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Research Article

Reevaluating Order Effects in the Binaural Bithermal Caloric Test

Elizabeth Burnette,[a] Erin G. Piker,[a,b] and Dennis Frank-Ito[a]

Purpose: The purpose of this study was to determine whether a significant order effect exists in the binaural bithermal caloric test.
Method: Fifteen volunteers (mean age = 24.3 years, range = 18–38 years) with no history of vestibular disorder, hearing loss, concussion, or neurological disease underwent caloric testing on 3 occasions. Irrigations were randomized using 8 possible order combinations. The parameters of interest included unilateral weakness, directional preponderance, total response from the right ear, and total response from the left ear.
Results: Order effects were analyzed using 2 methods. The first analysis was done looking at the 8 possible orders. We also had an a priori established hypothesis that the first irrigation tested would influence the calculation of unilateral weakness more than the other 3 irrigations. To test this hypothesis, the 8 orders were condensed into 4 order conditions based on the first irrigation. The effect of order was determined using analysis of variance tests. Although the first irrigation tended to be the largest, no significant effects were observed.
Conclusions: This experiment demonstrated that while there is great inter-individual and intra-individual variability in caloric test results, the order of irrigations had no significant effect in the test. Future studies may explore the effects of nonphysiological factors on test results.

The binaural bithermal caloric test (caloric test) is one of the most widely used methods to study vestibular function and to diagnose vestibular disorders. During caloric testing, cool and warm water or air stimuli are delivered to the external ear canal in order to radiate a change in the temperature gradient of the horizontal semicircular canal of the inner ear. This temperature change induces a nystagmus response in the eyes called the vestibulo-ocular reflex (VOR). The VOR is observed and measured by videonystagmography (VNG), and the slow phase velocity (SPV) of the VOR is used to assess vestibular function (Anderson, 1995).

Clinically, a caloric test involves four irrigation conditions named based on the irrigated ear and temperature of the water: right ear, warm temperature (RW); left ear, warm temperature (LW); right ear, cool temperature (RC); and left ear, cool temperature (LC). The SPV from each of the four caloric irrigations will be measured and used to calculate unilateral weakness (UW), necessary for determining if there is an asymmetry in the peripheral vestibular system function, at the level of the horizontal semicircular canal between the right and the left ear. Typically, UW is calculated using Jongkees' formula, that is, the relative vestibular reduction is equal to the difference in the two ears' responses divided by the total response (TR): [(RW + RC) − (LW + LC)] / TR, where TR = [RW + RC + LW + LC] (Furman & Jacob, 1993). A UW value of zero indicates that responses from the right and left ears are equal. A positive UW value indicates a relative weakness in the left ear, and a negative value indicates a relative weakness in the right ear. There is a wide range of acceptable UW values that are considered normal; thus, a UW value above ± 25% is the commonly used threshold for clinical diagnosis of a vestibular impairment on one side (Barin, 2016). However, the use of Jongkees' formula assumes that there is no systematic difference across the four test conditions. Thus, there should be no order of test condition effects, such as temperature effects (i.e., warm or cool) and/or right/left ear effects.

When referring to an "order effect," we mean the order in which the four test conditions (i.e., RW, LW, RC, and LC) are administered may affect the calculated UW. For example, if the first administered condition always produced the largest nystagmus, this could bias the UW calculation, leading to a patient being wrongfully diagnosed (or undiagnosed) depending on which condition was administered first. Most commercial VNG equipment defaults to a particular test order. For example, the software used

[a]Duke University, Durham, NC
[b]James Madison University, Harrisonburg, VA
Correspondence to Elizabeth Burnette: e.burnette@duke.edu

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in our clinical laboratory defaults to an RW condition first, and this is typically the first condition administered to patients. In addition, many clinicians may choose to first irrigate the ear believed to be the impaired side. Thus, the order of caloric irrigations is not routinely randomized, and it is not known if we are biasing the caloric response by starting with a particular condition.

