Chapter 5

The effect of sustained centrifugation on the orientation of Listing's Plane

The orientation of Listing's plane in the head specifies the amount of ocular torsion in each gaze direction, which is known to be affected by gravity. In the search for physiological parameters reflecting the vestibular adaptation process, this chapter therefore investigates whether the orientation of Listing's plane is affected by sustained exposure to hypergravity. Non-astronaut subjects were exposed to the four centrifuge conditions described in Chapter 4. The orientation of LP was determined shortly before and after each centrifuge run, with the head erect and tilted in pitch. The results show that exposure to $3G_x$ for 90 min. induced a backward tilt of LP when the head was erect. Pitch head tilt induced a counter-pitch of LP, which was found to be less pronounced after centrifugation. The results are explained by a model indicating that sustained centrifugation decreases the effect of gravity on orientation responses.

In Chapter 3 it was described that the subjective vertical measurements did not provide evidence for a consistent bias in the internal representation of the vertical as related to the direction of the applied centrifugal load, nor for a decrease in otolith sensitivity. However, ocular orienting responses were found to be affected by sustained centrifugation (e.g., ocular counter rolling; Groen et al., 1996b). To investigate the effect of sustained centrifugation on orienting ocular responses in more detail, a study was performed that focused on the effect of sustained centrifugation on the orientation of Listing's plane (LP). Listing's law states that, during saccades and fixations, the amount of ocular torsion is determined by the gaze direction, thereby reducing the eye's three degrees of freedom to two. This can be visualised by describing all eye orientations by a particular rotation vector. This vector represents the rotation required to bring the eye from a certain chosen three dimensional reference position to the desired, three dimensional eye position[8]. Instead of filling up a 3D space, these axes, or rotation vectors, generally lie in a single plane, called a *displacement plane* (DP, Tweed and Vilis, 1990). Obviously, the displacement plane is dependent on the choice of the reference position: expressing the *same* eye positions with respect to a different reference position leads to a different DP (i.e., having a different orientation). Changing the orientation of the reference position by an angle of $2\alpha°$ upward or downward, leads to a change in the orientation of the displacement plane of $\alpha°$ in the same direction (Tweed et al., 1990). The term *Listing's plane* (LP) is generally reserved for the DP that is formed by rotation vectors expressed relative to a specific reference position called the 'Primary Position', which is, by definition, orthogonal to LP (Haslwanter, 1995, Tweed and Vilis, 1990).

Interestingly, the orientation of LP in the head is not fixed, but depends on the orientation of the head relative to gravity. Head tilts to the side induce a shift of LP along the torsional (or *x*-) axis while a pitch tilt of the head induces a counter rotation of LP (in monkey: Haslwanter et al., 1992; Hess and Angelaki, 2003; in human: Bockisch and Haslwanter, 2001; Furman and Schor, 2003). The dependence of the orientation of LP on gravity suggests that it is mediated by the otoliths. More specifically, Hess & Angelaki showed that the primary eye position is not governed by

[8] Here eye *position* refers to the eyes' three-dimensional orientation in the head.

the gravito-inertial acceleration, but by the estimate of gravity (Hess & Angelaki, 1997; 1999). Clarke & Haslwanter (2007) also investigated the effect of gravitaty on the orientation of LP, and observed a consistent immediate backward tilt of LP when entering the 0G phase in parabolic flight, which disappeared again in the subsequent 1G phase. This suggests that the orientation of LP is not only dependent on the direction of gravity (i.e. head tilt) but also on its magnitude. Regarding the study described in this chapter, it was thus hypothesized that centrifugation-induced otolith adaptation is reflected in the pitch orientation (elevation) of LP. Because LP forms the coordinate system for the oculomotor system (Crawford & Vilis, 1992; Crawford, 1994; Crawford et al., 1997), such changes would consequently affect oculomotor responses.

Displacement planes (DP, with the reference position straight ahead) were therefore obtained in different head orientations (–45, 0, and 45° pitch tilt) and it was investigated whether the elevation was changed after sustained centrifugation. Changes in the head pitch dependency could indicate a reduced reaction to head tilt (possibly a reduced otolith sensitivity), whereas changes in the absolute orientation of the DP, regardless of pitch head orientation, could indicate a shift in the spatial properties of the oculomotor coordinate system. Such a change could then be the consequence of a direction specific effect of centrifugation.

