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Assessment of *in vivo* and post-mortem mechanical behavior of brain tissue using magnetic resonance elastography

Jonathan Vappou [a,1], Elodie Breton [b], Philippe Choquet [b], Rémy Willinger [a], André Constantinesco [b,*]

[a] Institut de mécanique des fluides et des solides UMR 7507 CNRS-ULP, Strasbourg, France
[b] Service de Biophysique et médecine nucléaire, CHU Hautepierre, Hôpitaux Universitaires de Strasbourg et UMR 7507 CNRS-ULP, Strasbourg, France

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ABSTRACT

The knowledge of *in vivo* brain tissue mechanical properties is essential in several biomedical engineering fields, such as injury biomechanics and neurosurgery simulation. Almost all existing available data have been obtained *in vitro* by invasive experimental protocols. However, the difference between *in vivo* and post-mortem mechanical properties remains poorly known, essentially due to the lack of a common method that could measure them both *in vivo* and *ex vivo*. In this study, we report the use of magnetic resonance elastography (MRE) for the non-invasive assessment of *in vivo* brain tissue viscoelastic properties and for the investigation of their evolution after the death. Experiments were performed on seven adult male rats. Shear storage and loss moduli were measured *in vivo*, just after death and at post-mortem time of ≈ 24 h. A significant increase in shear storage modulus G' of approximately 100% was found to occur just after death ($p = 0.002$), whereas no significant difference was found between *in vivo* G' and G' at 24 h post-mortem time. No significant difference was found between shear loss modulus G'' *in vivo* and just after death, whereas a decrease of about 50% was found to occur after 24 h ($p = 0.02$). These results illustrate the ability of MRE to investigate some of the critical soft tissue biomechanics-related issues, as it can be used as a non-invasive tool for measuring soft tissue viscoelastic properties.

1. Introduction

For several decades, the interest in brain tissue mechanical behavior has been increasing, mostly as a consequence of the emergence of biomedical engineering fields such as head impact biomechanics and neurosurgery simulation. For such fields, the accurate knowledge of *in vivo* brain tissue mechanical response is essential in order to ensure realistic results. However, due to experimental limitations, almost all existing results have been obtained by *ex vivo* experiments performed on excised brain tissue samples. How the mechanical properties of dead brain tissue differ from those of the living tissue remains poorly known, and using *ex vivo* instead of *in vivo* brain tissue properties might lead to significant errors.

The alteration of brain tissue structural properties after death at a microscopic level has been well established (Perry et al., 1981; Fountoulakis et al., 2001). Thus, it is strongly expected that it also undergoes significant alteration regarding its mechanical properties. Several parameters, such as metabolic activity, perfusion, and intra-cranial pressure, have probably a significant influence on the mechanical properties of brain tissue (Bilston, 2002; Fallenstein et al., 1969); they are not taken into account when performing *ex vivo* experiments on extracted brain samples. Hence, there is much interest in knowing the *in vivo* brain tissue mechanical response, and also in evaluating the differences between living and dead brain tissue and the temporal evolution of its post-mortem mechanical response.

This has led to the development of experimental protocols capable of measuring both *ex vivo* and *in vivo* brain tissue mechanical properties. Invasive studies have been performed for this purpose, either using an expandable balloon inserted inside brain tissue (Metz et al., 1970), or using indentation techniques (Gefen and Margulies, 2004; Miller et al., 2000). However, the invasive and destructive nature of these methods, in particular which require the skull to be partially removed, do not allow reliable conclusions about the mechanical properties of brain tissue in its *in vivo*, non-altered, and natural environment.

Magnetic resonance elastography (MRE) (Muthupillai et al., 1995) is a non-invasive technique that was initially developed as a tool for the detection of abnormal stiffness differences in soft tissues. MRE is based on the detection of propagating shear waves

* Corresponding author. Tel.: +33 388127549; fax:+33 388128121.
E-mail addresses: jv2273@columbia.edu (J. Vappou), andre.constantinesco@chru-strasbourg.fr (A. Constantinesco).
[1] Current address: Biomedical Engineering Department at Columbia University, New York, NY, USA.

