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# Effects of Ginkgo Biloba Extract (EGb 761) on the Guinea Pig Vestibular System

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YABE, T., M. CHAT, E. MALHERBE AND P.-P. VIDAL. *Effects of Ginkgo biloba extract (EGb 761) on the guinea pig vestibular system.* PHARMACOL BIOCHEM BEHAV **42**(4) 595-604, 1992. – Previous studies have demonstrated that the administration of Ginkgo biloba extract (EGb 761) improves the compensation of the vestibular syndrome induced by transection of the VIIIth nerve. To investigate the mechanisms at play, the vestibular nuclei of alert guinea pigs were perfused with EGb 761. This perfusion always induced a stereotyped reversible postural syndrome that was the mirror image of the syndrome provoked by the unilateral lesion of the otolithical receptors. This result supports the hypothesis that EGb 761 has a direct excitatory effect on the lateral vestibular nuclei (LVN) neurons. In a second step, we quantified the horizontal vestibuloocular reflex (HVOR) of the normal guinea pig following IP injection of EGb 761. In normal guinea pig, IP administration of EGb 761 led to a reversible, dose-dependent decrease of the HVOR gain without affecting the phase of the reflex. These data help to explain the therapeutic effects of EGb 761 during vestibular syndromes and strongly suggest an impact at the neuronal level.

Vestibular nuclei Posture Vestibuloocular reflex Vertiginous syndromes

THREE lines of evidence suggest that Ginkgo biloba extract (EGb 761) can modulate vestibular function. First, by using radiolabeled extract it was demonstrated that EGb, or at least certain of its components (22), crosses the blood-brain barrier. Second, clinical studies have demonstrated that EGb 761 administration has a beneficial effect during vertiginous syndromes [see (5,14,23)]. Third, behavioral, anatomical, and electrophysiological studies have shown that EGb 761 can improve vestibular compensation after the VIIIth nerve section:

1. At a behavioral level, the static postural syndrome, the oculomotor deficits, and the dynamic postural impairments following a neurectomy in the cat are compensated in 5 weeks. EGb 761 shortens this delay to 3 weeks (11,19). A similar observation has been made in the neurectomized rat (7).
2. At the electrophysiological level, the regeneration of a normal resting discharge of the deafferented vestibular neurons is instrumental in the vestibular compensation (3,8, 9,20,27,36,37,44,48). EGb 761 administration appears to facilitate the return to a normal resting activity of the deafferented neurons (11,19).
3. At an anatomical level, it has been shown that, 1 week after vestibular neurectomy, the terminal aborization loss can amount to 35% of the synapses contacting the deafferented medial vestibular neurons (13,18,29). Then, a reactive sprouting takes place over 5 months that leads to an occupation of the vacant synaptic sites on the membrane of the deafferented neurons (8,9,11,19). It has been reported that chronic EGb 761 administration fastens the sprouting induced by the vestibular nerve lesion (11,19) and can also have a protective effect at the vestibular receptors level (26).

In summary, it has been well demonstrated that EGb 761 has an effect on the central and peripheral vestibular system but the mechanisms at play are unknown.

To investigate whether EGb 761 could have a direct impact at the neuronal level, we studied the effect of EGb 761 on the guinea pig vestibular system. This species has been a useful model for both in vivo and vitro electrophysiological recording techniques, and its vestibular system has been the object of intensive investigations (1,2,15,28,30,32,33,36-41,44-47). In the first part of this report, we present some evidence in favor of a direct excitatory effect of EGb 761 on the lateral

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vestibular nuclei (LVN) neurons. In the second part, we show that IP injection of EGb 761 induces a reversible, dose-dependent decrease of the gain of the horizontal vestibuloocular reflex (HVOR).

## METHOD

Pigmented male and female guinea pigs (*Cavia porcellus*) weighing between 400–600 g were used in these experiments.

### *Perfusion of the Vestibular Complex*

*Surgical procedure.* Guinea pigs were anesthetized with a nembutal (40 mg/kg)/droperidol (4 mg/kg) mixture delivered IP and prepared for vestibular field potentials recording.

To stimulate the vestibular nerve, trains of 100-ms pulses (duration: 300 ms, frequency: 250 Hz) were delivered through two Teflon-coated silver electrodes implanted over the round window of the middle ear cavity and over the horizontal and anterior semicircular ampullae, respectively. If nystagmic ocular movements were induced, the placement of the electrodes was assumed to be correct and the electrodes were fixed using dental cement. A craniotomy was performed over the ipsilateral hemicerebellum and a T-shaped screw was implanted along the midline for subsequent fixation of the cannula.

The guinea pig was then placed in a stereotaxic apparatus to determine the boundaries of the vestibular nuclei. The extracellular vestibular field potential was evoked by single shocks applied through the labyrinthine electrodes and mapped by extracellular field recordings with glass microelectrodes filled with 2 M NaCl (resistance between 1 and 2 MΩ). As first described by Shimazu and Precht (35) in the cat, the vestibular field is composed of an early positive or positive-negative wave (P), a large sharp negative wave (N1), and a delayed negative component (N2). In guinea pigs, the latencies of the P presynaptic wave, N1 monosynaptic wave, and N2 polysynaptic wave are approximately 0.8, 1, and 1.3 ms, respectively (2,45). A metal cannula of 0.3 mm diameter (arrow 2 in Fig. 1A) was lowered into the brainstem at coordinates corresponding to the center of the vestibular nuclei complex, defined as the site of the maximal amplitude of the N1 wave. The cannula was fixed to the T-shaped screw with dental cement at a depth corresponding to the onset of the vestibular field, that is, at the superior border of the vestibular complex.

On the 3 following days, the posture and eye position of the operated guinea pig were observed. Animals that exhibited a postural asymmetry or an oculomotor deviation were discarded because it was assumed that these signs revealed a vestibular nuclei lesion caused by the cannula implantation.

