

## NEUROSCIENCES AND NEUROANAESTHESIA

# Cross-frequency coupling during isoflurane anaesthesia as revealed by electroencephalographic harmonic wavelet bicoherence

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### Editor's key points

- This study investigates the EEG wavelet bicoherence patterns under isoflurane general anaesthesia.
- Anaesthesia with isoflurane produced two peaks along the diagonal line of the bicoherence matrix in the  $\alpha$  and  $\delta$  regions, and also the coupling of these two waves.
- The  $\alpha$  peak slowed with higher concentrations of isoflurane.

**Background.** Fourier bicoherence has previously been applied to investigate phase coupling in the EEG in anaesthesia. However, there are significant theoretical limitations regarding its sensitivity in detecting transient episodes of inter-frequency coupling. Therefore, we used a recently developed wavelet bicoherence method to investigate the cross-frequency coupling in the EEG of patients under isoflurane anaesthesia; examining the relationship between the patterns of wavelet bicoherence and the isoflurane concentrations.

**Methods.** We analysed a set of previously published EEG data, obtained from 29 patients who underwent elective abdominal surgery under isoflurane anaesthesia. Artifact-free, 1 min EEG segments at different isoflurane concentrations were extracted from each subject and the wavelet bicoherence calculated for all pairs of frequencies from 0.5 to 20 Hz.

**Results.** Isoflurane caused two peaks in the  $\alpha$  (6–13 Hz) and slow  $\delta$  (<1 Hz) regions of the bicoherence matrix diagonal. Higher concentrations of isoflurane shifted the  $\alpha$  peak to lower frequencies [11.3 (0.9) Hz at 0.3% to 7.1 (1.2) Hz at 1.5%], as has been previously observed in the power spectra. Outside the diagonal, we also found a significant  $\alpha$  peak that was phase-coupled to the slow  $\delta$  waves; higher concentrations of isoflurane shifted this peak to lower frequencies [10.8 (1.2) to 7.7 (0.7) Hz].

**Conclusions.** Isoflurane caused cross-frequency coupling between  $\alpha$  and slow  $\delta$  waves. Increasing isoflurane concentration slowed the  $\alpha$  frequencies where the coupling had occurred. This phenomenon of  $\alpha$ – $\delta$  coupling suggests that slow cortical oscillations organize the higher  $\alpha$  band activity, which is consistent with other studies in natural sleep.

**Keywords:** electroencephalographic signals; isoflurane anaesthesia; wavelet bicoherence

Accepted for publication: 24 August 2012

General anaesthetic drugs are thought to block consciousness by depressing the central nervous system. Some of the resultant changes in the brain's spontaneous electrical activity are manifest in the EEG. Anaesthetic-related changes in the EEG are well studied,<sup>1</sup> and have some commonality with natural sleep processes.<sup>2</sup> In general, the low-voltage, high-frequency pattern of wakefulness transits to high-amplitude, low-frequency waves, and finally to the burst-suppression pattern as anaesthetic drug concentration increases.<sup>3–4</sup> As yet, the physiological mechanisms that underlie EEG activity during anaesthesia remain largely unclear.

The effects of general anaesthetics on the cerebral cortex and the thalamus<sup>5</sup> cause a multitude of different EEG oscillations.<sup>6</sup> Evidence from intracellular recordings in animals<sup>7–8</sup>

and EEG studies in humans<sup>9–10</sup> indicate that the cortically generated slow oscillation (<1 Hz) has the ability to trigger and group a variety of faster brain rhythms. The two classical slow rhythms ( $\delta$ : 1–4 Hz and spindle: 7–15 Hz) are predominant in non-rapid eye movement sleep, whereas the two fast oscillations ( $\beta$ : 20–30 Hz and  $\gamma$ : 30–60 Hz)<sup>6</sup> are more commonly a feature of wakefulness and rapid eye movement sleep. However, it is unresolved as to whether the modulation of higher-frequency brain activities by slow oscillations is correlated with the level of unconsciousness when induced by general anaesthesia.<sup>11</sup> Molaei-Ardekani and colleagues investigated the amplitude modulation of the  $\alpha$  oscillation by the  $\delta$  oscillation in children during desflurane anaesthesia. They found that the POM (phase of modulation,

i.e. the  $\delta$  phase at which the  $\alpha$  amplitude is maximal) in the high  $\delta$  band (1.8–4 Hz) showed promise in distinguishing different depths of anaesthesia.<sup>11</sup>

Bicoherence analysis is an alternative way of quantifying the degree of quadratic phase coupling (QPC) among different frequency components of a signal, and is largely independent of the amplitude of the signal.<sup>12</sup> It has been successfully used in a variety of EEG studies,<sup>13–19</sup> including anaesthesia.<sup>19–26</sup> The EEG bicoherence spectrum has been found to track the concentration of anaesthetic drugs. Specifically, the bicoherence values in the  $\alpha$  (at around 10 Hz) and  $\delta$ – $\theta$  (at around 4 Hz) bands along the diagonal line of the bicoherence matrix were found to correlate well with the concentrations of isoflurane or sevoflurane.<sup>20–22</sup> Hayashi and colleagues<sup>23</sup> also found that deeper anaesthesia shifted all the peaks to lower frequencies and caused increased bicoherence in the  $\delta$ – $\theta$  region. Recently, Pritchett and colleagues<sup>26</sup> investigated bicoherence parameters across equal band (i.e.  $\delta$ – $\delta$ ) and unequal band (i.e.  $\delta$ – $\theta$ ) bifrequency regions, during different anaesthetic levels in routine clinical anaesthesia, and found that bicoherence estimates for the  $\delta$ – $\theta$  region were more sensitive to anaesthetic changes, when compared with other bifrequency regions.