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using motion-encoding gradients added into the MRI sequence (Lewa, 1991). The observed shear wave patterns allow the determination of underlying mechanical properties. In particular, MRE has recently been applied to the *in vivo* characterization of brain tissue mechanical behavior. Several brain MRE studies have determined the spatial distribution of brain elasticity *in vivo* (Manduca et al., 2001; Hamhaber et al., 2007; Kruse et al., 2008) under the assumption of linear Hookean elasticity. Very recently, *in vivo* brain tissue viscoelastic properties have been measured non-invasively (Larrat et al., 2007; Sack et al., 2008). Such results represent a considerable advance for brain biomechanics. However, comparing viscoelasticity measured by MRE and viscoelasticity measured *in vitro* by mechanical techniques is not straightforward, due to the strong dependence on the experimental protocol. Results obtained by such different methods can not be directly compared, and no conclusion regarding possible differences between *ex vivo* and *in vivo* mechanical properties can therefore be established from these results. In this context, there is much interest in proposing a method that would allow the determination of brain tissue mechanical properties both *in vivo* and post-mortem, in similar experimental conditions.

This study reports the use of MRE combined with a wave inversion algorithm for the determination of *in vivo* brain tissue viscoelastic properties on rat subjects. Experiments were also performed *ex vivo* under similar conditions in order to quantitatively evaluate brain tissue mechanical alteration after death.

2. Material and methods

2.1. General protocol

Experiments were performed on seven Sprague–Dawley male rats aged from 8 to 9 weeks (weighting between 300 and 350 g), in accordance with French regulations (authorizations A67-482-20 and 67-104). The following experimental protocol was applied:

- *In vivo* experiments: Rats were placed in an MR-compatible technical cell (described below) and were anesthetized with 2% Isoflurane pushed by air.
- *Ex vivo* experiments, *post-mortem* time $\approx$ 30 min (PM0.5 h). This time corresponds approximately to the time needed for euthanasia and placing the body inside the magnet + half of the time needed for the imaging process. Rats were sacrificed by lethal intracardiac injection of Pentothal after gaseous anaesthesia (Isoflurane).
- *Ex vivo* experiments, *post-mortem* time $\approx$ 24 h (PM24 h): Rats were stored in refrigerator (5 °C) until the following day. Prior to experiments, rats were let out at magnet gap temperature ($\approx$ 27 °C) during $\approx$ 1 h 30 in order to allow temperature equilibration.

In addition to this protocol, one subject was used as a control for an estimation of the global error: For this subject, MRE experiments were performed *in vivo* five times at four different days. Delay between two consecutive measurements was 24 h and first measurement was performed twice. The purpose of this control study was to estimate a global measurement error of the method, which would include the variation due to differences in rat head positioning, the error related to the wave inversion algorithm itself, as well as the influence of the varying phase signal-to-noise