*Justification of the concentration of the perfused EGb 761.* The 10-mg/ml concentration was chosen for two reasons: a) It is the optimal solubility in saline and b) it compares favorably with the intravenous injection performed in the clinic, which is twice higher.

*Experimental protocol.* After 3 days of rest, the totally unrestrained guinea pigs were subjected to perfusion of the vestibular nuclei by connecting the outer extremity of the cannula to a slow infusion pump by a Teflon catheter. EGb 761 was prepared by Henri Beaufour Institute (Les Ulis, France). It is a well-defined and complex product extracted from green leaves of *Ginkgo biloba*. The leaves are dried and subjected to a 15-step extraction procedure, beginning with an acetone-water mixture under partial vacuum. The final extract is standardized to contain 24% flavonoid glycosides (ginkgo flavone glycosides) and 6% terpene lactones.

Two solutions were perfused at a rate of 1 μl/5 min: a) a saline solution as a control and b) a solution of EGb 761 (10 mg/ml) to test osmolarity. The dried extract was diluted in saline and neutralized to pH 7.3. The osmolarity of that solution was measured using cryoscopy and found to have an osmolarity of 310 mosm, which is in the range of isotonic solutions.

The posture of animals during and after the perfusion was monitored by employing an X-ray photographic method; top and profile view X-ray exposures were taken before and one half hour after the perfusion. Exposures were obtained from above with the X-ray tube 60 cm away from the film and from the side at a distance of 90 cm. As the perfusion rate of the infusion pump was constant, the amount of drug injected in the vestibular nuclei could be approximately estimated at any time during the experiment.

*Data analysis.* To quantify the postural syndrome induced by the EGb 761 perfusion, guinea pig X-ray pictures were analyzed as previously described by de Waele et al. (44,47). In that protocol, because of the presence of a massive nystagmus no attempt was made to quantify the HVOR.

Thoracic and lumbar vertebrae rotation about the longitudinal axis was estimated by attributing, on top view X-ray exposures, a coefficient of rotation (CR) for each vertebra. Values in arbitrary units (A.U.) were attributed according to the following criteria. If the spiny process crest was central relative to the vertebral body, that is, in the absence of any rotation about the longitudinal axis (refer to the arrow in Fig. 1B), it was assigned a zero CR. If it was eccentric (corresponding to a vertebra rotation of about 45° relative to the longitudinal axis) or if it was visualized near the edge of the vertebral body (rotation of about 90°), values of CR 1 and 2 were assigned, respectively. Finally, if the spiny process crest was invisible (in case of a vertebrae rotation larger than 90°; see, e.g., arrow 2 in Fig. 1C) a CR of 3 was given. The CR was assumed to be positive if the vertebrae rotated toward the side of the perfusion and negative if they rotated toward the opposite side. By summing the CR of different adjacent vertebrae, we could estimate the global CR of the thoracic and lumbar column.

*Histological procedures.* At the end of the experiments, guinea pigs were killed with a nembutal overdose. Brains were then removed and stored in 10% formaldehyde solution. Transversal sections, cut at 75 μm thickness on a freezing microtome, were then processed and stained with cresyl violet acetate for visualization of the vestibular nuclei. Sections were examined for signs of degeneration of vestibular nuclei neurons. They also served to reconstruct the cannula tracks and determine the exact location of the cannula tips.

### *HVOR and Electroencephalogram Recording in Normal and Hemilabyrinthectomized Guinea Pigs*

*Surgical procedure.* Under nembutal (40 mg/kg)/droperidol (4 mg/kg) anesthesia, the subsequent surgical procedure was followed: a) A coil made of two turns (diameter: 9 mm, weight with the connecting lead: 3 mg) of Teflon-coated seven-stranded stainless steel wire (0.23 mm of diameter, A-M Systems, Inc., Everett, WA) was implanted on the eyeball using fine sutures with 7-0 silk to measure eye movements with the search coil method (12). b) Three stainless steel T-shaped bolts were cemented in the temporal and occipital bones to secure the fixation of the head holder and record the electrocorticogram signal. c) A head holder was cemented stereotaxically onto the skull to restrain the animal's head during the recording session. The stereotaxic plane was determined by a head rotation around the interaural axis until the calvarium was horizontal between anterior 6 and 14 mm from the stereotaxic 0.

