

Fig. S1. Location of recordings in *C. perspicillta*'s auditory cortex. (a) Coronal sections of the frontal auditory field (FAF; top) and the auditory cortex (AC; bottom) of *C. perspicillata*. In the zoomed-in insets for each location, an in-scale schematic of the electrode positioning in shown. For the case of the auditory cortex, layer borders are demarcated (see also rightmost inset of the panel); for the case of the FAF, layer borders were difficult to determine and are therefore not shown. Note, in the rightmost inset of the panel, the grouping for the dPTE analyses relative to electrode depth and AC laminar distribution. (b) Laminar recordings in the AC were made mostly in the high frequency fields. In the panel, the approximate position of each recording is illustrated. Each circle corresponds to a single penetration, each being of three possible colours depending on the animal where experiments were performed. Areas delimited by orange, black or blue dashed lines (each colour corresponding to a specific animal) depict the approximate extent of the trepanations. A total of 20 independent penetrations pairs (i.e. n=20 penetrations in FAF and AC) were performed.



Fig. S2. Pre-vocal power changes associated with the production of echolocation and communication calls. (a) Percentage pre-vocal power change across LFP frequency bands (δ, 1-4 Hz; θ, 4-8 Hz; α, 8-12 Hz; β₁, 12-20 Hz; β₂, 20-30 Hz; γ₁, 30-60 Hz; γ₂, 60-120 Hz; γ₃, 120-200 Hz), relative to a no-voc baseline, across all cortical depths in FAF (left) and AC (right). Pre-vocal power change values related to echolocation utterances (n = 138) are depicted in blue: those related to communication utterances (n = 734) are depicted in orange. Data shown as mean ± sem. (b) Significance matrices depicting p-values statistical tests to determine whether changes shown in panel a were significant (i.e. significantly different than 0% change for each channel and frequency band; two-sided, FDR-corrected Wilcoxon signed rank tests). The colour scale in the figures indicates the log10 of the corrected p-values (significance when $p_{corr} < 0.05$). (c) Example GLM fitted with pre-vocal power change data from an FAF channel located at 450 mm from the cortical surface, in the g2-band. Power changes in this band significantly predicted ensuing call type on a trial-by-trial basis (Wald test, $p = 3.02 \times 10^{-9}$), with moderate effect size $R^2m = 0.11$. (d) Example GLM fitted with prevocal power change data from the AC, same electrode depth as in c, and also in the g_2 frequency band. Relative power changes in this frequency band and brain region did not significantly predict ensuing vocal type (Wald test, p = 0.72). Source data are provided as a Source Data file.



0 400 θ 0 200 a B₁ 100 -50 100 β2 0 -20

AC

δ



GLM - HF vs. LF communcation <u>AC</u> 0.2 0.15 0.1 ъ 6 B. Y 0.05 0 h.s 300 550750 depth [μm] 300 5507 depth [μm] 50

Fig. S3. Pre-vocal LFP power distinguishes between ensuing HF-communication and echolocation calls. (a) Normalized average power spectral density (PSD) of echolocation (blue), HF-communication (orange), LF-communication (gray), and all communication calls (black, dashed). (b) Percentage pre-vocal power change across LFP frequency bands (δ , 1-4 Hz; θ, 4-8 Hz; α, 8-12 Hz; β₁, 12-20 Hz; β₂, 20-30 Hz; γ₁, 30-60 Hz; γ₂, 60-120 Hz; γ₃, 120-200 Hz), relative to a no-voc baseline, across all cortical depths in FAF (left) and AC (right). Prevocal power change values related to echolocation utterances (n = 138) are depicted in blue; those related to HF-communication utterances (n = 155) are depicted in orange. Data shown as mean ± sem. Pre-vocal LFP power differences between HF-communication and echolocation conditions resemble the patterns shown in Fig. 1. (c) Same as in b, but the data shown corresponds to the pre-vocal power change across LFP bands associated to the vocalization of HF-communication (orange) and LF-communication (gray, n = 579) calls. Note that differences across HF- and LF-communication calls are barely perceptible. Interestingly, pre-vocal LF-communication calls appeared higher than for HF-communication calls, strengthening the notion that the utterance of high frequency sounds does not necessarily imply higher power in pre-vocal LFPs. (d) Pre-vocal power change in frontal and auditory regions predict whether animals vocalize HF-communication or echolocation calls. Effect size (R²_m) of GLMs considering all frequency bands and channels, both in frontal and auditory cortices. Effect sizes were considered small when $R^2_m < 0.1$, and medium for $R^2_m >= 0.1$. For illustrative purposes, effect size values from non-significant models were set to 0. (e) Same as in d, but depicting GLM outcomes for classifying across the HF- and LF-communication conditions. Models in FAF and predict ensuing vocal type poorly ($R^{2}_{m} \ll 0.1$, i.e. small effect sizes). Source data are provided as a Source Data file.



