

N1 Magnitude of Auditory Evoked Potentials and Spontaneous Functional Connectivity between Bilateral Heschl's Gyrus are Coupled at Inter-individual Level

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Abstract: N1 component of auditory evoked potentials (AEPs) is extensively used to investigate the propagation and processing of auditory inputs. However, the substantial inter-individual variability of N1 could be a possible confounding factor when comparing different individuals or groups. Therefore, identifying the neuronal mechanism and origin of the inter-individual variability of N1 is crucial in basic research and clinical applications. This study is aimed to use simultaneously recorded electroencephalography (EEG) and functional magnetic resonance imaging (fMRI) data to investigate the coupling between N1 and spontaneous functional connectivity (FC). EEG and fMRI data were simultaneously collected from a group of healthy individuals during a pure-tone listening task. Spontaneous FC were estimated from spontaneous BOLD signals that were isolated by regressing out task evoked BOLD signals from raw BOLD signals, and then were correlated to N1 magnitude across individuals. It was observed that spontaneous FC between bilateral Heschl's gyrus (HG) was significantly and positively correlated with N1 magnitude across individuals (Spearman's $R = 0.829$, $P < 0.001$). The specificity of this observation was further confirmed by two whole-brain voxel-wise analyses (voxel-mirrored homotopic connectivity analysis and seed-based connectivity analysis). These results enriched our understanding of the functional significance of the coupling between event-related brain responses and spontaneous brain connectivity, and hold the potential to increase the applicability of brain responses as a probe to the mechanism underlying pathophysiological conditions.

Acronyms

AAS	-	Average artifact subtraction
ACC	-	Anterior cingulate cortex
AEP	-	Auditory evoked potential
BCG	-	Ballistocardiograph
BOLD	-	Blood-oxygen-level dependent
EEG	-	Electroencephalogram
FC	-	Functional connectivity
FDR	-	False discovery rate
fMRI	-	Functional magnetic resonance imaging
GLM	-	General linear model
GSR	-	Global signal regression
HG	-	Heschl's gyrus
IFG	-	Inferior frontal gyrus
IPC	-	Inferior parietal cortex
MCC	-	Middle cingulate cortex
ROI	-	Regions of interest
sFC	-	Spontaneous functional connectivity
SNR	-	Signal-to-noise ratio
STG	-	Superior temporal gyrus
VAS	-	Visual analog scale
VMHC	-	Voxel-mirrored homotopic connectivity

Introduction

Auditory evoked potentials (AEPs) are stimulus locked electrical activities originating from the brain in response to auditory stimuli (Burkard et al. 2007). AEPs recorded using electroencephalogram (EEG) are comprised of several distinct components, each of which captures different neural origins and functional relevance (Joos et al. 2014). In particular, N1 component in AEPs, a negative peak occurring approximately 100 ms after stimulus onset which is generated mainly around primary/secondary auditory cortex and auditory association areas (Meyer et al. 2006), is suggested to play an important role in encoding auditory information, and it has been extensively used to investigate the propagation and processing of acoustic sensory inputs (Ford and Mathalon 2004). It is well known that N1 component in AEPs has substantial inter-individual variability (Liem et al. 2012). That is, being evoked by auditory stimuli with identical sensory perception, the magnitude of N1 vary greatly among individuals. Identifying the mechanisms and origins of this inter-individual variability is crucial, since the relevant knowledge will help in ruling out possible confounding factors when comparing N1 between different individuals or groups (Boatman et al. 2008). The inter-individual variability of N1 has been related to brain structure (Liem et al. 2012), demographic characteristics, such as age (Anderer et al. 1998), socio-cultural factors, such as musical training (Baumann et al. 2008), and neurological disorders, such as schizophrenia (Rosburg et al. 2008). However, the mechanism underlying N1 variability is still poorly understood. Even within the relationships mentioned above, experimental results were not consistently reported. For example, while larger N1 for elderly was reported in (Anderer et al. 1998; Herrmann et al. 2013; Rufener et al. 2014), lower N1 was reported in (Papanicolaou et al. 1985; Tremblay et al. 2004).

Importantly, functional connectivity (FC), i.e., the inter-regional correlation of neural activity (Fox and Raichle 2007), of spontaneous brain activities has been recently recognized as an important type of neural determinant of inter-individual difference of event-related brain responses (Mennes et al. 2010). Here, spontaneous brain activities refer to the neural activities not induced by any external tasks or stimulations (Fox and Raichle 2007; Raichle 2006). Based on this definition, we further define the FC measured on spontaneous brain activities as spontaneous FC (sFC) (Song et al. 2008). Spontaneous FC has been shown to be able to predict an individual's brain responses evoked by a variety of sensory stimuli, such as visual stimuli, or cognitive tasks, such as those used for studying inhibition (Mennes et al. 2010; Tian et al. 2012). Particularly, a recent EEG study (Kühnis et al. 2014) demonstrated that N1 magnitude was negatively correlated with the inter-hemispheric FC (as indexed by lagged phase synchronization at beta band) between bilateral Heschl's gyrus (HG), which is a region suggested to be the main generator of N1 (Liem et al. 2012; Reite et al. 1994). However, due to the limited spatial resolution of EEG and ill-posedness of the EEG source inverse problem, the localization of N1 sources (i.e., bilateral HG) and the reconstruction of source signals could be problematic (Wendel et al. 2009), and it still remains unknown that whether N1-correlated sFC is specific to bilateral HG.

