Cold Thermal Irrigation Decreases the Ipsilateral Gain of the Vestibulo-Ocular Reflex

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Objectives: During head rotations, neuronal firing rates increase in ipsilateral and decrease in contralateral vestibular afferents. At low accelerations, this "push-pull mechanism" is linear. At high accelerations, however, the change of firing rates is nonlinear in that the ipsilateral increase of firing rate is larger than the contralateral decrease. This mechanism of stronger ipsilateral excitation than contralateral inhibition during high-acceleration head rotation, known as Ewald's second law, is implemented within the nonlinear pathways. The authors asked whether caloric stimulation could provide an acceleration signal high enough to influence the contribution of the nonlinear pathway to the rotational vestibulo-ocular reflex gain (rVOR gain) during head impulses.

Design: Caloric warm (44°C) and cold (24, 27, and 30°C) water irrigations of the left ear were performed in 7 healthy human subjects with the lateral semicircular canals oriented approximately earth-vertical (head inclined 30° from supine) and earth-horizontal (head inclined 30° from upright).

Results: With the lateral semicircular canal oriented earth-vertical, the strongest cold caloric stimulus (24°C) significantly decreased the rVOR gain during ipsilateral head impulses, while all other irrigations, irrespective of head position, had no significant effect on rVOR gains during head impulses to either side.

Conclusions: Strong caloric irrigation, which can only be achieved with cold water, reduces the rVOR gain during ipsilateral head impulses and thus demonstrates Ewald's second law in healthy subjects. This unilateral gain reduction suggests that cold-water caloric irritation shifts the set point of the nonlinear relation between head acceleration and the vestibular firing rate toward a less acceleration-sensitive zone.

Key words: Caloric stimulation, Ewald's second law, Nystagmus, Vestibular, Video-oculography.

INTRODUCTION

The six semicircular canals (SCCs) of the two labyrinths measure angular accelerations of the head about any axis in three-dimensional space. Within each cupula, this information is transformed into neuronal signals that are sent to the brainstem along the vestibular nerve. A major projection of vestibular signals feeds to a network that drives the rotational vestibulo-ocular reflex (rVOR). The rVOR ensures clear vision by eliciting compensatory eye movements in the opposite direction of head rotation with approximately the same angular velocity as the head. In normal-life conditions, head accelerations often occur with high frequencies, that is, greater than 1 Hz, for example, during locomotion (Grossman et al. 1988). To ensure a robust performance of the rVOR, elements of the SCCs and their afferents are specialized in transmitting information about head rotations of high angular acceleration to the vestibular nuclei (Fernandez & Goldberg 1971).

During low angular accelerations, the SCCs operate in a linear push-pull fashion. For instance, during a head rotation in the horizontal plane to the right, the firing rate of afferent neurons from the right lateral SCC increases approximately by the same magnitude as the neuronal firing rate from the left lateral SCC decreases. This push-pull mechanism is realized in the linear vestibular pathways from both labyrinths to the brainstem and the cerebellum (Lasker et al. 1999, 2000; Minor et al. 1999; Minor & Lasker 2009). Properties of the rVOR for sinusoidal head movements in the low-to-middle frequency range were successfully modeled using linear transfer functions (Paige 1983; Robinson 1981). These traditional linear models, however, could not sufficiently explain rVOR properties observed at higher frequencies and velocities (with gain being higher than expected), as emphasized by Minor et al. (1999). These authors experimentally characterized the dynamics of the rVOR at high frequencies and proposed a new model that took into account the limitations of the vestibular organs when high head accelerations are applied (Lasker et al. 1999; Minor et al. 1999). Specifically, at high accelerations and high frequencies, the change of firing rates is nonlinear in that the ipsilateral increase is larger than the contralateral decrease (Lasker et al. 1999; Minor et al. 1999). The additional excitatory signal from the ipsilateral labyrinth travels within the nonlinear pathways from both labyrinths to the brainstem and the cerebellum (Galiana et al. 2001; Halmagyi et al. 1990; Lasker et al. 1999, 2000; Minor & Lasker 2009; Minor et al. 1999). This mechanism of stronger ipsilateral excitation than contralateral inhibition of ampullary hair cells during high-acceleration head rotation forms the basis of Ewald's second law (Ewald 1892). By taking both linear and nonlinear pathways into account, Lasker et al. (1999) could experimentally reproduce Ewald's second law in canal-plugged squirrel monkeys.

