communications biology

A Nature Portfolio journal

Article

https://doi.org/10.1038/s42003-025-08170-0

Modified specific components of conspecific advertisement calls influence behavioral and neural responses in music frogs

Check for updates

Tao Qing[1,2,3], **Di Shen**[1,2,3], **Yanzhu Fan**[1,2], **Suxin Shi**[1], **Lidi Lu**[1,2], **Yue Wu**[1,2] & **Guangzhan Fang**[1,2]

Vocal communication plays a critical role in the transfer and exchange of information among animals. However, it remains unclear how modifications to specific call components simultaneously affect behavioral and neural responses. To address these issues, we conducted phonotaxis experiments and neural signal recordings in Emei music frogs (*Nidirana daunchina*), exposing them to auditory stimuli with varying degrees of information coherence violations. During the electrophysiological recordings, we also presented stimuli with altered physical properties featuring rising intonation. The phonotaxis experiments showed that females exhibited reduced attraction to altered calls with potential information coherence violations, suggesting that information coherence may influence female choice. Similarly, the electrophysiological experiments indicated a correlation between the amplitudes of the N400 and late positive components (LPC) with information incongruity and altered physical properties, respectively. Notably, the N400 amplitudes increased proportionally with the extent of potential information coherence violations. Given that N400 is a well-established neural indicator for prediction error in perceptual processes, including semantic processing in humans, and considering the significant evolutionary conservation of brain structure and function among vertebrates, these findings suggest that information coherence contained in the calls plays a crucial role in anuran vocal communication.

Animal communication is a fundamental aspect of animal life, acting as a critical channel for the transfer and exchange of information among individuals[1,2]. Animals utilize a variety of communication modalities, which can be classified based on their transmission medium, including visual, auditory, olfactory, and tactile signals[2–5]. Information conveyed by a sender is considered biologically significant if it induces a behavioral change in the receiver[6]. Among the various communication forms, auditory signals are particularly notable for their efficiency and immediacy in information transmission[3,7,8]. The inherent properties of sound waves, such as their ability to propagate over long distances, rapid transmission speed, and lack of persistent effects[7], render sound signals a prominent and widespread mode of communication across many animal species.

Vocal communication, comprising various components, serves multiple functions, including facilitating courtship and reproduction[6,9], deterring predators[10–12], and defending food resources[13] and territories[14]. These findings suggest that animal vocalizations may function as complex signals encoding a wide range of information types[15–17]. Additionally, the time-frequency properties of animal vocalizations, alone with their structural elements (e.g. notes, elements or syllables), often exhibit significant variation among individuals[18–22] and between different species[23,24]. This supports the notion that such signals are crucial for species discrimination and individual recognition. Consequently, different components of acoustic signals may have distinct functions in vocal communication. For instance, in zebra finches, 'macro-structural' variation (i.e., spectral and temporal characteristics) determines mate choice between subspecies, while 'micro-structural' variation (i.e., syllable type) influences mate choice within subspecies[25,26]. Similarly, in the tokay gecko (*Gekko gecko*), phases I and II of advertisement calls are reported to facilitate for individual identification and species recognition, respectively[22]. In natural environments, sound signaling is influenced by various factors, including environmental noise and calls from

---

[1]Chengdu Institute of Biology, Chinese Academy of Sciences, No.23, Qunxian South Street, Tianfu new area, Chengdu, Sichuan, China. [2]University of Chinese Academy of Sciences, 19A Yuquan Road, Beijing, China. [3]These authors contributed equally: Tao Qing, Di Shen. e-mail: fanggz@cib.ac.cn

other individuals[27]. These factors can create unfavorable conditions, such as the interruption or overlap of sound signals, potentially affecting the behavioral response of animals[28]. Previous research has shown that female Mediterranean field crickets (*Gryllus bimaculatus*) exhibit a significant increase in choice latency and a marked reduction in preference for male calls when playback is accompanied by concurrent white noise[29], indicating that disruptions in call components has a detrimental effect on female choice. However, it remains unclear how modifications to specific call components simultaneously affect behavioral and neural responses[10,30].

In comparison to other vocal animals, vocal communication in most anurans serves as the primary sensory modality, exhibiting strong channel specificity and minimal cross-modal interference[31,32]. These characteristics position anurans as powerful model systems for investigating the fundamental questions in auditory processing outlined above. In anuran species, males engage in territorial competition and mate attraction via advertisement calls, while females evaluate male quality and/or associated resources by parsing these conspecific calls[33,34]. Research has revealed that male Túngara frogs (*Physalaemus pustulosus*) emit advertisement calls comprising two distinct elements: the "whine" and "chuck"[35]. These elements serve unique functions, with the whine primarily facilitating caller identity recognition (e.g., "what") and the chuck exerting a greater influence on determining caller location (e.g., "where"), indicating distinct call components may fulfill different roles in acoustic signal recognition[35]. Similarly, analyses of acoustic parameters and electrophysiological studies have shown that the initial notes of the Emei music frog's (*Nidirana daunchina*) advertisement calls play a crucial role in individual recognition and acoustic communication, compared to other subsequent notes[36,37]. These findings suggest that different components of advertisement calls convey varying biological information. As with other vocal taxa, disruptions in call structures detrimentally affect female choice in anurans. For instance, female grey treefrog (*Hyla versicolor*) exhibit increased recognition errors when conspecific call structures are masked by heterospecific calls[28]. Male serrate-legged small tree frogs (*Kurixalus odontotarsus*) exhibit comparable responses to conspecific calls and their modified versions containing noise-overlapped notes. In contrast, females demonstrate a significant aversion to such noise-degraded advertisement calls, indicating that signal degradation selectively impairs female mate choice decisions[38]. Collectively, these findings suggest that discrete acoustic components within complex vocalizations may serve distinct functions and hold specific biological significance in certain species.

Event-related potentials (ERPs) refer to time-locked brain responses elicited by specific sensory, perceptual, or motor events. These ERPs are characterized by their amplitudes and latencies, providing insights into the temporal dynamics and efficiency of information processing in the brain[39,40]. In humans, the N400 component is characterized by a negative deflection that peaks approximately 400 ms post-stimulus onset, with its amplitude increasing in response to greater degrees of stimulus mismatch[40,41]. This component is recorded at the scalp level and engages various brain regions and neural resources, encompassing the anterior medial temporal lobe, middle and superior temporal areas, interior temporal areas, and prefrontal areas in both hemispheres[40]. Numerous studies have demonstrated that the N400 reflects semantic violations[40–43]; however, other researches indicate that the N400 can also be elicited by non-semantic mismatched stimuli, including images[44,45], gestures[46,47], facial expressions[48,49], and environmental cues[42,50]. Consequently, the N400 has been implicated in both semantic and non-semantic processing, with theories explaining its underlying mechanisms emphasizing concepts of prediction error and surprise[42,43]. Late positive components (LPC) are indicative of the reanalysis process of acoustic signals, with their amplitude closely associated with the violation of expectations within a given context, such as unintended alterations in physical properties[51]. Additionally, LPCs are implicated in the integrative processing of these violations[37,52,53]. In humans, substituting the last word of a sentence with a different word alters both the semantics and physical properties, thereby affecting the amplitudes of N400 and LPC[51,54]. Conversely, modifications solely in physical attributes, such as a sudden change in the voice of the last word from male to female (i.e. rising intonation), do not affect the N400 amplitude but do influence the LPC amplitude. Thus, the LPC serves as a control counterpart to the N400, aiding in the differentiation of N400 elicited by semantics violation from that caused by physical incongruity[54]. Similar auditory ERP components, exhibiting comparable stimulus responses and time windows, have been documented across various species, including monkeys[55], cats[56], dogs[57,58], dolphins[59], rabbits[60], rats[61] and frogs[36,62–65]. Given the high degree of evolutionary conservation in brain structure and function among vertebrates[66], the presence of analogous ERP components across species suggests potential similarities in brain functions[67], particularly in auditory cognitive processes such as auditory perception of information coherence.

Our previous studies have demonstrated that different call notes may play distinct roles in individual recognition and auditory perception in music frogs[34,35]. Furthermore, a preliminary experiment employing the N400 paradigm, analogous to the paradigm used in humans, revealed that replacing the last note of a call with another evoked an N400 response in this species, suggesting that music frog calls possess information coherence. These findings establish the music frog as a suitable animal model for investigating how modifications to specific call components simultaneously affect behavioral and neural responses. Therefore, this study examines the perceptual and behavioral responses in the Emei music frog by manipulating advertisement calls and conducting phonotaxis and electrophysiological experiments. Specifically, analogous to altering the last word of a sentence in humans, we modified the final component of the conspecific call to deviate from its typical pattern, enabling the preceding notes to establish a specific context and thereby inducing violations of information coherence[68]. To elaborate, we employed a series of acoustic stimuli that included an original call with six notes (OC), OC with the last note reversed in situ (OR, i.e. the last note was played backward; altering acoustic properties and inducing information coherence violations), OC with the last note replaced by silence (OS, modifying acoustic properties and creating an expectation for information input), OC with the last note replaced by a call note from another species (*Quasipaa boulengeri*; OQ, altering acoustic properties and violating information coherence), and OC with the last note replaced by white noise (OW, modifying acoustic properties and violating information coherence; Table 1). Variants of OC, OQ, and OW were created by increasing the pitch of the sixth component (OCP, OQP, and OWP, respectively; introducing changes in physical properties). For each of these eight stimuli, we generated corresponding control stimuli by reversing each of the first five notes in situ (OCC, ORC, OSC, OQC, OWC, OCPC, OQPC, and OWPC; i.e. each of the first five notes was played backward in sequence; see Supplementary Figs. 1 and 2). In the phonotaxis experiment with female frogs, we utilized treatment stimuli (OC, OCP, OR, OS, OQ, OW) and control stimuli (OCC, OCPC, ORC, OSC, OQC, OWC) to assess whether female preference is influenced by information coherence rather than simple acoustic properties. In the electrophysiological experiments, we employed treatment stimuli (OC, OCP, OQ, OQP, OW, OWP) and control stimuli (OCC, OQC, OWC, OCPC, OQPC, OWPC) to investigate the effects of information coherence and simple acoustic properties on neural responses in frogs. The initial three control stimuli were used to evaluate whether the replacement of the last note elicits an N400 response, while the latter three controls were designed to confirm that the induced N400 is due to information coherence violations rather than changes in simple physical attributes of the sixth component. We recorded the phonotaxis behavior of females and electroencephalogram (EEG) signals from different brain regions, including the left and right sides of the telencephalon (LT and RT), diencephalon (LD and RD), and mesencephalon (LM and RM) of both males and females. The aim of this study was to explore possible information coherence in the Emei music frog's calls and investigate the associated perception mechanisms through the analysis of behavioral results and ERPs. The study hypothesized the following: (1) Females would less frequently select stimuli exhibiting altered information coherence (OR, OS, OQ, OW) compared to the original advertisement call (OC); (2) Variants with the last note replaced (OQ, OQP, OW, OWP) are anticipated to generate greater

Table 1 | The acoustic stimuli used in phonotaxis and electrophysiological experiments

Treatment stimuli			Control stimuli		
Stimuli	**Manipulation methods**	**Predictions**	**Stimuli**	**Manipulation methods**	**Predictions**
For phonotaxis experiment					
OC	Original call with six notes	Females prefer OC	OCC	Each of the first five notes of OC is reversed in situ respectively	No preference in females due to lack of information coherence
OCP	Increasing the pitch of the 6th note of OC	Changing acoustic properties of the 6th note and having no effect on female preference	OCPC	Manipulation similar to OCC	Consistent with OCC
OR	Reversing the 6th note of OC in situ	Reducing female preference due to information coherence violations and acoustic properties changes	ORC	Manipulation similar to OCC	Consistent with OCC
OS	Replacing the 6th note of OC by silence	Leading to anticipation of information input and having no effect on female preference	OSC	Manipulation similar to OCC	Consistent with OCC
OQ	Replacing the 6th note of OC by a sympatric species' call note	Consistent with OR	OQC	Manipulation similar to OCC	Consistent with OCC
OW	Replacing the 6th note of OC by white noise	Consistent with OR	OWC	Manipulation similar to OCC	Consistent with OCC
For electrophysiological experiment					
OC	The same as above	Eliciting smaller N400 and LPC	OCC	The same as above	Eliciting smaller N400 and LPC
OQ	The same as above	Eliciting greater N400 and LPC	OQC	Manipulation similar to OCC	Eliciting smaller N400 and LPC
OW	The same as above	Eliciting greater N400 and LPC	OWC	Manipulation similar to OCC	Eliciting smaller N400 and LPC
OCP	The same as above	Eliciting greater LPC	OCPC	Manipulation similar to OCC	Eliciting smaller N400 and LPC
OQP	Increasing the pitch of the 6th component of OQ	Eliciting greater LPC compared to OQ	OQPC	Manipulation similar to OCC	Eliciting smaller N400 and LPC
OWP	Increasing the pitch of the 6th component of OW	Eliciting comparable LPC compared to OW due to minor difference between OWP and OW	OWPC	Manipulation similar to OCC	Eliciting smaller N400 and LPC

N400 amplitudes than the original advertisement call (OC), as this component signifies a mismatch between auditory stimuli and expectations, with amplitude increasing proportionally to prediction error[40,41]; (3) Compared to control stimuli, where each of the first five notes was reversed in situ (denoted as OQC, OQPC, OWC and OWPC, respectively), the non-reversed acoustic stimuli (OQ, OQP, OW, OWP) were expected to produce higher N400 amplitudes due to larger violations of information coherence. This expectation is based on the premise that revised sequences may lack coherence and display minimal corresponding violations; (4) The rising intonation version (OCP), as opposed to the original advertisement call (OC), is predicted to elicit higher LPC amplitudes, reflecting this ERP component's association with processing unexpected changes in the physical properties of acoustic stimuli[51].

