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Plasticity Mechanisms in Vestibular Compensation in the Cat Are Improved by an Extract of Ginkgo Biloba (EGb 761)

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LACOUR, M., L. EZ-ZAHER AND J. RAYMOND. *Plasticity mechanisms in vestibular compensation in the cat are improved by an extract of Ginkgo biloba (EGb 761).* PHARMACOL BIOCHEM BEHAV **40**(2) 367–379, 1991.—The effects of administration of an extract of Ginkgo biloba (EGb 761) on vestibular compensation was studied in unilateral vestibular neurectomized cats. This experimental model of CNS plasticity was investigated by using behavioral tests (postural disorders compensation, locomotor balance recovery), electrophysiological (spontaneous and evoked neck muscle activity) and neurophysiological (spontaneous firing rate recovery of deafferented vestibular cells) recordings, and immunocytochemical methods (synaptic loss and synaptic reoccupation within the deafferented vestibular nuclei). In all experiments, EGb 761 was administered over 30 days at daily doses of 50 mg/kg IP. The results showed a faster recovery in the EGb-treated group of cats as compared to an untreated control group. EGb administration strongly accelerated postural and locomotor balance recovery. Concomitantly, spontaneous neck muscle activity, vestibulo-collic reflexes and spontaneous firing rate of vestibular units located on the lesioned side were restored earlier. Morphological correlates characterized by a more rapid synaptic reoccupation were found in the deafferented medial vestibular nucleus by means of immunoreactive labelling using an antibody against a synaptic vesicle-associated protein (synaptophysin), but they displayed a longer time-constant in comparison with the behavioral and neurophysiological data. These results clearly demonstrate that EGb 761 acts on recovery mechanisms considered as key processes in vestibular compensation. They suggest that this substance would possess neurotrophic and/or neuritogenic properties improving functional recovery after CNS injury.

Extract of Ginkgo biloba (EGb 761) Animal model of CNS plasticity Vestibular compensation Posture Locomotor balance Neck muscle activity Vestibulo-collic reflex Vestibular nuclei Spontaneous firing rate restoration Synaptic loss Synaptic reoccupation Cat

UNILATERAL lesion of the peripheral vestibular receptors (hemilabyrinthectomy) or of the vestibular nerves (vestibular neurectomy) results in well-known postural, locomotor and oculomotor deficits. All these disorders progressively vanish with time, leading to a total or near complete functional recovery which illustrates that basic property of the central nervous system (CNS): neuroplasticity [see (31,56)]. This remarkable functional recovery, referred to as vestibular compensation, has long been recognized as a suitable experimental model for studying the plasticity mechanisms which underly postlesion recovery in the CNS.

On the basis of electrophysiological and biochemical investigations, it has been shown that both static and dynamic syndromes observed during the acute stage could result from changes in the neuronal properties of the second-order vestibular cells, and particularly from imbalanced tonic and phasic nuclear activity. Extracellular unit recordings performed in cats and guinea pigs showed a decrease in the spontaneous firing rate of vestibular units located in the medial and superior nuclei on the lesioned side (39, 57, 58, 60), associated with an increase in resting discharge on the opposite side (57,59). Asymmetrical spontaneous activity was also found in otolith-related neurons in the cat (33, 49, 75) and, concomitantly, both canal and otolith vestibular cells on the deafferented side exhibited a loss of their dynamic sensitivity to head rotation in the frontal plane (51, 60, 75). Metabolic activity changes within the vestibular nuclei, evidenced by using the deoxyglucose method in the rat (37), the frog (19), and the cat (46), were also found to be closely correlated with the neurophysiological deficits. As a rule, the tonic and dynamic neuronal properties of the deafferented cells were partially restored in compensated animals (49, 52, 55, 61, 75), a result which has also been corroborated by the deoxyglucose method (19, 37, 46). In addition, a more recent study in the cat indicated that recovery of postural and locomotor equilibrium functions strictly parallels the functional reorganization time-course described in the deafferented vestibular units, underlying the functional implication of such neurophysiological mechanisms in the adaptive process (35).

Present-day knowledge does not provide a clear explanation as to the origin of this restoration of neuronal properties, even though different recovery-promoting mechanisms such as deafferentation hypersensitivity, synaptic derepression and reactive

synaptogenesis of remaining sensory inputs have been proposed. An increase in synaptic efficacy of commissural (11), cerebellovestibular (12) and visual (73) inputs was evidenced in the frog and in the cat. Structural remodelling, a rather well-documented neuroplasticity mechanism in the CNS, received only little attention in vestibular compensation and still remains poorly supported at present (21,29). However, a recent immunocytochemical study performed in the deafferented medial vestibular nucleus in the cat strongly suggested that it could be very active in the recovery process, since the lesion-induced synaptic loss was followed by a complete synaptic reoccupation in compensated animals, five months after the vestibular lesion (54).

Pharmaceutical treatments used to improve functional recovery following vestibular lesion have only been investigated recently as noted in a review by Peppard (48). The aim of the present study was to evaluate the effects of an extract of Ginkgo biloba (EGb 761) on vestibular compensation in the cat.

The EGb is prepared from leaves of one of the most ancient trees, which have been used in traditional Chinese medicine over several thousand years (45). The standardized extract of Ginkgo biloba leaves (24% flavonol heterosides and 6% terpenes: EGb 761) has been marketed in Europe (Tanakan, Rokan) and is particularly effective in patients exhibiting cardiovascular disorders (46). It also improves short-term memory in normal human beings (64), cognitive deficits (68) and vigilance (22) in geriatric patients. In addition to these clinical observations, experimental data clearly demonstrated that EGb 761 administration reduces the effects of arachidonic acid-induced cerebral infarction in the rat (8), of hypobaric hypoxia in the mouse (9), and decreases ischemia as well as experimental embolism-induced edema in mice and gerbils (63). EGb 761 also attenuates the effects of experimentally induced stress in the rat (41) when it displays neither anxiolytic, antidepressant nor analgesic activity (50).

Two other properties of EGb 761 must be considered with respect to vestibular compensation. The first concerns the cholinergic system, which has been reported to be heavily implicated in the recovery process after unilateral vestibular injury. Acetylcholine agonists and antagonists induce postural decompensation and over-compensation, respectively, in totally compensated frogs and guinea pigs (5, 19, 20, 56). Such results have been interpreted as resulting from hypersensitivity and/or an increased number of the acetylcholine receptors. Interestingly enough, chronic oral administration of EGb 761 enhances the number of muscarinic acetylcholine receptors in the hippocampus of the rat, with more pronounced effects in the old than in the young (65). The second interesting property concerns the changes in brain energy metabolism, which are characterized in the rat by glucose and deoxyglucose uptake improvement (28) and by ATP cerebral level increase (47). The biochemical modifications that have been reported above during the time-course of vestibular compensation, closely connected with the neurophysiological changes, constitute a second argument supporting the general idea that EGb could positively influence the recovery process. In fact, previous results obtained by our team in the unilateral vestibular neurectomized cat (UVN) clearly demonstrated the beneficial effects of EGb 761, which induces a faster compensation of both postural, locomotor and equilibrium disorders (14). Similar results have been obtained in the rat (10). Our purpose here was to correlate this EGb-induced improvement of posture and gait with eventual concomitant neurophysiological and morphological changes in the deafferented vestibular nuclei, and with the vestibulospinal balance recovery at the neck muscle level. This work was an attempt to specify the way in which EGb 761 acts on the recovery mechanisms, to determine its target sites and, more generally, to define its neurotrophic and/or neuritogenic properties.