In humans, the existence of the nonlinear vestibular pathway, and hence Ewald's second law, becomes manifest when applying the head-impulse test (Halmagyi & Curthoys 1988) in patients with unilateral vestibular loss (Halmagyi et al. 1990). When the head is rapidly rotated to the affected side, the gain (that is, eye-velocity divided by head velocity) of the rVOR is lower than when the head is rotated to the unaffected side. Such deficient rVOR results in a catch-up saccade that is triggered by the visual system and that can be depicted by the clinician.

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To demonstrate Ewald's second law in healthy subjects, we attempted to suppress or enhance the nonlinear pathway unilaterally with caloric irrigation, and then apply rapid head rotations, because these nonlinear mechanisms are elicited only for head accelerations above ~100°/s^2 (Baloh et al. 1977). The nonlinear pathway has been found to be especially sensitive to changes in temperature (Park et al. 2010), and in particular cold temperature both lowers the resting discharge rate and decreases the sensitivity to head velocity. The rVOR evoked by ipsilateral head impulses will thus be driven predominately by the linear pathway. Conversely, warm stimulation could push the nonlinear pathway into its more sensitive operating range, and so should lead to an enhancement of the rVOR.

MATERIALS AND METHODS

Test Subjects

Seven healthy naïve individuals (4 men, 3 women, mean age 37 years, range 19 to 51 years) with no history of otologic or cochleo-vestibular disorders were recruited. The study was approved by the institutional review board of the Petz Aladár Hospital, Györ, Hungary (PAMOK Hospital Protocol number 76-1-18/2015), and written informed consent was obtained from all participants. The protocol was in accordance with the ethical standards laid down in the 1964/2013 (7th revision) Declaration of Helsinki for research involving human subjects. Before the measurements, the left eardrum was examined and the head-impulse test was carried out in a sitting position. All subjects had a normal eardrum and normal gain values for the horizontal rVOR.

Stimulation and Measurement Methods

Before warm- and cold-water caloric irrigation, baseline head-impulse testing (HIT) was obtained in the plane of the lateral SCCs. Video HIT (vHIT) was carried out with the ICS Impulse® video goggles (GN Otometrics, Taastrup, Denmark) (MacDougall et al. 2009; Weber et al. 2009). For head impulses, the position of the right eye was recorded with 250 samples/s. Caloric stimulation was done with the lateral SCCs in an earth-vertical position, so the subject's head was inclined by 30° from supine, with the Reid's baseline elevated 60°. Subjects were tested in a well-lit room (to ensure a small pupil) with an eye-level target at a minimum distance of 1 m in front of them, which was positioned to allow a straight fixation direction during the head impulses. Caloric irrigation lasted 1 min and was carried out in the left ear in all subjects and at temperatures 24, 27, 30, and 44°C. After 1 min of irrigation, 5 high-acceleration head impulses to the left and to the right (peak head velocity >160°/s, which translates to accelerations above approximately 3000°/s^2) were administered over a time interval of 30 sec in a random order. This block of 2 times 5 impulses (both left and right) was repeated 5 times over 150 sec resulting in 5 blocks. The gain values of impulses were averaged per block, thereby creating one data point per subject every 30 sec. The subjects relaxed for 10 min between caloric irrigations. To minimize effects of possible caloric habituation due to repetitive caloric irrigation (Lidvall 1961), irrigation at different temperatures was in a different random order for each subject.

After caloric irrigation with the lateral canals in the earth-vertical position, the experiment was repeated with the lateral canals earth-horizontal (Reid's baseline pitched 30° down),

so the head was pitched forward 30° from upright. Because in this position, convectional thermal endolymphatic movements along the canal are missing, if there is any change in the rVOR, it should be caused only by direct cooling or heating of the end organ or the vestibular nerve afferents.