Results

Female frogs prefer calls with normal acoustics

To investigate the impact of modifications to specific call components on female choice, the original call (OC) was paired with each of other treatment stimuli to form 5 stimulus pairs. When the OC and OR pair was played back antiphonally, the females demonstrated a significant preference for the former (25 vs. 9; binomial test: $p = 0.009$; Fig. 1A). The females significantly preferred the OC when the stimulus pair OC and OW was played back antiphonally (24 vs. 10; binomial test: $p = 0.024$; Fig. 1A). However, when the OC and OCP pair (19 vs. 15; binomial test: $p = 0.608$), OC and OS pair (17 vs. 17; binomial test was not available due to the equal number of choices), or OC and OQ pair (19 vs. 15; binomial test: $p = 0.608$) was played back antiphonally, there was no significant difference in female choice. These comparable choices between OC and the other three stimuli may result from the preserved information coherence in OCP and the frequency encounters with OS and OQ in natural conditions, potentially arising from interruptions or overlaps with calls from the sympatric anuran species *Quasipaa boulengeri*. Therefore, the information coherence in auditory signals and its familiarity may collectively influence female choice. For the control counterpart, there were no significant differences in the number of choices for all control stimulus pairs (binomial test: all $p \geq 0.229$; Fig. 1C). In addition, there were no significant differences in latencies for all treatment stimulus pairs (independent samples t-test: all $p \geq 0.119$; Fig. 1B) and all control stimulus pairs (independent samples t-test: all $p \geq 0.053$; Fig. 1D). Given the prolonged latency period observed in animals with regard to behavioral dimensions and the relatively much shorter response time within tens of milliseconds with regard to neural processes, the employment of electrophysiological tools is of paramount importance.

The results of N400 amplitudes and latencies

The analysis of N400 amplitudes revealed significant main effects for the factors "stimulus group" ($F_{1,15} = 5.789$, partial $\eta^2 = 0.278$, $p = 0.029$) and "stimulus type" ($F_{5,75} = 8.429$, partial $\eta^2 = 0.360$, $p < 0.001$), but not for "brain area" ($F_{5,75} = 2.158$, $\varepsilon = 0.438$, partial $\eta^2 = 0.126$, $p = 0.128$) (Fig. 2B and Table 2). Moreover, a significant triple interaction was observed among these three factors ($F_{25,375} = 3.540$, $\varepsilon = 0.228$, partial $\eta^2 = 0.191$, $p = 0.004$). Subsequent simple effect analysis of the treatment stimuli revealed that the N400 amplitudes evoked by OC and OCP were significantly smaller than those evoked by the note-replaced versions of OC (OQ, OQP, OW, and OWP; all $p \leq 0.011$ for significant differences; Fig. 2B and Table 3), although some differences did not reach statistical significance. In contrast, no significant differences in N400 amplitudes could be found between the original advertisement call (OC) and its rising intonation version (OCP) for all brain areas (all $p \geq 0.087$), between the note-replaced versions OQ and OQP (all $p \geq 0.663$), or between the note-replaced versions OW and OWP (all $p \geq 0.111$). Furthermore, no significant differences in N400 amplitudes were observed among the six control stimuli for each brain area (all $p \geq 0.144$; Fig. 2A and Table 3). In general, for all brain areas, the N400 amplitudes evoked by the note-replaced versions of OC (OQ, OQP, OW, and OWP) were significantly greater than those evoked by their control counterparts (OQC, OQPC, OWC, and OWPC; all $p \leq 0.043$ for significant differences; Fig. 2 and Table 3), although some differences did not reach statistical significance. These findings suggested that information coherence violations but not changes in the physical properties of acoustic stimuli could elicited a significant N400 response. There were no significant differences in N400 amplitudes among the six brain areas for each treatment stimulus (all $p \geq 0.063$; Fig. 2B and Table 3) and for each control stimulus (almost all $p \geq 0.185$; Fig. 2A and Table 3). For all brain areas except the left mesencephalon, the N400 amplitudes evoked by OCP were significantly smaller than those evoked by its control counterpart (OCPC; all $p \leq 0.028$; Fig. 2 and Table 3), potentially due to the absence of information coherence violations in OCP. In addition, the difference in N400 amplitude evoked by OQC had a p-value of less than 0.05 ($p = 0.011$; Table 3), however, no significant difference could be found via multiple comparison.

With regard to the latency of the N400, no significant main effects were observed for the factors "stimulus group" ($F_{1,15} = 3.115$, partial $\eta^2 = 0.172$, $p = 0.098$), "stimulus type" ($F_{5,75} = 0.539$, partial $\eta^2 = 0.035$, $p = 0.746$), and "brain area" ($F_{5,75} = 1.431$, $\varepsilon = 0.579$, partial $\eta^2 = 0.087$, $p = 0.247$; Table 2). Furthermore, no significant interactions were observed among these factors (all $p \geq 0.219$).

The results of LPC amplitudes and latencies

The analysis of LPC amplitudes revealed significant main effects for the factors "stimulus type" ($F_{5,75} = 10.023$, partial $\eta^2 = 0.401$, $p < 0.001$) and "brain area" ($F_{5,75} = 6.648$, $\varepsilon = 0.418$, partial $\eta^2 = 0.307$, $p = 0.004$), but not for the factor "stimulus group" ($F_{1,15} = 2.388$, partial $\eta^2 = 0.137$, $p = 0.143$; Fig. 3B and Table 2). Additionally, a significant interaction was observed between "stimulus group" and "stimulus type" ($F_{5,75} = 7.594$, partial $\eta^2 = 0.336$, $p < 0.001$). In the case of treatment stimuli, the simple effect analysis showed that LPC amplitudes evoked by OCP were significantly greater than those evoked by other acoustic stimuli, with the exception of OQP ($p = 0.001$; Fig. 3B and Table 4), indicating changes in the physical properties of acoustic stimuli without information coherence violations could elicited a significant LPC response. Concerning the control stimuli, the LPC amplitudes evoked by OCC and OCPC were significantly greater than that evoked by OWC ($p = 0.011$; Fig. 3A and Table 4). Furthermore, the LPC amplitudes evoked by OCP and OQP were significantly greater than those evoked by their control counterparts (OCPC and OQPC; all $p \leq 0.031$), while the LPC amplitudes evoked by OQ were significantly smaller than that evoked by its control counterpart OQC ($p = 0.017$; Fig. 3 and Table 4). The findings indicate that the brain is capable of rapidly identifying unintended changes in physical properties of stimuli.

As for the latency of the LPC, there were no significant main effects observed for the factors "stimulus group" ($F_{1,15} = 0.050$, partial $\eta^2 = 0.003$, $p = 0.826$), "stimulus type" ($F_{5,75} = 1.791$, partial $\eta^2 = 0.107$, $p = 0.125$), and "brain area" ($F_{5,75} = 0.948$, partial $\eta^2 = 0.059$, $p = 0.455$; Table 2). Additionally, there were no significant interactions among these factors (all $p \geq 0.105$).

Discussion

The N400 is an ERP component associated with both semantic and non-semantic processing of mismatched visual or auditory stimuli. Its amplitude increases with the degree of incongruity in information coherence[40,41]. Specifically, when a stimulus is semantically appropriate or matches other information, the N400 amplitude is smaller; conversely, when a stimulus is semantically inappropriate or mismatched, the N400 amplitude is larger. This is attributed to the fact that processing unexpected stimuli requires more neural resources than processing expected ones[40]. Theories explaining the N400's mechanisms often involve concepts such as prediction error and surprise[42,43]. Consistent with this, our findings indicate that the amplitudes of N400 elicited by the original call (OC) and its rising intonation version (OCP) were significantly smaller than those evoked by the last note-replaced versions of OC (OQ, OQP, OW, and OWP), although some differences did not reach statistical significance. Previous studies on the music frog have suggested that brain resources allocation for perception varies among various notes, with each note potentially playing a role in individual recognition