METHOD

Experiments were performed in 31 adult cats weighing 3–4 kg, submitted to a unilateral vestibular neurectomy (UVN) on the left side. Of these, 6 were used in behavioral studies devoted to quantifying the compensation of postural and locomotor disorders and the recovery of equilibrium function. Thirteen cats were used in electrophysiological investigations dealing with the recovery of head postural control, by recording the spontaneous and reflex electromyographic (EMG) activity of neck muscles, and with the spontaneous firing rate restoration of the deafferented vestibular cells, by means of unitary extracellular recordings in fully conscious animals. The remaining 12 cats were utilized in an immunocytochemical study in order to quantify the synaptic surface loss within the deafferented vestibular nuclei and the subsequent synaptic reoccupation, as a function of the postoperative survival time.

Data from this multidisciplinary approach were collected from the first postoperative day until complete compensation.

Effects of EGb 761 on the recovery process at both the behavioral, EMG, neuronal and morphological levels were assessed by comparing the recovery profiles in two groups of cats: one control group, without any pharmacological treatment after vestibular neurectomy, and one experimental group treated daily with 50 mg/kg/day of an extract of Ginkgo biloba (EGb 761, IP) for 30 postoperative days. The standardized extract was the same as that used in commercial preparation, Tanakan (IPSEN Laboratory, France).

Vestibular Neurectomy

Unilateral vestibular neurectomy was performed on the left side by using a method described in a previous paper (72). It was carried out under sterile conditions and performed under visual control through a dissecting microscope on cats anesthetized with pentobarbital sodium (40 mg/kg). The vestibular nerves were sectioned at a postganglion level after mastoidectomy, partial destruction of the bony labyrinth and surgical exposure of the internal auditory canal. Presence of both spontaneous nystagmus and classic postural and locomotor disorders immediately after surgery were used as criteria indicating the effectiveness of the lesion. The animals were maintained postoperatively under antibiotics until recovery from the surgical effects (2–3 days).

Experimental Procedures

Behavioral study. Experiments were performed on 3 control (untreated) and 3 experimental (EGb-treated) cats. Recovery of postural and locomotor balance functions was described by means of clinical observations and quantified by using the rotating beam test.

Clinical observations. Daily measurements were made to quantify the postural and locomotor disorders occurring during the acute stage of the lesion and their subsequent compensation with time (chronic and compensated stages). Observation platforms based on the number of falls to the operated side per unit of time and on the degree of postural-locomotor asymmetry were used for this quantification. Posture and gait asymmetries were evaluated in the cats by measuring the deviation during locomotion (in cm/m), the support surface while standing erect (in cm^2) and the head-tilt (in degrees). Results were reported on a 4-points scale with, by convention, the state I corresponding to the most acute disorders observed just after surgery and the IV representing a near complete compensation. States II and III were defined as intermediate stages leading to 30% and 60% of recovery, respectively.

Equilibrium function recovery. It was quantified by using the

rotating beam test described in a previous paper (72). Prior to surgery, the cats were trained to walk on a cylindrical beam (3 m long, 12 cm diameter, 1.2 m above ground) connecting two compartments, which could be rotated about its longitudinal axis at variable speeds (tangential linear velocity from 0 to 35 m/min, i.e., angular velocity from 0 to 750°/s). The performance of the cat was evaluated daily and defined as the highest speed of rotation of the beam which did not lead to a fall in 4 consecutive trials. As a rule, performance increased according to the training sessions (1 hour once daily) and, within 8–12 training periods, all animals had reached their maximum performance (Max. P) which was highly comparable from one cat to another (range: 21–33 m/min). Data obtained after surgery were expressed for each cat as a percentage of its preoperative Max. P, each animal being taken as its own control.

Statistical evaluation. Results from this behavioral study were tested with the Student's *t*-test, by comparing the mean postoperative times (i.e., the averaged number of days) required to achieve a full posture and equilibrium recovery in both groups of cats.

Electrophysiological study.

Neck muscle electromyographic activity. EMG activity from 4 control (untreated) and 4 experimental (EGb-treated) cats was recorded using chronic EMG electrodes bilaterally implanted in the splenius capitis muscles (lateral flexor and dorsal extensor of the head). These intramuscular bipolar electrodes were made of two teflon-coated silver wires with bared tips (2–3 mm), separated by 1 cm in each muscle. The wires from the bilateral muscles were chronically implanted under sterile conditions on cats anesthetized with pentobarbital sodium (40 mg/kg) 2 weeks before vestibular neurectomy; they were led subcutaneously to a plug sealed to the cat's head. This classical technique permits stable recordings over a period of about 2 months. A metal implant was fixed stereotaxically onto the skull with dental acrylic and anchored by stainless steel screws. Three bolts fixing this implant to the head-holder ensured head restraint during the recording sessions. The cats were also chronically prepared for vertical and horizontal eye movement recordings by using Ag-AgCl electrodes implanted in the zygomatic arches above and below one of the eyes. EOG recordings helped to evaluate the influence of eye movements on neck muscle activity.

The cats were tested before surgery (control data) and once a day during the entire postoperative period (6 weeks). Each experimental session included two successive subsessions, devoted to the recordings of spontaneous and evoked neck muscle EMG activity, respectively. The cat was placed in a stereotaxic frame mounted on a platform, its head fixed to a holder mounted on the frame, and its trunk tightly wrapped in a hammock secured to the table so as to minimize body and limb movements. The stereotaxic frame together with the head holder were pitched 23° nose down, thus bringing the horizontal semicircular canals into the horizontal plane (7).

Spontaneous EMG recordings were performed with the cat's longitudinal axis lying in the horizontal position (0°). Polyunitary EMG activity recorded on both sides was sampled through a band pass filter (100–1000 Hz), rectified and integrated (time constant: 10 ms) over 10-s time periods using on-line computation (HP RS 25). A mean value (mV) was calculated for both ipsilateral and contralateral splenius muscles. EMG recordings were coupled with eye movement monitoring in the vertical and horizontal planes in order to eliminate the EMG modulations induced by spontaneous saccades or eye position shifts [cf. (66)].

Evoked neck muscle activity was recorded during static tilts of the platform in the roll and pitch directions, in the range $\pm 30°$ relatively to the standard horizontal position (0°), using 10° steps. By convention, sign + corresponds to a roll rotation towards the left side (which will be the operated side) and to a nose down pitch rotation, while sign − indicates opposite static tilts. In the normal cat, roll and pitch rotations lead to asymmetrical and symmetrical EMG modulations, respectively, which tend to restore the head horizontal position. Such evoked EMG activities constitute the behavioral expression of the vestibulocollic reflexes involved in head postural control. Data processing was based on the same protocol as previously defined for the spontaneous EMG activity. However, due to the strong imbalance observed in neck muscle spontaneous activity after UVN (ipsilateral increase and contralateral decrease), EMG reflex responses were also expressed by their module (M). The module was calculated by subtracting the mean DC level from the total amplitude (A) of the EMG response ($M = A - DC$).

Extracellular neuronal activity. Neuronal events from 3 experimental (EGb-treated) and 2 control (untreated) cats were analyzed and compared to those obtained in a normal group (unlesioned) used in previous studies [cf. (35,74)]. Prior to the experiments the animals were prepared for chronic recording of vestibular nuclei units. A craniotomy (70–80 mm^2) was performed under aseptic conditions on anesthetized cats (pentobarbital sodium: 40 mg/kg), leaving the dura intact, and a plastic chamber was stereotaxically implanted on the skull in a parasagittal plane 23° from the vertical to allow access to the brain stem. A needle was cemented on the midline, close to the chamber at the zero antero-posterior coordinates, as a reference point for the microelectrodes tracks. A metal implant was also fixed onto the skull in order to ensure the head restraint (cf. neck muscle activity). During the recording sessions, the dura was carefully removed under local anesthesia (lidocaine 2%), and a recording tungsten microelectrode (9–12 MΩ at 100 Hz) was vertically driven through the cerebellum with a micromanipulator fixed to the stereotaxic frame mounted on the platform. The extracellular neuronal signals were recorded from the vestibular nuclei on the side of the lesion, delimited on the basis of stereotaxic coordinates. They were fed through a cathode follower, filtered, preamplified and displayed on a oscilloscope. Action potentials were passed through a window discriminator transforming the isolated spikes into standard impulses.

