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PLASTIC CHANGES UNDERLYING VESTIBULAR COMPENSATION IN THE GUINEA-PIG PERSIST IN ISOLATED, *IN VITRO* WHOLE BRAIN PREPARATIONS

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Abstract—Vestibular compensation for the postural and oculomotor deficits induced by unilateral labyrinthectomy is a model of post-lesional plasticity in the central nervous system. Just after the removal of one labyrinth, the deafferented, ipsilateral vestibular nucleus neurons are almost silent, and the discharge of the contralateral vestibular nucleus neurons is increased. The associated static disorders disappear in a few days, as normal activity is restored in both vestibular nuclei. In this study, we searched for traces of vestibular compensation in isolated whole brains taken from adult guinea-pigs. The electrophysiological responses evoked in control brains were compared to those evoked in brains taken from animals that had previously been labyrinthectomized. Guinea-pigs compensated for an initial labyrinthectomy within three days. *In vivo*, subsequent deafferentation of vestibular nucleus neurons on the intact side triggered "Bechterew's phenomenon": a new postural and oculomotor syndrome appeared, similar to the one induced by the first lesion, but directed to the newly deafferented side. These disturbances would be caused by the new imbalance between the discharges of neurons in the two vestibular nuclei triggered by the second deafferentation. Experiments were designed to search for a similar imbalance *in vitro* in brains taken from labyrinthectomized animals, where the intact vestibular nerve is cut during the dissection. Isolated whole brains were obtained from young guinea-pigs at various times (one to seven days) following an initial labyrinthectomy. An imbalance between the resting activities of medial vestibular nucleus neurons on both sides of the brainstem was revealed in brains taken more than three days after the lesion: their discharge was higher on the compensated, initially lesioned side than on the newly deafferented side. In some cases, an oscillatory pattern of discharge, reminiscent of the spontaneous nystagmus associated *in vivo* with Bechterew's syndrome, appeared in both abducens nerves. These data demonstrate that most of the changes underlying vestibular compensation persist, and can thus be investigated in the isolated whole brain preparation.

Brains removed only one day after the lesion displayed normal commissural responses and symmetric spinal inputs to vestibular nucleus neurons. However, an unusually large proportion of the neurons recorded on both sides of the preparation had very irregular spontaneous discharge rates. These data suggest that the first stages of vestibular compensation might be associated with transient changes in the membrane properties of vestibular nucleus neurons. Brains taken from compensated animals displayed a significant, bilateral decrease of the inhibitory commissural responses evoked in the medial vestibular nucleus by single-shock stimulation of the contralateral vestibular nerve. The sensitivity of abducens motoneurons on the initially lesioned, compensated side to synaptic activation from the contralesional vestibular nucleus neurons was also decreased. Both changes may explain the long-term, bilateral decrease of vestibular-related reflexes observed following unilateral labyrinthectomy. Spinal inputs to vestibular nucleus neurons became progressively asymmetric: their efficacy was increased on the lesioned side and decreased on the intact one. This last modification may support a functional substitution of the deficient, vestibular-related synergies involved in gaze and posture stabilization by neck-related reflexes. © 1999 IBRO. Published by Elsevier Science Ltd.

Key words: vestibulo-ocular reflex, abducens nerve, postural control, post-lesional plasticity.

In order to stabilize gaze and posture, the multiple internal representations of an event (e.g., head rotation) obtained through different sensory modalities (mostly the visual, vestibular and proprioceptive ones) are matched into a single frame of reference in which appropriate motor commands can be coded. Such complex sensorimotor transformations are highly sensitive to pathological damage, excessive natural stimulations and/or exposure to conflicting sensory information, and therefore display a high degree of plasticity.[55]

A remarkable example of this plasticity can be observed following unilateral removal of one labyrinth. The strong oculomotor and postural syndrome induced by such a lesion recovers to a large extent, in a process known as vestibular compensation (see Refs 9, 10, 44 and 49 for reviews). The static deficits, which include major distortions of posture, associated with an ocular nystagmus with quick phases directed towards the intact side, disappear over a few days in most species.[57] The dynamic deficits include a reduced gain and an abnormal timing of the vestibulo-ocular and vestibulo-spinal reflexes. They also improve over a period of several weeks, though this recovery is partial, and is limited to the low and middle frequency ranges of head movements.[52]

The neuronal substrates of vestibular compensation (Fig. 1) have been studied extensively *in vivo*. Following unilateral labyrinthectomy, the ipsilesional vestibular nucleus neurons (VNns) are deprived of the massive excitatory input coming from the labyrinthine sensory afferents and lose their resting discharge, which averages about 30–40 spikes/s in alert vertebrates.[30,38] The VNns on the contralesional side increase their firing rate, following suppression of the commissural inhibition normally exerted by the deafferented VNns

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Abbreviations: AHP, afterhyperpolarization; CV, coefficient of variation; D*n*, day *n* (i.e. the number of days between the two lesions); IWB, isolated *in vitro* whole brain; MVN, medial vestibular nucleus; VNn, vestibular nucleus neuron.

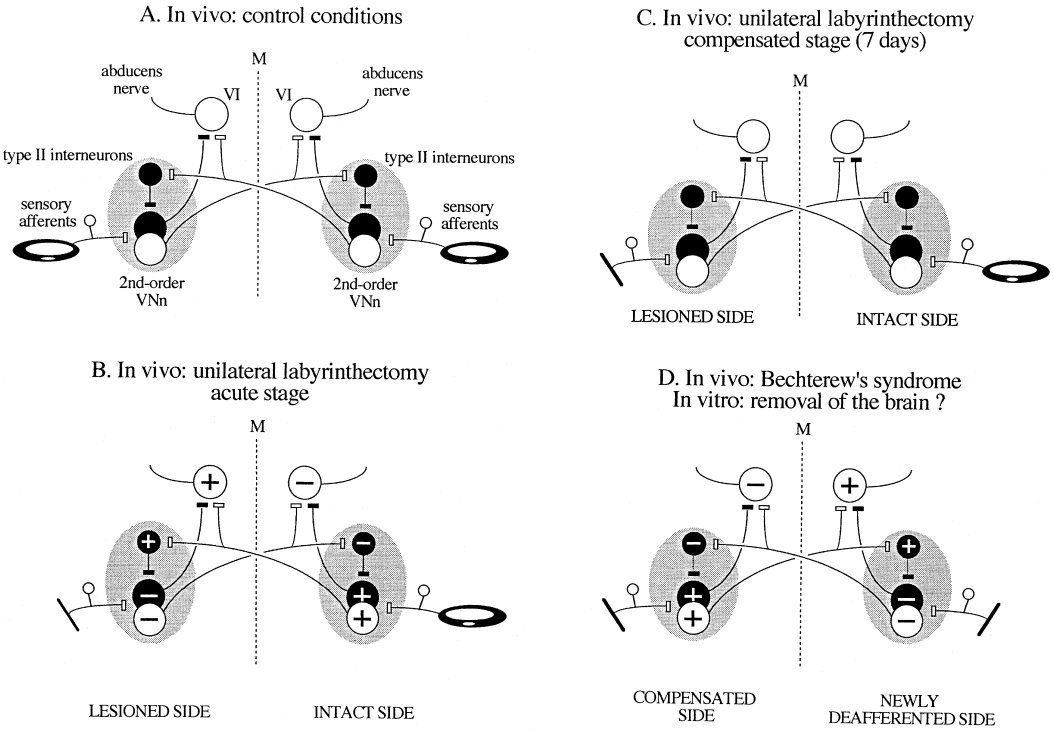

Fig. 1. Evolution of spontaneous neuronal activity in vestibular-related pathways during vestibular compensation and after subsequent deafferentation of the intact side (Bechterew's phenomenon). The excitatory neurons and synapses are shown in white, whereas the inhibitory neurons and synapses are drawn in black. In A and C, the absence of signs inside the neurons indicate balanced levels of spontaneous activity between both sides of the brain. In B and D, the + signs indicate the neurons displaying a significantly higher than normal spontaneous activity, while the − signs indicate those displaying a lower than normal discharge rate. M, the median line of the brain; VI, abducens motoneurons.

(Fig. 1A, B). The resulting imbalance between the mass discharges of the VNns in the right and left vestibular nuclei is responsible for the static deficits. During the few days following the lesion, the recovery of a normal resting discharge by the deafferented VNns[38,56] plays a key role in the disappearance of the static syndrome (for reviews see Refs 9, 37 and 49; Fig. 1C) by restoring the balance between the spontaneous activities of neurons in the two vestibular nuclei. Since the labyrinthine sensory afferents on the lesioned side remain silent,[20,46] one of the main questions about vestibular compensation is: how do the ipsilesional second-order VNns recover a normal spontaneous activity?

The cellular events underlying this recuperation are still controversial.[9,10,30,31,49,55] They may include changes in the intrinsic membrane properties of central vestibular neurons and/or modifications of their sensitivity to neurotransmitters. In addition, various studies suggest that the suppression of the sensory vestibular input on one side would be partly compensated by changes in the efficacy of other synaptic inputs to the deafferented VNns. The other sensory information reaching the vestibular nuclei, mainly from the visual and spinal proprioceptive pathways, may play an important role in both the induction and maintenance of vestibular compensation. Several authors also suggested an involvement of (i) the inhibitory afferents coming from the cerebellum or (ii) the commissural fibers linking together the two vestibular complexes in the compensation process. Similarly, the partial recovery of the dynamic vestibular reflexes could rely on functional modulations of the efficacy of the spinal, visual, cerebellar and/or cortical pathways that still converge on the ipsilesional VNns (for reviews see Refs 9, 10, 49 and 55).

Since most of these parameters are difficult to quantify *in vivo*, the first aim of this study was to test whether the isolated, *in vitro* whole brain of adult guinea-pig (IWB)[28] was suitable to investigate these changes. Several arguments favor the use of the IWB: (i) the guinea-pig has been used extensively to study vestibular plasticity;[55] (ii) on the IWB, the recorded neurons can be identified through their synaptic connections, which is mandatory to relate the data collected previously *in vivo* with the *in vitro* results obtained on slices; (iii) we recently demonstrated the viability of vestibular-related pathways in the IWB, and assessed some of their main physiological and pharmacological properties.[3] The results obtained in IWBs taken from control guinea-pigs were thus used as a reference to search whether traces of the plastic, cellular changes underlying vestibular compensation could be retained in IWBs taken from previously labyrinthectomized animals.

The second aim of this study was to investigate whether, following unilateral labyrinthectomy, the new balance reached between the spontaneous activity of VNns on both sides of the brain was dependent on a continuous inflow of substituting sensory signals, or could become at least partially independent of visual and spinal proprioceptive information. Indeed, the IWB offers an interesting opportunity to study this problem, since the sensory inputs reaching the second-order VNns are all suppressed in this totally deafferented preparation, and thus would not be responsible for the possible persistence of changes related to the compensation process.

On the other hand, this complete deafferentation prevented us from using intact vestibular-related networks (i.e. still connected to a functional labyrinth) as references, since the "control" IWBs taken from normal animals are acutely bilabyrinthectomized preparations. Therefore, we lesioned

the vestibular apparatus of guinea-pigs on one side at various times (one, three, five and seven to eight days) before the preparation of the IWB. This allowed us to compare the properties of vestibular-related networks that had a different history *vis-à-vis* the lesion. In one half-brain (called the "compensated side"), vestibular-related neurons had one, three, five or seven to eight days to compensate for the unilateral labyrinthectomy. On the other side (called the "newly deafferented side"), the same structures were only disconnected from the ipsilateral labyrinth when the brain was removed.

Previous *in vivo* experiments demonstrated that, following compensation for an initial, unilateral labyrinthectomy, such an acute deafferentation of the contralesional VNns triggers a new oculomotor and postural syndrome, known as the Bechterew phenomenon.[4,44] This syndrome is similar to the one induced by the first labyrinthectomy, but directed towards the side of the second lesion. The second deafferentation would disrupt the new balance reached between the spontaneous activity of neurons in the two vestibular nuclei after the initial lesion (Fig. 1C, D). On the side of the second lesion, the newly deafferented VNns are less excited because they lose their labyrinthine afferents. In contrast, the discharge of the first deafferented VNns (which recovered a normal activity during the course of compensation) increases because the commissural inhibition coming from the newly deafferented side is reduced (Fig. 1D).

Since we were expecting to deal with an *in vitro* equivalent of this Bechterew syndrome, a complementary behavioral study was done to evaluate the intensity of the Bechterew phenomenon induced *in vivo*, in age-matched guinea-pigs, during the same first week of compensation. The time-course of the network modifications observed *in vitro* in the IWB was compared with the time-course of the *in vivo* Bechterew phenomenon triggered.