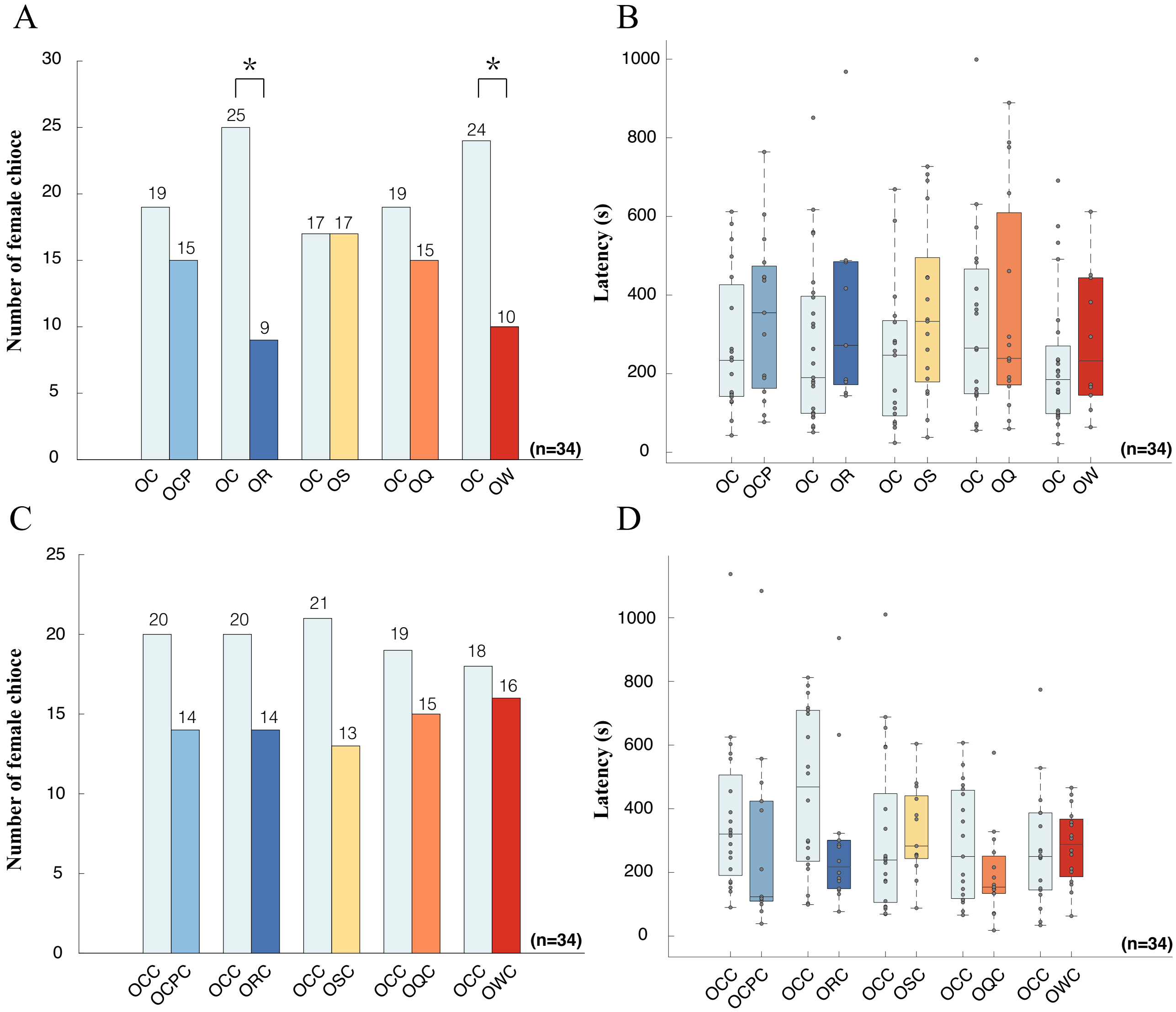

Fig. 1 | The number and latencies of female chioces. The number and latencies of female choices for the treatment stimuli (**A**, **B**) and control stimuli (**C**, **D**) (n = 34 in each group). OC the original advertisement call with six notes, OCP the rising intonation version of OC (where the pitch of the sixth note was increased by 15%), OR the sixth note of OC was reversed in situ, OS the sixth note of OC was replaced by silence, OQ the sixth note of OC was replaced by the sixth note of an advertisement call of *Quasipaa boulengeri* (a sympatric anuran species), OW the sixth note of OC was replaced by white noise. The control stimuli for treatment stimuli are denoted as OCC, OCPC, ORC, OSC, OQC, and OWC respectively, where the first five notes of each treatment stimulus were reversed in situ while the sixth component remained unchanged. *$p < 0.05$.

and auditory perception, though the first note may be particularly critical. This indicates that different notes of calls may contain distinct biological information[36,37], contributing to overall information coherence. The last element of the note-replaced versions violated information coherence compared to OC, eliciting prediction error and significantly greater N400 amplitudes. In contrast, there were no information coherence violations between OC and OCP, resulting in similar N400 amplitudes for these two stimuli. Additionally, no significant differences in N400 amplitudes were observed when control stimuli were played back, due to fewer information coherence violations. Furthermore, the results revealed that the N400 amplitudes elicited by the note-replaced versions of OC (OQ, OQP, OW, and OWP) were significantly higher than those elicited by their control counterparts (OQC, OQPC, OWC, and OWPC). This discrepancy can be attributed to the retention of information coherence in the first five syllables of the note-replaced versions, resulting in greater information coherence violations between the last replaced component and the preceding five ones, thus eliciting larger N400 amplitudes. Conversely, the control stimuli, featuring a temporal reversal in situ of each of the first five syllables, lost all biological information and information coherence, reducing the incongruity of information coherence between the last syllable and the preceding five ones, and consequently resulted in smaller N400 amplitudes. Furthermore, the LPC, which reflects the reanalysis process of acoustic signals, is highly sensitive to unintended changes in physical properties[53]. The present results support this notion, as the LPC amplitudes elicited by OCP were generally greater than those evoked by other treatment stimuli. These findings suggest that potential violations in information coherence rather than alterations in the physical properties of note-replaced sounds may elicit the N400 component, which is associated with the processing of information incongruity. Analogous to humans, the rising intonation of specific notes did not affect the coherent properties of calls and did not elicit the N400 component. Collectively, these findings suggest that frogs are highly sensitive to modifications of specific components of advertisement calls, suggesting that information coherence contained in the calls plays a crucial role in vocal communication.

In alignment with these findings, our behavioral results indicate that female music frogs exhibit a preference for the original advertisement calls

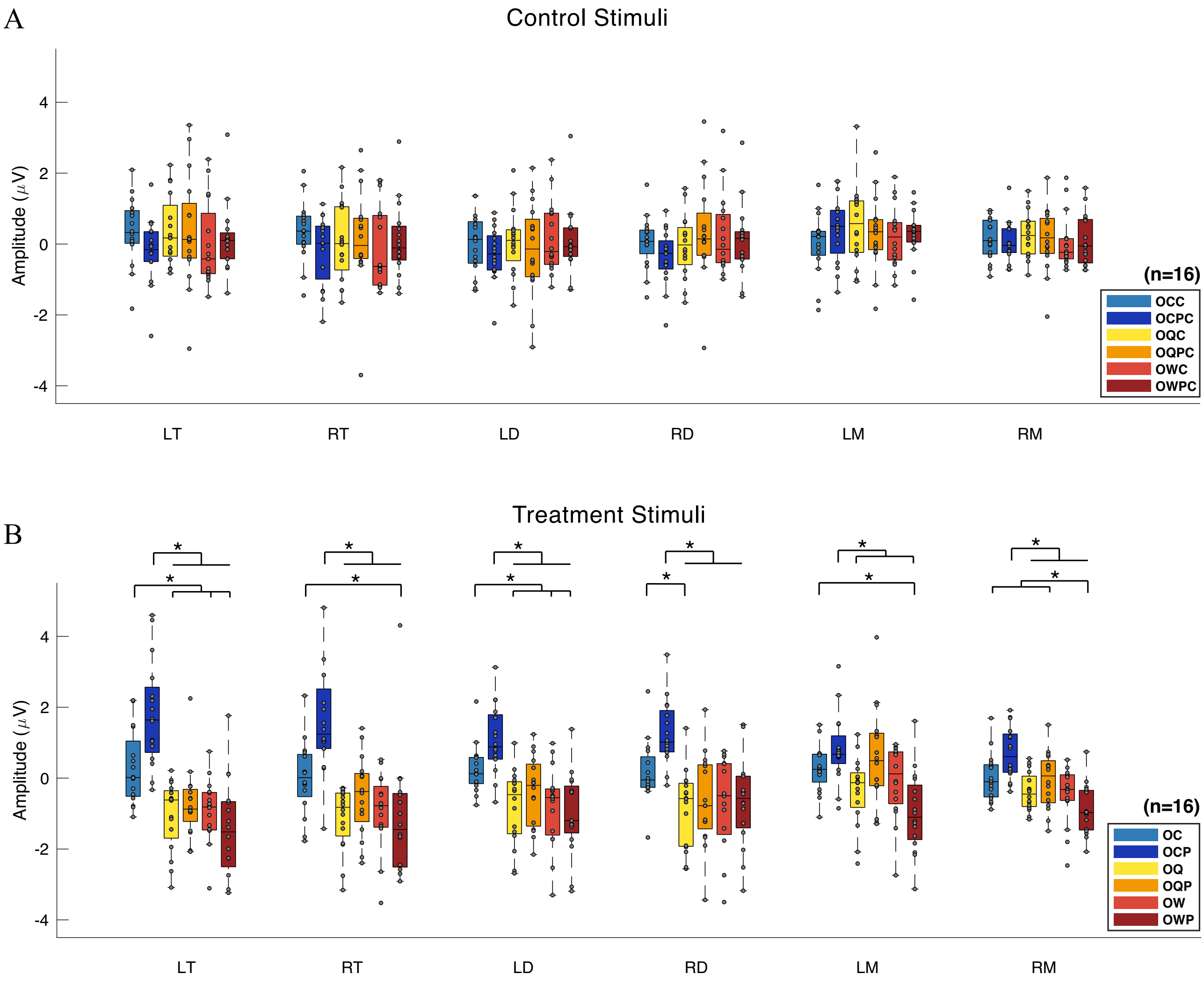

Fig. 2 | The average amplitudes of N400. The average amplitudes of N400 for the control (**A**) and treatment (**B**) stimuli (n = 16 in each group). LT and RT the left and right sides of the telencephalon, LD and RD the left and right sides of the diencephalon, LM and RM the left and right sides of the mesencephalon, OC the original advertisement call with six notes, OCP the rising intonation version of OC (where the pitch of the sixth note was increased by 15%), OQ the sixth note of OC was replaced by the sixth note of an advertisement call of *Quasipaa boulengeri* (a sympatric anuran species), OQP the rising intonation version of OQ (where the pitch of the sixth component of OQ was increased by 15%), OW the sixth note of OC was replaced by white noise, OWP the rising intonation version of OW (where the pitch of the sixth component of OW was increased by 15%). The control stimuli for treatment stimuli are denoted as OCC, OCPC, OQC, OQPC, OWC, and OWPC, respectively, where the first five notes of each treatment stimulus were reversed in situ while the sixth component remained unchanged. *$p < 0.05$.

over modified versions where the last note was temporally reversed or replaced with white noise. Given that neither the reversed sixth note nor white noise occur naturally, the observed aversion to stimuli OR and OW in females may stem from the novelty of these stimuli. This suggests that both the intrinsic information coherence within the call and its familiarity may play crucial roles in female selection. These observations corroborate prior research on syllable alterations in the serrate-legged small tree frog, which demonstrated that disruptions in call structure negatively impact on female choice[38], likely due to the violation of information coherence in the modified advertisement calls. Contrary to the predictions, females displayed no preference between original advertisement calls and revised versions where the last note was replaced by silence (OS) or a note from a sympatric anuran species (OQ). This indicates that behavioral and electrophysiological data may not be always align. Variations in female behavioral preferences seem to arise from differing acoustic properties, such as varying degrees of information coherence violations and novelty, across stimuli. Firstly, future research is required to ascertain if sound familiarity accounts for the differences between behavioral and electrophysiological responses, given that the two revised versions (OS and OQ) are frequently encountered in natural conditions due to interruptions or overlaps. Secondly, behavior and electrophysiology represent distinct metrics on different time scales; the brain may perceive differences in stimuli that are not reflected in observable behavior[69]. Lastly, there are inherent differences in detection parameters between the current behavioral and electrophysiological experiments, as it is challenging to directly ascertain whether frogs detect information coherence violations behaviorally.

Syllables constitute the fundamental components of vocalizations across numerous animal species, with each syllable potentially conveying distinct pieces of information within a vocalization. Both non-human primates[14] and birds[70] utilize vocalizations to transmit information concerning external environmental conditions or the presence of predators, referred to as context-specific signals[30,71]. For example, avian calls can communicate information about predators[10–12], food discovery and quality[13], risks of brood parasitism[72,73], and social interactions[14]. Notably, bird alarm calls can induce similar behavioral responses within the same population, as well as across different populations of the same species or even among different but sympatric species[74], whereas distinct alarm calls trigger varied behavioral reactions[70]. This suggests that alarm and

Table 2 | The results of ANOVAs for the amplitudes and latencies of N400 and late positive component (LPC) with respect to the three factors

	For the amplitude(1,15)(5,75)(5,75)					For the latency(1,15)(5,75)(5,75)				
	F	***ε***	***P***	**partial η^2**	**Bonferroni**	***F***	***ε***	***P***	**partial η^2**	**Bonferroni**
N400										
stimulus group	5.789	NA	0.029*	0.278	TS > CS	3.115	NA	0.098	0.172	NA
stimulus type	8.429	NA	0.000**	0.360	OQ,OW,OWP >OCP; OWP > OC	0.539	NA	0.746	0.035	NA
brain area	2.158	0.438	0.128	0.126	NA	1.431	0.579	0.247	0.087	NA
stimulus group* stimulus type	13.025	0.568	0.000**	0.465	See Table 3	1.442	NA	0.219	0.088	NA
stimulus group* stimulus type* brain area	3.540	0.228	0.004*	0.191	See Table 3	1.147	0.351	0.335	0.071	NA
LPC										
stimulus group	2.388	NA	0.143	0.137	NA	0.050	NA	0.826	0.003	NA
stimulus type	10.023	NA	0.000**	0.401	OCP > OQ,OW,OWP	1.791	NA	0.125	0.107	NA
brain area	6.648	0.418	0.004*	0.307	LT > RD,RM; RT > RM	0.948	NA	0.455	0.059	NA
stimulus group* stimulus type	7.594	NA	0.000**	0.336	See Table 4	0.702	NA	0.624	0.045	NA

