MRI-related anxiety can induce slow BOLD oscillations coupled with cardiac oscillations

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Abstract
Objective: Although about 1–2% of MRI examinations must be aborted due to anxiety, there is little research on how MRI-related anxiety affects BOLD signals in resting states.
Methods: We re-analyzed cardiac beat-to-beat interval (RRI) and BOLD signals of 23 healthy fMRI participants in four resting states by calculation of phase-coupling in the 0.07–0.13 Hz band and determination of positive time delays (pTDs; RRI leading neural BOLD oscillations) and negative time delays (nTDs; RRI lagging behind vascular BOLD oscillations). State anxiety of each subject was assigned to either a low anxiety (LA) or a high anxiety (HA, with most participants exhibiting moderate anxiety symptoms) category based on the inside scanner assessed anxiety score.
Results: Although anxiety strongly differed between HA and LA categories, no significant difference was found for nTDs. In contrast, pTDs indicating neural BOLD oscillations exhibited a significant cumulation in the high anxiety category.
Conclusions: Findings may suggest that vascular BOLD oscillations related to slow cerebral blood circulation are of about similar intensity during low/no and elevated anxiety. In contrast, neural BOLD oscillations, which might be associated with a central rhythm generating mechanism (pacemaker-like activity), appear to be significantly intensified during elevated anxiety.

Significance: The study provides evidence that MRI-related anxiety can activate a central rhythm generating mechanism very likely located in the brain stem, associated with slow neural BOLD oscillations.

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1. Introduction

Symptoms of anxiety have been associated with elevated arousal and physiological activity in diverse laboratory studies (e.g., Egloff et al., 2002; Pointer et al., 2012; Tumati et al., 2021) and have been related to distinct resting state functional connectivity (RSFC) changes (e.g., Takagi et al., 2018 Tumati et al., 2021). Furthermore, even minor increases in anxiety during sitting in a quiet environment have been related to comparably small respiratory changes...
Pacemaker neurons play an important role in the central nervous system (see review, Ramirez et al., 2004). One well-documented example of pacemaker neurons is the pre-Bötzinger complex responsible for respiratory rhythm generation in two brainstem regions (Katz et al., 1994; Dewey et al., 2007) and 1–2% of these examinations must be aborted due to claustrophobia (Munn et al., 2015). Presumably, that anxiety is normally distributed, it could be assumed that in addition to the 1–2% of extreme high anxiety resulting in termination of scanning, the majority of MRI participants may display intermediate levels of anxiety and a minority no/low anxiety. Importantly, anxiety may impact the findings of brain imaging in multiple ways. Although many high-level studies on MRI resting state are available (e.g., Shokri-Kojori et al., 2018; Sobczak et al., 2020; Valenza et al., 2020), to the authors’ knowledge none examined MRI-related anxiety. One possible reason for this could be the complexity of the BOLD signal (Buxton et al., 2004; Murphy et al., 2013), including the neurovascular and neurometabolic coupling. Importantly, neural activity potentials provoke both an increase in blood flow (vascular BOLD) due to neurovascular coupling and an increase in oxygen consumption due to metabolic coupling (neural BOLD; Logothetis et al., 2001; Arthurs and Boniface, 2002; Huneau et al., 2015). An example for metabolic or neuro-BOLD coupling (NBC) is the entrainment of arteriolar vasomotor fluctuations by neural activity with an NBC time of 2.6 s in awake mice (Mateo et al., 2017). Noteworthy, vascular BOLD oscillations have their origin not only in rhythmic neural activity fluctuations, but preferentially in the baroreflex loop driven by cerebral blood flow velocity (CBFv) oscillations in the main cerebral arteries (Diehl et al., 1998). Those precede blood pressure and cardiac interval oscillations by ~1–2 s and dominate the resting state activity (Tong and Frederick, 2014; Shokri-Kojori et al., 2018).

