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RESEARCH ARTICLE

Auditory neural networks involved in attention modulation prefer biologically significant sounds and exhibit sexual dimorphism in anurans

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ABSTRACT

Allocating attention to biologically relevant stimuli in a complex environment is critically important for survival and reproductive success. In humans, attention modulation is regulated by the frontal cortex, and is often reflected by changes in specific components of the event-related potential (ERP). Although brain networks for attention modulation have been widely studied in primates and avian species, little is known about attention modulation in amphibians. The present study aimed to investigate the attention modulation networks in an anuran species, the Emei music frog (*Babina daunchina*). Male music frogs produce advertisement calls from within underground nest burrows that modify the acoustic features of the calls, and both males and females prefer calls produced from inside burrows. We broadcast call stimuli to male and female music frogs while simultaneously recording electroencephalographic (EEG) signals from the telencephalon and mesencephalon. Granger causal connectivity analysis was used to elucidate functional brain networks within the time window of ERP components. The results show that calls produced from inside nests which are highly sexually attractive result in the strongest brain connections; both ascending and descending connections involving the left telencephalon were stronger in males while those in females were stronger with the right telencephalon. Our findings indicate that the frog brain allocates neural attention resources to highly attractive sounds within the window of early components of ERP, and that such processing is sexually dimorphic, presumably reflecting the different reproductive strategies of males and females.

KEY WORDS: Auditory attention, Brain network, Granger causal connectivity analysis (GCCA), Event-related potential, Music frog

INTRODUCTION

Virtually all living creatures encounter a rich variety of acoustic stimuli in their natural environment, such as humans at a cocktail party (Carlile, 2015) or frogs in a reproductive lek (Höglund and Alatalo, 2014). In most cases, only a few stimuli in such noisy environments have biological relevance and need to be salient to individuals. Accordingly, individuals must be able to selectively allocate

processing resources to such important stimuli (Bronkhorst, 2015; Koch et al., 2011). These processes involve the allocation of attention.

Attention involves both voluntary attention (top-down goal-directed processes) and reflexive attention (bottom-up stimulus-driven mechanisms) and is the ability to focus awareness on one stimulus, thought or action while ignoring other irrelevant ones (Gazzaniga et al., 2014). Attention allocation in humans has been shown to be modulated by the frontal cortex (Baluch and Itti, 2011; Grabenhorst and Rolls, 2010), which modifies the activity of the primary auditory cortex, thereby influencing auditory perception in the auditory system (Bidet-Caulet et al., 2007; Kauramäki et al., 2007). Changes in attention allocation are reflected by electrophysiological changes in specific components of the event-related potential (ERP). For example, when subjects were asked to pay attention to target sounds, the elicited N1 ERP components (a negative peak occurring ~100 ms after the stimulus onset) were enhanced compared with those elicited by sounds the subjects were not told to attend to (Hillyard et al., 1973; Woldorff and Hillyard, 1991). Moreover, perceptual functions are carried out through brain networks (Bullmore and Sporns, 2009; De Vico Fallani et al., 2009). Thus, it is reasonable to hypothesize that the neural networks underlying these specific ERP components reflect attention allocation during the early stages of auditory perception. Notably, although studies have investigated brain networks for attention allocation (Alho et al., 2015; Salmi et al., 2009), few have identified attention allocation networks associated with the neural events occurring within the time window of specific ERP components.

Forebrain-based attention modulation, including that related to auditory perception, has been found in mammalian (Birrell and Brown, 2000; Schafer and Moore, 2007) and songbird (Sridharan et al., 2014) species. Many vertebrate forebrain systems have been shown to have been conserved during evolution, including the auditory system of land vertebrates (Butler and Hodos, 2005). In view of the fact that land vertebrates are derived from an amphibian ancestor, it seems reasonable to hypothesize that auditory attention modulation exists in amphibians and that investigation of these mechanisms can shed light on the general principles of auditory functioning. Thus, the present study sought to identify the network for attention allocation in the brain of an anuran species, the Emei music frog, *Babina daunchina*.

Male music frogs produce advertisement calls from underground nest burrows. Male call acoustics are modified by burrow resonance such that both males and females can discriminate calls produced from inside burrows from those produced in open fields. Females prefer calls from inside nests which are of high sexual attractiveness (HSA) and males are more likely to vocally compete with other males calling from inside burrows rather than in response to calls of low sexual attractiveness (LSA) produced from outside burrows (Cui et al., 2011; Fang et al., 2014a; Xue et al., 2015).

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Electrophysiological studies have shown that call playback stimuli elicit N1 ERP components significantly different from those elicited by white noise (WN) (Fang et al., 2015), consistent with the idea that music frogs allocate attention on the basis of the biological significance of sounds insofar as N1 is not solely determined by sensory processing but is modulated by attention (Gazzaniga et al., 2014). Moreover, both resting-state brain networks (Xue et al., 2016b) and functional brain networks associated with auditory perception (Xue et al., 2016a) have been identified in the music frog, suggesting that brain functions such as attention are mediated by specific brain networks. Taken together, these studies strongly support the idea that brain networks associated with the allocation of attention resources exist in music frogs and are related to the reproductive strategies of males and females.

Granger causal connectivity analysis (GCCA) is a mathematical tool based on linear regression modeling of stochastic processes (Granger, 1969). It calculates the statistical causality between data series by implementing a statistical and predictive notion of causality whereby causes help to predict their effects in the time and frequency domains (Gao et al., 2015; Seth et al., 2015). In the present study, we aimed to investigate the brain networks related to specific ERP components in music frogs by GCCA, which has been used in previous studies of mammalian species including humans to identify functional brain networks related to attention modulation (Friston et al., 2013; Gregoriou et al., 2009; Ozaki, 2011). To do this in music frogs, we presented two conspecific advertisement calls (HSA and LSA) and WN stimuli to frogs and recorded electroencephalogram (EEG) signals at the same time. By analyzing these signals within the time window of ERP components with the GCCA method, we sought to build causal networks elucidating the functional connections between brain areas. Based on the results of previous neural studies in this species, we predicted that (1) brain networks processing stimulus information within the time window of ERP components are lateralized and strongest for biologically important sound stimuli, and (2) these brain connections are sexually dimorphic.