The resting discharge of each neuron tested was evaluated directly by the computer from recordings of 100 to 1000 action potentials, depending on the spontaneous firing rate of the cell, sampled at 5 KHz. The data processing program provided the mean level of instantaneous discharge (M), the standard deviation (Σ) and the coefficient of variation ($CV = \Sigma/M$) for each unit. This later parameter gives a good estimate of the regularity (or irregularity) of the neuronal discharge.

Neuronal recordings were performed with the cat's longitudinal axis in the horizontal position (0°). At the end of each recording session, the exposed surface of the cerebellum was covered with a sterile animal placental membrane and the plastic chamber implanted on the skull was cleaned and filled with cotton wool.

Identification of the recording sites was sometimes done on line by means of field potential measurements induced by electrical stimulation of the contralateral labyrinth [cf. (74)]. In most of the cases, postmortem histological identifications were performed using dye marks, a method described in previous studies (74). Most responsive cells were found to be located in and around the lateral vestibular nucleus, which is well known to heavily project at all the spinal levels and to play a major role in head and body postural control (69–71).

Statistical evaluation. Average EMG values recorded daily in the standard horizontal position (0°) were referred to as the mean DC level for both the left (ipsilateral) and right (contralateral) splenius muscles in the two groups of cats. These mean sponta-

neous activities, expressed in terms of muscular energy (surface of the integrated EMG per unit of time), were amalgamated over each of the six postoperative weeks. These weekly mean postoperative data were expressed in percent of the controls and statistically tested using the Student's *t*-test. Evoked neck muscle activity recorded during static tilts in the frontal (roll) and sagittal (pitch) directions were submitted to the same data processing. Results from the neurophysiological study (spontaneous firing rate and coefficient of variation) were also tested with the Student's *t*-test according to the same general procedure.

Immunocytochemical study. This investigation was conducted on a population of 12 cats subdivided into one EGb-treated experimental group (N = 7) and one untreated control group (N = 5). Immunocytochemical study was performed by using an antibody against synaptophysin, a synaptic vesicle-associated protein, in order to quantify the synaptic loss and the subsequent synaptic reoccupation. Preparation of tissues, immunocytochemical staining and quantimetric analysis of immunoreactive surfaces within the vestibular nuclei have been previously described in detail [see (54)].

Tissue preparation. Animals were deeply anesthetized with pentobarbital sodium (50 mg/kg) and sacrificed at various survival intervals: 1 week (acute stage), 3 weeks (compensatory stage), 3 and 5 months (in compensated cats). They were perfused with a fixative containing 4% paraformaldehyde and 0.2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. The brainstem containing the vestibular nuclei was dissected and cut into serial 300 μm thick frontal sections. The ipsilateral and contralateral vestibular nuclei were identified, dissected out of each section, embedded in Epon and semithin sections (1 μm) were cut at corresponding frontal levels in both the deafferented (left) and normally afferented (right) vestibular structures.

Immunocytochemical staining. After removing Epon (solution of 2 g KOH in 10 ml absolute methyl alcohol and 5 ml propylene oxide) and rinsing, the sections were treated for immunoperoxidase staining by sequential incubations in: 17% normal swine serum in Triton buffer (0.3% Triton X 100, 0.45 M NaCl in sodium phosphate buffer, pH 7.4); affinity-purified antibodies against synaptophysin diluted 1:16 in Triton buffer containing 1% normal swine serum, for 15 h at room temperature; swine antirabbit IgG (Dako patts, 1:50); rabbit peroxidase-antiperoxidase complex (Biosys, 1:100); and diaminobenzidine/hydrogen peroxide.

Quantification of synaptophysin immunoreactive surfaces. Twenty sections from each animal were analyzed at different rostro-caudal levels in the ipsilateral and contralateral nuclei and computed by means of an image analyzing system. The percentage of immunoreactive puncta surface was measured in the medial vestibular nuclei, which exhibited the most homogeneous immunocytochemical staining distribution, under 25 × lens. Thinness of the sections allowed precise focussing on peroxidase reaction product with unambiguous detection of the synaptic profiles. The ratio between the immunoreactive area and the total area of tissue (blood vessels excluded) did not differ from one section to adjacent section. Synaptic surface loss and synaptic reoccupation were evaluated by comparing the immunoreactive staining in the deafferented to that of the normally afferented medial nucleus referred to as the control level. It was verified that the synaptic surface remained unchanged in the contralateral vestibular nuclei of lesioned cats, whatever the survival period tested, and that these synaptic profiles did not differ significantly from those made in unlesioned cats. This indicates that the contralateral vestibular nuclei constitute a good reference for evaluating the lesion-induced changes in the ipsilateral vestibular structures (Fig. 1A,B).

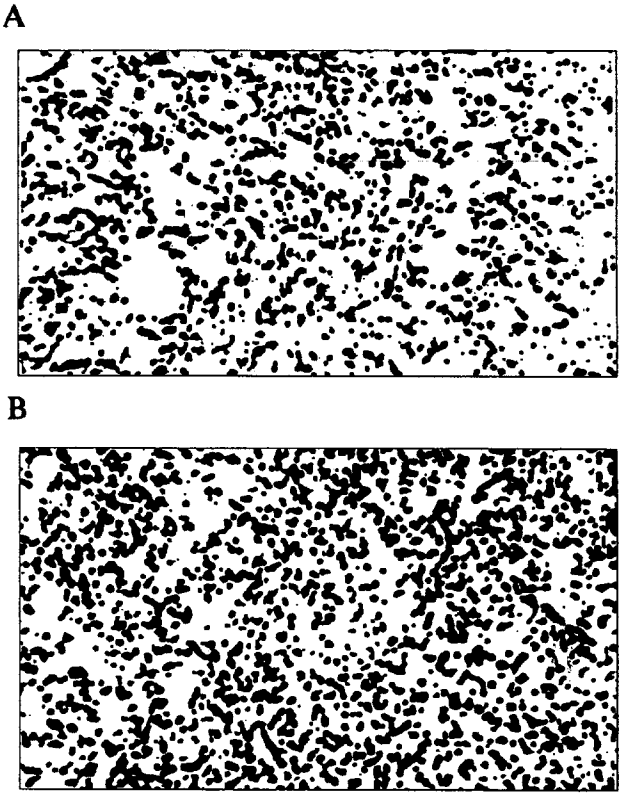

FIG. 1. Digitized images of synaptic densities in the medial vestibular nuclei. Antisynaptophysin immunostaining on semithin sections of the ipsilateral (A) and contralateral (B) medial vestibular nuclei recorded from a unilateral vestibular neurectomized cat at the 7-day survival period. The synaptic density averages 27.97% in the nucleus located on the intact side (B), a value close to that found in the normal cat, while it is significantly decreased (18.30%) in the nucleus located on the lesioned side (A).

Statistical evaluation. Data from the deafferented medial nucleus were expressed in percent of the values calculated in the contralateral structure for each survival period, both measurements being made at stereotaxic planes of the nucleus. They were expressed as the mean (± SD), the differences between the two sides, the two groups of cats and the survival periods being analyzed statistically using the Student's *t*-test.

RESULTS

Behavioral Study: Effects of EGb 761 on Postural and Equilibrium Functions Recovery

Postural compensation. Postural and locomotor disorders are progressively compensated for with time in both the EGb-treated (T) and the untreated (NT) groups.

In the NT group, the initial period of maximum impairment lasts several days (State I). States II and III of recovery are reached at the end of the first and second postoperative weeks, respectively, while an almost complete functional recovery is achieved on the average 19 days postoperatively (± 2.1) (Fig. 2B).