Analysis of Caloric Nystagmus

To assess the intensity of the caloric nystagmus during the head impulses, the nystagmus beats occurring in the time frame of individual head impulses were counted off-line. The ICS software saved 750 msec of data for each head impulse, beginning 100 msec before the start of the impulse. Usually the intensity of the caloric nystagmus is characterized by the maximal slow-phase velocity of the nystagmus. With the software used in this experiment, we could not retrieve these values from the dataset. Because there is a high correlation between the slow-phase velocity and nystagmus frequency (Hinchcliffe 1968; Mulch et al. 1978), we decided to characterize the effect of irrigation using the frequency of nystagmus beats. Nonetheless, nystagmus beats could be clearly depicted and when more than one beat was registered per trial, the nystagmus frequency (beats/s) was calculated (Fig. 1). This was easily possible when the ensuing nystagmus had a direction opposite to the compensatory saccades (Fig. 2, right panel). To differentiate caloric nystagmus beats from compensatory saccades induced by HIT when the two occurred in the same direction (Fig. 2, left panel), only those nystagmus beats were counted that occurred immediately before an impulse or later than 200 msec after the impulse (i.e., at a period when vHIT-induced compensatory saccades have already stopped). In cases where caloric nystagmus quick phases and vHIT-induced compensatory saccades were expected to beat into opposite directions, this restriction was not needed. Beat frequency was only calculated, when at
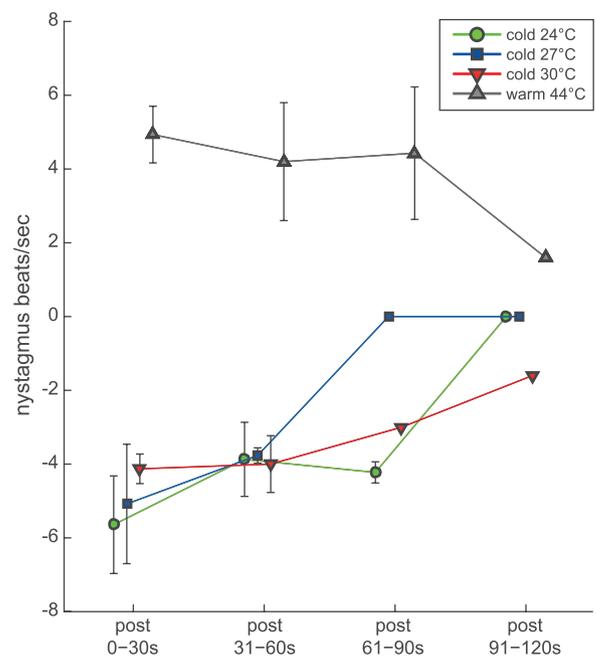

Fig. 1. Nystagmus frequency after caloric irrigation (earth-vertical orientation of the lateral semicircular canal; 7 subjects; mean nystagmus frequency ±1 SD) during video-head-impulse testing. See inset for definition of symbols used.
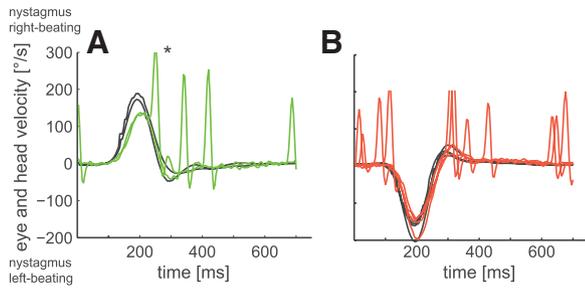
Fig. 2. Head impulses 0–30 sec after cold (24°C) water irrigation of the left ear in a typical subject (#6), inducing a right-beating caloric nystagmus. For illustrative purposes of the relation between the beating direction of the caloric nystagmus (to the right) and the rVOR response, we mirrored both eye and head-velocity traces in B. Eye movements to the right were positive, and eye movements to the left were negative. Note that head-velocity traces in A and B were mirrored to allow direct comparison with the eye-velocity traces and that traces with quick phases during the critical period used for gain calculation were excluded. A, Head impulses to the left (in black) triggering an eye movement (rVOR, in green) to the right with decreased gain (compared with the head-velocity trace). *A quick phase to the right during the descending portion of the rVOR response that was likely a compensatory saccade. B, Head impulses to the right (in black), eliciting an rVOR response to the left (in red, i.e., with a negative sign) that did not show any decrease in gain or compensatory saccades. The right-beating fast phases depicted in B are consistent with caloric nystagmus beats. rVOR indicates rotational vestibulo-ocular reflex.

least two nystagmus beats could be identified unanimously. Recall that testing was done in the light; so fixation of visual targets likely suppressed the nystagmus slow phases and the beat frequency.