MATERIALS AND METHODS
Animals
Fifteen Emei music frogs, *Babina daunchina* (Chang 1933) (nine males and six females), were collected from the Emei Mountain area (29.60°N, 103.36°E, elevation of 1315 m above sea level), Sichuan, China. Animals were separated by sex and housed in two opaque plastic tanks (45 cm×35 cm and 30 cm deep) containing ~3 cm water with ~5 cm mud in height around the walls so that the frogs could shelter themselves. The tanks were placed in a room under controlled temperature (23±1°C) and relative humidity (70–80%) with a 12 h:12 h light:dark cycle (lights on at 08:00 h) using a fluorescent lamp. The animals measured 4.5±0.05 cm in body length and 9.7±0.45 g (mean ±s.e.m.) in mass at the time of surgery. The animals were fed live crickets (bought from a pet food shop) every 3 days. The experimental protocols were approved by the Animal Care and Use Committee of Chengdu Institute of Biology under license number 2016005 and carried out according to international standards of animal care and use.

Surgery
All experiments were conducted after the reproductive season had ended for this species. Surgical procedures have been described in detail in a previous study (Fang et al., 2011). In brief, four cortical EEG electrodes, composed of miniature stainless steel screws (diameter 0.8 mm), were implanted on the frog skull after anesthesia, above the left and right sides of the telencephalon and mesencephalon. As shown in Fig. 1, sites R1, R2, R3 and R4 refer to electrodes for the left telencephalon (LT), right telencephalon (RT), left mesencephalon (LM) and right mesencephalon (RM), respectively, and were referenced to the electrode above the cerebellum (P). After the operation, animals recovered individually in their home cages for 2 days with the same housing conditions as before the experiments. Intraperitoneal injections of gonadotropin-releasing hormone (GnRH-A6, Xingwang, Sichuan, China; 1.25 µg per animal) were used to bring animals into the reproductive stage as described in a previous study (Fang et al., 2011). After administration, the reproductive status of males was determined by recording call activity and that of females was determined based on phonotaxis behavior (i.e. approaching a speaker broadcasting male advertisement calls).

At the end of the experiments, the animals were killed by intraperitoneal injection of sodium pentobarbital, and hematoxylin dye was injected through the skull holes in which the electrodes were implanted to confirm recording electrode sites.
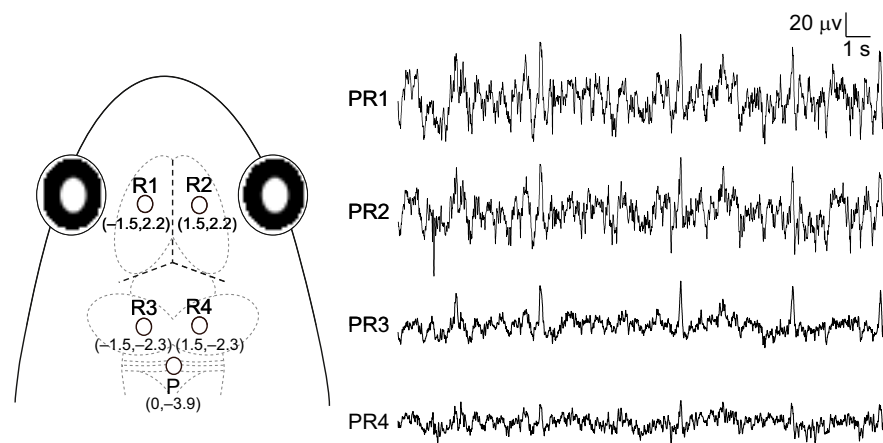

Fig. 1. Electrode placement (left) and 20 s of typical electroencephalographic (EEG) tracings for each channel (right). The intersection of the three black dashed lines on the frog head denotes the intersection of the lambdoid suture corresponding to lambda. R1–R4 indicate electrodes for the left telencephalon, right telencephalon, left mesencephalon and right mesencephalon, respectively, which were referenced to the electrode above the cerebellum (P). Adapted from Fang et al. (2011) with permission.

Stimulation and apparatus

Three stimuli of different biological significance were used: HSA calls, LSA calls and WN (Fig. 2) (Fang et al., 2015). HSA and LSA calls that contained five notes recorded from the same individual were chosen randomly from the data set of our laboratory. WN was synthesized with temporal features that resemble a typical call consisting of five notes, with the duration of each note equal to the average note duration of the HSA and LSA calls, and for which each note was shaped with rise and fall time sinusoidal periods of 7.5 ms by Adobe Audition (Adobe, San Jose, CA, USA). All stimuli were equalized for intensity measured at the center of the tank (re. 20 μPa, 65 dB SPL; AWA6291, Aihua, Hangzhou, China).

An opaque plastic tank (80×60 cm and 55 cm deep) containing mud and water was used for the experiments and was placed in a soundproof and electromagnetically shielded chamber (background noise, 23.0±1.7 dB). An infrared camera with a motion detector was mounted centrally about 1 m above the tank for monitoring the locomotor activity of the subjects. Two speakers (SME-AFS, Saul Mineroff Electronics, Elmont, NY, USA) were placed 1.7 m apart, equidistant from the opposite ends of the tank with a rectangular hole (20 cm×15 cm) at the lower central wall area of each end. Before the experiments, we used a 1000 Hz tone to calibrate the peak output intensity of each speaker to 70 dB SPL (measured at the center of the tank).