In this study, to overcome the intrinsic limitations of EEG in estimating sFC while to retain the possibility of acquiring N1 component, EEG and functional magnetic resonance imaging (fMRI) signals were simultaneously sampled from 19 healthy individuals during a pure-tone listening task. As compared with EEG, blood oxygenation level dependent (BOLD) signal recorded using fMRI could provide a much higher spatial resolution and is more reliable for the estimation of

sFC. Hence, simultaneous EEG-fMRI recording would allow us to assess the relationship between N1 magnitude and sFC in an accurate and comprehensive way. We hypothesize that an individual's magnitude of AEP N1 is coupled with the fMRI-derived sFC between bilateral HG. Unlike most of previous studies where sFC was directly measured under resting-state condition, in the current study we isolated spontaneous BOLD signals from the original signals recorded during task, and sFC was measured based on the isolated spontaneous BOLD signals. This FC analysis technique, usually referred to as task-regressed FC analysis, allows us to analyze spontaneous FC on event-related fMRI data, which is capturing increased interest in recent literature (Fair et al. 2007; Kucukboyaci et al. 2013). After sFC was estimated, we correlated sFC between bilateral HG with N1 magnitude across individuals. A strong positive coupling between N1 magnitude and sFC of bilateral HG was observed (Spearman's $R = 0.829$, $P < 0.001$). To avoid possible bias introduced by the analysis based on the pre-selected regions of interests (i.e. bilateral HG), two data-driven whole-brain voxel-wise analyses, i.e., voxel-mirrored homotopic connectivity (VMHC) analysis (Zuo et al., 2010) and seed-based analysis, were performed. These analyses confirmed that the observed N1-sFC coupling was specific to bilateral HG.

Materials and Methods

Participants

Nineteen healthy volunteers (11 males and 8 females) aged 21.1 ± 2.4 years (mean $\pm$ SD, range = 19 - 26 years) participated in this study. All volunteers gave their written informed consent. The Ethics Committee of the Faculty of Psychology, Southwest University, Chongqing, China, approved the experimental procedures, which were in accordance with the standards of the Declaration of Helsinki.

Stimulation and Experimental Design

Forty identical diotic auditory stimuli consisted of brief 800 Hz pure tones (500 ms duration, 5 ms rise and fall times, inter-stimulus interval: 16.5 - 22.5 s) were delivered via pneumatic tubing to earphones inserted inside the acoustic canals. The earphones were mounted inside ear defenders, which could effectively reduce MRI acoustic noise. Individuals were asked to focus their attention on the stimulation with eyes open and relax their muscles. Individuals were instructed to move a slider to rate the intensity of loudness elicited by the auditory stimulus 9 - 12 s after each stimulus, using an electronic 0 - 10 visual analog scale (VAS: the left anchor was “no sound” and the right anchor was “unbearable sound”). At the end of each trial, the slider automatically returned to the midpoint (VAS = 5). The rating operation ensures that the individuals were attending to the auditory stimuli during the experiment. To ensure high audibility of the auditory stimuli, the sound pressure level of auditory tones was individually determined prior to data collection such that a VAS of 6 out of 10 was obtained.

Data Collection

EEG and fMRI data were simultaneously collected during the experiment. EEG were recorded using 32 Ag-AgCl electrodes (including the reference channel) placed on the head with an MR-compatible cap (Brain Products GmbH, Munich, Germany; pass-band: 0.01 - 100 Hz; sampling rate: 5000 Hz; reference: FCz) according to the international 10-20 system. All electrode impedances were kept below 10 k Ω . Electrocardiographic signal was also recorded using an electrode attached to the individual's upper back to monitor ballisto-cardiographic (BCG) signals, which was used as a reference signal for BCG artefact removal.

Functional MRI data were acquired using a Siemens 3.0 Tesla Trio scanner with a standard head coil. A whole-brain gradient-echo, echo-planar-imaging sequence was used for functional scanning with a repetition time (TR) of 1500 ms (29 ms echo time, 25 5.0 mm-thick axial slices with 0.5 mm-thick inter-slice gaps, 3 $\times$ 3 mm in-plane resolution, field of view 192 $\times$ 192 mm, image matrix 64 $\times$ 64, flip angle 90 $^\circ$) over 330 volumes (8.25 min). A high-resolution T1-weighted structural image (1 mm³ isotropic voxel magnetization-prepared rapid acquisition with gradient echo) was acquired for anatomical registration after functional imaging.

EEG Data Preprocessing

MRI gradient artefacts were removed from EEG using the averaged artefact subtraction method (AAS) (Allen et al. 2000). The timings of heartbeat occurrence were estimated by detecting QRS complex from BCG signal through a combined adaptive thresholding algorithm (Niazy et al. 2005) automatically, and the result was checked by an experienced data analyst manually. The information of heartbeat timing was used to remove BCG artefact through AAS method.

Continuous EEG data were band-pass filtered (1 - 30 Hz, FIR filter) and down-sampled to 500 Hz. EEG artefacts (including eye-blink artefacts and BCG artefacts) were further eliminated using the independent component analysis based method in EEGLAB (Delorme and Makeig 2004). Across all individuals, 2 to 4 components (mean $\pm$ SD: 3.1 ± 0.74) were removed for each individual, and the number of components rejected does not covary with the magnitude of N1 across individuals (Spearman's $R = 0.150$, $P = 0.593$). EEG data were segmented into epochs (-1000 - 1000 ms), and epochs were baseline-corrected using the pre-stimulus interval (-500 - 0 ms). Finally, EEG epochs were re-referenced to a common average reference, and the epochs with a peak-peak magnitude exceeding $140 \mu\text{V}$ were discarded (mean $\pm$ SD: 1.87 ± 3.38). AEPs waveform for each individual was obtained by averaging all remaining EEG epochs and N1 magnitude was measured as the minimum of AEPs waveform (75 – 125 ms) at Cz (Fig. 1). Individuals ($n = 2$) with a signal-to-noise ratio (SNR) of N1 smaller than 3 were discarded, where the SNR is defined as the ratio between N1 magnitude and the standard deviation of AEPs waveform within the pre-stimulus interval (Hu et al. 2010). It should be noted that in the current study we used magnitude (positive value) instead of amplitude (negative value) to characterize N1. Despite the equivalence of the two measures in characterizing N1, we prefer to use N1 magnitude in the current study because it would be more intuitive to understand the correlation between positive variables.