Rhythmic neural activity either in cortical, subcortical or brainstem regions can control cardiac function, such as beat-to-beat intervals (RRI) very fast (see review, Thayer and Lane, 2009) and become manifest also in RRI signals. Neural activity oscillations precede RRI oscillations for a short period of time (<0.3 s), but are associated with clearly delayed neural BOLD oscillations due to NBC. Investigations of the subject-specific maximal time delay between RRI and BOLD oscillations revealed a mean (+SD) of 2.2 ± 0.2 s (Pfurtscheller et al., 2020).

Pacemaker neurons play an important role in the central nervous system (see review, Ramirez et al., 2004). One well-documented example of pacemaker neurons is the pre-Bötzinger complex responsible for respiratory rhythm generation in two brainstem areas (Menuet et al., 2020). Another example of rhythmic burst activity are reticular neurons in the brain stem (Lambertz and Langhorst, 1998). This type of bursting activity acts as a generator for the “0.15–Hz rhythm” (Perlitz et al., 2004) and may be identical with the “pacemaker-like activity” related to Mayer waves (Julien, 2006) and a recently postulated central pacemaker in brainstem responsible for control of cardiovascular-respiratory oscillations (Pfurtscheller et al., 2020). However, it is well possible that there are several pacemakers in the brainstem region acting in a frequency range around 0.15 Hz. Such a central pacemaker in the brainstem is associated with rhythmically changing local field potentials and thus, is a major determinant of BOLD oscillations (Logothetis et al., 2001; Arthurs and Boniface, 2002; Huneau et al., 2015). Support for the existence of a 0.15–Hz rhythm came from the study by Keller et al. (2020). They reported correlations of BOLD signals in the 0.12–0.18 Hz band in insula and secondary somatosensory area (regions related to interoceptive perception) with HRV power in the mid-frequency band 0.12–0.18 Hz.

At least three issues should be acknowledged for MRI-related anxiety studies: (i) to analyze not only BOLD signals but also cardiac interval or pulse interval fluctuations, due to the close interaction of brain and heart (see review, Thayer and Lane, 2009), (ii) not to restrict MRI resting state BOLD signals to frequencies below 0.1 Hz (Shokri-Kojori et al., 2018; Sobczak et al., 2020; Valenza et al., 2020) as suggested by the Washington University School of Medicine (Snyder and Raichle, 2012) and (iii) to study cardiac or pulse interval fluctuations not only in the recommended standard low and high frequency bands (Task Force 1996), but also in intermediate bands, in order to enable investigation of the cardiovascular 0.15-Hz rhythm (Perlitz et al., 2004).

Our main aim was to analyze BOLD and RRI oscillations during a first-time exposure of healthy subjects to fMRI scanning and to relate the time delays between the two signals to state anxiety assessed by questionnaire. In our first studies about phase coupling between RRI and BOLD oscillations, either only four regions of interest (ROIs) (precentral cortex and midcingulum, right and left) or two resting states (R1, R2) were considered (Pfurtscheller et al., 2017, 2018). Furthermore, in these prior studies some RRI time series still contained some MRI-artefacts due to high scanning rate (Kugel et al., 2003). Therefore, a further revision of the RRI time series by KUBIOS software (Tarvainen et al., 2014) was realized. In the present paper, re-processed phase-locking values (PLVs) in the band 0.07–0.13 Hz for 24 cortical and subcortical ROIs were used for 23 subjects and 4 resting states separately for low/no anxiety and moderate anxiety, respectively.

More specifically, the goals of this research were (i) to investigate the impact of MRI-related anxiety on slow BOLD oscillations in cortical and subcortical regions with a particular focus on low/no and moderate anxiety and (ii) to investigate the relationship between low/no anxiety and BOLD oscillations resulting only from cerebral blood oscillations (vascular BOLD).