Data acquisition

The animal was placed in the experiment tank and connected to the signal acquisition system (RM6280C, Chengyi, Sichuan, China) and allowed to habituate for about 24 h before the playback experiments. As it is extremely difficult to implement operant conditioning paradigms in frogs, only reflexive attention (stimulus-driven processes) was considered in the present experiment. The procedure was based on the equal-frequency paradigm. Specifically, each stimulus was presented a total of 100 times to each animal for a total of 300 stimulus presentations for each subject. Stimuli were presented from the two speakers simultaneously and broadcast in a random order within three blocks (100 stimulus presentations each), with a 1.5 s inter-stimulus interval between each stimulus presentation (Fang et al., 2015). Each stimulation block was followed by a 5 min silent period. Electrophysiological signals were recorded from the start of the experiments to the end. The band-pass filter was set to 0.16–100 Hz for filtering EEG signals with a hardware notch filter to eliminate possible interference at 50 Hz and improve the signal-noise ratio. A sampling rate of 1000 Hz was used.

Data processing

EEG recordings were filtered by multitaper filtering, which removed the 50 Hz mains-electricity line-noise without disturbing the Granger causality (GC) between signals (Mitra and Bokil, 2007). EEG signals were then divided into epochs with a duration of 1700 ms, including a pre-stimulus baseline of 200 ms. All epochs were inspected visually and those with artifacts and electrode drifts were removed from any further processing. The EEG signal within three 100 ms time windows corresponding to three previously identified auditory ERP components in this species (Fang et al., 2015) was analyzed further: the time window of 30–130 ms after stimulus onset was defined as ERP component N1, the 150–250 ms window was defined as P2 and the 290–390 ms window was defined as P3. The epochs of a given ERP component from an individual were then extracted and concatenated sequentially to build a new data set. Therefore, for each individual, three data sets were derived corresponding to the N1, P2 and P3 ERP components.

As the length of epochs acquired from various trials was equal, the data set was then processed as multi-trial data using the GCCA
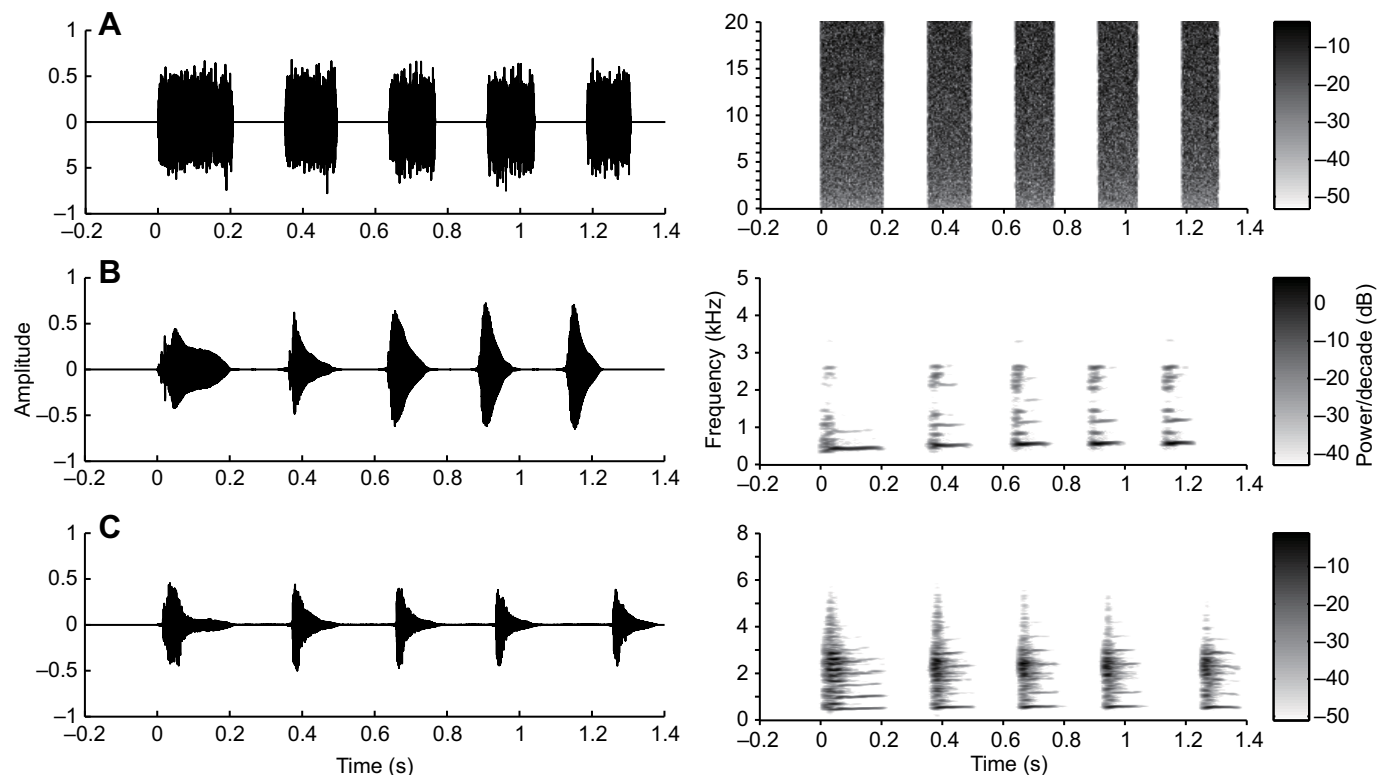

Fig. 2. Waveforms (left) and spectrograms (right) of the three stimuli. (A–C) White noise (WN), the high sexual attractiveness (HSA) call and the low sexual attractiveness (LSA) call, respectively. Adapted from Fang et al. (2015) with permission.