Functional MRI Data Preprocessing

The fMRI data were firstly preprocessed using SPM8 (Wellcome Trust Center for Neuroimaging, London, UK) with routine procedures (Ashburner et al. 2012), including slice-timing and head motion correction, spatial normalization (voxel size = $3 \times 3 \times 3$ mm), spatial Gaussian smoothing

(FWHM = 8 mm), and grand mean scaling. Then, we assessed BOLD activation pattern to auditory stimuli following a conventional analysis pipeline in SPM8. Specifically, voxel-wise BOLD activity for each individual was modelled with a general linear model (GLM) using a theoretical stimulus-evoked BOLD time-series (constructed by canonical hemodynamic functions at stimulus onsets) and other nuisance factors (e.g., head motion) as the regressors, and statistical significance of GLM coefficients of the first regressor were assessed at group level with one-sample t-test (corrected with false discovery rate correction [FDR]).

To ensure reliable estimation of sFC, we further applied the following steps to remove remaining noise from BOLD signals. First, nuisance effects, including head motion, white matter (WM), and cerebrospinal fluid signals (CSF), were removed from BOLD signal for each voxel. Specifically, WM and CSF signals were extracted by averaging the voxels within pre-defined WM and CSF template provided by the REST toolbox (Song et al. 2011). The WM and CSF signals, together with head motion parameters estimated by head motion correction step, were then used as regressors to account for the variance contributed by these nuisance factors for each voxel through multiple linear regression (Yan and Zang 2010). The residuals (i.e., signals which cannot be explained by the nuisance factors) of the multiple linear regression were kept for subsequent analysis. Second, task-regressed FC analysis (Kucukboyaci et al. 2013) was performed in order to estimate sFC from the BOLD signal recorded during auditory task. More specifically, task-evoked BOLD time-course were constructed with canonical hemodynamic functions, and the time-course as well as its first derivative (modelling the slight temporal drift across brain regions) were regressed out from each voxel to eliminate possible effect of auditory task on that voxel (Fair et al. 2007) (see “*Methodological Considerations*” of Discussion for

more discussion). Third, global signal regression (GSR; i.e., removing whole-brain-averaged BOLD signal from each voxel), followed by a 2nd order Butterworth filter (0.01 - 0.1 Hz), was further performed, which is effective in eliminating typical fMRI noise that cannot be completely removed by previous steps (for methodological considerations, see Discussion). Fourth, to further eliminate the influence of excessive transient head motion, outlier volumes were identified in the following way: (1) calculating frame-wise displacement (FD) value for each volume, which is defined as the sum of the absolute values of the derivatives of the six realignment parameters ; (2) labeling those volumes with FD larger than 0.5 as outlier volumes. The identified outlier volumes were discarded in the following analyses. Individuals (n = 2) with excessive head motion (larger than 3 mm of peak-peak translational and 3 degrees of peak-peak rotational movement) were discarded. After screening of EEG and fMRI data, 15 (out of 19) individuals (8 males and 7 females; aged 21.6 ± 2.4 years) were kept for the following analyses.

Coupling between N1 and sFC

To comprehensively investigate the coupling between N1 magnitude and sFC, sFC was estimated by three analysis approaches: (1) *ROI-based connectivity*, i.e., calculating Fisher's Z-transformed Pearson's correlation coefficient between the time courses of left and right HG (defined according to Anatomical Automatic Labeling atlas (Tzourio-Mazoyer et al. 2002)); (2) *voxel-mirrored homotopic connectivity (VMHC)*, i.e., calculating Fisher's Z-transformed Pearson's correlation coefficients between all voxels in one hemisphere and their mirrored counterparts in another hemisphere (Zuo et al. 2010); and (3) *seed-based connectivity*, i.e., calculating Fisher's Z-transformed Pearson's correlation coefficients between the seed time courses (left and right HG) and the BOLD signals of all voxels. The sFC estimated by the first

approach (ROI-based connectivity) was calculated at ROI level between bilateral HG, which was used to be correlated with the magnitude of N1 to test the hypothetical N1-sFC coupling. On the other hand, the sFCs estimated by the latter two whole-brain voxel-wise analysis approaches (VMHC and seed-based connectivity) were calculated at voxel level covering the whole-brain, allowing us to test the specificity of the hypothetical coupling (i.e., whether N1 was coupled with the sFC between brain regions other than bilateral HG or not) in a more data-driven way. The coupling between N1 magnitude and sFC estimated by the above three approaches was estimated respectively as the Spearman's rank correlation coefficient. Here, we chose Spearman's rather than Pearson's correlation coefficient mainly because of the following reasons: (1) there is growing evidence suggesting that neurovascular coupling could be nonlinear (Hoffmeyer et al. 2007; Liu et al. 2010; Sheth et al. 2004), so Spearman's correlation coefficient is a better tool for our analysis, as it only assumes monotonicity of the relationship and hence is able to capture nonlinear relationship; (2) Spearman's correlation coefficient is much more robust than Pearson's correlation coefficient, which is especially important for whole-brain voxel-wise analyses because it is not easy to accurately identify all outliers in these analyses. Prior to the coupling analyses, inter-individual variability in rating of loudness (5.34 ± 0.91) was partialled out by linear regression to eliminate possible confounds. Multiple testing correction was performed on the statistical results of whole-brain analyses (VMHC and seed-based connectivity analyses) with the AlphaSim method implemented in REST toolbox ($P_{\text{corrected}} = 0.05$) (Song et al. 2011). This method achieves multiple testing correction by jointly applying two thresholds to the results, including a specified p-value threshold, and a cluster size threshold estimated by Monte-Carlo simulations (Ledberg et al. 1998).