2. Material and method

2.1. Subjects and experimental paradigm

A total of N = 23 participants (12 female, 22 right-handed) between 19–34 years (M = 24, SD = 3.2 years) took part in the study. Participants were naïve to the purpose of the study, had no former MRI experience and were without any record of neurological or psychiatric disorders (assessed by self-report). All participants gave informed written consent to the protocol of the study, which had been approved by the local Ethics Committee at the University of Graz.

The experimental design consisted of two sessions separated by about 50 minutes and four resting states (R1, R2, R3, R4). Due to the time-consuming scanning procedure and the necessity to restrict movements to the absolute minimum, a break seemed mandatory. The first session started with the first questionnaire to assess state anxiety score (AS) followed by the first resting state recording (R1). We decided to obtain anxiety ratings prior to the beginning of the recordings to capture also anticipatory anxiety. In the subsequent resting states, we were primarily interested in the experience of anxiety during the scanning. Thereafter, two movement tasks (self-paced button press and visual stimulus-paced button press in 10-s intervals, each lasting 600 s) were performed. The purpose of the movement tasks was to examine whether self-paced movement (movement at free-will) is associated with slow cortical excitability fluctuations and whether stimulus-paced movement at the resonance frequency of the baroreflex loop results in elevated responses. The first session ended with the second resting state (R2) and the second assessment of AS. The second session was a duplicate of the first one, with two resting states (R3, R4) and two questionnaires. Individuals were requested to keep their eyes open, to stay awake, and to...
avoid movements during the resting state recordings. Each resting state had a duration of 350 s and each AS assessment about 300 s.

AS was assessed with the state-trait anxiety and depression inventory (STADI; Laux et al., 2013). The STADI is an instrument constructed to assess both state and trait aspects of anxiety and depression. It is based on the State-Trait Anxiety Inventory (Spielberger et al., 2009). The items were presented on a screen within the scanner. Items were answered via a trackball.

2.2. Cardiac beat-to-beat intervals and respiration

The electrocardiogram (ECG) was recorded inside the scanner using the Siemens Physiological ECG Unit. For the positioning of the ECG electrodes on the thorax, standard channels (Siemens Standard, lead 1) were used. The respiratory data were acquired by using a pneumatic cushion, which is connected via an air hose to a pressure sensor on the PERU-unit. The cushion was attached to the subject by using a respiration belt. The sampling rate was 400 Hz. The fMRI plug-in for EEGLAB (Niazy et al., 2005) was used to detect ECG beat-to-beat complexes. Within this tool, the FASTR algorithm (for removal of gradient-induced artifacts) and the beat-to-beat detection algorithm were used in succession, resulting in beat-to-beat (RRI) time courses. ECG recording during MRI with high scanning rate results in reduced quality of the cardiac signal (Kugel et al., 2003). Therefore, the RRI signals were re-processed using the KuBioS HRV Premium Package (Kubios Ltd. Finland; version 3.0.2; Tarvainen et al., 2014). RRI's were then interpolated to the same sampling frequency as the BOLD acquisitions (1/871 ms⁻1). Finally, all signals were resampled at 10 Hz.

2.3. fMRI recording and ROI-based BOLD analyses

Functional images were acquired with a 3 T scanner (Magneton Skyra, Siemens, Erlangen, Germany) using a multiband GE-EPI sequence (Moeller et al., 2010) with a simultaneous six-band acquisition with TE/TR = 34/871 ms, 52° flip angle, 2 × 2 × 2 mm³ voxel size, 66 contiguous axial slices (11 × 6), acquisition matrix of 90 × 104 and a FOV of 180 × 208 mm². For further details see Pfurtscheller et al. (2017). The AAL atlas (Tzourio-Mazoyer et al., 2002) was used to extract time courses from 24 ROIs: precentral gyrus (1, 2), middle frontal gyrus (7, 8), middle frontal gyrus, orbitaL part (9, 10), supplementary motor area (19, 20), superior medial frontal gyrus (23, 24), medial frontal gyrus, orbitaL part (25, 26), insula (29, 30), anterior cingulum (31, 32), midcingulum (33, 34), posterior cingulum (35, 36), amygdala (41, 42), and precuneus (67, 68). The numbers indicate the ROI labels according to the AAL atlas. Odd and even numbers denote left and right hemispheres, respectively. In addition, a few ROIs from pons/brain stem (95, 93, 103) were also extracted.