A review of the literature shows that there is no consensus regarding whether or not an order effect in response to binaural bithermal caloric stimulation exists. Several studies have attempted to show an order effect with relation to the order of temperature (i.e., a temperature effect). It has been shown that the magnitude of nystagmus emanating from warm water irrigations is consistently larger than the magnitude from cool irrigations (Zapala, Olsholt, & Lundy, 2008). However, the bithermal caloric test is still able to distinguish between normal and abnormal ears. Additional investigators examined whether administering the warm caloric irrigations first and the cool irrigations last biased the response. Results are not conclusive. Olóriz et al. (2007) reported a temperature order effect and presented findings that showed testing that began with warm irrigations, regardless of ear, followed by cool irrigations produced more pathological results (i.e., UW greater than 24% for their study protocol). Conversely, Noaksson, Schulin, Kovacsovics, and Ledin (1998) found no effect of temperature order on a sample of 118 patients. However, it should be noted that both of these studies were completed on dizzy patients, who arguably have a greater likelihood of presenting with an abnormal UW compared to normal controls and, thus, may not be the most valid group in which to assess temperature order effects of the caloric test. As with temperature effects, the literature is unclear whether a definite right/left irrigation order effect exists. One of the first investigations to examine the order of caloric irrigations was conducted by Ford and Stockwell (1978). They concluded that there was "response habituation" in the caloric response, which means that the first response was the biggest and subsequent irrigations would result in smaller responses. Similarly, Furman and Jacob (1993) also observed response habituation and concluded that this phenomenon would bias the calculation of UW. They went on to recommend a correction factor be used to prevent such a bias. Conversely, Lightfoot (2004) did not observe any response habituation and concluded that there was no order effect on the caloric response. It should be noted that Lightfoot (2004) utilized both electronystagmography (ENG) and VNG technology simultaneously to confirm the contribution of a testing technique to a possible order effect, whereas Ford and Stockwell (1978) and Furman and Jacob (1993) used ENG only. It is quite possible that ENG, which is dependent upon the corneo-retinal potential (CRP), may produce an order effect due to calibration. The findings from Lightfoot (2004), showing the absence of an order effect when VNG is used, have not been replicated.

Given the aforementioned conflicting results regarding order effects on caloric irrigation response, the purpose of this study is to determine in healthy volunteer participants whether an order effect can be observed on calculated UW, the right ear total response (RT; right warm condition + right cool condition), the left ear total response (LT; left warm condition + left cool condition), and the directional preponderance (DP) of the observed nystagmus. Based on the findings from Lightfoot (2004), we hypothesized that there would be no significant order effects when using VNG. We also had an a priori established hypothesis that the first irrigation tested, regardless of temperature or ear, would influence the calculation of UW more than the other three irrigations.

Method

Subjects

Fifteen healthy volunteers were recruited (10 women and five men; mean age = 24.3 years, age range = 18–38 years) after the Duke University Health System Institutional Review Board approval. Inclusion criteria for participation required no history of vestibular disorders, hearing loss, concussion, or neurological disease. In addition, all subjects were confirmed to have no occluding cerumen in the external auditory canals and intact tympanic membranes. Every subject underwent binaural bithermal caloric irrigations on three separate occasions, with at least 24 hr between test sessions. Eight different orders of caloric irrigations were included (see Table 1), and the orders were randomized within subjects using a modified balance incomplete Latin square design.

Subjects were tested while lying in the "standard caloric test position" (i.e., supine with the head elevated by 30°, Ford & Stockwell, 1978) and wearing goggles that both denied vision and tracked eye movement via VNG. All subjects were calibrated before testing began. The stimuli consisted of 250 mL of water irrigated into either the right or the left ear canal at either 30 °C (i.e., cool irrigation) or 44 °C (i.e., warm irrigation) for a period of 30 s. The subjects' eye movements were recorded during the irrigation and for an additional 2 min following the irrigation. While recording, subjects were tasked with simple word games to ensure an alert state.

