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Supplementary Material

Supplement to Material and Methods

Physical parameters of US waves

A sketch of an experimental setup for TFUS is shown in Figure 2A, using the stimulation of a rat as example. The ultrasound pressure wave is generated by an ultrasound transducer and delivered to the target through a guide filled with acoustic gel. At any point in space, the ultrasound pressure is a sinusoidal temporal wave (Figure 2B) with a certain center frequency (f_c). It is usually applied at frequencies f_c between 200 kHz to 650 kHz in order to allow the soundwave to pass through the skull without being completely absorbed. At the parameter ranges interesting for human application, distortions of the sinusoidal wave shape due to non-linear effects are negligible. This can be easily seen when estimating the amount of non-linearity, as e.g. described in the International Electrotechnical Commission (IEC) standards for ultrasound measurements [1, 2]. A burst of waves of duration TBD (tone burst duration) is triggered, followed by an inactive period (Figure 1B). The distance between two bursts is called PRP (pulse repetition period) and a batch of consecutive bursts is a sonication (with a particular SD, sonication duration). The ratio between the TBD and the PRP defines the duty cycle (DC), usually expressed in percentage. A stimulation can consist of one or more sonications, repeated at a certain ISI (inter sonication interval). At a specific position in space, the instantaneous intensity (Figure 2C) is given by

$$I_i(t) = \frac{p^2(t)}{\rho c}, \quad (1)$$

where $p(t)$ is the acoustic pressure, ρ is the mass density of the medium and c is the speed of sound in the medium. The intensity can be averaged over the entire time interval (I_{ta}, temporal average intensity) or only over the TBD (I_{pa}, pulse average intensity). If these values are calculated at the position of the spatial maximum, they are called I_{spta} (spatial peak temporal average intensity) and I_{sppa} (spatial peak pulse average intensity).

I_{spta} can be mathematically expressed as

$$I_{spta} = \frac{1}{PRP}\int_0^{PRP} I_i(t, \vec{r}_{max})dt, \quad (2)$$

where $\vec{r}_{max}$ denotes the position of maximum intensity. For an arbitrary pulse waveform, I_{sppa} can be expressed as

$$I_{sppa} = \frac{1}{PD}\int_{t_{10\%}}^{t_{90\%}} I_i(t', \vec{r}_{max})dt', \quad (3)$$

where $t_{10\%}$ and $t_{90\%}$ are the time points where 10% and 90% of $\int_0^{PRP} I_i(t, \vec{r}_{max})dt$ is reached. PD (pulse duration) is defined as 1.25 × ($t_{90\%} - t_{10\%}$). In the papers considered in this review, I_{spta} is calculated as I_{sppa} × DC during the sonication (the exception is [3], which calculated I_{spta} as I_{sppa} × SD/ISI).

The above measures are usually determined from measurements of the TFUS pressure profile using a hydrophone in a water-tank. Skull samples are used as standard procedure to estimate the TFUS intensity in the brain after transmission through the skull bone. As this cannot account for interindividual variability, the attenuation through the temporal bone window (the thinnest area of the lateral skull) of a sample could for example be used as the worst-case scenario. This is a conservative strategy to ensure safety for all skull regions, but it will lead to low intensities in many subjects with thicker skulls. Alternatively, computer simulations have been employed, but their accuracy and precision require further testing [4, 5].

