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Methodological aspects of elicitation and analysis of vestibulo-spinal reflexes

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ABSTRACT

Vestibulospinal reflexes can be elicited in humans by low amplitudes direct (galvanic) currents lasting tens of milliseconds and applied across the two mastoids bones, which can be delivered by particular stimulators. This stimulus induces a perception of body sway and a postural response appropriate to counteract the perceived sway. Both the direction of the perceived and of the actual body sway are modulated by the orientation of the head with respect to the body. This modulation depends on the integration of vestibular and neck signals and allows to correctly infer the direction of body sway. In the present study we show that labyrinthine stimulation can be performed also by using train of pulses of 1 msec duration, which can be delivered by virtually all stimulators allowed for human use. Moreover, we developed a simple technique for visualising the time course of the changes in the direction of the postural response, based on the evaluation of the velocity vector of subject's centre of pressure. All together, the results improve the suitability of VS reflex elicitation to clinical practice by indicating stimulation characteristics which can be easily reproduced in both physiological and pathological condition, and by describing a simple, reliable method to analyse the CoP movement elicited by vestibular reflexes.

Key words

Vestibulospinal reflexes • Electrical stimulation • Human • Centre of pressure

Introduction

Vestibulospinal (VS) reflexes elicited by stimulation of labyrinthine receptors stabilise posture (Wilson and Melvill Jones, 1979) and their evaluation is relevant for both basic and clinical research. In animals, VS reflexes can be elicited by natural stimulation of the vestibular apparatus obtained by imposing whole-body rotational stimuli (Wilson and Melvill Jones, 1979). In humans this technique is very uneasy, since it requires bulky and costly mechanical devices with sophisticated control systems. An easier technique able to activate the labyrinth described by Johann Purkynje (1819), consists of applying electrical currents across the mastoid bones. In order to activate limb muscles and produce body sway, direct (galvanic) currents of 10 msec-few sec of duration, delivered by stimulus isolation units commercially available or assembled by the experimenters can be utilized (Baldissera et al., 1990; Britton et al., 1985; Day et al., 1997, Lund and Broberg, 1983). Shorter stimuli are not effective on limb muscles, although with high stimulation intensities they can modify the EMG activity of jaw (Deriu et al., 2003) and neck muscles (Watson et al., 1998) However, only few stimulators allowed for human use can deliver current pulses larger than 1-2 msec.

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In blindfolded humans, galvanic stimulation of the labyrinth elicits a postural sway in the direction of the anode (Britton et al., 1993; Day et al., 1997; Lund and Broberg, 1983), which seems appropriate to counteract the effects of the fall in the direction of the cathode perceived by the subject when he is held still (Fitzpatrick et al., 1994). The time course of the postural response has been evaluated by observing the corresponding changes in EMG activity (Baldissera et al., 1990; Britton et al., 1993; Day et al., 1997). However, this method fails to provide an overall description of the phenomenon, which is provided by stabilometric platforms (Bizzo et al., 1985), able to record, in the sagittal and in the frontal plane, the instantaneous position of the centre of pressure (CoP), which is the point where the reactive force of the support base against the body weight is applied.

When the subject is facing forward, the direction of postural sway lays within the frontal plane, while it occurs in the sagittal plane when the head is rotated. Indeed, a key feature of the postural response to electrical labyrinthine stimulation is the fact that the orientation of the head with respect to the trunk modulates the direction of body sway (Lund and Broberg, 1983). This modulation is due to the fact that labyrinthine signals monitor the direction of head displacement, while the direction of the perceived sway is related to the body, whose position in space is stabilized by VS reflexes. As a consequence, a stimulus-driven vestibular signal must be transformed in different perceptions and postural responses according to the orientation of the head (Manzoni, 2007).

Vestibular information processing and integration with the neck input, which takes place at cerebellar level (Manzoni, 2007), allows 1) to infer the correct direction of body sway from labyrinthine signals and 2) to generate VS reflexes that displace the body in a direction which is opposite to that of the perceived body sway. When this transformation occurs appropriately and there are no deficits in the neck proprioceptive input, subjects sway in the frontal plane toward the anode (Britton et al., 1985; Day et al., 1997, Lund and Broberg, 1983). For these reasons, a reliable evaluation of the orientation of body sway may have a remarkable impact in clinical evaluations of VS reflexes and of their modulation by neck proprioception.

The direction of body sway elicited by the labyrinthine stimulation can be evaluated on the basis of the changes observed in the X and Y coordinates of the CoP (Lund and Broberg, 1983). This measure however is biased by instability and drift in the base line CoP position, leading to large fluctuation in the directions of CoP displacement observed at a given head-to-body position (see Fig. 1D of Lund and Broberg, 1983). A far more accurate approach was recently proposed by Mian and Day (2005), based on the use of a cumulant density function (a time-domain measure equivalent to cross-correlation) of the stochastic current signal (used for labyrinthine stimulation) with the output of the force plate where the subject was standing. Nonetheless, this procedure is complex and requires an equipment adequate to generate stochastic voltage time signals.

Thus, the goal of the present investigation was to develop 1) a protocol of electrical stimulation of the labyrinth, based on trains of short-duration pulses to be used in substitution of the classical galvanic currents, which would allow the use of stimulus isolation units delivering pulses of 1-2 msec, and 2) a simple and reliable method of signal analysis aimed at evaluating the direction of postural sway induced by labyrinthine volleys and, potentially, by any other stimulation modulating posture.

Methods

Subjects and labyrinthine stimulation

12 healthy volunteers (age, 19-24 yrs), after a cardiologic, neurological and orthopaedic evaluation, were enrolled in the study. All of them signed an informed consent approved by the Ethics Committee of the University of Pisa.

All the subjects stood on a stabilometric platform (Dune, model 2000; Dune S.A.R.L., Neyenheim, France). The experimental session began with the fixation of a point positioned at 90 cm far from the subject, at the eyes level, followed by the eyes closure. Then the labyrinth was stimulated and the effects of CoP displacement analysed according to four different experimental protocols. In all the experiments the labyrinth was activated by using long duration (300 msec) trains of constant current pulses (1 msec), applied to plate electrodes located over the mastoid bones. The interstimulus interval used corresponded