Caloric Irrigation

All caloric irrigations were performed using an open-loop water irrigation device (Aqua Stim Water Caloric Irrigator, Micromedical Technologies, Chatham, IL), and eye movements were recorded using VNG goggles (I-Portal VOG 100-Hz Eye Tracker, Neurokinetics, Inc., Pittsburg, PA) and analyzed using commercial software (I-Portal VEST VNG Software, Neurokinetics, Inc., Pittsburg, PA).

All irrigation orders alternated between the right and left ears, but the temperatures and starting ear were randomized using a modified balance incomplete Latin square design. Previous studies tended to only examine order effects in "Cool, Cool, Warm, Warm" (CCWW) versus "Warm, Warm, Cool, Cool" (WWCC) orders (Furman & Jacob, 1993; Lightfoot, 2004; Noaksson et al., 1998; Olóriz et al.,

Table 1. Order name, corresponding order, average unilateral weakness (UW%), right total (RT), left total (LT), and directional preponderance (DP%) as well as their standard deviations (*SD*) for each of the eight order conditions.

Order name and corresponding order[a]	*N*	Mean UW% (*SD*)	RT (*SD*)	LT (*SD*)	Mean DP% (*SD*)
1: RW, LW, RC, LC	6	0.2 (9.8)	34.2 (11.2)	33.7 (11.6)	8.5 (19.5)
2: LW, RC, LC, RW	5	−8.2 (11.6)	22.9 (8.5)	26.2 (7.3)	−8.3 (21.4)
3: RC, LC, RW, LW	5	0.5 (10.8)	34.3 (4.6)	34.2 (6.8)	−5.6 (8.6)
4: LC, RW, LW, RC	6	9.7 (14.2)	32.0 (18.1)	27.8 (21.2)	−11.0 (8.3)
5: RW, LC, RC, LW	5	8.9 (10.0)	28.3 (4.2)	23.6 (3.8)	−5.8 (14.0)
6: LW, RW, LC, RC	5	0.0 (9.8)	30.8 (12.0)	30.2 (9.5)	−6.8 (13.9)
7: RC, LW, RW, LC	5	5.7 (9.5)	28.3 (6.5)	26.1 (9.2)	−7.4 (12.3)
8: LC, RC, LW, RW	5	7.3 (4.2)	32.6 (7.1)	28.6 (8.5)	−5.7 (9.6)
ALL	42	3.2 (10.6)	30.5 (9.5)	28.8 (10.2)	−5.0 (13.9)

Note. RW = right ear, warm temperature; LW = left ear, warm temperature; RC = right ear, cool temperature; LC = left ear, cool temperature.
[a]Orders were randomized by temperature and side, although all orders alternate between the right and left ears.

2007). The current investigation expands on other studies' observations by testing CWCW and WCWC orders of administration as well as the previously tested CCWW and WWCC orders. Thus, the experimental design is balanced for order, ear, and temperature sequences. Participants were given a minimum of 2 min between irrigations, longer if they felt symptomatic.

The caloric parameters of interest include UW, DP, total response from the right ear (i.e., RW + RC = right total; RT), and total response from the left ear (i.e., LW + LC = left total; LT). The UW quantifies the difference between the caloric responses from the right ear (i.e., RT) and the left ear (i.e., LT) and was calculated as follows:

$$\text{UW\%} = (RT - LT)/(RT + LT) * 100. \quad (1)$$

Thus, a negative number indicated the UW was to the right ear. The DP of the nystagmus quantifies the difference between right-beating (i.e., RW and LC conditions) and left-beating (i.e., LW and RC conditions) caloric responses and was calculated as follows:

$$\text{DP\%} = (RW + LC) - (LW + RC)/(RW + LW + RC + LC) * 100. \quad (2)$$

Statistical Analysis

Statistical analyses were completed using SPSS Statistics 24 software (SPSS Inc., Chicago, IL). An initial statistical analysis was completed using a repeated-measures analysis of variance to evaluate the effect of a test session on the four caloric parameters of interest (UW, DP, RT, and LT). The independent variables were the three separate test sessions (three levels), and the dependent variables were the four caloric parameters. The results indicated no significant main effect of the test session on the caloric UW ($F = 0.898$, $df = 2$, $p = .414$), the RT ($F = 0.228$, $df = 2$, $p = .801$), the LT ($F = 0.035$, $df = 2$, $p = .964$), or the DP ($F = 0.104$, $df = 2$, $p = .902$). The results were then collapsed across test sessions for all further statistical analysis.