toolbox for Matlab (The MathWorks Inc., Natick, MA, USA) (Seth, 2010). Specifically, each data set was processed with detrending and ensemble demeaning. The covariance stationarity of each epoch in a data set was checked by the augmented Dickey–Fuller test (Hamilton, 1994) and Kwiatkowski–Phillips–Schmidt–Shin test (Kwiatkowski et al., 1992) for multi-trial data. As many of the epochs failed to pass the tests, first-order differencing was applied to all epochs to address the non-stationarity covariance. Those epochs were excluded if they failed to pass the tests after applying first-order differencing. Insofar as this processing can induce some acute variations of power spectra at lower frequencies, which would be problematic for the analysis of frequency-dependent GC (Seth, 2010), only time-dominant GC was applied in the present study. The optimal model order for each data set was automatically selected using the Akaike information criterion (mean±s.e.m.=26.64±0.50) (Akaike, 1974). The Durbin–Watson test was used to determine whether the residuals were uncorrelated (Durbin and Watson, 1950) and all data sets passed this test. The consistency test assesses the portion of data captured by the multivariate autoregressive model with a threshold of 70% (Ding et al., 2000); all data sets passed this test.

GC values were calculated with a significance threshold $P=0.01$, Bonferroni corrected (Seth et al., 2015). The electrodes were defined as the nodes and every node pair represented two GC connections with values indicating connection strengths (i.e. GC connections). For example, in the Results, LT–LM stands for the GC connection from the left telencephalon (LT) to the left mesencephalon (LM), while LM–LT stands for the GC connection from the left mesencephalon (LM) to the left telencephalon (LT). As four electrode sites were involved in a particular condition (i.e. stimulus type×reproductive status), a data set with 12 GC connections was computed for each ERP component of each subject. For each component, the GC connections from all individuals were organized into a matrix for statistical analysis according to sex, stimulus type and reproductive status. For each matrix, the GC connections were categorized into three groups according to their flow direction: from telencephalon to mesencephalon (T–M), from mesencephalon to telencephalon (M–T) and between corresponding brain areas bilaterally (L–R).

Statistical analysis

The data groups were normality distributed and their variances were homogeneous according to the Shapiro–Wilk W test and Levene's test, respectively. Repeated measures ANOVA with the within-subject factors 'connection', 'stimulus' and 'reproductive status' and the between-subjects factor 'sex' was employed for each group. Greenhouse–Geisser epsilon (ε) values were employed to correct the P-values if the data could not be applied to Mauchly's test of sphericity or failed to pass it. Simple and simple–simple effects analyses with the least-significant difference (LSD) were applied for significant dual and triple interactions, respectively.

Effect size was determined with Cohen's d for t-tests and partial η^2 for ANOVA (Cohen's d or partial $\eta^2=0.20$ is set as small, 0.50 as medium and 0.80 as large effect sizes). SPSS software (release 19.0) was utilized for these statistical analyses. A significance level of $P<0.05$ was used in all comparisons (Utts and Heckard, 2005).

RESULTS

The mean (±s.e.m.) number of epochs used for GC analysis was 66.09±0.93 for the N1 component, 69.77±0.87 for the P2 component and 67.52±0.82 for the P3 component. There was neither a main effect nor an interaction with respect to the factor 'reproductive status'; therefore, the results were based on the average over the two reproductive stages.

The HSA calls elicit greater GC brain connections

The factor 'stimulus' showed significant main effects in several data groups. HSA calls yielded significantly higher GC values than the LSA and WN stimuli in the L–R group for the N1 component ($F_{2,26}=8.238$, $P<0.01$, partial $\eta^2=0.388$) (Fig. 3C). For the T–M

Table 1. Results of simple effect analysis for the factors 'connection' and 'sex' for different groups and ERP components

| Component | GC connection | Based on connection | | | | m/f | Based on sex | | | |
		$F_{1,13}$	P	Partial η^2	LSD		$F_{3,24}/F_{3,15}$	P	Partial η^2	LSD
N1	LT–LM	0.427	0.525	0.032	NA	m	6.281	0.003*	0.440	LT–LM>LT–RM/RM–LT
	LT–RM	4.592	0.052	0.261	NA					
	RT–LM	9.059	0.010*	0.411	f>m	f	4.957	0.014*	0.498	RT–RM/RT–LM>LT–LM/LT–RM
	RT–RM	6.349	0.026*	0.328	f>m					
P2	LT–LM	1.496	0.243	0.103	NA	m	7.049	0.016*	0.468	LT–LM>LT–RM
	LT–RM	3.841	0.072	0.228	NA					
	RT–LM	7.819	0.015*	0.376	f>m	f	3.450	0.044*	0.408	RT–RM>LT–LM/LT–RM
	RT–RM	6.357	0.026*	0.328	f>m					
	LM–LT	0.077	0.786	0.006	NA	m	12.902	0.000*	0.617	LM–LT>RM–RT>RM–LT LM–RT>RM–LT
	LM–RT	4.395	0.056	0.253	NA					
	RM–LT	7.438	0.017*	0.364	f>m	f	6.043	0.018*	0.547	LM–RT>LM–LT/RM–LM/RM–RT
	RM–RT	0.011	0.916	0.001	NA					
P3	LT–LM	6.033	0.029*	0.317	m>f	m	9.559	0.000*	0.544	LT–LM>LT–RM/RT–LM/RT–RM
	LT–RM	5.564	0.035*	0.300	f>m					
	RT–LM	9.493	0.009*	0.422	f>m	f	3.401	0.045*	0.405	RT–RM/RT–LM>LT–RM/LT–LM
	RT–RM	5.963	0.030*	0.314	f>m					
	LM–LT	0.156	0.699	0.012	NA	m	14.259	0.000*	0.641	LM–LT>RM–RT>RM–LT LM–RT>RM–LT
	LM–RT	6.697	0.023*	0.340	f>m					
	RM–LT	8.630	0.012*	0.399	f>m	f	11.256	0.000*	0.692	LM–RT>LM–LT>RM–RT LM–RT>RM–LT
	RM–RT	0.148	0.706	0.011	NA					