Results

Auditory Evoked Brain Responses

Fig. 1 shows AEPs waveforms at Cz and the scalp topography of N1 component. Similar to previous EEG studies (Mouraux and Iannetti 2009; Zhang et al. 2013), the scalp topography of N1 was maximal at the fronto-central region, and extended bilaterally towards temporal regions. Across individuals, N1 magnitude was $8.35 \pm 3.96 \mu\text{V}$. The estimated SNR of N1 magnitude across individuals was 9.08 ± 5.09 (mean $\pm$ SD).

Fig. 2 shows the cortical regions significantly activated by auditory tones. These activated regions mainly included bilateral superior temporal gyrus (STG), bilateral insular, anterior/mid-cingulate cortices (ACC/MCC), inferior frontal gyrus (IFG), and bilateral inferior parietal cortices (IPC) ($P_{\text{FDR}} < 0.05$). Significant voxels could also be observed within corpus callosum (CC). We believe these significant voxels do not indeed reflect auditory-related activity in CC, but the activity in surrounding regions of CC (e.g., MCC) which are distorted towards the boundary of CC due to a slight bias in fMRI image normalization and smoothing (Reimold et al. 2006). This is supported by our observation that no matter how large a p-value threshold was chosen (up to $p = 0.05$ uncorrected), significant voxels within CC were always located at the boundary of CC, and the center of their belonging cluster converged around MCC and posterior cingulate cortex.

Coupling between N1 and sFC

ROI-based Connectivity Analysis. At inter-individual level, N1 magnitude ($8.35 \pm 3.96 \mu\text{V}$, expressed in positive value) and sFC of bilateral HG (0.97 ± 0.31) were significantly and positively correlated (Spearman's $R = 0.829$, $P < 0.001$; Fig. 3).

Voxel-mirrored Homotopic Connectivity (VMHC) Analysis. Fig. 4A shows the cortical regions that N1 magnitude and VMHC were significantly correlated at inter-individual level ($P_{\text{AlphaSim}} < 0.05$; uncorrected P-value threshold = 0.01; estimated cluster size threshold = 2538 mm^3). Significant positive Spearman's correlations were mainly observed at bilateral medial HG (marked in red in Fig. 4A).

Seed-based Connectivity Analysis. Fig. 4B shows the cortical regions that N1 magnitude and seed-based sFC were significantly correlated at inter-individual level for two seeds (left and right HG), respectively ($P_{\text{AlphaSim}} < 0.05$; uncorrected P-value threshold = 0.01; estimated cluster size threshold: *left* = 6102 mm^3 , *right* = 4833 mm^3). Significant positive Spearman's correlations were only observed at the homotopic regions contralateral to the seeds (marked in red in Fig. 4B), while no correlation was observed at regions ipsilateral to the seeds.

Discussion

The present study provided converging evidence indicating the strong and specific inter-individual relationship between N1 magnitude of AEPs and sFC of bilateral HG. ROI-based analysis showed that N1 magnitude of AEPs was positively correlated with sFC between bilateral HG at inter-individual level (Spearman's $R = 0.829$, $P < 0.001$; Fig. 3). VMHC analysis and seed-based analysis confirmed the relationship between N1 magnitude and sFC of bilateral HG, and ruled out the possible contribution of the variability in N1 magnitude from sFC between other bilateral homotopic regions (Fig. 4A), as well as between HG and any other heterotopic regions (Fig. 4B).

Validity of the Coupling between N1 and sFC

Although the coupling between N1 and BOLD-sFC was not directly investigated in previous studies, clues of such coupling could still be found in the literature. First, it was reported that both N1 magnitude and inter-hemispheric structural connectivity between bilateral auditory cortices were commonly affected by musical training (Elmer et al. 2014; Shahin et al. 2003). Second, even limited by the spatial resolution, a previous EEG study (Kühnis et al. 2014) indicated that N1 magnitude was positively correlated with EEG-based FC between bilateral HG within a musical group. However, there are also discrepancies between our results and those reported in literature, possibility due to differences in neuroimaging modality, experimental design, or analysis strategy. In our study, a strong positive N1-sFC correlation was observed within the the healthy group, while in (Kühnis et al. 2014), no significant N1-sFC correlation (also positive but not significant) was observed within healthy control but a significant negative

correlation was observed within a musical group. Such discrepancies may imply a different mechanism underlying the observed N1-sFC couplings. The coupling in (Kühnis et al. 2014) may capture inter-individual difference in musical training effect, while the coupling in our study may capture other aspects of inter-individual difference (e.g., saliency-detection as was discussed in the following). But such an interpretation should be made carefully. Given that EEG is a more direct measurement of neural activity with a much higher temporal resolution than fMRI, it is possible that the FC measured by EEG and fMRI represent different aspects of synchrony between brain regions.