Conversely, the T group exhibits a faster recovery, with postural and locomotor disorders compensated for within the second postoperative week. State I is shortened while states II and III

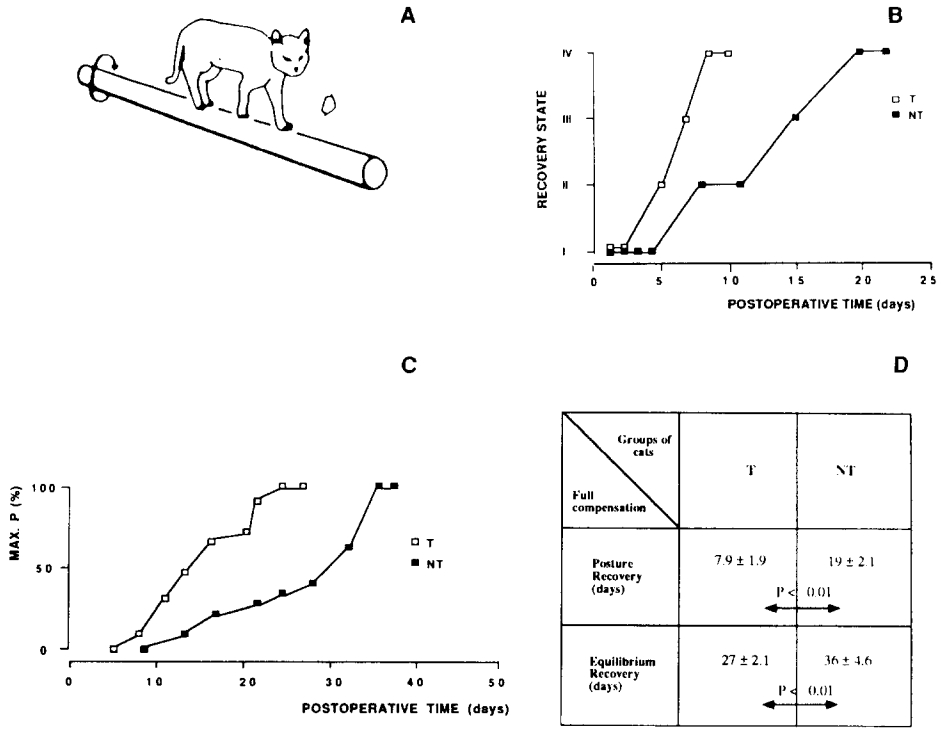

FIG. 2. Effects of the extract of Ginkgo biloba (EGb 761) on posture and locomotor balance recovery. (A) The rotating beam test, used to quantify locomotor equilibrium recovery in the cat. (B) Postural function recovery in the treated (T: N = 3) and untreated (NT: N = 3) groups of cats. Mean recovery curves showing the clinical state of recovery (States I–IV: on the ordinates) as a function of the postoperative time (in days, on the abscissae). State I corresponds to the maximal disorders induced by unilateral vestibular nerve lesion. States II and III to intermediate states with 30% and 60% recovery, respectively, and State IV to a near-complete recovery. (C) Locomotor balance recovery in the same groups of cats. Mean recovery curves showing the maximal performance (Max. P) of the cats on the rotating beam (on the ordinates) as a function of the postoperative time in days (abscissae). Max. P is expressed in percent of the preoperative values. (D) Table indicating the mean postoperative time-period (in days, with the standard deviation) required for achieving postural and locomotor balance recovery, and the statistical evaluation (*t*-test).

of behavioral recovery are attained as early as 5 and 8 days postoperatively. An almost complete compensation of the deficits, except a head tilt towards the lesioned side, is seen 7.9 days (± 1.9) after surgery (cf. Fig. 2B).

As described in a previous paper [cf. (14)], oculomotor recovery, as tested by disappearance of the spontaneous vestibular nystagmus, is also achieved more rapidly in the EGb-treated animals when tested in both light and darkness conditions.

Locomotor equilibrium recovery. Locomotor balance recovery was evaluated by using the rotating beam test (Fig. 2A). It develops in both groups in close relationship with compensation of the postural deficits, the cats remaining unable to walk on the rotating beam, even at the lowest speed of rotation, as long as they show an intense postural syndrome corresponding to state I. Locomotor balance on the beam occurs when state II of recovery is reached, corresponding to a delay averaging 5 and 9 days in the T and NT groups, respectively.

Recovery of equilibrium function, expressed in percent of the maximal performance of each animal before surgery, is illustrated in Fig. 2C. In the NT group of cats, recovery first develops slowly with a mean Max. P corresponding to 25% and 50% of the control at 20 and 30 postoperative days, respectively. It goes faster during the following days, as indicated by a return towards Max. P (100% of the control preoperative values) at the end of the fifth postoperative week (36 ± 4.6 days). By contrast, locomotor balance recovery is achieved more rapidly in the T-group. Max. P averaging 50% of the control preoperative level is seen as early as 13 days after surgery, and 100% is reached at the end of the third postoperative week. An almost linear relationship between Max. P and postoperative time is found in this group, contrasting with the delay observed in the curve concerning the NT group.

Conclusions. Statistical analysis shows that EGb significantly accelerates ($p<0.01$) both the postural and equilibrium function recovery (Fig. 2D), in comparison with the untreated group. The beneficial effects of an EGb postoperative treatment are illustrated by a mean time benefit of approximately 11 and 9 days for the postural disorders compensation and the locomotor balance recovery, respectively.

Electrophysiological Study: Effects of EGb 761 on Spontaneous Neck Muscle Activity Restoration and Vestibulo-Collic Recovery

Spontaneous EMG activity. In the normal cat, the spontaneous EMG activity recorded in the horizontal position (0°) (cf. Fig. 3A) from the left and right splenius capitis muscles is symmetrical, with values averaging 68.51 ± 29.14 mV (SD) and 77.23 ± 28.07 mV, respectively ($p>0.05$, NS). Interindividual