Head-Impulse Gain Measurements

The head-impulse gain was calculated off-line with a custom algorithm implemented in Matlab (The MathWorks, Natick, MA). Due to the experimental paradigm, eye-velocity traces were prone to two distinct biases. First, caloric irrigation produces nystagmus and offsets in baseline eye velocity before the head-impulse test were recorded. Second, nystagmus beats (saccades) during the head impulses complicates gain calculation. To overcome the first bias, we removed any eye-velocity offsets present in the period before the head impulse before gain calculation. To overcome the latter bias, velocity gain was calculated only during the ascending part of the response (i.e., before the expected onset of any compensatory saccades), over a period of 52 msec, while traces with quick phases during the critical period used for gain calculation were excluded. For our special experimental conditions, this traditional velocity gain calculation was preferred over the cumulative position algorithm (Macdougall et al. 2013) provided by the manufacturer of the ICS Impulse® Goggles. Although this method is more prone to goggle-slip artifacts around peak head acceleration, these artifacts are likely to be constant across all conditions, so that they cannot explain differences between blocks. Furthermore, it emphasizes the higher frequency response compared with the standard cumulative position algorithm provided by the manufacturer. Noteworthy, this approach is also more sensitive to lags in eye velocity relative to head velocity encountered in some traces, resulting in overall lower gains than compared with the approach provided by the manufacturer.

Statistical analysis was carried out using Graphpad Software. Average gain values at different times after irrigation were compared using nonparametric, one-way analysis of variance (Friedman test, with Dunn's multiple comparison post-test).

RESULTS

Caloric Nystagmus

With the plane of the lateral SCCs positioned earth-vertical (the subject's head inclined by 30° from supine), we first confirmed the presence of caloric nystagmus evoked by irrigation of the left ear. For all subjects, Figure 1 shows the frequency and direction of nystagmus as a function of time after the end of the irrigations (average ±1 SD, bins of 30-sec duration). Water temperature above 37°C (44°C) led to caloric nystagmus in the direction of the irrigated ear (positive; slow phases: contralateral to irrigation). Thus warm-water irrigation corresponds to a long-lasting (or low-frequency) excitation of the lateral SCC on the ipsilateral side. Accordingly, water temperature below 37°C (30, 27, and 24°C) led to caloric nystagmus in the contralateral direction (negative; slow phases: ipsilateral to irrigation). Over time, nystagmus frequency decreased, but was still present in most conditions 90 sec after the end of irrigation. No caloric nystagmus was elicited when ear irrigations were performed with the plane of the lateral SCC oriented earth-horizontal (the subject's head inclined by 30° from upright; data not shown).

Figure 2 shows eye-velocity traces for a typical subject during head impulses to the left (Fig. 2A) and to the right (Fig. 2B) after the caloric irrigation of the left ear. Note that the head-velocity traces are mirrored for comparison with the rVOR. For cold-water (24°C) caloric irrigation of the left ear, irrigation-related nystagmus quick phases will beat to the contralateral side (i.e., rightwards). When applying head impulses to the left, the rVOR response and the irrigation-related nystagmus will point in the same (rightward) direction (Fig. 2A), whereas for head impulses to the right, the leftward rVOR response and the (rightward) irrigation-related nystagmus will point into opposite directions (Fig. 2B). Note that in Figure 2, rightward eye movements are by definition always with a positive sign.

Head Impulses

Gains of the rVOR elicited by head impulses were recorded before (baseline) and after ear irrigation (during caloric nystagmus). Baseline rVOR gains for head turns to the left were somewhat smaller than those to the right (Fig. 3). Most likely, this is related to the fact that the rVOR was always recorded from the right eye, and traditional velocity gains show higher values with head impulses in the direction of the eye that is recorded (Weber et al. 2008).

