Book Chapter

Real-Time Multifrequency MR Elastography of the Human Brain Reveals Rapid Changes in Viscoelasticity in Response to the Valsalva Maneuver

Helge Herthum¹, Mehrgan Shahryari², Heiko Tzschätzsch², Felix Schrank², Carsten Warmuth², Steffen Görner², Stefan Hetzer³, Hennes Neubauer², Josef Pfeuffer⁴, Jürgen Braun¹ and Ingolf Sack²*

¹Institute of Medical Informatics, Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Germany
²Department of Radiology, Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Germany
³Berlin Center for Advanced Neuroimaging (BCAN), Germany
⁴Application Development, Siemens Healthcare GmbH, Germany

*Corresponding Author: Ingolf Sack, Department of Radiology, Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany

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**Abstract**

Modulation of cerebral blood flow and vascular compliance plays an important role in the regulation of intracranial pressure (ICP) and also influences the viscoelastic properties of brain tissue. Therefore, magnetic resonance elastography (MRE), the gold standard for measuring *in vivo* viscoelasticity of brain tissue, is potentially sensitive to cerebral autoregulation. In this study, we developed a multifrequency MMRE technique that provides serial maps of viscoelasticity at a frame rate of nearly 6 Hz without gating, i.e., in quasi-real time (rt-MMRE). This novel method was used to monitor rapid changes in the viscoelastic properties of the brains of 17 volunteers performing the Valsalva maneuver (VM). rt-MMRE continuously sampled externally induced vibrations comprising three frequencies of 30.03, 30.91, and 31.8 Hz were over 90 s using a steady-state, spiral-readout gradient-echo sequence. Data were processed by multifrequency dual elasto-visco (MDEV) inversion to generate maps of magnitude shear modulus $|G^*|$ (stiffness) and loss angle $\phi$ at a frame rate of 5.4 Hz. As controls, the volunteers were examined to study the effects of breath-hold following deep inspiration and breath-hold following expiration. We observed that $|G^*|$ increased while $\phi$ decreased due to VM and, less markedly, due to breath-hold in inspiration. Group mean VM values showed an early overshoot of $|G^*| 2.4 \pm 1.2$ s after the onset of the
maneuver with peak values of 6.7 ± 4.1% above baseline, followed by a continuous increase in stiffness during VM. A second overshoot of $|G^*|$ occurred 5.5 ± 2.0 s after the end of VM with peak values of 7.4 ± 2.8% above baseline, followed by 25-s sustained recovery until the end of image acquisition. $\phi$ was constantly reduced by approximately 2% during the entire VM without noticeable peak values. This is the first report of viscoelasticity changes in brain tissue induced by physiological maneuvers known to alter ICP and detected by clinically applicable rt-MMRE. Our results show that apnea and VM slightly alter brain properties toward a more rigid-solid behavior. Overshooting stiffening reactions seconds after onset and end of VM reveal rapid autoregulatory processes of brain tissue viscoelasticity.

**Keywords**

Real-Time Multifrequency MRE; Cerebral Autoregulation; Valsalva Maneuver; Stiffness; Viscoelasticity

**Introduction**

A balance of intracranial mechanical properties is of crucial importance for normal brain function [1-4]. Shear modulus and bulk modulus of brain tissue influence cerebrovascular compliance and pulsatility as well as intracranial pressure (ICP) [5-8]. While shear modulus can be measured non-invasively by magnetic resonance elastography (MRE) [9], there is currently no method for direct ICP measurement without an intervention or without making model assumptions [2]. In complex multiphasic mechanical systems such as the brain, shear modulus and pressure are linked through poroelastic interactions between the fluid and solid spaces [10-12]. Thus, it is likely that regulation of ICP, which is one of the most important vital functions of intracranial mechanics, also affects shear viscoelasticity [13-15]. However, this mechanical component of cerebral autoregulation is largely unstudied due to a lack of imaging techniques that can measure cerebral shear modulus *in vivo* with high spatial and temporal resolution.
In the past, cerebral MRE was used to study a wide variety of physiological effects or diseases which affect the *in vivo* shear modulus of brain tissue [16,17]. It has been shown that the brain becomes softer during normal aging [18,19] or pathophysiological processes such as neuroinflammation [20,21], demyelination [22], or neurodegeneration [23,24]. In patients, brain softening has been observed in a wide set of neuronal disorders including multiple sclerosis [25,26], Alzheimer’s disease [27-29], Parkinson’s disease [30,31] and normal pressure hydrocephalus [32-34]. Brain tumors can be either softer or stiffer than normal tissue [35-37], while malignant tumors have reduced viscosity [38-40]. A higher stiffness of neural tissue has been associated with increased perfusion pressure [41-44], ICP [45,46], formation of cytotoxic edema in dying animals [47,48], proliferation of neurons [49], neuronal activity [50,51], and brain maturation [52]. All of these studies have revealed that brain viscoelasticity can change within minutes (perfusion alterations), weeks (brain maturation in mice), or years (aging, disease progression). However, requiring several minutes of data acquisition, conventional MRE is limited in resolving non-periodic rapid processes such as cerebral autoregulation and ICP alterations which cannot be consistently repeated in volunteers.

Faster techniques including time-harmonic ultrasound elastography [53,54] and real-time MRE (rt-MRE) [55] have been introduced recently. While cerebral ultrasound elastography is limited by acoustic windows and cannot generate detailed maps, rt-MRE has the potential to map viscoelasticity with both high spatial resolution and high frame rates. However, feasibility of rt-MRE has as yet only been demonstrated with a small field of view in the lower extremities [55] and has never been tested in the brain.

Therefore, we here introduce real-time multifrequency MRE (rt-MMRE) for applications in the human brain. Multifrequency extension of rt-MRE was motivated by previous work on multifrequency wavefield inversion promising higher stability and consistency of parameter maps than single-frequency direct inversion [56,57]. Moreover, rt-MRE builds on continuous
stroboscopic sampling of harmonic vibrations [58], which can be spectrally decomposed into multifrequency vibrations without extra scan time. As such, rt-MMRE is a natural extension of rt-MRE that yields, at no extra cost, consistent viscoelasticity maps at relatively high frame rates in the order of 6 Hz depending on the repetition time (TR). Since rt-MRE does not require gating and provides multiple viscoelasticity maps per second, we consider this method as a real-time imaging technique.

Using rt-MMRE, we investigate rapid viscoelastic changes during cerebral autoregulation associated with the Valsalva maneuver (VM). The VM is a standard maneuver to voluntarily increase ICP by forceful breathing against the closed airway with abdominal muscle contraction at the same time. VM will be compared with normal breath-holds in inspiration (BH-in) and expiration (BH-ex). To address frequency dispersion and to test the overall consistency of the values measured in association with the VM, the experiment is repeated with a second set of drive frequencies.

Overall, this study has two aims: first, we introduce rt-MMRE based on three simultaneous excitation frequencies to acquire hundreds of viscoelasticity maps within less than 1 min of scan time. Second, we explore cerebral autoregulation with the unprecedentedly high spatiotemporal resolution offered by rt-MMRE.