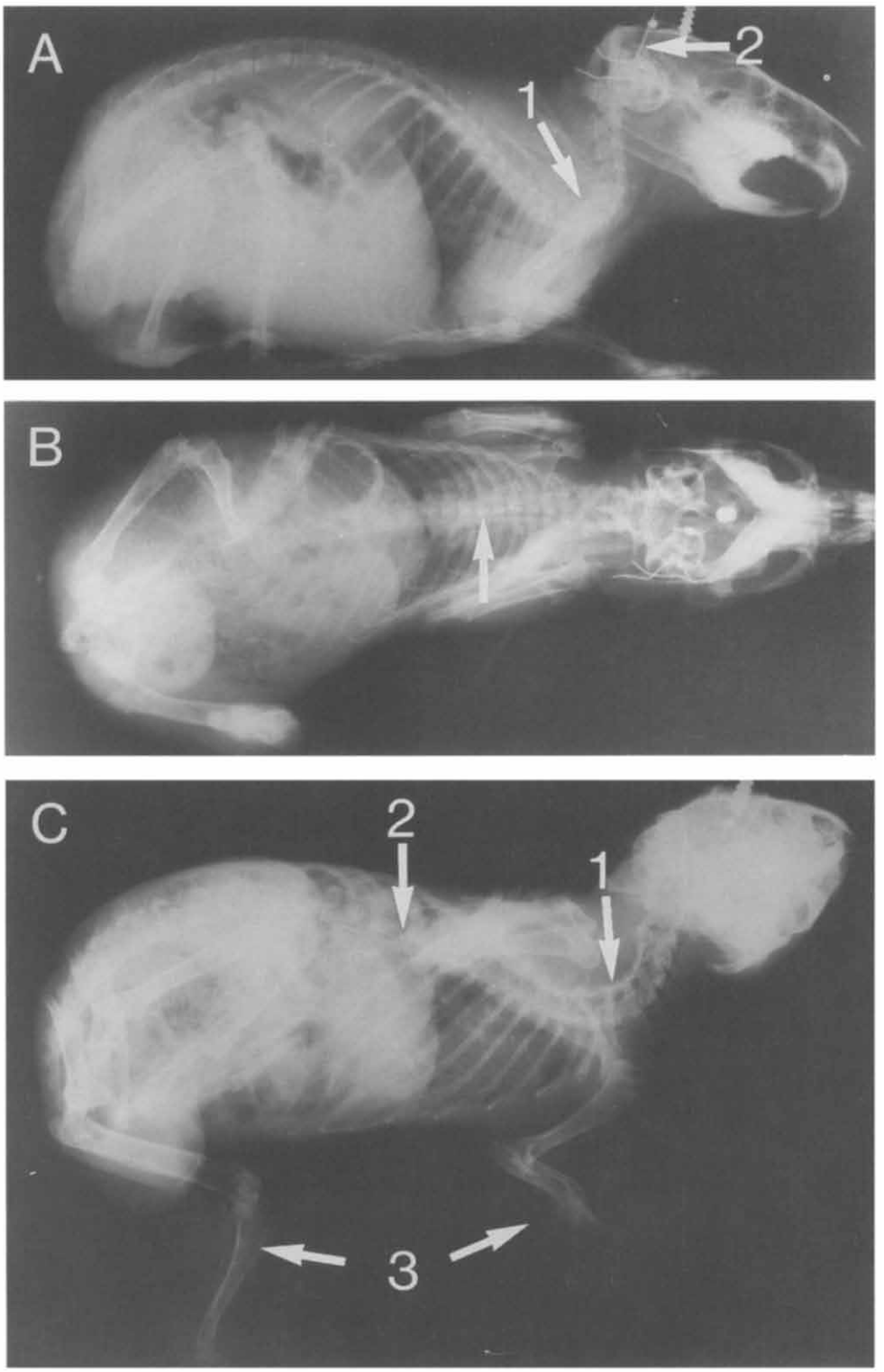


FIG. 1. Skeletal geometry before and after EGb 761 perfusion of the vestibular nuclei. (A) Lateral X-ray exposure of a saline-perfused guinea pig at rest. Note the vertical orientation of the cervical column and the physiological curvature of the cervicothoracic junction (arrow 1). Arrow 2 indicates the position of the perfusion cannula. (B) Top view exposure of the same control animal. The spiny process crests of the thoracic vertebrae are well centered, indicating the absence of rotation of the thoracolumbar column (arrow). (C) Top view exposure of the same animal after EGb 761 perfusion. The head neck ensemble is rotated en bloc on the side contralateral to the perfused side. The cervicothoracic junction (arrow 1) is viewed from the side as a consequence of the forced rotation of the thoracic vertebrae toward the contralateral side. Arrow 2 indicates the disappearance of the spiny process crests of the rotated thoracic and lumbar vertebrae. Notice also the forced extension of the forelimb and hindlimb ipsilateral to the perfused side and the forced flexion of the contralateral limbs (arrow 3).

*Recording conditions.* The HVOR was studied after 1 week of recovery from surgical procedures. Each recording session lasted 1 h and the testing program never exceeded 2 weeks. Alertness was continuously monitored using the electroencephalogram (EEG), and the data recorded during episodes of slow waves were discarded.

For the HVOR recording, the guinea pig was placed on a servo-controlled turntable for vestibular stimulation. Its body was unrestrained and its head was secured to the head holder in a 35° nose-down position to orient the horizontal semicircular canals in the horizontal plane (6). The head holder was fixed to the turntable such that the guinea pig's eyes were positioned in the center of a magnetic field generated by two pairs of field coils set up at right angles on the turntable. In that location, the current generated by the magnetic field in the eye coil was linearly related to the rotation of the eye (volume of linearity: 40 $cm^3$). The horizontal and vertical components of the eye position were recorded by a SKALAR (Delf, The Netherlands) magnetic search coil system with a band-width of DC to 200 Hz (3 dB).

Calibration of the eye movements was obtained by rotating the field coils around the animal in steps of 10° in the horizontal and vertical planes. Resolution of the eye position was estimated to have an accuracy of 1°.

*Justification of the concentration of the IP injection.* The 10- mg/ml concentration was chosen because, in the clinic, therapeutical doses amount to 3 mg/kg/24 h. It is generally accepted that there is a factor of 7 at play when one wants to compare the dose administered in humans and in guinea pigs (25). In that condition, the 25- and 50-mg/kg IP injected solutions compare to the dose used in humans and twice that dose, respectively.

*Experimental protocol.* Three noninjected guinea pigs were tested with the protocol described above to control whether the repetition of the VOR measurement in a relatively short period of time could lead to a vestibular habituation. Five animals were injected IP with 25 mg/kg EGb 761 diluted in a saline solution. Three were injected with 50 mg/kg EGb 761 diluted in a saline solution. Both saline-diluted solutions were neutralized to pH 7.3 and were found to be in the range of isotonic solutions (270–328 mosm) when their osmolarity was checked by cryoscopy.

The horizontal and vertical components of the eye position, the turntable position and velocity signal, and the EEG were recorded on an FM magnetic tape (band width: DC to 1 KHz) for off-line analysis. Each animal was subjected to five frequencies (0.05, 0.1, 0.5, 1, and 2 Hz) of horizontal sinusoidal rotations in darkness at a peak angular velocity of 40°/s before and 30 min, 1, 2, 24, 48, and 72 h after IP injections of EGb 761.