Figure 3 summarizes the results in all subjects. Cold-water irrigation (24°C) significantly decreased the rVOR gain of ipsilateral head-impulse tests during the second time period (31 to 60 sec after the end of irrigation; $p < 0.001$, one-way nonparametric analysis of variance; Fig. 3A). Thus, low-frequency inhibition of the lateral SCC by cold-water irrigation significantly reduced the velocity of high-frequency excitation of the same canal by ipsilateral head impulses (i.e., head impulses to the left). The rVOR gain of contralateral (i.e., rightward) head impulses, however, was not significantly influenced by cold-water irrigation (Fig. 3B). Likewise, rVOR gains of head-impulse tests to
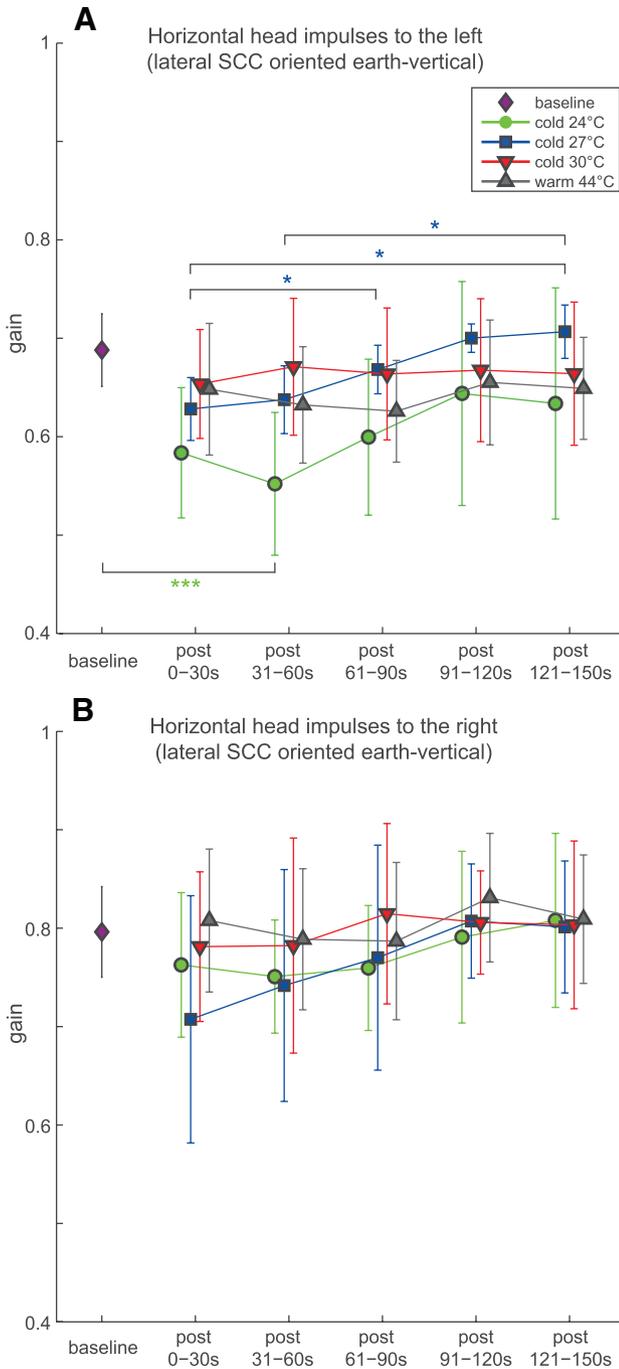
Fig. 3. rVOR gains (n = 7, mean ± 1 SD) for head impulses to the left (A) and to the right (B) after cold- and warm-water caloric irrigation of the left ear with the head inclined by 30° from supine (i.e., the lateral SCCs are oriented earth-vertical) over periods of 30 sec each. Results for the different temperatures are shown separately (see inset for explanation of symbols). To improve visualization, results for the different conditions are slightly shifted along the x axis. Significant differences are marked by brackets and stars (***p < 0.001; *p < 0.05), using the same color coding as for the different measurements after caloric irrigation, that is, green for 24° cold-water caloric irrigation and blue for 27° cold-water caloric irrigation. Nonparametric one-way ANOVA showed no significant differences between other parameters. Baseline measurements before caloric irrigation: purple diamond. ANOVA, analysis of variance; rVOR, rotational vestibulo-ocular reflex; SCC, six semicircular canal.
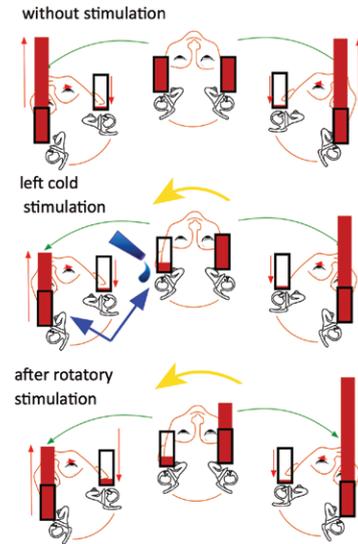