Parts of the results presented here have been published as preliminary reports.[53,54]

EXPERIMENTAL PROCEDURES

Animals: surgical procedures

Experiments were performed on young, adult pigmented guinea-pigs (*Cavia porcellus*) of both genders, weighing 130–300 g (Elevage de la Garenne, Saint-Pierre d'Exideuil, France). All studies were carried out in accordance with the European Communities Council directive of 24 November 1986, and followed the procedures issued by the French Ministère de l'Agriculture. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Unilateral labyrinthectomies were performed under halothane anesthesia with the aid of an operating microscope. The semicircular canals, utricle and saccule were exposed via a retroauricular approach. The ampullae of all three semicircular canals and the otolithic maculae were drilled, and further destroyed using suction. The animals were then let free to compensate in normal visual conditions until their brain was dissected out, or a second labyrinthectomy was performed on the intact side (for the *in vivo* study of Bechterew's phenomenon).

In vitro *experiments*

The data reported in the present paper were collected in 65 IWBs taken from guinea-pigs which had been unilaterally labyrinthectomized 20–24 h (one day, $n=14$, D1 brains), three days ($n=26$, D3 brains), five days ($n=10$, D5 brains) or seven to eight days ($n=15$, D7 brains) before.

Because no significant difference was observed between D5 and D7 brains, results obtained in these brains were pooled together as data collected in D5–D7 brains. Similarly, in the many cases where similar results were obtained in IWBs prepared three or more days after the first lesion (i.e. D3, D5 and D7 brains), they were grouped together as data collected in D3–D7 brains. The rationale for this grouping was that, in contrast to D1 brains, behavioral compensation for the static deficits induced by the initial labyrinthectomy was complete in all D3–D7 brains.

Given the difficulty of the surgical procedure, not all experiments yielded usable data. Brains were only used if the amplitude of the field potential evoked in the vestibular nuclei by stimulation of the ipsilateral vestibular nerve, and/or of the response evoked in the abducens nerves by stimulation of the contralateral vestibular nerves, reached minimal values of 0.35 and 0.5 mV, respectively. These reference values were taken from our recent study of vestibular-related networks in the IWBs of control guinea-pigs.[3] The low minimal amplitude retained for the field potential evoked in the vestibular nuclei also takes into account the decrease in the size of this field potential observed in D3–D7 brains (see below).

Isolation and maintenance of the in vitro *whole brain.* The methods used to isolate the brain were modifications[3] of those originally described by Mühlethaler *et al.*[28] Following anesthesia, the animal was transcardially perfused for 0.5–1 min with Ringer solution at room temperature, and then for 5 min with cold (8–12°C) Ringer. Subsequently, the animal was then decapitated, and the bones covering the brain and spinal cord were broken and removed. The exposed brain was cut in the front at the level of the olfactory bulbs, and in the back at the level of the C1–C2 cervical segments. The brain was isolated from the skull by severing the different cranial nerves, the carotid, hypothalamic and vertebral arteries, and the first spinal cord roots. It was then placed in cold saline and transferred to the incubation/recording chamber.

In the chamber, the brain was submerged in saline and first maintained at 13°C. Once the brain was fixed by rubber strings, one of the vertebral arteries was separated from the surrounding tissue. A fine, stainless steel cannula (external diameter 0.4 mm) connected to the perfusion system was inserted inside and fixed with knots of fine silk thread. The second vertebral artery was closed and the perfusion rate was increased from its initial rate of 0.8–1.0 ml/min to 2.0–2.5 ml/min.

The next step was to close the two carotid arteries, which were the major source of leaks of perfusate from the brain vascular system. The hypophyseal, labyrinthine, spinal and other small arteries, which had been severed during the removal of the brain, were then all sutured. Following elimination of major leaks from the arterial system of the brain, the temperature of the chamber and perfusate was gradually increased to 29–30°C. The perfusion rate was increased progressively during rewarming, to reach 4.5–5.5 ml/min. After rewarming, the average survival time of isolated brains was 6–7 h.

A peristaltic pump (Gilson Minipuls 3) was used to drive the perfusion system and to evacuate fluid from the chamber. The brain perfusate went through a pressure transducer (Druck), a bubble trap and a filtering unit (Millipore, 0.22 μm pore diameter). The temperature of the IWB was controlled by a thermoregulation unit (Biomedical Engineering, Thornwood, U.S.A.).

The physiological Ringer solution, used for (i) transcardial perfusion of the animal, (ii) moistening of the brain during the dissection and (iii) initial filling of the chamber, had the following composition (in mM): NaCl 130, $NaHCO_3$ 20, $MgSO_4$ 1.3, KH_2PO_4 1.25, KCl 5, $CaCl_2$ 2.4, glucose 10. The saline used for perfusion of the brain had a slightly different composition and contained (in mM): NaCl 126, $NaHCO_3$ 26, $MgSO_4$ 1.3, KH_2PO_4 1.2, KCl 3, $CaCl_2$ 2.4, glucose 15. This solution included 1.5–3.0% of Dextran 70 (Macrodex® 70, Pharmacia, Sweden) to increase the osmotic pressure of the perfusate. All solutions were bubbled continuously with a mixture of 95% O_2 and 5% CO_2.

Electrophysiological experiments. Extracellular recordings from the abducens nerves and the area of the medial vestibular nucleus (MVN; defined as in Ref. 3) were used to evaluate the spontaneous activity of vestibular-related networks on each side of the IWB. We also quantified the latency and amplitude of the responses evoked in the MVN area and abducens nerve by stimulation of the ipsilateral vestibular nerve, the contralateral vestibular nerve and the spinal cord.

The spinal cord and both vestibular nerves were stimulated with bipolar, metallic electrodes. The electrodes for stimulation of the vestibular nerves (interpolar distance 1 mm) were made using stainless steel microelectrodes (0.2–0.3 mm thick; FHC, Brunswick, ME, U.S.A.) and inserted in the anterior (vestibular) branch of the vestibulo-cochlear

nerve. The electrode for stimulation of the spinal cord was made of stainless steel wires (0.4–0.5 mm thick). Its large interpolar distance (about 2.5 mm) was adjusted to ensure massive, bilateral stimulation of the tissue.

Recordings of abducens nerves were obtained through smooth, fire-polished, glass suction electrodes. Their tips were specifically designed to match the size of the nerve stumps. Great care was taken not to damage the abducens nerves during the application of these suction electrodes. Extracellular field potential and single-unit recordings in the vestibular nuclei were made using glass microelectrodes, filled with a 2 M solution of NaCl (resistance: 2–8 MΩ).

Rectangular pulses of 0.2 ms duration were used for all stimulations. The threshold stimulating intensity was determined for each response. The maximal intensities used were not bigger than three to five thresholds, and did not exceed 800–900 μA.

Conventional electrophysiological equipment was used for recording and storage of information. The signals from suction electrodes were amplified by a low input resistance d.c./a.c. amplifier (Tektronix AM-502, U.S.A.). The microelectrode signals were recorded in the current-clamp mode with an Axoclamp 2A amplifier (Axon Instruments, U.S.A.). The signals were bandpassed at d.c./0.1 Hz–3/10 kHz, stored on videotapes using a Neurocorder (NeuroData Instruments, U.S.A.) interface and simultaneously displayed on a digital storage oscilloscope.

The recorded signals were replayed off-line, digitized through an A/D converter, and stored as PC-compatible files using a home-made data acquisition system (Data Acquisition Multi I/O, SICMU, Geneva, Switzerland) and its dedicated software. The latencies, amplitudes and other parameters of responses were then quantified. All characteristics of the responses were measured, unless stated otherwise, at the sites of their largest amplitude.

The mean rate and regularity of the spontaneous activity were evaluated, for each extracellularly recorded unit, from five samples of 10–30 successive spikes. The coefficient of variation (CV), defined as the ratio between the standard deviation and the mean value of the inter-spike interval, was taken as an index of discharge regularity.

Statistical analysis. The first labyrinthectomy, performed before removal of the brain, was usually done on the left side. Similar data were obtained in the few cases where the initial lesion was performed on the right side, and all results were therefore pooled together. Calculations of medians or means ± S.D. and further processing of all results were carried out using StatWorks and CricketGraph software (Cricket Software Inc., Philadelphia, PA, U.S.A.) on an Apple Macintosh computer. The normality of all data distributions was checked for each parameter (Kolmogorov–Smirnov test,[22] 10% confidence interval).

Normally distributed parameters. Normality was achieved for most of the quantified parameters, allowing us to use standard, parametric statistical tests. In each set of data, one-way ANOVA for independent samples (5% confidence interval) was first used to search for significant differences between brains taken from control animals ("control" brains; data from Ref. 3) and those taken from previously labyrinthectomized guinea-pigs (D1, D3, D5 and D7 brains). Separate tests were run for the compensated and newly deafferented sides, assuming that control brains were symmetric (the data obtained previously in control brains were used as reference for both sides of the IWBs taken from lesioned guinea-pigs). When no significant variation was found between D3, D5 and D7 brains, ANOVA was run between control, D1 and D3–D7 ("compensated") brains. When a significant difference was revealed, unpaired Student's *t*-tests (two-tailed, 5% confidence interval) were used for further two by two comparisons between groups. In brains taken from labyrinthectomized animals, symmetry between the two sides was tested using paired or unpaired Student's *t*-test, depending on whether paired observations were obtained for the parameter in question.

Other parameters. Statistical comparisons between the spontaneous discharges and CVs of second-order VNns, for which significant deviations from normality were observed, were done with non-parametric tests. For each side of the brain, separate Kruskal–Wallis one-way ANOVA for independent samples (5% confidence interval) showed whether the discharge rate was significantly modified over time. Mann–Whitney *U*-tests for independent samples (5% confidence interval) were then used for further two by two comparisons of data obtained for each group of brains, and to test the symmetry of discharges between both sides of the preparations.

In vivo *experiments; assessment of Bechterew's phenomenon*

Nine animals were used for this *in vivo* study. Following a first, left side labyrinthectomy, the intact labyrinth was lesioned after either 20–24 h ($n=3$, D1 animals), three days ($n=3$, D3 animals) or seven days ($n=3$, D7 animals) of compensation. Each guinea-pig was X-rayed in resting position between 2 and 5 h after this second labyrinthectomy. Exposures were obtained both from above and from the side, with the X-ray tube 90 cm away from the animal.

For each animal, the frequency of the spontaneous nystagmus induced by the second lesion was measured as the mean number of quick phase beats occurring in the light over three to five periods of 30 s, with the guinea-pig unrestrained. The mean number of beats per minute was derived from these measures at 1, 2, 4, 6 and 24 h following the lesion.

Statistical analysis was performed using the same software as for the *in vitro* results. Once the normality of each data sample was checked (normality test, 10% confidence interval), one-way ANOVAs for independent samples were performed to search for significant differences between the spontaneous nystagmus induced in D1, D3 and D7 guinea-pigs. Unpaired Student's *t*-tests (two-tailed, 5% confidence interval) were then used for further two by two comparisons between groups.

RESULTS

In vivo *experiments on Bechterew's phenomenon*

All guinea-pigs completely recovered from gaseous anesthesia in about 45 min following the end of the second labyrinthectomy (performed on the right side).

Postural disturbances. In D7 animals (where seven days elapsed between the two lesions), the second labyrinthectomy induced a strong postural syndrome directed towards the newly deafferented side. Just after the lesion, animals laid on one side and displayed episodes of violent ipsiversive rolling around the body's longitudinal axis. Two to five hours later (Fig. 2C), the head was still strongly tilted towards the newly deafferented side because of a lateral tilt of the cervical column in the frontal plane. The head was also rotated in the stereotaxic horizontal plane around the tilted axis of the cervical column, because of a distributed rotation along the first cervical joints. Abnormal positions of the limbs were observed: the forelimb ipsilateral to the second lesion and the contralateral hindlimb were flexed, while the contralateral forelimb and ipsilateral hindlimb were extended. Altogether, this syndrome was the mirror image of the one observed in the opposite direction following the initial labyrinthectomy performed on the other side seven days before.[57] It disappeared, however, in about 24 h, whereas compensation for the first lesion was only achieved after three days.

In D3 animals (where three days elapsed between the two lesions), the second labyrinthectomy induced similar postural deficits, including a head rotation towards the newly deafferented side in both the horizontal and frontal planes, and abnormal positions of the limbs (Fig. 2B). The intensity of the postural syndrome, however, appeared lower than in D7 guinea-pigs. In particular, spontaneous episodes of rolling towards the newly deafferented side were never observed, and all postural disturbances disappeared in about 10 h.