METHODS

This experiment was carried out as part of the experiment described in Chapter 3, where a detailed description of the study design can be found. In short, 12 non-astronaut subjects were exposed to four different centrifuge conditions on four different days. The centrifuge conditions differed in G-load and duration and consisted of a 45 or 90 min. exposure to $2G_x$ or $3G_x$ (denoted by 2G45, 2G90, 3G45 and 3G90, respectively). DP recordings were performed within 30 min. before and within 45 min. after the centrifuge run. Centrifugation procedures have been described in Chapter 2.

Eye movement recordings

Binocular eye movements were recorded using video-oculography (VOG, Eye Tracking Device, Chronos Ltd, Berlin), at a sampling rate of 100 Hz. The subject was seated and head position in space was fixed by means of a personal bite board (see Figure 5.1). This bite board was attached to a standard that could be adjusted to the desired head position: erect, 45° backward or 45° forward tilt. For calibration purposes, a small laser was attached to the bite-board, projecting a cross-hair (extending 3.0° up, down, left, and right) in front of the subject. By locating this device between the eyes, the reference position was always the straight ahead position. This allowed for calibration in all head orientations.

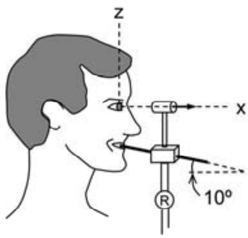

Figure 5.1: Schematic drawing of the experimental setup (calibration 'Head-fixed'). The bite-board was oriented 10° upward, to ensure a comfortable "head erect" position. A laser was positioned in between the eyes, projecting calibration targets along the line of sight. The whole device could be rotated in the sagittal plane about 'R' into a 45° forward or backward position, inducing the desired head tilts.

Due to technical problems with the calibration device during the first 2 days of the experiment, an additional recording was made using a slightly different set-up. The set-up described above will be referred to as 'Head-fixed' and the set-up described below will be referred to as 'Earth-fixed'. In the latter, the subject was seated 60 cm from a backlit projection screen with the head in the erect position (head fixation as described above). Predefined calibration targets (7.0° up, down, left and right) were presented for the left and right eye separately. Because the screen was Earth-fixed, this set-up only allowed for measurements with the head in the erect position.

First, a DP recording was performed using the 'Earth-fixed' method (head erect only). After calibration, eye movements were elicited by a visual target jumping over the screen within a range of ± 15º horizontally and vertically. Second, (when possible) the recordings were performed with the 'Head fixed' method. The screen was removed and the head was randomly positioned into one of the three tilt conditions. Subsequently, calibration was performed with the laser device projecting calibration targets parallel to the line of sight. After calibration, the subject was to make voluntary eye movements for about 45 s. This procedure was then repeated for all head tilt conditions. All measurements were performed in the dark, with only the (calibration) targets visible.