The degrees of freedom corresponding to the three factors and their interaction are indicated within the brackets on the first line. The symbol ">" signifies that the amplitudes on the left side of ">" are significantly larger than those on the right side, while there are no significant differences among the corresponding conditions on the same side of ">". *F* represents the *F*-value from ANOVA, and partial η^2 is the effect size for the ANOVA. NA indicates that the information is not applicable. LT and RT represent the left and right sides of the telencephalon, LD and RD represent the left and right sides of the diencephalon, and LM and RM represent the left and right sides of the mesencephalon. OC refers to the original advertisement call with six notes; OCP represents the rising intonation version of OC, where the pitch of the sixth note was increased by 15%; OQ indicates modified version of OC, where the sixth note was replaced by the sixth note of an advertisement call from a sympatric anuran species, *Quasipaa boulengeri*; OQP represents the rising intonation version of OQ, with a 15% increase in the pitch of the sixth component; OW denotes the altered version of OC, with the sixth note replaced by white noise; and OWP represents the rising intonation version of OW, with a 15% pitch increase in the sixth component. In addition, CS and TS refer to the control and treatment stimuli, respectively. $^{*}p < 0.05$ and $^{**}p < 0.001$.

recruitment calls in birds may possess referential or semantic attributes[10–12,70,75], invoking mental representations in the receivers' cognition regarding events or predator information[76], which might indicate the presence of semantic elements in animal vocal communication. Consequently, these calls are hypothesized to represent a rudimentary form of human language[77]. For example, Japanese great tits (*Parus major minor*) and willow tits (*Poecile montanus*) form mixed-species flocks during the non-breeding season, using specific calls as recruitment signals. The "D" call of Japanese great tits and the "Tää" call of willow tits both function as recruitment signals, with the "Tää" call also capable of recruiting Japanese great tits[78,79]. When the "D" call was substituted with the "Tää" call, Japanese great tits exhibited similar responses to the "ABC-Tää" call and the "ABC-D" call, whereas no behavioral response was observed to the "Tää-ABC" and "D-ABC" calls[74]. In alignment with these findings, our current study demonstrated no significant difference in female preference when the last note of the original call was replaced with a note from a sympatric species ("OC v.s. OQ"). Similarly, a mismatch effect has been observed in dogs' frontal lobes with a latency akin to the human N400 after playback of the acoustic stimulus with an incorrect objective word[57]. Moreover, errors involving familiar words evoked a stronger mismatch effect, further supporting the existence of semantic communication in non-human mammals. Our results indicated that the modified versions of OC with the last note replaced (OQ, OQP, OW, and OWP) elicited a significant N400 response, suggesting the potential for semantic communication in frogs; however, additional research is required to substantiate this hypothesis.

In summary, the current findings underscore the role of each component of conspecific call in vocal communication, providing evidence that the advertisement calls of music frogs may embody information coherence, as indicated by behavioral response and the involvement of the N400 and LPC components. These components are indicative of cognitive processes related to violations of information coherence and unexpected changes in physical attributes, respectively. Furthermore, these results suggest that frogs can distinguish between calls with inconsistencies in both physical attributes and information coherence. However, it is crucial to recognize the limitations of this study, particularly as the discussion of the biological significance of N400/LPC components is extrapolated from findings in humans and dogs, attributed to the high evolutionary conservation of brain structure and function among vertebrates[66]. Nonetheless, interspecies variations in behavior and physiological activity exist. Previous research has identified that in humans, the N400 is predominantly elicited in the left temporoparietal cortex[39], while in dogs, it is mostly observed in the frontal lobe[57]. In contrast, our study reveals that in frogs, the N400 is present across all brain regions. Since acoustic communication is critical for male competition and female choice in frogs, and given the relative simplicity of the frog brain, the entire brain is engaged in auditory perception. This interspecific difference may indicate a progression of brain specialization throughout animal evolution. Collectively, further research is necessary to thoroughly understand the similarities and differences in brain functions between mammals and anurans, and to determine the extent to which these are reflected in each ERP component and brain region, at both the behavioral and electrophysiological levels.

Methods

Animals

All experiments were conducted during the reproductive season of the species. Fifty adult music frogs, consisting of eight males and forty-two females, were captured from Emei Mountain, located in Sichuan, China (29.60° N, 103.36° E, 1315 m). Thirty-four females of them, with an average body weight of 10.02 ± 1.17 g and an average body length of 5.1 ± 0.16 cm, were used for phonotaxis experiments that were conducted in the field near the capture site. They were released after all experiments were finished. The other sixteen frogs (eight males and eight females) for electrophysiological experiments were then housed separately by sex in two opaque tanks, with dimensions of 54 cm × 40 cm and a depth of 33 cm, containing a moderate amount of mud and water. They were placed in a controlled environment with a 12:12 light-dark cycle, where the lights were turned on at 08:00 in the morning. The temperature was maintained at a constant 22 ± 1 °C. Prior to the surgical procedures, the subjects had an average mass of 10.9 ± 2.5 g and an average body length of 4.9 ± 0.33 cm. To sustain their nutrition, the frogs were provided with live crickets as their diet every three days. Throughout the entire study, we took great care to minimize both the number of frogs used and any potential suffering they might experience. All experimental procedures conducted in this study received approval from the Animal Care and Use Committee of the Chengdu Institute of Biology, Chinese Academy of Sciences (permit number: 20191203) to ensure compliance with ethical standards.

Table 3 | Results of simple effect analysis for the amplitudes of N400 with respect to the three factors

	F	*P*	partial η^2	Bonferroni	*F*	*P*	partial η^2	Bonferroni
For the Stimulus type (TS)					For the Stimulus type (CS)			
Stimulus type |LT	10.453	0.001*	0.826	OQ,OW,OWP > OC; OQ,OQP,OW,OWP > OCP	1.131	0.400	0.340	NA
Stimulus type |RT	5.294	0.010*	0.706	OW > OC; OQ,OQP,OW,OWP > OCP	1.409	0.294	0.390	NA
Stimulus type |LD	6.205	0.006*	0.738	OQ,OW,OWP > OC; OQ,OQP,OW,OWP > OCP	1.235	0.356	0.360	NA
Stimulus type |RD	6.996	0.004*	0.761	OQ > OC; OQ,OQP,OW,OWP > OCP	2.085	0.144	0.487	NA
Stimulus type |LM	5.163	0.011*	0.701	OWP > OC; OQ,OWP > OCP	0.789	0.579	0.264	NA
Stimulus type |RM	7.730	0.002*	0.778	OWP > OC,OQP; OQ,OQP,OW,OWP > OCP	0.204	0.954	0.085	NA
For the Brain area (TS)					For the Brain area (CS)			
Brain area |OC/OCC	1.214	0.365	0.365	NA	1.494	0.269	0.404	NA
Brain area |OCP/ OCPC	2.426	0.102	0.524	NA	1.841	0.185	0.456	NA
Brain area |OQ/OQC	2.952	0.063	0.573	NA	5.184	0.011*	0.702	NA
Brain area |OQP / OQPC	2.217	0.126	0.502	NA	0.401	0.838	0.154	NA
Brain area |OW/OWC	1.443	0.284	0.396	NA	1.238	0.355	0.360	NA
Brain area |OWP /OWPC	1.359	0.311	0.382	NA	0.141	0.979	0.060	NA
For the stimuli OC and OCC					For the stimuli OCP and OCPC			
Stimulus group |LT	0.034	0.857	0.002	NA	28.757	0.000**	0.657	OCPC > OCP
Stimulus group |RT	1.646	0.219	0.099	NA	19.520	0.000**	0.565	OCPC > OCP
Stimulus group |LD	0.986	0.336	0.062	NA	21.361	0.000**	0.587	OCPC > OCP
Stimulus group |RD	0.153	0.701	0.010	NA	23.471	0.000**	0.610	OCPC > OCP
Stimulus group |LM	1.694	0.213	0.101	NA	2.254	0.154	0.131	NA
Stimulus group |RM	0.386	0.544	0.025	NA	5.947	0.028*	0.284	OCPC > OCP
For the stimuli OQ and OQC					For the stimuli OQP and OQPC			
Stimulus group |LT	15.370	0.001*	0.506	OQ > OQC	7.734	0.014*	0.340	OQP > OQPC
Stimulus group |RT	9.523	0.008*	0.388	OQ > OQC	1.971	0.181	0.116	NA
Stimulus group |LD	8.251	0.012*	0.355	OQ > OQC	0.348	0.564	0.023	NA
Stimulus group |RD	7.772	0.014*	0.341	OQ > OQC	4.207	0.058	0.219	NA
Stimulus group |LM	10.047	0.006*	0.401	OQ > OQC	0.455	0.510	0.029	NA
Stimulus group |RM	10.781	0.005*	0.418	OQ > OQC	0.528	0.479	0.034	NA
For the stimuli OW and OWC					For the stimuli OWP and OWPC			
Stimulus group |LT	4.999	0.041*	0.250	OW > OWC	14.431	0.002*	0.490	OWP > OWPC
Stimulus group |RT	3.552	0.079	0.191	NA	5.375	0.035*	0.264	OWP > OWPC
Stimulus group |LD	5.179	0.038*	0.257	OW > OWC	10.101	0.006*	0.402	OWP > OWPC
Stimulus group |RD	4.875	0.043*	0.245	OW > OWC	13.026	0.003*	0.465	OWP > OWPC
Stimulus group |LM	1.036	0.325	0.065	NA	21.404	0.000**	0.588	OWP > OWPC
Stimulus group |RM	5.206	0.038*	0.258	OW > OWC	30.166	0.000**	0.668	OWP > OWPC

The symbol ">" indicates that the amplitudes on the left side of ">" are significantly greater than those on the right side. Furthermore, there is no significant difference among the corresponding conditions on the same side of ">" for each case. In the analysis of variance (ANOVA), *F* represents the *F*-value, while partial η^2 denotes the effect size. NA indicates that the information is not applicable. LT and RT refer to the left and right sides of the telencephalon, while LD and RD refer to the left and right sides of the diencephalon, and LM and RM refer to the left and right sides of the mesencephalon. OC refers to the original advertisement call with six notes; OCP represents the rising intonation version of OC, where the pitch of the sixth note was increased by 15%; OQ indicates modified version of OC, where the sixth note was replaced by the sixth note of an advertisement call from a sympatric anuran species, *Quasipaa boulengeri*; OQP represents the rising intonation version of OQ, with a 15% increase in the pitch of the sixth component; OW denotes the altered version of OC, with the last note replaced by white noise; and OWP represents the rising intonation version of OW, with a 15% pitch increase in the last component. The control stimuli for treatment stimuli are denoted as OCC, OCPC, OQC, OQPC, OWC, and OWPC respectively, where the first five notes of each treatment stimulus were reversed in situ while the sixth component remained unchanged. In addition, CS and TS refer to the control and treatment stimuli, respectively. $^{*}p < 0.05$ and $^{**}p < 0.001$.