Order effects were analyzed using two different methods. The first analysis was done looking at the eight possible orders (see Table 1). We also had an a priori established hypothesis that the first irrigation tested would have a larger influence on the calculation of UW than the other three irrigations. To test this hypothesis, the eight orders were condensed into four order conditions in which either the RW, the LW, the RC, or the LC irrigation was performed first. Thus, in the second analysis, two separate orders existed within each of the four order conditions, but the two orders both had the same first irrigation and were thus combined based on the starting condition (see Table 2). The effect of order on the caloric UW, RT, LT, and DP was determined using a series of analysis of variance.

Results

The average UW, RT, LT, and DP for each of the eight possible orders is summarized in Table 1. The largest UW was observed with order 4 (9.7%) and the smallest with order 6 (.01%). However, results were not significant, indicating no statistically significant main effect of order on UW ($F = 1.532$, $df = 7$, $p = .196$), RT ($F = 0.647$, $df = 7$, $p = .714$), LT ($F = 0.575$, $df = 7$, $p = .770$), or DP ($F = 0.817$, $df = 7$, $p = .581$). The data are depicted in Figure 1. Although results were not significant, the variation between conditions can be seen in Figure 1.

The average UW, RT, LT, and DP for each of the four first-order conditions is summarized in Table 2. The UW value was a negative number, indicating that the vestibular response was weaker in the right ear, only when the LW test condition was first indicating a possible bias. We wanted to test the hypothesis that the first irrigation was significantly larger than the subsequent irrigations. Figure 2 shows the mean SPV for each irrigation for each of the first orders. Figure 2 illustrates that, on average, when the RW and LW irrigations were the first to be administered, they resulted in the largest SPV of that session. The same effect was not observed for the RC and LC. However, the SPV only differed by a few degrees between conditions and would not be considered clinically significant. Furthermore,

Table 2. Order name and corresponding order for the second analysis, which combined orders from the first analysis that had the same starting condition resulting in four possible orders, the average unilateral weakness (UW%), right total (RT), left total (LT), and directional preponderance (DP%) as well as their standard deviations (*SD*) for the first four order conditions.

Order name and corresponding order	*N*	Mean UW% (*SD*)	RT (*SD*)	LT (*SD*)	Mean DP% (*SD*)
1 RW first: (RW, LW, RC, LC) and (RW, LC, RC, LW)	11	4.5 (10.4)	31.2 (8.5)	28.7 (9.7)	1.3 (17.7)
2 LW first: (LW, RC, LC, RW) and (LW, RW, LC, RC)	10	−3.6 (10.8)	27.2 (10.7)	28.4 (8.4)	−7.5 (16.4)
3 RC first: (RC, LC, RW, LW) and (RC, LW, RW, LC)	10	3.4 (9.8)	30.9 (6.3)	29.8 (8.9)	−6.6 (10.2)
4 LC first: (LC, RW, LW, RC) and (LC, RC, LW, RW)	11	8.4 (9.3)	32.3 (12.1)	28.2 (14.3)	−8.1 (8.9)
ALL	42	3.2 (10.6)	30.4 (9.5)	28.7 (10.1)	−5.0 (13.9)

Note. RW = right ear, warm temperature; LW = left ear, warm temperature; RC = right ear, cool temperature; LC = left ear, cool temperature.

although the RW and LW were the largest when they were the first administered, the effect of the first irrigation order on the caloric response parameters was not statistically significant. Results indicated no significant effect of order on UW ($F = 2.235$, $df = 3$, $p = .103$), RT ($F = 6.468$, $df = 3$, $p = .707$), LT ($F = 0.037$, $df = 3$, $p = .799$), or DP ($F = 0.966$, $df = 3$, $p = .420$). The data are depicted in Figure 3.