The above studies were all based on the Fourier bicoherence. Owing to the non-stationary nature of the EEG data, the Fourier bicoherence may be inadequate for the detection of the characteristically intermittent and transient coupling that is often observed in the raw EEG during general anaesthesia; because its basis functions (sines and cosines) do not decay with time. This problem can be addressed by the wavelet transform which, with a suitable choice of wavelet function, can give a better time and frequency resolution. For these reasons, a wavelet-derived bicoherence measure should be a better estimate of transient phase coupling in EEG signals.<sup>27–30</sup> Recently, we proposed an improved wavelet bicoherence method,<sup>31</sup> which exploits phase randomization to eliminate spurious information in the bicoherence values, thus obtaining a reliable estimate for the cross-frequency interactions. The general harmonic wavelet<sup>32–33</sup> was chosen to overcome the drawbacks of the frequently used Morlet wavelet. (The Morlet wavelet transform needs a relatively complex algorithm and some arbitrary parameter choices in implementation.) In the original study, this method was applied to the analysis of slow wave to rapid eye movement natural sleep transitions. The present study analysed the EEG during isoflurane anaesthesia, examining how different isoflurane concentrations affected the bicoherence spectrum, especially focusing on the cross-frequency coupling of slow oscillation and high-frequency oscillations.

## Methods

### Protocol and data recordings

In this study, we re-analysed EEG data from a previously reported study.<sup>20</sup> The data were obtained from 29 patients (nine men and 20 women, age 34–77 yr, ASA physical

status I–II) who underwent elective abdominal surgery under isoflurane general anaesthesia combined with epidural anaesthesia, after obtaining institutional approval (Osaka Prefectural Habikino Hospital, Osaka, Japan) and written informed consent from all participants. None of these participants had any neurological or psychiatric disorders, nor were they receiving medication with any drugs known to influence anaesthetic or analgesic effects.

Thirty minutes before the admission to the operating theatre, each patient received i.m. premedication with 0.5 mg atropine. An epidural catheter was placed at the appropriate spinal location (T7/8, T8/9, T9/10, or T10/11). After confirming the effect of epidural analgesia, which was administered to minimize the influence of surgical stress on EEG during surgery, anaesthesia was induced with 3 mg kg<sup>−1</sup> thiopental. After tracheal intubation, anaesthesia was maintained with isoflurane, oxygen, and nitrogen. Vecuronium was given as required. Lidocaine 1% (80–110 mg h<sup>−1</sup>; initial dose, 90–100 mg) was administered epidurally. Patients received controlled ventilation to maintain adequate oxygenation and normocapnia. To keep mean arterial pressure at 60 mm Hg, as required, 2–5  $\mu$ g kg<sup>−1</sup> min<sup>−1</sup> dopamine was administered.

Five EEG electrodes (A<sub>1</sub>, A<sub>2</sub>, FP<sub>1</sub>, FP<sub>2</sub>, and FPz; according to the International 10–20 System) were attached to the patients before induction of anaesthesia. FPz was used as body ground. EEG data were collected from a single lead FP<sub>1</sub>–A<sub>1</sub> using a 514X-2 EEG telemetry system (GE Marquette, Tokyo, Japan). EEG signals in the waking state were obtained before the induction of anaesthesia. To minimize the influence of thiopental, all data, except those gathered with patients in the awake state, were collected at least 1 h after the induction of anaesthesia. To achieve a steady state, the end-tidal concentration of isoflurane was purposefully maintained at set levels (1.5%, 1.3%, 1.1%, 0.9%, 0.7%) for 30 min, before being changed to another concentration. To more strongly differentiate the effect of the different end-tidal concentrations of isoflurane on brain activities during the lightening phase of anaesthesia, the EEG segments at 0.3% and 0.5% isoflurane were also collected immediately after the operation for comparison. The expired concentration of isoflurane was continuously monitored and recorded using a Capnomac (GE Healthcare, Helsinki, Finland) monitor. This device was calibrated by means of the calibration gas that was provided by the manufacturer every month. The EEG segments with the ‘burst-suppression’ pattern present were excluded from our analysis.

### Data processing

The EEG data were sampled at 512 Hz and then downsampled to 128 Hz by running *resample.m* in Matlab. Raw EEG data segments of 1 min at different isoflurane concentrations were extracted from each patient to calculate the general harmonic wavelet transform (GHWT)-based wavelet bicoherence for further studies. The detailed algorithms of the GHWT-based wavelet bicoherence are shown in the Appendix. Each 1 min segment of EEG data was visually

inspected, and any segments with excessive artifacts were excluded from further analysis.

During the computation of bicoherence, it is difficult to determine from a single epoch whether phase coupling is present in the signal.<sup>34</sup> Therefore, the signal can be divided into a series of epochs, and a reliable bicoherence estimate can be obtained by averaging the wavelet bicoherence of all epochs.<sup>35</sup> The signals were divided into a series of 10 s epochs, with an overlap of 75%. For each epoch, bicoherence values were computed in all pairs of frequencies from 0.5 to 20 Hz, with a step of 0.5 Hz, and a bandwidth of 1 Hz. For each 1 min segment of EEG signal, we can thus obtain the wavelet bicoherence matrix,  $b$ , with each element,  $b(f_p, f_q)$ , denoting the bicoherence value at bifrequency pair  $(f_p, f_q)$ , where  $0.5 \leq f_p, f_q \leq 20$  Hz.

The growth patterns in the GHWT-based wavelet bicoherence were investigated in the following two aspects. First, the bicoherence values in the diagonal line ( $f_p = f_q$ , denoted as  $\text{diag\_bic}$ ) were examined. The  $\text{diag\_bic}$  is different from the simple power spectrum, which measures the amplitude at each frequency in a signal. The  $\text{diag\_bic}$  quantifies the QPC between components at a frequency and its doubled frequency, and a non-linear phase coupling between components at frequency  $f_q$  and its harmonics of  $2f_q$  will produce a peak in the plot of  $\text{diag\_bic}$ . The maximum bicoherence value in the diagonal line between 6 and 15 Hz was detected as the  $\text{diag\_bic}$   $\alpha$  peak. Similarly, the maximum bicoherence value between 0.5 and 4 Hz was detected as the  $\text{diag\_bic}$   $\delta$  peak. The coupling of slow  $\delta$  oscillations ( $f_q = 0.5$  Hz and 1 Hz) with other higher oscillations was calculated by the summed bicoherence [ $b(f_p, 0.5) + b(f_p, 1)$ ], denoted as  $\text{low\_bic}$ . The maximum bicoherence value in the  $\text{low\_bic}$  between 6 and 15 Hz was denoted as the  $\text{low\_bic}$   $\alpha$  peak.