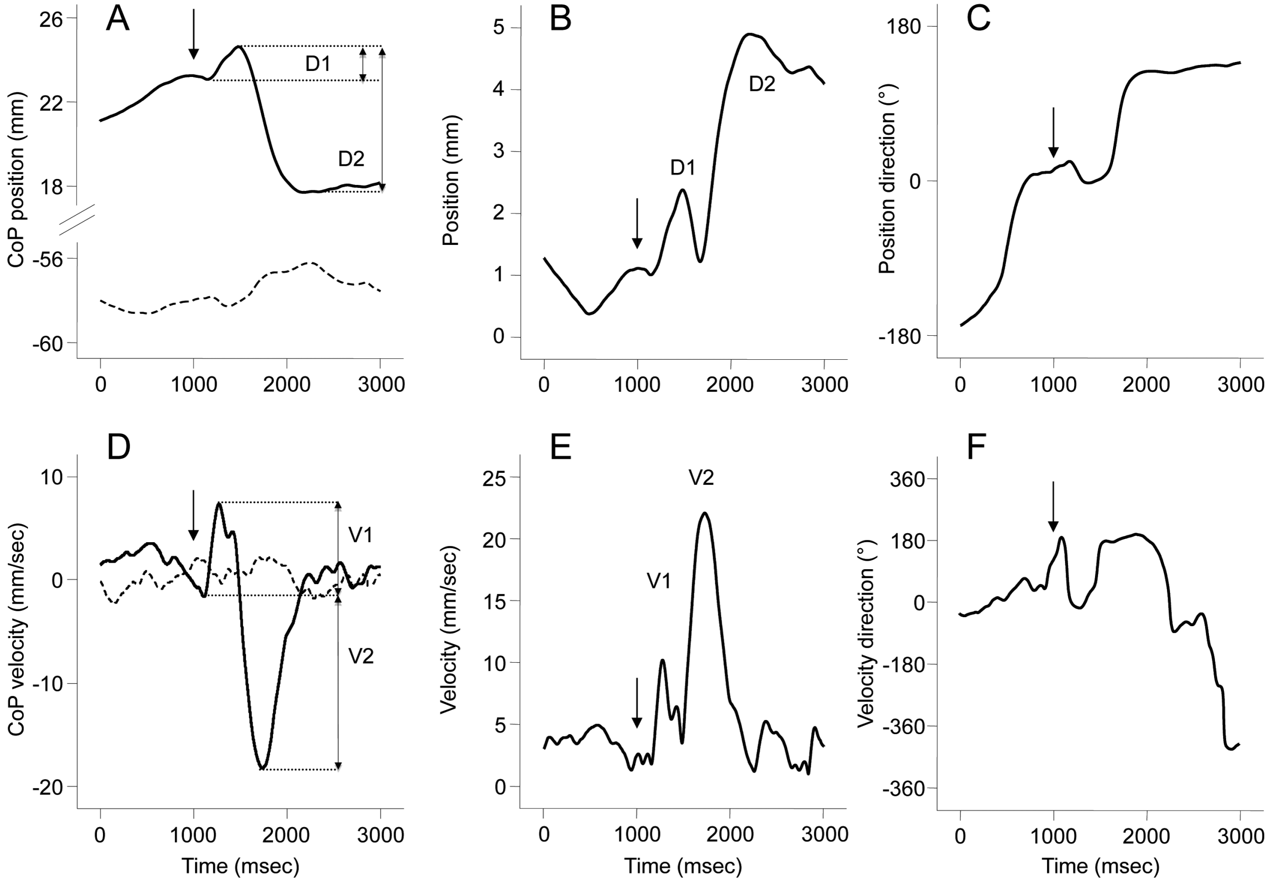

Fig. 1. - Changes in CoP position and velocity induced by labyrinthine stimulation.

A. X (continuous trace) and Y (dotted trace) coordinates of CoP position before and after stimulation of the labyrinth (1.2 times the threshold, train pulses of 300 msec, 1 msec pulse duration, 40 μsec inter-pulse interval, cathode on the right mastoid process) beginning at the time indicated by the arrow. The traces are obtained from a subject with the head forward and are the average of 30 successive sweeps. Upper and lower displacements of the X coordinate represent displacement of the CoP to the right and the left side, respectively. Upper and lower displacements of the Y coordinate represent forward and backward displacements of the CoP, respectively. The amplitude of the first (D1) and second (D2) stimulus-induced displacements of CoP were evaluated as indicated in the figure. The origin of X and Y coordinates correspond to the average position of the CoP during the whole testing period.

B. Stimulus-induced changes in the module of the CoP displacement vector elicited by labyrinthine stimulation (arrow). The module (M) was evaluated point by point from the data of panel A according to the following formula: M = $\sqrt{X^2 + Y^2}$.

C. Direction (Dir) of the CoP displacement vector evaluated from the data shown in panel A according to the formula Dir = $\tan^{-1} Y/X$. The direction values are referred to a body centred reference frame where 0 and 180° represent displacements along the traverse axis towards the right and left side, respectively, while +90 and -90 refer to forwards and backward displacements, respectively. The arrow marks the beginning of the stimulus.

D. X (continuous trace) and Y (dotted trace) coordinates of CoP velocity before and after stimulation of the labyrinth at the time indicated by the arrow. The traces are obtained by differentiation of those illustrated in A. The amplitude of the first (V1) and second (V2) peak velocities were evaluated as indicated in the figure.

E. Stimulus-induced changes in the module of the CoP velocity vector elicited by labyrinthine stimulation (arrow). The module (M) was evaluated as in B.

F. Direction of the velocity vector (evaluated as in C) observed for the same data illustrated in A-B. The arrow marks the beginning of the stimulus.

to 40 μsec in all the experimental protocols except the second one, where this parameter was changed from 40 μsec to 3 msec. The cathode was always placed on the right mastoid and the anode on the left one, except in the third experimental protocol, where the polarity was reversed in consecutive tests. Stimuli were delivered by an isolated, constant-current stimulator (Digitimer model DS7A) allowed for human use and driven by a master generator (Ortec, model 4650). In order to elicit unpredictable stimuli, the master generator was triggered by the R wave of the ECG. The stimulator was made refractory by an electronic circuit for a time window following the trigger, so to obtain a repetition rate of about 0,25 Hz. The intensity of the current delivered was checked at the end of the stimulation by measuring the voltage drop induced by the current across a 1 KΩ resistor. The stimulus intensity used was 1.2 × the lower stimulus intensity inducing the perception of a definite increase in body sway without autonomic symptoms and/or visual flash hallucinations. The threshold of sway perception varied between 0.9 and 1.6 mA among the different subjects.

Data recording and analysis

The position of the CoP was recorded through a normalized stabilometric platform (Dune, sampling rate 5 Hz), with the origin of the coordinates system placed at about the mid point of the inter-feet line.

In order to evaluate the parameters of the stimulus-locked CoP motion, the original signals from the force sensors were separately acquired and analyzed through a Lab-view software prepared ad hoc (sampling rate 2 KHz). This software calculated the time course of X and Y coordinates of the CoP starting from the output of platform's force sensors. The Lab-view software was used to calculate the peak amplitudes of the CoP displacements with respect to the pre-stimulus baseline. Averages traces of CoP position obtained with the Lab-view software underwent a further elaboration to obtain the first derivative (velocity). Both position and velocity were evaluated separately for X and Y axes on averaged traces (at least 30 sweep), synchronised by the stimulus and including a pre-stimulus period of 1.0 sec and a post-stimulus period of 2 sec. Initial and late changes in CoP position with respect to prestimulus levels were indicated as D1 and D2 (Fig. 1A). These traces were submitted to a smoothing procedure in which a given point (at the Nth position in the trace) was substituted by the average of all (101) the points included between N+50 and N-50. This procedure did not modify the time course of the traces and was applied also to velocity traces.