Fig. 4. Hypothetical effect of cold-water caloric irrigation (24°C) of the left ear during head impulses. Red arrows represent the magnitude of changes in overall vestibular-afferent activity, that is, nonlinear and linear pathway activity, during head impulses. Changes are identical in all paradigms. Upper panel, Without caloric irrigation. Columns represent the afferent discharge (spontaneous afferent activity approximately 90/s). Head impulses to the right and left: ipsilateral afferent activity increases, the contralateral afferent discharge decreases. Middle panel, During and after left cold caloric irrigation (blue arrows), the spontaneous afferent discharge rate decreases and the increase of the activity during ipsilateral head impulse elicits less asymmetric activity between both sides than without caloric stimulation; yellow arrow: slow phase direction of caloric nystagmus. Lower panel, When stopped after sustained rotation to the left (counter-clockwise; Mantokoudis et al. 2016), the endolymph movements elicit a change in the spontaneous activity as if the head would accelerate to the right (clockwise) and post rotatory nystagmus appears with a slow phase to the left (yellow arrow). In this case, slow phases of the post rotatory nystagmus add or subtract to the slow-phase eye velocity during the head impulses. The difference between caloric nystagmus and post rotatory nystagmus appears during the head impulse to the right: after caloric stimulation, there is no enhancement of the gain to the right, while there is an increase of the rVOR gain to the right after sustained rotation. rVOR, rotational vestibulo-ocular reflex.

either side were not affected by ipsilateral warm-water (44°C) caloric stimulation.

Control experiments with head-impulse tests applied to both sides after warm- and cold-water caloric stimulation with the plane of lateral SCC-oriented earth horizontal (the subject's head inclined 30° from supine) did not reveal changes of rVOR gains compared with the baseline (data not shown).

DISCUSSION

The main finding of this study was that a strong cold-water caloric stimulus (24°C) had a significant inhibitory effect on the ipsilateral horizontal high-acceleration rVOR gain, assessed with the head-impulse test. Water temperatures 7°C warmer or cooler than body temperature (44 and 30°C) were not strong enough to significantly influence the horizontal head-impulse rVOR gain, although they were effective enough to elicit a tonic vestibular asymmetry and horizontal nystagmus.

Strong cold-water caloric irrigation offers a promising method to study the effects of at least partial acute unilateral

vestibular loss in healthy human subjects. According to the model developed by Minor and Lasker (2009), vestibular signals encoding angular head rotations are transmitted to the vestibular nuclei along two parallel afferent streams. The linear pathways from both sides contribute equally during ipsilateral and contralateral rotations, even during high accelerations. In other words, a unilateral deficit of the linear pathways would lead to the same rVOR gain during head impulses on both sides. The lower gain on the affected side in case of unilateral peripheral vestibular loss is caused by a deficit of the nonlinear pathway that normally enhances the rVOR mainly during ipsilateral horizontal head impulses. Thus, in the model of Minor and Lasker, Ewald's second law only applies for high head accelerations and is implemented by the nonlinear pathway that is driven mainly by excitatory, that is, ipsilateral SCC input.