Data analysis: determination of the Displacement Plane

3D eye position (Fick angles) was obtained using dedicated software (Iris Tracker, Chronos Ltd, Berlin). Horizontal and vertical eye position was based on automatic pupil tracking. The torsional position (rotation about the line of sight) was computed by a polar cross correlation algorithm of iris segments (Clarke et al., 2002). Measured with an artificial eye, the measurement accuracy of the Chronos VOG system is 0.1° for the horizontal and vertical eye position and 0.4° for the torsional eye position (within a measurement range of ±20°, see Clarke et al., 2002). However, especially the torsional eye position is subject to measurement errors, that depend on the quality of the iris segments (i.e., the amount of structure present) and the exact location of the pupil centre. Where the first source of error is dependent on characteristics of the individual iris (and can thus not be accounted for), errors in torsional position due to misdefined pupil centres were accounted for by evaluating 36 iris segments for each video frame. These 36 local estimates were then subjected to an iterative sine-fit algorithm that resulted in a more veridical estimate of ocular torsion (Bos and De Graaf, 1994; Groen et al., 1996a). 3D eye position data were subsequently transformed into rotation vectors [r_x, r_y, r_z] (see Haustein, 1989; Haslwanter, 1995), expressed in a head fixed, right-handed, orthogonal coordinate system with the *x*-axis aligned with straight ahead gaze (see Figure 5.2). Each dot in Figure 5.2 represents the tip of a rotation vector, decribing one particular eye position. For example, using the right hand rule, an upward gaze direction with no ocular torsion component is represented by a rotation vector that is aligned with the negative y-axis in Figure 5.2 (left panel), with the vector magnitude equal to the angle of rotation. The DP was obtained from a least squares planar fit ($r_x = ar_y + br_z + c$) through the data. Pitch tilt (elevation, β) of the plane was defined as the tangent of b and is the angle between the plane fit and the z-axis (see Figure 5.2, right panel). Thickness of the DP, which can be taken as a measure of accuracy, was defined by the standard deviation of the distance from the data to the fitted plane. Planes of which the ratio between the thickness and the vertical range exceeded 0.08 °/° (matching the average thickness divided by 15° assumed to be a useful range) were not taken into account for further analysis. Furthermore, elevation values deviating more than 2·SD from average were considered outliers too. For statistical analysis DP elevations of both eyes were averaged.

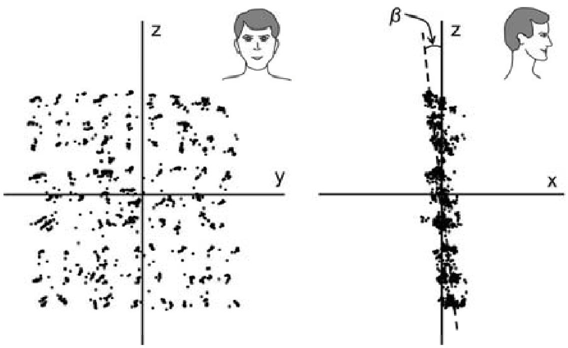

Figure 5.2: Example of recorded data. Eye position is expressed as rotation vectors relative to the straight ahead position. The front view (left panel) shows the gaze directions while the side view (right panel) shows that torsional position is restricted to a displacement plane (DP). DP-elevation is characterized by the angle β, the tilt of the plane relative to the z-axis.

RESULTS

Of the data recorded with the 'Head-fixed' method (i.e., head tilted –45, 0 and +45°), 12.5% was missing due to technical problems (see Methods) while 8% did not meet the inclusion-criteria. The 'Earth-fixed' data set (i.e., head erect only) was complete and allowed an assessment of the repeatability of the DP-elevation in general. The average variability within subjects, as expressed by the standard deviation of DP-elevation of the four consecutive individual pre-tests, equalled 1.6°. The intra-class correlation coefficient for these four repetitions was 0.92. DP-elevation differed considerably between subjects, ranging from –6.2 to +8.0° in the pre-tests (mean 0.0°, SD 2.9°, head erect). The correlation between DP-elevation obtained with the two calibration methods (i.e., 'Head-fixed' and 'Earth-fixed', see Methods) was 0.66 ($p<0.0001$). Average thickness of DP equalled 1.2° (SD 0.4).

Effect of head tilt on DP-elevation

DP-elevation significantly depended on head tilt as indicated by a within subject main effect ANOVA on the data of the pre-tests ($F(2, 147)=12.09$, $p<.001$): the DP tilted backward when the head tilted forward and vice versa (see Figure 5.3, open symbols). To assess the effect of the different centrifuge conditions on this tilt-dependency we calculated the slope of the regression line (° DP tilt/° head tilt) through the available data for each subject and condition. This data was then submitted to a within subjects, 4 (centrifuge condition) × 2 (session) ANOVA, where session refers to the pre- and post-test. Despite the small number of subjects having a full data-set on slope ($n=5$), the effect of session was significant ($F(1,4)=8.2$, $p=.046$). Averaged over all data, mean slope changed from –0.02°/° (SD = 0.02) in the pre-test to –0.01°/° (SD = 0.02) in the post-test (see Figure 5.3, filled symbols). The analysis did not reveal significant differences between the effects of the four centrifuge conditions on slope. Because this could be due to the limited number of subjects having a full dataset on slope, an additional analysis was performed, using a main effect ANOVA with subject as random factor and centrifuge condition and session as fixed factors. An interaction term centrifuge condition × session was added to the model. However, no differences between the effects of centrifuge condition could be demonstrated. Again, only the effect of session appeared significant ($F(1, 59) = 4.22$, p=.044).