Wavelet transform coherence was applied to the BOLD and RRI time series using the “Cross Wavelet and Wavelet Coherence toolbox” (Grinsted et al., 2004). Based on a Morlet wavelet the phase-locking value (PLV) was calculated for frequencies between 0.07 and 0.13 Hz (Lachaux et al., 1999). The obtained PLV values were the basis for the distinction between negative (nTDs) and positive time delays (pTDs). The percentages of significant (p < 0.05) time samples, or bins (%sigbins), which indicate the total length of a significant phase-locking episode, were also computed in each case. A time delay (TD) was considered reliable, when the associated significant length of phase coupling (sigbin, given in %) was equal to, or larger than 10% (percentage of samples within the time series; arbitrary threshold). For further details see Pfurtscheller et al. (2017).

3. Results

3.1. State anxiety

Anxiety score (AS; possible range of 10–40) declined from the first (R1: AS = 19.96 ± 4.52) to the last resting state (R4: AS = 14.04 ± 4.02) with a considerable variability not only between participants, but also intra-individually between resting states (Fig. 1A). As Fig. 1A indicates, not all subjects showed a decline to AS = 10. About 20% of the participants still displayed a moderate anxiety in R4. In order to obtain a better insight into the dynamics of AS, two anxiety categories were generated based on the highest and lowest score of each individual across the four resting states. Specifically, the lowest score of each individual was assigned to a low anxiety (LA) category (AS = 13.5 ± 3.1) (Fig. 1B) and the highest score of each individual was assigned to a high anxiety (HA) category (AS = 20.9 ± 4.8) (Fig. 1C). For each subject, only resting states with the highest and the lowest anxiety rating were analyzed. It should be noted that the distributions of LA and HA categories differed. A compressably small range (AS = 10–15) was observed within the LA category (Fig. 1B) and a comparably broad range (AS = 15–30) within the HA category (Fig. 1C). Of note, given a possible range of scores from 10 to 40, AS in the HA category indicated rather moderate levels of anxiety as there were no scores in the range 30–40. Noteworthy, in a minority of the subjects (10–20%) no anxiety decline was observed.

3.2. Relationship between slow cardiac interval (RRI) and BOLD oscillations

The association between slow BOLD and cardiovascular oscillations is of current interest in studies of fMRI and autonomic network activity (e.g., Shokri-Kojori et al., 2018; Keller et al., 2020; Valenza et al., 2020). However, studies analyzing RRI intervals and unfiltered BOLD signals with a high scanning rate are limited, because it is not possible to entirely remove all cardiac artefacts and confounds (Kugel et al., 2003; Murphy et al., 2013). Fig. 2 shows RRI time series and unfiltered BOLD signals of 100 s duration from one characteristic subject and two resting states (R1, R2) with elevated anxiety (Fig. 2A and C) and low anxiety (Fig. 2B and D), respectively. Corresponding HR spectra from the total recording period of 300 s duration are displayed in Fig. 2C and D.