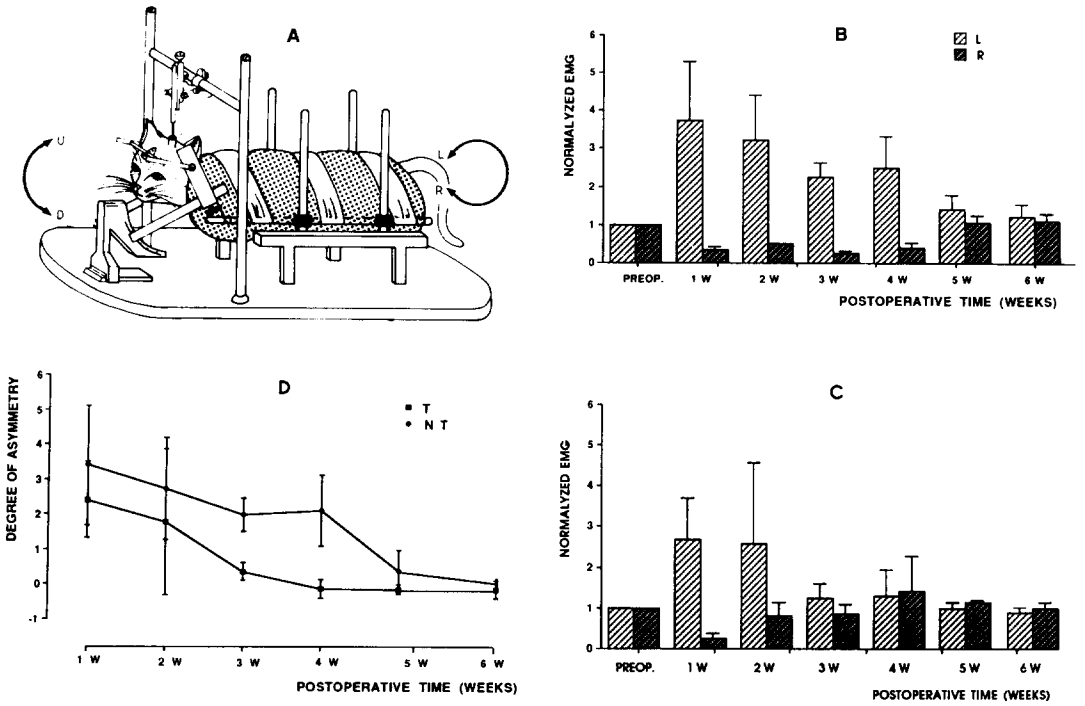

FIG. 3. Effects of the extract of Ginkgo biloba (EGb 761) on head posture recovery. (A) Experimental set up used for EMG (neck muscle) and extracellular unit (vestibular nuclei) recordings in the alert cat. (B) Spontaneous EMG activity restoration in the ipsilateral (left: L) and contralateral (right: R) splenius capitis muscles. Histograms showing the development on the mean EMG activity (ordinates), normalized with respect to the controls (preop) as a function of the postoperative time in weeks (abscissae) in the untreated control group of cats (N=4). Standard deviation is reported as vertical solid lines. (C) Results from the EGb-treated group of cats (N=4). Same conventions as above. (D) Mean curves indicating the degree of asymmetry between the left and right splenius capitis neck muscles (ordinates) as a function of the postoperative time in weeks (abscissae), in the treated (squares) and untreated (circles) groups of cats. Asymmetry is evaluated by the difference between left and right average spontaneous EMG activity, normalized with respect to the controls (referred to as zero ± SD). Standard deviations are reported as vertical lines.

variations are observed (range: 37.14–149 mV for the left muscle, 34.24–127.84 mV for the right muscle) but, as a rule, each animal exhibits stable spontaneous neck muscle activity from one experimental session to another. This is reflected by the left/right ratio which reaches on average 0.92 ± 0.16, a value very close to unit and constituting a good index of a balanced spontaneous EMG activity on both sides [cf. (30)].

Unilateral vestibular neurectomy induces strong asymmetry in the spontaneous EMG activity of the neck muscles, characterized by marked hyperactivity of the ipsilateral (left) splenius muscle associated with a strong hypoactivity of the contralateral (right) splenius muscle. This imbalance in EMG activity, responsible for the head tilt on the lesioned side, is found in both the T and NT groups of cats. However, the recovery time-course of a balanced EMG activity differs according to the two groups (Fig. 3B,C). In the NT control group, a strong asymmetry remains until the end of the fourth postoperative week, even though a slight attenuation is observed during this period, due essentially to a decrease in the spontaneous hyperactivity of the ipsilateral muscle. Normalized values with respect to the controls, recorded during the first postoperative month, show a reduction from 3.56 ± 1.61 to 2.51 ± 0.88 in the left muscle, but no significant change in the right muscle (0.30 ± 0.08 to 0.38 ± 0.15). Return towards normal and symmetrical EMG values is found at the end of the fifth postoperative week, with mean normalized values of 1.35 ± 0.40 and 0.87 ± 0.19 in the left and right muscles, respectively (Fig. 3B). This recovery time-course is also reflected by the development of the degree of asymmetry, decreasing from 3.40 ± 1.71 (first week) to 0.37 ± 0.59 (fifth week: cf. Fig. 3D).

By contrast, restoration of a balanced EMG activity is strongly accelerated in the T group of cats (Fig. 3C). Normalized EMG values recorded during the first postoperative week from the left (2.79 ± 1.05) and right (0.25 ± 0.14) splenius muscles do not differ significantly from those encountered in the untreated animals. This asymmetry decreases slightly as early as the second postoperative week, because of restored spontaneous EMG activity in the contralateral right muscle. A balanced neck muscle activity is regained later on, as early as the third postoperative week, as illustrated by mean normalized values close to the controls. This faster recovery time-course is also illustrated in Fig. 3D, which compares the development of the asymmetry index in both groups of cats. A two weeks lead is clearly evidenced in the EGb-treated group.

Vestibulo-collic reflexes. Unilateral vestibular neurectomy also leads to a deep disorganization of the vestibulo-collic reflexes, which are totally lacking during static tilts in both roll and pitch directions. This means that head stabilization in space is completely missing following UVN, a result supported by many behavioral observations.

The time-constants of vestibulo-collic reflex restoration encountered in the two populations of cats correlate rather well

with those found in the above investigations concerning recovery of spontaneous EMG activity: near complete compensation needs around five weeks in the NT control group while it requires only three weeks in the T experimental group of cats.

Figure 4 summarizes these results as a function of the postoperative time in weeks and of the degree of static tilt, during frontal (roll: Fig. 4A–C) and sagittal (pitch: Fig. 4B–D) tilts in the NT (Fig.4A,B) and T (Fig. 4C,D) populations. Control data recorded in the normal cat before UVN are reported for comparison (open squares).

In all cases, no neck muscle EMG modulation can be seen during the first postoperative week. In the NT group, EMG modulations reappear during the third postoperative week in the ipsilateral splenius muscle, while subnormal responses are present one week later in the contralateral muscle (Fig. 4A,B). Near normal vestibulo-collic responses as compared to the controls are observed in both roll and pitch directions at the end of the fifth postoperative week, i.e., at a time-delay comparable to that found for spontaneous EMG activity recovery. In the T group, EMG modulations in the contralateral splenius muscle are also lacking during the first postoperative week but, interestingly, almost normal responses in terms of modulation are recorded ipsilaterally in spite of the high DC level induced by the vestibular lesion in the left splenius muscle (Fig. 4C,D). This means that the central processing of position signals provided by the intact labyrinth is less perturbated in this group. Bilateral but subnormal (particularly on the contralateral side) EMG responses are found as early as the second week. Amplitude of the modulations develops later on, leading to preoperative patterns of response on both sides at a time-delay also comparable to that previously described for spontaneous EMG activity recovery (i.e., 3 weeks postoperative).

Conclusions. Head postural control is again restored significantly more rapidly under postoperative treatment with an extract of Ginkgo biloba. A time benefit of around 2 weeks is observed in the recovery process of a balanced static and dynamic EMG activity in the neck muscles.