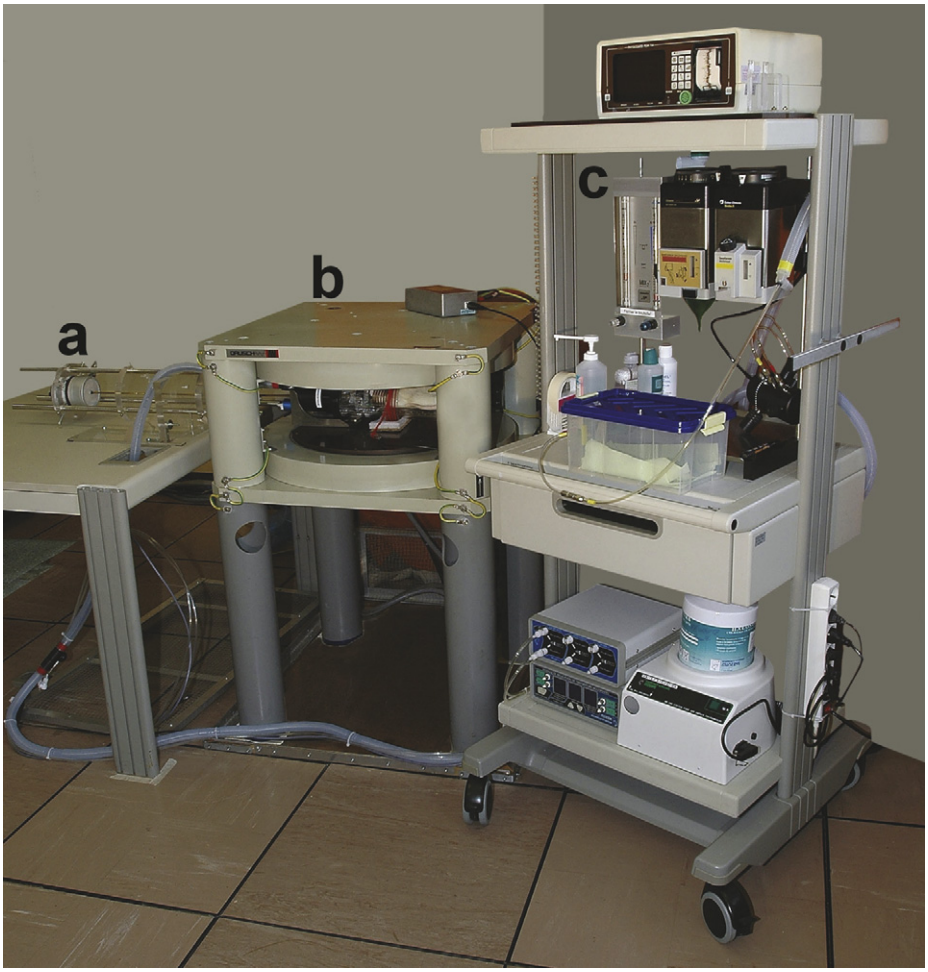

Fig. 1. Experimental setup for rat MRE: modal exciter (a), horizontal magnet (b), and anaesthesia device (c).

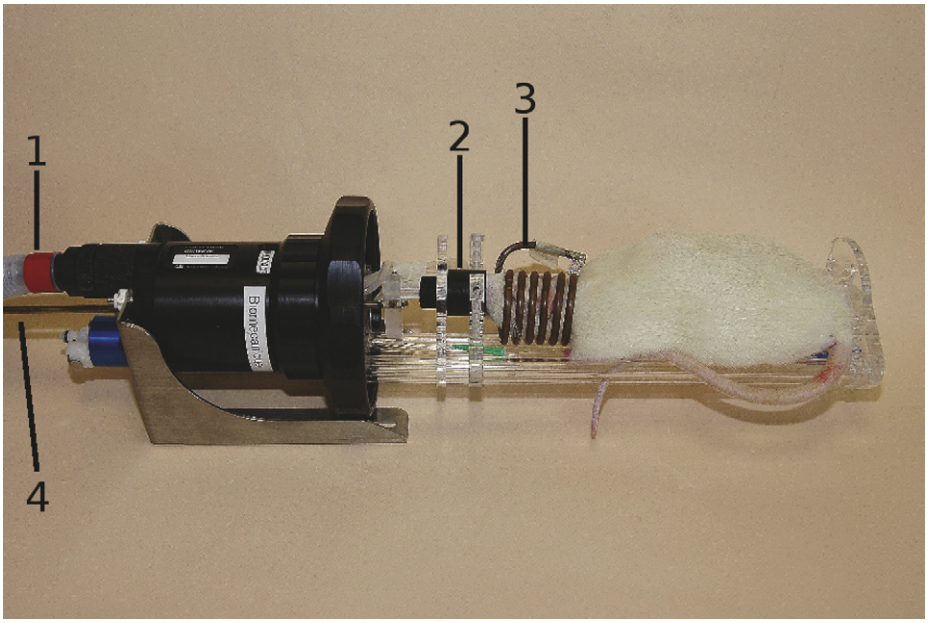

Fig. 2. Illustration of Minerve technical cell, featuring anesthesia gas pipe (1), anesthesia mask (2), RF coil (3), and excitation transmission rod (4).

(S/N) ratio, and the possible intrinsic variation of brain tissue mechanical properties from one day to another.