Stimulus and procedure for phonotaxis experiments

For the phonotaxis experiments, six types of treatment stimuli (Table 1 and Fig. 4) were used: the original advertisement call containing six notes (OC), a modified version of OC with the pitch of the sixth note increased by 15% (OCP), a modified version of OC with the sixth note reversed in situ (OR, i.e. the sixth note was played backward), a modified version of OC with the sixth note replaced by silence (OS), and two modified versions of OC with the sixth note replaced by the sixth call note of a sympatric anuran species (*Quasipaa boulengeri*, OQ) or by white noise (OW). We predict that females would exhibit an aversion for OR, OQ, and OW over OC due to violations of information coherence and varying degrees of acoustic properties alterations. In contrast, females are expected to show comparable behavioral responses between OC and OCP (which alters acoustic properties but does not disrupt information coherence) and between OC and OS (which modifies acoustic properties and creates an expectation for information input but does not disrupt information coherence). To further assess the presence of information coherence in the original call, corresponding control stimuli were generated by reversing each of the first five notes in situ for each treatment stimulus (OCC, OCPC, ORC, OSC, OQC, and OWC; i.e. each of the first five notes was played backward in sequence; see Table 1 and Supplementary Fig. 1). The aim was to disrupt the information coherence embedded in the first five original notes while preserving the information in the last one. This approach minimized incongruity in information coherence between the last component and the preceding five reversed notes, as

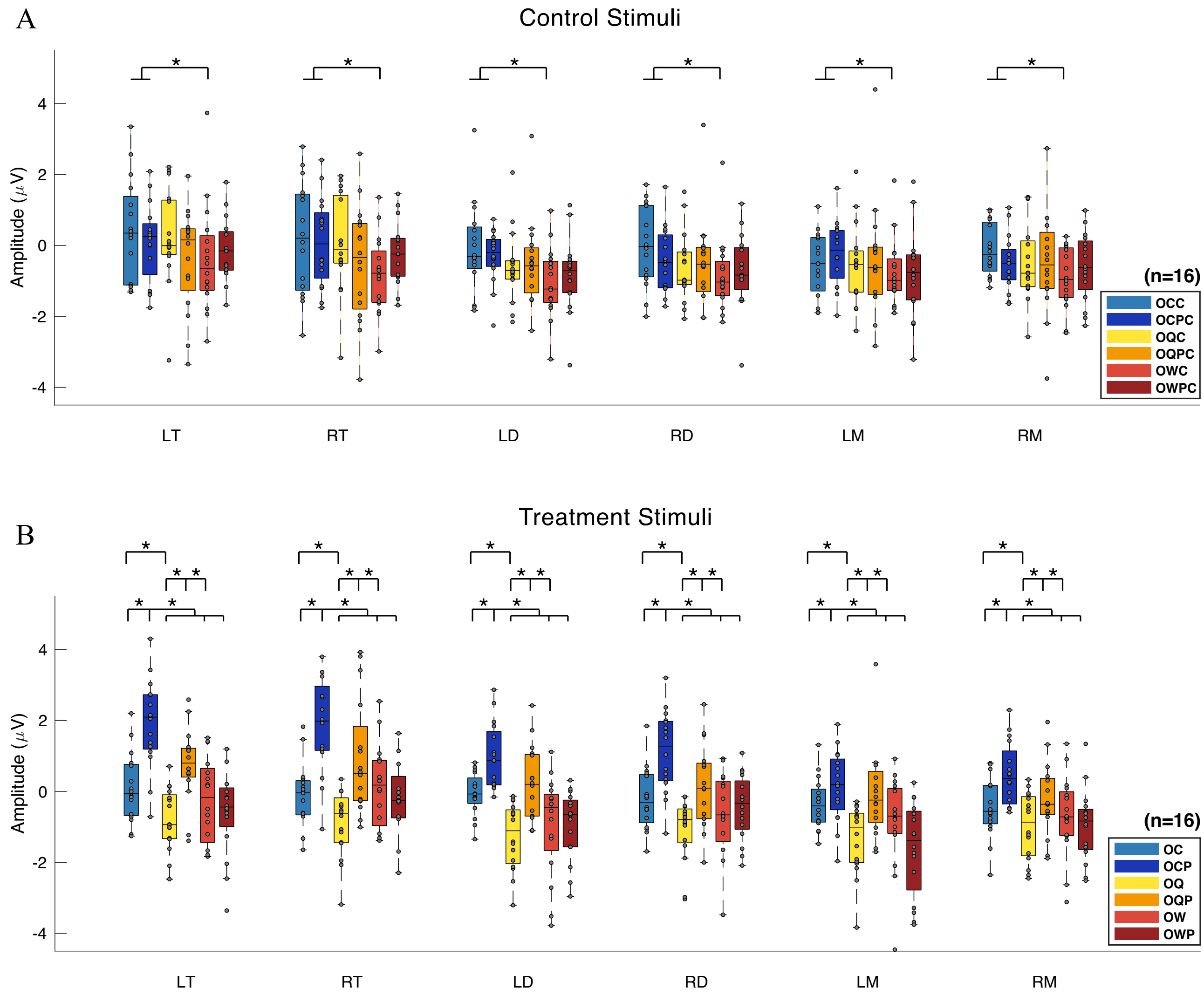

Fig. 3 | The average amplitudes of the late positive component. The average amplitudes of the late positive component for the control (**A**) and treatment (**B**) stimuli (n = 16 in each group). LT and RT the left and right sides of the telencephalon, LD and RD the left and right sides of the diencephalon, LM and RM the left and right sides of the mesencephalon, OC the original advertisement call with six notes, OCP the rising intonation version of OC (where the pitch of the sixth note was increased by 15%), OQ the sixth note of OC was replaced by the sixth note of an advertisement call of *Quasipaa boulengeri* (a sympatric anuran species), OQP the rising intonation version of OQ (where the pitch of the sixth component of OQ was increased by 15%), OW the sixth note of OC was replaced by white noise, OWP the rising intonation version of OW (where the pitch of the sixth component of OW was increased by 15%). The control stimuli for treatment stimuli are denoted as OCC, OCPC, OQC, OQPC, OWC, and OWPC, respectively, where the first five notes of each treatment stimulus were reversed in situ while the sixth component remained unchanged. $^*p < 0.05$.

the control stimuli lacked coherence. Accordingly, we hypothesize that females would exhibit comparable behavioral responses among OCC and the other control stimuli. If the results align with these predictions, they would indicate that female preference is driven by violations of information coherence rather than changes in the simple physical attributes of the last note.

To avoid the potential impact of pseudoreplication on statistical analysis in animal behavior and neuroscience studies[80–83], we controlled for these possible effects by using multiple stimulus exemplars. Specifically, we randomly selected four conspecific and four heterospecific advertisement calls recorded from four conspecific and four heterospecific individuals, respectively. Both conspecific and heterospecific calls possessed similar durations, and the temporal and frequency parameters of the calls were close to the average values of the corresponding populations. Using Adobe Audition 3.0 software (San Jose, California, USA), we generated five variants of each conspecific call. The duration and amplitude of white noise (OW), with 10 ms rise and fall times, matched the duration and amplitude of the corresponding call note. Accordingly, four treatment stimulus sets were created, each containing one OC and five modified versions (OCP, OR, OS, OQ and OW). For each treatment stimulus set, the modified acoustic stimuli were paired with OC respectively to form five stimulus pairs. Similarly, four control stimulus sets were created, each containing six control stimuli (OCC, OCPC, ORC, OSC, OQC and OWC). Each of the last five control stimuli was paired with OCC respectively to form five additional stimulus pairs. The intensity of each stimulus was adjusted to 65 ± 0.5 dB SPL (the average intensity of the natural call amplitude) at the center of the experimental tank using a sound pressure meter (relative to 20 μPa, C-weighting and fast time-weighting; Aihua, AWA6291; Hangzhou, China). Each treatment stimulus set and its corresponding control stimulus set was intended to be presented to an equal number of naïve females who had not previously been exposed to the stimuli.

To minimize the influence of visual stimulation and environmental noise, phonotaxis experiments for females were conducted in ambient light conditions between 20:30 and 03:00 (the next day). During the

Table 4 | Results of simple effect analysis for the amplitudes of LPC for the stimulus group and stimulus type

	F	*P*	partial η^2	Bonferroni
Stimulus type |TS	10.476	0.001*	0.826	OCP > OC,OQ,OW,OWP; OC > OQ; OQP > OQ,OW
Stimulus type |CS	5.155	0.011*	0.701	OCC,OCPC > OWC
Stimulus group |OC	0.437	0.519	0.028	NA
Stimulus group |OCP	50.347	0.000**	0.770	TS > CS
Stimulus group |OQ	7.132	0.017*	0.322	CS > TS
Stimulus group |OQP	5.633	0.031*	0.273	TS > CS
Stimulus group |OW	0.208	0.655	0.014	NA
Stimulus group |OWP	0.320	0.580	0.021	NA

The symbol ">" indicates that the amplitudes on the left side of ">" are significantly greater than those on the right side. The symbols "|" denote the conditions on the left side of "|" under the conditions on the right side of "|". The *F*-value from the analysis of variance (ANOVA) is denoted as *F*. The effect size for ANOVA is represented by partial η^2. NA signifies that the information is not applicable. Additionally, OC refers to the original advertisement call with six notes; OCP represents the rising intonation version of OC, where the pitch of the sixth note was increased by 15%; OQ indicates modified version of OC, where the sixth note was replaced by the sixth note of an advertisement call from a sympatric anuran species, *Quasipaa boulengeri*; OQP represents the rising intonation version of OQ, with a 15% increase in the pitch of the sixth component; OW denotes the altered version of OC, with the last note replaced by white noise; and OWP represents the rising intonation version of OW, with a 15% pitch increase in the last component. The control stimuli for OC, OCP and OW are denoted as OCC, OCPC and OWC respectively, where the first five notes of each treatment stimulus were reversed in situ while the last component remained unchanged. In addition, CS and TS refer to the control and treatment stimuli, respectively. $^{*}p < 0.05$ and $^{**}p < 0.001$.

experiments, the ambient temperature and relative humidity ranged from 16.7 to 25.8 °C and from 67.4 to 90%, respectively. An open metal tank (270 cm × 95 cm × 100 cm) containing approximately 5 cm of mud on the bottom was placed outdoors. Small holes (5 mm in diameter, spaced 2 cm apart) were drilled along the four tank walls, and the inner walls were covered by sound-absorbing cotton with 5 cm in thickness to reduce acoustic reverberation. Two speakers (SME-AFS, Saul Mineroff Electronics, Elmont, NY, USA) were positioned at the opposite ends of the tank. Before the experiments, a pure tone of 1,000 Hz was used to calibrate each speaker's output intensity to 70 dB SPL (measured 1 m from the speaker). One female selected randomly was covered by a transparent semicircular plastic cup with a diameter of 20 cm in the center of the tank and allowed to habituate for approximately 2 min in darkness. Then, one of the stimulus pairs was selected randomly and played back antiphonally with a 3 s inter-stimulus interval (ISI), which is approximately equal to the mean of inter-call intervals in nature. After 3 min of playbacks, the plastic cup was lifted remotely to allow the female to move freely in the arena. The behaviors of each subject were monitored through two infrared cameras with motion detection (WAPA, BL-CS7200IB-P(ND)WL-I2SH; Shenzhen, China), mounted at opposite ends of the tank. A positive preference was scored if the female approached a location within 10 cm from one of the speakers, and then the trial was stopped. A "no preference" score was assigned if the animal failed to choose within 20 min after the cup was lifted. The scores and latencies (the interval between the onset of lifting the cup and the moment that the female completed selection between the two stimuli) were recorded for each animal. To control for possible spatial biases, the assignments between the stimuli and speakers were varied randomly across subjects. Additionally, the subjects' body mass and snout-to-vent length were measured after the experiments.

Surgery for electrophysiological experiments

Our previous studies provide detailed descriptions of surgical procedures[84,85]. Prior to surgery, the frog was anesthetized using 0.1% tricaine methanesulfonate (MS-222). Subsequently, six stainless steel screws, each with a diameter of 0.8 mm, were implanted on both sides of specific regions of the brain: the telencephalon (LT and RT), diencephalon (LD and RD), and mesencephalon (LM and RM). Additionally, we implanted a reference electrode above the cerebellum (C) (Fig. 5). To attach the electrodes, one exposed end of formvar-insulated nichrome wire was securely wrapped around each screw, while the other end was soldered to an electrical connector. Dental cement was used to affix the electrodes to the skull, and pain-relieving ointment (CVS pharmacy, Woonsocket, RI, USA) was uniformly applied to the wound to prevent infection and alleviate discomfort. To ensure waterproofing, the connector was protected with a self-sealing membrane (Parafilm®M; Chicago, IL, USA). Each subject was individually housed for a recovery period of 7 days before proceeding with subsequent experiments. Upon completion of the experiments, the animal was euthanized using an overdose of anesthetic. To determine that all data used for analysis was derived from the intended brain regions, hematoxylin dye was injected into the corresponding sites of the skull where the electrodes had been previously implanted.