Although the RRI and BOLD signals are composed of waves of varying duration, the signals indicate similar patterns for both HA and LA. However, there were anxiety-related differences between the two resting state recordings with respect to the time shift between RRI and BOLD signals (indicated by stippled lines in Fig. 2A and B) and the peak maxima in the HR spectra. Basically, for HA the BOLD signal lags the RRI signal (characteristic for pTD) and the maximal spectral power is above 0.1 Hz (Fig. 2C). In contrast, for LA the BOLD leads RRI signals (characteristic for nTD) and the maximal spectral peak is concentrated below 0.1 Hz (Fig. 2D). Such patterns with clear RRI and BOLD signals and HRV spectra with dominant LF components in Fig. 2 were only found in few subjects,
Fig. 1. (A) Intraindividual trajectories of anxiety scores (AS) of 23 healthy subjects across resting states. Indicated (circle) are 3 subjects with AS ≥ 20 in all resting states. (B) Distribution of state anxiety of 23 healthy subjects with low/no anxiety (LA) and (C) for moderate anxiety (termed high anxiety category). Note, all subjects in the high anxiety (HA) category reported AS between 15–30 (ellipse of stippled lines) and 20 subjects in the LA (category ellipse of stippled lines) reported AS between 10–15. Three healthy subjects out of 23 (circle) indicated in no resting state a low anxiety.

Fig. 2. Examples of slow beat-to-beat interval (RRI) time series [s] from thorax and BOLD oscillations [arbitrary units, a.u.] from left cingulum (see arrow to insert E) of one characteristic subject (s18) from two resting states (R1, R2). (A) data during elevated anxiety (AS = 28) and (B) data during low anxiety (AS = 13). (C) Heart rate (HR) spectrum for elevated anxiety with a dominant peak at 0.11 Hz (calculated by Kubios HRV Premium Package). (D) HR spectrum for low anxiety with a dominant peak at 0.09 Hz. The color indicates the ranges 0.06–0.1 Hz (gray), 0.1–0.14 Hz (red) and 0.14–0.5 Hz (green). In the case of RRI and BOLD signal, coherent peaks are connected by stippled lines.
but significant positive cardiac-BOLD phase coupling (elevated neural BOLD components) in about 50% of the sample (see, Pfurtscheller et al., 2020).

3.3. Distributions of TDs for LA and HA categories

After considering all available, reliable TD values, we used subgroups of TDs to form histograms for both anxiety categories (Fig. 3A and B). We compared the means of pTDs and nTDs between anxiety categories using t-tests (Table 1). While nTDs did not differ between anxiety categories (t(344) = 0.549, p = .584), pTDs were significantly higher for HA as compared to LA (t(311.70) = 2.64, p = .009).

In a next step, Hartigan’s dip test statistic was used to analyze unimodality for LA and HA categories separately. For LA, the test statistic D was not significant (D = 0.019, p = .554), thus suggesting unimodality of TDs. Importantly, for HA, there was strong evidence for multimodality (D = 0.035, p = .001), indicating that TDs were composed of different distributions with a difference of 2.3 s (see Table 1).

4. Discussion

Studying phase-coupling between RRI time courses and BOLD signals in more than one resting state revealed a bimodal distribution of TDs characterized by two peaks separated by ~2.3 s in the case of moderate anxiety (HA category). This indicates that slow vascular oscillations precede neural oscillations by ~2.3 s. In contrast, in the case of low/no anxiety (LA category), the distribution of TDs was unimodal with a dominance of vascular BOLD oscillations (as indicated by predominant nTDs).

4.1. Anxiety in the scanner

Being placed in a narrow, noisy space within the scanner can induce compromised emotional wellness, feelings of fear and even claustrophobia (Chapman et al., 2010; Munn et al., 2015), particularly in individuals exposed to MRI scanning for the first time in their life. The LA category with a comparably small range may be considered typical for low/no anxiety and situations without fear or minimal negative affect. Importantly, the HA category covered the range from moderate to more severe anxiety, without including extraordinary high levels of anxiety (i.e., none of the subjects indicated a AS > 30). Thus, caution is warranted when generalizing findings to high or even clinical levels of anxiety (or claustrophobia). The difference in TDs between LA and HA categories suggests that with moderately elevated anxiety, additional physiological mechanisms or processes are engaged possibly facilitating the processing of unpleasant emotions. Hence, it could be expected that considerable changes in resting state brain activity may occur from one resting state to another. A representative example is depicted in Fig. 2 with AS = 28 (elevated anxiety) and pTD (neural BOLD oscillations) in the first resting state R1 and AS = 13 (low anxiety) and nTD (vascular BOLD) in R2.