In D1 animals, and in contrast with what happened in D3 and D7 guinea-pigs, the second labyrinthectomy only induced very transient postural deficits. These disturbances, which included a slight lateral head tilt towards the newly deafferented side and sometimes a small horizontal head deviation,

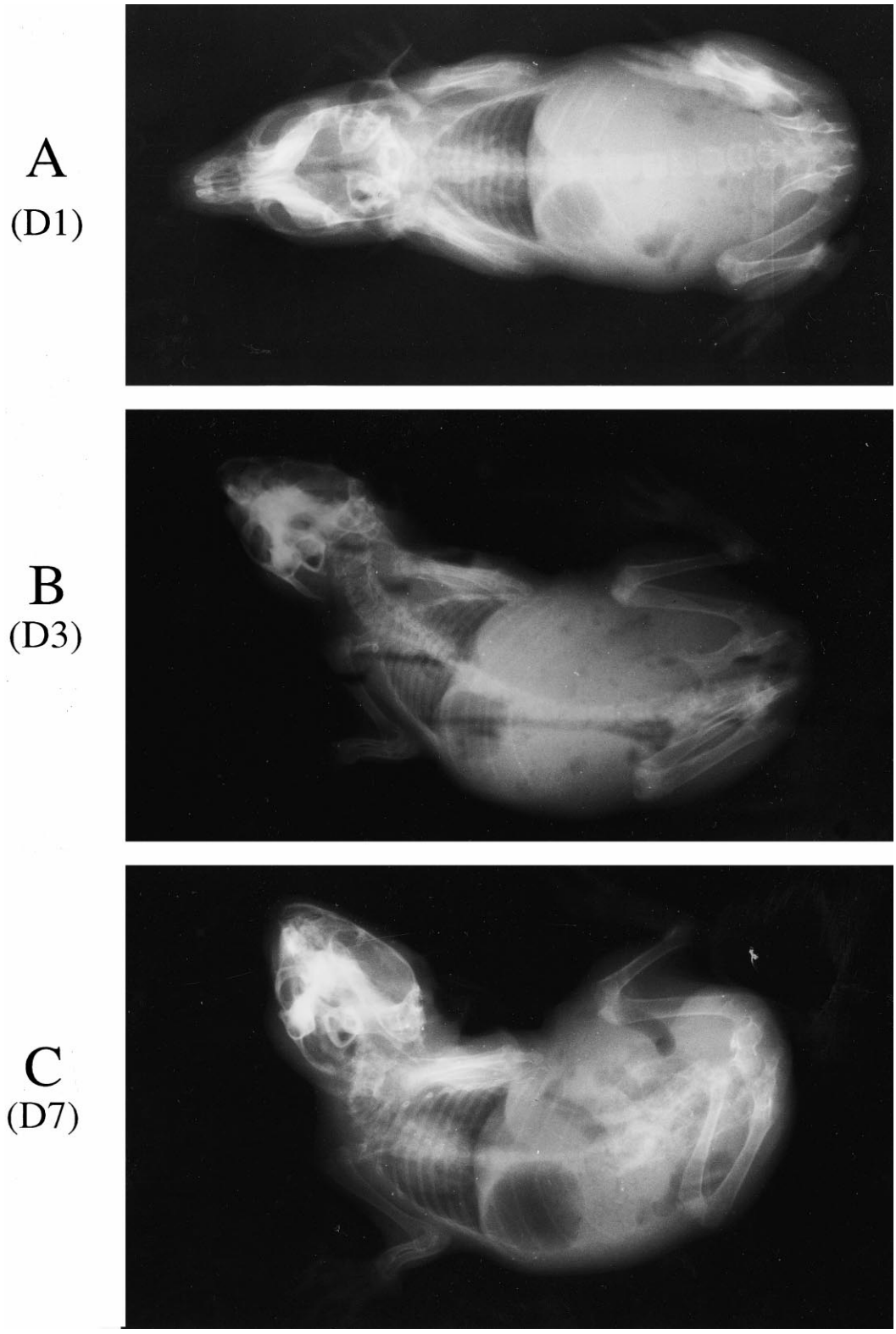

Fig. 2. Radiographs showing the postural impairments associated with Bechterew's phenomenon *in vivo*. X-rays displaying examples of the postural syndromes observed *in vivo* in D1 (A), D3 (B) and D7 (C) guinea-pigs 2–5 h after the second labyrinthectomy. Note the absence of Bechterew's syndrome in D1 animals.

disappeared in less than 1 h. Therefore, on X-rays taken 2–5 h following the second lesion, D1 animals had a normal resting posture (Fig. 2A).

Spontaneous nystagmus. In all lesioned animals, the postural deficits characterizing Bechterew's phenomenon were associated with a spontaneous ocular nystagmus with quick phases directed towards the side of the initial lesion, i.e. the compensated side (Fig. 3). Significant differences were found between the nystagmus intensities

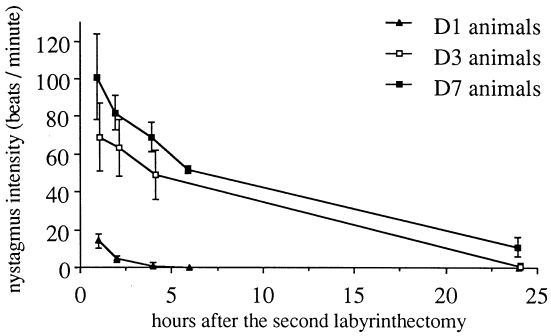

Fig. 3. Evolution of the spontaneous nystagmus associated *in vivo* with Bechterew's phenomenon. Diagram displaying the mean intensities (±S.D.) and time-course of the spontaneous nystagmus associated with Bechterew's phenomenon in D1, D3 and D7 animals.

obtained in D1, D3 and D7 animals at each given time following the lesion.

In D7 animals, the mean number of beats per minute averaged 101±23 1 h after the lesion, 69±8 after 4 h and was still 11±4 after 24 h.

In D3 animals, the nystagmus intensity tended to be lower, even if this difference was not always significant. The mean number of beats per minute was 69±18 after 1 h ($P=0.14$ related to D7 animals), 49±13 after 4 h ($P=0.09$) and fell to 1±2 after 24 h ($P=0.03$). Finally, in D1 animals, only a transient and much weaker nystagmus was observed. The number of beats per minute averaged only 14±3 1 h after the lesion ($P=0.007$ related to D3 animals), and fell to 1±1 after 4 h ($P=0.003$). The spontaneous nystagmus completely disappeared after 5 h.

In vitro *experiments*

Preliminary remarks. In a recent study,[3] we described the characteristics of vestibular-related pathways in IWBs taken from normal guinea-pigs, with both labyrinths intact until the removal of the brain. As pointed out in the introduction, these data will be considered as control values, and quoted as collected in control brains. Since both vestibular nerves are cut during removal of the brain, these control IWBs are acutely bilabyrinthectomized preparations.

In contrast, the brains taken from previously lesioned animals had undergone two vestibular deafferentations that were separated by either one, three, five or seven to eight days. In the rest of the paper, we will refer to the side of the IWB ipsilateral to the initial labyrinthectomy as the "compensated" side, whereas the contralateral side deafferented during removal of the IWB will be called the "newly deafferented" side (Fig. 1D). For each parameter, "symmetry" and "asymmetry" will indicate whether similar or different values were collected on both sides of the IWB. The symmetry status is independent of whether or not each of the two mean values is in the range of control values obtained in control brains.

As already detailed in the Experimental Procedures, data collected in D5 and D7 brains were pooled together as results obtained in D5–D7 brains. Moreover, when similar results were obtained in IWBs prepared three or more days after the first lesion (i.e. D3, D5 and D7 brains), they were grouped together as data collected in D3–D7 brains.

Spontaneous activity of identified, second-order vestibular neurons. Extracellular recordings were obtained from the region of the MVN, with the MVN boundaries defined according to the same criteria as in our previous study in control brains.[3] The recorded neurons were identified as second-order VNns on the basis of their response to stimulation of the ipsilateral vestibular nerve. Only those neurons that generated spikes at latencies of 2.1 ms, suggesting a monosynaptic activation (see discussion in Ref. 3), were retained for analysis. The proportion of second-order neurons was not precisely quantified in brains taken from previously lesioned animals, because it was apparently not significantly different from control brains.

On the other hand, we checked whether the latency of activation of second-order VNns by ipsilateral vestibular nerve stimulation was modified in D1 or D3–D7 brains. No significant asymmetry was found for this parameter between both sides of the preparations, and none of the values obtained on each side was significantly different from the value of 1.71±0.29 ms ($n=92$) obtained in control brains.

Spontaneous discharge rates displayed by second-order vestibular nucleus neurons. Significant deviations from normality were observed for several distributions of the spontaneous discharge rates displayed by each second-order VNn (Fig. 4), particularly on the newly deafferented side, where high proportions of silent neurons were observed (see below). Non-parametric, ordinal statistical tests comparing the medians (and not the means) of each sample were therefore used to search for significant modifications of this parameter. Thus, the silent neurons were always included.

In control brains, the mean resting discharge of second-order VNns amounted to 8.7±6.9 spikes/s, with a median of 7.9 spikes/s; only 12% of these 89 extracellularly recorded, second-order VNns was silent at rest.

No significant asymmetry was observed for the spontaneous activity of second-order VNns in D1 brains (Fig. 4), where both median values were well in the control range. Accordingly, the proportion of silent neurons, which was 7% on both sides, was unchanged.

On the other hand, the spontaneous discharges of second-order VNns (Fig. 4) were asymmetric both in D3 ($P=0.047$) and D5–D7 brains ($P<0.001$). The spontaneous activity of second-order VNns was similar on the newly deafferented side of D3 and D5–D7 brains ($P=0.66$), and in both cases was well below the control value ($P=0.03$ for D3–D7 brains). In contrast, on the compensated side, the discharge of second-order VNns was within the control range in D3 brains, and became larger than normal only in D5–D7 brains ($P=0.01$). In summary, the asymmetry was significantly stronger in D5–D7 brains than in D3 brains. Consistent with these data, the proportion of silent neurons was strongly increased on the newly deafferented side of D3–D7 brains, reaching 24% in D3 brains (versus 6% on the compensated side) and 34% in D5–D7 brains (versus 4% on the compensated side).

Coefficient of variation of the spontaneous discharge of second-order vestibular nucleus neurons. In control brains, the regularity of the discharge of second-order VNns has been linked with their intrinsic membrane properties.[3] Since vestibular compensation might rely on modifications of the membrane properties of central vestibular neurons, we searched whether the CV characterizing the spontaneous activity of second-order VNns was modified in brains taken from previously lesioned animals (Fig. 5A). As for the

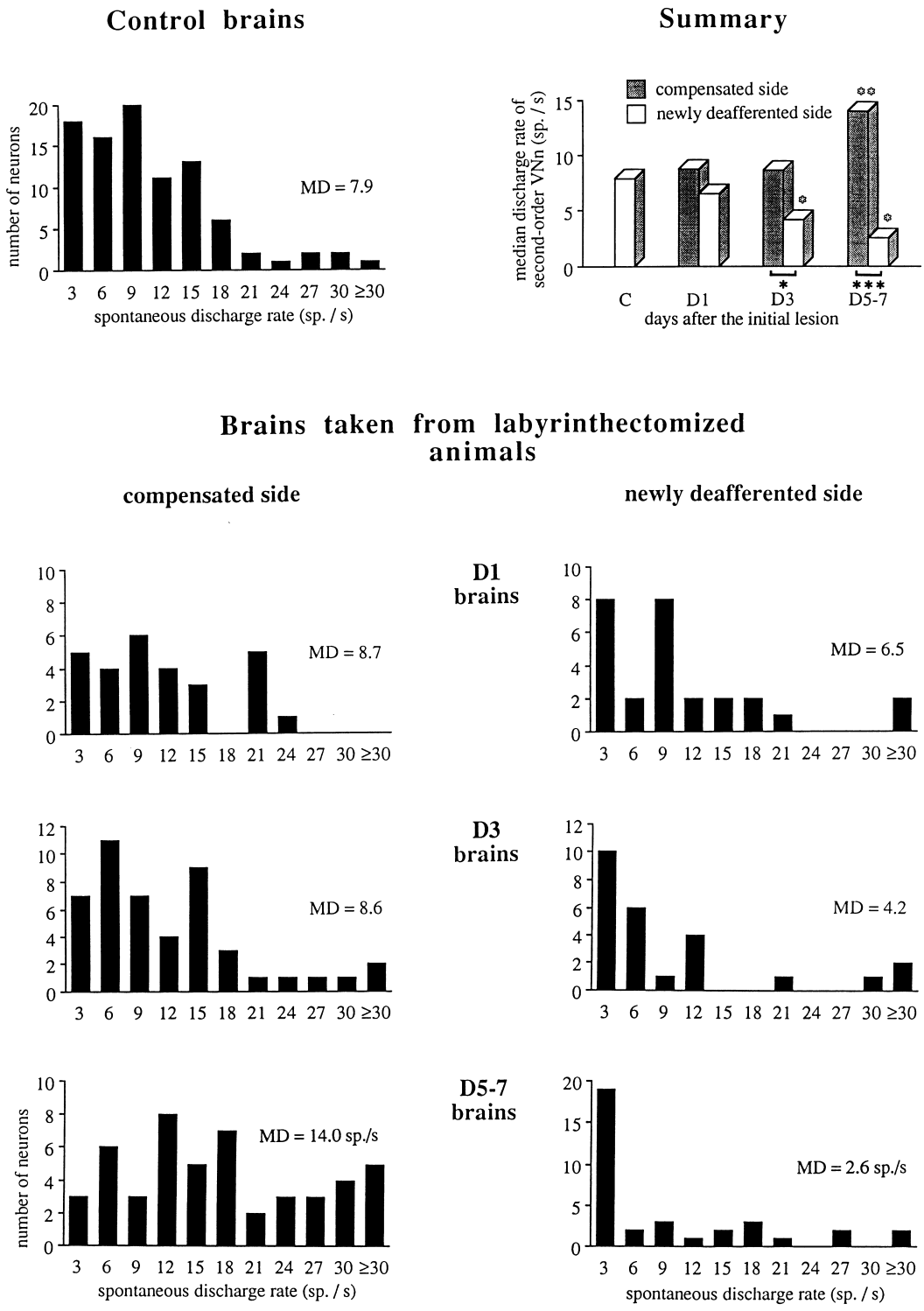

Fig. 4. Spontaneous activity of second-order vestibular nucleus neurons. The seven black histograms show the distributions of second-order VNns recorded in control brains and in brains taken from previously labyrinthectomized animals according to their spontaneous discharge rates. The column labeled 3 gives the number of neurons whose spontaneous activity was strictly lower than 3 spikes/s, the column labeled 6 includes those units whose activity was between 3 and 6 spikes/s, and so on. MD is the median discharge rate given for each distribution. The top right histogram summarizes the median discharges obtained for these seven groups of second-order VNns. The white asterisks indicate the values which were significantly out of the control range, while the black ones show the groups of isolated brains for which a significant asymmetry appeared between both sides of the brainstem (*$P<0.05$, **$P<0.01$, ***$P<0.001$).