As shown in Fig. 1D, the average velocity trace showed two peaks V1 and V2, related to the D1 and D2 displacements observed on the average position traces. Finally, the averaged traces of the X and Y components of position and velocity were converted to polar coordinates so to obtain module and direction values of the CoP position (Fig. 1B, C) and velocity (Fig. 1E, F) vectors. For this purpose, the X-Y coordinates of CoP position were referred to the average CoP position maintained by the subject during the whole period of stimulation (2 min). The relation between Cartesian (X, Y) and polar (module, direction) coordinates is the following: module $= \sqrt{X^2 + Y^2}$; direction $= \tan^{-1} Y/X$. Two bell-shaped increments in the position (D1 and D2) and velocity module (V1 and V2) were always observed after the stimulus. For both position and velocity vector, the direction values at the peaks of D1/V1 pointed nearly in the opposite direction with respect to D2/V2. The orientation of the vectors was related to a body centred reference system where 0° and 180° represent displacements along the latero-lateral axis towards the right and left side, respectively, while +90° and -90° refer to forwards and backward displacements, respectively.

Experimental protocols and statistical analysis (SPSS.13)

The first experimental protocol (7 subjects, head forward) was aimed at assessing whether the stimulus over the mastoid bone was effective in eliciting CoP displacement in subjects standing on a stabilometric platform with the head forward (HF) and whether the effect was specific, in that it disappeared with sham stimulation obtained by applying the galvanic stimulation at neck levels. The order of the two stimulations (electrodes on mastoid or on the neck) was randomised among subjects. The changes in CoP mean position in the frontal plane and its velocity were analysed through a 2-electrode placement (mastoid bones, neck) × 3 times (t0, t1, t2) repeated measure ANOVA. T0, t1 and t2 were intervals of 250 msec corresponding to the prestimulus period (t0) and to the peaks of the CoP displacement (t1→D1, t2→D2:) and velocity (t1→V1, t2→V2). These intervals are shown in Figs. 2, 3 and 5.

The second protocol (4 subjects) tested the effects of the electrodes polarity changes in subjects with head forward through repeated measures ANOVA (3 times × 2 electrodes polarities) applied to the CoP position and velocity in the same intervals as in the above protocol.

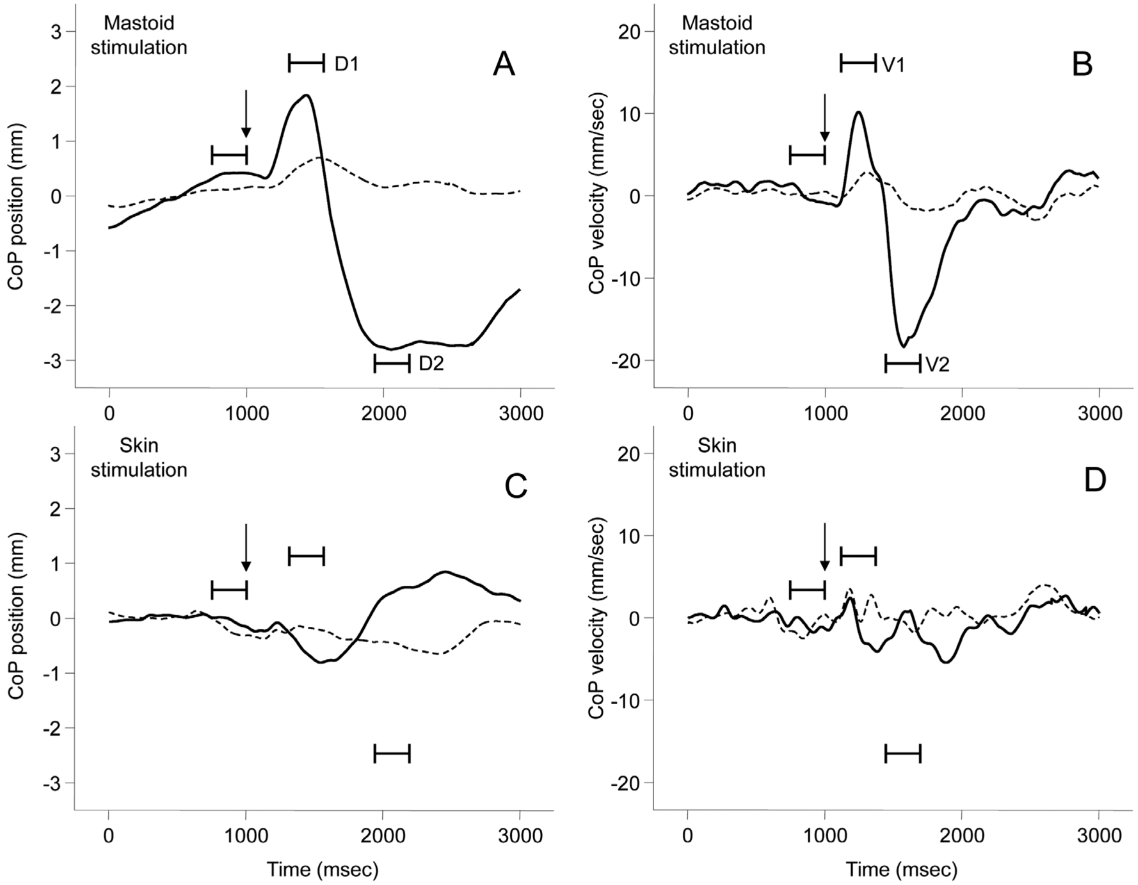

Fig. 2. - Effects of electrodes displacement from the mastoid bone to the neck skin.
A. X (continuous trace) and Y (dotted trace) coordinates of CoP position before and after stimulation of the labyrinth (1.2 times the threshold, train pulses of 300 msec, 1 msec pulse duration, 40 μsec inter-pulse interval, cathode on the right mastoid process) at the time indicated by the arrow. The traces are the grand average obtained from five subject (30 sweeps averaged for each subject) with the head forward. Upper and lower displacements of the X coordinate represent displacement of the CoP to the right and the left side, respectively. Upper and lower displacements of the Y coordinate represent forward and backward displacements of the CoP, respectively. The origin of X and Y coordinates correspond to the average position of the CoP during the prestimulus levels. Note, along the X axis, the former displacement of the CoP (D1) towards the right side (cathode) and the latter (D2) towards the left (anode).
B. X (continuous trace) and Y (dotted trace) coordinates of CoP velocity before and after stimulation of the labyrinth at the time indicated by the arrow. The traces are obtained by differentiation of those illustrated in A. Note the two successive peaks of X component velocity, V1 and V2, corresponding to D1 and D2 in panel A.
C. X (continuous trace) and Y (dotted trace) coordinates of CoP position before and after electrical stimulation of the neck skin at the time indicated by the arrow, with the same parameters as in A. The traces are the grand average obtained from the same five subject displayed in A (30 sweeps averaged for each subject), while keeping the head forward. The coordinate system is the same as in A.
D. X (continuous trace) and Y (dotted trace) coordinates of CoP velocity before and after stimulation of the neck skin at the time indicated by the arrow, with the same stimulus parameters and electrodes position as in C. The traces are obtained by differentiation of those illustrated in A.
In A-C, the segments indicate the time frames utilised in order to evaluate the average values of CoP position and velocity.

The third protocol (5 subjects, head forward) investigated the effects of different interpulse intervals (40 μsec -3 msec) randomly administered.

The fourth protocol (7 subjects) was performed in subjects having their head directed forward (HF), rightward (HR) and leftward (HL), in randomised order. In the two head-rotated positions, the head was maintained at 90° with respect to the body and visually controlled all over the session Between consecutive tests participants were allowed to sit for 3 min with their eyes open. Analysis of the CoP velocity and position along the anterior-posterior (Y) axis was performed through a 2 times (t1, t2) x 3 head positions (HF, HR, HL) repeated measures ANOVA applied to the same time intervals as in the above protocols. In all statistical evaluations CoP position and velocity data were expressed as a difference with respect to the whole prestimulus period (1 sec).