4.2. Dominance of nTDs with low/no anxiety

The selection of resting state sessions from all 23 subjects with LA (AS: M = 13.5 ± 3.1) revealed a unimodal TD distribution (Fig. 3A) with a dominant peak at nTD = −1.0 ± 0.7 s. Because this category showed a unimodal distribution and also included three subjects with AS = 10 (the lowest level in the scale used), this category perhaps matches “normal”, anxiety-free BOLD recordings and situations without any fear (Fig. 1C), characterized by a dominance of vascular oscillations (nTD). Notably, the lead of the BOLD signal before cardiac changes, which is characteristic for nTD, is confirmed by the observation of Shokri-Kojori et al. (2018) that slow fluctuations of pulse time interval differences follow BOLD signals in the band 0.01–0.09 Hz by −1.2 rad ± 1.7 rad (1 rad × 180/π = 57.3°) in resting states. This −1.2 rad corresponds to −1.9 s in the case of 0.1 Hz and is similar to the nTD of −1.0 s (Table 1) in our study. The unimodal TD distribution during low-anxiety or anxiety-free BOLD recordings (Fig. 3A) supports the findings of Tong and Frederick (2014, page 6) that “physiological low frequency oscillations in BOLD are mostly – perhaps even solely – associated with spatial patterns that result from cerebral blood circulation.”

The histograms in Fig. 3A and B illustrate that all TDs are within −3 s and +3 s, which is reasonable for nTDs, because the range of 0.1-Hz oscillations in heart rate and blood pressure is between zero and −90° (corresponding to nTD = −2.5 s during 0.1 Hz) and limited due to cerebral autoregulation (Zhang et al., 1998). Diehl et al. (1998) reported a delay between cerebral blood flow velocity (CBFv) in the middle cerebral artery (MCA) and arterial blood pressure waves in the finger of 70.5 ± 29° during slow stimulus-paced breathing at 6/min (resonance breathing). Beside nTDs, pTDs are also limited within −3 s and +3 s due to the NBC time of 2–3 s (Mateo et al., 2017; Pfurtscheller et al., 2020).

Notably, in the specific example in Fig. 2B the RRI time course seems to correlate more strongly with the BOLD signal for the LA condition as compared to the HA condition. This is not unexpected because during low or no anxiety the vascular BOLD oscillations are dominant, whereas during elevated anxiety beside vascular also neural BOLD oscillations appear.

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**Fig. 3.** Histograms of time delays between slow spontaneous BOLD and beat-to-beat interval (RRI) oscillations in the band 0.07–0.13 Hz for negative (nTD) and positive time delays (pTDs). Data from 23 subjects, 24 regions of interest (ROIs) and four resting states are displayed for low anxiety (LA) category (Fig. 3A) and for high anxiety (HA) category (Fig. 3B). Note, the limitation of TDs within −3 s and +3 s in both histograms and the difference between means of pTD (1.34 s) and mean nTD (−0.97 s) for HA category of 2.3 s.

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Anxiety category (AC), state anxiety (AS), positive and negative time delays (pTD, nTD) in (s) (means (M) and standard deviation (SD) for “high anxiety” (HA) category and “low anxiety” (LA) category; N number of time delays; p values in the bottom row indicate the significance of differences (Diff) between HA and LA categories. The difference between pTD and nTD for the high anxiety category (HA) is 2.3 s.