*Data analysis.* The EEG, horizontal and vertical components of eye position, turntable position, and velocity were displayed on paper using an electrostatic chart recorder (Gould ES 1000, Cleveland, OH). Calculations were performed by land. The quick phases were eliminated from the record of the horizontal component of the eye position by constructing the cumulative slow phase curve (CSPC): Using a transparent sheet of paper, the sinusoidal slow phases were redrawn, omitting the segments of the record corresponding to the quick phases. To avoid empty gaps on the sample, each time a quick phase was bypassed the paper was shifted by hand to the left so that the two segments corresponding to two successive slow phases were in continuity. Then, the following calculations were performed from the original records and from the CSPC: a) peak angular velocity and position of the table; b) gain, defined as the peak amplitude of the eye movement on the CSPC divided by the peak amplitude of the table position; and c) phase shift was calculated on the original record of the horizontal component of the eye position—it was defined as 0° when the eye position was 180° out of phase with the table position.

*Statistical analysis.* Regarding the dose-treated groups, all results are expressed as a mean value ± SD of absolute data in comparison with the respective time 0. Statistical analysis was performed using the two-way $F$ test of variance: *, **, and *** indicate $0.01 < p \leq 0.05$, $0.001 < p \leq 0.01$, and $p \leq 0.001$, respectively.

## RESULTS

### *Saline and EGb 761 Perfusion of the Vestibular Nuclei*

*Saline perfusion.* After the perfusion of up to 15 μl saline in the vestibular complex, the resting posture of the three guinea pigs was normal. This is illustrated in Fig. 1, which shows an X-ray view taken from the side (Fig. 1A) and from above (Fig. 1B) of guinea pig 1 (cf. Table 1). As previously described (43), on an X-ray view from the side (Fig. 1A) the cervical column is oriented vertically and is aligned with the gravity vector. The animal holds its head such that the plane of the horizontal semicircular canals is held tilted at about 20° above the horizontal plane. The absence of head rotation in the horizontal and frontal plane can be detected by the superposition of the two tympanic bullae. On the X-ray view from above, the spiny process crests of the thoracic vertebrae (see arrow in Fig. 1B) are well centered. This indicates a lack of rotation of the thoracic and lumbar vertebrae about the longitudinal axis of the animal.

*EGb 761 perfusion in the vestibular complex.*

*Postural syndrome.* By contrast, in the same guinea pig (1 in

TABLE 1

EFFECTS OF EGb 761 PERFUSION ON SKELETAL GEOMETRY

| Guinea pig | Gb injected (μl) | Gb quantity (mg) | Latency (min) | Thoracic CR (A.U.) | Lumbal CR (A.U.) | Duration (h) |
|---|---|---|---|---|---|---|
| 1 | 3 | 0.03 | 3.0 | −9 | −9 | 1 |
| 2 | 12 | 0.12 | 3.5 | −9 | −9 | 3 |
| 3 | 5 | 0.05 | 4.0 | −9 | −9 | 1.5 |

This table indicates, for the three tested guinea pigs, the total amount injected, the quantity of EGb 761, the latency of the effects of EGb 761 perfusion, the CR of the thoracic vertebrae, the CR of the lumbar vertebrae, and the duration of the induced vestibular syndrome. Negative CR values indicate a rotation of the vertebrae toward the side contralateral to the perfused vestibular nucleus.