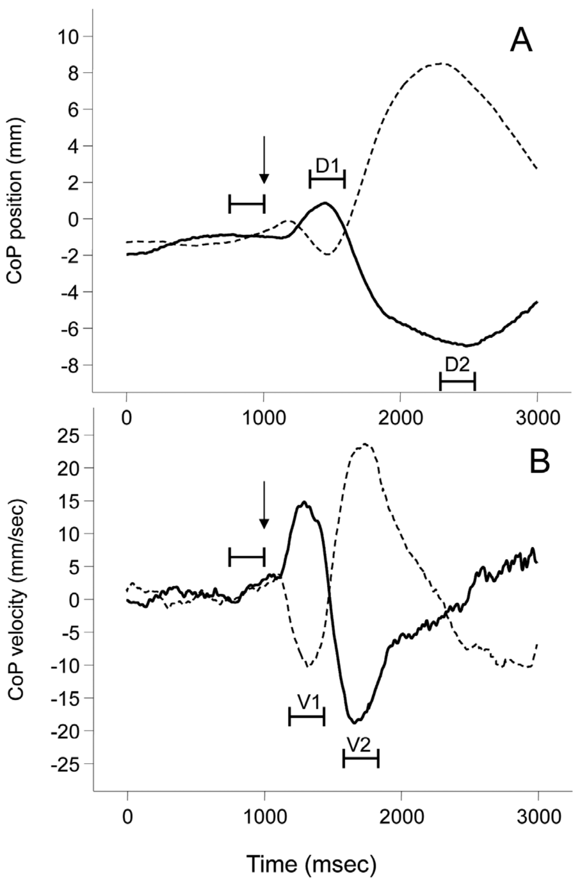

Fig. 3. - Effects of changing electrodes polarity on stimulus-induced changes in CoP position and velocity A. Time course of the X coordinate of CoP position before and after stimulation of the labyrinth (1.2 times the threshold, train pulses of 300 msec, 1 msec pulse duration, 40 μsec inter-pulse interval) at the time indicated by the arrow. Data represented by continuous lines were obtained with the cathode on the right mastoid process, while the dotted line refers to cathode on the left process. The traces are the grand average obtained from four subjects with the head forward (30 sweeps averaged for each subject). Upper and lower displacements represent CoP motion to the right and the left side, respectively. The origin of X and Y coordinates correspond to the average position of the CoP during the prestimulus period. Note that both the former (D1) and latter displacement (D2) of the CoP reversed their sign by changing the polarity of the electrodes.
B. Changes in the X component of CoP velocity before and after stimulation of the labyrinth at the time indicated by the arrow. The traces are obtained by differentiation of those illustrated in A. As in A, the continuous line was obtained with the cathode on the right mastoid process, the dotted line with the cathode on the left process. Upper and lower displacements represent CoP motion to the right and the left side, respectively. Note that the two successive peaks (V1 and V2) reversed their orientation by exchanging the polarity of the electrodes. In both A and B, the segments indicate the time frames utilised in order to evaluate the average values of CoP position and velocity.

Results

Specificity of the stimulus

In 7 subjects standing in HF condition, the described galvanic stimulation elicited a stimulus-locked postural sway in the frontal plane, with small or absent components in the sagittal plane (Fig. 2A). The CoP displacement was biphasic, began at about 180-200 msec from stimulus onset and was initially directed towards the right (cathode) side (D1). Then the direction of CoP movement changed and was directed towards the left (anode) side (D2). The behaviour of CoP velocity is shown in Fig. 2B for the same subjects. In all the subjects, the described effects was not observed any longer after displacing the stimulating electrodes to a lower position on the neck (Fig. 2C, D). For each postural variable considered, a significant effect of electrodes placement × time (position: $F = 22.47$, $n = 2$, $p < 0.001$; velocity: $F = 9.29$, $n = 2$, $p < 0.019$) was found. Its decomposition revealed that differences between t0 (pre-stimulus) and t1/t2 (post stimulus) were always significant for real (position: $t0 \rightarrow t1$ $p < 0.024$, $t0 \rightarrow t2$ $p < 0.002$; velocity: $t0 \rightarrow t1$ $p < 0.002$, $t0 \rightarrow t2$ $p < 0.026$), but not for sham stimulation, which modified significantly only velocity at t1 (post hoc, $p < 0.048$). At this time, however, the change induced by real stimulation was three times larger (post hoc, $p < 0.005$) (Fig. 2B).

Effects of the electrodes polarity

With the cathode on the right side, D1 and D2 were directed towards the right and the left side, respectively, while the directions reversed for placement of the cathode on the left side (Fig. 3A). In other words, D1 and D2 were always directed towards the cathode and the anode, respectively. Decomposition of the significant time x electrodes polarity inter-

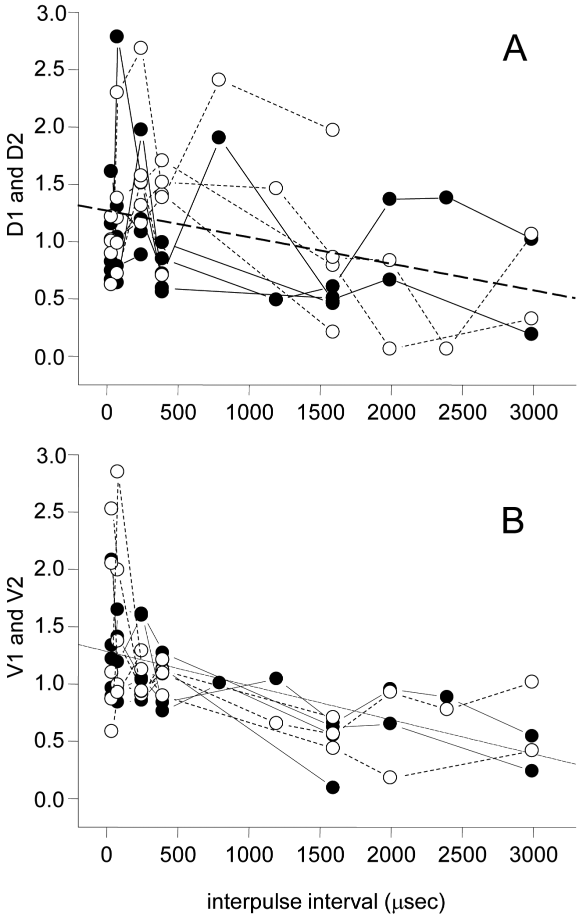

Fig. 4. - Relation between amplitude and velocity of CoP displacement and duration of the inter-pulse interval of stimulus train.
In five subjects, indicated by the different symbols, the interpulse interval was varied between 0,040-3 msec. The subject head was always oriented forward. The stimulus intensity was kept constant at 1.2 times the perceptive threshold and the cathode was on the right side. The values of D1/D2 (A) and V1/V2 (B) have been plotted as a function of the inter-pulse interval. In all the panels, data relative to a given subject have been normalized with respect to the corresponding average value. In both A and B dots (continuous lines) and open circles (small-dashed lines) correspond to the first and second CoP displacement, respectively. Dashed straight lines are regression lines for all plotted points (see text).

action observed for the CoP position ($F = 32.27$, $n = 2$, $p < 0.001$) and velocity ($F = 28{,}042$, $n = 2$, $p < 0.01$) (Fig. 3B) indicated significant differences for D1 ($p < 0.0005$), D2 ($p < 0.016$), V1 ($p < 0.022$) and V2 ($p < 0.011$) between right and left cathode placements.