Discussion

The results from the current investigation showed that when using VNG, there are no significant effects of irrigation order on the caloric response. Specifically, the UW, RT, LT, and DP are not significantly affected by the order in which the caloric irrigations are performed. There is great variability in the caloric response parameters, but it does not appear to be due to order effects. Although the RW and LW tend to be larger when administered first, the difference in SPV between irrigations is minimal and not statistically or clinically significant. In other words, when the right ear is irrigated first, we would not expect a bias for diagnosing left-sided vestibular hypofunction, and vice versa.

These results suggest that the order of irrigation during binaural bithermal caloric testing does not play a clinically significant role or significantly affect the resulting UW, RT, LT, or DP of the caloric response. Thus, the order of caloric irrigation should not result in a bias for the diagnosis of vestibular hypofunction. Furthermore, by utilizing multiple different order combinations, we were also able

Figure 1. Series of box-and-whisker plots showing unilateral weakness (UW%; Figure 1A), right total (RT; Figure 1B), left total (LT; Figure 1C), and directional preponderance (DP%; Figure 1D) as a function of each of the eight order conditions. Each of the eight conditions is specified on the *x*-axis. The upper and lower boundaries of each box represent the 25th and the 75th percentile, respectively, and the whiskers connect the highest and lowest points above or below the box. The line in the middle of the box represents the median for that condition, and the line going through the middle of the graph indicates the mean from all order conditions, allowing you to visualize whether the median for that order was above or below the total mean. In addition, individual dots outside the box indicate outliers that are > 2 box lengths above or below the 75th or the 25th percentile.

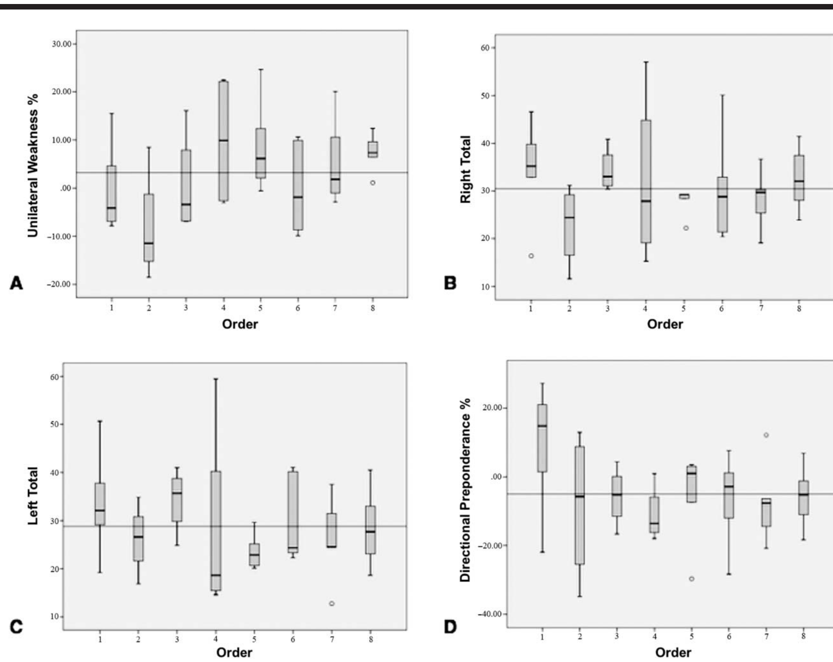
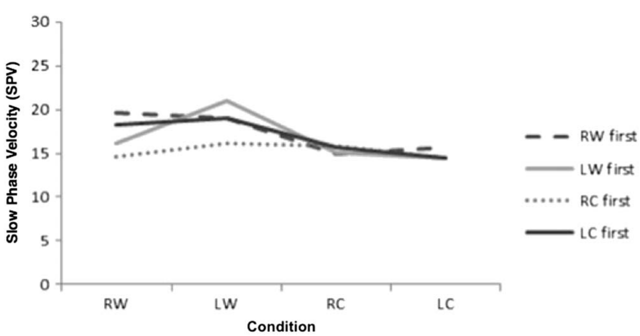