spontaneous discharge rates, non-parametric statistical tests had to be used. In control brains, the mean CV of second-order VNns was 0.38 ± 0.26, with a median of 0.35.

On the compensated side of D1 brains, the median CV increased to 0.58, which was significantly higher than in control brains ($P<0.001$). A similar trend towards an increase in discharge irregularity was found on the newly deafferented side, even if this increase was not significant because of the large variance of the distribution. Altogether, D1 brains displayed symmetric values of CVs in both vestibular nuclei ($P=0.13$).

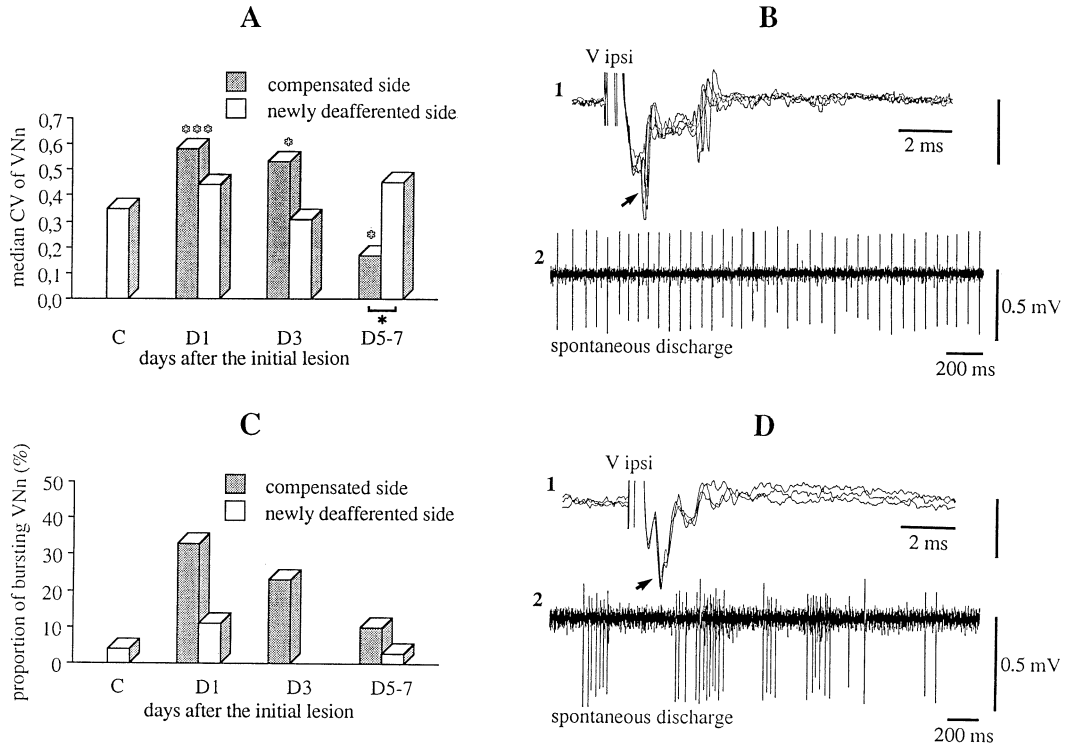

Fig. 5. Regularity of the spontaneous discharge of second-order VNns. (A) Histogram showing the medians of the CV characterizing the spontaneous activity of second-order VNns in control brains, and on each side of brains taken from previously labyrinthectomized animals. Again, in this and all the following histograms, the white asterisks indicate the values which were significantly out of the control range, while the black ones show the groups of isolated brains for which a significant asymmetry appeared between both sides of the brainstem. (B) Example of a regular VNn recorded on the compensated side of a D7 brain. This cell, identified as a second-order VNn by ipsilateral vestibular nerve stimulation (arrow on trace 1), displayed a regular spontaneous discharge (2). Trace 2 was bandpass filtered from 100 Hz to 3 kHz. (C) Histogram displaying the proportion of bursting, second-order VNns recorded in brains taken from control and labyrinthectomized animals. (D) Example of a bursting VNn recorded on the compensated side of a D1 brain. This neuron, identified as a second-order VNn by ipsilateral vestibular nerve stimulation (arrow on trace 1), was endowed with a very irregular spontaneous discharge, including bursts of spikes (2). Again, trace 2 was bandpass filtered from 100 Hz to 3 kHz.

On the compensated side of D3 brains, the median CV was still higher than in control brains ($P=0.03$), while the CVs obtained on the newly deafferented side were still well within the range of control values. Nevertheless, the CVs were not significantly asymmetric between both sides of D3 brains because of the large variances of both samples.

On the compensated side of D5–D7 brains (see example in Fig. 5B), the median CV was significantly lower than in control brains ($P=0.01$), while on the newly deafferented side, the median CV was within the range of control values, as in D1 and D3 brains. Altogether, the CVs obtained in D5–D7 brains became significantly asymmetric ($P=0.04$), being lower on the compensated side than on the newly deafferented one.

Proportion of spontaneously bursting, second-order vestibular nucleus neurons. In control brains, about 4% of the second-order VNns (Fig. 5C) displayed spontaneous bursts of two or more spikes separated by less than 20 ms (spiking frequency ≥ 50 spikes/s). We observed that the increase of the median CV of VNns observed in D1 and D3 brains was mainly due to a large increase in the proportion of these very irregular neurons (which often displayed CVs greater than 1; see example in Fig. 5D).

Indeed, in D1 brains, the proportion of bursting neurons reached 33% on the compensated side and 11% on the newly deafferented side. It still amounted to 23% on the compensated side of D3 brains, but fell to 0% on their newly deafferented side. Finally, in D5–D7 brains, the proportion of bursting neurons further decreased to 10% on the compensated side, while amounting to 3% on the newly deafferented side.

Recordings of spontaneous activity in the abducens nerves. To investigate whether the asymmetry between the spontaneous discharges of VNns was reflected further up in the vestibulo-oculomotor networks, both abducens nerves were simultaneously recorded in 41 IWBs taken from labyrinthectomized animals (10 at D1, 16 at D3, six at D5 and nine at D7) and their spontaneous activities quantified (Fig. 6A, B). Paired statistical tests were therefore used to check the symmetry of their discharges in each group of IWBs. The spontaneous level of activity was defined in each nerve as the maximum peak-to-peak amplitude of the spontaneous discharges observed over three to five separate periods of 1.2 s.

In control brains, the mean spontaneous activity recorded in abducens nerves was 0.23 ± 0.11 mV ($n=39$). In D1 brains, the spontaneous activities recorded in both abducens nerves were symmetric, and both were in the control range.

In contrast, in the 31 compensated brains (D3–D7 brains), a prominent asymmetry appeared between the spontaneous activities of both abducens nerves ($P<0.001$). The level of asymmetry of the discharges did not vary between D3, D5 and D7 brains. Altogether, the values obtained in D3–D7 brains were 26% higher than in control brains in the nerve excitable

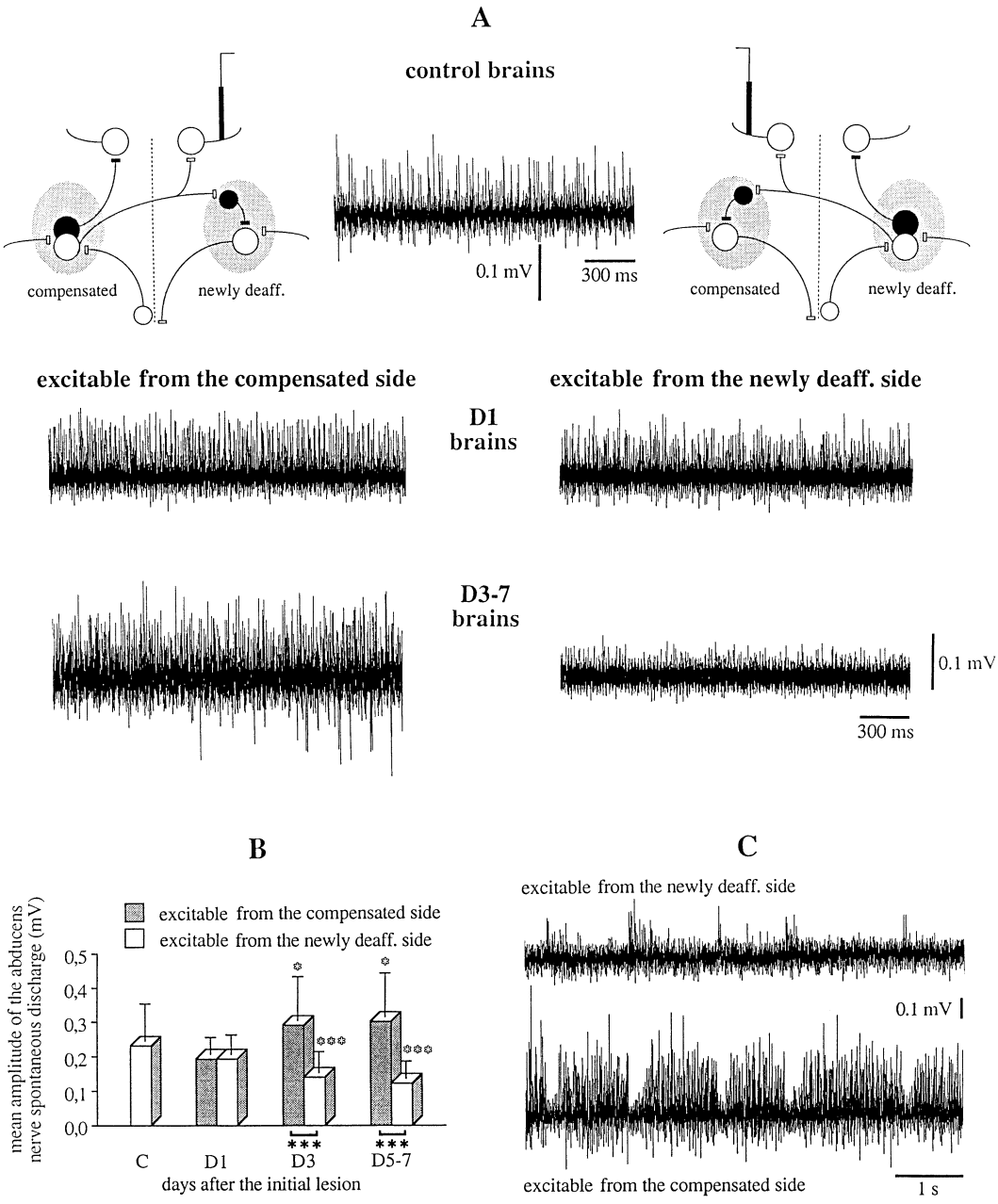

Fig. 6. Spontaneous activities recorded in the abducens nerves. (A) Examples of spontaneous activities recorded from the abducens nerves of brains taken from control and previously labyrinthectomized guinea-pigs; each panel displays three to five superimposed traces. The left and right schemes of the upper row give the positions of the recording electrodes used to obtain the responses shown in the left and right columns of the panel, respectively. Some of the elements of the neuronal networks have been omitted for the sake of clarity. Note that the traces displayed on the left were obtained from the abducens nerve excitable from the compensated side, i.e. on the contralateral, newly deafferented side. Similarly, the traces displayed for the newly deafferented side were obtained from the abducens nerve excitable from the newly deafferented side. (B) Summary of the mean amplitude (±S.D.) of the spontaneous discharges of abducens nerves observed in brains taken from control and labyrinthectomized animals. For the meaning of the black and white asterisks, see Fig. 5A. (C) Example of a spontaneous nystagmus recorded in both abducens nerves of a D3–D7 brain. The long bursts of discharge corresponding to slow phases were observed in the nerve excitable from the compensated side, i.e. on the newly deafferented side. In A and C, all traces were bandpass filtered from 100 Hz to 3 kHz.

from the compensated side ($P=0.047$), and 43% lower in the nerve excitable from the newly deafferented side ($P<0.001$).