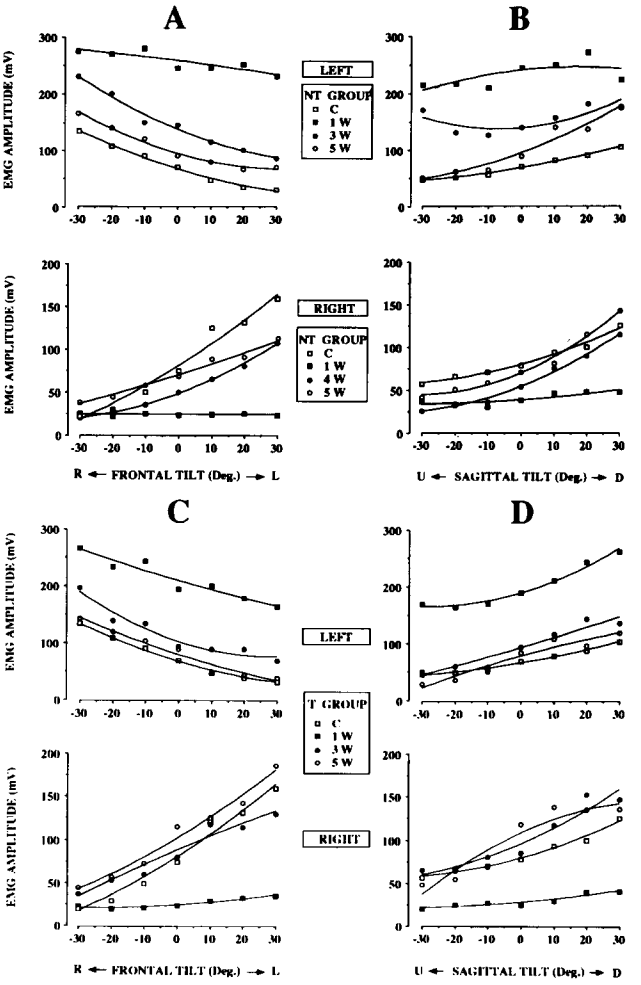

FIG. 4. Effects of the extract of Ginkgo biloba (EGb 761) on vestibulocollic reflex recovery in response to static tilts in the frontal and sagittal planes. (A) EMG modulations recorded in the left (ipsilateral: upper graphs) and right (contralateral: lower graphs) splenius capitis muscles in the untreated cats (N=4) are expressed on the ordinates (mV) as a function of the degree of static tilt (±30°: abscissae) in the frontal plane. Sign + and − indicate a tilt towards the left (L) and right (R) sides, respectively, with respect to the horizontal position referred to as 0 degree. Mean modulations from the normal unlesioned cats are reported for comparison (open squares). The average EMG modulations recorded during the first (filled squares: 1 W), the third (filled circles: 3W) and the fifth (open circles: 5W) postoperative weeks are plotted on each graph. In the contralateral muscle (lower graphs), results from the third week have been omitted and replaced with those from the fourth postoperative week (filled circles), since data recorded at the three weeks postoperative time did not differ significantly from those of the first week. The solid lines through the data show the best fitting curves (second order polynomial curves). (B) Results from the same untreated group of cats, during static tilt in the sagittal plane (signs + and − mean nose down and nose up tilts, respectively). Same conventions as in A. (C,D) Results from the EGb-treated cats (N=4), during static tilts in the frontal (C) and sagittal (D) planes. Same conventions as in A and B.

Neurophysiological Study: Effects of EGb 761 on Spontaneous Firing Rate Recovery Within the Deafferented Vestibular Nuclei

In the awake normal cat, the mean spontaneous firing level of vestibular units located in the Deiters' nucleus is 27.10±18.10 imp/s. This activity is strongly reduced in both groups of cats after UVN.

Figure 5A illustrates the postoperative time-course of the spontaneous activity recorded from Deiters' cells located on the deafferented side, in the NT group of cats [cf. (35)]. The mean firing level decreases to 6.35±4.91 imp/s during the first postoperative week, thus representing an average reduction of 77% as compared to the controls ($p<0.001$). Most of the tested cells (57%) show a firing rate ranging from 0 to 5 imp/s, while spontaneous firing rates higher than 15 imp/s were never encountered. Correlatively, the coefficient of variation (CV) increases significantly (1.23±0.36 instead of 0.73±0.3 in the controls: $p<0.001$), attesting to very irregular firing rates (Fig. 5C). Mean spontaneous activity and CV do not change until the fourth postoperative week during which some signs of recovery are present. But five weeks are needed for a consistent restoration of the spontaneous firing level. At this time, the frequency discharge averages 14.22±10.12 imp/s, corresponding to a 51% restoration, and the recorded units display a more gaussian distribution with a mode centered in the range 10–15 imp/s for 27.8% of the cells. The average CV decreases in parallel with resting discharge recovery and regains near normal value (0.82±0.37). Recordings performed during the sixth postoperative week point to similar results (resting discharge = 14.47±7.81 imp/s; CV=0.90±0.22, Fig. 5C).

Results from the T group of cats are illustrated in Fig. 5B. As a rule, similar deficits are found just after UVN. The aver-

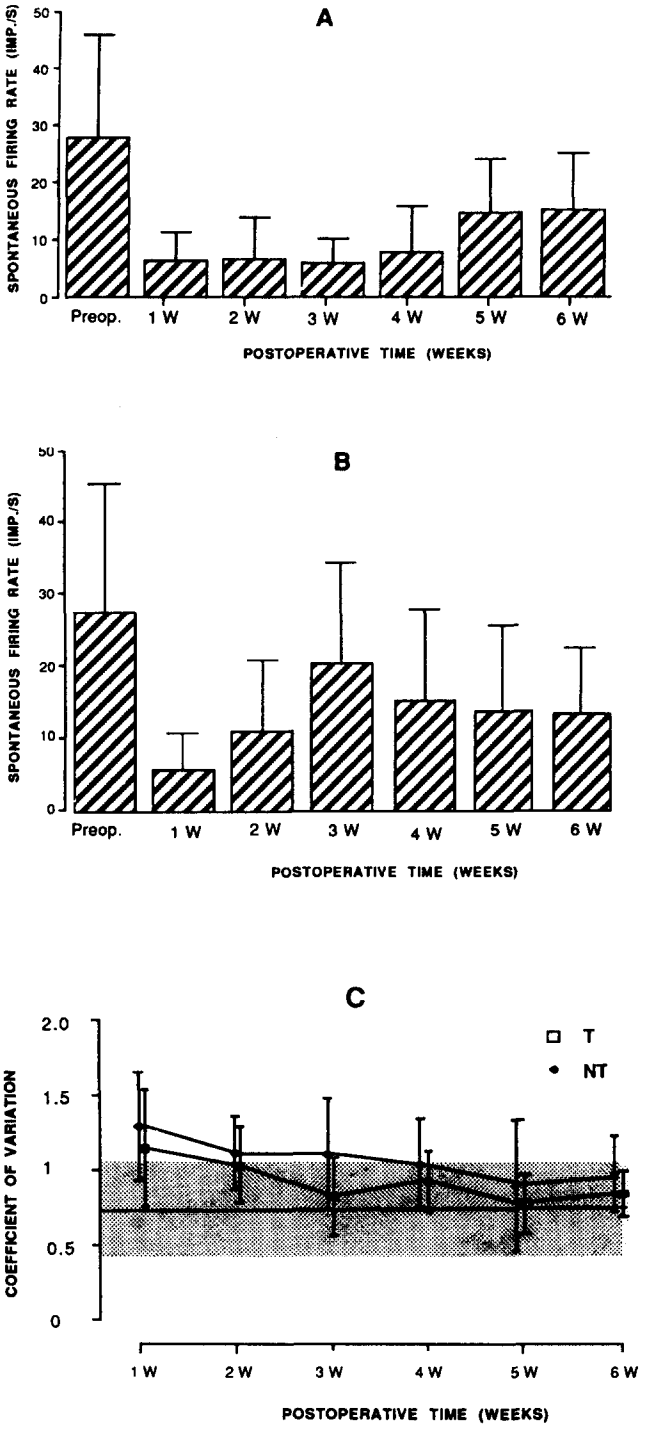

FIG. 5. Effects of the extract of the Ginkgo biloba (EGb 761) on the spontaneous firing rate recovery of vestibular cells located on the lesioned side. (A) Histograms illustrating the mean spontaneous resting discharge (in imp./s: ordinates) of deafferented vestibular cells within the Deiters' nucleus (n = 178 units) as a function of the postoperative time in weeks (abscissae) in the untreated group of cats (N = 2). Results from a control unlesioned population [cf. (74)] are reported for comparison (preop). Vertical lines represent the standard deviations of the mean values. (B) Histograms from the EGb-treated group of cats (N = 3 cats; n = 165 units). Same conventions as in A. (C) Modifications of the coefficient of variation (CV: ordinates) during the time-course of vestibular compensation (in weeks: abscissae). The CV recorded in intact cats is reported as the horizontal solid line (mean value) and the dashed area (±SD).

age resting discharge (5.99 ± 4.97 imp/s) corresponds to a reduction of 78.4%, which does not differ significantly from that calculated in the NT population. Most of the units also fire in the range 0–5 imp/s (50%) and the CV is again increased relatively to the controls (1.09 ± 0.39). However, the postoperative time-course of recovery displayed by this treated group is strongly accelerated. Improvement is underlined by a slight restoration observed as early as two weeks after the lesion, with mean values of 11.25 ± 9.82 imp/s and of 0.98 ± 0.25 for the resting discharge and the CV, respectively. This process progressively goes on since the average firing level increases to 20.48 ± 13.9 imp/s, while the CV decreases to 0.77 ± 0.26 during the third postoperative week (Fig. 5C). These values do not change significantly later on: the mean spontaneous activity corresponds to a 55% restoration, a gaussian-like distribution is regained with a mode in the 10–15 imp/s range for 33% of the units, and the CV remains comparable to the controls (0.87 ± 20), indicating that most of the tested units exhibit a regular firing behavior.