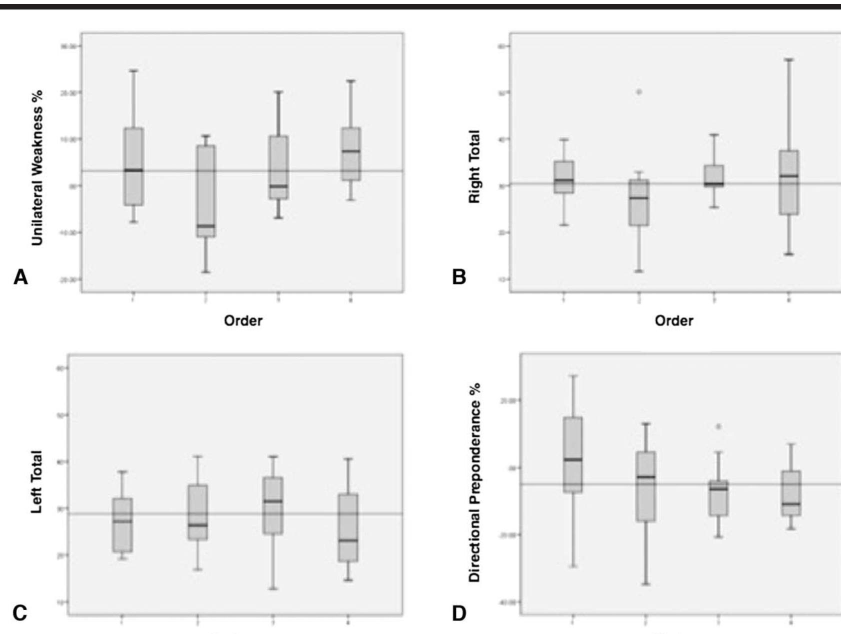
Figure 2. The mean slow phase velocity (SPV) for each irrigation for each of the first orders. On average, when the right warm (RW) and left warm (LW) irrigations were the first to be administered, they resulted in the largest SPV of that session. RC = right ear, cool temperature; LC = left ear, cool temperature.



to systematically show that there was no systematic difference in the responsiveness of one ear compared to the other (i.e., no right/left effect) and no consistent difference between the response to warm and cold stimuli (i.e., no temperature effect).

In contrast to our findings, the early work from Ford and Stockwell (1978) showed the presence of response habituation in which the observed nystagmus intensity declined over time, suggesting the possibility of an order effect. Furman and Jacob (1993) directly assessed the possibility of an order effect by examining the influence of the right/left order in which the caloric stimuli were delivered. As with Ford and Stockwell (1978), they also observed a consistent declining trend in the response from the first caloric to the fourth caloric. More importantly, the reduced caloric response across administrations resulted in a significant bias in the calculation of the UW. Specifically, Furman and Jacob (1993) showed a bias toward right-sided UW being diagnosed 2.4 times more often than a left-sided UW when the order of irrigation was left cool, right cool, left warm, and right warm. They suggested including a correction factor in Jongkees' formula in which they subtracted the average UW for the laboratory from each patient's score. However, it is important to take note that both of these investigations measured the SPV of the VOR using ENG, which requires the use of electrodes to measure the CRP of the eye. We know that the magnitude of the CRP, and subsequently the magnitude of the measured caloric response, is affected by nonpathologic variables including ambient room light and that the CRP requires frequent calibration to ensure the signal is not reduced (Jacobson & Newman, 1993). In contrast, more modern eye recordings utilize VNG to measure the SPV of the nystagmus, a technique that does not require frequent calibrations. Lightfoot (2004) performed simultaneous ENG along with VNG and did not observe a significant order effect with VNG. Instead,