In six (19%) of the D3, D5 or D7 brains, beginning 1–2 h after the end of rewarming, an oscillatory pattern of discharge appeared spontaneously in both abducens nerves (Fig. 6C). Long bursts of increasing activity in the nerve excitable from the compensated side ended abruptly to alternate with short, brisk discharges in the nerve excitable from the newly deafferented side. This pattern was strongly reminiscent of the pattern of discharges recorded *in vivo*, in the abducens nerves of acute cats, during an ocular nystagmus triggered by high-frequency stimulation of one of the vestibular nerves[23,24] (with the quick phases of the nystagmus directed towards the compensated side of the IWB).

Orthodromic field potentials evoked in the region of the medial vestibular nucleus by ipsilateral vestibular nerve stimulation. The functionality of the asymmetric vestibulo-abducens pathways characterizing D3–D7 brains was tested using single-shock stimulations of both vestibular nerves. As

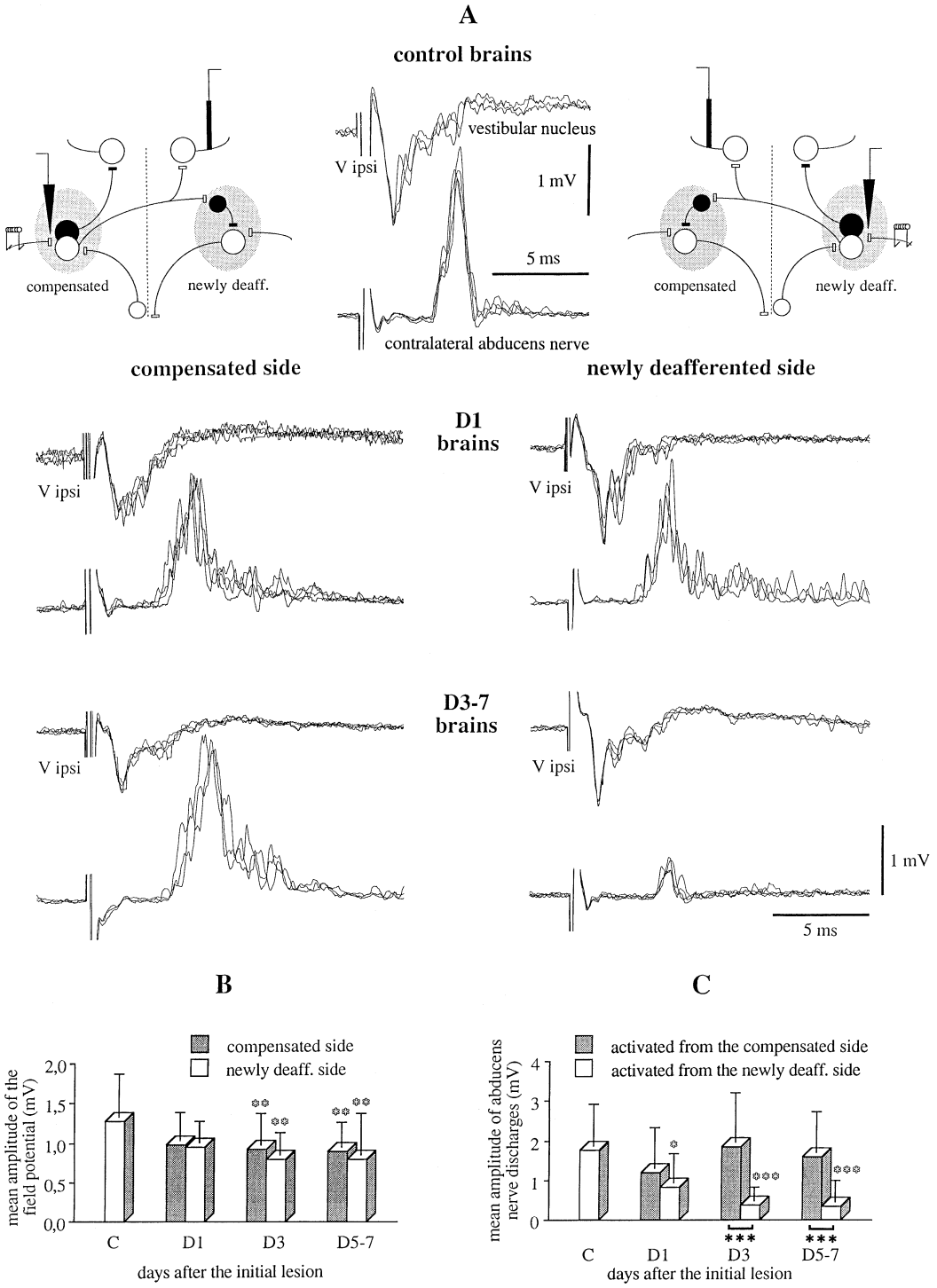

Fig. 7. Responses evoked by vestibular nerve stimulation in the ipsilateral MVN area and in the contralateral abducens nerve. (A) Examples of the field potentials evoked in the ipsilateral MVN area and in the contralateral abducens nerve by stimulation of the vestibular nerves in brains taken from control and labyrinthectomized guinea-pigs. The left and right schemes of the upper row give the positions of the recording electrodes used to obtain the responses shown in the left and right columns of the panel, respectively. (B) Histogram displaying the mean amplitudes (±S.D.) of the field potentials evoked in brains of control and labyrinthectomized animals by ipsilateral vestibular nerve stimulation. (C) Histogram showing the mean amplitudes (±S.D.) of responses evoked in the abducens nerves of brains taken from control and labyrinthectomized animals by contralateral vestibular nerve stimulation. See Fig. 5A for the meaning of the black and white asterisks in B and C.

in control brains, single-shock stimulation of the ipsilateral vestibular nerve evoked a characteristic, polyphasic field potential[3] in the region of the MVN on both sides of D1, D3, D5 and D7 brains (Fig. 7A). For each group of brains taken from previously lesioned animals, the latency and amplitude of the monosynaptic component of the field potential (defined as in Ref. 3) were therefore compared to the mean values observed in control brains (1.2 ± 0.2 ms and 1.28 ± 0.54 mV, respectively). The polysynaptic components of the field were not studied in detail, because they could be variably expressed according to the exact location of the electrode in the vestibular complex. In addition, close visual

inspection did not reveal any significant variations of these polysynaptic waves between control IWBs and those taken from lesioned animals.

Because only one MVN was investigated at a time, paired observations on both sides of the brains could not be obtained. Unpaired statistical tests were therefore used to check the symmetry of data obtained in each group of IWBs.

Latency of the monosynaptic component of the field potential. No significant asymmetry was found between the latencies obtained on both sides of D1 or D3–D7 brains (Fig. 7A). In the newly deafferented MVN, the mean latency of the field potential was in the range of control values in all cases. Similarly, no significant variation was observed in the MVN on the compensated side.

Amplitude of the field potential. Neither D1 nor D3–D7 brains displayed asymmetric amplitudes of the field potential evoked on each side by stimulation of the ipsilateral vestibular nerve (Fig. 7A, B). The amplitudes obtained in D1 brains were both in the control range. In contrast, the amplitude of the field potentials recorded in D3–D7 brains were about 30–35% lower than in control brains on both the compensated and newly deafferented sides (respective P values of 0.004 and 0.001).

Recordings of vestibular-evoked responses in the abducens nerves.

Response evoked by stimulation of the contralateral vestibular nerve. In addition to the field potential evoked in the region of the ipsilateral MVN, we have demonstrated that stimulation of the vestibular nerve elicits, in control brains, a synchronized discharge in the contralateral abducens nerve, with an average latency of 3.8 ± 0.7 ms and a peak amplitude of 1.75 ± 1.06 mV.[3]

In D1 brains, both the latency and peak amplitude of the responses evoked in each abducens nerve from their respective contralateral vestibular nerve were symmetric (Fig. 7A, C). The latency of the discharge was in the range of control values in both the abducens nerve activated from the compensated side ($P=0.17$) and the abducens nerve activated from the newly deafferented side ($P=0.07$). The peak amplitude of the response was significantly lower than normal in the nerve activated from the newly deafferented side ($P=0.02$), and was also decreased but nevertheless stayed in the control range in the nerve activated from the compensated side (ANOVA between control, D1 and D3–D7 brains gave $P=0.33$).

In contrast, D3–D7 brains displayed a strong asymmetry with respect to both the latencies ($P<0.001$) and amplitudes ($P<0.001$) of the responses evoked in both abducens nerves (Fig. 7A, C). This asymmetry did not vary with time between D3, D5 and D7 brains. In the abducens nerve activated from the compensated side, neither the latency (3.5 ± 0.6 ms) nor the peak amplitude of the discharge were significantly different from control values. On the other hand, responses evoked in the abducens nerve activated from the newly deafferented side had a five-fold lower amplitude than in control ($P<0.001$) and D1 ($P=0.02$) brains, while their latency (4.8 ± 1.7 ms) was 26% longer than in control brains ($P=0.003$).

Response evoked by stimulation of the ipsilateral vestibular nerve. In control brains, ipsilateral vestibular nerve stimulation did not produce any short-latency response in the abducens nerve. It induced a late (latency 22.3 ± 9.6 ms), usually desynchronized, discharge (amplitude 0.36 ± 0.17 mV) in 53% of the preparations, most probably triggered by a post-inhibitory rebound in abducens motoneurons.[3]

These late discharges were observed in both abducens nerves of D1 brains, more frequently ($n=7$, 70%) in the nerve ipsilateral to the newly deafferented side (i.e. excitable from the compensated side) than in the one ipsilateral to the compensated side and excitable from the newly deafferented side ($n=2$, 20%). No significant asymmetry was otherwise observed between either the latencies or amplitudes of these responses, which were similar to those obtained in control brains.

Late rebound discharges were also recorded in 18 of the 31 D3–D7 brains (58%), but only in the abducens nerve ipsilateral to the newly deafferented side (i.e. excitable from the compensated side). Their latency and amplitude were in the range of the values obtained in control and D1 brains.

Responses evoked in the medial vestibular nucleus area by contralateral vestibular nerve stimulation. The characteristics of the field potentials evoked in the MVN area by stimulation of the contralateral vestibular nerve, and the proportion of extracellularly recorded second-order VNns recruited by that stimulation, were used as respective indices of the efficacy of the inhibitory and excitatory commissural connections linking together the two MVN areas in brains taken from previously lesioned animals (Fig. 1).

In control brains, single-shock stimulation of the contralateral vestibular nerve evoked a positive field potential in the MVN area, with an average latency of 2.8 ± 0.3 ms and a mean amplitude of 0.30 ± 0.07 mV (Fig. 8A). Intracellular recordings revealed that this stimulation evoked inhibitory postsynaptic potentials in 75% of the second-order VNns, and excitatory postsynaptic potentials in the remaining cells.[3] Therefore, the positive field potential evoked by contralateral vestibular nerve stimulation reflected the summation of these two inputs, with a large predominance of the inhibitory component.

Latency of the field potential evoked from the contralateral vestibular nerve. The latency of the positive field potential (Fig. 8A) remained symmetric between both sides of D1 ($P=0.07$) as well as D3–D7 brains. No significant difference was ever observed with control values (ANOVA between control, D1 and D3–D7 brains gave $P=0.09$ and 0.16 for the newly deafferented and compensated sides, respectively).

Amplitude of the field potential evoked from the contralateral vestibular nerve. The amplitudes of field potentials recorded in D1 brains were also symmetric (Fig. 8A, B), and in the range of control values. In contrast, one of the striking characteristics of D3–D7 brains was a 75%, symmetric decrease of the amplitude of the positive field potentials evoked by contralateral vestibular nerve stimulation in both MVN areas (Fig. 8A, B). No difference was observed between D3, D5 and D7 brains. This decrease was highly significant when compared to both control ($P<0.001$ on both sides) and D1 brains ($P=0.04$ and 0.002 on the newly deafferented and compensated sides, respectively). In several cases, the usual positive field potential was totally suppressed, or even replaced by a small, negative field potential.