Effects of different interstimulus interval

As shown in Fig. 4A, the amplitudes of D1 and D2 tended to decrease when the inter-pulse interval was increased from 40 μsec to 3 msec. Similar results were obtained for V1 and V2 (Fig. 4B). The correlation between the response amplitude and inter-pulse interval duration was statistically significant (D1/D2: $R = 0.36$, $y = -0{,}232x + 1.27$, $p < 0.004$; V1/V2: $R = 0.546$, $y = -0.299x +1.285$, $p < 0.0005$).

Effects of head rotation

CoP displacement and velocity changes in 7 subjects with the head forward are shown in Fig. 5A and B, respectively. As expected (Britton et al., 1993; Day et al., 1997; Fitzpatrick et al., 1994, Lund and Broberg, 1983) turning the head to the left (HL) or to the right side (HR) dramatically changed the pattern of the stimulus-induced CoP motion from the frontal to the sagittal plane (Fig. 5CE-F). The initial direction of CoP movement was always towards the cathode, forward (positive values) in HL (Fig. 5C, D) and backward (negative values) in HR (Fig. 5E, F). Values of CoP position and velocity changes are given in Table I for both X and Y coordinates. Statistical analysis highlighted a significant time x head position effects along the Y axis (position: $F = 21.61$, $n = 1$, $p < 0.004$; velocity: $F = 63.89$, $n = 1$, $p < 0.0005$), post-hoc comparison indicating significant differences between HR and HL for both position (D1: $p < 0.026$; D2: $p < 0.0005$) and velocity (V1: $p < 0.022$; V2: $p < 0.006$).

Vectorial analysis of CoP velocity

This analysis was performed in the same subjects tested for the effects of neck rotation. As described in the methods section, stimulus-induced changes in CoP position and velocity were elaborated in polar coordinates (Fig. 1). As shown in Fig. 6, representing the modifications in both position and velocity vectors elicited by the stimulus in all subjects in HR, during the pre-stimulus interval the directions were changing continuously, reflecting the random oscillation of the CoP around its mean position. At about 200 msec after the stimulus, independently upon the pre-stimulus values, the direction of both position and velocity vectors started to drift toward a value of about 270°/-90°, that corresponded to a backward direction (D1/V1). Such a value, once gained was maintained for about 300 msec, then, the vectors

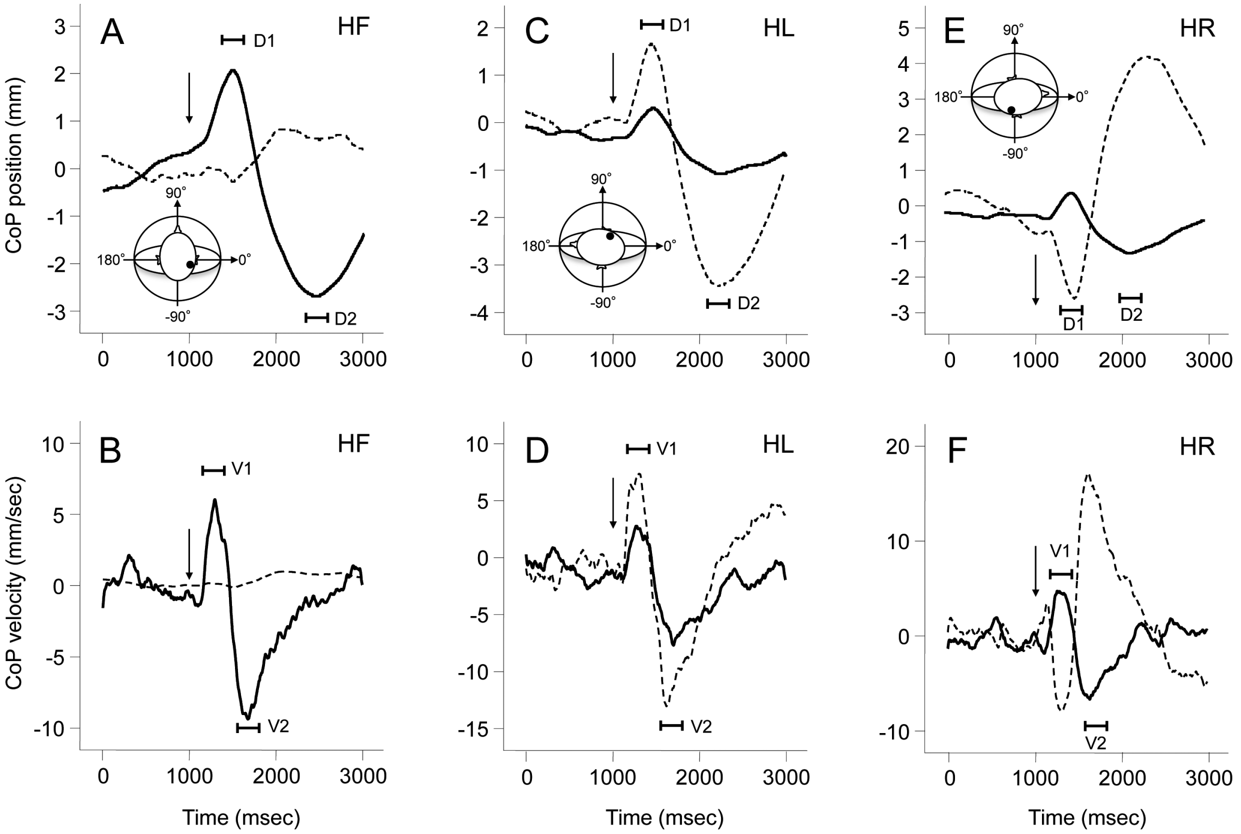

Fig. 5. - Modulation of the stimulus-induced CoP modifications by head rotation.
Grand average of CoP position (A, C, E) and velocity (B, D, F) changes elicited in six subjects by a train of pulses (1.2 times the threshold, 300 msec, 1 msec pulse duration, 40 μsec inter-pulse interval, cathode on the right mastoid process), whose beginning is indicated by the vertical arrows. For each subject 30 superimposed sweeps were averaged. Upper and lower displacements of the X traces represent right and left movements of the CoP, while upper and lower displacements of the Y traces correspond to forwards and backwards movements. The position of the head during each test is represented by the inserted figures, where the dot corresponds to the cathode position.
A-B. When the head was oriented forward (HF) the stimulus induced changes in the CoP position and velocity along the X axis (continuous trace), but was almost uneffective along the Y axis (dotted trace).
C-D. When the head was rotated to the left by about 90° (HL), the same stimulus became effective in displacing the CoP position along the Y axis (upper trace). For this head orientation, D1 and V1 were oriented forward, while D2 and V2 backward. On the other hand, the X component of CoP displacement (and velocity) was reduced with respect to HF.
E-F. When the head was rotated to the right by about 90° (HR), the stimulus displaced the CoP position mainly along the Y axis (dotted trace), but in the opposite direction with respect to HI. Now D1 and V1 were oriented backward, while D2 and V2 forward. As in HL, the X component of CoP displacement (and velocity) was reduced with respect to HF.
In all the panels, the segments indicate the time frame utilized in order to evaluate the average values of CoP position and velocity. In all position traces, the origin of X and Y coordinates corresponds to the average position of the CoP during the prestimulus period.

rapidly rotated forward (90°/-270°), generating a second peak (D2/V2) in the module trace.