| AC      | AS  | Positive Time Delay | Negative Time Delay |
|---------|-----|---------------------|---------------------|
|         | M   | SD                  | M   | SD | N   | M   | SD |
| HA      | 20.87 | 4.83               | 1.34 | 0.65 | 221 | −0.97 | 0.56 | 168  |
| LA      | 13.48 | 3.07               | 1.14 | 0.76 | 161 | −1.01 | 0.65 | 178  |
| Diff    | 7.39  | 0.20               | 0.20 | 0.009 | 0.009 | 0.06 | 0.584 |
| p       | <0.001 |                   |                   |                   |                   |

### 4.3. Dominance of nTDs and pTDs with elevated levels of anxiety

The bimodal distribution for the HA category with moderate anxiety was further supported by a statistical test indicating different distributions of TDs. Each peak in the histogram suggests the dominance of BOLD oscillations either leading (nTD) or lagging behind (pTD) RRI signals. Julien (2006) suggested that pacemaker-like activity of an autonomous oscillator in central nervous system structures could generate slow waves at ~0.1 Hz independent of baroreflex influences. Evidence for such a central pacemaker comes from (a) the observation that in patients with left ventricular assist devices low frequency oscillations are independent of any effects of blood pressure regulation (Cooley et al., 1998), (b) the findings of Lambertz and Langhorst (1998) and Perlitz et al. (2004) on rhythms emerging in neurons of the reticular formation in brain stem, and (c) recent research on BOLD signals in pons/brain stem (Pfurtscheller et al., 2020). In this respect, the discussion on the basic phenomenon of respiratory sinus arrhythmia (RSA) and the mechanisms of its generation appears to be particularly interesting (Eckberg and Karemaker, 2009). This discussion points out that not only the baroreflex, but also a central mechanism may play an important role. We suggest that pTDs characterize oscillations independent from the baroreflex loop, presumably originating from a central pacemaker.

In the HA category with moderate anxiety scores (AS = 14–29), pTDs were significantly enhanced as compared to LA. Notably, already a moderate level of anxiety seems to be associated with the engendering of a central pacemaker, thus suggesting that even mild anxiety symptoms could be sufficient to activate the pacemaker. In a recently published study (Pfurtscheller et al., 2020) it was shown for the first time that BOLD oscillations in pons/brainstem can be phase-coupled with RRI signals in the band 0.10–0.15 Hz (pTDs) and in addition, that this coupling was especially dominant in the HA category (using the same anxiety categories as in this report). It was hypothesized that this pattern could “hint toward a neural pacemaker operating as a kind of emergency system in threat-evoking situations, when the organism is in need of additional resources” (page 11, Pfurtscheller et al., 2020).

### 4.4. Slow vascular BOLD oscillations precede slow neural BOLD oscillations

In our view, a notable finding is the difference between the two TD peaks in the histogram of the HA category of ~2.3 s (cf., Fig. 3B and Table 1). This finding of two separable peaks may be of physiological relevance. An explanation could be the existence of two independent mechanisms responsible for rhythm generation, the baroreflex loop and a central pacemaker (Julien, 2006; Eckberg and Karemaker, 2009). One goal of the co-activation (i.e., synchronization) of both mechanisms could be the generation of large waves in RRI signal, thus increasing heart rate variability (HRV). A comparably large HRV seems to be a prerequisite for a successful processing of negative emotions (see review papers, Thayer and Lane, 2009; Schwerdtfeger et al., 2020). An important component of HRV is the low frequency (LF) band dominated by slow oscillations generated by baroreflex and central mechanisms. These signals have different sources and different modalities – blood flow and neural activity. The superposition of both signals assumes the time delay between vascular and neural BOLD oscillations (TD peak difference) equals the NBC time of ~2.5 s or 90° in the case of 0.1-Hz oscillations (Mateo et al., 2017; Pfurtscheller et al., 2020).