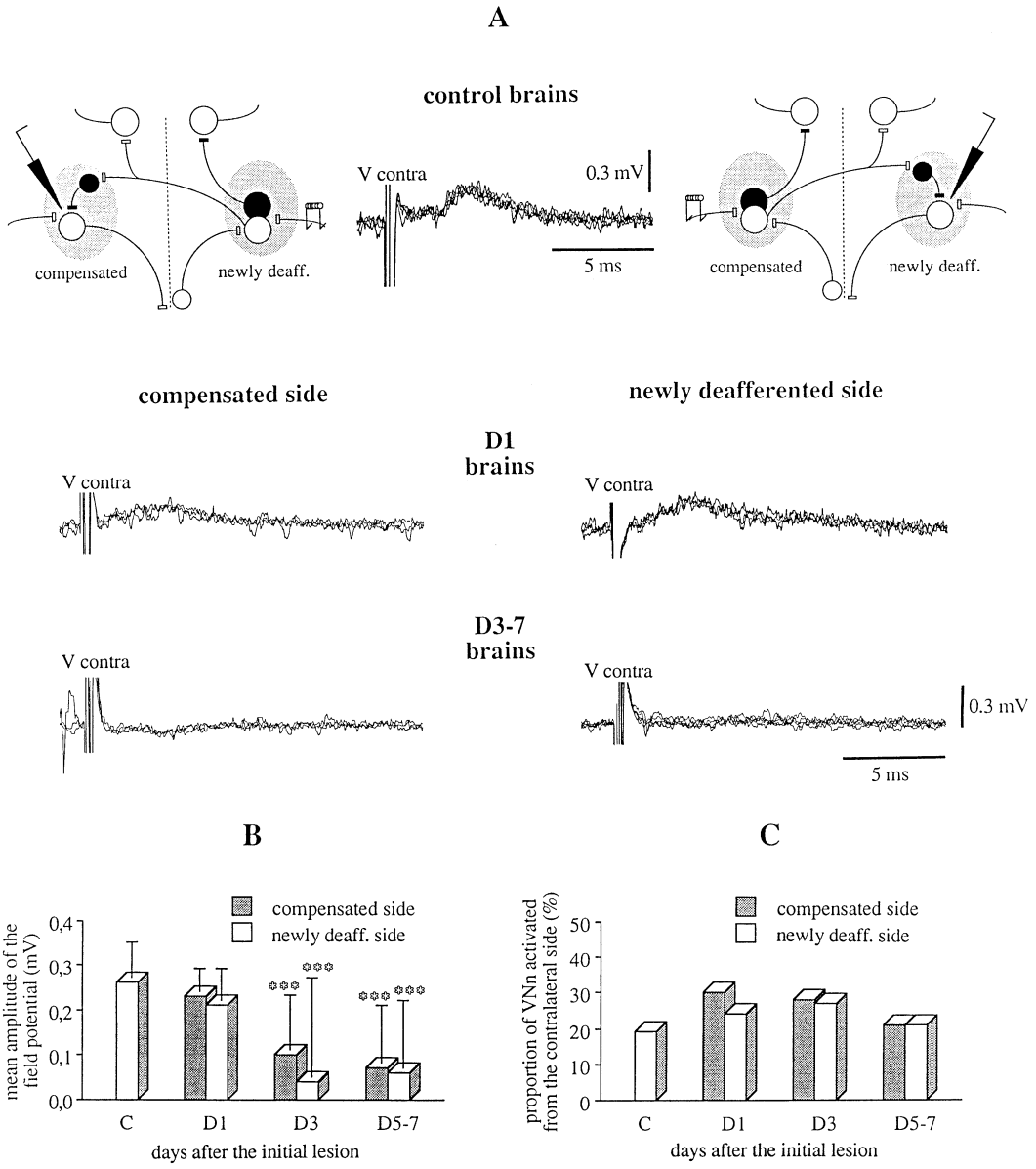

Fig. 8. Responses evoked by vestibular nerve stimulation in the contralateral MVN area. (A) Examples of the field potentials evoked in the contralateral MVN area by stimulation of the vestibular nerves in brains taken from control and labyrinthectomized guinea-pigs. The left and right schemes of the upper row give the positions of the recording electrodes used to obtain the responses displayed in the left and right columns of the panel, respectively. (B) Histogram displaying the mean amplitudes (±S.D.) of the field potentials evoked in the MVN area of brains taken from control and labyrinthectomized animals by contralateral vestibular nerve stimulation. For the meaning of the asterisks, see Fig. 5A. (C) Histogram showing the proportion of second-order VNns recruited by stimulation of the contralateral vestibular nerve brains taken from control and labyrinthectomized animals.

Second-order vestibular nucleus neurons recruited by contralateral vestibular nerve stimulation. Nineteen percent of the second-order VNns recorded extracellularly in control brains was activated through commissural pathways, with a mean latency of 4.3±2.0 ms. This percentage fits well with the finding that excitatory postsynaptic potentials were evoked following stimulation of the contralateral vestibular nerve in 24% of the intracellularly recorded, second-order VNns.

The proportion of second-order VNns activated from the contralateral side did not vary much in either D1 or D3–D7 brains (Fig. 8C).

Antidromic responses evoked in the medial vestibular nucleus area by stimulation of the spinal cord. Recordings of the antidromic field potential evoked in the MVN area by bilateral stimulation of the spinal cord and quantification of the number of antidromically activated, second-order VNns were used to determine whether the compensation process was associated with significant modification of the vestibulo-spinal pathways.

Antidromic field potential evoked in the medial vestibular nucleus area by spinal cord stimulation. In control brains, stimulation of the spinal cord evoked an initial, sharp negativity in the region of the MVN, followed by a slower and longer-lasting negativity.[3] The first negative wave had a mean latency of 0.84±0.11 ms, an average amplitude of 0.27±0.16 mV and was not depressed by high-frequency stimulations. These characteristics suggested that it reflected antidromic activation of vestibulo-spinal neurons.

Table 1. Quantitative characteristics of responses evoked in vestibular-related pathways by bilateral spinal stimulation (means ± S.D.)

Parameter	Control brains	D1 brains		D3 brains		D5–D7 brains	
		Compensated side	Newly deafferented side	Compensated side	Newly deafferented side	Compensated side	Newly deafferented side
Recordings from the region of the MVN							
Antidromic responses							
Field potential latency (ms)	0.74 ± 0.14	0.89 ± 0.17	0.84 ± 0.11	0.82 ± 0.11	0.68 ± 0.12	0.75 ± 0.19	0.69 ± 0.21
Field potential size (mV)	0.35 ± 0.20	0.26 ± 0.08	0.33 ± 0.07	0.40 ± 0.15	0.47 ± 0.33	0.30 ± 0.16	0.27 ± 0.11
No. of activated, second-order VNns (%)	11	7	12	2	8	14	9
Latency of VNn activation (ms)	0.74 ± 0.18	0.82 ± 0.11	0.83 ± 0.14	0.78 ± 0.00	0.63 ± 0.18	0.75 ± 0.19	0.80 ± 0.10
Orthodromic responses							
Field potential latency (ms)	1.7 ± 0.3	2.7 ± 1.9	1.6 ± 0.4	2.0 ± 0.8	2.8 ± 2.5	1.7 ± 0.5	2.2 ± 0.8
Field potential size (mV)	0.23 ± 0.10	0.27 ± 0.06	0.35 ± 0.12*	0.38 ± 0.17*	0.20 ± 0.18	0.38 ± 0.27*	0.14 ± 0.13
					as		as
No. of activated second-order VNns (%)	27	27	23	33	25	43	9
Latency of VNn activation (ms)	3.0 ± 1.6	4.0 ± 2.2	4.1 ± 2.4	3.0 ± 1.2	2.6 ± 0.9	3.0 ± 1.2	7.5 ± 7.7*
							as
Abducens nerves recordings: spinal-evoked responses							
Discharge latency (ms)	3.0 ± 0.8	3.8 ± 4.5	7.1 ± 6.8	2.7 ± 1.3	4.6 ± 3.1	3.9 ± 3.2	4.9 ± 3.6
			as				
Discharge amplitude (mV)	0.56 ± 0.32	0.90 ± 0.84	0.64 ± 0.72	0.82 ± 0.61	0.16 ± 0.20*	0.81 ± 0.52	0.23 ± 0.30*
					as		as

For recordings obtained from the abducens nerves, the values indicated on the compensated side refer to the responses obtained from the nerve activated from (and contralateral to) the compensated side. Similarly, the values given for the newly deafferented side correspond to the responses obtained from the nerve activated from the newly deafferented side. Asterisks indicate which values were significantly different from those obtained in control brains; “as” is added below the values when a significant asymmetry was found between both sides of the IWB.

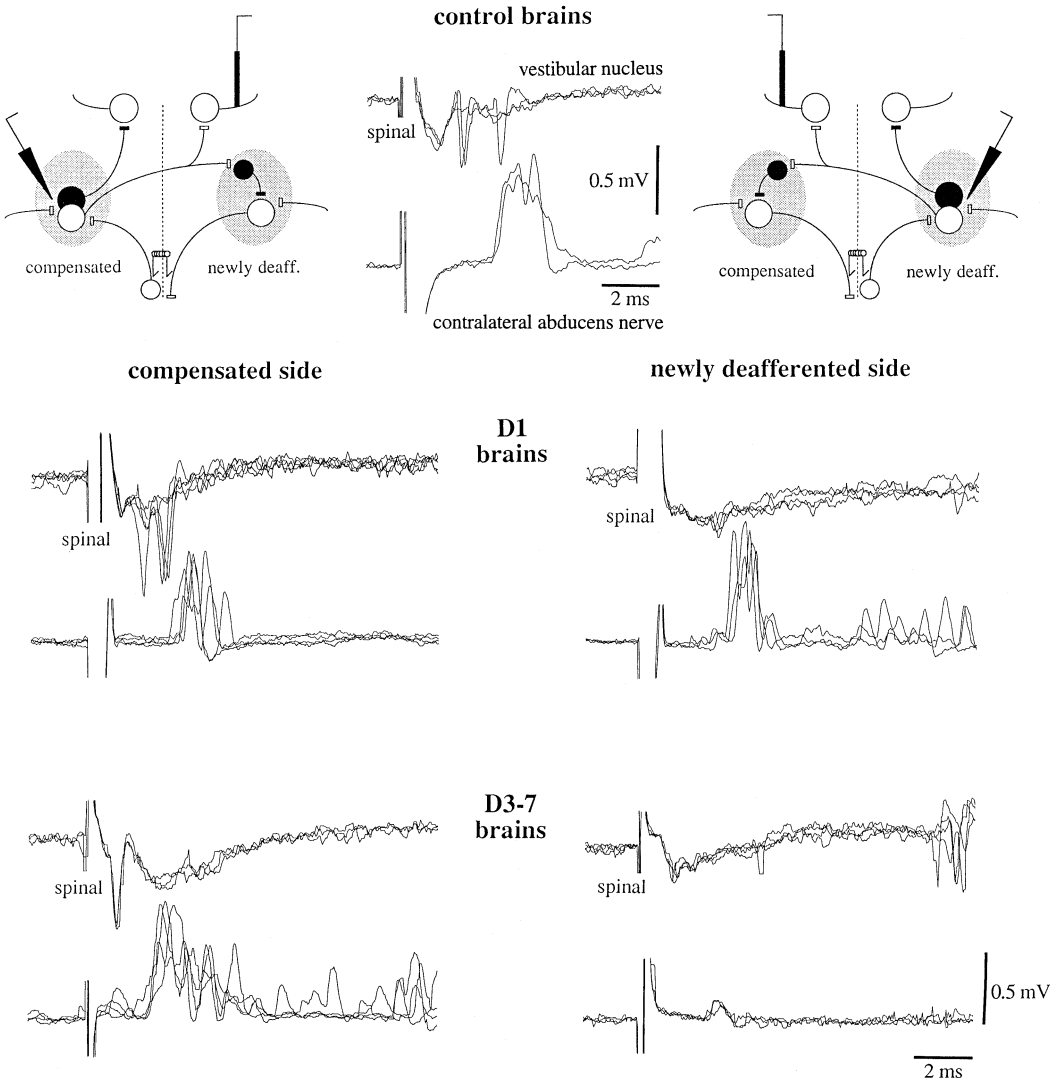

Fig. 9. Responses evoked in vestibular-related pathways by bilateral spinal stimulation. Examples of the field potentials evoked in the MVN area and in the contralateral abducens nerve by bilateral stimulation of the spinal cord in brains taken from control and labyrinthectomized guinea-pigs. The left and right schemes of the upper row give the positions of the recording electrodes used to obtain the responses displayed in the left and right columns of the figure, respectively.