2.2. Animal handling and MRE

Experiments were performed on a 0.1 T resistive magnet (Bouhnik SAS, Velizy-Villacoublay, France) and excitation was generated by a modal exciter (Prodera, France) and transmitted to rat head through a non-magnetic rod. Fig. 1 illustrates the experimental setup. Rats were placed in an MR-compatible technical cell (Fig. 2) for small animal imaging (Minerve SAS, Esternay, France). This cell includes a technical channel (used here for excitation transmission), a gaseous anaesthesia mask and a bed. Mechanical excitation was transmitted to the rat head through a bite-bar (see Fig. 3). Displacement magnitude was measured by accelerometry at the output of the bite-bar without the rat ($\approx 10\,\mu m$).

Typical MRI parameters used were: TR = 900 ms, TE = 60 ms, field of view (FOV) = 70 mm × 70 mm, slice thickness 7 mm, size of matrix 128 × 96 reconstructed 128 × 128, NEX = 4, and total acquisition time of approximately 6 min for one image. Experiments were performed at a mechanical excitation frequency of $f = 180$ Hz. No phase unwrapping was performed, the final value of the phase shift being in the $[-\pi, +\pi]$ range.

2.3. Inverse problem

The wave equation inversion approach proposed here has been used in several studies on *in vivo* breast (Sinkus et al., 2005) and brain (Sack et al., 2008) tissue and is explained in detail in Oliphant et al. (2001). The starting point is the Helmholtz equation:

$$\Delta\vec{u} + k^2\vec{u} = 0 \tag{1}$$

where k is the complex wave number and $\vec{u}$ the displacement vector. Time Fourier transform of one motion component u_z of $\vec{u}$ gives

$$\Delta \mathrm{FT}_t(u_z) + k^2 \mathrm{FT}_t(u_z) = 0 \tag{2}$$

where FT_t stands for the time Fourier transform. Eq. (2) gives

$$k = \sqrt{\frac{-\Delta \mathrm{FT}_t(u_z)}{\mathrm{FT}_t(u_z)}} \tag{3}$$

Complex resolution of Eq. (3) allows determination of real (k') and imaginary (k'') parts of k. Complex resolution of equation $G = \rho(\omega^2/k^2)$ allows final determination of shear and storage moduli G' and G'', defined by $G = G' + iG''$. Time steps necessary for the time Fourier transformation were obtained through the use of phase shifting between mechanical excitation and motion-sensitizing gradient. Eight values of phase shift were used ($\theta = 0 + n(\pi/4)$, $n = 0 \ldots 7$), resulting in a total experiment time of approximately 50 min. Prior to MRE sequence, rat positioning was accurately adjusted and checked through the use of a FAST 3D gradient echo imaging sequence. The component of the wavefield used for reconstruction was the direction parallel to motion of the actuator (phase direction). Incompressibility of brain tissue was supposed, neglecting therefore the contribution of compression waves, and density was chosen to be $\rho = 1000\,\mathrm{kg\,m^{-3}}$. The Laplacian operator was implemented in its 2D discretized form. A median filter (standard deviation = 2 pixels) was applied on the G' and G'' spatial distribution images.

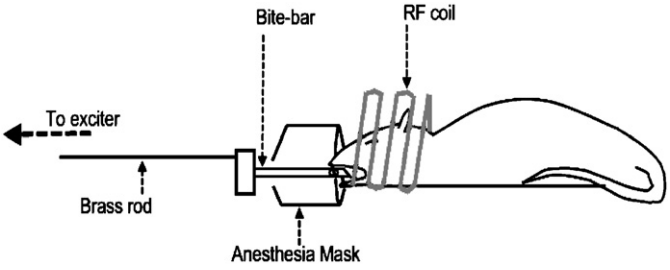

Fig. 3. Schematic representation of brass rod, anesthesia mask, and bite-bar, showing how mechanical excitation is transmitted to rat head.

2.4. Calculation of viscoelastic parameters and statistical analysis

Reported values of shear storage (G') and loss (G'') moduli were obtained by averaging inside a ROI. This ROI was selected manually on the magnitude image as the whole brain excluding brain stem and olfactive bulbs. The resulting region selection was recorded and automatically applied to G' and G'' images. Fig. 4 illustrates the procedure and shows an example of distribution maps obtained at $f = 180$ Hz *in vivo* in a rat.