In the whole-brain voxel-wise analyses, the results suggest that the observed N1-sFC coupling is specifically observed within bilateral HG. Bilateral HG has been identified as one of the major generators of AEP N1 in previous N1 localization studies, although other sub-regions within primary and secondary auditory cortices were also identified frequently (Lütkenhöner and Steinsträter 1998; Meyer et al. 2006; Pantev et al. 1996). The slight difference in the estimated N1 source localizations could be due to the limited spatial resolution and reliability of EEG source localization techniques, and the small size of bilateral HG compared with the possible inter-individual/-group variability in HG structure (Meyer et al. 2014; Morosan et al. 2001; Rademacher et al. 2001; Wong et al. 2008). Our result, by showing that N1 magnitude is correlated with the spontaneous FC between bilateral HG across individuals, suggest an important role of spontaneous FC of bilateral HG in the generation or modulation of N1.

FC of spontaneous brain activities, as a measurement of functional integration across brain regions, provides a powerful tool to capture the brain states (e.g., consciousness, attention) (Craddock et al. 2009; Martuzzi et al. 2010; Shirer et al. 2012). The brain states could influence

the performance of various sensory perception tasks through a top-down process (Gilbert and Sigman 2007). Therefore, the observation that N1 magnitude was strongly correlated with sFC between bilateral HG across healthy individuals may imply that inter-individual difference in baseline information integration of bilateral HG (as indexed by sFC) could greatly influence the strength of auditory evoked neural activity in bilateral HG (as indexed by N1 magnitude). Such inter-individual coupling could serve as a substantial neural mechanism underlying the processing of auditory information.

Importantly, VMHC analysis showed that N1 magnitude was correlated with sFC between bilateral medial HG, but not between bilateral lateral HG (Fig. 4A). Actually, medial HG and lateral HG belong to different sub-division of auditory cortex: while medial HG belongs to core regions of auditory cortex, lateral HG belongs to lateral belt regions (Schönwiesner and Zatorre 2008). From the perspective of hierarchical acoustic signal processing, acoustic input to the brain is transmitted through a pathway (from core regions to belt/para-belt regions of auditory cortex) along which acoustic features are sequentially processed by functionally specialized sub-regions of auditory cortex (Wessinger et al. 2001). It has been reported that auditory core regions are more sensitive to pure-tone stimuli, and belt/para-belt regions are more sensitive to complex sounds (Okada et al. 2010), which also applies to bilateral HG: with intracranial recording, it was reported that while medial HG is more sensitive to onset processing, lateral HG is more involved in processing pitch variation. (Schönwiesner and Zatorre 2008). The VMHC result seems to coincide with the functional role of medial HG (which is more sensitive to pure-tone onset processing), given that pure-tone stimulation was used in our experiment. In addition, previous studies demonstrated that N1 component, as a typical vertex potential, was involved in bottom-

up cognitive mechanisms of saliency-detection, arousal, or attentional reorientation (i.e., more related to onset processing of auditory information than pitch processing) (Downar et al. 2000; Mouraux and Iannetti 2009). Considering the functional similarity between N1 component and medial HG, it is not surprising that N1 magnitude and sFC of bilateral medial HG were highly coupled at inter-individual level, and their coupling could reflect inter-individual difference in onset processing of auditory information, e.g., the distinct ability of saliency-detection of different individuals.

The Significance of the Coupling between N1 Magnitude and sFC

The observation that N1 magnitude and sFC between bilateral HG were strongly coupled has two important practical implications.

First, it is important to consolidate event-related brain responses and ongoing neural activities in understanding cortical processing to sensory inputs and tasks, since event-related brain responses (e.g., N1 in AEPs) are not only determined by experimental operations (e.g., intensity of sensory inputs), but also influenced by the brain states (Olofsson and Polich 2007) as reflected by ongoing neural activities (e.g., sFC between bilateral HG). Similar to previous studies (Liu et al. 2011a; Mennes et al. 2010; Smith et al. 2009; Tian et al. 2012), our finding added to the scientific evidence concerning the inherent relationship between event-related brain responses and spontaneous brain connectivity. Investigating such a relationship could provide a more comprehensive description on an individual's cortical processing to sensory stimuli or tasks, and can enrich our understanding in the functional significance of the coupling between event-related brain responses and spontaneous brain connectivity *per se*.

Second, it is important to rule out possible inter-individual variability in brain responses when comparing different experimental groups. Intuitively, inter-individual variability in brain states is an important confounding factor in various pathological studies, since the brain states, as reflected by spontaneous brain activities/connectivity, could be quite different between patients and healthy individuals. If this confounding factor was not controlled, significant difference of event-related brain responses could reflect the entangled effects of experimental manipulations and the states of the brain. Possibly due to this reason, previous studies assessing the difference of N1 magnitude between different populations (e.g., between young and elderly) yielded inconsistent results: larger N1 magnitude was reported in (Anderer et al. 1998; Herrmann et al. 2013; Rufener et al. 2014), while lower magnitude was reported in (Papanicolaou et al. 1985; Tremblay et al. 2004). Spontaneous FC holds the potential to capture inter-individual variability in brain states. This information could be utilized to remove the variability in N1 magnitude induced by different brain states, thus increasing the reliability and applicability of brain responses as a probe to the mechanism underlying pathophysiological conditions. For this purpose, more work should be done in the future to extract information of brain states from spontaneous FC.

Methodological Considerations

To accurately and reliably assess the relationship between event-related EEG responses and spontaneous sFC, several methodological considerations should be highlighted.