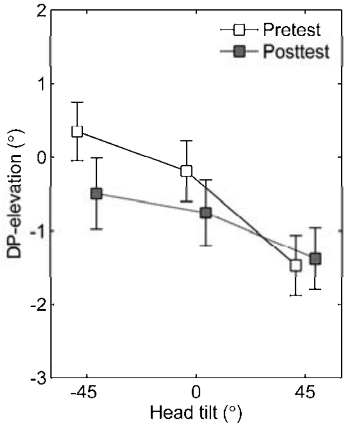

Figure 5.3: Mean DP-elevation as a function head tilt, averaged over the four centrifuge conditions (bars indicate standard error of mean). According the right-hand-rule, negative angles indicate backward tilt of the DP relative to the z-axis.

Absolute orientation of DP

The data in Figure 5.3 suggests that not only the effect of head tilt is affected by centrifugation, but also the absolute orientation of the DP in the head. Figure 5.4 shows the average difference between the pre- and post-tests for these head erect conditions for the four centrifuge conditions. Note that the difference depends on the applied centrifuge condition: a significant difference of −1.0° (SD 1.5°) was found in the 3G90 condition ($t(11) = -2.34$, p=.039), indicating a backward tilt of DP when the head was erect.

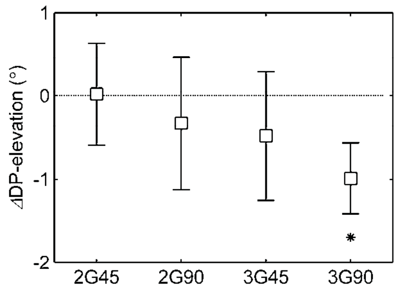

*Figure 5.4: Mean difference in DP-elevation between the pre- and posttest for the 4 centrifuge conditions (Bars indicate standard error of mean). A negative change indicates a backward tilt of DP relative to the pretest value. Statistical differences are indicated by *.*

DISCUSSION

In the current study displacement planes (DP, with the reference position defined as the straight ahead gaze) were recorded in different head orientations and it was investigated whether 1) the dependency of DP-elevation on pitch head tilt was affected by sustained centrifugation, and 2) whether sustained centrifugation induced changes in the spatial properties of the oculomotor coordinate system. The results indicate that both questions can be answered in the positive. Averaged over all conditions, centrifugation decreased the counter pitch of DP in response to head tilt. Furthermore, 90 minutes of $3G_x$ stimulation induced a small but significant backward tilt in the absolute orientation of DP when the head was erect. Shorter exposures or exposures at a lower G-level only induced marginal changes in the absolute orientation of DP.

Methodological issues

Average thickness of the displacement planes recorded in the current study was in the upper range of the values generally obtained for recordings made with scleral search coils (Bockisch & Haslwanter 2001:

0.4°; Furman & Schor, 2002: 1.0°; Haslwanter et al., 1994: <1°; Melis et al, 1997: 0.69°). This can be attributed to the fact that the determination of ocular torsion using video-based systems is slightly less accurate than obtained from scleral search coils (Houben et al., 2006, but see also Merfeld et al., 1996). Nevertheless, the day to day variability of DP-elevation was similar to the variability found by others (Clarke & Haslwanter 2007: 2.1°; Haslwanter et al., 1994: 3.4°; Melis et al., 1997: <3°). In addition, the effect of pitch head tilt on DP-elevation as found by Bockisch and Haslwanter (2001) and Furman and Schor (2003) was replicated. Therefore it is concluded that video-oculography is suitable to determine DP-elevation in humans.