One-way analysis of variance (ANOVA) was conducted on the G' and G'' data sets for the three situations, i.e., *in vivo*, at PM0.5 h and PM24 h. Tukey's HSD multiple comparison tests were performed in order to evaluate the differences between each pair of means and their significance. A p-value lower than 0.05 was considered significant.

3. Results

3.1. Control rat

Averaged values for the control rat were found to be $G' = 8450 \pm 410$ Pa and $G'' = 7140 \pm 610$ Pa, which indicates relatively good reproducibility (5% variability for G', 9% for G'') between the five experiments. Fig. 5 displays the values of G' and G'' for the five experiments.

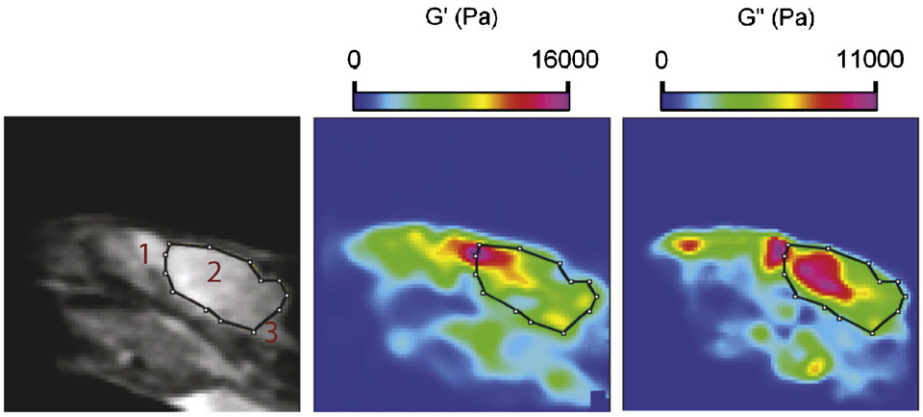

Fig. 4. Example of magnitude image (left) showing the olfactive bulbs (1), the brain (2), and the brainstem (3). Distribution maps of G' and G'', showing the ROI drawn manually on the magnitude image.

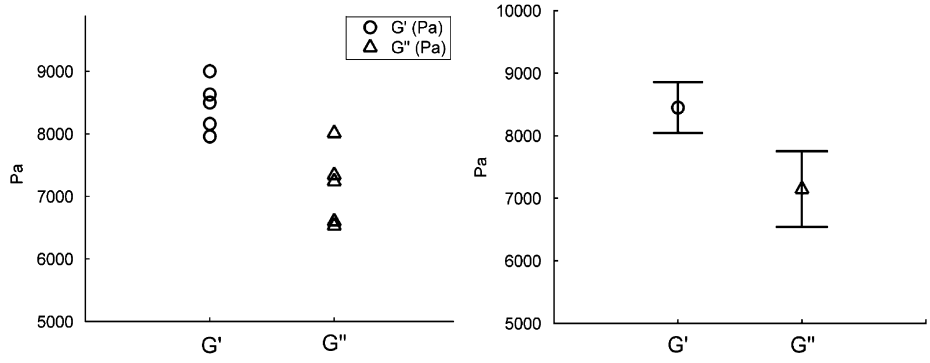

Fig. 5. Values of storage and loss moduli found at 180 Hz for control rat for the five experiments.

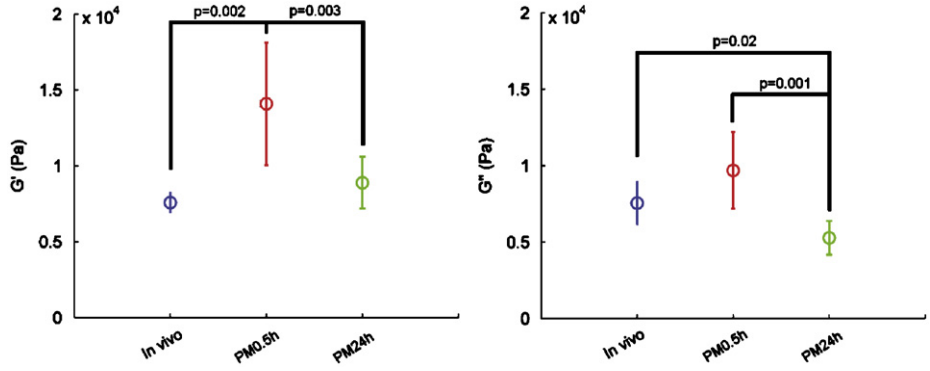

Fig. 6. Averaged values of storage and loss moduli at 180 Hz *in vivo*, at PM0.5 h and at PM24 h.

