electrodes, including their resting membrane potential, has been reported.

Long-term deafferentation induced a strong increase of the overshoot triggered by the steepest slopes for both types of MVNn. These data confirm results obtained by Ris et al. (2001c, 2002), who already observed a significant increase of the overshoot of the ipsilesional MVNn of guinea pigs 7 days after the lesion, which was, however, restricted to type B MVN neurons. Furthermore, contrary to what happened after 1 wk, the increase of the overshoot was associated with a higher sensitivity of the firing rate of MVN neurons (particularly type B MVNn) to current injection performed at the resting membrane potential (i.e., while the neurons were spontaneously active).

In summary, our data show that all the changes in the membrane and response properties of MVNn that were observed after 7–10 days of compensation were still present 3 wk later, i.e., 1 mo after the lesion. However, while these changes were restricted to type B MVNn after the first week of compensation, most of them concerned both the ipsilesional type A and B MVNn after 1 mo. No major discrepancy appeared despite the different stimulation and recording protocols used in the various studies. New modifications that were not present at all after just 7–10 days of
compensation were also observed, like the increase in the AHP amplitude and discharge regularity of type B MVNn or the increase of the sensitivity of the firing rate of MVN neurons to ramp-like current injections. As pointed out in the preceding text, the plastic processes triggered at the neuronal level by labyrinthectomy continue well after the first few days of compensation, long after the static deficits triggered by the lesion have disappeared. Because no data have been published about the responses to sinusoidal current injection displayed by ipsilesional MVNn after 7–10 days of compensation, we were not able to evaluate the time course of appearance of the modifications of these responses observed 1 mo after the lesion.

Functional implications of the long-term changes of the membrane properties of MVNn induced by UL

Because the vestibular nerve afferent fibers provide a permanent excitatory input to MVNn, the MVN neurons should be more depolarized in vivo than they are on slices. After the loss of vestibular nerve afferents induced in vivo by UL, the mean resting potential of the ipsilesional MVNn should then become more hyperpolarized. In the guinea pig, there is a complete in vivo recovery of the spontaneous discharge of the deafferented MVNn during the first week of compensation (Ris et al. 1995, 1997). On the other hand, the in vitro studies demonstrate only moderate modifications of the spontaneous resting discharge and resting membrane potential of the ipsilesional MVNn in slices taken after 1 wk of compensation, restricted to type B neurons (Him and Dutia 2001; Ris et al. 2001c, 2002). The general depolarization of the ipsilesional MVNn by 5–10 mV we report at 1 mo suggests that their resting discharge recovered in vivo is more sustained at that stage by changes in their intrinsic membrane properties. Because this depolarization is accompanied by a similar increase of the firing threshold of the neurons, the resting discharge recovered by the deafferented MVNn during the first week after the lesion in vivo would not be modified later on.

The long-term increase of the size of the AHP and regularity of discharge of type B neurons observed after 1 mo of vestibular compensation was associated with a trend for the proportion of type A MVNn, which displays a large, single component AHP, to augment after long-term deafferentation. All this could increase in vivo the stability of the spontaneous discharge recovered by the deafferented MVNn. Indeed, bigger AHPs should limit the sensitivity of MVNn to small amplitude synaptic inputs, as shown by Babalian et al. (1997). Stability of the spontaneous discharge is probably an essential requirement for a proper functioning of the vestibular system, and suppression of the labyrinthine afferents generates instability in central vestibular networks. Indeed, UL is associated with an increase in the irregularity of the spontaneous discharge of the ipsilesional, second-order MVNn recorded in isolated whole brains (Vibert et al. 1999a). Bilateral labyrinthectomy induces a strong instability of the resting posture and head position that decreases with time but never disappears (Ris and Godaux 1998). The increase of the size of the AHP and regularity of discharge of type B MVNn after long-term deafferentation was associated with a decrease of the strength of the double AHP displayed by these neurons. The proportion of type B MVNn displaying a double AHP also decreased. Altogether, after long-term deafferentation, the basic membrane properties of type B MVNn tended to become more similar to those of type A MVNn than in control slices.

In vivo studies have shown that UL induces major deficits of the vestibulospinal and vestibulooocular synergies. The horizontal vestibulooocular reflex (HVOR) improves over several weeks in the low- and middle-frequency range of head movements, between 0.1 and ~10 Hz, both in guinea pigs and monkeys (Gilchrist et al. 1998; Lasker et al. 2000; Vibert et al. 1993). However, the quality of the recovery decreases with the amplitude of the movement, and there is no dynamic recovery for high acceleration impulses corresponding to high-frequency stimuli.