Safety regulations for diagnostic US

To quantify the possible harmful effect of cavitation and temperature increase in tissues, two main indices have been introduced. An estimation of the likelihood of inertial cavitation, which is a threshold phenomenon, is given by the mechanical index (MI), defined as

$$MI = \frac{p_r}{\sqrt{f_c}}, \quad (4)$$

where p_r is the peak rarefaction pressure expressed in MPa, and f_c is the center frequency expressed in MHz. MI is dimensionless, so a multiplication of a factor $\sqrt{1\ MHz}/_{1\ MPa}$ is implied (it should be noted that equation 4 is used as stated in TFUS studies, while p_r is usually derated by 0.3 dB cm^{-1} MHz^{-1} in diagnostic US to account for the difference between in-water and in-tissue acoustic attenuation). This is complemented by a thermal index (TI) to characterize the steady-state temperature increase in soft tissue during continuous sonication

$$TI = \frac{W_p}{W_{deg}}, \quad (5)$$

where W_p is the time-averaged acoustic power of the source and W_{deg} is the power needed to raise the temperature in the target tissue by 1°C, based on thermal models that are specific to the tissue type. When the

bone is close to the transducer surface, the ultrasound power is assumed to be completely absorbed by the skull and a different formulation of the thermal index (called TIC, thermal index for cranial bone) is used [6]:

$$TIC = \frac{W_0/D_{eq}}{40\ mW\ cm^2}. \quad (6)$$

W_0 is the total output power from the ultrasound source in mW, and D_{eq} is the equivalent aperture diameter given by $\sqrt{\frac{4}{\pi}A_{aprt}}$ (A_{aprt} is the aperture area of the source) [7].

The safety guidelines for diagnostic ultrasound devices published by the FDA (Food and Drug Administration) [8] are based on MI, TI, I_{spta} and I_{sppa}. Table 1 states the limits for those indices. It should be noted that there are further guidelines for other ultrasound applications, having different limits. For example, IEC standard 60601-2-5 for physiotherapy US equipment sets an upper limit for the "effective intensity'', defined as the ratio of acoustic output power to effective radiating area, of 3 W/cm^2. The standard also states that this value should only be reached for short times to prevent substantial heating. The "effective intensity'' of 3 W/cm² is usually interpreted as upper limit for I_{spta} [9, 10, 11]. Lee and colleagues [9] compare the intensities used in their study against this limit rather than using the FDA guidelines for diagnostic US.

Estimations of temperature increases caused by TFUS

Complementary to TI, the theoretical temperature increase at the TFUS focus point has been reported. For that, the following simplified formula was used that is based on the assumption that no heat is lost by conduction, convection or other heat removal processes [12]:

$$\Delta T_{max} = \frac{\dot{Q}\Delta t}{C_v} \quad (7)$$

Variable Δt is the time duration of exposure and C_v is the medium's heat capacity per unit volume ($C_v = \rho\ C_p$, with ρ being the mass density and C_p the heat capacity per unit mass). $\dot{Q}$ is the rate of heat generation per unit volume and is given by

$$\dot{Q} = 2\alpha I_{TA}, \quad (8)$$

where α is the ultrasonic amplitude absorption coefficient and I_{TA} is the temporal-average intensity. In order to find the maximum of $\dot{Q}$, it should be calculated using the I_{SPTA}. It is not clear which parameter of the TFUS should be regarded as the time duration of exposure, since two studies used TBD [13, 14], some others SD [15, 9, 16, 11], one SD x duty cycle [17], and one 1/10*SD x duty cycle [18]. A more accurate model for the temperature distribution that accounts for heat conduction and the effects of blood flow is the bio-heat equation [19], used in a recent preprint work [20].

Risk of bias

In this paragraph, we summarize possible causes of bias in the findings on TFUS safety, in terms of the treatment given to the sample, the sample itself and the method to assess safety. In most of the cases, safety assessment was performed on small animals (rodents, in particular rats), and the need to extrapolate the findings from animals to humans might bias our interpretation of the safety of stimulation settings used in humans, because of the different thickness of skull and the reverberation that might occur inside skull cavities in small animals. The length of follow-up might also be a source of bias, especially for unwanted long-term effects. In addition, within the same species, different sets of sonication parameters were applied (**Error! Reference source not found.**), and their influences on safety assessment were not confirmed by repeated experiments and damage was not assessed for all subjects. These confounds, together with possible human limitations in reading results (for example from histology) or limitation of the employed methods (e.g. spatial resolution of MRI) can give false negatives. On the other hand, false positives can occur when damages do not arise from TFUS treatment. For this reason, studies using animal models of stroke or epileptic activity [11, 21] performed histological analysis in control subjects, who underwent only TFUS treatment (**Error! Reference source not found.**). Regarding animal experiments, the environment, housing, and management are not likely to influence the results concerning anatomical changes or damage, but might affect the outcome of behavioral assessment. In human experiments, follow-up questionnaires and interviews cause a response bias that can be countered using sham stimulation in blinded trials, preferably double-blinded. Moreover, it is worth noting that 22 out of 79 papers eligible for this review didn't assess safety or harmful effects in any way (**Error! Reference source not found.**), which represent a lack of data.