In conclusion, this study also points to a faster recovery of the neuronal properties of the deafferented vestibular cells in the group of cats submitted to a postoperative treatment with EGb 761, as compared to the untreated population. Time-constant of vestibular compensation is shortened under EGb in a way similar to that described in the above studies.

Immunocytochemical Study: Effects of EGb 761 on Synaptic Reoccupation Within the Deafferented Vestibular Nuclei

As described in a previous paper (54), immunoreactive synaptic surfaces quantified in the normal cat show a higher density and a more homogeneous distribution in the medial vestibular nuclei as compared to the others, the reason why this study was conducted at this level.

In both the NT and T groups of cats, antisynaptophysin immunostainings performed on semi-thin sections in the contralateral medial vestibular nucleus (used as the reference) show a synaptic density averaging 30.73 ± 3.57% of the total tissue surface (Fig. 6A). No significant differences were noticed as a function of the tested survival time periods in the antisynaptophysin immunoreactivity, which always resembles widely distributed terminals boutons.

In the NT group, synaptic density measured in the ipsilateral nucleus decreases significantly one week after UVN, with an average immunoreactive surface representing 19.75 ± 3.88% of the total tissue surface. This corresponds to a mean synaptic surface loss of 35.25 ± 13.83% (Fig. 6B). At the three weeks and three months survival postoperative periods, a significantly increased synaptic density is observed, as reflected by the corresponding synaptic surface loss values (14.00 ± 13.25% and 13.70 ± 17.13%, respectively). Such results point to a synaptic reoccupation reaching 60.28% on the average, as compared to the previous short survival period ($p<0.001$: Fig. 6C). Synaptic labelling returns towards normal immunoreactive density five months after the lesion. The mean value recorded at this survival period (26.93 ± 4.45%) does not differ significantly from

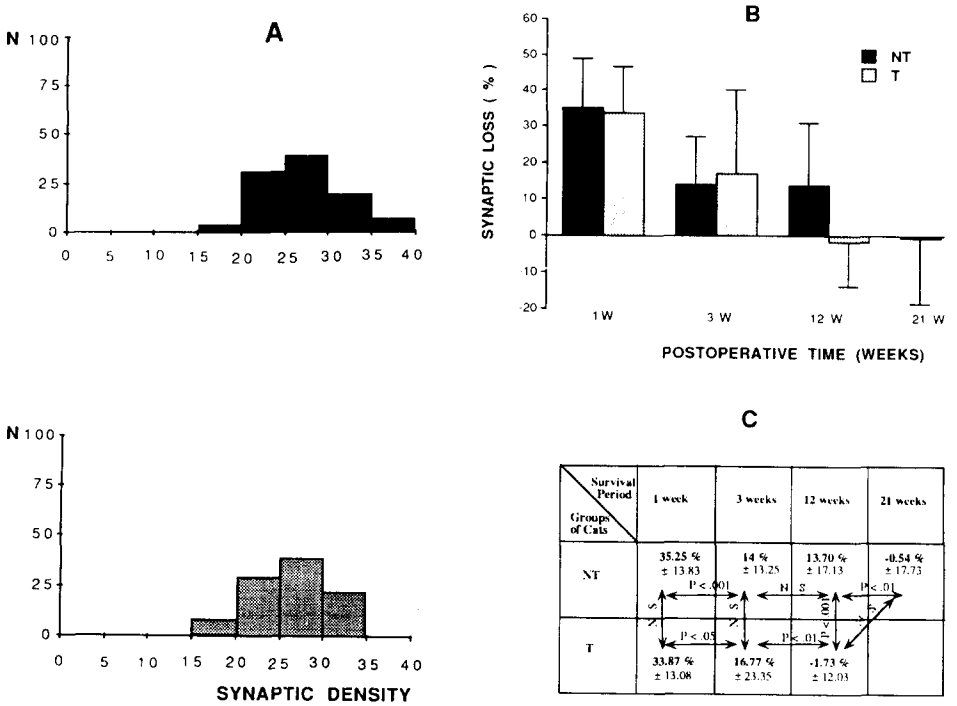

FIG. 6. Effects of the extract of Ginkgo biloba (EGb 761) on the synaptic reoccupation within the medial vestibular nucleus on the lesioned side. (A) Synaptic density distribution in the control (contralateral) medial vestibular nucleus in the untreated (upper graph) and EGb-treated (lower graph) groups of cats. Histograms illustrating the immunoreactive surfaces expressed in percent of total tissue surface by class intervals of 5% (abscissae). The ordinates show the number of values in each class expressed in percent of the total number of measurements. (B) Histograms showing the mean synaptic loss in the ipsilateral medial vestibular nucleus, expressed in percent of the immunoreactive surface calculated in the contralateral nucleus (ordinates) as a function of the survival period (1 week, 3 weeks, 12 weeks and 21 weeks: abscissae). Results from the untreated group of cats (N = 5) are represented as filled histograms, while those from the EGb-treated population (N = 7) are shown as open histograms. Standard deviation is reported as vertical lines. Note the faster complete synaptic reoccupation in the treated group (12 weeks) as compared to the untreated one (21 weeks). (C) Table illustrating the average values (± SD) of synaptic loss in both groups as a function of the survival periods, and the statistical analysis (*t*-test).

the controls (26.44 ± 2.83%), pointing to a total synaptic reoccupation (average synaptic surface loss: −0.54 ± 17.73%).

Quantification of the synaptic density in the T group shows similar deficits one week after the lesion, with an average immunolabelling value corresponding to a 33.87 ± 13.08% synaptic loss. Data recorded at the three-weeks survival period are again comparable to those from the NT group (Fig. 6B,C): the immunoreactive surface corresponds to a less important synaptic loss (16.77 ± 23.25%) which, in turn, represents a 50.48% synaptic reoccupation. By contrast with the NT population, normal synaptic density within the deafferented medial vestibular nucleus is recovered earlier in this treated group, i.e., three instead of five months after UVN. The average immunoreactive surface recorded at this survival period (28.35 ± 3.32%) does not differ from the controls, bearing witness to complete synaptic reoccupation (mean synaptic surface loss: −1.73 ± 12.03%).

In conclusion, unilateral vestibular nerve lesion leads to comparable synaptic losses in the untreated and EGb-treated groups of cat, as gauged by the decreased immunoreactive labelling seen within the deafferented medial nucleus at the one-week survival period. Synaptic density recovers completely in both groups but occurs earlier in the treated cats (three months/five months), suggesting that the extract of Ginkgo biloba also improves the recovery process by accelerating the development of lesion-induced structural modifications.

DISCUSSION

Results reported in the present study provide experimental evidence of the beneficial effects of EGb treatment on functional recovery after vestibular injury. Not only does EGb 761 strongly accelerate the postural and locomotor balance recovery [as well as oculomotor compensation: cf. (14)], but it clearly induces a more rapid restoration of both head postural control and spontaneous discharge in the deafferented vestibular nuclei neurons. As illustrated in Fig. 7, the compensation of postural and locomotor deficits as well as of spontaneous and reflex neck muscle activity parallels the recovery of a rebalanced neuronal activity in both the untreated and the treated groups of cats, but the time-constant of the recovery process is considerably shortened under EGb treatment. Similar conclusions can be drawn from the immunocytochemical investigation even though a longer time-constant is observed in the structural remodelling process, which raises the question of the functional implication of such morpho-

logical changes in vestibular compensation.