Recording conditions for electrophysiological experiments

A soundproof and electromagnetically shielded chamber was utilized for the experimental setup. Inside this chamber, an opaque plastic tank with dimensions of 80 cm × 60 cm and a depth of 55 cm was placed. The tank contained a mixture of mud and water on the bottom. To capture the subjects' behaviors, an infrared camera with motion detection was mounted at a height of approximately 1 m above the tank. The EEG signals of the frog were recorded using a signal acquisition system (Chengyi, RM6280; Chengdu, China). The recorded signals were subjected to a band-pass filter ranging from 0.16 Hz to 100 Hz, with a sampling frequency of 1000 Hz.

Stimulus and procedure for electrophysiological experiments

In human ERP studies, employing the N400 component typically involves a "violation paradigm" to delineate various stages of semantic processing. Such studies often induce semantic violation by substituting the final word of a sentence with an incongruous word, thereby allowing the preceding words to establish a specific context[86–88]. Analogously, we investigated the last note of the advertisement call to determine whether disruptions in the informational coherence of acoustic stimuli could provoke variations in N400 amplitudes. We collected 46 conspecific advertisement calls, each comprising six notes, from 46 individuals of the same species. Following the methodology for generating acoustic stimuli in the phonotaxis experiments, six types of treatment stimuli (Table 1 and Fig. 6) were included in the electrophysiological experiments: OC, OCP, OQ, OQP (OQ with the pitch of the sixth component increased by 15%), OW, and OWP (OW with the pitch of the sixth component increased by 15%). We predict that the stimuli with replacement of the sixth component (OQ, OW, OQP, and OWP) would elicit greater N400 and LPC amplitudes compared to OC due to disruptions in information coherence and alterations in acoustic properties. Furthermore, the stimuli with rising intonation (OCP, OQP and OWP) are expected to elicit greater LPC amplitudes compared to their corresponding non-rising intonation versions (OC, OQ and OW)

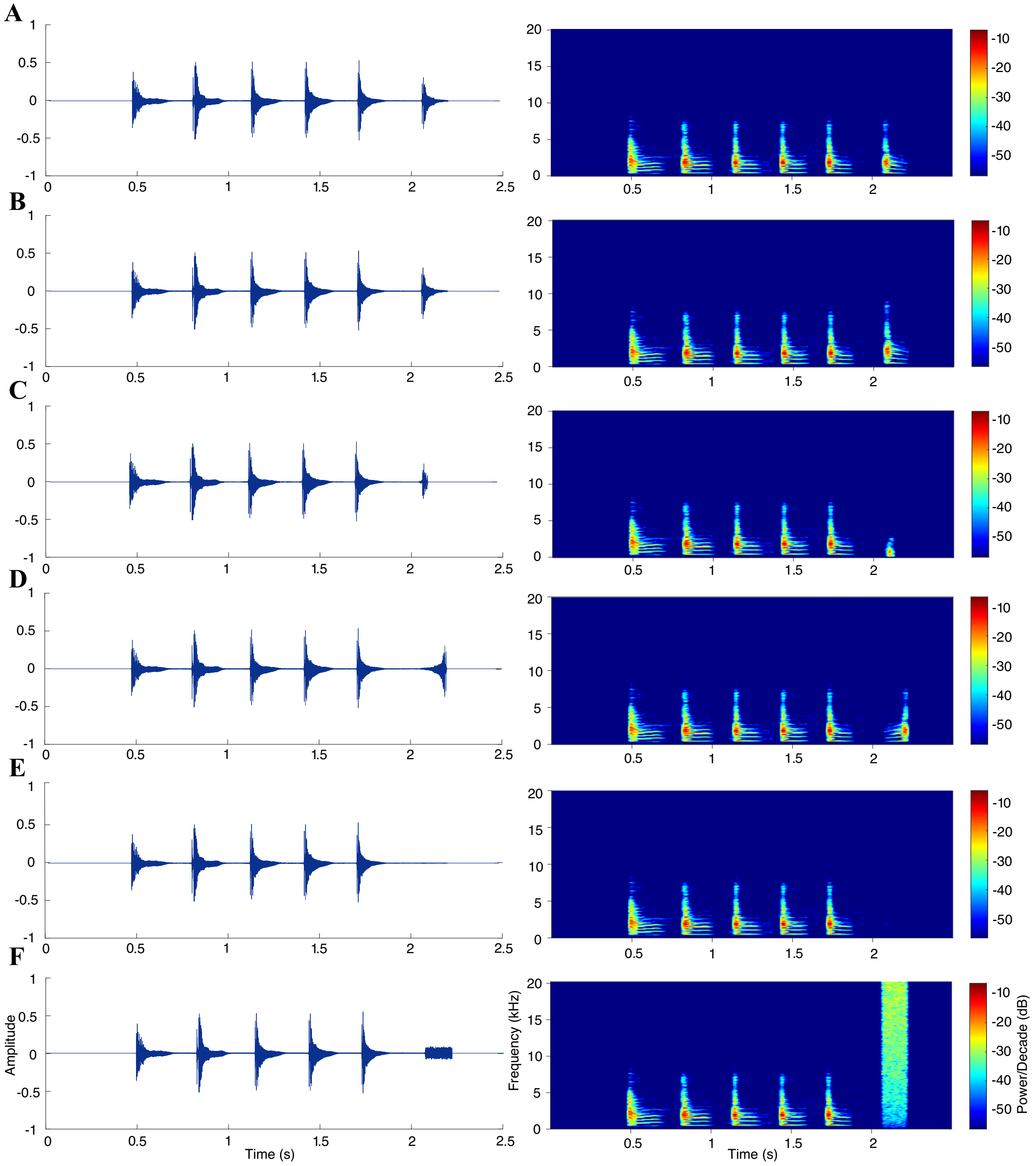

Fig. 4 | Waveforms and spectrograms of the six treatment stimuli for phonotaxis experiments. **A** represents the original advertisement call (OC), **B** illustrates the rising intonation version of OC, where the pitch of the sixth note was increased by 15% (OCP), **C** shows the modified version of OC, where the sixth note was replaced by the sixth note of an advertisement call from a sympatric anuran species, *Quasipaa boulengeri* (OQ), **D** presents a modified version of OC with the last note was reversed in situ (OR), **E** shows a modified version of OC with the last note replaced by silence (OS), **F** demonstrates the altered version of OC, with the last note replaced by white noise (OW). It is worth noting that the rising intonation in the last note (OCP) results in measurable alterations in the frequency spectrum profiles of the last components compared to the original version (OCP vs. OC).

because of greater alterations in acoustic properties. If the results align with these predictions, they would indicate that the induced N400 is driven by violations of information coherence rather than changes in the simple physical attributes of the last note. A total of 276 acoustic stimuli (46 × 6) were generated. Corresponding control stimuli (OCC, OCPC, OQC, OQPC, OWC and OWPC; Table 1 and Supplementary Fig. 2) were created by reversing each of the first five notes in situ while leaving the last note unchanged, thereby disrupting information coherence in the first five notes. We predict that the N400 evoked by these control stimuli, which lack information coherence, would be smaller due to reduced prediction error between the last note and the preceding five reversed ones. If significant

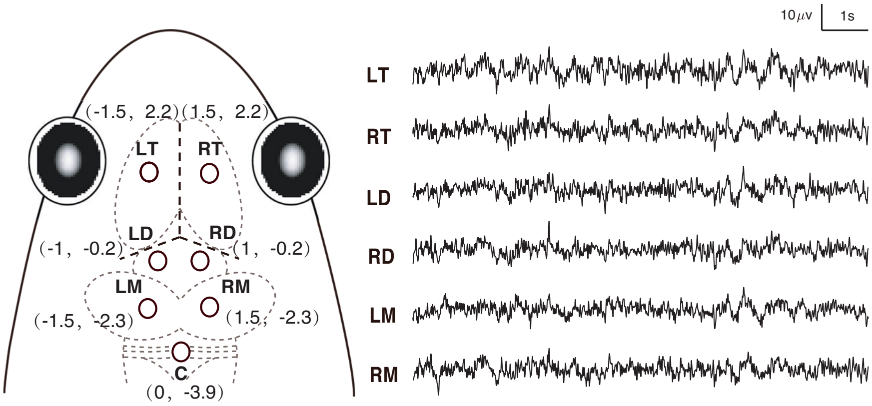

Fig. 5 | Electrode placements and 10-second samples of typical EEG tracings for each brain area. LT and RT the left and right sides of the telencephalon, LD and RD the left and right sides of the diencephalon, LM and RM the left and right sides of the mesencephalon, C the reference electrode implanted above the cerebellum. The intersection point of the three bold dashed lines in the frog's head represents the intersection of the suture lines.

differences in N400 amplitudes are observed between the treatment and control stimuli, this would suggest that disrupting the coherence of information in the preceding segments of calls influences the perception of subsequent segment. The two stimulus types (OS and OR) used in the phonotaxis experiments were excluded from the electrophysiological experiments due to specific limitations: OS (a modified version of OC with the last note replaced by silence) has zero amplitude in the final component, OR (a modified version of OC with the last note reversed in situ) has minimal amplitude in the initial section of the last reversed note, and averaging time points for ERP analysis is challenging for OR due to individual differences in auditory sensitivity.

Two portable field speakers were positioned equidistantly at opposite ends of the experimental tank. To form a quasi-free sound field of the sound level distribution at the bottom of the bank, each acoustic stimulus was played back simultaneously to the subjects via the two speakers at 65.0 ± 0.5 dB SPL. The sound pressure levels were measured at the center of the tank using the sound pressure meter. For each frog, 15 different original calls were selected randomly from the 46 calls and played back with an ISI of 3 s in order to create an acoustic scene. Subsequently, the 276 acoustic stimuli were played back randomly with ISI of 3 s. Following a 5-min break, the stimuli were replayed once again in a randomized order. The entire session lasted approximately 45 minutes. The control experiment followed a similar procedure to the experimental setup. All experimental procedures were carried out using a custom-made software written in C ++, which could save the order of the random stimulus stream automatically. A trigger pulse was sent to the signal acquisition system at every stimulus onset via the parallel port of a PC for further time-locking analysis.

Data acquisition and processing for electrophysiological experiments

Following a seven-day period of postoperative recovery, the subject was placed in the experimental tank and connected to the signal acquisition system for approximately one day of habituation before proceeding with the subsequent experiments. During the experiments, feeding was restricted to minimize potential influences of nutrient metabolism on brain activities. The EEG signals and behavioral data were recorded during playbacks of acoustic stimuli. To extract ERP components, the EEG recordings underwent filtering using a 50 Hz notch filter and a band-pass filter ranging from 0.25 to 25 Hz. Subsequently, the EEG signals were divided into epochs of 1700 ms duration, including a 200 ms pre-stimulation baseline relative to the onset of the last component of each stimulus. All epochs were inspected visually, and those with muscle artifacts and electrode drift were excluded for further analysis.

For each subject, accepted epochs were averaged based on stimulus type and brain area. The grand average waveforms, obtained from averaged waveforms across all subjects (see Supplementary Figs. 3 and 4), revealed the presence of N400 and LPC peaks for each brain area and each stimulus type. The latencies of these peaks were measured from the grand average waveforms, and the medians were calculated for each ERP component, regardless of brain area and stimulus type. Consequently, the time windows for N400 and LPC were defined as 310-610 ms and 610-910 ms respectively after the onset of the last component of acoustic stimuli with the medians as the midpoints. For each ERP component, we calculated the amplitude by averaging across the corresponding time window, and determined the latency using the "half-area algorithm"[89,90]. The half-area algorithm refers to calculating the area under the ERP waveform within the corresponding time window for each ERP component and determining the time point at which this area is divided into equal halves. In this way, the mean amplitude and latency of each ERP component could be acquired for each stimulus type, each brain area and each subject.