The main change induced by long-term deafferentation was a significant increase in the sensitivity of MVNn to both ramp-like and sinusoidal currents delivered at the resting membrane potential, i.e., in the presence of action potentials. In both cases, this increase was stronger for type B than for type A MVNn. The higher sensitivity of ipsilesional MVNn was not due to the fact that they were more depolarized than the MVNn recorded in control slices. Indeed, responses to sinusoidal currents delivered during steady-state depolarization show that in control slices, depolarization induces a decrease, and not an increase, of the sensitivity of MVNn neurons. The ramp-like currents we used were high-amplitude stimuli, but their main frequency component ranged from 0.2 Hz for the 5,000-ms ramps to 5 Hz for the 200-ms ramps. The fundamental frequency of ramps is therefore inside the low- and middle-frequency range of head movements, where the dynamic vestibular synergies recover in vivo (Gilchrist et al. 1998; Vibert et al. 1993). All this suggests that the increased overshoot displayed by the ipsilesional MVNn and the increased sensitivity of MVNn to ramp-like and sinusoidal currents might be involved in this recovery. This would be in accordance with the increased transmission by vestibuloocular pathways found in vivo by Broussard and Hong (1999). There was an apparent discrepancy between the higher sensitivity of the ipsilesional type B MVNn to ramp-like and sinusoidal currents and the increase of their AHP, which should make them less sensitive to current changes. However, while the increased AHP must give type B MVNn a greater stability and smaller sensitivity to small, random synaptic noise (see Babalian et al. 1997), it did not apparently alter their sensitivity to high-amplitude ramp-like and sinusoidal potential modulations that reach 5–10 mV or more in most cases.

Detailed analysis of the data obtained in control slices revealed that compared with type A MVNn, the peak frequency of resonance of type B MVNn was lower, whereas the amplitude of their resonance was similar or higher. In accordance with the model put forward by Minor et al. (1999) for the monkey HVOR, we have suggested (Ris et al. 2001b) that at high frequencies, type B MVNn might work *in a nonlinear way*, such as a signal detection mode, to amplify the contribution of vestibular nerve afferents. This should apply to guinea pigs because the HVOR dynamics and the way they are affected by UL appear roughly similar in all mammalian species (Broussard et al. 1999; Escudero et al. 1993; Gilchrist et al. 1998; Minor et al. 1999; Smith and Curthoys 1989). Because our sinusoidal stimulations were designed to evoke only linear responses, the decrease of the firing rate modulation displayed by type B MVNn >4 Hz might result from the progressive
involvement of nonlinear properties in the responses of type B MVNn at higher frequencies. In other words, type B MVNn would display linear responses over a smaller range of amplitude and frequencies than type A MVNn but would be the only cells able to respond to high-frequency, high-amplitude stimuli by working as signal detectors.

Remarkably, the increased sensitivity of the ipsilesional type B MVNn was associated with an increase of their peak frequency of resonance. However, in vivo studies in guinea pigs, monkeys, and humans have shown that the dynamic vestibular reflexes triggered by high-frequency stimuli like velocity steps would be definitively impaired on the ipsilesional side after UL (Curthoys 2000; Gilchrist et al. 1998; Halmagyi et al. 1990; Lasker et al. 2000). This discrepancy might be due to the fact that we used low-amplitude sinusoidal currents to keep the responses of MVNn in the linear range. The increase in the peak frequency of resonance of the ipsilesional type B MVNn suggests that the increased amplitude of their AHP is associated with an extension of the frequency range of their linear responses like in A MVNn. In accordance with the fact that the basic properties of type B MVNn come closer to those of type A MVNn after one month of compensation (see preceding text), the dynamic response profiles of type A and B neurons become more similar in control slices. This transformation of the ipsilesional type B MVNn might impair their nonlinear properties and thus could explain the suppression in vivo of the responses to high-amplitude, high-frequency head movements directed toward the ipsilesional side.

Conclusion

Because synaptic uncoupling conditions were not used, we cannot exclude that the changes in the membrane and response properties of MVNn we observed might indirectly be due to changes in neurotransmitter receptor activation. It is, however, unlikely because the increase in the discharge rate of extracellularly recorded, ipsilesional MVNn obtained on slices taken after 1 mo of compensation persisted in conditions of synaptic uncoupling (Vibert et al. 1999b).

As pointed out in the preceding text, only the membrane properties of type B MVNn were modified after 7–10 days of vestibular compensation. Even if most of these changes extended to type A MVNn 3 wk later, our data show that the type B MVNn were still more modified than type A MVNn by long-term deafferentation. In several respects, the static and dynamic membrane properties of type B MVNn became more similar to those of type A MVNn than in control slices, and the overall homogeneity of MVNn was increased. The fact that both the resting membrane potential and firing threshold of the neurons became more depolarized suggests that changes in active conductance compensated for the loss of excitatory afferents.

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