The direction of stimulus-induced body sway was evaluated in each subjects, by using two different measurements: first of all, we calculated the average direction values for both position and velocity vectors in the time frames where a constant value was reached and maintained. These time frames are indicated by the segments in Fig. 6 (C-D). Second, we evaluated the direction value at the peaks of D1/D2 and V1/V2. Since the two direction estimates had almost the same value for both velocity ($r = 0.998$, $p < 0.0005$ $Y = 0.978x - 0.33$) and position ($r = 0.978$, $p < 0.0005$ $Y = 0.961X + 2.28$) vectors, only on the second measurement was utilized for the analysis.

Table I - Average ± SD values obtained during the time frames illustrated in Fig. 5, for the Y coordinate of CoP position (D1-D2) and velocity (V1-V2), which correspond to the peak modifications elicited by labyrinthine stimulation.

	D1	D2	V1	V2
HF	-0.21 ± 2.37	0.63 ± 1.54	0.45 ± 5.22	-1.56 ± 13.15
HL	1.73 ± 1.81	-3.42 ± 3.04	7.07 ± 2.96	-15.32 ± 6.99
	↑	↑	↑	↑
	p < 0.031	p < 0.035	p < 0.010	p < 0.0005
	↓	↓	↓	↓
HR	-1.79 ± 1.76	4.15 ± 4.66	-5.01 ± 2.92	16.76 ± 5.68

As shown in Fig. 7 (A, B), for both position and velocity vectors peak directions were closely related to the direction of interaural axis, which corresponds to 0° in HF, 90° in HL and -90° in HR (Fig. 5). In Fig. 7 the direction relative to D2/V2 were referred to interaural axis orientation towards the anode (HF: 180°, HL: -90°, HR: 90°). The correlation between interaural axis and body sway orientation was very high for both position ($r = 0.95$, $p < 0.0005$, $Y = 1.206X -16.697$) and velocity data ($r = 0.986$, $p < 0.0005$, $Y = 0.998X +1.164$). The regression line of position data (but not that of velocity data) deviated significantly from the line $Y = X$ ($p < 0.022$), due to the large value of the intercept (-16.697).

As shown in Fig. 7 (C, D), the (absolute) differences between CoP position data and regression line values (average 33.4° ± 11.2°) were larger than those of velocity data (average 12.4° ± 12.1°) (ANOVA, F = 23,77, n = 1, $p < 0.0005$). Finally position data were also more scattered with respect to the corresponding mean values obtained at the different body-to-head position (Fig. 7A, B). In fact, the mean of the absolute difference between individual and average values was 9.3° ± 1.8° for velocity and 18.0° ± 2.1° for position (ANOVA, F = 12,51, n = 1, $p < 0.001$).

In conclusion, the direction of the velocity vector at the peaks of V1/V2 was less variable than that of the position vector and represented a more precise estimate of interaural axis orientation. The correspondence between velocity vector and interaural axis all over the post-stimulus interval can be appreciated in Fig. 8.

Discussion

The present report indicates that train pulses (0.8-1.5 mA) administered to the labyrinth can produce clear postural and perceptive effects. Postural effects were visualized by the changes in CoP position evaluated over averaged traces of stimulus-triggered CoP coordinates. This stimulation was particularly effective when the inter-pulse interval was reduced below 500 µsec. Interpulse intervals of 3000 µsec did not modify CoP position. This is consistent with previous results showing that train pulses of 30 msec (2-4 mA, 1 msec pulse duration) at 333 Hz were ineffective in eliciting a perception of body sway, although they could modify the activity of sympathetic post-ganglionic fibers innervating skeletal muscles (Voustianouk et al., 2006). In this respect, it has to be reminded that individual pulses (1-1.6 mAmp) of current failed to induce modification of EMG activity of leg and arm muscles when the pulse duration was shorter then 10 msec (Baldissera et al., 1990; Britton et al., 1993). Altogether, these findings suggest that, during trans-mastoid, galvanic stimulation of the labyrinth, the injected current is submitted to a capacitive-like distortion, so that low intensity, short duration pulses are ineffective in modifying the firing of vestibular afferents. According to this hypothesis, it has been observed that both masseter (Deriu et al., 2003) and neck muscles (Watson et al., 1998) can be activated by short duration (2 ms) pulses, provided that the current is raised to an higher intensity (4-5 mA). It is likely that the capacitive distortion is introduced by current flow within the extra-labyrinthine tissues, since the primary vestibular afferents can be activated by short current pulses of 50 µsec, when directly applied to the perilimphatic space (Goldberg et al., 1984). In conclusion, trains of low amplitude, short duration pulses applied between the two mastoid bones represent a suitable method to induce postural sway.

The labyrinthine origin of the observed response was supported by the present findings. Indeed, body