Magnitudes of the Granger causality (GC) connections to the left of '>' are significantly larger than those to the right. Note that there were no main effects and interactions with respect to the factor 'reproductive status'; the results in this table were based on the average over the reproductive and non-reproductive stages. There was no interaction between two factors in the M–T group of N1 components, which therefore are not present in the table. The F-values (from ANOVA) for the factor 'sex' are different between males and females and are therefore presented separately; the first F-value is for males and the second is for females. Partial η^2 is the effect size for ANOVA. LSD, least-significant difference; NA, not applicable; f, female; m, male; LT, left telencephalon; RT, right telencephalon; LM, left mesencephalon; RM, right mesencephalon. *$P<0.05$.

group, the P2 component showed higher GC values for HSA calls than for LSA or WN stimuli ($F_{2,26}$=4.525, $P<0.05$, partial η^2=0.258) (Fig. 4A). Both HSA and LSA calls yielded significantly greater P2 component GC values in the L−R group

than did WN stimuli ($F_{2,26}$=8.072, $P<0.01$, partial η^2=0.383) (Fig. 4C). The N1 component also yielded higher GC values in the T–M group for HSA calls; however, the difference only reached the marginal significance level (P=0.054) (Fig. 3A).

In other words, for both the N1 and P2 components, the GC connections in the T–M and L–R groups were stronger with respect to the HSA calls than to both the LSA and WN stimuli, despite the fact that the GC T–M connection associated with N1 was of
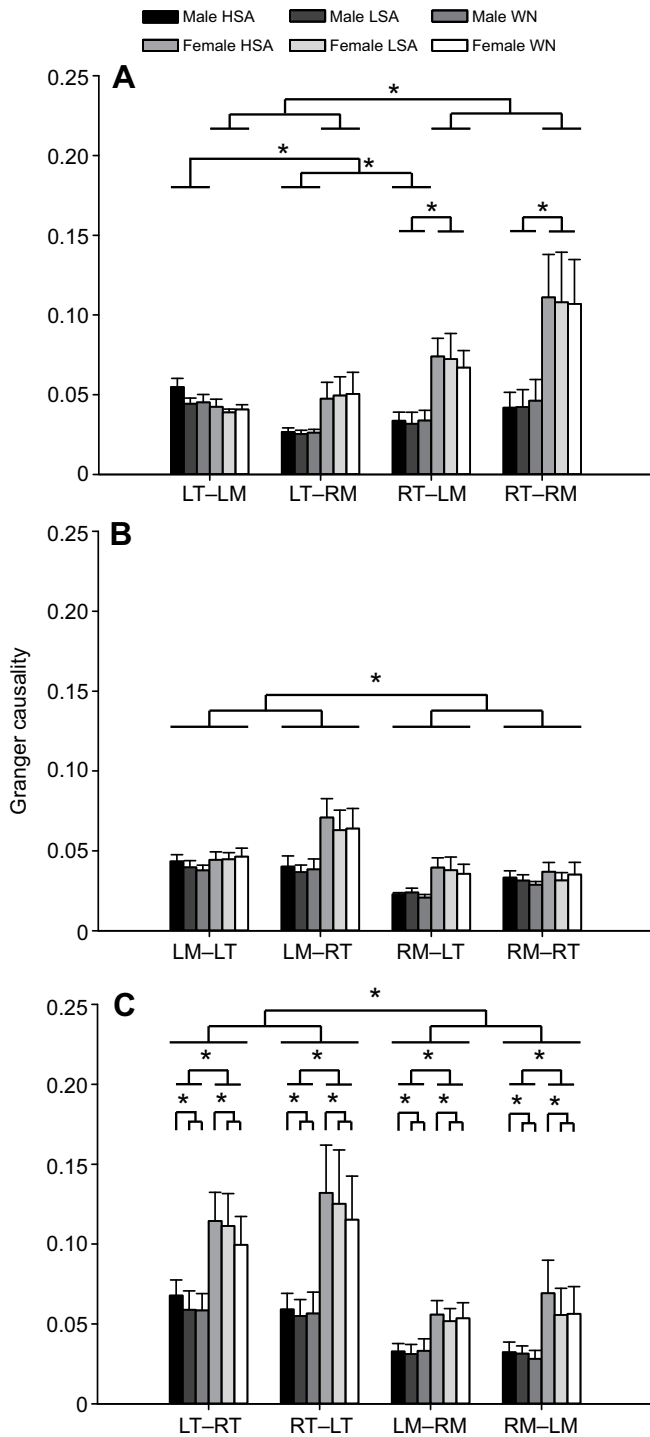