An alternative mechanism may be postulated based on findings that vestibular afferents change their sensitivity with temperature. Young and Anderson (1974) found that the sensitivity of regular afferents is positively correlated with temperature. Thus, cold-water irrigation could decrease the sensitivity of these neurons, resulting in decreased gain for ipsilateral

movements. Park et al. (2010) reported that irregular vestibular afferents show increased sensitivity with body-core temperature increases, from which we could likewise predict decreased sensitivity with cold stimulation. Note, however, that such a mechanism would also predict symmetrical changes in gain (increased ipsilateral gain with warm stimulation) in an earth-vertical position together with gain changes after caloric stimulation in an earth-horizontal position, which we did not observe.

A similar experiment with rotatory stimuli was recently conducted by Mantokoudis et al. (2016). They found that moderate degrees of post rotatory nystagmus (up to 30°/s) did not alter the rVOR gain of horizontal head impulses with high peak velocities. With lower peak-velocity head impulses and higher velocities of post rotatory nystagmus (higher than 30°/s), however, the rVOR gains increased or decreased as predicted by summation of the velocity of the post rotatory nystagmus and the velocity of the rVOR elicited by the head impulses. These results of Mantokoudis et al. are, in some respect, similar to ours: With caloric stimulation of moderate temperature difference (7°C warmer or cooler than body temperature), rVOR gains of horizontal head impulses in either direction did not change compared with
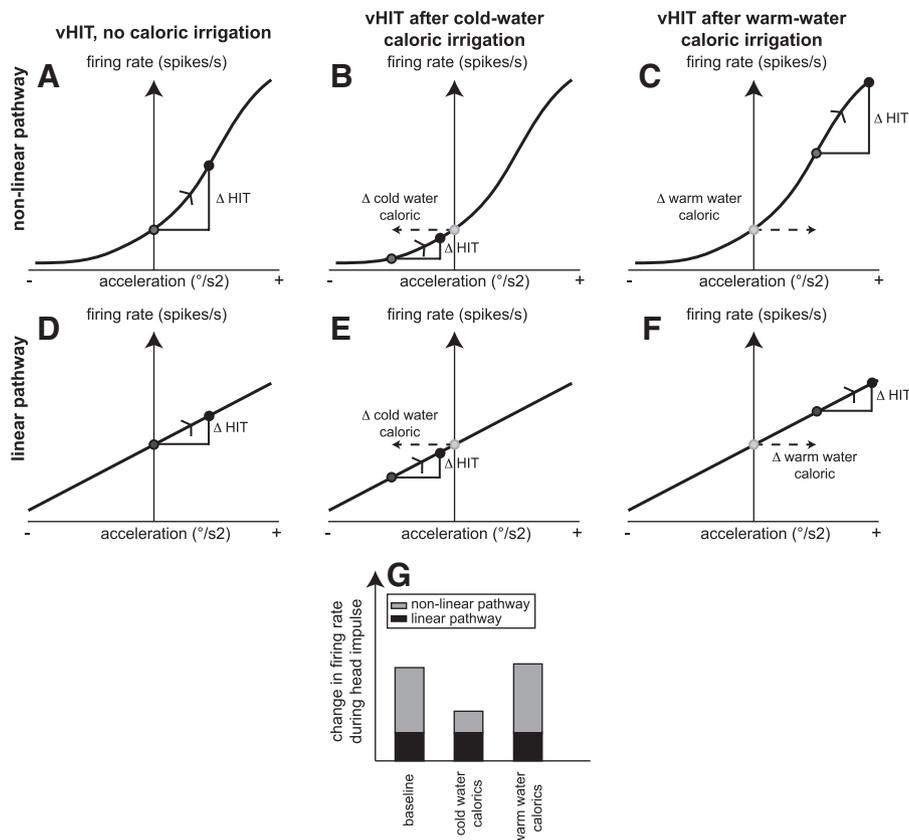