Also, the power spectra of the 1 min EEG data segments were calculated using the Welch method.<sup>36</sup> The signals were divided into a series of 10 s epochs, with an overlap of 75%. The epochs were windowed using a Hamming window, and the number of FFT points was set to be 2048. The maximum value of power between 6 and 15 Hz was detected as the spectral  $\alpha$  peak, and the maximum value of power between 0.5 and 4 Hz was detected as the spectral  $\delta$  peak.

### Statistical analysis

Data were exported to SPSS (Version 13.0, SPSS Inc., Chicago, IL, USA) for statistical analysis. As the samples at different concentrations of isoflurane were unbalanced, one-way analysis of variance with the Gabriel *post hoc* test were used to compare the peak frequencies and bicoherence or power values at these peaks. The Levene test was used in advance to check whether the variances were homogeneous across multiple groups. If the assumption of homogeneity of covariance was violated, the Games–Howell *post hoc* test was used. For all tests,  $P < 0.05$  was considered

**Table 1** Summary of levels of anesthesia with sample sizes

Isoflurane concentration (%)	Sample size	
	Number of observations	Number of subjects
0	36	13
0.3	16	8
0.5	63	9
0.7	287	19
0.9	363	24
1.1	280	23
1.3	193	16
1.5	116	10

statistically significant. Data are presented as mean [standard deviation (SD)].

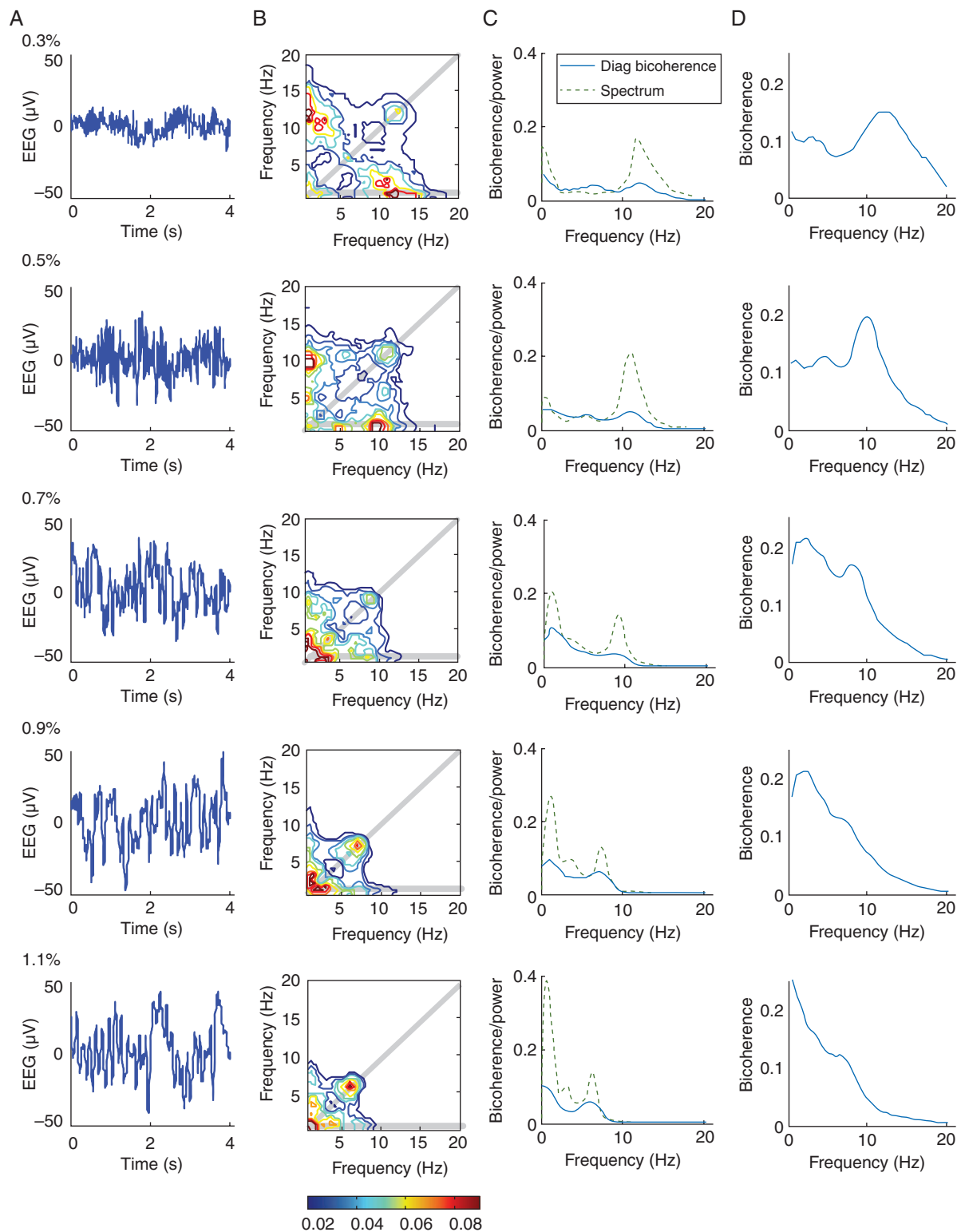
### Results

EEG signals from 29 patients were analysed in this study. As the data were recorded during routine clinical anaesthesia, and the majority of the recording for each subject was collected at the levels of anaesthesia best suited to surgery, not all the EEG data under each isoflurane concentration were observed in each subject, resulting in an uneven sample size for each isoflurane concentration, as shown in Table 1.