In all groups of brains taken from labyrinthectomized guinea-pigs, the mean latency and amplitude of the antidromic field potential were symmetric between both sides of the IWB and in the range of control values (Table 1).

Proportion of second-order vestibular nucleus neurons antidromically recruited from the spinal cord. In control brains, 11% of the second-order VNns were antidromically activated following stimulation of the spinal cord, with a mean latency of 0.74 ± 0.18 ms ($n=9$). In accordance with the lack of modification of the antidromic field potentials evoked by spinal stimulations, the proportion of antidromically activated second-order VNns did not vary in brains taken from labyrinthectomized guinea-pigs (Table 1). Accordingly, the average latencies of antidromic activation of second-order VNns were not modified either in comparison with control IWBs.

Orthodromic responses evoked in the medial vestibular nucleus area by stimulation of the spinal cord. The characteristics of the orthodromic field potential evoked by the bilateral spinal cord stimulation, and the proportion of second-order VNns recruited by that stimulation, were used as indices of the efficacy of the spinal afferents reaching the two MVN areas.

Orthodromic field potential evoked in the medial vestibular nucleus area by spinal cord stimulation. The second, negative wave of the spinal-evoked field potential (see above) was depressed by high-frequency stimulation, which indicated its synaptic origin. In control brains, this second component had a mean latency of 1.8 ± 0.2 ms and a mean amplitude of 0.22 ± 0.10 mV.[3]

The latencies of the orthodromic field potential (Fig. 9, Table 1) were symmetric between both sides of D1 and D3–D7 brains, and fell within the range of control values (ANOVA between control, D1 and D3–D7 brains gave $P=0.09$ on the newly deafferented side and $P=0.06$ on the compensated side).

In D1 brains, the amplitudes of the orthodromic field potentials evoked in both MVN areas were also symmetric ($P=0.10$), but tended to be larger than in control brains.

However, only the 40% increase observed on the newly deafferented side was significant ($P=0.02$).

In D3–D7 brains, in contrast, the mean amplitude of the spinal, orthodromic field potential was asymmetric ($P=0.003$). The mean amplitude measured on the newly deafferented side was in the control range ($P=0.19$). In contrast, the amplitude of the field potential recorded on the compensated side corresponded to a significant 73% increase ($P=0.03$) relative to the control value.

Proportions of second-order vestibular nucleus neurons orthodromically activated by spinal cord stimulation. In control brains, 27% of the second-order VNns were orthodromically activated by spinal stimulation, with an average latency of 3.0 ± 1.6 ms.

Similar proportions of second-order VNns were activated in D1 and D3 brains (Table 1). The latencies of orthodromic activation were also in the normal range on both the newly deafferented and compensated sides.

In D5–D7 brains, the proportion of second-order VNns orthodromically activated from the spinal cord reached 43% on the compensated side and fell to 9% on the newly deafferented side (Table 1). In addition, the latency of orthodromic activation was significantly larger on the newly deafferented side than on the compensated side ($P=0.01$).

Responses evoked in the abducens nerves by stimulation of the spinal cord. In addition to the field potentials evoked in both MVN areas, bilateral stimulation of the spinal cord evoked a biphasic response in the abducens nerves of control brains, with a latency of 3.0 ± 0.8 ms and a peak amplitude of 0.52 ± 0.34 mV.[3]

In the brains taken from previously lesioned guinea-pigs (Fig. 9, Table 1), paired observations were made from both nerves. D1 brains were characterized by the absence of any asymmetry between the amplitudes of the spinal-evoked discharges recorded in both nerves. In contrast, the latency of the response was slightly asymmetric between both sides ($P=0.04$). Despite this asymmetry, the latency and amplitude of the discharges evoked in the abducens nerve contralateral to the newly deafferented side (Table 1) fell within the range of control values (ANOVA between control, D1 and D3–D7 brains gave respective P values of 0.11 and 0.74). The same was true for the latency and amplitude of the responses recorded in the abducens nerve contralateral to the compensated side.

In D3–D7 brains, the responses evoked by stimulation of the spinal cord (Table 1) showed an asymmetry in terms of both their latencies ($P=0.03$) and amplitudes ($P<0.001$). This asymmetry did not vary between D3, D5 and D7 brains. In the abducens nerve contralateral to the compensated side, neither the latency nor the amplitude of the response were different from normal values. In the abducens nerve contralateral to the newly deafferented side, the amplitude of spinal-evoked responses was 63% lower than normal ($P<0.001$), even if their latency was still in the normal range.

DISCUSSION

Persistence of the asymmetries linked with Bechterew's syndrome in the isolated whole brain

In vivo data. The postural and oculomotor disturbances observed in D7 animals following the second lesion were very similar to those induced by a single, unilateral labyrinthectomy.[19,34,38,39,44,47,57] These static deficits were weaker and recovered faster in D3 animals, while the second labyrinthectomy only induced very transient symptoms in D1 animals. In guinea-pigs then, Bechterew's phenomenon became prominent only when the two labyrinthectomies were separated by at least three days, i.e. when behavioral compensation for the static deficits linked with the initial lesion was complete. These results are in good agreement with previous studies of Bechterew's syndrome in vertebrates. No asymmetric postural syndrome was ever observed following bilateral labyrinthectomy, i.e. when the two lesions were performed simultaneously.[25,44,50]

The progressive aggravation of Bechterew's syndrome with the increase of the delay between the two labyrinthine lesions followed the same time-course as the recovery of the resting discharge of the deafferented VNns after the initial labyrinthectomy. Indeed, in the guinea-pig,[38,39] the mean resting discharge of the ipsilesional, second-order VNns falls from 36 spikes/s in normal, alert animals to 7 spikes/s just after unilateral labyrinthectomy, and is still low (about 17 spikes/s) after one day of compensation. The spontaneous discharge averages 25 spikes/s after three days, and completely recovers only after one week. Therefore, our data show that the intensity of Bechterew's syndrome observed *in vivo* largely reflects the level of spontaneous activity recovered by the second-order VNns on the initially deafferented side at the time of the second lesion.

Persistence of the asymmetries in vestibular-related pathways of brains taken from labyrinthectomized guinea-pigs: a coherent pattern. The vestibular-related pathways of brains taken from compensated animals (i.e. D3–D7 brains) displayed a prominent asymmetry between both sides of the *in vitro* preparation. The pattern of this asymmetry was coherent with the known connectivity of the vestibulo-ocular network, and mimicked Bechterew's syndrome induced *in vivo* (Fig. 1D). Indeed, the second-order VNns recorded on the newly deafferented side were significantly hypoactive, whereas those recorded on the compensated side tended to be more active than in control brains. Accordingly, the abducens motoneurons contralateral to the newly deafferented VNns became almost silent, while the abducens motoneurons contralateral to the VNns on the compensated side were hyperactive. The asymmetry of neuronal activities between both vestibular nuclei was finally reflected in the responses evoked in the abducens nerves by single-shock, vestibular or spinal stimulation.

The asymmetry found in the vestibulo-ocular networks of IWBs taken from labyrinthectomized animals increased with the time elapsed between the initial lesion and the removal of the brain, and followed the same time-course as Bechterew's syndrome induced *in vivo*. It was indeed either absent or non-significant in D1 brains, and the imbalance between the spontaneous activities of second-order VNns increased from D3 to D5–D7 brains. It is therefore likely that the asymmetry characterizing the D3–D7 brains *in vitro* reproduced the imbalance between neuronal activities on both sides of the vestibular-related networks, which underlies Bechterew's phenomenon *in vivo* (Fig. 1D). This hypothesis is further ascertained by the occurrence of a "spontaneous nystagmus" with quick phases directed towards the compensated side[23,24]

in some of the D3–D7 brains, since a similar nystagmus was associated with Bechterew's phenomenon *in vivo*.

Consequences of the persistence of Bechterew's asymmetry in the isolated whole brain. The persistence of Bechterew's asymmetry in the IWB has allowed us to answer positively the two main questions raised in the introduction. First, the IWB does retain some of the plastic cellular changes underlying vestibular compensation. Second, after one day and during the first week of vestibular compensation, the CNS does not require any continuous inflow from substituting sensory afferents to maintain the new balance reached between the resting discharges of VNns on both sides of the brainstem. Indeed, during the first week of vestibular compensation, a similar Bechterew phenomenon is triggered (i) *in vitro* following the section of the intact vestibular nerve in IWBs taken from previously labyrinthectomized animals and (ii) *in vivo* by a second labyrinthectomy. In addition, we have seen above that, *in vivo*, the intensity of Bechterew's syndrome was linked with the level of spontaneous activity recovered by the initially deafferented VNns. These two points strongly suggest that, after one day, the physiological changes *in vivo* underlying the recovery of a normal resting discharge by the initially deafferented VNns persist in IWBs, despite the absence of any organized proprioceptive or visual input in this preparation.

This does not mean that the sensory, non-vestibular information reaching vestibular neurons (which mainly include proprioceptive, somatosensory and visual fibers)[55] does not play an essential role during the first 24 h of compensation (see Refs 9, 10 and 49 for reviews). Indeed, the static postural symptoms induced by unilateral labyrinthectomy do not compensate as long as guinea-pigs are lifted off the ground.[19,43] Furthermore, restraining the head and body of a guinea-pig in their normal, straight ahead position slows compensation for the static symptoms.[19,35,45] Finally, we cannot exclude that, even after the first day of compensation, asymmetric sensory information might be responsible for triggering the mechanisms allowing the deafferented, second-order VNns to go on recovering a normal spontaneous activity.

Our data extend the results obtained *in vivo* by Magnus[25] and Spiegel and Démétriades,[50] which demonstrated that Bechterew's syndrome still occurred in compensated cats and dogs after exclusion of optic signals, or section of the upper cervical dorsal roots. Bechterew's phenomenon was even observed following removal of the whole cerebrum or ablation of the cerebellum. In fully compensated animals then, an intact medulla oblongata appears sufficient to maintain the new balance reached between the mass discharges of second-order VNns on both sides, and to trigger Bechterew's syndrome.[44]

On the other hand, several authors have shown that section of the cervical dorsal roots or spinal transection induced a postural decompensation in guinea-pigs[2,19] and monkeys.[17] In contrast with our data, these experiments suggested[43] (see Ref. 49 for review) that spinal input was necessary to maintain the resting discharge of the deafferented, second-order VNns. This decompensation, which often lasted only a few hours, could, however, simply be due to a greater sensitivity of the deafferented VNns to diaschisis (i.e. neural shock[26]) induced by any additional deafferentation (see Ref. 49 for discussion of this point), and not the removal of the spinal inputs *per se*. The fact that spinal transection bilaterally depressed the spontaneous activity of identified, type I and type II VNns in normal as well as in compensated gerbils[30,32] (see Ref. 11 for the type I–type II classification of vestibular-related neurons) gives further support to this hypothesis.

The properties of vestibular-related pathways in isolated whole brains taken from labyrinthectomized guinea-pigs give insights into the neuronal mechanisms of vestibular compensation

We will now discuss to what extent the modifications of the vestibular- and spinal-evoked responses observed in IWBs taken from previously labyrinthectomized animals can shed some light on the neuronal mechanisms underlying the first week of the compensation process.

Responses evoked in the vestibulo-abducens pathways by single shock stimulations of the vestibular nerves

Field potentials evoked in the medial vestibular nucleus area by ipsilateral vestibular nerve stimulation. Our data first confirm that, despite their silence following labyrinthectomy, the ipsilesional, sensory vestibular neurons did not degenerate.[20,46] Until at least one week after the lesion, they retained their ability to respond to electrical stimulation and could still generate synaptic potentials in second-order VNns (see Refs 9, 10 and 49 for reviews).

Our data also suggest that a significant decrease in the efficacy of the synaptic transmission between the sensory afferent fibers and their target vestibular neurons occurs three days after the initial labyrinthectomy (Fig. 10). The amplitudes of the evoked vestibular field potentials were indeed significantly lower than normal on both sides of D3–D7 brains. On the newly deafferented side, this reduction might result from the large dysfacilitation of second-order VNns induced by the acute deafferentation (Fig. 1D). However, this explanation does not hold for the compensated side, where the resting discharge of VNns was in the normal range or even higher. This result may be linked with the transganglionic degeneration observed in the central terminals of sensory vestibular neurons after peripheral vestibular deafferentation.[16] In the spinal cord, peripheral nerve lesion is followed within a few days by a significant decrease in the conduction velocity of dorsal root myelinated axons, and in the amplitude of the related evoked field potential (see Ref. 1 for review).