**Materials and Methods**

**Subjects**

rt-MMRE was performed in 17 healthy volunteers without a history of neurological diseases (5 females, 36 ± 13 years, age range: 25–81 years, randomly selected). The study was approved by the ethics committee of Charité – Universitätsmedizin Berlin in accordance with the Ethical Principles for Medical Research Involving Human Subjects of the World Medical Association Declaration of Helsinki. Every participant gave written informed consent. Participant characteristics are summarized in Table 1. Group mean time curves of heart rate are given in Inline Supplementary Figure 1.
Table 1: Participant characteristics with abbreviations: body mass index (BMI), systolic blood pressure (BPsys), diastolic blood pressure (BPdis), and heart rate (HR).

| ID | Sex | Age in years | BMI in kg/m² | BPsys in mmHg | BPdia in mmHg | HR in bpm |
|----|-----|--------------|--------------|---------------|---------------|-----------|
| 1  | f   | 37           | 17.5         | 97            | 56            | 69        |
| 2  | m   | 29           | 24.2         | 130           | 77            | 80        |
| 3  | m   | 43           | 23.6         | 150           | 88            | 88        |
| 4  | m   | 46           | 26.3         | 134           | 85            | 62        |
| 5  | m   | 34           | 22.7         | 124           | 68            | 76        |
| 6  | m   | 25           | 20.8         | 120           | 70            | 70        |
| 7  | m   | 27           | 21.6         | 118           | 70            | 64        |
| 8  | m   | 30           | 26.3         | 126           | 78            | 78        |
| 9  | f   | 28           | 20.7         | 131           | 85            | 62        |
| 10 | m   | 36           | 19.9         | 77            | 50            | 54        |
| 11 | m   | 26           | 20.2         | 121           | 75            | 80        |
| 12 | f   | 26           | 20.5         | 113           | 60            | 55        |
| 13 | f   | 29           | 25.7         | 114           | 72            | 73        |
| 14 | m   | 51           | 20.7         | 130           | 85            | 62        |
| 15 | m   | 37           | 26.2         | 122           | 72            | 70        |
| 16 | f   | 27           | 31.6         | 140           | 78            | 90        |
| 17 | m   | 81           | 22.5         | 125           | 80            | 70        |

Mean (SD) – 36 (13) 23 (3) 122 (16) 74 (10) 71 (10)

Experimental Setup

All experiments were performed in a 3T MRI scanner (Siemens MAGNETOM Prisma, Erlangen) using a 32-channel head coil. Triple-harmonic vibrations in a narrowband frequency regimen were synchronously induced by four pressurized air drivers attached to a transmission plate and placed underneath the head. The applied frequencies were: 30.03, 30.91, and 31.8 Hz (hereinafter referred to as 31-Hz regimen). The two outmost drivers were operated at the highest frequencies with alternated phases relative to each other. The two inner drivers were operated with the same frequency, again with alternated phases. This way each frequency induced mainly lateral-rotational head
motion with minimized compression components. The setup is shown in Figure 1.

**Figure 1:** Experimental setup. (A) Image slice position (dashed yellow line) and region of interest (ROI) based on anatomical image (yellow solid line in the insert) for rt-MMRE of the brain. (B) Diagram of the four flask drivers with vibration frequencies and 180°-phase alterations between the drivers. (C) Top view of positioning of actuator setup in the 32-channel head coil. (D) Bottom view of driver setup.

Vibrations and radiofrequency (RF) excitation started 5 s before data acquisition to ensure establishment of steady states of time-harmonic oscillations and magnetization before start of each experiment. The following rt-MMRE experiments were performed:

i Valsalva maneuver (VM)
ii deep inspiration and breath-hold (BH-in)
iii expiration and breath-hold (BH-ex)

The VM experiment included four consecutive phases: 30 s baseline, 5 s breath-hold in inspiration, 20 s VM and 35 s recovery (total scan time: 90 s). Prior to the experiment, subjects
were trained to perform a moderate Valsalva maneuver that could be easily sustained for 20 s to prevent involuntary movement after deep breathing. This experiment was repeated with a second narrowband frequency regimen comprising 40.77, 41.67, and 42.55 Hz (hereinafter referred to as 42-Hz regimen) in order to check the overall consistency of MRE during VM and if there is a noticeable influence of frequency.

BH-in and BH-ex experiments consisted of 30-s baseline acquisition with the volunteer breathing normally, followed by a 25-s breath-hold in inspiration or expiration, and a final 35 s recovery phase (total scan time: 90 s).

A resting period of at least 30 s was observed between the experiments. Start and stop commands were given as visual signals to the volunteers. The finger pulse was continuously recorded to track changes in heart rate.

Additionally, anatomical images were acquired using a T1-weighted, turbo-spin echo (TSE) sequence.

**rt-MMRE Pulse Sequence**

Single-frequency rt-MRE using a 2D single-shot gradient echo MRE pulse sequence with spiral readout was recently introduced for directly mapping skeletal muscle function [55]. For rt-MMRE we used a similar prototype—a single-shot, gradient-echo sequence with dual-density spiral readout, which samples multifrequency vibrations in a stroboscopic fashion as illustrated in Figure 2. TR was 62 ms including RF excitation with 20° flip angle, 20 ms TE, 28 ms readout length, signal spoiling and fat suppression. For motion encoding, a single-cycle, bipolar motion-encoding gradient of 17.5-ms duration (57 Hz) and 40-mT/m amplitude was deployed within each TR according to the principle of fractional encoding [59]. Images were reconstructed using the SPIRiT non-Cartesian parallel imaging technique [60]. Three Cartesian motion components were encoded in an interleaved fashion within the series of consecutive TRs, yielding a sequence of 1,458 wave images. Collapsing these three components into a single viscoelasticity map resulted in a
total MRE frame rate of $3 \times TR = 186$ ms, or approximately 5.4 Hz.

Figure 2: Steady-state gradient echo timing diagram with spiral readout trajectory for single-shot multifrequency real-time MRE. From top to bottom: Experimental design with timing, harmonic vibrations at three frequencies over a period of $9 \times 3$ TRs with stroboscopic image acquisition below, interleaved wavefield encoding over $2 \times 3$ TRs, simplified sequence diagram with combined RF and x-gradients to illustrate fat saturation, RF excitation, motion encoding and spiral readout by x-gradients over a single TR period.

Data were acquired in a single transverse image slice with a field of view (FoV) of $192 \times 192$ mm$^2$ and $2 \times 2 \times 5$ mm$^3$ voxel size. The slice was automatically positioned using the localizer-based auto-align function at the level of the basal nuclei along the largest diameter of the lateral ventricles in the sagittal plane as shown in Figure 1.
Parameter Reconstruction