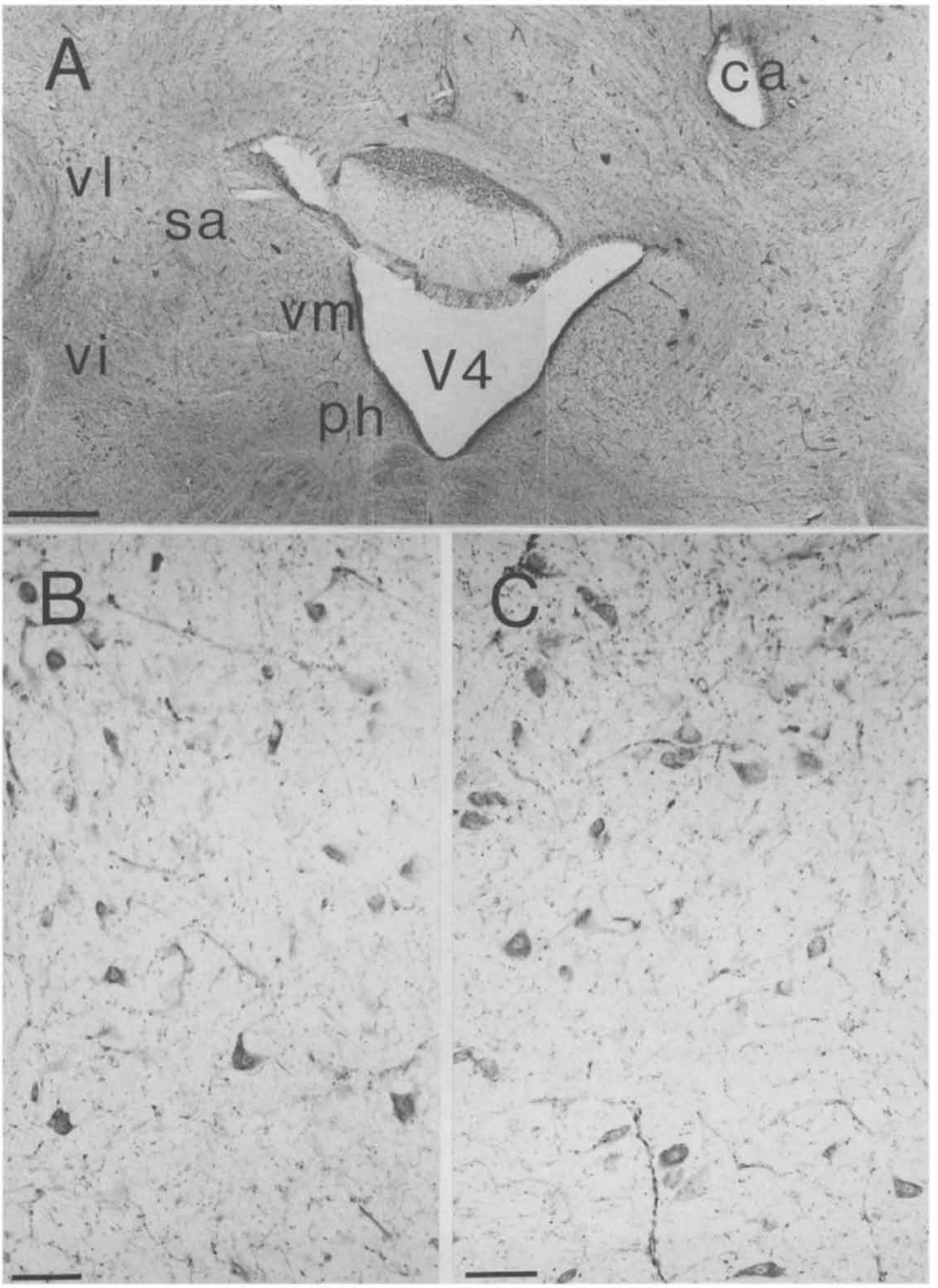


FIG. 2. Histological study of the lateral vestibular nucleus following perfusion with EGb 761 solution. (A) Frontal section of the tested guinea pigs' brainstem (2 in Table 2) after EGb 761 perfusion. Note the cannula tip track (ca) above the right side vestibular nuclei (VN) and the absence of ischemic signs in the ipsilateral VN (compare with the contralateral VN). Bar = 500 $\mu$m; cresyl violet stain. ph, prepositus hypoglossi nucleus; sa, stria acoustica; V4, the fourth ventricle; vi, inferior vestibular nucleus; vl, lateral vestibular nucleus; vm, medial vestibular nucleus. (B, C) High magnification of the left (intact side) and right (perfused side) lateral vestibular nuclei of Fig. 2A, respectively. No sign of either chromatolysis or gliosis is present. Bar = 100 $\mu$m.

Table 1), the perfusion of the vestibular complex with 3 μl EGb 761 diluted in saline (10 mg/ml) induced a massive and stereotyped postural syndrome, as illustrated in Fig. 1C, an X-ray view from above. The head neck ensemble is rotated en bloc on the side contralateral to the injection. This rotation explains why the physiological curvature of the cervicothoracic junction in the sagittal plane, which is easily seen on the lateral X-ray view (arrow 1 on Fig. 1A) but not detected on the X-ray view from above in the normal animal (Fig. 1B), is now visible on the X-ray view from above after the EGb 761 perfusion (arrow 1 in Fig. 1C). The spiny process crests of the thoracic and lumbar vertebrae normally seen on the X-ray from above before the perfusion (arrow in Fig. 1B) have disappeared after the perfusion (arrow 2 in Fig. 1C). This demonstrates the rotation of the thoracic vertebral bodies about the longitudinal axis of the animal at the origin of the bascule of the head-neck ensemble in the frontal plane. The rotation can also be detected by comparing the thoracic ribs in Figs. 1B and 1C. A quantified analysis of the vertebrae rotations indicate a maximal rotation (CR 9 in Table 1) for both the thoracic and lumbar columns in the three tested guinea pigs. As a result of the postural syndrome, it was impossible to obtain a clear lateral X-ray view.

Finally, the EGb 761 perfusion induced a forced extension of the forelimb and hindlimb ipsilateral to the side of the perfusion and the forced flexion of the limbs contralateral to the cannula (arrow 3 in Fig. 1C).

*Oculomotor syndrome.* In the three perfused guinea pigs, the oculomotor syndrome (not illustrated) followed the same time course of the postural syndrome. It was characterized by a tonic upward deviation of the eyeball on the ipsilateral side and a tonic downward deviation on the contralateral side. This abnormal eye position was accompanied by an ocular nystagmus with the slow phase oriented toward the contralateral side of the perfusion.

The postural and vestibular syndromes disappeared within a few hours after the perfusion (see Table 1). It should be noted that these postural and oculomotor syndromes are mirror images of the ones we described after unilateral selective lesion of the otolithical receptors (44). After an otolithical lesion, the head-neck ensemble is rotated ipsilaterally to the side of the lesion and the limbs are extended contralaterally.

*Histological study.* In the three perfused guinea pigs, histological study revealed that the inferior extremity of the cannula was localized at the superior border of the vestibular nuclei complex. The lack of gliosis under the cannula tract associated with an absence of chromatolysis strongly suggests that EGb 761 had no toxic effects on the vestibular neurons (Figs. 2A, B, and C).

### *EGb 761 IP Injection*

*HVOR gain immediately before and after EGb 761 IP injection.* Eye movements during sinusoidal rotations of the turntable in darkness at 0.05, 0.1, 0.5, 1, and 2 Hz are illustrated in Figs. 3 and 4 before and 2 h after IP injection of 50 mg/kg EGB 761. From top to bottom, the turntable position (i.e., the head position in the head-fixed animal) and the horizontal and vertical components of the eye movements are traced for each of the five frequencies tested. The horizontal component of the eye position induced by the sinusoidal table

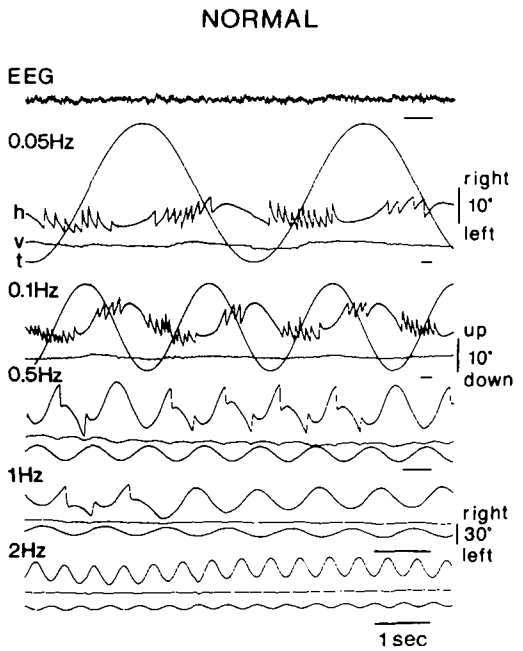


FIG. 3. Normal HVOR elicited by sinusoidal horizontal stimulation. From top to bottom: EEG recording, HVOR recordings at 0.05, 0.1, 0.5, 1, and 2 Hz. h, horizontal component of eye movements; v, vertical component of eye movements; t, table position; sec, second. Upper, middle, and lower calibration bars applied to the horizontal, vertical, and table position signals, respectively. Note that the compensatory horizontal sinusoidal slow-phase eye movements are interrupted by anticompensatory fast-phase eye movements.