### 4.5. What can be learned from elevated fMRI-related anxiety in healthy subjects?

In a recently published review paper Tumati et al. (2021) reported on fMRI studies in anxiety disorders (AD). Imaging studies in AD revealed abnormal neuro-cardiac phase coupling, decrease of serotonergic and noradrenergic activity and modulations in resting state functional connectivity (RSFC) in various networks. RSFC in the default mode network represents normal brain–heart interaction, i.e., normal neuro-cardiac phase coupling, and is characteristic for normal brain function. AD, including specific phobias, are characterized by decreased RSFC in the default mode network and increased RSFC in the salience and somatomotor networks. These RSFC modulations in AD are often serious and mark conditions requiring a long-lasting therapy. In contrast, in healthy subjects, anxiety does not reach clinical levels and in general, can be regulated effectively and successfully. Studies of fMRI-related anxiety in healthy subjects offer the unique possibility to investigate not only the effect of low and elevated anxiety on BOLD signals, but also their trajectories over time during scanning sessions. In clinical studies, assessment of fMRI-induced anxiety should not rely on the first sessions only, as in most subjects anxiety declines after the first sessions. Most importantly, connectivity in salience and somatomotor networks, which are active during anxiety processing, affects especially neural BOLD signals with frequency components > 0.1 Hz.

### 5. Limitation and future prospects

Although findings appear promising, several unresolved questions need to be acknowledged. First, the choice of the frequency band for PLV analyses, either broad (e.g., 0.07–0.13 Hz, as in the current study) or narrow, needs to be discussed. The functional meaning of broad cardiac LF components (standard 0.04–0.15 Hz) is less well understood as compared with high frequency (HF) components (standard 0.15–0.4 Hz). In the latter case, the parasympathetic (vagal) influence is dominating while in the former case the sympathetic influence has been challenged (Reyes Del Paso et al., 2013; Schwerdtfeger et al., 2020). Research on LF power spectra between RRI and blood pressure found evidence for two distinct spectral components at 0.08 ± 0.01 Hz (below 0.1 Hz) and 0.12 ± 0.02 Hz (above 0.1 Hz), thus suggesting two separate rhythms within the LF-band (Kuusela et al., 2003). Resting state fMRI analyses also confirmed the existence of narrow bands below and above 0.1 Hz by findings of intrinsic frequency clusters centered at 0.08 Hz and 0.15 Hz (Yuen et al., 2019). Interestingly,
Keller et al. (2020) reported on the intermediate band (0.12–0.18 Hz) with correlations between cardiac and BOLD signals in regions related to interoceptive perception. Therefore, further research is advised to focus more strongly on bands below and above 0.1 Hz.

Second, beside the distinct changes observed between resting states (Pfurtscheller et al., 2017), Rassler et al. (2018) reported cessation of RSA meaning an abnormal coupling pattern with increase of RR intervals (HR deceleration) during inspiration and decrease of RRI during expiration identified by wave-to-wave analyses. Such a cessation of RSA was found in a minority of healthy subjects with moderate anxiety and pTD. Whether the cessation of RSA is related to the pacemaker-like activity needs further research.

Third, it would be of clinical interest to use well-designed fMRI-trials in order to examine to which extent rhythmic brain stem oscillations may occur in individuals with thoroughly diagnosed anxiety disorders (e.g., generalized anxiety disorders, panic disorders, specific phobias, post-traumatic stress disorders, obsessive–compulsive disorders or social anxiety disorders).

6. Conclusions

(i) It was shown that anxiety during MRI-examinations is coupled with slow neural BOLD oscillations in cortical and subcortical regions in healthy young subjects. While low/no anxiety was associated with dominant slow vascular BOLD oscillations, elevated anxiety was accompanied by both vascular and neural BOLD oscillations.

(ii) It was shown that already moderately elevated anxiety could engender neurophysiological processes possibly associated with the regulation of unpleasant emotions.

(iii) Moderately elevated anxiety was reported in 95% of the sample in the first resting state, which seems reasonable. Notably, moderately elevated anxiety was reported in 70% of the sample in the second resting state about 30 min later and still in 20% of the sample in the final resting state.

Declaration of Competing Interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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