The 1,458 raw, complex-valued MR images were smoothed with a Gaussian filter ($\sigma = 0.65$ px) and subsequently unwrapped using gradient unwrapping [9]. The three vibration frequencies were decomposed by temporal Fourier transformation. Due to stroboscopic undersampling of vibrations in rt-MMRE, the frequencies appeared at aliased positions in the spectrum (see Figure 3A). The frequencies were selected by three Gaussian bandpass filters ($\sigma = 0.1$ Hz) each of which centered at the expected (aliased) frequency of the fundamental drive frequency. These filters were used for inverse Hilbert transformation to compute complex-valued wave fields (wave images) for each vibration frequency, separately yielding 4,374 ($1,458 \times 3$ vibration frequencies) time-resolved wave images [58]. Nine wave images of three Cartesian field components and three vibration frequencies (see Figure 3B) were fed into multifrequency dual elasto-visco inversion [56], yielding 486 ($4,374/3$ encoding components/3 vibration frequencies) consecutive maps of stiffness ($|G^*|)$ and loss angle ($\phi$) with 5.4-Hz frame rate over the entire examination time. While $|G^*|$ is a measure of stiffness, $\phi$ describes the ratio of elastic to viscous tissue properties indicating fluid properties as explained in Streitberger et al. [38] $|G^*|$ and $\phi$ maps from the beginning and end of the series were discarded within 5-s margins to minimize transient effects introduced by periodic boundary conditions of the Hilbert transform. Consequently, the final observation window was 80 s. All data processing was done in MATLAB (version 2020a). The inversion pipeline is publicly available at https://bioqic-$apps.charite.de [61]. Main results are given in Table 1, Table 2 and in Supplementary Tables 1a–c. Raw data can be made available upon request without restrictions.
Figure 3: Representative Fourier power spectra with three aliased excitation frequencies for one motion-encoding component above and wave deflections for three encoding components and three vibration frequencies below. (A) Power spectra for vibrations at 31-Hz regimen. Color coding indicates the respective vibration frequency with Gaussian bandpass filter used for Hilbert transformation. The frequency axis is scaled from 0 to the Nyquist frequency in Hz, which is determined by the sampling rate of 5.4 Hz. Stroboscopic sampling of multiharmonic vibrations causes all frequencies to be aliased within this limited frequency window. (B) Representative wave images after frequency decomposition for the three encoding components using the 31-Hz regimen (30.03, 30.91, 31.8 Hz). [ʘ,↔,↕ denote deflections through-plane (head-to-feet), left-right, and up-down (anterior-posterior), respectively].

Table 2: Mean $|G^*|$ (SD) in Pa and mean $\phi$ (SD) in rad for each phase and participant in the Valsalva maneuver experiments using the 31-Hz regimen (30.03, 30.91, 31.8 Hz).

| ID  | Mean $|G^*|$ (SD) in Pa | Mean $\phi$ (SD) in rad |
|-----|------------------------|------------------------|
|     | BSL | ESM | LRM | REC | BSL | ESM | LRM | REC |
| 1   | 1230(5) | 1230(5) | 1230(5) | 1230(8) | 0.64(0.001) | 0.64(0.001) | 0.64(0.001) | 0.64(0.001) |
| 2   | 1324(10) | 1324(10) | 1324(10) | 1324(11) | 0.67(0.001) | 0.79(0.001) | 0.79(0.001) | 0.79(0.001) |
| 3   | 1351(11) | 1415(14) | 1406(8) | 1351(9) | 0.75(0.001) | 0.79(0.001) | 0.79(0.001) | 0.79(0.001) |
| 4   | 1400(12) | 1530(20) | 1406(8) | 1400(8) | 0.75(0.001) | 0.77(0.001) | 0.81(0.001) | 0.79(0.001) |
| 5   | 1441(9) | 1494(24) | 1406(14) | 1441(13) | 0.75(0.001) | 0.79(0.001) | 0.79(0.001) | 0.79(0.001) |
| 6   | 1520(4) | 1534(7) | 1506(2) | 1520(1) | 0.75(0.001) | 0.79(0.001) | 0.79(0.001) | 0.79(0.001) |
| 7   | 1321(9) | 1263(17) | 1350(14) | 1321(11) | 0.61(0.001) | 0.61(0.001) | 0.63(0.001) | 0.62(0.001) |
| 8   | 1495(5) | 1519(20) | 1605(9) | 1543(6) | 0.79(0.001) | 0.79(0.001) | 0.79(0.001) | 0.79(0.001) |
| 9   | 1510(7) | 1624(9) | 1613(9) | 1543(1) | 0.69(0.001) | 0.78(0.001) | 0.78(0.001) | 0.78(0.001) |
| 10  | 1395(4) | 1431(9) | 1473(9) | 1405(8) | 0.78(0.001) | 0.78(0.001) | 0.78(0.001) | 0.78(0.001) |
| 11  | 1237(0) | 1293(11) | 1231(16) | 1243(9) | 0.69(0.001) | 0.69(0.001) | 0.69(0.001) | 0.69(0.001) |
| 12  | 1321(11) | 1350(12) | 1310(11) | 1321(11) | 0.75(0.001) | 0.79(0.001) | 0.79(0.001) | 0.79(0.001) |
| 13  | 1503(4) | 1510(12) | 1532(2) | 1505(6) | 0.69(0.001) | 0.69(0.001) | 0.69(0.001) | 0.69(0.001) |
| 14  | 1375(8) | 1407(14) | 1380(9) | 1375(4) | 0.79(0.001) | 0.77(0.001) | 0.79(0.001) | 0.79(0.001) |
| 15  | 1295(6) | 1421(26) | 1407(13) | 1295(15) | 0.74(0.001) | 0.74(0.001) | 0.74(0.001) | 0.74(0.001) |
| 16  | 1305(7) | 139(19) | 1422(2) | 1303(18) | 0.81(0.001) | 0.79(0.001) | 0.80(0.001) | 0.80(0.001) |
| 17  | 1157(9) | 1192(9) | 1170(12) | 1213(6) | 0.73(0.001) | 0.73(0.001) | 0.73(0.001) | 0.73(0.001) |

BSL, Baseline; ESM, established maneuver; LRM, late response maneuver; REC, recovery.
Parameter Analysis and Statistical Tests

For every time frame, $|G^*|$ and $\phi$ were quantified by averaging values over the same region of interest (ROI). ROIs were manually drawn based on anatomical T1-weighted images, as shown in Figure 1A. Furthermore, these ROIs were refined by empirical thresholds of 10 (time-averaged MRE signal magnitude) and of 950 Pa (time-averaged $|G^*|$ map) to remove ventricles and larger sulci similar to Shahryari et al. [62] (see Figure 4).

Figure 4: Representative rt-MMRE MRE magnitude, CSF masks, $|G^*|$ and $\phi$ maps of the in vivo human brain. Time-averaged MRE magnitude, derived CSF masks, $|G^*|$ and $\phi$ maps of one volunteer over the four phases [baseline (BSL), established maneuver (ESM), late response maneuver (LRM) and recovery (REC)] of the VM experiment using the 31-Hz regimen (30.03, 30.91, 31.8 Hz). The number of CSF associated voxels for each phase was BSL: 1079, ESM: 1128, LRM: 1069, REC: 1074. The $|G^*|$ maps show slightly elevated values throughout the slice. The region of interest (ROI) is indicated by white lines. The same ROI was used for all phases and for the $\phi$ maps as well.
The same ROI was also used to determine magnetization signal-to-noise ratio (SNR) and wave displacement SNR (WSNR) for every time frame. WSNR was derived using the blind noise estimation method proposed by Donoho et al. [63] as outlined and previously applied to MRE data in Bertalan et al. [64] and Schrank et al. [55] This noise estimation method is suited for wave image analysis since the spatial frequencies of MRE waves and noise are well separated in the wavelet domain [65,66].

To test if multifrequency inversion yields more stable values than single-frequency inversion we determined the coefficient of variation (CV) during the baseline phase prior to VM, BH-in and BH-ex for both $|G^*|$ and $\phi$ in all volunteers. The same raw data was used, but for the single-frequency inversion only one frequency from the temporal Fourier spectrum was selected.