The present data demonstrate that recovery of the spontaneous discharge within the deafferented vestibular nuclei represents a basic mechanism and a key process in the compensation of the vestibular syndrome. This general statement had already been supported by electrophysiological recordings of canal- (39, 51, 55, 57, 58, 60) and otolith- (49,75) related vestibular units in compensated animals and during decompensation manoeuvers (1, 2, 3, 27), but it had never been demonstrated in totally awake preparations tested daily during the course of compensation. Regain of balanced neuronal activity leads to symmetrical tonic and dynamic vestibulospinal influences exerted on both neck, body and limb muscles by the lateral and medial vestibulospinal tracts (69–71). Most of the data encountered in literature can be supported by this model. Postural stability in cat (72) and man (4), locomotor balance function in cat (72) and squirrel monkey (25), spinal motoneuronal pool excitability in baboon (34), righting reflexes in cat (23) and dynamic otolith-spinal reflexes in baboon (32) are recovered within a similar time period (40 days, i.e., around 5 weeks). Compensation for static oculomotor imbalance (spontaneous nystagmus) in cat (42), monkey (15) and man (16) also requires a comparable time delay interval. The only exception concerns the VOR gain recovery, which remains incompletely compensated for in all tested species (15, 16, 42). Restoration of tone in the vestibular nuclei on the lesioned side, responsible for the reduction of the static vestibular asymmetries, can result from an intrinsic activity developing within the vestibular nuclei themselves, and/or from changes in synaptic efficacy of the remaining inputs (11, 12, 58, 61, 73). Such mechanisms would also explain the restoration of the dynamic vestibular deficits.

The hemodynamic and metabolic properties of the extract of Ginkgo biloba (EGb 761: cf. Introduction) are consistent enough to provide a first interpretation level able to support the faster recovery observed in the treated group of cats. EGb-induced brain metabolism (28) and ATP synthesis (47) changes are compatible with a neurotrophic hypothesis. As the metabolic activity of a neural cell is closely correlated to its functional electrical activity (62), and the biochemical modifications of the deafferented vestibular units parallel their spontaneous discharge recovery (19, 37, 40), the EGb 761 could improve the development of intrinsic activity on the lesioned side. Changes in the cell membrane properties are probably involved in this process. Recent works suggest that the vestibular lesion leads to hyperpolarization of the deafferented second-order cells which could result from membrane ion permeability modifications (67). Since activity-dependent energy metabolism reflects sodium pump activity (43) and EGb modifies Na+ flux across the cell membrane (13), such cellular mechanisms could constitute the target site of the extract of Ginkgo biloba.

Such EGb properties could also improve cholinergic neurotransmission, whose functional implication in vestibular compensation is rather well-documented (5, 6, 20, 56, 58). Cellular energy metabolism enhancement may induce an increase in A-coenzyme acetyl synthesis from glucose and pyruvine. As EGb facilitates glucose uptake and pyruvate concentration (28), this substance can be considered as a direct precursor of A-coenzyme acetyl and could, thereby, lead to an improvement of the acetylcholine synthesis. This EGb-induced property seems unlikely to result from an inhibitory action of the acetylcholinesterase enzyme. A recent study performed in aged rats indicates that chronic administration of Ginkgo biloba restores the number of muscarinic acetylcholine receptors, in a proportion similar to

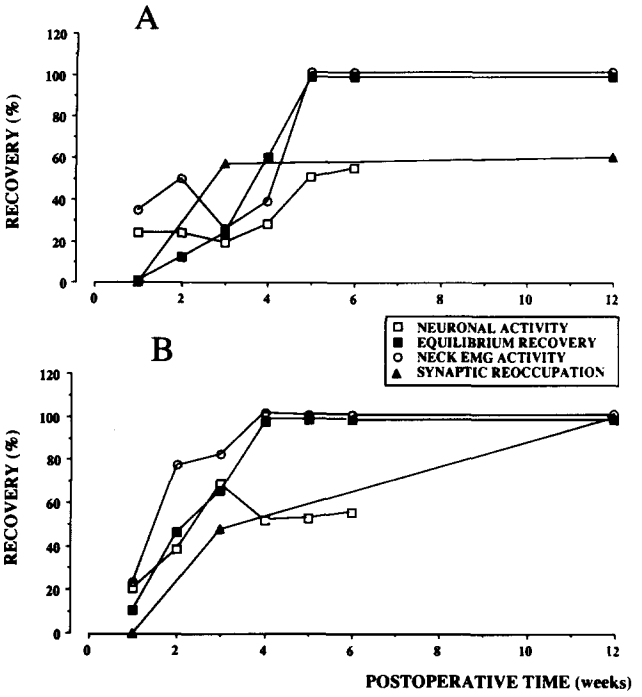

FIG. 7. Effects of the extract of Ginkgo biloba (EGb 761) on the time-constant of the vestibular compensation. Diagrams comparing the time-course of recovery as illustrated by the behavioral (filled squares), EMG (open circles), neuronal (open squares) and immunocytochemical (filled triangles) data in the untreated (A) and the EGb-treated (B) groups of cats. All the results are expressed in percent of the control preoperative values (ordinates), as a function of the postoperative time in weeks (abscissae). Data reported here concern the period running from the first to the twelfth postoperative week. Note the parallel development of all these parameters and their shortened time-constant under EGb postoperative treatment.

that found in the young (65). Such a direct influence of EGb, which remains, however, to be demonstrated, would result from an agonist-like effect on acetylcholine synthesis (or its precursors) involving the terpenes (ginkgolides and bilobalides) contained in this substance.

However, the effects of EGb 761 can be also explained in terms of structural modifications. In this second hypothesis, EGb would influence the structural remodelling in the deafferented vestibular nuclei by the way of neuritogenic properties. The protective effect of EGb 761 from decompensation manoeuvers using anesthetics in hemilabyrinthectomized and totally compensated rats (10) supports this hypothesis. Moreover, more recent experiments conducted on the same animal model using quantitative autoradiographic methods (P. Bustany and P. Denise, personal communication) pointed to a significant EGb-induced increase of the protein biosynthesis in the medial and superior vestibular nuclei located on the lesioned side. This observation corroborates previous findings (47) showing a higher level of catecholamines, indoleamines and their metabolites in the brain of mice and rats following acute and chronic EGb administration. Protein biosynthesis increase would influence neuronal plasticity phenomenon and, more generally, all the memory and learning processes. The acceleration of the vestibular compensation pro-

cess seen in monkeys (24,26) and frogs (17, 18, 20, 38) treated with neuropeptides can be interpreted according to such an hypothesis, since hormonal and neuro-hormonal factors can directly affect the protein biosynthesis. Results from our immunocytochemical study have clearly shown a faster synaptic reoccupation under EGb treatment, indicating that the structural remodelling within the deafferented vestibular nuclei is facilitated by this substance. This suggests that EGb could act either on the sprouting of new synapses (neuritogenic property) and/or on the synaptic efficacy of the remaining inputs (neurotrophic property: increased number of postsynaptic membrane receptors, protein biosynthesis enhancement, for instance). The immunoreactive labelling used here (antibody against synaptophysin, a synaptic vesicle-associated protein) is unable to distinguish between these two recovery mechanisms. Further experiments are needed to elucidate the possible neuritogenic effect of EGb 761. However, even though sprouting is involved, its functional implication and its causal relationship with behavioral recovery still remains unclear, since the postoperative time-course of such structural changes differs from that found in our behavioral and neurophysiological investigations. It may be hypothesized that the early phase of partial synaptic reoccupation could contribute to a rebalance of the vestibular nuclei activity on both sides, while the second phase of synaptogenesis would represent the final structural adjustment of the neuronal networks implicated in vestibular compensation. Such adjustments would be expected to occur more rapidly in the treated group of cats, which earlier experiences sensorimotor interactions in its postoperative environment in comparison with the control group.

The clinical implications of pharmacological treatments are of crucial importance in human pathology since drugs which influence the CNS repair capacity can lead to a faster and/or more optimal functional recovery in the human being. Therefore, assuming that plasticity mechanisms are basically similar in all types of CNS injury, the extract of Ginkgo biloba (EGb 761) could theoretically be used as a valuable therapy in the general framework of brain function recovery.

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