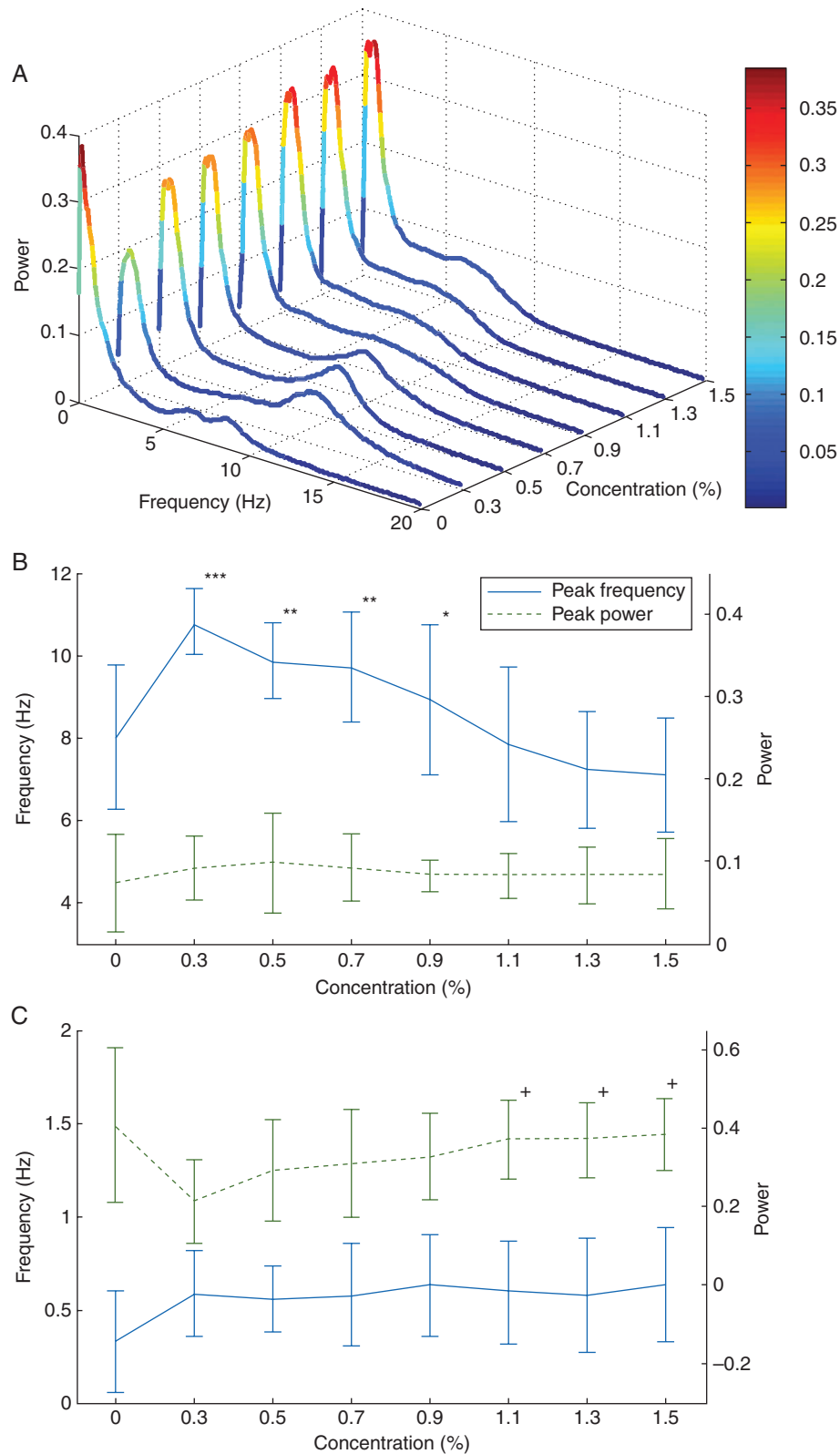
Figure 1A and B shows typical EEG signals and the contour plots of corresponding wavelet bicoherence matrix at different isoflurane concentrations. As can be seen from the figure, the bicoherence patterns change with increasing isoflurane concentrations; the changes are more distinct in the diagonal line and along the horizontal lines at slow frequencies. Figure 1C and D shows the bicoherence values in the diagonal line ( $\text{diag\_bic}$ ) and the summed bicoherence values along the horizontal line at slow frequencies of 0.5 and 1 Hz ( $\text{low\_bic}$ ). In the  $\text{diag\_bic}$ , it is obvious that isoflurane caused two main peaks at  $\alpha$  and slow  $\delta$  bands, and that higher concentrations of isoflurane shifted the  $\alpha$  peak to lower frequencies. These observations were consistent with the changes observed in the power spectrum with deeper isoflurane concentration. The shift of the  $\alpha$  peak also appeared in the plot of  $\text{low\_bic}$ . The summed bicoherence values at  $\alpha$  peaks also changes, as clearly seen in Figure 1D.

### Power spectral changes

Figure 2A shows the mean power spectra for each study period from all the 29 subjects, whereas Figure 2B and C summarizes the spectral peak frequencies and power values at these peaks in the  $\alpha$  and slow  $\delta$  bands during changes in isoflurane concentration. Isoflurane of 0.3% caused a significant  $\alpha$  peak at 10.8 (0.8) Hz, and when the isoflurane concentration increased, the peak moved to a lower region, resulting in peaks at about 7 Hz at 1.3% and 1.5% ( $F_{7,114} = 8.043$ ,  $P < 0.001$ ). *Post hoc* analysis showed that the



**Fig 1** Typical bicoherence patterns from a sample EEG in a 49-yr-old man undergoing removal of leiomyosarcoma of stomach with isoflurane anaesthesia combined with epidural anaesthesia. (A) Raw signal epochs, representative of EEG recorded during 1 min with increasing isoflurane concentrations of 0.3%, 0.5%, 0.7%, 0.9%, and 1.1%. (B) Corresponding GHWT-based wavelet bicoherence matrix. (C) The bicoherence along the diagonal line of the bicoherence matrix (solid line), and corresponding power spectra (dashed line). (D) The summed bicoherence values at slow wave [ $b(f_p, 0.5) + b(f_p, 1)$ ] along the horizontal line of the bicoherence matrix [shading in (B)].