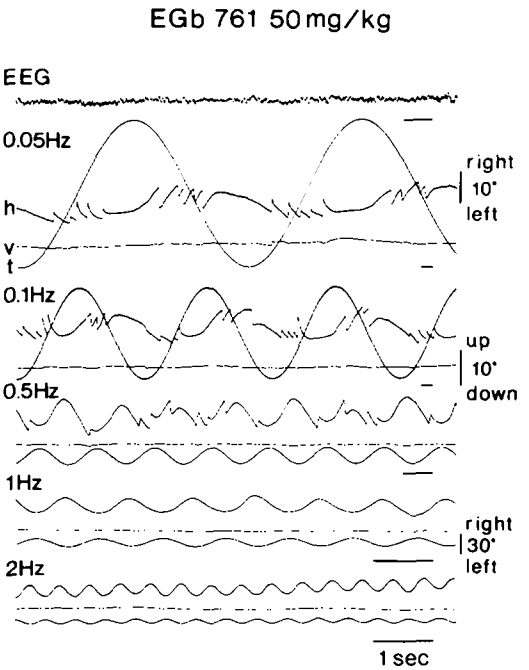


FIG. 4. HVOR at 2 h after IP injection of 50 mg/kg EGb 761. Same legend as in Fig. 3. Notice the decrease in the number of fast phases and the diminution of the overall amplitude of the slow phases compared with the HVOR illustrated in Fig. 3. Note also that there are no remarkable changes in the EEG recording compared with that in Fig. 3.

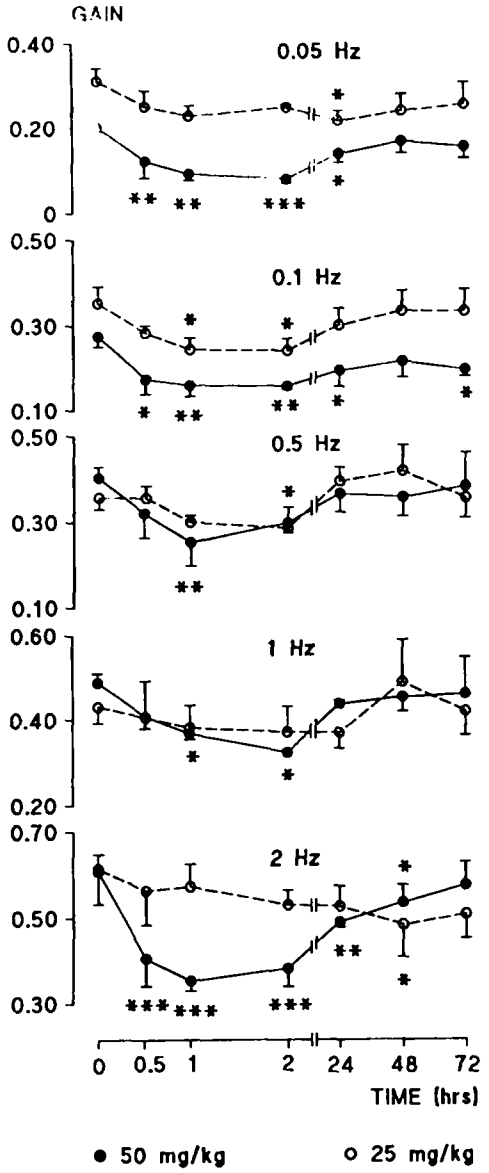


FIG. 5. Averaged HVOR gain decrease following the injection of 25 mg/kg ($n$ = 5) and 50 mg/kg ($n$ = 3) of EGb 761 in normal guinea pigs. Dotted line indicates the results of the injection of 25-mg/kg and the solid line shows the results of the 50-mg/kg injection. Each point corresponds to a mean value of the HVOR gain tested before (time 0), and one half, 1, 2, 24, 48, and 72 h after the EGb 761 injection. Vertical bar indicates the standard deviation of each value. Statistical analysis was performed using the two-way $F$ test of variance. *$0.01 < p \leq 0.05$, **$0.001 < p \leq 0.01$, ***$p \leq 0.001$.

rotations can be depicted as a slow sinusoidal eye movement oriented contralaterally to the table rotation that stabilized the gaze. The slow phases are periodically interrupted by quick phases toward the ipsilateral side that reorient the eye in the orbit. Comparing the two figures, it can be seen that, at every tested frequency, the number of quick phases and the overall amplitude of the slow phases are diminished in Fig. 4. This indicates that EGb 761 has induced an important depression of the HVOR.

The vertical component of the eye movement is virtually flat because the head is positioned (35° nose down) such that the plane of the horizontal semicircular canal coincides with the plane of the vestibular stimulation. In that head orientation, the plane of the vertical semicircular canal is approximately parallel to the axis of rotation and therefore the ampullae of the vertical semicircular canals are hardly stimulated.

The five graphs illustrated in Figure 5 correspond to the mean values and standard deviations of the HVOR gain tested at 0.05, 0.1, 0.5, 1, and 2 Hz immediately before (time 0), and one half, 1, 2, 24, 48, and 72 h after the injection of 25 and 50 mg/kg EGb 761. Statistical analysis was performed using the two way $F$-test of variance: *, ** and *** indicate $0.01 < p \leq 0.05$, $0.001 < p \leq 0.01$ and $p \leq 0.001$, respectively. Clearly, the EGb 761 effect on the HVOR was dose dependent and reversible. It was already noticeable 30 min after the injection, reached a peak 1 or 2 h later, and then slowly returned to subnormal values in 48–72 h. The gain of the HVOR showed a statistical difference for the 50-mg/kg EGb 761 injection at all tested frequencies 1 and 2 h after the injection when compared to the preinjection values.