First, it should be made clear that we didn't estimate FC directly using the original BOLD signals, but using the spontaneous BOLD signals isolated from the original signals. To stress this

point, we used sFC instead of FC to refer to our estimated FC values. Under the resting-state condition, sFC could be directly measured by the correlation coefficients between spontaneous BOLD activities, as the resting-state paradigm guarantees the “spontaneous” nature of observed signals. Under task conditions, however, sFC could not be directly estimated because the recorded BOLD signals reflect a mixed effect of spontaneous and task-induced brain activities. And in addition, it is important to analyze sFC instead of FC in event-related studies, because if FC is calculated between original signals recorded during task, it would contain entangled information from evoked responses and spontaneous signals, making interpretation difficult. Fortunately, strong evidence has indicated recently that BOLD signal recorded during task could be well approximated by the linear superposition of spontaneous and task-induced BOLD activity (Fox et al. 2006). This finding suggested that we are able to isolate spontaneous BOLD signals by subtracting task-induced BOLD variations from original BOLD recording. Therefore, in order to estimate sFC from our data, firstly, we modelled task-induced BOLD activities using canonical hemodynamic function, and removed the effect of task-induced BOLD activities by multiple linear regression (Fair et al. 2007). After that, sFC was calculated based on the task-regressed spontaneous BOLD signals. This method, usually termed “task-regressed FC analysis” in the literature, allows us to analyze spontaneous FC on event-related fMRI data, which extended the applicability of FC analysis, and is gaining support from recent literature (Fair et al. 2007; Kucukboyaci et al. 2013). It should be noted that previous studies have suggested altered FC under continuous cognitive/perception tasks (Hampson et al. 2002; Liu et al. 2011b; Lowe et al. 2000; Xu et al. 2006). Therefore, even evoked BOLD responses have been effectively removed by task-regressed FC analysis, spontaneous BOLD fluctuations (and hence the sFC strength) could still be modulated by the engagement of the auditory tasks. However, there is

also evidence showing that event-related tasks do not substantially influence FC strength (Morgan and Price 2004), probably due to the relatively lower cognitive load required for these tasks. Given the long inter-stimulus interval and low cognitive load of our event-related auditory task, we believe that the sFC estimated in our study largely reflects the synchronization of resting-state spontaneous brain activities.

Second, GSR was performed prior to connectivity analysis, since it can efficiently reduce the influence of inter-individual difference caused by typical fMRI artefacts, e.g., cardiac, respiratory, and head motion artefacts (Fox et al. 2009). Although GSR was criticized for its tendency to bias BOLD-FC to negative values (Murphy et al. 2009), our analyses were focused on sFC between homotopic regions which is normally positive, and, thus, were minimally influenced by the distortion effect of GSR (Fox et al. 2009).

Conclusion

In this study, we observed a strong coupling between the magnitude of AEP N1 and sFC of bilateral HG with simultaneous EEG-fMRI recording. The result may imply that inter-individual difference in baseline information integration of bilateral HG could influence the strength of auditory evoked neural activity in bilateral HG. This finding could reflect important neural mechanism underlying auditory information processing, which holds the potential to increase the applicability of brain responses as a probe to the mechanism underlying pathophysiological conditions.

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Disclosure Statement

The authors declare no competing financial interests.