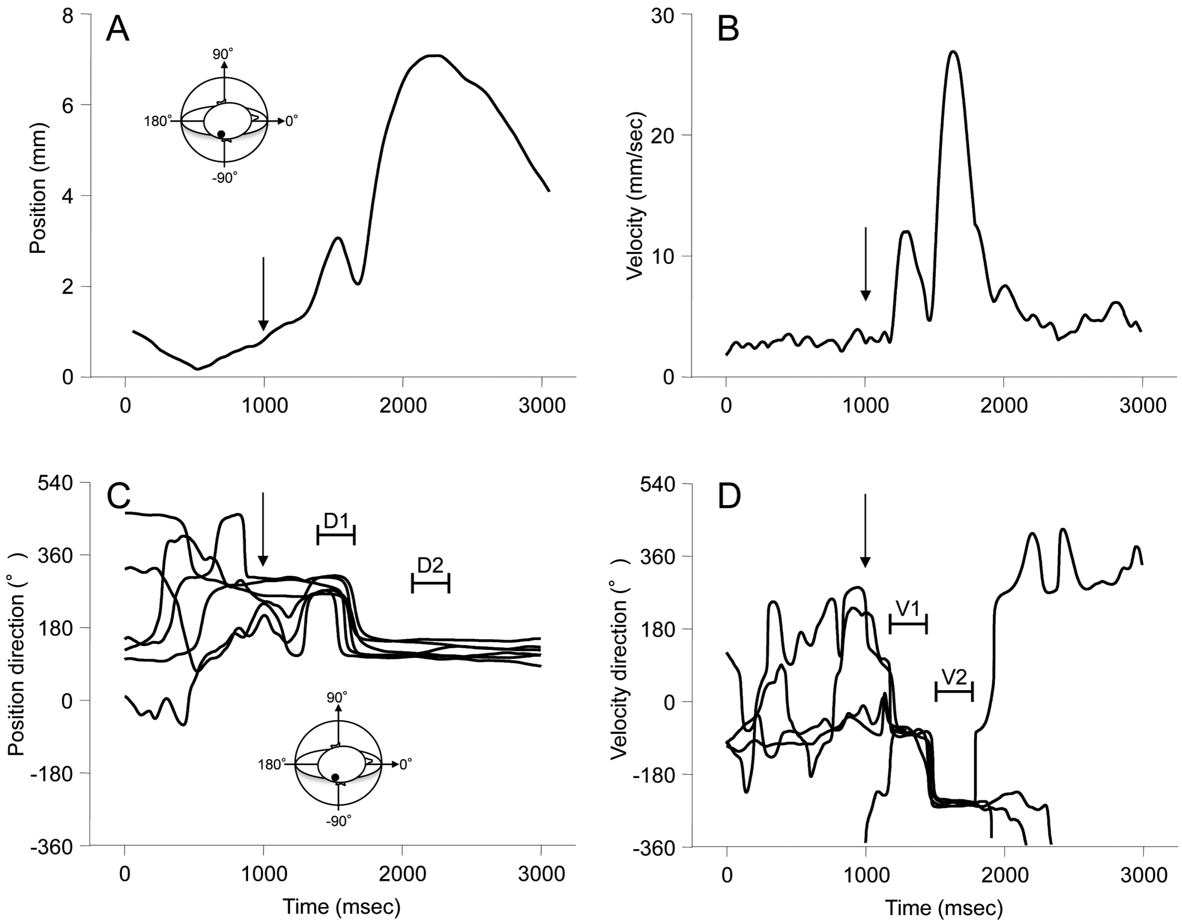

Fig. 6. - Time course of the changes in the direction of CoP position and velocity vectors induced by labyrinthine stimulation in individual subjects
A. Average changes in the module of The CoP position vector elicited in six subjects with the head oriented to the right side (HR, see the inset in C) by stimulation of the labyrinth (1.2 times the threshold, train pulses of 300 msec, 1 msec pulse duration, 40 µsec inter-pulse interval, train frequency 0.42 Hz), performed at the time indicated by the arrow. The cathode was on the right mastoid process (black dot in the inset of panel C).
B. Average changes in the module of The CoP velocity vector elicited in the same subjects illustrated in A by the same stimulus and in the same head position. The arrow indicates the stimulus beginning.
C. Changes in the direction of CoP position vector elicited by labyrinth stimulation at the time indicated by the arrow in the six subjects illustrated in A-B. Note that, independently upon the prestimulus values, vector orientation shifted abruptly, following the stimulus first towards the cathode (-90°/270°) and then towards the anode (90°/-270) side.
D. Changes in the direction of CoP velocity vector elicited by stimulation of the labyrinth at the time indicated by the arrow have been shown for the same subjects illustrated in A-C. Similarly to the direction of position vector, whatever the prestimulus values could be, vector orientation shifted abruptly, following the stimulus, first towards the cathode and then towards the anode side.
In C and D the segments correspond to the time frames utilised in order to evaluate the average direction during D1/D2 and V1/V2.

sway was not elicited when the electrodes were displaced from the mastoid bone to the neck skin. This procedure did not abolish stimulation of cutaneous receptors localized under the electrode, but makes the flow of electric current far from labyrinthine structures.

Moreover, the direction of CoP displacement was reversed by changing the polarity of the electrodes (Britton et al., 1993) and the trajectory of CoP was modified by the head position, as expected (Lund and Broberg, 1983). Finally, the direction of both earlier and later CoP displacement in HR and HL were opposite.

In the present experiments, the time course of CoP movement revealed an initial displacement towards the cathode and a later one towards the anode: this

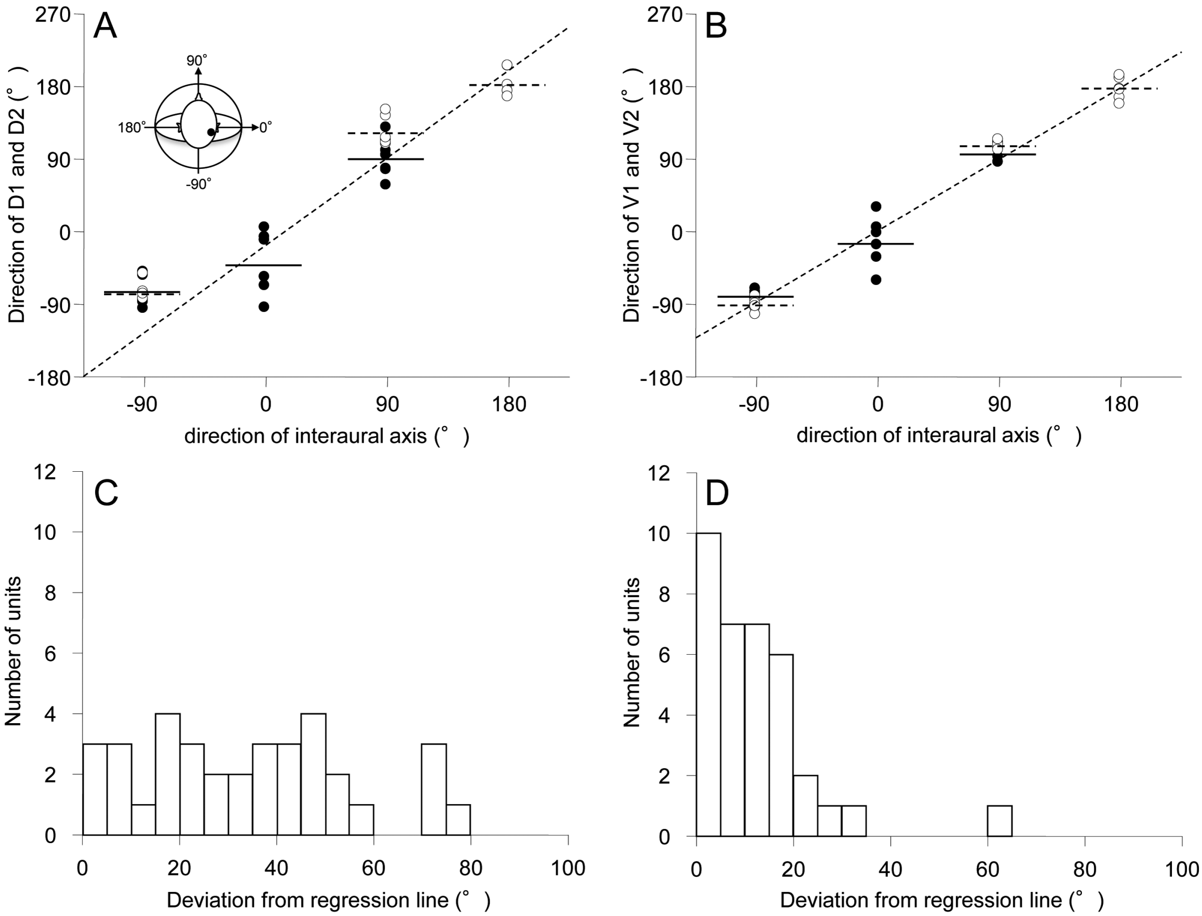

Fig. 7. - Relation between direction of position/velocity vectors and interaural orientation.
A-B: relation between the direction of the position (A) and velocity vectors (B) and the orientation of the interaural axis, obtained in six subjects. Circles and dots refers respectively to the initial (D1, V1) and late (D2, V2) CoP displacements. D2 and V2 have been referred to an interaural axis orientation towards the anode. The dotted lines are obtained by linear regression. The horizontal continuos and dotted segments represent the average value obtained for D1/V1, and D2/V2, respectively. The direction of position and velocity vectors has been evaluated with respect to the reference frame illustrated in the inset.
C-D: Distribution of the scatter from the regression line observed for the directions of CoP position (C) and velocity (D) vectors illustrated in A-B.