Asymmetry of the discharges evoked in the contralateral abducens nerve. The low size of the response evoked by stimulation of the vestibular nerve on the compensated side in the contralateral abducens nerve on the newly deafferented side suggests that, *in vivo*, vestibular compensation must be associated with a reduction in the efficacy of synaptic transmission between the second-order VNns on the intact side and the contralateral abducens motoneurons on the lesioned side (Fig. 10). Indeed, while stimulation of the vestibular nerve on the newly deafferented side only evoked 35% smaller than normal excitatory field potentials in the ipsilateral MVN area, the response obtained in the contralateral abducens nerve was decreased by about 80%. In D5–D7 brains, this decrease could be due to the increased activity of the VNns on the compensated side, which exert a

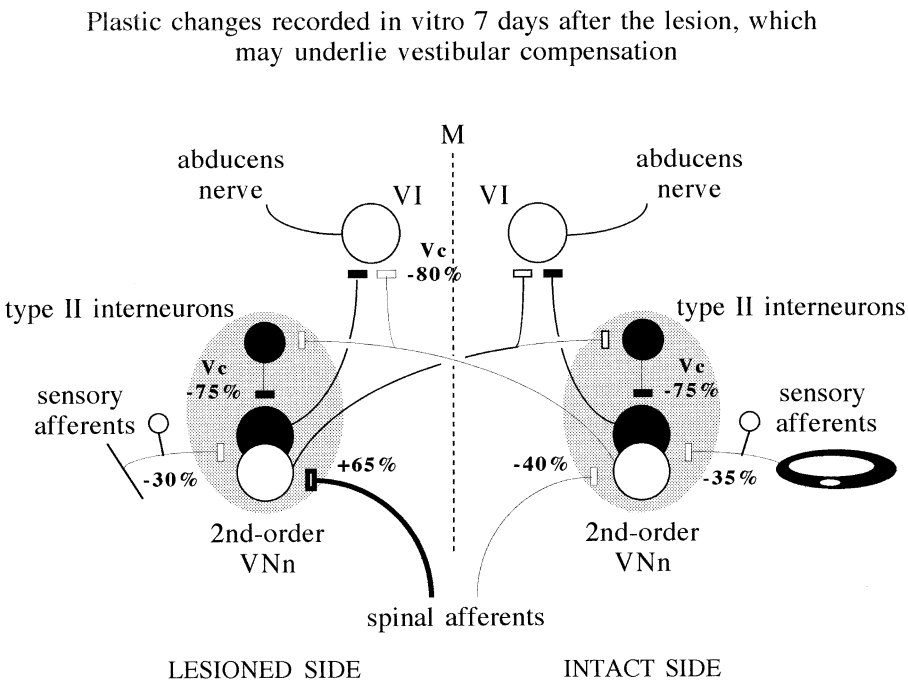

Fig. 10. Plastic changes recorded *in vitro* seven days after the lesion, which may underlie vestibular compensation *in vivo*. We used the drawing of Fig. 1C to summarize the putative changes of synaptic efficacy which have taken place in the vestibular-related pathways of compensated guinea-pigs, one week after a unilateral labyrinthectomy. The approximative percentages of modification of the response to single-shock stimulation of the afferent input are shown in each case, while the thickness of the pathways has been reduced or increased accordingly. In the case of ambiguity, Vc indicates that the modified response was induced by stimulation of the contralateral vestibular nerve. As in Fig. 1, the excitatory neurons and synapses are shown in white, whereas the inhibitory neurons and synapses are drawn in black. The absence of signs inside the neurons indicate balanced levels of spontaneous activity between both sides of the brain in these compensated guinea-pigs. M, the median line of the brain; VI, abducens motoneurons.

more powerful inhibition on the ipsilateral abducens motoneurons (Fig. 1D). However, this explanation does not hold in D3 brains, where the spontaneous activity of the VNns on the compensated side was in the normal range. Therefore, this large *in vitro* decrease of the responses evoked in the abducens nerve on the compensated side following stimulation of the contralateral vestibular nerve is probably linked with the compensation process.

In contrast, a normal response was evoked by stimulation of the vestibular nerve on the compensated side in the abducens nerve on the newly deafferented side, despite the fact that stimulation of the contralateral vestibular nerve on the compensated side evoked 30% smaller than normal excitatory field potentials in the ipsilateral MVN area (Fig. 10). This could be due to the decreased activity of the newly deafferented VNns, which must exert a less powerful inhibition on the ipsilateral abducens motoneurons (Fig. 1D).

The decreased efficacy of the neuronal pathways linking the intact labyrinth with its target motoneurons could contribute to the bilateral reduction of the gain of the horizontal vestibulo-ocular reflex observed following unilateral labyrinthectomy in the guinea-pig,[52] as well as in other vertebrate species (see Refs 9, 10 and 49 for reviews). This result has important clinical implications: following unilateral loss of the sensory vestibular afferents (due to unilateral neurectomy, trauma, vestibular neuritis), human patients experience strong vestibular-related deficits for head rotations towards both the ipsi- and contralesional sides (see Ref. 9 for review). At least one clinical study suggests that the ocular responses triggered by caloric stimulations of the intact labyrinth are also modified on a long-term basis, and go on changing for several years after the lesion.[12]

Irregularity of the resting discharge of second-order vestibular nucleus neurons: a consequence of membrane properties changes? A prominent characteristic of D1 brains was the presence in both MVN areas of a high proportion of irregularly discharging neurons, which often displayed bursts of spikes. This behavior was not related to variations in the spontaneous discharge of second-order VNns. The irregularity progressively disappeared in D3–D7 brains, though at a faster rate on the newly deafferented, previously intact side than on the compensated, initially lesioned side. The regularity of the spontaneous activity of second-order VNns in D5–D7 brains is in agreement with the recent *in vivo* results obtained by Ris and Godaux[40] in awake animals.

These data indicate that the first days of vestibular compensation are associated with a disruption of the discharge regularity of second-order VNns, mostly on the lesioned side (as already suggested by *in vivo* data; Curthoys I. S. *et al.*, personal communications), but also on the intact one. Interestingly, similar patterns of irregular activity characterize the "injury discharges" displayed at the acute stage by the deafferented sensory vestibular neurons in the ipsilesional Scarpa ganglion.[46]

This increase in the irregularity of VNns suggests that unilateral labyrinthectomy might provoke a decrease of their afterhyperpolarization (AHP), particularly on the lesioned side. Indeed, in control brains, the regularity of second-order VNns has been linked with the amplitude of the AHP which follows each spike.[3] Since the amplitude of the AHP sets the sensitivity of sensory vestibular neurons to external stimulation,[14] this diminution could increase the synaptic sensitivity of the ipsilesional second-order VNns. Such a process might facilitate substitution of the sensory vestibular afferents by the remaining inputs converging on

these deafferented cells. At least, our results indicate that transient changes in the membrane properties of second-order VNns might be associated with the initial stages of the vestibular compensation process. In accordance with this hypothesis, Cameron and Dutia[8] have recently reported that, in slices, the spontaneous activity of VNns recorded in the rostral one-third of the ipsilesional MVN was increased for the first 24 h following the lesion.

Changes in the efficacy of vestibular-related commissural pathways in isolated whole brains taken from labyrinthectomized guinea-pigs. As pointed out in the Results, the positive field potentials evoked in each MVN area by stimulation of the contralateral vestibular nerve were used to evaluate the strength of the commissural connections in response to single-shock stimulations.

D3–D7 brains, in contrast to control and D1 brains, were characterized by a massive, bilateral decrease (by about 75%) of the positive field potentials linked with the commissural inhibition of second-order VNns. Interestingly, the small excitatory component of commissural connections was left unaffected, since the proportion of second-order VNns activated from the contralateral side was not modified.

This large, bilateral decrease of the commissural field potentials could be linked to the bilateral decrease of the response of second-order VNns to ipsilateral vestibular nerve stimulation observed in the same D3–D7 brains (Fig. 10). The amplitude of the field potentials induced in the MVN area was, however, only 30–35% lower than in normal IWBs. On the newly deafferented side of D3–D7 brains, the low spontaneous firing rate of the acutely deafferented VNns may have masked the effects of additional hyperpolarizing inputs (Fig. 1D). However, this explanation does not hold on the compensated side, where the level of activity of second-order VNns was either increased or in the normal range.

Our data therefore indicate that, *in vivo*, after three days of compensation, the commissural inhibition evoked on the lesioned side following single-shock stimulation of the intact side is decreased (Fig. 10). However, most of the previous electrophysiological studies indicate that the influence of this commissural inhibition on the resting discharge of the ipsilesional VNns is not modified during *in vivo* vestibular compensation.[29,48,49] Hence, we may have to differentiate (i) the tonic activity of commissural pathways setting the balance between the mass discharges of the two vestibular complexes at rest from (ii) the kinetic responses evoked by head movements or single-shock stimulation, which were bilaterally decreased in D3–D7 brains.

The decreased sensitivity of the inhibitory commissural pathways to single-shock stimulation might be involved in the bilateral reduction of the gain of the vestibulo-ocular reflex observed in all vertebrates following unilateral labyrinthectomy.[9,10,49,52] Indeed, there is general agreement that the commissural pathways are necessary for an effective compensation of the dynamic deficits (see Refs 18, 36 and 49 for reviews), while in mammals, their role in the disappearance of the static deficits is controversial.

Modifications of spinal-related pathways in isolated whole brains taken from labyrinthectomized animals

Spinal-evoked responses in the medial vestibular nucleus area. In D1 brains, the orthodromic field potentials evoked in the MVN area from the spinal cord were increased bilaterally. Since the proportion of second-order VNns activated orthodromically by the same spinal stimulation was not increased accordingly, the potentiation of spinal-evoked responses may mainly involve the VNns polysynaptically activated from the ipsilateral vestibular nerve.

In D3–D7 brains, the orthodromic responses of spinal origin (related to synaptic activation of VNns from proprioceptive and somatosensory pathways[3,6,41,42]) were increased in the MVN area on the compensated side, and tended to decrease on the newly deafferented side. This asymmetry did not arise from the general asymmetry prevailing in the vestibular-related pathways of these IWBs (Fig. 1D). Indeed, the antidromic responses evoked by spinal stimulations were similar on both sides. Furthermore, symmetric field potentials were recorded in the MVN areas following stimulation of the ipsilateral vestibular nerve. Therefore, we believe that the excitability of second-order VNns remained similar between both sides of the preparations.

The spinal influence would hence become progressively asymmetric after three days of compensation: the efficacy of spinal input would increase on the lesioned side and would be reduced on the intact side (Fig. 10). Since these asymmetric modifications appeared when behavioral compensation for the static deficits was already completed, they should be mainly involved in dynamic vestibular compensation.

These changes may support the functional substitution of the deficient, vestibular-related synergies by spinal-related reflexes such as the cervico-ocular and cervico-collic reflexes.[5,13,21,27] Indeed, the cervico-ocular reflexes appear to be potentiated following unilateral labyrinthectomy,[7,15,33,51] though in humans, the reported modifications were highly variable between subjects (see Refs 9, 10 and 49 for reviews). In the compensated gerbil, spinal transection provokes a strong, asymmetric decrease of the gain of type I, second-order VNns on the deafferented side to natural vestibular stimulation.[32]

Discharges evoked in abducens nerves. Discharges evoked in both abducens nerves by spinal stimulation displayed the same asymmetries as the responses evoked by contralateral vestibular nerve stimulation. Their reduction in the abducens nerve ipsilateral to the compensated side was in accordance with the hypothesis that, *in vivo*, vestibular compensation is associated with a strong decrease of the transmission between the second-order VNns on the intact side and the contralateral abducens motoneurons on the lesioned side (see above and Fig. 10).

CONCLUSIONS

The data presented in this paper give some insights into the mechanisms underlying vestibular compensation. The persistence in the isolated brain of asymmetries linked with Bechterew's phenomenon was demonstrated. Our data show that, from one day on, the spontaneous discharge recovered by the deafferented second-order VNns can be maintained in the absence of any functional vestibular, visual, somatosensory or proprioceptive sensory afferent. In contrast, the induction of that recovery probably requires the presence of asymmetric sensory inputs.

Different mechanisms appear to be involved according to

the compensation time. (1) D1 brains (taken after 20–24 h of compensation) were not very different from the control ones. For instance, they displayed a normal efficacy of the commissural pathways. The large proportion of irregular and bursting neurons found on both sides may reflect an increased sensitivity of second-order VNns to synaptic inputs and/or changes in the intrinsic membrane properties underlying their spontaneous discharge. A slight, bilateral increase of the sensitivity of second-order VNns to spinal stimulations was also observed. (2) D3–D7 brains were removed at a stage where behavioral compensation for the static deficits was completed. Results obtained on these IWBs suggest that vestibular compensation is associated at that point (Fig. 10) with (i) a bilateral decrease in the efficacy of the commissural inhibition in response to single-shock stimulation, (ii) a large decrease of the efficacy of the synaptic transmission between the VNns on the intact side and the contralateral abducens motoneurons, and (iii) an asymmetry of the spinal input reaching the second-order VNns, which is increased on the lesioned side and tends to decrease on the intact side.

Further investigations are needed to elucidate the cellular basis of these modifications.

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