*HVOR phase immediately before and after EGb 761 IP injection.* The results are summarized in Table 2. It should first be noted that the phases of the HVOR are similar to those calculated previously from a larger sample of guinea pigs (10). Second, no statistical differences (confidence interval 0.05) were observed before and after injection of EGb 761.

*Alertness level after EGb 761 IP injection.* The alertness level was monitored before and during the HVOR recording sessions using the EEG as an index. No systematic tendency to a decrease of the level of alertness was observed in the eight tested guinea pigs after EGb 761 IP injection.

*HVOR gain and phase in three control guinea pigs.* To check whether the repeated measurements of the VOR in a short period of time could have some effect on the VOR gain, three noninjected guinea pigs were submitted to the same protocol as injected animals. The result of this test is shown in Table 3. Statistical analysis was performed using the two-way $F$ test of variance, which clearly demonstrates that no statistically significant differences took place when the VOR was tested repetitively.

## DISCUSSION

We shall first discuss the results of the vestibular complex perfusion and then focus on the effect of IP injection on the HVOR.

### *Perfusion of the Vestibular Nuclei*

EGb 761 perfusion induced a massive but reversible postural syndrome in the three guinea pigs we tested. This is strongly suggestive of an excitatory effect of EGb 761 on vestibular neurons. A similar protocol has been used to study the NMDA (47) and $H_2$ receptors (49). It has allowed us to predict the presence of these receptors in the vestibular nuclei, receptors that were then proven to be effectively present at the level of the vestibular nuclei by our two subsequent in vitro studies (31,34).

A precise assessment of the perfused EGb 761 diffusion would have required a radioactively tagged compound that is unfortunately not presently available. Hence, it cannot be ascertained whether the behavioral effects of the perfusion were solely due to the effect of the EGb 761 on the LVN or whether neighboring structures were also responsible. However, a series of arguments tend to indicate that the EGb 761-

TABLE 2

MEAN VALUES AND STANDARD DEVIATIONS OF HVOR PHASE BEFORE (NORMAL) AND AFTER (50 mg/kg) THE EGb 761 INJECTION

| | Frequency | | | | |
|---|---|---|---|---|---|
| Dose | 0.05 Hz | 0.1 Hz | 0.5 Hz | 1 Hz | 2 Hz |
| Normal ($n$ = 3) | 45.2 ± 3.7 | 37.9 ± 2.6 | 14.7 ± 3.0 | 9.9 ± 2.0 | 5.6 ± 0.8 |
| 50 mg/kg ($n$ = 3) | 41.1 ± 2.1 | 37.5 ± 2.2 | 16.1 ± 0.6 | 8.9 ± 2.2 | 5.8 ± 0.5 |

There is no statistical significant difference ($p < 0.05$) between the values recorded before and after EGb 761 injection.

induced postural syndrome was only mediated through its impact on the vestibular nuclei:

1. The histological reconstructions demonstrated that the three cannulae were implanted above the vestibular nuclei and the latency of the postural effect was short (in the range of minutes).
2. The syndromes observed were not of mechanical origin since the EGb 761 solution was isotonic and injections of up to 15 $\mu$l saline were without effect in the three control guinea pigs.
3. The postural deficits were not due to cellular lesions since they were all reversible in a few hours. Moreover, histological examination of the vestibular nuclei did not reveal any sign of neuronal necrosis (Fig. 2).
4. The effects and diffusion of EGb 761 appeared to be limited to the vestibular nuclei. Respiration, thermoregulation, vigilance, and feeding behaviors were all normal.
5. It is unlikely that our perfusions led to a rapid diffusion of the EGb 761 in the ventricular system for at least two reasons: First, the position of the cannula was checked during implantation (no CSF coming out of the cannula), and after the experiment the histological materials showed that the cannulae were placed above the center of the vestibular complex, quite remote from the ventricular cavity. Second, a major diffusion of the compound in the ventricular cavity should have not produced a major asymmetric static vestibular syndrome. It should have left the posture unaffected as was the case with the IP injection since the vestibular complex on both sides would have been affected.
6. Finally, our study shows that the postural and oculomotor syndromes induced by the EGb 761 perfusion were the mirror image of syndromes we have described that follow the unilateral lesion of the otolithical receptors (44). Both these syndromes followed a similar time course and can be attributed to increased activity of the LVN neurons. Indeed, after unilateral selective otolithical destruction the postural and the oculomotor deficit is induced by an imbalance between the resting discharges of the LVN neurons of the two sides. The neurons located in the nucleus on the lesioned side are suddenly deprived of a massive excitatory input because of the loss of their otolithical afferents. Consequently, their resting discharge decreases considerably (20,48). It is therefore reasonable to hypothesize that an imbalance of the resting discharge of the lateral vestibular neurons is also at the root of the EGb 761-induced syndromes. However, the deficits were oriented contralaterally to the deficits observed after the otolithical lesion. Therefore, one can reasonably make the hypothesis that EGb 761 had probably provoked an imbalance of the resting discharge of the lateral vestibular neurons bilaterally by inducing a hyperactivity of the LVN neurons on the side of the perfusion.