Fig. 3. Granger causality (GC) connections of the N1 components of each network group for males and females under different stimulus conditions. (A–C) Connections from the telencephalon to the mesencephalon (T–M), from the mesencephalon to the telencephalon (M–T) and between the left hemisphere and right hemisphere (L–R), respectively (nine males and six females). Data are means±s.e.m. The asterisks denote a significant difference for repeated-measures ANOVA ($P<0.05$). LT, left telencephalon; RT, right telencephalon; LM, left mesencephalon; RM, right mesencephalon.
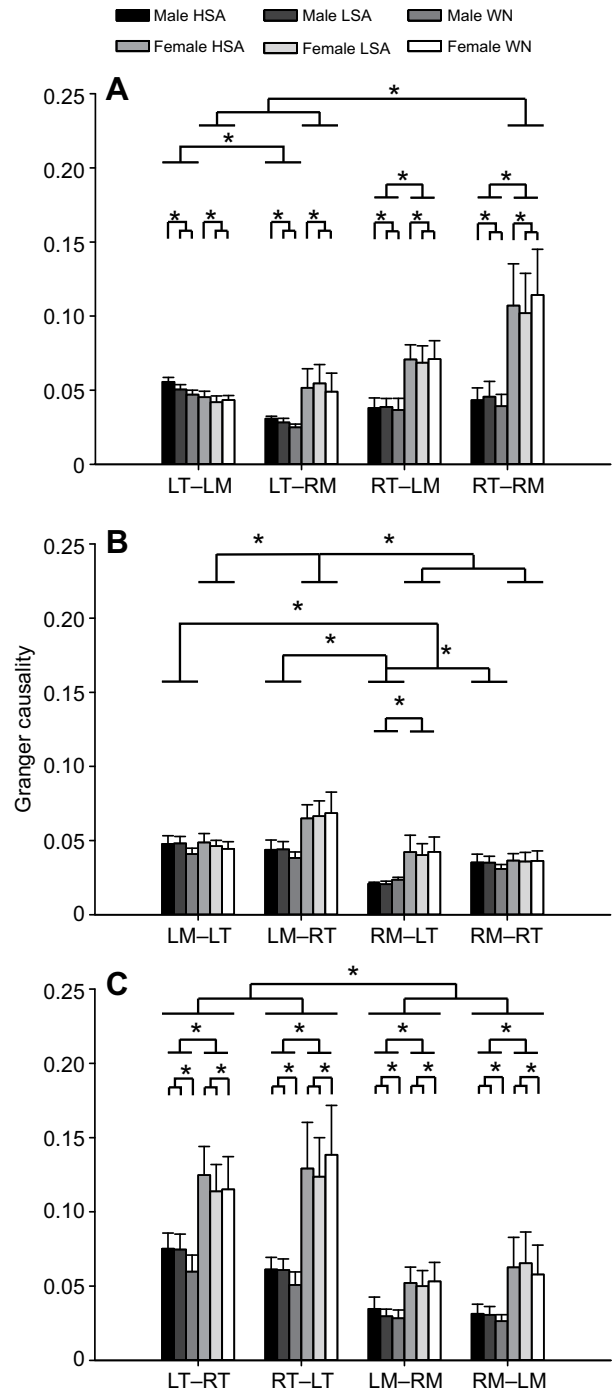

Fig. 4. GC connections of P2 components of each group for males and females under different stimulus conditions. (A–C) Connections in the T–M, M–T and L–R groups, respectively (nine males and six females). Data are means±s.e.m. The asterisks denote a significant difference for repeated-measures ANOVA ($P<0.05$).

marginal significance. Overall the results support the idea that the network prefers the HSA calls.

The brain networks are asymmetric and sexually dimorphic

In the T–M group, all three ERP components yielded significant interactions between the factors 'connection' and 'sex' ($F_{3,39}$=5.846, $P<0.01$, partial η^2=0.310 for N1 components; $F_{3,39}$=5.182, $P<0.05$, partial η^2=0.285 for P2; and $F_{3,39}$=6.710, $P<0.05$, partial η^2=0.340 for P3). According to simple effect analysis, despite differences among the GC connections, the GC values in males for LT–LM were consistently higher for all ERP components while in females the GC values of RT–RM were highest (Table 1). In addition, the GC values of RT–RM and RT–LM were significantly higher in females than in males for all ERP components. Notably only the LT–LM for P3 components yielded higher GC values in males than in females (Table 1).

In the M–T group, both P2 and P3 components yielded significant interactions between the factors 'connection' and 'sex' ($F_{3,39}$=3.461, $P<0.05$, partial η^2=0.210 for P2 components and $F_{3,39}$=3.563, $P<0.05$, partial η^2=0.215 for P3). Despite the particular order of GC connections, simple effect analysis showed that the GC values of LM–LT were significantly higher in males while the GC values of LM–RT were significantly higher in females. In addition, the GC values of RM–LT for the P2 component and the GC values of LM–RT and RM–LT for the P3 component were significantly higher in females than in males (Table 1). In the M–T group, the GC values for the N1 component for LM–LT and LM–RT were significantly greater than those for RM–LT and RM–RT ($F_{3,39}$=13.008, $P<0.01$, partial η^2=0.500) (Fig. 3B).

In the L–R group for all ERP components, the factor 'connection' yielded significant effects. The values of LT–RT and RT–LT were significantly greater than those of LM–RM and RM–LM [$F_{3,39}$=23.900, $P<0.01$, partial η^2=0.648 for N1 (Fig. 3C); $F_{3,39}$=42.909, $P<0.01$, partial η^2=0.767 for P2 (Fig. 4C); and $F_{3,39}$=41.382, $P<0.01$, partial η^2=0.761 for P3 (Fig. 5C)].

The results show ascending GC connections from the left mesencephalon in the M–T groups were always stronger than those from the right mesencephalon. The results also show that the descending connections in the T–M groups were sexually dimorphic and that the connections between the two sides of the telencephalon were stronger than those with the mesencephalon in the L–R groups, revealing asymmetry in the functional organization of these brain networks. When the results of the T–M and M–T groups are combined, it may be concluded that both the ascending and descending connections involving the left telencephalon (i.e. LT–LM and LM–LT) are relatively strong in males, while in females, connections involving the right telencephalon (i.e. RT–RM and LM–RT) are strongest.