3.2. Comparison in vivo–ex vivo

The values of shear moduli at $f = 180$ Hz *in vivo*, at PM0.5 h and at PM24 h, are represented averaged among all subjects (Fig. 6). The ANOVA test led to two statistically significant conclusions:

- G' (PM0.5 h) was found significantly different from both G' (*in vivo*) ($p = 0.002$) and G' (PM24 h) ($p = 0.003$). This suggests that the storage modulus increases dramatically just after death ($\approx 100\%$), and decreases again at PM24 h.
- G'' (PM24 h) was found significantly different from both G'' (*in vivo*) ($p = 0.02$) and G'' (PM0.5 h) ($p = 0.001$). This suggests that the loss modulus decreases after death.

4. Discussion

In this study, an MRE-based method was used for the determination of *in vivo* mechanical properties of rat brain tissue, and the question of the evolution of brain tissue mechanical properties after death was addressed. This issue has a strong importance in brain biomechanics, and its investigation requires comparison to be made between living and dead brain tissue with the same protocol. For this purpose, MRE experiments were performed *in vivo* on anesthetized rats and *ex vivo* on same rats just after death and 24 h after death.

Two significant results were found: first, the shear storage modulus was found to increase dramatically ($\approx 100\%$) right after death. A possible explanation of this result could be the increase in brain volume and intracranial pressure due to vascular-related

causes. Rats were sacrificed with an intracardiac injection of barbiturate, causing chemically induced apnea and blood rush into vital organs like brain. This may lead to an increase in brain volume, blood fraction, and intra-cranial pressure. However, further investigation is necessary in order to test accurately this hypothesis, and comparison should be made between results obtained by different modes of euthanasia or with different experimental protocols. The second major result is the decrease in shear loss modulus ($\approx 50\%$) after 24 h. However, several limitations of this direct comparison should be discussed. Comparison at PM24 h was made at a non-controlled temperature that was only estimated to be close to magnet gap temperature (approximately 25 °C), which is in any case lower than *in vivo* temperature. Therefore, this temperature difference does not allow a direct comparison in exactly similar conditions. Storage conditions (refrigeration) could also possibly have an influence on the kinetics of tissue alteration and, thus, on the viscoelastic response of brain tissue after 24 h. Again, additional experiments with different storage conditions should be carried out in order to evaluate the influence of this parameter.

These results can be compared to those reported in studies that have measured living and dead brain tissue mechanical properties using invasive methods, although caution must be used because of the obvious differences in the experimental protocols. Metz et al. (1970) measured the pressure and the expansion of a cylindrical balloon filled with water and inserted inside brain tissue, defining a pumping modulus that represents the global mechanical resistance of the tissue. A successive decrease in the pumping modulus was reported between living, 5, 20, and 40 min after death, suggesting that brain tissue softens after death. However, the qualitative nature of the pumping modulus and the missing information on the reproducibility of these results make direct comparison difficult. More recently (Gefen and Margulies, 2004), brain tissue relaxation responses were tested by indentation on porcine subjects ($n = 10$) *in vivo*, *in situ* (just after death, brain still in the braincase), and *in vitro* (following *in situ* experiments, brain was extracted from braincase). The statistically significant conclusions were that both short-term and long-term shear moduli decreased between *in situ* and *in vitro* conditions, and that the long-term time relaxation constant decreased after death. General trends can be inferred from the results of both studies despite the differences in the protocols used. The significant increase at PM0.5 h is not found in the invasive study, which would corroborate the hypothesis of an intracranial pressure-related cause for the high stiffness observed. Also, a decrease in brain tissue viscosity after death is suggested by both studies, which could possibly be related to the absence of perfusion. As suggested previously, additional experiments such as MRE experiments on extracted brains could help to identify the causes of the results observed.