Interestingly, the magnitude of DP counterpitch found in the pre-tests seems to exceed the effect reported by Furman and Schor (2003), who measured the orientation of Listing's plane in response to whole body tilts up to 30°. The effect of body tilt on the orientation of Listing's plane found by Bockisch and Haslwanter (2001) also seemed somewhat smaller, although it is hard to judge the data based on their figures and the limited amount of subjects. Because both studies used whole body tilt instead of head tilt, as was the case in our study, it is tempting to ascribe differences to the contribution of the neck. Influences of the neck on ocular orientation responses (OCR) have, for example, also been described by Bles et al. (1998b). Furthermore, it may be assumed that a head-tilt paradigm represents a more natural situation than a body tilt paradigm. The neck provides additional information about the orientation of the head relative to the vertical and may therefore affect the orientation of oculomotor responses.

Are the changes in DP orientationrelated to vestibular adaptation?

Do the results of the current study reflect changes in the oculomotor system that can be related to adaptation-induced phenomena? The centrifugation induced effects were small and it does not seem plausible that such small changes would be the cause of major behavioural changes that are observed after sustained centrifugation. Given the large intra-subject variability of DP-elevation, the effects are in any case not large enough to monitor adaptation at an individual level. Nevertheless, the results may contribute to our understanding of vestibular adaptation in general. In the next paragraphs a hypothesis is proposed that might provide an explanation for these results.

The decreased DP-counterpitch found after sustained centrifugation suggests a decreased sensitivity or gain of the orientation response. This is in accordance with findings of Groen et al. (1996b), who measured the otolith driven ocular response (Ocular Counter Roll, OCR) to lateral body tilt and found that the gain of this response was decreased after a 60 min exposure to 3G. Interestingly, as was already mentioned in Chapter 3, a similar decrease in OCR gain was generally also observed after spaceflight (Dai et al., 1994; Hoffstetter-Degen et al., 1993; Vogel & Kass, 1986; Young & Sinha, 1998; but see also Moore et al., 2001). This down-scaling of orientation-responses may be a common reaction of the system to deal with novel gravitational states. Instead of the gravitational cue, the body or the visual environment is taken as a spatial reference. Associated to this is Mittelstaedt's concept of the idiotropic vector (Mittelstaedt, 1983), which is the tendency to take the longitudinal body axis as a reference for verticality. It is already known from spaceflight that astronauts tend to shift to a body-centric frame of reference and interestingly, there is also evidence that this occurs after the transition to hypergravity as well. Jenkin and colleagues (2005) measured the perceived direction of 'up' in the different phases of parabolic flight and found that *both* in the micro- and hypergravity phase subjects shifted towards a body-centric frame of reference.

This shift towards a more body-centric frame of reference could also apply to Listing's plane (LP) Let us assume that LP takes a certain orientation in the head, which is modulated by the direction of gravity relative to the head. As such, the orientation of LP is determined by a head-fixed component and a space-fixed component. This can be visualized by denoting the head-fixed component by the vector $\mathbf{LP_h}$, comparable to an 'idiotropic vector', and the space-fixed component by the vector $\mathbf{LP_g}$. The resulting elevation of LP follows from the addition of these two vectors (see Figure 5.5, left panel). Modulation of LP-elevation by head tilt is accomplished by varying the direction of $\mathbf{LP_g}$ relative to $\mathbf{LP_h}$, resulting in the counterpitch of LP during head tilt. The strength of this modulation is given by the length of $\mathbf{LP_g}$ relative to $\mathbf{LP_h}$: If there would be no effect of head tilt on LP-elevation, $\mathbf{LP_g}$ would be 0, whereas if LP would perfectly orient to gravity, $\mathbf{LP_g}$ would be large relative to $\mathbf{LP_h}$. Now the effect of centrifugation can be understood by decreasing the effect of gravity on LP-elevation, in favour of the head-fixed orientation $\mathbf{LP_h}$. Decreasing the length of $\mathbf{LP_g}$ results in a backward tilt of LP (see Figure 5.5, right panel), together with a smaller effect of head tilt on LP-elevation. This is accordance with the results of the current study.