finding is consistent with previous investigations recording CoP position (Day et al., 1997; Horak and Hlavacka, 2001; Marsden et al., 2002) or else vertical and lateral forces exerted over two separate foot support plates (Day et al., 1997; Marsden et al., 2002). At variance, only a later displacement towards the anode is observed when body motion is evaluated by measuring the movement of body markers (Day et al., 1997; Marsden et al., 2002), or the output of accelerometers fixed to the body (Britton et al., 1993). The later, anode-oriented displacement, which persists all along the stimulus duration can be therefore attributed to the sustained sway elicited by stimulation. On the other hand, the initial CoP displacement towards the cathode has been attributed to the initial forces that propels the body towards the anode side (Hlavacka and Horak, 2006): this is consistent with the observation that, in the present experiments, when subjects were asked to lean towards a side and their movement was impeded, the CoP was displaced towards the opposite side.

In conclusion, the biphasic time course of CoP motion observed in our experiments was fully consistent with the data described in the literature further documenting the vestibular origin of postural response.

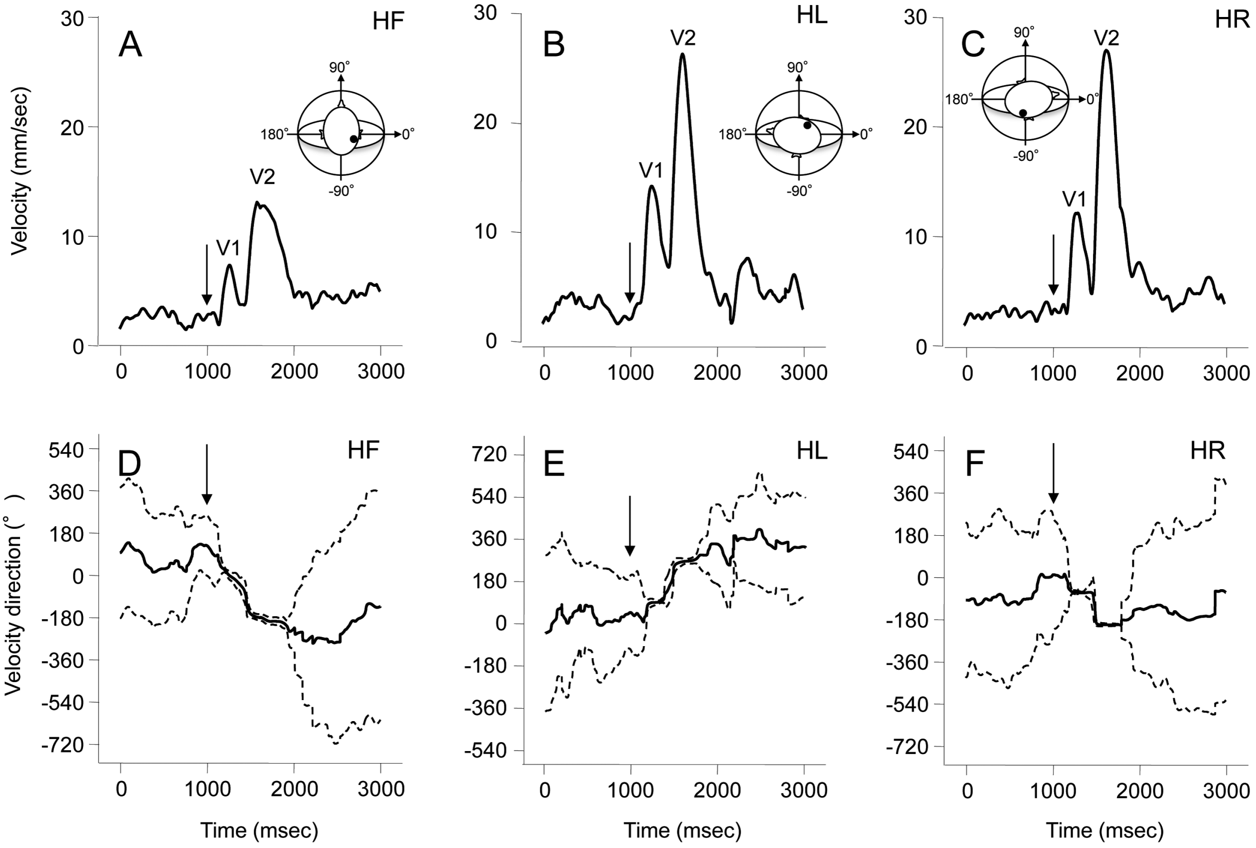

Fig. 8. - Time course of the average changes in the direction of CoP velocity vector induced by labyrinthine stimulation. A-C. Time course of the average changes in the module of CoP velocity vector elicited in six subjects standing in HF (A), HL (B) and HR (C) positions by stimulation of the labyrinth (1.2 time the threshold, train pulses of 300 msec, 1 msec pulse duration, 40 µsec inter-pulse interval, train frequency 0.42 Hz, cathode on the right mastoid process) at the time indicated by the arrow. The inserted figure represents the head positions during the tests.
D-F. Time course of the average changes (continuous lines) ± SD (dotted lines) in the direction of CoP Position vector elicited in the subjects standing as illustrated in A-C. Please note the abrupt decrease in SD of vector orientation following the delivery of stimulus, which corresponds to D1 and D2 peaks. As in A-C the arrows mark the beginning of the stimulus.

The second novel outcome of the present study is the indication of a new method for the analysis of the CoP position and movement. In fact, we have shown that the direction of body sway could be captured by monitoring the direction of both position and velocity vector. Both measurements were highly correlated to the relative orientation of the head, but velocity data showed a better fit and a lower scatter of the individual values (Fig. 6). This finding could be attributed to the fact that the position vector was centred on the average position maintained by the subject during the whole testing period, while stimulus-induced body sway begin, in the individual traces, from points that did not correspond to the origin of coordinates, particularly in subjects showing baseline drift in CoP position. On the other hand, the direction of velocity vector reflects the change in direction of CoP motion with respect to the actual position of latter: it seems, therefore, more appropriate for evaluating the direction of stimulus-elicited body sway and, as a consequence, the accuracy of neck proprioception in monitoring the relative body-to-head position

In conclusion, the present data clearly indicate that train of pulses administered at short inter-pulse intervals (40 µsec) are an efficient method to activate the labyrinth and that evaluation of the velocity vector allow a reliable monitoring of the orientation of postural responses. This method could be exploited in order to the test the efficacy of neck proprioceptive information in modifying the reference frame for processing vestibular signals (Manzoni, 2007) in both physiological and pathological condition.

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