This study is among the first attempts to determine viscoelastic properties of brain tissue *in vivo* by MRE. The first published results on viscoelastic properties have been presented very recently. In their study, Sack et al. (2008) have measured the linear viscoelastic properties of brain tissue on six healthy human subjects at two frequencies, $f = 25$ and 50 Hz. Shear storage modulus G' was reported to be 1.17 ± 0.03 and 1.56 ± 0.07 kPa and shear loss modulus G'' to 0.49 ± 0.06 and 1.07 ± 0.06 kPa at, respectively, $f = 25$ and 50 Hz. Although no direct comparison can be made because of the different frequencies studied, a significant difference can be expected between the two studies, since these results at $f = 50$ Hz are approximately five times lower than our values at $f = 180$ Hz. Such differences can be explained not only by the fact that experiments were performed on different species, but also by the different slice directions (axial slice in their study compared to sagittal in ours). Since the final values of the shear moduli result from an average inside a ROI in the 2D image, they should depend on the slice direction because of brain heterogeneity. Larrat et al. (2007) have also reported preliminary MRE results on viscoelastic properties of the rat brain, in the 200–1000 Hz frequency range. Although no statistical information regarding the number of subjects is provided, the reported values of approximately $G' \approx 600$ and $G'' \approx 300$ Pa at $f = 200$ Hz differ considerably from our results. Again, a direct comparison between those two studies is not straightforward, not only because of the slice orientation (coronal in their study), but also because of different parameters used such as the slice thickness. Such parameters, which are related to the acquisition and the inversion algorithm, may influence the results obtained, as discussed more in detail below.

Although this MRE method has been previously shown to be in relatively good agreement with mechanical rheometry on *in vitro* porcine brain tissue (Vappou et al., 2007), other parameters may have had an important influence in the present study that had a more limited effect in large, *in vitro* porcine brains. Neglecting compression waves and reflected waves interfering with incident waves might not be justified, especially in a confined, small geometry like the rat brain. This is particularly true here since the wavelength is similar to the dimension of the whole brain itself. Increasing the excitation frequency—and hence decreasing the wavelength—could partially overcome this limitation, but in our case, this would result in an increase in the attenuation and, thus, in a decrease in the phase S/N ratio. Other parameters may affect the results obtained, such as the use of a 2D Laplacian instead of its 3D formulation, and the effect of the slice thickness, which results in an averaging effect of the displacement over the entire slice depth. In addition, the choice of the ROI may affect the final values, as a consequence of the considerable variability that was found inside the same brain slice in terms of G' and G''. However, this source of variability was limited by selecting the same ROI, i.e., the whole brain without the olfactive bulbs and the brain stem. All these parameters may have had a significant influence on the values that have been reported in this study. Studying them in detail goes beyond the scope of this paper, and ongoing work is currently focused on quantitatively evaluating their effects as well as determining phase S/N threshold values. This investigation is being performed on both numerical and physical phantoms. However, the purpose of this study was to compare the viscoelastic properties between *in vivo* and *ex vivo* brain at different post-mortem times using the same method. Thus, as a first approximation, possible errors resulting from aforementioned limitations have a systematic nature and should not affect the basic conclusions regarding relative comparisons.

5. Conclusions

Presently, MRE provides a unique means for non-invasively measuring the viscoelastic properties of brain tissue. This study addresses the question of possible alteration of brain tissue mechanical properties after death by using MRE. Preliminary results concerning the evolution of shear storage and loss moduli during the first 24 h post-mortem were presented here. Many additional experiments can be proposed in order to more accurately investigate this issue, including longitudinal studies that would allow us to evaluate the evolution of brain tissue mechanical response versus post-mortem time. More generally, this study also shows the ability of MRE to investigate essential questions of soft tissue biomechanics by providing the possibility of non-invasively measuring their viscoelastic properties under different conditions.

Conflict of interest statement

All authors disclose any financial and personal relationships with other people or organizations that could inappropriately have influenced their work.

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