We further analyzed difference $|G^*|$ and $\phi$ values relative to mean baseline values given as $\Delta |G^*| = |G^*|_{(t)} - |G^*|_{(\text{baseline})}$ (correspondingly for $\Delta \phi$) in order to quantify individual parameter changes. In addition, peak viscoelasticity values and their temporal delays relative to the onset and end of VM were identified and tabulated for each volunteer.

Finally, group statistics was applied to the absolute values of $|G^*|$ and $\phi$, after temporal averaging over the following experimental phases for each participant:

1. Baseline (BSL): 2.5–22.5 s
2. Established maneuver (ESM): 32.5–47.5 s
3. Late response maneuver (LRM): 52.5–57.5 s
4. Recovery (REC): 70–80 s.

Of note, these time intervals were given by the aforementioned study design (30-25-35 s for baseline-breathhold/VM-recovery) minus 2.5 s transition phases at the beginnings and ends of these phases including an additional late-response phase. The transition phases were discarded from our analysis in order to minimize transients resulted by the frequency bandpass filter. Also, 5 s BH (25–30 s) and 10 s of post-VM (60–70 s) were
considered as transition phases and henceforth not included in our group statistical analysis. All phases are demarcated in Figure 5.

Figure 5: Time courses of group mean values of $\Delta|G^*|$ (top of subfigures) and $\Delta\phi$ (bottom of subfigures) using the 31-Hz regimen (30.03, 30.91, 31.8 Hz). The gray areas show 95% confidence intervals. For Valsalva maneuver (VM), timing was as follows: breath-hold in inspiration (BH-in) at 25 s, start of VM at 30 s, stop of VM at 50 s. For breath-hold (BH) experiments, timing was as follows: start of BH at 25 s, stop at 50 s. (A) Valsalva maneuver (VM). (B) Breath-hold in inspiration (BH-in). (C) Breath-hold in expiration (BH-ex).

To test possible deformations of lateral ventricles due to VM as reported previously [67], we applied automatic segmentation of cerebral spinal fluid (CSF) to the temporal averaged MRE magnitude images of the different experimental phases using SPM12 (Penny et al., [68]; see Figure 4). CSF probability maps were thresholded at 0.5 to generate logical CSF-associated voxel masks. A linear mixed-effects model with varying intercept was employed. CSF volume was used as dependent variable and the individual phases as independent variables. Participants were assigned as random effect, and $P$-values were calculated using Tukey’s *post hoc* test with Bonferroni correction for multiple comparisons. To test for significant changes in $|G^*|$ and $\phi$ between phases (1)–(4), a linear mixed-effects model with varying intercept was employed. $|G^*|$ and $\phi$ were used as dependent variables and the individual phases as independent variables. Participants were assigned as random effect, and $P$-values were calculated using Tukey’s *post hoc* test with Bonferroni correction for multiple comparisons. This test does not account for inter-individual slope variations of $|G^*|$ and $\phi$ but analyzes the significance of temporal changes of these
parameters. SNR and viscoelastic parameters were correlated using a linear mixed model with $|G^*|$ and $\phi$ as dependent variables and SNR or WSNR as fixed effects with subjects as random factor. All statistical analysis was done in R (version 3.6.2). Unless otherwise stated, errors are given as standard deviation (SD). Correlations between viscoelastic baseline values as well as individual peak responses and participant characteristics (see Table 1) were analyzed using Pearson’s correlation coefficient. $P$-values below 0.05 were considered statistically significant.

**Results**

Variation in baseline $|G^*|$ and $\phi$ was smaller when using multifrequency inversion (CV = 0.74%, 0.51%) than single frequencies (CV = 0.99%, 0.77%, $P < 0.001$).

Figure 4 shows representative time-averaged MRE magnitude images, automatically segmented CSF masks, as well as $|G^*|$ and $\phi$ maps acquired during the four phases of the experiment. A slight increase in $|G^*|$ was visible in the late VM response, whereas no response of $\phi$ was apparent in individual maps. Group statistics revealed no significant change of CSF-associated voxels between the different states of the maneuver. A descriptive statistic for the individual phases of the VM experiment in the 31-Hz regimen and for each participant is given in Supplementary Table 2.

**Relative $|G^*|$ and $\phi$ Changes**

Individual analysis of $|G^*|$ showed an increase (6.7 ± 4.1%, $P < 0.001$) at approximately 2.4 ± 1.2 s after start of VM and 5.5 ± 2.0 s after end of VM (7.4 ± 2.8%, $P < 0.001$). $\phi$ decreased during ESM (−2.1 ± 1.4%, $P < 0.001$). Averaged time courses of $\Delta|G^*|$ and $\Delta\phi$ are presented in Figure 5. An early peak of $\Delta|G^*|$ showed a difference of 69 ± 50 Pa ($P < 0.001$) from baseline values. After a short drop, $\Delta|G^*|$ steadily increased during ESM. The second overshoot differed from baseline by 82 ± 42 Pa ($P < 0.01$). $\Delta|G^*|$ recovered toward baseline values once the
volunteers returned to normal breathing. $\Delta \phi$ was constantly decreased during ESM ($-0.018 \pm 0.012$ rad, $P < 0.01$).

The BH-in experiment showed an increase in $\Delta|G^*|$ after $3.0 \pm 1.0$ s ($18 \pm 16$ Pa, $P < 0.001$) with a maximum at $17.0 \pm 2.0$ s after start of BH-in ($32 \pm 29$ Pa, $P < 0.001$). $\Delta \phi$ decreased during ESM ($-0.006 \pm 0.004$ rad, $P < 0.001$) reaching a minimum at $17 \pm 2$ s after start of BH-in ($-0.009 \pm 0.007$ rad, $P < 0.001$).

The BH-ex experiment showed no clear peak, neither in $\Delta|G^*|$ nor $\Delta \phi$. $|G^*|$ increased continuously with onset of BH-ex and reached a maximum $2.5 \pm 1.5$ s after the end of BH-ex ($26 \pm 23$ Pa, $P < 0.001$).

### Absolute $|G^*|$ and $\phi$ Changes

Figure 6 shows boxplots with median effects for different states of the maneuver for $|G^*|$ and $\phi$. The significance levels, indicated by asterisks, were determined from a linear mixed model analysis with varying intercept and participants as random effect. For the VM, different individual effect sizes were observed; however, all subjects showed an increase in $|G^*|$ and a decrease in $\phi$ due to the maneuver. Averaged $|G^*|$ values changed between all phases of the experiment (range: 1,370–1,446 Pa) with significance levels indicated in the figure. Averaged $\phi$ values changed both from BSL to ESM and again from ESM to LRM (range: 0.784–0.800 rad).

By contrast, $|G^*|$ only changed at the start and end of the maneuver in BH-in (range: 1,338–1,372 Pa), whereas $\phi$ changed between BSL and ESM as well as between ESM and LRM (range: 0.783–0.792 rad). In the BH-ex experiment, $|G^*|$ changed between LRM and REC (range: 1,348–1,371 Pa) while $\phi$ changed between ESM and LRM (range: 0.786–0.791 rad).