**Fig 2** (A) Averaged power spectra at each study period: steady state with no drug (0) and isoflurane concentrations of 0.3–1.5%, from all the 29 subjects. (B) The spectral peak frequencies and power values at these peaks regarding  $\alpha$  band during changes in isoflurane concentration. \*\*\*Significant difference in the peak frequency compared with those at 0 and 1.1%, 1.3%, 1.5%; \*\*significant difference compared with those at 1.1%, 1.3%, 1.5%; \*significant difference compared with at 1.3%. (C) The spectral peak frequencies and power values at these peaks in the  $\delta$  band during changes in isoflurane concentration. +Significant difference in the power values at peak frequency compared with those at 0.3%.



spectral  $\alpha$  peak frequencies at lower isoflurane concentrations were significantly higher than those at higher isoflurane concentrations, and also before any isoflurane was administered ( $P < 0.05$ ). In contrast, the power of the spectral  $\alpha$  peak showed no significant difference ( $P > 0.05$ ).

It can be clearly seen from Figure 2c that the induction of isoflurane seemed to shift the spectral peak at about 0.3–0.6 Hz; and the power of the  $\delta$  peaks tended to increase with increases in isoflurane concentration from 0.3% to 1.5%. Multiple comparison of the spectral power values over the seven periods from 0.3% to 1.5% indicated significant difference ( $F_{6,101} = 2.787$ ,  $P < 0.05$ ), with the power values at 1.1–1.5% significantly higher than those at 0.3% ( $P < 0.05$ ).

### Bicoherence changes

Figure 3A shows the mean bicoherence values in the diagonal line at each study period, and Figure 3B and C summarizes the diag\_bic peak frequencies and bicoherence values at these peaks around  $\alpha$  and slow  $\delta$  bands with changes in isoflurane concentration. Consistent with the power spectra, the significant  $\alpha$  peak at 11.3 (0.9) Hz tended to decrease to 7.1 (1.2) Hz, with increases in isoflurane concentration from 0.3% to 1.5% ( $F_{7,114} = 12.416$ ,  $P < 0.001$ ); the diag\_bic  $\alpha$  peak frequencies at lower isoflurane concentrations were significantly higher than those at deeper isoflurane concentrations, and also than the no isoflurane condition ( $P < 0.05$ ).

Figure 4A shows the mean summed bicoherence values in the slow  $\delta$  bands (low\_bic) for each study period, whereas Figure 4B summarizes the changes in the low\_bic peak frequencies and peak bicoherence values around  $\alpha$  band with changes in isoflurane concentration. Again a significant  $\alpha$  peak appeared at a frequency of 10.8 (1.2) Hz at 0.3% isoflurane, which slowed to 7.7 (0.7) Hz at 1.5% isoflurane ( $F_{7,114} = 6.767$ ,  $P < 0.05$ ). As the isoflurane deepened, the summed bicoherence values tended initially to increase in amplitude, followed by a decrease ( $F_{7,114} = 14.162$ ,  $P < 0.001$ ). Post hoc analysis showed that the low\_bic  $\alpha$  peak frequencies at lower isoflurane concentrations (0.3–0.9%) were significantly higher than those at deeper isoflurane concentrations (1.1–1.5%), and also than during the period before induction (no isoflurane) ( $P < 0.05$ ). These results indicated that isoflurane caused QPC between  $\alpha$  waves and slow  $\delta$  waves, and that both the  $\alpha$  coupling strength and frequency changed with increasing isoflurane concentrations. The shift of the low\_bic peak frequency was also consistent with that of the spectral  $\alpha$  peak in power spectrum.

### Discussion

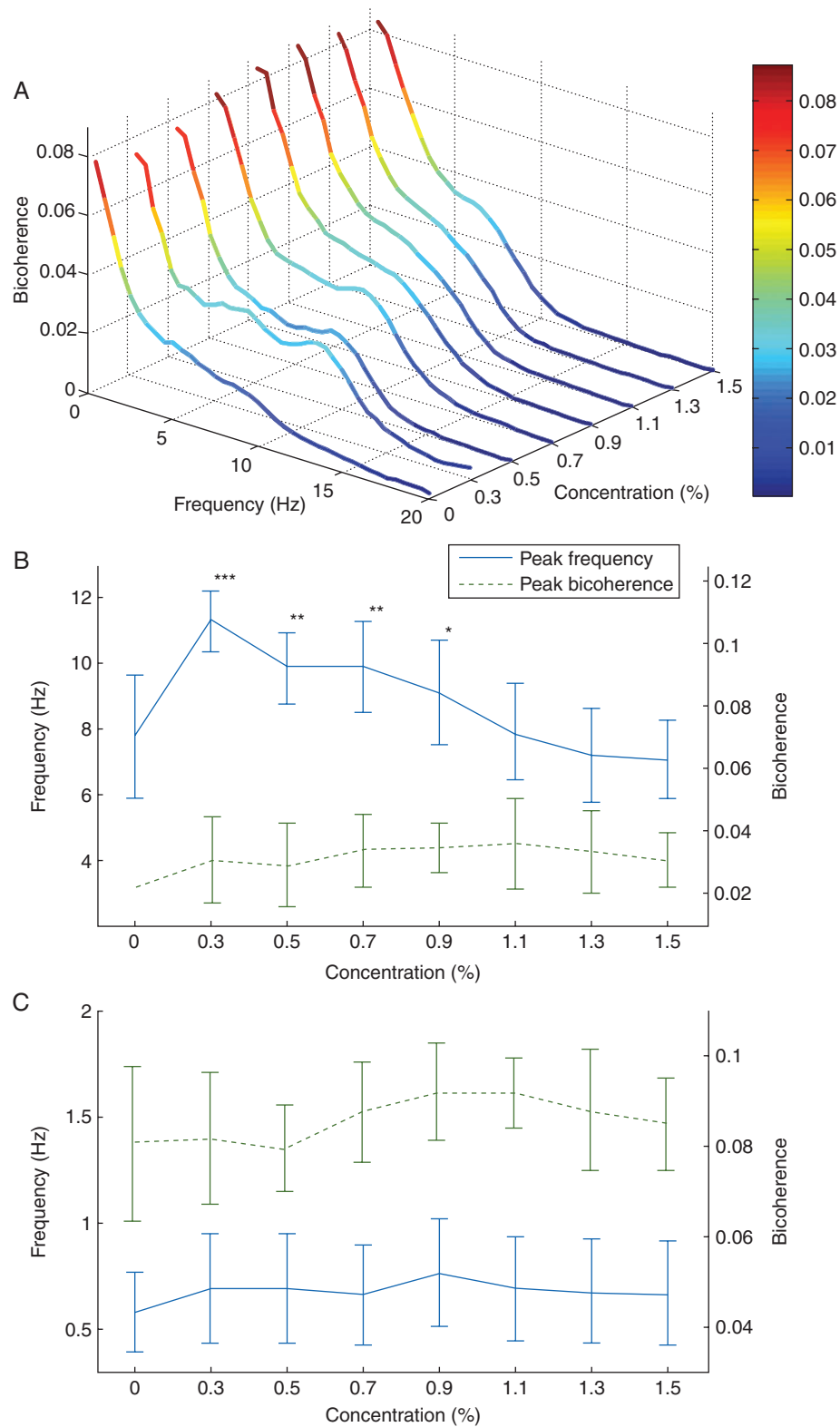
We investigated the EEG wavelet bicoherence patterns under isoflurane general anaesthesia combined with epidural anaesthesia. Isoflurane caused peaks in  $\alpha$  and  $\delta$  segments of the diagonal line of the bicoherence matrix, and higher concentrations of isoflurane shifted the diag\_bic  $\alpha$  peak to lower frequencies. This is consistent with the power spectral observations. Furthermore, the  $\alpha$  and  $\delta$  waves were coupled