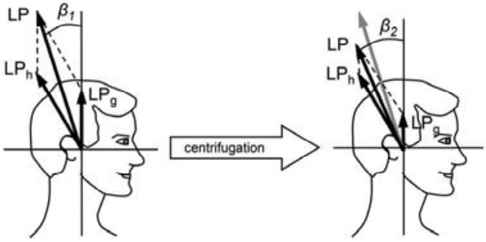

Figure 5.5: Proposed model determining LP-elevation. The orientation of LP (β) results from vector addition of a head-fixed vector $\boldsymbol{LP_h}$*, combined with a vector* $\boldsymbol{LP_g}$ *that is parallel to gravity (left panel). The length of* $\boldsymbol{LP_g}$ *denotes the strength of the gravitational modulation. If the head is tilted, the vector* $\boldsymbol{LP_g}$ *induces a counterpitch of LP. If the effect of gravity on LP-elevation is decreased after centrifugation (right panel), this would result in a backward tilt of LP* ($\beta_1<\beta_2$)*, together with a decrease of head tilt induced modulation of LP-elevation.*

Furthermore, the model also predicts a backward tilt of LP in 0G: in absence of $\mathbf{LP_g}$, LP would be equal to $\mathbf{LP_h}$. However, the extent of this effect has been shown to exceed the effect of body tilt on the orientation of LP (Clarke & Haslwanter, 2007), which is not (yet) reproduced by the model. Possibly, a zero gravity condition leads to a qualitatively different response than conditions where a gravity vector is present.

Interestingly, the hypothesis that LP-elevation is determined by a head-fixed and a space-fixed component also matches experimentally obtained results on the effect of head pitch on LP-elevation. These studies reported on the effect of a full revelation of head tilt on LP-elevation and showed an asymmetric response (e.g., Bockisch and Haslwanter, 2001; Haslwanter et al., 1992). That is, elevation when the head is erect differs from elevation in the up-side-down position, and forward head tilt often leads to greater changes than backward head tilt, which is qualitatively simlar to the effect of pitch tilt on LP-elevation as shown in Figure 5.6. This figure shows the orientation of LP (OLP) that is predicted from the proposed model, and clearly shows this asymmetrical behaviour. Nevertheless, it is clear that this hypothesis requires further elaboration. Considering the large variability of LP-elevation within, and between subjects, a larger amount of data is required for a quantitative analysis.

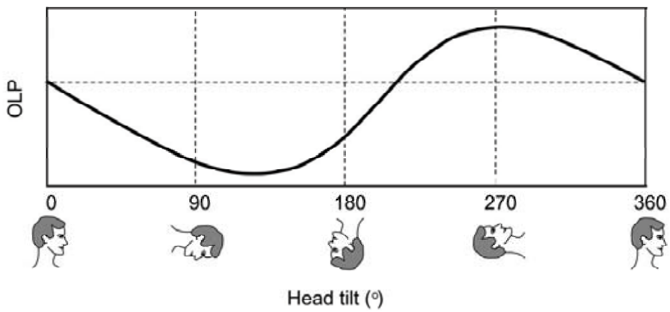

Figure 5.6: Predicted elevation of Listing's plane (OLP) as a function pitch head tilt. Note that this type of behaviour is also observed experimentally (Bockisch & Haslwanter, 2001; Haslwanter et al., 1992)

Alternatively, the two effects of sustained centrifugation could also be caused by two separate mechanisms: 1) a direction-specific bias in the estimate of the gravitational vertical, responsible for the backward tilt of LP when the head is erect, and 2) a decreased effect of head tilt on the spatial behaviour of LP. The hypothesis mentioned above is elegant in that it links these two effects to a single cause: a shift towards a more body centred frame of reference.

Conclusion

It was shown that after centrifugation the DP tilts backward, and the effect of head tilt on DP-elevation is decreased. This suggests that the gain of orientation responses is decreased after sustained centrifugation, which can be understood as a shift towards a more head-centric frame of reference. Nevertheless, because the effects were small relative to the within-subject variability, DP-elevation was not considered to be informative about otolith adaptation to hypergravity at an individual level.