DISCUSSION

Many species including frogs exhibit behaviors referred to as 'attention' (Brush and Narins, 1989), which is modulated in mammals by the frontoparietal attention system (Gazzaniga et al., 2014). In anurans, purely perceptual processes such as stimulus recognition and localization are mostly completed at the level of the auditory midbrain (Wilczynski and Endepols, 2007). Because important neuroanatomical features have been conserved during vertebrate brain evolution (Allman, 2000; Finlay et al., 2001; Northcutt, 2002), it is reasonable to speculate that the GC connections associated with the early telencephalic electrophysiological activity evoked by the acoustic stimuli
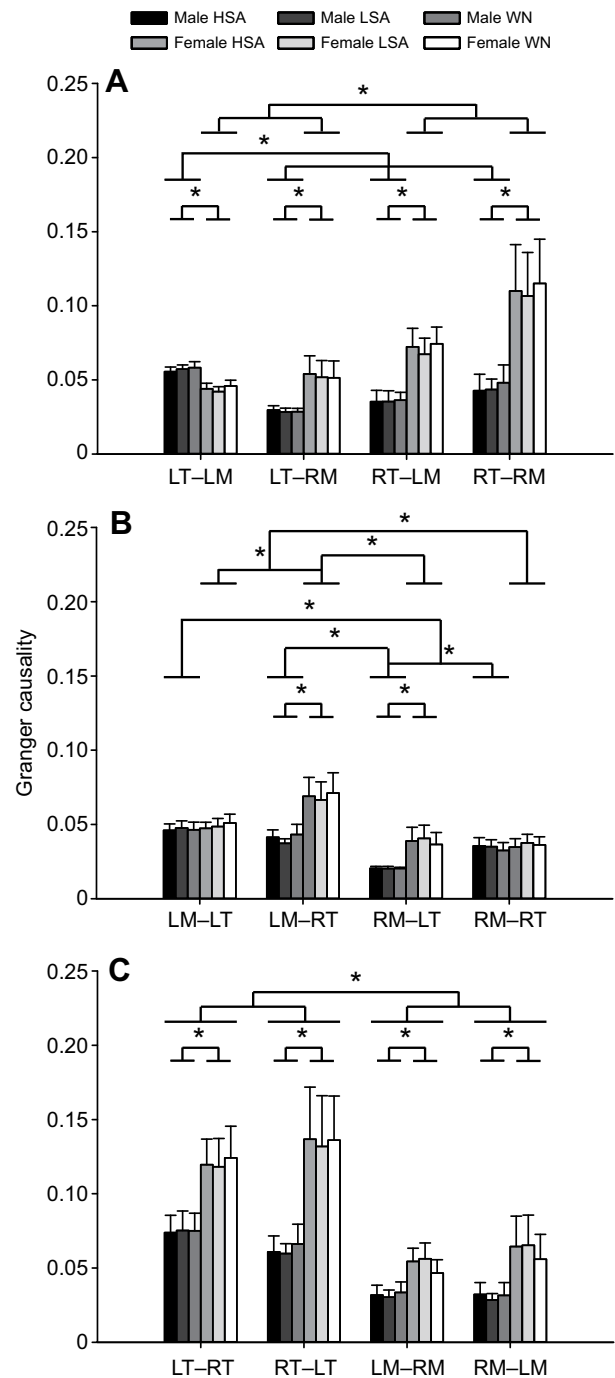

Fig. 5. GC connections of P3 components of each group for males and females under different stimulus conditions. (A–C) Connections in the T–M, M–T and L–R groups, respectively (nine males and six females). Data are means±s.e.m. The asterisks denote a significant difference for repeated-measures ANOVA (*P*<0.05).

(especially top-down connections) are, in all likelihood, attributable to attention.

In amphibians, forebrain structures are far less differentiated than in mammals (Butler and Hodos, 2005). For this reason, the neural networks associated with the early attentional period in frogs cannot readily be explained by comparison with networks previously identified in mammals (Alho et al., 2015; Salmi et al., 2009; Woldorff et al., 1993). Nevertheless the study of frog brain networks for attention can provide insights into how the organization of these

networks enables animals to become adapted to their environment. In particular, the strengths of GC causal brain network connections in music frogs within the time window of the N1, P2 and P3 ERP components varied with the acoustic stimulus type and were sexually dimorphic. The results of the present study also show that the factor 'reproductive status' has neither main effects nor interactions with other factors, consistent with previous studies revealing that reproductive status does not affect the amplitude and latency of auditory ERP components elicited by male advertisement calls and white noise (Fang et al., 2015). The GC connections therefore represent relatively stable network properties not readily susceptible to seasonal hormonal influences.

Attention is influenced by the biological significance of stimuli

The present results show that HSA calls elicit stronger GC connections involving the telencephalon during the ERP component time windows, implying that the telencephalon plays a key role in processing information about these stimuli during these time periods. In humans, the frontal cortex, including the frontal eye fields and inferior frontal gyrus, plays critical roles during auditory attention modulation (Knight et al., 1981; Osnes et al., 2012; Thomsen et al., 2004; Yamaguchi et al., 2000). Similarly, these frontal cortical areas have also been shown to be the core areas for attention modulation in non-human primates (Schafer and Moore, 2007; Wardak et al., 2006) and rodents (Birrell and Brown, 2000) for both auditory and visual stimuli, insofar as attention modulation for different sensory modalities is completed by common neural networks (Shinn-Cunningham, 2008). No equivalent experiments using electrical micro-stimulation to shift attention have been carried out in avian species. Nevertheless, the arcopallial gaze field in birds has been proposed to exhibit the same anatomical connections, functions and physiological responses as the frontal eye field in mammals and has been proposed as the counterpart structure for attention modulation in avian species (Sridharan et al., 2014). This similarity across species suggests that the role of the mammalian frontal cortex in attention modulation reflects a conserved brain function that arose early in vertebrate evolution (Allman, 2000; Finlay et al., 2001; Northcutt, 2002). Our previous ERP study in music frogs has shown that the amplitudes of the N1 and P2 components to conspecific calls show the greatest variation in the telencephalon, especially for the right telencephalon (Fang et al., 2015). In view of the fact that the N1 and P2 components reflect attention allocation in humans (Näätänen, 1992), we hypothesize that GC causal connections involving the telencephalon identified in the present study, which are manifest within the time windows of the N1 and P2 components, reflect the fact that the telencephalon plays the same role in attention modulation in music frogs as the frontal cortex in mammalian species.