after isoflurane induction, and the coupling strength changed when the isoflurane concentration deepened. A previous study used Fourier methods to quantify the bicoherence but failed to detect significant  $\alpha$ – $\delta$  coupling in the same data set. This comparison suggests that wavelet bicoherence is a more sensitive method to quantify transient episodes of inter-frequency coupling than traditional Fourier transform-based algorithms, and hence significant coupling episodes may be easily ‘lost in the background EEG noise’.

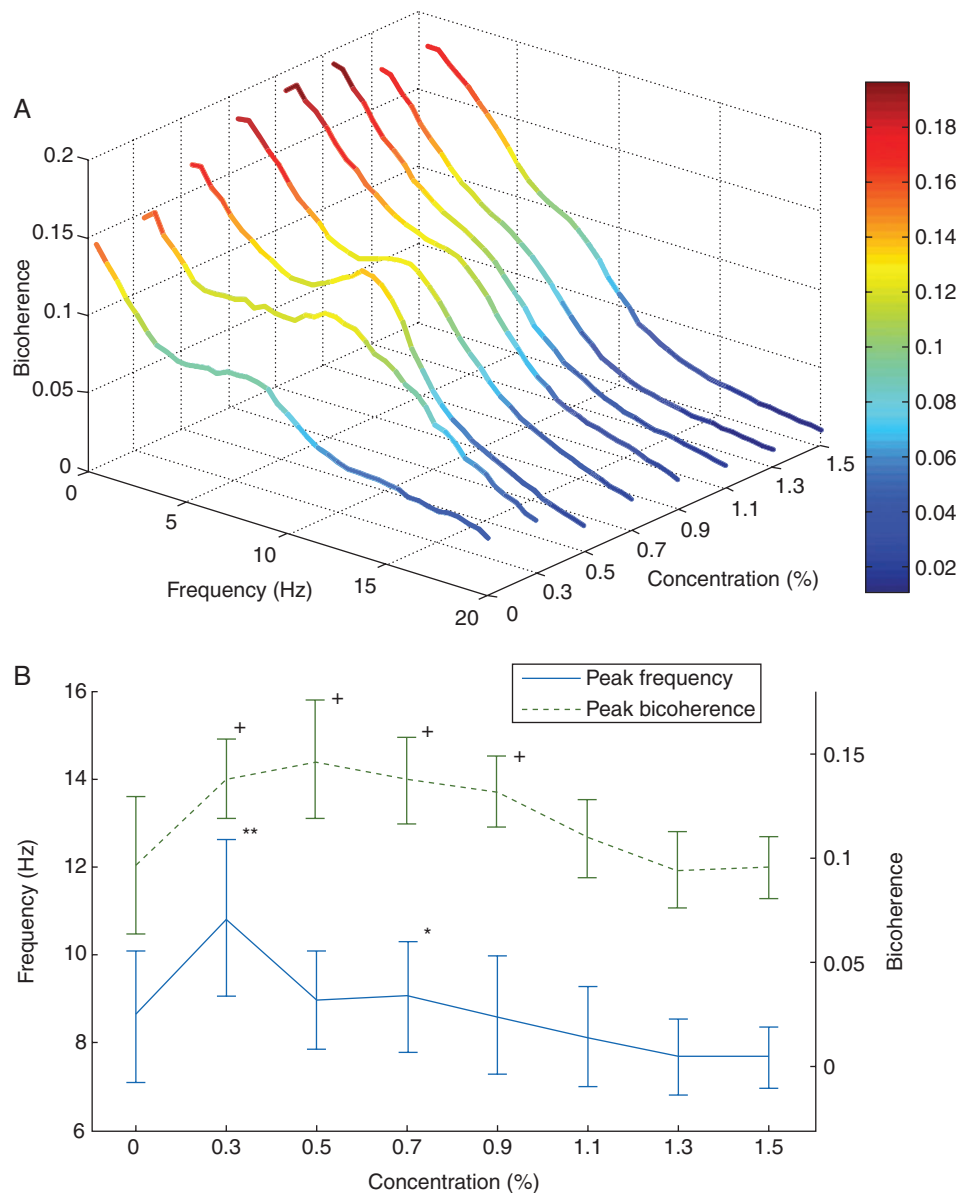
The observed slowing in the diag\_bic  $\alpha$  peak frequency (Fig. 3A and B) is compatible with previous studies that used Fourier bicoherence during sevoflurane or isoflurane anaesthesia.<sup>20 22 23</sup> However, we did not find significant differences in the bicoherence values at diag\_bic  $\alpha$  peaks with increasing isoflurane concentrations, nor did we detect a significant peak in  $\delta$ – $\theta$  area along the diagonal line of the bicoherence matrix as in Hagiwara and colleagues.<sup>20</sup> Instead, in addition to the previously mentioned diag\_bic  $\alpha$  peak, we found a clear peak in the  $\delta$  region (Fig. 3A and C), which was coincident with the power spectral analysis (Fig. 2), and most importantly, we detected the cross-frequency coupling between the  $\alpha$  waves and slow  $\delta$  waves. We would suggest that three important details of the signal processing are responsible for the increased sensitivity of our technique. First, the wavelet bicoherence is more suitable for detecting the transient episodes of phase coupling that are inherent in the non-stationary EEG signals. Its basis function (i.e. the wavelet function) decays with time; thus, it is capable of detecting temporally local structures. In contrast, the basis functions of Fourier transform (sines and cosines) do not change with time and thus are not optimized to detect temporally local structures.<sup>27</sup> Secondly, we used an improved wavelet bicoherence method to estimate the QPC. Namely, a phase randomization process was used to eliminate the spurious values brought by the segment-averaging operation and the surrogate test used to ensure reliable bicoherence values.<sup>31</sup> Thirdly, the previous work relied on an average of bicoherence values taken over a broader area around the diagonal.<sup>20</sup> We only used values lying directly on the diagonal.