Results of the second VM experiment performed using the 42-Hz regimen are presented in Supplementary Material. No significant differences in viscoelastic responses between the 31-Hz and 42-Hz regimen were observed ($P = 0.24$).
Figure 6: Group values as boxplots for the absolute values of $|G^*|$ (top) and $\phi$ (bottom) in each phase illustrate the changes in viscoelastic properties induced by the different maneuvers using the 31-Hz regimen for each phase [baseline (BSL), established maneuver (ESM), late response maneuver (LRM), recovery (REC)]. (A) Valsalva maneuver (VM). (B) Breath-hold in inspiration (BH-in). (C) Breath-hold in expiration (BH-ex). Asterisks at the top demarcate significant changes in $|G^*|$ and $\phi$ which were determined from a linear mixed-effects model with varying intercept. $|G^*|$ and $\phi$ assigned dependent variables and the individual phases as independent variables. Participants were assigned as random effect, and $P$-values were calculated using Tukey’s post hoc test with Bonferroni correction for multiple comparisons. (*$P < 0.05$, ***$P < 0.001$).

Descriptive statistics of $|G^*|$ in Pa and $\phi$ in rad for the individual phases of the VM experiment and for each participant are summarized in Table 2A. Correspondingly, statistical results for the breath-hold and VM experiments performed with the 42-Hz regimen are presented in Supplementary Tables 1a–c. Participant characteristics did not correlate with $|G^*|$ or $\phi$.

**SNR Analysis**

Time-averaged SNR and WSNR values did not change significantly across volunteers and over time ($P = 0.43$). Mean SNR was $29 \pm 2$ dB across all volunteers with minor and insignificant variations of $\pm 0.5$ dB over the course of the
experiment. Mean WSNR was 36 ± 2 dB with minor and insignificant variations of ± 1 dB over the course of the experiment. Significant correlation between group mean $|G^*|$ and $\phi$ was observed (31-Hz regimen: $R = -0.4$, $P < 0.001$, 42-Hz regimen: $R = -0.5$, $P < 0.001$).

Discussion

This paper presents a novel rt-MMRE technique for the in vivo measurement of rapid and non-periodic changes in brain viscoelasticity in humans. MRE exploiting stroboscopic sampling of multifrequency harmonic vibrations revealed the viscoelastic response of brain tissue to the Valsalva maneuver. Overall, the extension of rt-MRE to rt-MMRE by simultaneous excitation of multifrequency oscillations has increased the consistency of our measurements without adding scanning time. Probably for this reason, all subjects consistently showed an increase in $|G^*|$ and a decrease in $\phi$ with VM, resulting in high statistical significance. This basic finding is remarkable, since the VM is known to induce variability by subjective pressure generation. To further discuss our results we start by briefly reviewing the basic effects of VM on cerebral perfusion and ICP.

Physiological Effects of VM on Cerebral Blood Flow, ICP and MRE

In this study, elevation of intrathoracic pressure during VM was induced by deep inspiration following and increased abdominal pressure similar to the maneuver used in Ipek-Ugay et al. [69]. With onset of VM and elevated intrathoracic pressure, arterial blood pressure (ABP) increases [70,71]. Intrathoracic pressure is communicated through the vascular tree into the cranial cavity, leading to a transient increase in ICP and obstruction of venous outflow from the brain with, thus, increased venous pressure [72]. Reduced venous return to the heart causes ABP to decrease. Hence, cerebral perfusion pressure is reduced, leading to a reduction in cerebral blood flow (CBF). Cerebral autoregulation is a mechanism to maintain constant CBF. For this reason, cerebral autoregulation, after the decrease in CBF, immediately responds to reduce vascular resistance by dilating the cerebral
arteries in order to facilitate blood flow and maintain stable CBF. At the same time, the heart rate is increased through the baroreflex [73,74], which restores normal ABP and accumulation of blood in the brain, since venous return is still diminished. Constant influx of blood with reduced outflow steadily increases ICP. With release of intrathoracic pressure, there is a significant drop of ABP [75], and ICP returns to normal. As a result, normal venous return is restored and more blood flows back into the heart, leading to a transient increase in cardiac output and overshoot in ABP. Since vascular resistance is still low, CBF overshoots as well.

The time curves of MRE parameters presented in Figure 7 suggest that stiffness (|G*|) correlates with ICP while viscosity-related φ correlates with reduced venous outflow or cerebral perfusion pressure. Perfusion pressure is proportional to CBF normalized by mean vascular diameter [43] and, thus, decreases upon vasodilation with constant CBF. The ramp-up of |G*| during the continuing VM phase seems to reflect the increasing heart rate and steady accumulation of blood in the brain, which drives ICP. By contrast, φ remains low throughout the VM phase as if viscous damping in brain tissue is lower when perfusion pressure is reduced. It is an intriguing result that possible ICP changes during VM can be indirectly monitored using rt-MMRE since non-invasive ICP measurement are still an unsolved problem. These findings could help to relate pathologically increased ICP to overall brain stiffness for clinical applications.

In previous work we observed an increase in φ of the brain due to hypercapnia (2% increase) [43] and arterial pulsation (0.5% increase) [58]. In both studies, there was an increase in CBF with a concomitant increase in perfusion pressure while, as explained above, CBF in VM is, due to cerebral autoregulation, associated with a fairly constant CBF and reduced perfusion pressure. Together, the two rt-MMRE parameters, |G*| and φ, provide complementary information on the concert of physical parameters involved in ICP autoregulation.
Overall, our baseline parameters of brain viscoelasticity are in good agreement with previously reported values acquired in similar frequency ranges [42,43,58]. We observed no significant differences in the responses of rt-MMRE parameters to VM between the 31 and 42 Hz regimens. This consistency of multifrequency data further validates the technique of rt-MMRE. Furthermore, this observation indicates that the poroelastic response of brain tissue [15] is similar at 30 and 40 Hz [14]. Additional validation of rt-MMRE was obtained by reference experiments performed during breath-holds but without sustained VM. BH-in induced a similar increase in stiffness and decrease in $\phi$ as observed during VM. Thus, from an MRE perspective, deep inspiration followed by breath-holding induces effects similar to a light VM. Otherwise, no such changes were observed in BH-ex, rendering this maneuver neutral with regard to ICP. Nevertheless, even BH-ex had some small effect on MRE parameters, which, notably, were not correlated to changes in either SNR or WSNR. Also, analysis of CSF volume and ventricle size did not reveal any significant correlation with VM. Previous work by us and others showed that total brain volume increases due to VM by approximately 3% while ventricle volume shrinks by 20% [67,77]. In contrast to these studies, our
subjects were instructed to perform a moderate Valsalva maneuver to minimize variations in thoracic pressure, muscle strain, and head position.

In our previous work we used ultrasound time-harmonic elastography in a temporal bone window to acquire VM-induced rapid changes in shear wave speed in the temporal lobes of healthy volunteers [53]. Effect sizes in that region were higher (10.8 ± 2.5%) than revealed by MRE in the full brain tissue slice. It should be noted that the regions covered by our current study do not correspond to the medial temporal gyrus addressed by transtemporal time-harmonic elastography, which makes a direct comparison of effect sizes between the two studies difficult. To analyze the spatial representation of viscoelasticity changes we performed automatic image segmentation using MNI-based registration as well as voxel-wise correlation analysis based on a boxcar function. No significant patterns of viscoelasticity changes could be detected. A more detailed analysis of the spatiotemporal representation of brain viscoelasticity in response to the VM is warranted.