What could be the neuronal substrate of this hyperactivity? The present experiment did not allow us to choose one of the two following possibilities: Either EGb 761 has a direct depolarizing effect on the LVN neurons or this compound has a hyperpolarizing effect on inhibitory interneurons contacting them. One hypothesis would be that EGb 761 augments the acetylcholine level in the vestibular nuclei; it has been shown that EGb 761 can accelerate the precursor synthesis of that neuromediator (42). Moreover, acetylcholine is known to induce a large depolarization of the LVN (16,17) and this neuromediator is implicated in the vestibular compensation process (4). Alternatively, EGb 761 could have an effect on the

TABLE 3

HVOR GAIN VALUES OF THREE NONINJECTED CONTROL GUINEA PIGS

| | Time (h) | | | | | | |
|---|---|---|---|---|---|---|---|
| Frequency | 0 (M ± SD) | 0.5 (M ± SD) | 1 (M ± SD) | 2 (M ± SD) | 24 (M ± SD) | 48 (M ± SD) | 72 (M ± SD) |
| 0.05 Hz | 0.32 ± 0.07 | 0.34 ± 0.06 | 0.32 ± 0.08 | 0.35 ± 0.08 | 0.34 ± 0.09 | 0.30 ± 0.04 | 0.30 ± 0.07 |
| 0.1 Hz | 0.45 ± 0.05 | 0.43 ± 0.06 | 0.45 ± 0.08 | 0.44 ± 0.07 | 0.46 ± 0.01 | 0.38 ± 0.05 | 0.44 ± 0.08 |
| 0.5 Hz | 0.48 ± 0.05 | 0.42 ± 0.09 | 0.53 ± 0.05 | 0.51 ± 0.04 | 0.46 ± 0.06 | 0.47 ± 0.04 | 0.53 ± 0.06 |
| 1 Hz | 0.56 ± 0.06 | 0.51 ± 0.08 | 0.57 ± 0.10 | 0.56 ± 0.04 | 0.54 ± 0.08 | 0.56 ± 0.05 | 0.53 ± 0.08 |
| 2 Hz | 0.65 ± 0.08 | 0.62 ± 0.11 | 0.68 ± 0.06 | 0.68 ± 0.04 | 0.69 ± 0.05 | 0.65 ± 0.06 | 0.66 ± 0.09 |

There is no statistical significant difference between the values of the HVOR gain in noninjected guinea pigs during the repeated measurements of the VOR. Statistical analysis was performed using the two way $F$ test of variance. M, mean value. ±SD, standard deviation.

voltage-sensitive conductances of the vestibular neurons. Our results may also explain why EGb 761 administration accelerates the compensation of the static postural syndrome following the VIIIth nerve section (7,11,19): EGb 761 probably speeds the reequilibration of the resting discharge of the two lateral vestibular nuclei neurons by increasing the firing of the deafferented LVN neurons.

It remains to be seen whether EGb 761 has some influence on the other vestibular nuclei neurons. Our paradigm cannot exclude that possibility:

1. First, a unilateral lesion of the anterior and posterior canal input was shown to leave the resting posture of the guinea pig unaffected (de Waele, in preparation); therefore, even if EGb 761 had induced an asymmetry of the discharge of the superior and inferior vestibular nuclei that mediate these inputs it probably would not have been detected.
2. Second, none of the three guinea pigs exhibited a rotation of the head about the longitudinal axis of the cervical column. Such a rotation would have been a clear sign of modification of the neuronal activity in the horizontal vestibulospinal pathway (44); however, this does not exclude the possibility that EGb 761 had an impact on the medial vestibular nuclei neurons for at least two reasons: It may well be that the modifications induced by this compound on both the excitatory and inhibitory neuronal discharge of this nucleus cancel each other. It may also be that the massive postural syndrome made it impossible to detect a discrete head rotation in the horizontal plane.

*Effect of IP Injection on the HVOR*

As discussed above, the EGb 761 when perfused locally tends to have an excitatory effect on the LVN neurons. When injected IP, it decreases the gain of the HVOR. These results are not in contradiction. First, the LVN neurons control the vestibular reflexes in the lateral and frontal planes while the HVOR is controlled by the medial vestibular neurons. EGb 761 could have effects of opposite polarities in these two nuclei. Second, it may be impossible to compare the effects of a localized perfusion of a drug with the effects of its IP injection; in the latter case, the active compound diffuses in all the structures of the CNS. A similar example of effects of opposite polarities between a localized vestibular perfusion and an IP injection had also been demonstrated for thioperamide, an $H_3$ agonist (49).

Before injection, HVOR performances of all tested guinea pigs were considered normal since they were identical to those of a larger control group ($n$ = 18) of animals tested previously in our laboratory (10). Therefore, our results shows clearly that EGb 761 IP injection decreases the HVOR gain. Despite the intrinsic and intersubject fluctuations of the HVOR, 25 mg/kg was sufficient to demonstrate statistically the effect of EGb 761. At 50 mg/kg, the effect is particularly clear. This effect is most probably due to a rather specific effect of EGb 761 on the CNS since: a) It has been demonstrated that at least some of the derivatives of that compound cross the blood-brain barrier; b) the repetition of the protocol in three noninjected guinea pigs showed that the repetition of the HVOR measurements in a relatively short period of time did not induce per se a diminution of the gain; c) continuous EEG monitoring negates the possibility that the diminution of the HVOR gain was due to a vigilance decrease. In fact, EGb 761 has been shown to raise the alertness level in humans (24). Finally, and not surprisingly, the time course of the HVOR gain modulation was reminiscent of the time course of the EGb 761 blood concentration, which peaks 1½ h after administration and is minimal 72 h later (21).

Insofar as some extrapolations are possible, the amount of the injected drug was in the range (see the Method section) of therapeutic doses in the clinic. Hence, our results could partly explain why EGb 761 administration improves the condition of vertiginous patients: It may decrease the dynamic responses of the neuronal network (which included the medial vestibular nuclei neurons) implicated in detecting head motion in the horizontal plane. This result is not in contradiction with a putative augmentation of the resting discharge of the deafferented LVN neurons discussed above, which could help to improve the static vestibular responses.

## CONCLUSION

Our results provide some evidence of a direct action of EGb 761 on the neurons of the CNS. They may provide some clues to understanding the beneficial effect of EGb 761 on the compensation of the vestibular deficit and during vertiginous syndromes. Further studies at the in vitro level are necessary to elucidate which components of EGb 761 are active in the CNS and what cellular mechanisms are at play.

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