As shown in Figure 2, two peaks in the  $\alpha$  and  $\delta$  area appeared in the power spectrum and shifted with increasing isoflurane concentrations. Alpha activity is thought to be related to the rhythmic influence of the thalamic pacemaker cells when the nucleus reticularis (RE) is hyperpolarized in the presence of GABAergic general anaesthesia. These pacemaker neurones oscillate at 8–12 Hz and regulate and synchronize the excitability of cells in the thalamocortical pathways.<sup>1 37</sup> Anaesthetic augmentation and prolongation of the inhibitory postsynaptic potentials causes the RE neurones to inhibit the thalamic pacemaker neurones, slowing the mean frequency of the oscillators from the  $\alpha$  band towards the  $\theta$  band (4–8 Hz).<sup>37</sup>

The slow oscillation (~0.5–1 Hz) is generally viewed as primarily originating from neocortical networks and then



**Fig 3** (A) Averaged bicoherence values in the diagonal line ( $f_p=f_q$ ) at each study period from all the 29 subjects. (B) The diag\_bic peak frequencies and power values at these peaks regarding the  $\alpha$  band during changes in isoflurane concentration. \*\*\*Significant difference in the peak frequency compared with those at 0 and 0.9%, 1.1%, 1.3%, 1.5%; \*\*significant difference compared with those at 0 and 1.1%, 1.3%, 1.5%; \*significant difference compared with those at 1.3%, 1.5%. (C) The diag\_bic peak frequencies and power values at these peaks in the  $\delta$  bands during changes in isoflurane concentration.



**Fig 4** (A) Averaged summed bicoherence values at slow  $\delta$  bands [ $b(f_p, 0.5) + b(f_p, 1)$ ] at each study period from all the 29 subjects. (B) The low\_bic peak frequencies and bicoherence values at these peaks regarding  $\alpha$  band during changes in isoflurane concentration. \*\*Significant difference in the peak frequency compared with all those except at 0.5%; \*significant difference compared with those at 1.3%. +Significant difference in the bicoherence values at peak frequency compared with those at 0 and 1.1%, 1.3%, 1.5%.

secondarily imposed on recipient thalamic territories.<sup>6</sup> This rhythm is made up of a prolonged depolarizing ('up') phase, followed by a long-lasting hyperpolarizing ('down') phase, and was detected using intracellular recordings in anaesthetized cats<sup>7</sup> and EEG recordings during natural sleep and anaesthesia in humans and animals.<sup>7 38–40</sup> The slow oscillation, with a clear-cut hyperpolarizing phase, appears during slow-wave sleep and disappears in wakefulness and rapid eye movement sleep—when hyperpolarizations are erased and the cortical neurones are tonically depolarized.<sup>6 7</sup>

Bicoherence is a signal-processing technique capable of tracking changes in any reentry system. It is a method of investigating phase relations between two input signals with frequencies of  $f_1$  and  $f_2$  by examining an output signal with a frequency of  $f_1 + f_2$ .<sup>12</sup> With non-linear modulation—such as may be seen in the thalamocortical reverberating system—the output signal from the reverberating circuit is expected to reenter into the system as the input signal and cause self-modulated characteristics. Because this results in QPC between input signal components, bicoherence is expected to grow in these frequency components. Therefore,



when a certain rhythm is dominantly formed in a thalamo-cortical reverberating network, a phase-coupled peak will often appear in the corresponding bicoherence.<sup>23</sup> As shown in Figures 2 and 3, the frequency shift in the diag\_bic is coincident with the peak frequency shift in the power spectrum.

An interesting finding in this study is that isoflurane can induce a coupling of the slow oscillation with the  $\alpha$  oscillation (Fig. 4). In natural sleep, a number of studies<sup>7 8 39</sup> have demonstrated that the cortically generated slow oscillation acts through corticocortical and corticothalamic drives to organize the faster rhythms; for example, spindle waves typically occur during the so-called 'up' states of the slow oscillation.<sup>6</sup> During the depolarizing phase of the slow oscillation, the synchronous firing of neocortical neurones impinges upon thalamic RE pacemaking neurones, thus creating conditions for the formation of spindles, which are transferred to thalamocortical relay neurones and up to the cortex; at which level, the spindles shape the tail of the slow oscillatory cycle.<sup>6</sup> This combination also results in the K-complex, and spectral analysis has shown the periodic recurrence of human K-complexes, with main peaks at 0.5–0.7 and 12–15 Hz.<sup>41</sup> The phase coupling that is observed between slow  $\delta$  and  $\alpha$  oscillations is most likely a manifestation of this process, and points to some commonality between the states of natural sleep and general anaesthesia.<sup>2 42</sup> The fact that the  $\delta$ – $\alpha$  phase coupling tended to show a secondary decrease at very high concentrations of isoflurane is probably due to the loss of a sharp peak in  $\alpha$  oscillations with the approach of burst suppression (Figs 3 and 4), and hence decreased phase coupling.