Our study has limitations. The nature of stroboscopic sampling of vibrations by steady-state single-shot acquisitions limited our technique to 2D wave field sampling including three encoding components. This intrinsic limitation of rt-MMRE can currently not be overcome by a multishot variant because VM is a non-periodic event and cannot be repeated with enough temporal reliability. Consequently, our multi-frequency inversion technique was entirely 2D, which may have led to variability due to different slice positioning and oblique intersection of 3D shear wavefields rendering our values as effective viscoelasticity parameters. Nevertheless, our conclusions are drawn from group values in two different frequency regimens. The fact that these values changed with statistical significance in VM, while neither SNR nor anatomy changed, emphasizes the robustness of the observed MRE effects. Furthermore, our data could be used for suppression of bulk waves based on the in-plane curl component. However, this curl-analysis did not provide more consistent values than our standard MDEV inversion with respect to confidence intervals and statistical power. Finally, 2D brain
MRE has a long tradition in disease detection [25,26,29,30,31,38,78,79] as well as in the study of brain physiology [18,58,80,81]. It remains to be determined whether single-frequency 3D MRE can provide similarly consistent clinical and physiological brain data. Instead, as shown herein, unintentional breath-holds may affect 3D MRE due to long scan times. Generally, current MRE techniques cannot account for poroelasticity, heterogeneity, hyperelasticity, anisotropy and temporal variations of brain tissue at the same time. Therefore, to date all values measured by brain MRE should be considered as effective parameters.

In summary, we studied the viscoelastic response of the human brain to breathing and the Valsalva maneuver using a novel real-time multifrequency MRE technique. Significant increases in brain stiffness and decreases in $\phi$ due to VM were observed with use of two different frequency regimens. Control experiments showed that breath-holds after inhalation induce a response similar to VM but with a smaller effect size. By contrast, breath-holds after exhalation had the smallest effects on cerebral MRE parameters. The time courses we report here provide a reference for the VM response in healthy subjects and might be of value for studying dysfunctional autoregulation as associated with various neurological diseases. rt-MMRE is a fast technique which can provide consistent imaging markers of brain viscoelasticity within a fraction of a minute.

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Supplementary Material

We present here the results of the Valsalva maneuver (VM) experiment at a second narrowband frequency regimen comprising 40.77, 41.67, 42.55 Hz (hereinafter, referred to as 42-Hz regimen) as a complementary evaluation of our findings. We did so in order to validate the overall consistency of MRE during the VM and to check if there is a noticeable influence of frequency. Despite different absolute values, the repetition of the VM experiment gave similar results compared to those described in the main manuscript at 31-Hz regimen. Moreover we present the group mean heart rate (HR) and additional descriptive statistics for the VM, breath-hold in inspiration (BH-in) and breath-hold at expiration (BH-ex).

Supplementary figure 1 shows group mean HR for each experiment. Qualitatively the course of the HR for the VM experiment at both frequency regimens was the same (see subfigure A and D). HR increased with deep inspiration (25 s) and dropped with onset of the VM (30 s). Next, HR starts to rise (30 – 50 s). With the end of the maneuver HR returned to normal
or even below pre-exercise baseline values. BH-in showed a slight increase in HR similar to the VM (25 s) with deep inspiration. HR normalized throughout the breath-hold phase and is slightly lowered after the exercise (subfigure B). In the BH-ex experiment the HR did not show any changes with onset of the exercise but is slightly lowered with release and return to normal breathing as it can be observed in all experiments.

Supplementary Figure 1: Group mean heart rate in bpm for each experiment. A) VM using the 31-Hz regimen (30.03, 30.91, 31.8 Hz). B) BH-in using the 31-Hz regimen. C) BH-ex using the 31-Hz regimen. D) VM using the 42-Hz regimen (40.77, 41.67, 42.55 Hz).

Supplementary figure 2 shows the spectral power of aliased driving frequencies of the 42-Hz regimen in addition to the 31-Hz regimen with wave images of three Cartesian field component and three vibration frequencies below. The higher frequencies contained less spectral power and with smaller wave amplitudes.
Supplementary Figure 2: Representative Fourier power spectra with three aliased excitation frequencies for one motion-encoding component above and wave deflections for three encoding components and three vibration frequencies below. A) Power spectra for vibrations using the 31-Hz (left) and 42-Hz regimen (right). Color coding indicates the respective vibration frequency with Gaussian bandpass filter used for Hilbert transformation. The frequency axis is scaled from 0 to the Nyquist frequency in Hz, which is determined by the sampling rate of 5.4 Hz. Stroboscopic sampling of multiharmonic vibrations causes all frequencies to be aliased within this limited frequency window. B) Representative wave images after frequency decomposition for the three encoding components using the 31-Hz (left) and the 42-Hz regimens (right). (☉, [], ↔ denote deflections through-plane [head-to-feet], left-right, and up-down [anterior-posterior], respectively).

Supplementary figure 3 shows representative |G*| and φ maps in the 42-Hz regimen in addition to the 31-Hz regimen during the four phases of the experiment. Average |G*| was higher at 42-Hz than at 31-Hz as expected from the viscoelastic dispersion while φ was lower at higher frequencies.
Supplementary Figure 3: Representative rt-MMRE |G*| and φ maps of the in vivo human brain. A) Time-averaged |G*| and φ maps of one volunteer over the four phases (baseline (BSL), established maneuver (ESM), late response maneuver (LRM) and recovery (REC)) of the VM experiment using the 31-Hz regime (30.03, 30.91, 31.8 Hz). B) Averaged |G*| and φ maps at 42-Hz regimen (40.77, 41.67, 42.55 Hz). Similar stiffening during the Valsalva maneuver (increases in most |G*| values) as in (A) is visible. The region of interest (ROI) is indicated by white lines. The same ROI was used for all phases, for the φ maps and for the 42-Hz regimen as well.

Averaged time courses of Δ|G*| and Δφ at 42-Hz regimen in comparison the 31-Hz regimen are presented in supplementary Figure 4. As reported before, peak viscoelastic responses did not differ significantly in the two narrowband regimens. In accordance, qualitative time courses match well.
For 42-Hz regimen, group mean VM values showed an early overshoot of $|G^*| 2.4 \pm 1.2$ s after the onset of the maneuver with peak values of $4.4 \pm 3.8\%$ (in average: $61 \pm 63$ Pa, $P < 0.001$). A second overshoot of $|G^*|$ occurred $5.5 \pm 2.0$ s after the end of the VM with peak values of $7.2 \pm 3.4\%$ (in average: $124 \pm 72$ Pa, $P < 0.01$) above baseline. $\phi$ was reduced by $-2.2 \pm 2.1\%$ (in average: $-0.015 \pm 0.014$ rad, $P < 0.01$) during the entire VM without noticeable peak values.

**Supplementary Figure 4:** Time courses of group mean values of $\Delta |G^*|$ (top of subfigures) and $\Delta \phi$ values (bottom of subfigures). The gray areas show 95% confidence intervals. For Valsalva maneuver (VM), timing was as follows: breath-hold in inspiration (BH-in) at 25 s, start of VM at 30 s, stop of VM at 50 s. **A)** VM using the 31-Hz regimen (30.03, 30.91, 31.8 Hz). **B)** VM using the 42-Hz regimen (40.77, 41.67, 42.55 Hz).