Fig. 5. Schematic relationship between changes in head acceleration (*x* axis) and subsequent changes in the firing rate of the vestibular afferents (*y* axis) during head impulses. The firing rate at zero head acceleration is referred to as the "set point." Regular afferents (bottom row) and irregular afferents (top row) are shown separately. Note that the relationship between firing rate and head acceleration is linear for the regular vestibular afferents (i.e., the linear pathway), while it is nonlinear for the irregular vestibular afferents (i.e., the nonlinear pathway). The baseline condition (no caloric irrigation before the head impulse, A and D) is compared with an inhibitory condition (cold-water caloric irrigation before the head impulse, B and E) and an excitatory condition (warm-water caloric irrigation before the head impulse, C and F). Cold-water caloric irrigation ("inhibitory condition") shifts the set point (light grey circle) to the left (as indicated by the dashed arrow), while warm-water caloric irrigation ("excitatory condition") results in a shift to the right (as indicated by the dashed arrow). When applying a head impulse, the acceleration results in an increase in firing rate (Δ discharge rate). Due to the linear relationship, Δ discharge rate is independent from the initial firing rate. This, however, is not the case for the irregular afferents: when applying cold-water caloric irrigation, the firing rate is shifted to an area where the slope of the curve is less steep. For warm-water caloric irrigation, the firing rate is shifted to an area where the slope of the curve is almost linear. Resulting overall changes in firing are shown in G.

baseline. With strong caloric stimulation, which was obviously only possible with below-body water temperature (24°C), we also found an effect on rVOR gains of horizontal head impulses. Because there was no effect of caloric nystagmus when irrigating the lateral SCC in earth-horizontal position and no changes of rVOR gains for horizontal head impulses, we conclude that the present and absent effects on the rVOR gains in vertical position are related to the degree of ampullofugal deflection of the cupula due to thermal endolymph pressure and not by direct cooling of the ampulla and its afferents (Valli et al. 2002).

In the experiments of Mantokoudis et al. (2016), strong post rotatory nystagmus (nystagmus slow-phase velocity >30°/sec) increased the rVOR gain of horizontal head impulses when both nystagmus slow-phases and head-impulse rVOR pointed in the same direction and decreased when they were directed oppositely. In our study, only the strong cold caloric stimulus (24°C) had a significant decreasing effect on the rVOR gain of ipsilateral (i.e., leftward) horizontal head impulses, that is, when slow phases of caloric nystagmus and head-impulse VOR pointed in opposite directions, but there was no change of rVOR gain during contralateral (i.e., rightward) head impulses, as illustrated in Figure 4.

The fundamental difference between the experiments of Mantokoudis et al. (2016) and those reported in this study is the nature of the underlying vestibular signal. While post rotatory nystagmus is a post deceleration velocity signal in the linear pathways coming from the SCCs of both labyrinths and is then partly integrated by the velocity-storage mechanism, caloric nystagmus is elicited by an ongoing acceleration signal driven by cupular deflection in the lateral SCC only on the side of the irrigation. The acceleration signal of a strong cold-water caloric stimulus could therefore drive the irregularly firing afferents in an area where they are relatively insensitive to high acceleration. These afferents are asymmetric in nature because they show large excitatory responses, but only small inhibitory responses (Minor & Lasker 2009). They constitute the nonlinear pathway of the rVOR and implement Ewald's second law for high head accelerations. As a result of being driven into a zone less sensitive to acceleration, nonlinear neurons are prevented from effectively firing during ipsilateral rapid head rotations. We hypothesize that this is the cause for the diminished rVOR gains in the ipsilateral horizontal direction. These distinct effects of caloric irrigation on the linear and the nonlinear pathway when applying head impulses are explained in Figure 5. While, for the linear pathway, the change in the vestibular-afferent firing rate caused by an ipsilateral head impulse immediately after caloric irrigation will be independent from the initial firing rate (i.e., the "set point"), the change in firing rate in the nonlinear pathway depends critically on the initial discharge rate due to the nonlinear relationship between acceleration and neuronal discharge. The overall change in firing rate will therefore be lower for head impulses applied after cold-water caloric irrigation than after warm-water caloric irrigation, explaining the lower vHIT gain (Fig. 5G).

In summary, our findings emphasize the importance of the nonlinear pathway when probing the vestibular organs with high-acceleration stimuli. Combining caloric irrigation and head-impulses may therefore provide helpful for further studies as a model of transient impairment of the rotational vestibulo-ocular reflex.

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