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	#1 – the review is a systematic review without meta-analysis of studies in both animals and humans, as indicated in the title
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	#1- the applicable information are listed in the abstract section. 'Data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis method; study appraisal and synthesis methods' are summarized in the 'methods' section. 'Limitations; conclusions and implications of key findings' are in the 'conclusion' section. Given the early stage of TFUS intervention and the available data on its putative harmful effects, a systematic review registration number is not available and the authors decided not to register it.
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	#1 and 2-the rationale is described in the 'Introduction' section
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	#1 and 2- the objective are indicated in the last paragraph of the 'introduction' method. Given the early stage of TFUS intervention, a comparison with known techniques or several TFUS parameters was not possible. The authors also added an overview on how the stimulation parameters are defined, and an explanation on the technique used to assess safety, because it might be helpful to further investigate TFUS safety.
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	Given the early stage of TFUS intervention and the available data on its possible harmful effect, an existing systematic review registration number was not available and the authors decided not to register it.
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Explained in 'Literature review on the safety of TFUS' (#2) and Figure 1 (#3).

Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Explained in 'Literature review on the safety of TFUS' (#2) and Figure 1 (#3).
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Explained in 'Literature review on the safety of TFUS' (#2).
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Explained in 'Literature review on the safety of TFUS' (#2) and Figure 1 (#3).
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Explained in 'Literature review on the safety of TFUS' (#2), tables 2 and 3 (#4-8)
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Explained in 'Literature review on the safety of TFUS' (#2) and showed in tables 2 and 3 (#4-8)
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Given the early stage of the investigation of TFUS safety, a systematic risk of bias assessment was not possible. However, we summarize the possible risks of bias in 'risk of bias' (#4 of Supplementary materials)
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Not applicable: meta-analysis not performed
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	Not applicable: meta-analysis not performed
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Given the early stage of the investigation of TFUS safety, a systematic risk of bias assessment was not possible. However, we summarize the possible risks of bias in 'risk of bias' (#4 of Supplementary materials)
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Not applicable: meta-analysis not performed
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Described in 'studies screened in this review' (#10) and Figure 1 (#3).
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and	Described in 'studies screened in this review' (#10) and Table 2 and 3 (#4-8).

		provide the citations.	
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Given the early stage of the investigation of TFUS safety, a systematic risk of bias assessment was not performed.
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Not applicable: meta-analysis not performed. A general overview on the data extracted is in Tables 2 and 3 (#4-8) and the results extracted in the sections ' BBB opening', 'bleeding',' Cell damage or death, 'change of neural activity',' Animal behavior', 'Temperature', ' Findings from human studies'. (#10-12)
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Not applicable: meta-analysis not performed
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Given the early stage of the investigation of TFUS safety, a systematic risk of bias assessment was not possible.
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Not applicable: meta-analysis not performed
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	This is discussed in the section 'Discussion and Conclusions' (#12-13)
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	This is discussed in the section 'Discussion and Conclusions' (#12-13)
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	This is discussed in the section 'Discussion and Conclusions' (#12-13)
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	#13: Described in 'acknowledgements'

Table S1: This table shows details on our implementation of the PRISMA procedure for reporting systematic reviews [22, 23].

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