Supplementary figure shows boxplots with median effects for different states of the maneuver for $|G^*|$ and $\varphi$ at 42-Hz regimen in addition to 31-Hz regimen. The significance levels, indicated by asterisks, were determined from a linear mixed model analysis with varying intercept and participants as random effect. In the VM, averaged $|G^*|$ values changed in all phases of the experiment (range: 1953 to 2072 Pa) with significance levels indicated in the figure. Averaged $\varphi$ values changed in both cases from BSL to ESM and again from ESM to LRM (range: 0.674 to 0.686 rad). Descriptive statistics for the repetition of the VM experiment in the 42-Hz regimen and BH-experiments are given in Supplementary table 2a-c. A negative correlation between
BSL-\(|G^*|\) values and age at 42-Hz regimen (0.8% per year, \(R = -0.64, P < 0.005\)) was observed.

**Supplementary Figure 5:** Group values as boxplots for the absolute values of \(|G^*|\) (top) and \(\phi\) (bottom) in each phase illustrate the changes in viscoelastic properties induced by VM during the two frequency regimens for each phase (baseline (BSL), established maneuver (ESM), late response maneuver (LRM), recovery (REC)). **A)** Valsalva maneuver (VM) using the 31-Hz regimen. **B)** VM using the 42-Hz regimen. Asterisks at the top demarcate significant changes in \(|G^*|\) and \(\phi\) which were determined from a linear mixed-effects model with varying intercept. \(|G^*|\) and \(\phi\) assigned dependent variables and the individual phases as independent variables. Participants were assigned as random effect, and P values were calculated using Tukey’s post hoc test with Bonferroni correction for multiple comparisons. (*\(P < .05\), ***\(P < .001\))

Supplementary table 1a-c present mean \(|G^*|\) and \(\phi\) values for the individual phases and each participant for the experiments: VM in the 42-Hz regimen (1a), BH-in (1b) and BH-ex (1c) similar to Table 2 in the main manuscript.
| ID | BSL (162) | ESM (15) | LRM (21) | REC (26) | BSL (16) | ESM (19) | LRM (22) | REC (27) |
|----|-----------|----------|----------|----------|-----------|----------|----------|----------|
| 1  | 1947 (7)  | 1965 (19)| 1997 (8) | 1942 (16)| 0.731 (0.002)| 0.733 (0.005)| 0.738 (0.002)| 0.727 (0.002)|
| 2  | 1887 (17)| 1879 (8) | 1948 (14)| 1812 (18)| 0.688 (0.002)| 0.696 (0.009)| 0.679 (0.001)| 0.701 (0.005)|
| 3  | 1933 (15)| 1957 (16)| 2040 (8) | 1895 (10)| 0.681 (0.001)| 0.653 (0.003)| 0.675 (0.007)| 0.677 (0.003)|
| 4  | 2086 (12)| 2171 (36)| 2166 (5) | 2118 (12)| 0.756 (0.004)| 0.735 (0.003)| 0.75 (0.002)| 0.751 (0.002)|
| 5  | 2125 (5) | 2120 (43)| 2293 (35)| 2161 (11)| 0.68 (0.002)| 0.659 (0.002)| 0.683 (0.002)| 0.674 (0.001)|
| 6  | 2091 (17)| 2161 (23)| 2260 (18)| 2094 (17)| 0.675 (0.003)| 0.662 (0.001)| 0.678 (0.004)| 0.672 (0.002)|
| 7  | 1866 (7) | 1975 (16)| 2027 (13)| 1876 (17)| 0.705 (0.001)| 0.688 (0.008)| 0.7 (0.006)| 0.699 (0.003)|
| 8  | 2139 (4) | 2140 (37)| 2449 (17)| 2192 (10)| 0.663 (0.002)| 0.646 (0.001)| 0.662 (0.001)| 0.669 (0.002)|
| 9  | 2198 (7) | 2272 (15)| 2303 (10)| 2212 (15)| 0.688 (0.004)| 0.653 (0.008)| 0.667 (0.004)| 0.678 (0.004)|
| 10 | 2016 (6) | 1990 (12)| 2105 (16)| 2003 (18)| 0.657 (0.002)| 0.656 (0.003)| 0.653 (0.001)| 0.654 (0.002)|
| 11 | 1819 (8) | 1793 (26)| 1928 (14)| 1829 (24)| 0.693 (0.002)| 0.69 (0.002)| 0.687 (0.003)| 0.689 (0.002)|
| 12 | 1855 (15)| 1817 (25)| 1943 (17)| 1859 (14)| 0.654 (0.003)| 0.67 (0.001)| 0.659 (0.001)| 0.664 (0.003)|
| 13 | 2142 (6) | 2136 (22)| 2216 (7) | 2128 (12)| 0.685 (0.002)| 0.682 (0.007)| 0.678 (0.001)| 0.673 (0.003)|
| 14 | 1919 (6) | 1954 (21)| 2014 (3) | 1921 (12)| 0.668 (0.001)| 0.655 (0.003)| 0.67 (0.003)| 0.666 (0.001)|
| 15 | 1786 (3) | 1927 (23)| 1928 (19)| 1837 (14)| 0.704 (0.004)| 0.675 (0.006)| 0.702 (0.003)| 0.693 (0.002)|
| 16 | 1901 (11)| 1890 (18)| 1988 (14)| 1893 (14)| 0.695 (0.001)| 0.699 (0.003)| 0.693 (0.002)| 0.697 (0.004)|
| 17 | 1488 (6) | 1606 (15)| 1614 (21)| 1559 (5) | 0.643 (0.004)| 0.608 (0.003)| 0.627 (0.004)| 0.646 (0.003)|
| Mean (SD) | 1953 (170)| 1986 (162)| 2072 (190)| 1961 (167)| 0.686 (0.027)| 0.674 (0.031)| 0.682 (0.028)| 0.683 (0.026)|

Supplementary Table 1b: Mean |G*| (SD) in Pa and mean φ (SD) in rad for each phase and participant during the BH-in experiment using the 31-Hz regimen (30.03, 30.91, 31.8 Hz). Baseline (BSL), established maneuver (ESM), late response maneuver (LRM), recovery (REC).