There are a number of provisos to our results. First, we used epidural anaesthesia to suppress surgical stimuli, which blocks the nociceptive neuronal inputs, which would disturb the EEG pattern. The feasibility of using this method in clinical settings has been discussed in detail,<sup>20</sup> but the effect of epidural anaesthesia has to be taken into account when considering the relation between EEG bicoherence and isoflurane concentration. Secondly, the analysis of the EEG 'burst-suppression' pattern was not investigated in this study, although this pattern was found in the EEG recordings at 1.3% isoflurane for five patients and at 1.5% for one other. The episodic nature of burst-suppression patterns is likely to be well suited to wavelet bicoherence analysis, but is the subject of a future study. Thirdly, as indicated by the large variations in figures, the absolute values of the EEG bicoherence values in the awake state seemed to be dependent on the physiological status of the subject.<sup>20</sup>

In conclusion, we investigated how the GHWT-based wavelet bicoherence of EEG signals changes during isoflurane anaesthesia combined with epidural anaesthesia. We found that anaesthesia with isoflurane produced two peaks along the diagonal line of the bicoherence matrix in the  $\alpha$  and  $\delta$  regions, and also the coupling of these two waves, and that the  $\alpha$  peak slowed with higher concentrations of isoflurane.

## Declaration of interest

None declared.

## Funding

This research was partly supported by National Natural Science Foundation of China (61025019, 61203210) and Specialized Research Fund for the Doctoral Program of Higher Education in China (20101333110006).

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## Appendix: GHWT-based bicoherence

The bicoherence, as the normalized form of bispectrum, is a measure of the amount of QPC that occurs in a signal. The wavelet bicoherence can be seen as a generalization of the Fourier bicoherence. It can detect a temporally intermittent correlation due to its improved time resolution.<sup>27</sup> In both cases of Fourier bicoherence and wavelet bicoherence, if a signal contains three components with frequencies of  $f_p$ ,  $f_q$  and  $f_p + f_q$ , and the corresponding phases satisfy the relationship  $\varphi(f_p) + \varphi(f_q) = \varphi(f_p + f_q) + \text{const}$ , the three waves are quadratic phase-coupled, and the bicoherence value should be equal to 1, which will produce a peak in the bicoherence matrix at the intersection between  $f_p$  and  $f_q$ ; if no such relation is satisfied, the value is 0.

In this study, an improved wavelet bicoherence method<sup>31</sup> was used, and its implementation is as follows:

- (1) The signal  $x$  is segmented into  $M$  overlapping  $N$ -point epochs,  $x_k(n)$ , where  $k=1, 2, \dots, M$ ,  $n=1, 2, \dots, N$ .
- (2) For each epoch  $x_k(n)$ ,  $k=1, 2, \dots, M$ , the GHWT<sup>33</sup> is calculated to obtain the wavelet coefficients  $a_k(f_p, n)$ ,  $n=1, 2, \dots, N$  corresponding to the signal component at frequency  $f_p$ . The GHWT combines the advantages of the short-time Fourier transform and continuous wavelet transform. Through the GHWT, the signal can be arbitrarily interpolated along the frequency axis at constant defined points along the time axis, thus the instantaneous phase of a signal component can be accurately estimated. This is a prerequisite, if the resultant bicoherence is to reliably detect transient coupling information in EEG data.
- (3) The phase randomized wavelet bicoherence. The normalized squared wavelet bicoherence, which characterizes the QPC between components with frequencies  $f_p$  and  $f_q$  in  $x_k(n)$ , is calculated by<sup>27</sup>

$$b_k(f_p, f_q) = \frac{|B_k(f_p, f_q)|^2}{\sum_{n=1}^N |a_k(f_p, n)a_k(f_q, n)|^2 \sum_{n=1}^N |a_k(f_p + f_q, n)|^2} \quad (\text{A1})$$

where the phase randomized wavelet bispectrum is given by the expression

$$B_k(f_p, f_q) = \sum_{n=1}^N a_k(f_p, n)a_k(f_q, n)a_k^*(f_p + f_q, n)e^{jR\phi_k(f_p, f_q, n)} \quad (\text{A2})$$

where  $R \in (-\pi, \pi]$  is a random variable, and  $\phi_k(f_p, f_q, n)$  is

the biphasic, which can be calculated by:

$$\phi_k(f_p, f_q, n) = \phi_k(f_p, n) + \phi_k(f_q, n) - \phi_k(f_p + f_q, n) \quad (A3)$$

where  $\phi_k(f_p, n)$  denotes the instantaneous phase that is estimated from the wavelet coefficients  $a_k(f_p, n)$ . The addition of the random variable  $R$  can effectively retain the QPC information, or reduce spurious coupling information; so the resultant wavelet bicoherence is indeed close to the 'true' QPC value. If QPC occurs at the time  $n$  [i.e. the biphasic  $\phi_k(f_p, f_q, n) = 0$ ],  $R$  has no effect on the bispectrum; in contrast, if QPC does not occur [i.e.  $\phi_k(f_p, f_q, n) \neq 0$ ],  $R$  considerably reduces the bispectrum.

- (4) Significance testing of the wavelet bicoherence. A surrogate method was used to obtain a reliable measure of wavelet bicoherence of statistical significance. First, replacing  $\phi_k(f_p, f_q, n)$  with a new biphasic  $\phi'_k(f_p, f_q, n) = \phi_k(f_p, f_q, n) + \theta$  in equation (A2), where  $\theta \in (-\pi, \pi]$  is a random variable, a surrogated

bicoherence value can be derived by equation (A1). This procedure is run  $N_{\text{sum}}$  ( $N_{\text{sum}} > 19$ ) times and  $N_{\text{sum}}$  bicoherence values can be obtained at frequency pair  $(f_p, f_q)$ . The 95% statistical threshold is determined by mean plus 1.96 times  $\text{SD}$ . Finally, the original bicoherence value greater than this threshold will be preserved, otherwise is set to zero.

- (5) The wavelet bicoherence values from multiple epochs are averaged to derive a robust bicoherence estimate. To remove the effect of the transients and random noises in the signal, Q-test is used to remove the outlier bicoherence values in the segment-averaging procedure. This operation is capable of estimating reliable bicoherence for a data with small sample sizes.

The detailed algorithm of the improved GHWT-based wavelet bicoherence can be found in Li and colleagues.<sup>31 43</sup>

Handling editor: M. M. R. F. Struys