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Figure Captions

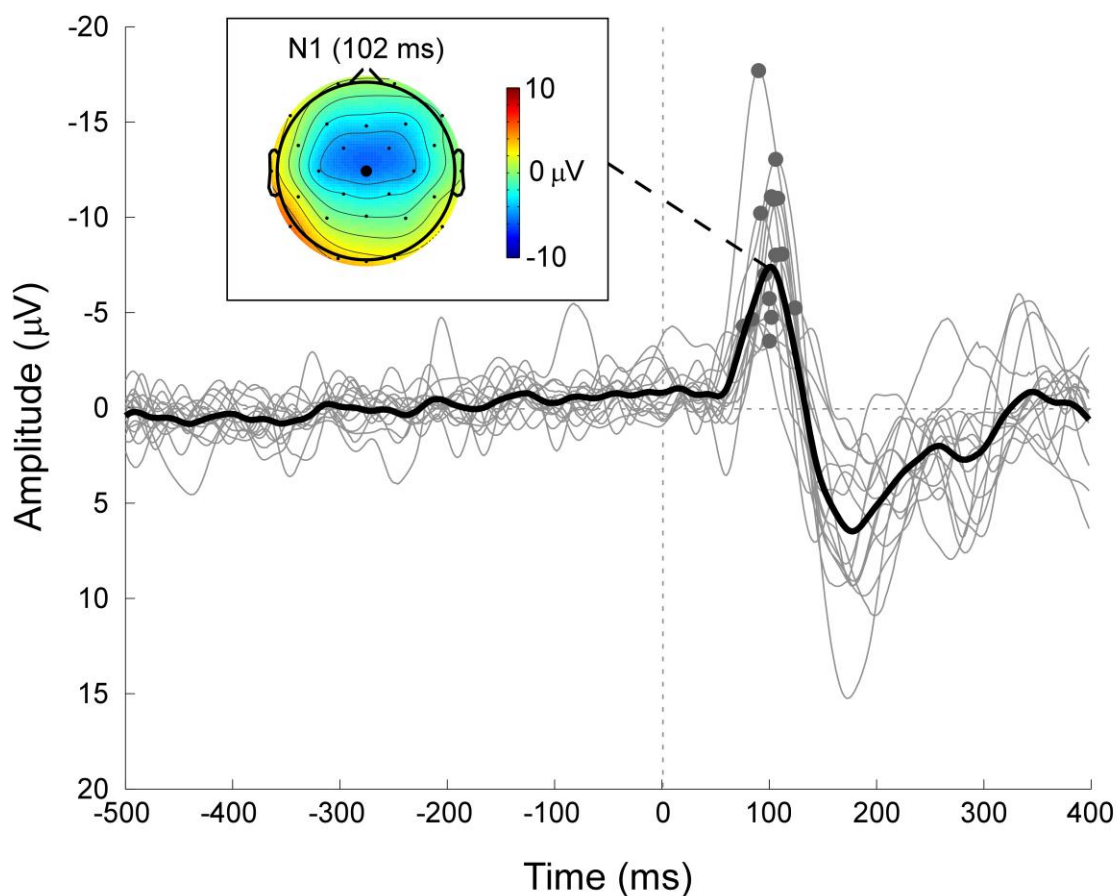


Fig. 1. Auditory evoked potentials waveforms and N1 scalp topography. Displayed signals were recorded from Cz (average reference). Single-individual average waveforms are superimposed (grey), and the group average waveform is marked with black thick line. The scalp topography of N1 component is displayed at its peak latency (102 ms). The peaks of N1 component, which show substantial inter-individual variability in magnitude, are marked using grey dots.

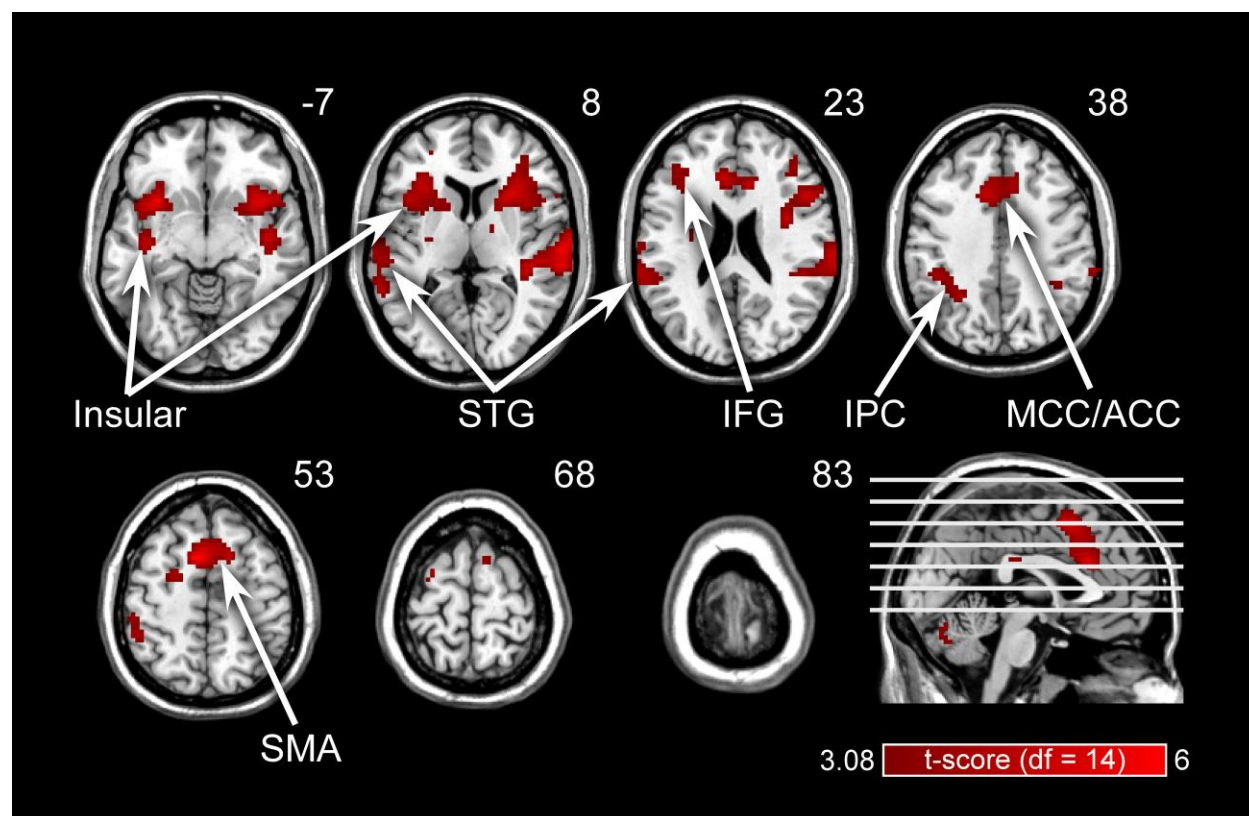


Fig. 2. Brain regions activated by auditory stimuli ($P < 0.05$, false discovery rate corrected). Bilateral superior temporal gyrus (STG), bilateral insular, anterior/mid-cingulate cortices (ACC/MCC), inferior frontal gyrus (IFG), and bilateral inferior parietal cortices (IPC) were activated by auditory stimuli (indicated in red).