| ID | BSL (162) | ESM (15) | LRM (21) | REC (26) | BSL (16) | ESM (19) | LRM (22) | REC (27) |
|----|-----------|----------|----------|----------|-----------|----------|----------|----------|
| 1  | 1313 (5)| 1313 (7)| 1332 (1)| 1309 (13)| 0.8 (0.004)| 0.795 (0.002)| 0.797 (0.002)| 0.806 (0.001)|
| 2  | 1530 (7)| 1531 (14)| 1550 (2)| 1524 (3)| 0.78 (0.002)| 0.762 (0.005)| 0.77 (0.003)| 0.781 (0.004)|
| 3  | 1541 (7)| 1551 (12)| 1542 (3)| 1503 (2)| 0.801 (0.002)| 0.795 (0.002)| 0.794 (0.001)| 0.804 (0.002)|
| 4  | 1396 (12)| 1434 (10)| 1431 (4)| 1399 (12)| 0.767 (0.004)| 0.762 (0.003)| 0.765 (0.001)| 0.77 (0.002)|
| 5  | 1237 (9)| 1237 (2)| 1233 (2)| 1211 (4)| 0.806 (0.004)| 0.801 (0.001)| 0.808 (0.001)| 0.813 (0.003)|
| 6  | 1299 (10)| 1356 (13)| 1350 (1)| 1270 (10)| 0.756 (0.006)| 0.744 (0.002)| 0.75 (0.001)| 0.767 (0.003)|
| 7  | 1528 (3)| 1545 (5)| 1545 (1)| 1537 (4)| 0.794 (0.002)| 0.794 (0.001)| 0.794 (0.001)| 0.791 (0.001)|
| 8  | 1223 (10)| 1291 (12)| 1271 (6)| 1220 (8)| 0.815 (0.006)| 0.803 (0.001)| 0.812 (0.001)| 0.816 (0.001)|
| 9  | 1292 (8)| 1315 (3)| 1314 (1)| 1296 (7)| 0.844 (0.001)| 0.834 (0.002)| 0.85 (0.002)| 0.849 (0.001)|
| 10 | 1353 (7)| 1396 (26)| 1379 (4)| 1336 (5)| 0.792 (0.001)| 0.782 (0.002)| 0.791 (0.002)| 0.797 (0.004)|
| 11 | 1363 (8)| 1389 (9)| 1385 (1)| 1355 (3)| 0.789 (0.001)| 0.781 (0.003)| 0.784 (0.0)| 0.784 (0.001)|
### Supplementary Table 1c: Mean $|G^*|$ (SD) in Pa and mean $\varphi$ (SD) in rad for each phase and participant in the BH-ex experiment using the 31-Hz regimen (30.03, 30.91, 31.8 Hz). Baseline (BSL), established maneuver (ESM), late response maneuver (LRM), recovery (REC).

| ID | Mean $|G^*|$ (SD) in Pa | Mean $\varphi$ (SD) in rad |
|----|-----------------------|----------------------------|
|    | BSL | ESM | LRM | REC | BSL | ESM | LRM | REC |
| 1  | 1306 (4) | 1302 (8) | 1317 (5) | 1313 (12) | 0.799 (0.003) | 0.799 (0.003) | 0.804 (0.003) | 0.821 (0.005) |
| 2  | 1536 (11) | 1541 (15) | 1568 (4) | 1538 (3) | 0.776 (0.003) | 0.773 (0.003) | 0.788 (0.003) | 0.779 (0.002) |
| 3  | 1520 (6) | 1533 (4) | 1522 (3) | 1506 (8) | 0.805 (0.003) | 0.803 (0.002) | 0.805 (0) | 0.798 (0.001) |
| 4  | 1386 (5) | 1422 (6) | 1436 (1) | 1428 (8) | 0.771 (0.003) | 0.757 (0.002) | 0.764 (0) | 0.758 (0.001) |
| 5  | 1234 (11) | 1251 (6) | 1249 (3) | 1227 (6) | 0.801 (0.004) | 0.796 (0.006) | 0.805 (0.001) | 0.806 (0.002) |
| 6  | 1292 (19) | 1327 (5) | 1322 (3) | 1277 (11) | 0.763 (0.007) | 0.759 (0.003) | 0.761 (0.002) | 0.771 (0.003) |
| 7  | 1542 (5) | 1557 (5) | 1572 (1) | 1561 (8) | 0.782 (0.003) | 0.785 (0.001) | 0.789 (0) | 0.785 (0.002) |
| 8  | 1232 (13) | 1300 (11) | 1302 (5) | 1229 (4) | 0.815 (0.006) | 0.804 (0.001) | 0.813 (0.002) | 0.815 (0.002) |
| 9  | 1293 (5) | 1331 (7) | 1344 (1) | 1307 (12) | 0.845 (0.001) | 0.844 (0.003) | 0.852 (0.001) | 0.842 (0.001) |
| 10 | 1362 (6) | 1381 (8) | 1359 (3) | 1356 (11) | 0.782 (0.001) | 0.794 (0.003) | 0.806 (0) | 0.781 (0.001) |
| 11 | 1360 (7) | 1373 (3) | 1366 (5) | 1368 (5) | 0.788 (0.001) | 0.79 (0.001) | 0.789 (0.001) | 0.778 (0.002) |
| 12 | 1364 (5) | 1345 (2) | 1366 (7) | 1338 (4) | 0.765 (0.005) | 0.774 (0.004) | 0.775 (0) | 0.771 (0.003) |
| 13 | 1326 (6) | 1336 (3) | 1318 (6) | 1287 (6) | 0.821 (0.002) | 0.824 (0) | 0.832 (0.001) | 0.82 (0.004) |
| 14 | 1153 (4) | 1153 (7) | 1161 (3) | 1165 (7) | 0.728 (0.002) | 0.722 (0.003) | 0.726 (0.001) | 0.725 (0.002) |
| 15 | 1338 (7) | 1342 (6) | 1357 (1) | 1325 (6) | 0.766 (0.003) | 0.77 (0.002) | 0.764 (0.001) | 0.768 (0.001) |
| 16 | 1537 (6) | 1533 (4) | 1561 (5) | 1559 (10) | 0.754 (0.007) | 0.761 (0.004) | 0.76 (0.002) | 0.77 (0.004) |
| 17 | 1235 (14) | 1302 (9) | 1312 (6) | 1244 (6) | 0.772 (0.005) | 0.784 (0.001) | 0.8 (0.004) | 0.775 (0.004) |
| Mean (SD) | 1354 (115) | 1372 (109) | 1378 (113) | 1355 (119) | 0.784 (0.027) | 0.785 (0.027) | 0.79 (0.029) | 0.786 (0.027) |
Supplementary table 2 presents the number of CSF associated voxels for the individual phases and each participant for the VM using the 31-Hz regimen derived from automatic segmented CSF masks as shown in Figure 4 in the main manuscript.

**Supplementary Table 2:** Number of CSF associated voxels derived from temporal averaged MRE magnitude images for each phase and participant during the Valsalva experiment using the 31-Hz regimen (30.03, 30.91, 31.8 Hz). Automatic segmentation was done using SPM12. CSF probability maps were thresholded at 0.5 to generate logical CSF masks. Group statistics using a linear-mixed model revealed no significant change of the CSF associated voxels between the different states of the maneuver. Baseline (BSL), established maneuver (ESM), late response maneuver (LRM), recovery (REC).

| ID | BSL | ESM | LRM | REC |
|----|-----|-----|-----|-----|
| 1  | 940 | 889 | 892 | 876 |
| 2  | 1070 | 1205 | 1040 | 1106 |
| 3  | 1079 | 1128 | 1069 | 1074 |
| 4  | 1091 | 1019 | 939 | 1016 |
| 5  | 1078 | 860 | 1170 | 1141 |
| 6  | 1030 | 1028 | 1067 | 1102 |
| 7  | 1079 | 1129 | 855 | 1011 |
| 8  | 1284 | 935 | 1106 | 1236 |
| 9  | 1001 | 1064 | 907 | 1201 |
| 10 | 918 | 894 | 821 | 910 |
| 11 | 1037 | 1056 | 1023 | 982 |
| 12 | 889 | 1143 | 850 | 955 |
| 13 | 909 | 1204 | 1012 | 950 |
| 14 | 866 | 1112 | 944 | 1114 |
| 15 | 985 | 998 | 1151 | 1007 |
| 16 | 985 | 888 | 1065 | 1104 |
| 17 | 1178 | 1043 | 1198 | 1098 |
| **Mean (SD)** | **1025 (108)** | **1035 (112)** | **1006 (117)** | **1052 (100)** |