In humans, when individuals are asked to attend to particular stimuli, such stimuli elicit greater N1 amplitude than stimuli which individuals do not attend to, suggesting that N1 amplitude is a reflection of the allocation of attention to specific stimuli (Hillyard et al., 1987, 1973; Woldorff and Hillyard, 1991). Behavioral studies in music frogs show that both males and females preferentially respond to HSA calls, and neurophysiological experiments demonstrate enhanced N1 amplitudes to this stimulus as well (Cui et al., 2011; Fang et al., 2014a). Clearly, HSA calls are more biologically significant than LSA calls and WN. The amplitudes of the P2 components are sensitive to the spectral complexity of sounds (Shahin et al., 2007), which is consistent with the fact that conspecific calls elicit higher P2 amplitudes than WN in music frogs

(Fang et al., 2015). Thus, HSA calls exhibit features which would be expected to elicit both higher N1 and higher P2 amplitudes.

These properties explain why the strongest telencephalic GC connections are associated with the presentation of HSA stimuli during the N1 and P2 windows. These results also imply that the modulation of auditory attention in the frog telencephalon is strongly related to the biological significance of acoustic stimuli, which is consistent with the idea that attending to these kinds of stimuli is adaptive for the individuals (Akre et al., 2011; Brown and Kotler, 2004; Schmidt et al., 2008). However, it is not possible to completely rule out the potential effects of acoustic sensory filtering processes on these putative brain attention networks using the present experimental design, although we believe this effect would be slight. Future research is needed to resolve this.

A sexually dimorphic lateralized attention modulation network in frogs

In the M–T group, the ascending connections from the left mesencephalon were stronger than those from the right mesencephalon for all ERP components. This finding, in conjunction with previous results indicating that processing of auditory stimuli is lateralized in this species, implies that the left mesencephalon is dominant for perception of biologically significant auditory stimuli (Fang et al., 2014b; Xue et al., 2015). In addition, the data show that GC connections in the left telencephalon are stronger in males while those in the right telencephalon are stronger in females. These data support the idea that the lateralization of auditory forebrain networks exhibits sexual dimorphism in music frogs and possibly reflects differences in male and female reproductive strategies (Xue et al., 2016a).

Left-hemisphere dominance in the perception of conspecific calls has been demonstrated in many vertebrate species (Ocklenburg et al., 2013; Rogers et al., 2013; Vallortigara et al., 2011; Vallortigara and Versace, 2017), including music frogs (Fang et al., 2014b; Xue et al., 2015; present study). In male leopard frogs (*Rana pipiens*), lesions of the left but not the right hemisphere reduce vocal activity significantly, suggesting the left hemisphere is also dominant for vocal communication in males (Bauer, 1993). In male music frogs, the left hemisphere shows greater activation following repeated presentation of HSA versus LSA calls (Xue et al., 2016a), consistent with the fact that male music frogs prefer to compete vocally with HSA calls (Fang et al., 2014a). In the present study, the stronger GC connections in the left telencephalon during the early attention period suggest that male music frogs may begin preparing to compete with other potential competitors, especially those producing HSA calls, quite quickly.

In avian species, males rely on local spatial information cues more than females do, and exhibit stronger encoding of this information in the left than the right hemisphere (Della Chiesa et al., 2006; Gülbetekin et al., 2007; Tommasi and Vallortigara, 2004). For music frogs, advertisement calls are also cues for sound localization; however, males need little local spatial information for competition. Nevertheless, the use of spatial information may contribute to the fact that the left hemisphere is more active than the right in males insofar as local information is necessary for males to decide whether to compete or not. However, studies have shown that, in humans, the right prefrontal cortex is less active in males than in females during the presentation of acoustic stimuli, consistent with the idea that males modulate attention less than females during presentation of stimuli (Ruytjens et al., 2007). In the present study, causal connections in males originating from the right telencephalon were significantly lower than those in females

(Table 1), suggesting that male frogs engage in less attention allocation than females. This result suggests that males do not allocate substantial attention resources for discriminating conspecific sounds produced by each individual, perhaps because, in many anuran species, male courtship behavior serves mainly to advertise male quality or resources in order to attract females, rather than to overpower a competitor (Cui et al., 2011; Wells and Schwartz, 2006).

In anuran species in which males compete in leks, females eavesdrop on the males' vocal competition and choose the best mate (Andersson and Simmons, 2006). To achieve this, in most cases females need to evaluate multiple male calls at the same time for assessment. Females must therefore be able to dynamically allocate attention to multiple targets. The right telencephalon has been proposed to be the site for rapid control of attention modulation (Evans et al., 2000; Yamaguchi et al., 2000), enabling individuals to efficiently acquire or change targets. In the present study, the right telencephalon was found to play the dominant role in female music frogs during the early attention period, consistent with the idea that the right telencephalon dominates in processing global spatial information (Della Chiesa et al., 2006; Gülbetekin et al., 2007; Tommasi and Vallortigara, 2004). This is also consistent with studies showing that females dynamically monitor and assess conspecific calls in the environment rather than focus on only one or a few advertisement calls (Wells, 2010).

Author contributions
Conceptualization: J.C., S.E.B., Y.T., G.F.; Methodology: Y.T., G.F.; Software: G.F.; Formal analysis: F.X.; Investigation: F.X., X.Y., Y.F.; Resources: G.F.; Data curation: X.Y.; Writing - original draft: F.X.; Writing - review & editing: S.E.B., Y.T., G.F.; Supervision: J.C., S.E.B., G.F.; Project administration: Y.T.; Funding acquisition: J.C., G.F.

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