Figure 3. Series of box-and-whisker plots showing unilateral weakness (UW%; Figure 3A), right total (RT; Figure 3B), left total (LT; Figure 3C), and directional preponderance (DP%; Figure 3D) as a function of the first four order conditions. Each of the four conditions is specified on the *x*-axis. The upper and lower boundaries of each box represent the 25th and the 75th percentile, respectively, and the whiskers connect the highest and lowest points above or below the box. The line in the middle of the box represents the median for that condition, and the line going through the middle of the graph indicates the mean from all order conditions, allowing you to visualize whether the median for that order was above or below the total mean. In addition, individual dots outside the box indicate outliers that are > 2 box lengths above or below the 75th or the 25th percentile.



he observed a significant change in the CRP across caloric irrigations that affected the ENG calibration, thus appearing as response habituation across caloric administrations. VNG is not affected by the CRP, and he found no evidence of a physiological adaptation of the caloric response using VNG.

The current results are in agreement with those presented by Lightfoot (2004) who observed little physiological habituation in the caloric response across irrigations when the VOR was measured using VNG. This study expands upon previous findings by including additional orders allowing for the systematic evaluation of order effects, temperature effects, and right/left effects.

Interestingly, Lightfoot (2004) asked participants to subjectively rate the perception of vertigo after each caloric irrigation. Although he did not observe an order effect on the SPV of the nystagmus, he did observe an order effect on the subjective assessment of vertigo. That is, subjects reported the greatest degree of vertigo during the first irrigation compared to the following three irrigations. This was the case for all orders and was not dependent upon which condition came first. In the current study, the RW and LW irrigations resulted in the largest SPV of that test session when they were the first condition. Although results were not statistically significant across orders, we did not ask participants whether their subjective perception of vertigo was greater in these conditions. It may be that other factors, such as anxiety, played a role in increasing the subjective response to the first irrigation. In some cases, this may increase the SPV and cause what appears to be a slight order effect that has nothing to do with physiological habituation. In the current study, participants were provided a detailed explanation regarding what the caloric test entailed and were told what the effects of the test would feel like in an effort to curb a markedly enhanced response to the first irrigation. Future research should focus on the effects of nonphysiological factors on the caloric response and the likelihood that such effects can bias the calculation of a UW.

There are several limitations to the current investigation. First, our sample size was relatively small. It is possible that the study was too underpowered to determine whether a true order effect existed. Second, we did not examine order effects in a patient population. We felt it was pertinent to first understand behavior in normal functioning subjects and to establish a baseline. It would be beneficial for future investigations to explore the limitations of this analysis and how it might hold in patients with peripheral or central disorders, anxiety, motion intolerance, atypical perception, etc., in order to determine how these results may be used clinically and within the general population. Specifically, future studies should focus on evaluating patients with known caloric asymmetries, as well as those with central integration issues such as migraines or traumatic brain injuries, to see if these results remain true in a disordered population.

In conclusion, the current investigation found no evidence of physiological adaptation or response habituation in the caloric response across test conditions. A correction factor does not seem necessary nor does it seem necessary to randomize caloric irrigations for clinical purposes. Of note, the current study demonstrated great inter-individual and intra-individual variability in caloric test results. This is not a novel finding. The caloric test is often considered the "gold standard" test for the assessment of the peripheral vestibular system, but responses between ears may differ between 20% and 30% before they are considered abnormal (Barin, 2016). The variability in the caloric response has been attributed to a number of factors including end organ function, temporal bone anatomy, patient state (e.g., anxiety) during testing, technical error (e.g., calibration), and nonphysiological factors (Davei, Harpur, & Jabeen, 1982; Proctor & Glackin, 1985). The results from the current investigation suggest that when conducting caloric testing using VNG, habituation of the response and subsequent order effects should not greatly contribute to such variability.

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