Statistics and reproducibility

For the phonotaxis experiments, the binomial test was used to analyze the number of female choices. There was no significant difference for the variable 'stimulus set', consistent with the idea that the four stimulus sets were not significantly different at eliciting behavioral responses from the subjects. The normality and homogeneity of variance for the latencies of female response were assessed using the Shapiro-Wilk W test and Levene's test, respectively. Independent samples t-test was used for comparison of differences in latencies. For the electrophysiological experiments, the normality of distribution and homogeneity of variances for the amplitudes and latencies of each ERP component were assessed using the Shapiro-Wilk W test and Levene's test, respectively. A four-way repeated measures ANOVA was conducted to analyze the amplitudes and latencies of each ERP component, considering the variables of "stimulus group" (treatment stimuli and control stimuli), "stimulus type" (the six types of acoustic stimuli), "sex" (female and male), and "brain area" (the six brain regions). As no significant main effect or interaction was found for the "sex" factor, a three-way repeated measures ANOVA was performed with the remaining three variables. Multiple comparisons with Bonferroni correction and simple effect analysis were performed when ANOVAs returned a significant difference and the interaction effects were significant, respectively. If the assumption of sphericity was violated, Greenhouse-Geisser ε values were employed. The effect size was determined using the partial η^2 value, with values of 0.20, 0.50, and 0.80 indicating small, medium, and large effect sizes, respectively. In the electrophysiological experiments, each stimulus type was presented 92 times. The sample size and the number of replications were determined by referencing previous studies in the same field. All statistical analysis was carried out using SPSS software (version 21.0; SPSS Inc., Chicago, IL, USA), with a significance level set at $p < 0.05$.

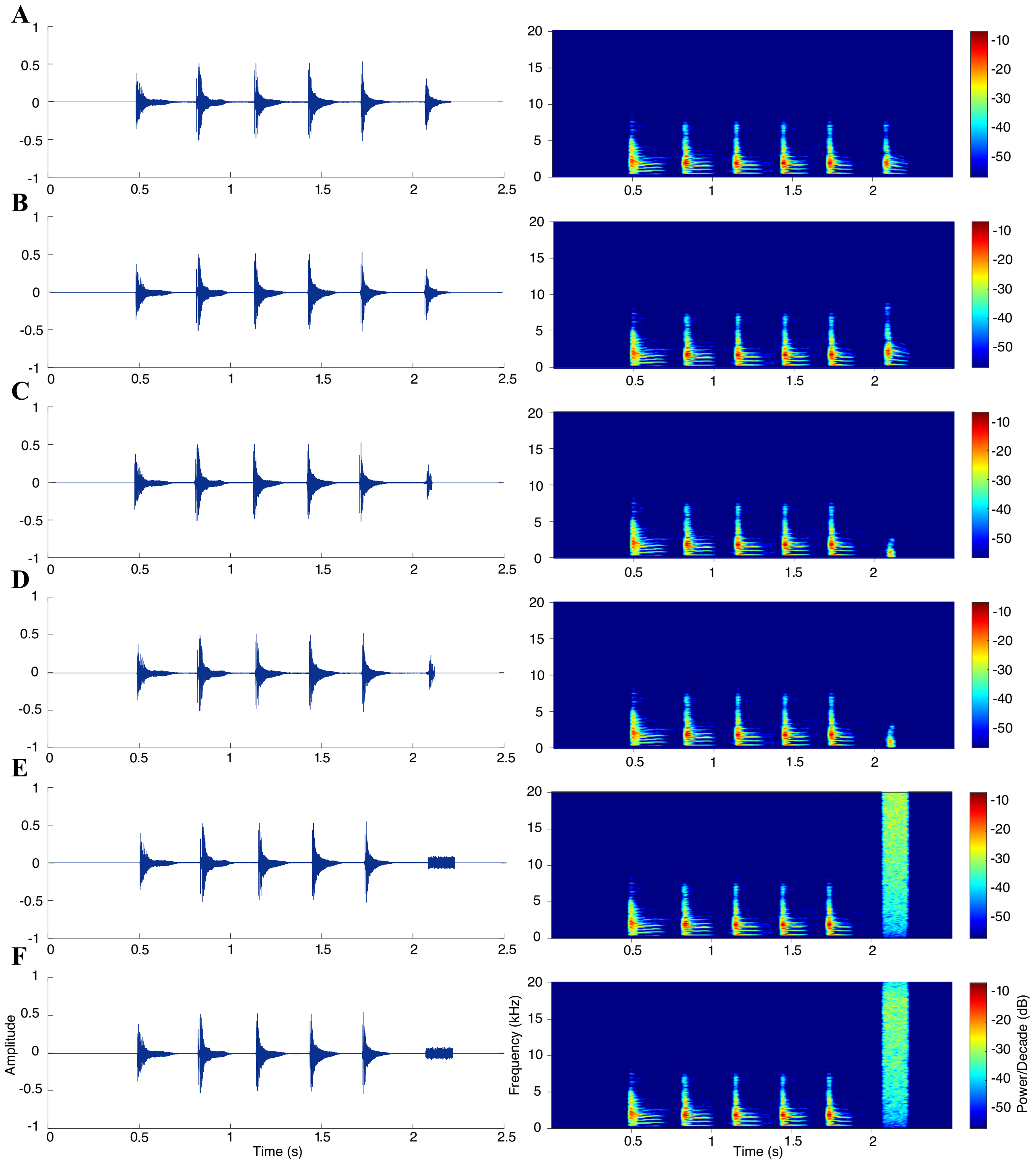

Fig. 6 | Waveforms and spectrograms of the six treatment stimuli used for electrophysiological experiments. A represents the original advertisement call (OC), **B** illustrates the rising intonation version of OC, where the pitch of the sixth note was increased by 15% (OCP), **C** shows the modified version of OC, where the sixth note was replaced by the sixth note of an advertisement call from a sympatric anuran species, *Quasipaa boulengeri* (OQ), **D** presents the rising intonation version of OQ, with a 15% increase in the pitch of the sixth component (OQP), **E** demonstrates the altered version of OC, with the last note replaced by white noise (OW), **F** shows the rising intonation version of OW, with a 15% pitch increase in the sixth component (OWP). It is worth noting that the rising intonation in the last components (OCP and OQP) results in measurable alterations in the frequency spectrum profiles of the last components compared to their corresponding original versions (OCP vs. OC and OQP vs. OQ). However, only minor alterations of the last components were observed for OWP and OW.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The dataset generated and analyzed in the current study is available at Qing, tao (2025), "Data for the music frog", Mendeley Data, V1, https://doi.org/10.

17632/zwgfyw33wx.1. Source data are also provided in Supplementary Data 1-3.

Received: 2 May 2024; Accepted: 5 May 2025;
Published online: 21 May 2025

References

1. Laidre, M. E. Principles of animal communication. *Anim. Behav.* **83**, 865–866 (2012).
2. Lacey, E., Patton, J. & Cameron, G. Life underground: the biology of subterranean rodents. *Aust. Mammal.* **23**, 75–76 (2001).
3. Cui, J., Wang, Y., Brauth, S. & Tang, Y. A novel female call incites male–female interaction and male–male competition in the Emei music frog, *Babina daunchina*. *Anim. Behav.* **80**, 181–187 (2010).
4. Clark, D. L. & Clark, R. A. Neutral point testing of color vision in the domestic cat. *Exp. Eye Res.* **153**, 23–26 (2016).
5. Ruiz-Olmo, J., Such-Sanz, A. & Piñol, C. Substrate selection for urine spraying in captive wildcats. *J. Zool.* **290**, 143–150 (2013).
6. Bergman, T. J. & Beehner, J. C. Information Ecology: an integrative framework for studying animal behavior. *Trends Ecol. Evol.* **38**, 1041–1050 (2023).
7. Schulz, T. M., Whitehead, H., Gero, S. & Rendell, L. Overlapping and matching of codas in vocal interactions between sperm whales: insights into communication function. *Anim. Behav.* **76**, 1977–1988 (2008).
8. Hodges-Simeon, C. R., Gaulin, S. J. & Puts, D. A. Voice correlates of mating success in men: examining “contests” versus “mate choice” modes of sexual selection. *Arch. Sex. Behav.* **40**, 551–557 (2011).
9. Kerwin, P., Yuan, J. & von Philipsborn, A. C. Female copulation song is modulated by seminal fluid. *Nat. Commun.* **11**, 1430 (2020).
10. Suzuki, T. N. Semantic communication in birds: evidence from field research over the past two decades. *Ecol. Res.* **31**, 307–319 (2016).
11. Zuberbühler, K. Syntax and compositionality in animal communication. *Philos. T. R. Soc. B.* **375**, 20190062 (2020).
12. Zuberbühler, K. Combinatorial capacities in primates. *Curr. Opin. Behav. Sci.* **21**, 161–169 (2018).
13. Clay, Z. & Zuberbühler, K. Food-associated calling sequences in bonobos. *Anim. Behav.* **77**, 1387–1396 (2009).
14. Arnold, K. & Zuberbühler, K. Semantic combinations in primate calls. *Nature* **441**, 303–303 (2006).
15. Briefer, E., Osiejuk, T. S., Rybak, F. & Aubin, T. Are bird song complexity and song sharing shaped by habitat structure? An information theory and statistical approach. *J. Theor. Biol.* **262**, 151–164 (2010).
16. Brillet, C. & Paillette, M. Acoustic signals of the nocturnal lizard *Gekko gecko*: analysis of the ‘long complex sequence. *Bioacoustics* **3**, 33–44 (1991).
17. Doupe, A. J. & Kuhl, P. K. Birdsong and human speech: common themes and mechanisms. *Annu. Rev. Neurosci.* **22**, 567–631 (1999).
18. Suzuki, T. N., Wheatcroft, D. & Griesser, M. Experimental evidence for compositional syntax in bird calls. *Nat. Commun.* **7**, 10986 (2016).
19. Williams, H. & Staples, K. Syllable chunking in zebra finch (*Taeniopygia guttata*) song. *J. Comp. Psychol.* **106**, 278–286 (1992).
20. De Lucia, M., Clarke, S. & Murray, M. M. A temporal hierarchy for conspecific vocalization discrimination in humans. *J. Neurosci.* **30**, 11210–11221 (2010).
21. Slabbekoorn, H. & Smith, T. B. Habitat-dependent song divergence in the little greenbul: an analysis of environmental selection pressures on acoustic signals. *Evolution* **56**, 1849–1858 (2002).
22. Yu, X. et al. Geographic variation in the advertisement calls of *Gekko gecko* in relation to variations in morphological features: implications for regional population differentiation. *Ethol. Ecol. Evol.* **23**, 211–228 (2011).
23. Mooney, R. Birdsong. *Curr. Biol.* **32**, R1090–R1094 (2022).
24. Griffiths, C. S. & Aaronson, N. L. Analysis of vocal communication in the genus *Falco*. *Sci. Rep.* **13**, 1846 (2023).
25. Clayton, N. S. Assortative mating in zebra finch subspecies, *Taeniopygia guttata guttata* and *T. g. castanotis*. *Philos. Trans. R. Soc. Lond. B.* **330**, 351–370 (1990).
26. Clayton, N. S. Subspecies recognition and song learning in zebra finches. *Anim. Behav.* **40**, 1009–1017 (1990).
27. Bee, M. A. Sound source segregation in grey treefrogs: spatial release from masking by the sound of a chorus. *Anim. Behav.* **74**, 549–558 (2007).
28. Marshall, V. T., Schwartz, J. J. & Gerhardt, H. C. Effects of heterospecific call overlap on the phonotactic behaviour of grey treefrogs. *Anim. Behav.* **72**, 449–459 (2006).
29. Bent, A. M., Ings, T. C. & Mowles, S. L. Anthropogenic noise disrupts mate choice behaviors in female *Gryllus bimaculatus*. *Behav. Ecol.* **32**, 201–210 (2021).
30. Suzuki, T. N. Other species’ alarm calls evoke a predator-specific search image in birds. *Curr. Biol.* **30**, 2616–2620 (2020).
31. Gomez, D., Théry, M., Gauthier, A. L. & Lengagne, T. Costly help of audiovisual bimodality for female mate choice in a nocturnal anuran (*Hyla arborea*). *Behav. Ecol.* **22**, 889–898 (2011).
32. Halfwerk, W., Page, R. A., Taylor, R. C., Wilson, P. S. & Ryan, M. J. Crossmodal comparisons of signal components allow for relative-distance assessment. *Curr. Biol.* **24**, 1751–1755 (2014).
33. Pröhl, H. & Hödl, W. Parental investment, potential reproductive rates, and mating system in the strawberry dart-poison frog, *Dendrobates pumilio*. *Behav. Ecol. Sociobiol.* **46**, 215–220 (1999).
34. Narins, P. M., Feng, A. S., Fay, R. R., Popper, A. N. Eds., *Hearing and sound communication in amphibians* (Springer, New York, 2006).
35. Farris, H. E., Rand, A. S. & Ryan, M. J. The effects of spatially separated call components on phonotaxis in túngara frogs: evidence for auditory grouping. *Brain Behav. Evol.* **60**, 181–188 (2002).
36. Yue, X. et al. The first call note plays a crucial role in frog vocal communication. *Sci. Rep.* **7**, 10128 (2017).
37. Chen, Q., Cui, J., Fang, G., Brauth, S. E. & Tang, Y. Acoustic analysis of the advertisement calls of the music frog, *Babina daunchina*. *J. Herpetol.* **45**, 406–416 (2011).
38. Zhang, H. et al. Females and males respond differently to calls impaired by noise in a tree frog. *Ecol. Evol.* **11**, 9159–9167 (2021).
39. Kutas, M. & Hillyard, S. A. Event-related brain potentials to semantically inappropriate and surprisingly large words. *Biol. Psychol.* **11**, 99–116 (1980).
40. Kutas, M. & Federmeier, K. D. Thirty years and counting: finding meaning in the N400 component of the event-related brain potential (ERP). *Annu. Rev. Psychol.* **62**, 621–647 (2011).
41. Hagoort, P., Hald, L., Bastiaansen, M. & Petersson, K. M. Integration of word meaning and world knowledge in language comprehension. *Science* **304**, 438–441 (2004).
42. Dudschig, C., Mackenzie, I. G., Leuthold, H. & Kaup, B. Environmental sound priming: does negation modify N400 cross-modal priming effects?. *Psychon. Bull. Rev.* **25**, 1441–1448 (2018).
43. Höltje, G. & Mecklinger, A. Benefits and costs of predictive processing: how sentential constraint and word expectedness affect memory formation. *Brain Res.* **1788**, 147942 (2022).
44. Friedrich, M. & Friederici, A. D. N400-like semantic incongruity effect in 19-month-olds: processing known words in picture contexts. *J. Cogn. Neurosci.* **16**, 1465–1477 (2004).
45. Nigam, A., Hoffman, J. E. & Simons, R. F. N400 to semantically anomalous pictures and words. *J. Cogn. Neurosci.* **4**, 15–22 (1992).
46. Özyürek, A. Hearing and seeing meaning in speech and gesture: insights from brain and behaviour. *Philos. T. R. Soc. B.* **369**, 20130296 (2014).
47. Fabbri-Destro, M. et al. Interaction between words and symbolic gestures as revealed by N400. *Brain Topogr.* **28**, 591–605 (2015).