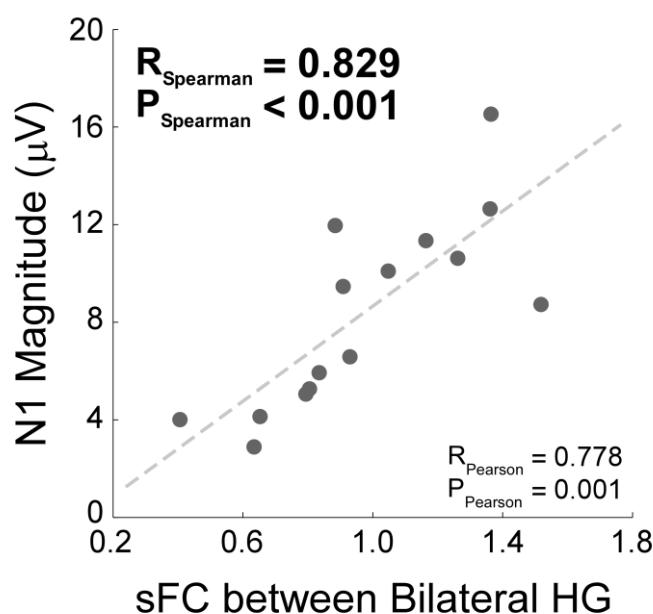


Fig. 3. Relationship between N1 magnitude and sFC of bilateral HG (Spearman's $R = 0.829$, $P < 0.001$). Each point represents the data from one individual. Spearman's correlation coefficient was calculated to assess the relationship. The best fitting line (dash line) as well as its associated Pearson's correlation coefficient are shown just for illustrative purpose.

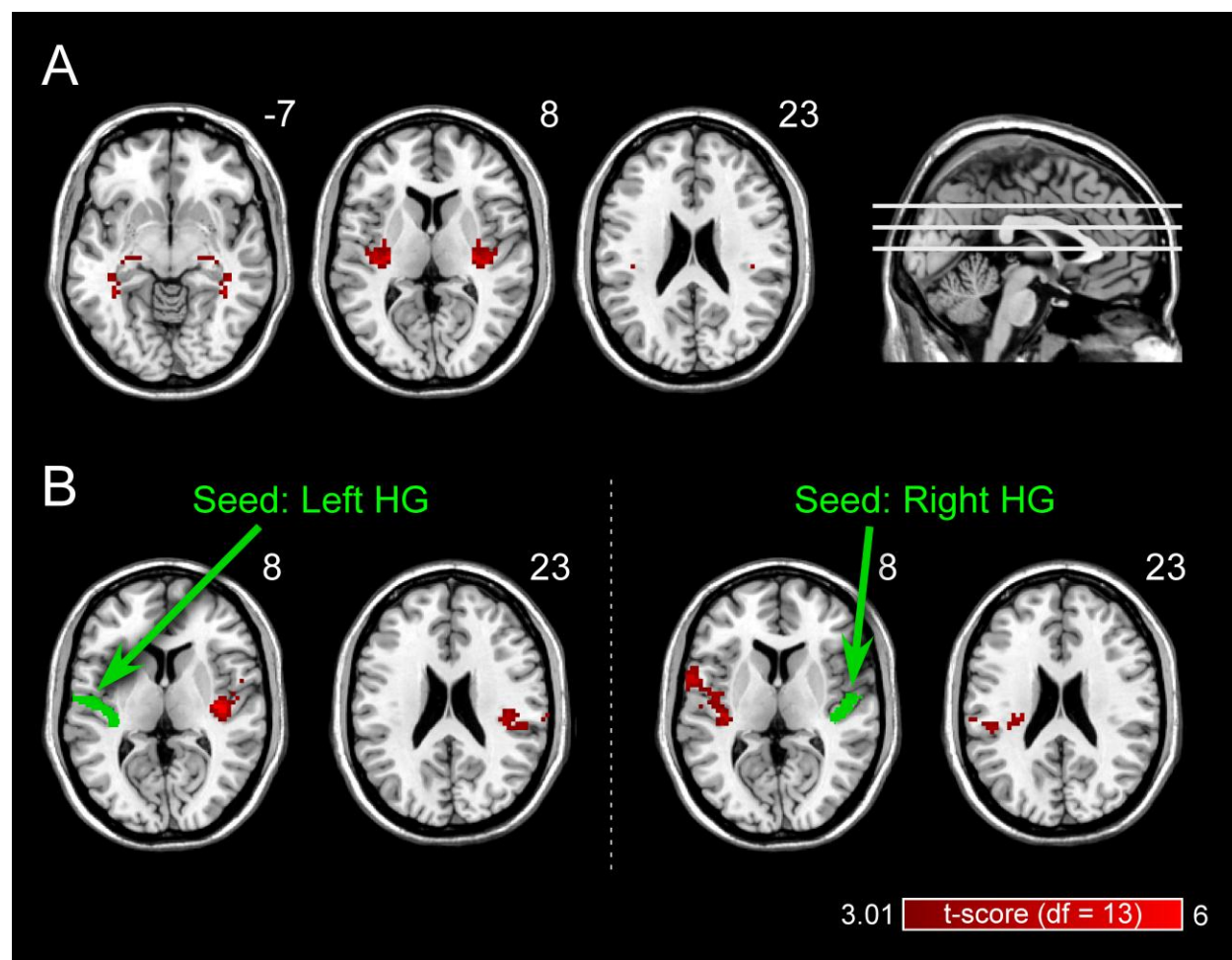


Fig. 4. The coupling between N1 magnitude and sFC that was estimated using voxel-mirrored homotopic connectivity (VMHC) analysis and seed-based connectivity analysis. (A) Brain regions showed significant Spearman's correlation between N1 magnitude and VMHC at inter-individual level. Significant positive correlations were observed at bilateral medial Heschl's gyrus (HG) (marked in red). (B) Brain regions showed significant correlation between N1 magnitude and seed-based sFC at inter-individual level. *Left:* left HG was used as the seed (marked in green). *Right:* right HG was used as the seed (marked in green). Significant positive

correlations were only observed at the homotopic regions contralateral to the seeds (marked in red), and no significant correlation was observed at regions ipsilateral to the seeds.