48. Olivares, E. I., Iglesias, J., Saavedra, C., Trujillo-Barreto, N. J. & Valdés-Sosa, M. Brain signals of face processing as revealed by event-related potentials. *Behav. Neurol.* **2015**, 514361 (2015).
49. Yu, L. et al. Emotional violation of faces, emojis, and words: evidence from N400. *Biol. Psychol.* **173**, 108405 (2022).
50. Uddin, S., Heald, S. L. M., Van Hedger, S. C. & Nusbaum, H. C. Hearing sounds as words: neural responses to environmental sounds in the context of fluent speech. *Brain Lang.* **179**, 51–61 (2018).
51. Besson, M. & Faïta, F. An event-related potential (ERP) study of musical expectancy: comparison of musicians with nonmusicians. *J. Exp. Psychol. Human.* **21**, 1278–1296 (1995).
52. Besson, M. & Schön, D. Comparison between language and music. *Ann. N. Y. Acad. Sci.* **930**, 232–258 (2001).
53. Kaan, E., Harris, A., Gibson, E. & Holcomb, P. The P600 as an index of syntactic integration difficulty. *Lang. Cogn. Process.* **15**, 159–201 (2000).
54. Mccallum, W. C., Farmer, S. F. & Pocock, P. V. The effects of physical and semantic incongruities of auditory event-related potentials. *Electroencephalogr. Clin. Neurophysiol.* **59**, 477–488 (1984).
55. Ehlers, C. L. EEG and ERP responses to naloxone and ethanol in monkeys. *Prog. Neuro-Psychoph.* **13**, 217–228 (1989).
56. Pincze, Z., Lakatos, P., Rajkai, C., Ulbert, I. & Karmos, G. Separation of mismatch negativity and the N1 wave in the auditory cortex of the cat: a topographic study. *Clin. Neurophysiol.* **112**, 778–784 (2001).
57. Boros, M. et al. Neural evidence for referential understanding of object words in dogs. *Curr. Biol.* **34**, 1750–1754 (2024).
58. Bálint, A., Eleőd, H., Magyari, L., Kis, A. & Gácsi, M. Differences in dogs' event-related potentials in response to human and dog vocal stimuli; a non-invasive study. *R. Soc. Open. Sci.* **9**, 211769 (2022).
59. Schusterman, R. J., Thomas, J. A., Wood, F. G., Schusterman, R. Eds., *Dolphin cognition and behavior* (Psychology, New York, 1986).
60. Wang, Y. P., Kawai, Y. & Nakashima, K. Rabbit P300-like potential depends on cortical muscarinic receptor activation. *Neuroscience* **89**, 423–427 (1999).
61. Morzorati, S. L. & Stewart, R. B. Development of acute tolerance during steady-state arterial alcohol concentrations: a study of auditory event-related potentials in rats. *Alcohol. Clin. Exp. Res.* **29**, 347–352 (2005).
62. Fang, G. et al. Sound classification and call discrimination are decoded in order as revealed by event-related potential components in frogs. *Brain Behav. Evol.* **86**, 232–245 (2015).
63. Fan, Y. et al. Hierarchical auditory perception for species discrimination and individual recognition in the music frog. *Curr. Zool.* **68**, 581–591 (2021).
64. Fan, Y. et al. Preference of spectral features in auditory processing for advertisement calls in the music frogs. *Front. Zool.* **16**, 13 (2019).
65. Yang, P. et al. Auditory sensitivity exhibits sexual dimorphism and seasonal plasticity in music frogs. *J. Comp. Physiol. A.* **204**, 1029–1044 (2018).
66. Krauzlis, R. J., Bogadhi, A. R., Herman, J. P. & Bollimunta, A. Selective attention without a neocortex. *Cortex* **102**, 161–175 (2018).
67. Attaheri, A. et al. EEG potentials associated with artificial grammar learning in the primate brain. *Brain Lang.* **148**, 74–80 (2015).
68. Hernández-Gutiérrez, D. et al. Do discourse global coherence and cumulated information impact on sentence syntactic processing? An event-related brain potentials study. *Brain Res.* **1630**, 109–119 (2016).
69. Steinfeld, R., Tacão-Monteiro, A. & Renart, A. Differential representation of sensory information and behavioral choice across layers of the mouse auditory cortex. *Curr. Biol.* **34**, 2200–2211.e2206 (2024).
70. Suzuki, T. N. Communication about predator type by a bird using discrete, graded and combinatorial variation in alarm calls. *Anim. Behav.* **87**, 59–65 (2014).
71. Wheeler, B. C. & Fischer, J. Functionally referential signals: a promising paradigm whose time has passed. *Evol. Anthropol.* **21**, 195–205 (2012).
72. Gill, S. A. & Bierema, A. M.-K. On the meaning of alarm calls: a review of functional reference in avian alarm calling. *Ethology* **119**, 449–461 (2013).
73. Lawson, S. L., Enos, J. K., Gill, S. A. & Hauber, M. E. Eavesdropping on referential yellow warbler alarm calls by red-winged blackbirds is mediated by brood parasitism risk. *Front. Ecol. Evol.* **9**, 706170 (2021).
74. Suzuki, T. N., Wheatcroft, D. & Griesser, M. Wild birds use an ordering rule to decode novel call sequences. *Curr. Biol.* **27**, 2331–2336 (2017).
75. Engesser, S., Ridley, A. R. & Townsend, S. W. Meaningful call combinations and compositional processing in the southern pied babbler. *Proc. Natl. Acad. Sci.* **113**, 5976–5981 (2016).
76. Suzuki, T. N. Alarm calls evoke a visual search image of a predator in birds. *Proc. Natl. Acad. Sci.* **115**, 1541–1545 (2018).
77. Manser, M. B. Semantic communication in vervet monkeys and other animals. *Anim. Behav.* **86**, 491–496 (2013).
78. Suzuki, T. N. Long-distance calling by the willow tit, *Poecile montanus*, facilitates formation of mixed-species foraging flocks. *Ethology* **118**, 10–16 (2012).
79. Suzuki, T. N. & Kutsukake, N. Foraging intention affects whether willow tits call to attract members of mixed-species flocks. *R. Soc. Open. Sci.* **4**, 170222 (2017).
80. Kroodsma, D. E., Byers, B. E., Goodale, E., Johnson, S. & Liu, W.-C. Pseudoreplication in playback experiments, revisited a decade later. *Anim. Behav.* **61**, 1029–1033 (2001).
81. Freeberg, T. M. & Lucas, J. R. Pseudoreplication is (still) a problem. *J. Comp. Psychol.* **123**, 450–451 (2009).
82. Schank, J. C. & Koehnle, T. J. Pseudoreplication is a pseudoproblem. *J. Comp. Psychol.* **123**, 421–433 (2009).
83. Lazic, S. E. The problem of pseudoreplication in neuroscientific studies: is it affecting your analysis?. *BMC Neurosci.* **11**, 5–5 (2010).
84. Niu, W. et al. Possible event-related potential correlates of voluntary attention and reflexive attention in the Emei music frog. *Biology* **11**, 879 (2022).
85. Fang, K., Tang, Y., Zhang, B. & Fang, G. Neural activities in music frogs reveal call variations and phylogenetic relationships within the genus *Nidirana*. *Commun. Biol.* **5**, 550 (2022).
86. Carrus, E., Pearce, M. T. & Bhattacharya, J. Melodic pitch expectation interacts with neural responses to syntactic but not semantic violations. *Cortex* **49**, 2186–2200 (2013).
87. Kutas, M. & Hillyard, S. A. Reading senseless sentences: brain potentials reflect semantic incongruity. *Science* **207**, 203–205 (1980).
88. Wang, X. et al. Semantic violation in sentence reading and incongruence in chord sequence comprehension: an ERP study. *Heliyon* **9**, e13043 (2023).
89. Handy, T. Ed., *Event-related potentials: a handbook* (MIT Press, Cambridge, MA, 2005).
90. Luck, S. Ed., *An introduction to the event-related potential technique* (MIT Press, Cambridge, MA, 2015).

Acknowledgements

The authors would like to express their sincere gratitude to Jing Yang and Jiangyan Shen from the Animal Behavior & Bionics Group of Chengdu Institute of Biology, Chinese Academy of Sciences for their valuable discussions and suggestions. This work was supported by the grants from the National Natural Science Foundation of China (Nos. 32170504 and 31970422) to Guangzhan Fang.

Author contributions

Conceptualization: G.F. Methodology: G.F., Y.F. Investigation: D.S., T.Q., S.S., L.L., Y.W. Visualization: T.Q., D.S. Supervision: G.F. Writing—original draft preparation: T.Q., D.S. Writing—review & editing: G.F.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s42003-025-08170-0.

Correspondence and requests for materials should be addressed to Guangzhan Fang.

Peer review information *Communications Biology* thanks Heidi Lyn, Adriano Lameira and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Primary Handling Editors: Julio Hechavarría and Benjamin Bessieres. A peer review file is available.

Reprints and permissions information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

© The Author(s) 2025