Fig. S4. Average dPTE matrices during pre-vocal, post-vocal, and no-voc periods. (a) Schematic representation of channel depth and cortical region associated with channel numbers in the panel. (b) Mean pre-vocal directed phase transfer entropy (dPTE) across LFP frequency bands (δ , θ , α , β_1 , γ_2) and conditions (echolocation utterance, top; communication utterance, bottom); 500 repetitions each). (c) Same as in b, with dPTE data corresponding to post-vocal periods. (d) Similar to b and c, illustrating average dPTE matrices corresponding to no-voc periods. Each matrix in the figure (i.e. panels b-d) illustrates the average dPTE across 500 repetitions calculated using 50 trials corresponding to echolocation, communication (both pre- and post-vocal), or no-voc related LFP segments. A cell (*i*, *j*) in a matrix shows the average dPTE value related to the information flow between channels *i* and *j*, which occurs in the $i \rightarrow j$ direction for dPTE values > 0.5 (red colours), and in the $j \rightarrow i$ direction for dPTE values < 0.5 (blue colours).



dPTE (cond1) > dPTE (cond2) ······ dPTE (cond2) > dPTE (cond1)

→ FAF → AC preferred directionality ← AC → FAF preferred directionality → Within structure preferred directionality

Fig. S5. Information flow differences in the FAF-AC circuit between vocalization and no-

voc conditions. (a, b) (Top) Graphs illustrating the differences between pre-vocal directionality and no-voc periods (a, echolocation vs. no-voc; b, communication vs. no-voc), across frequency bands. Edges were shown if three conditions were met: (i) the differences were significant (two-sided FDR-corrected Wilcoxon rank sum tests, $p_{corr} < 0.05$), (ii) the effect size was large (|d| > 0.8), and (iii) edges had already shown significant directionality (see edges in Fig. 2). Edge thickness is weighted according to the effect size of the comparison. Continuous lines indicate dPTEs for the first condition considered (see labels) higher than dPTEs for the second condition. Dashed lines indicate the opposite. (Bottom) Net information outflow (DInet) from FAF (blue bars) and AC (orange bars), in the two conditions considered (a, echolocation vs. no-voc; b, communication vs. no-voc). Significant differences across conditions are marked with stars (two-sided FDR-corrected Wilcoxon rank sum tests; * pcorr < 0.05, ** $p_{corr} < 0.01$, *** $p_{corr} < 0.001$, n.s.: not significant; n = 500 repetitions). Grey numbers in the panels indicate effect sizes (d; not shown for non-significant differences). Values were considered independently of whether there was previous significant directionality in any of the two conditions. Data shown as mean ± sem. (c, d) Same as a-b, but comparisons were made between post-vocal and no-voc periods (i.e. c, echolocation vs. no-voc; d, communication vs. no-voc). Source data are provided as a Source Data file.



pre-vocal (echolocation) vs. pre-vocal (communication)



Fig. S6. dPTE differences across vocalization conditions during pre-vocal and post-vocal periods. (a) Differences in pre-vocal directionality of information flow between vocalization conditions (echolocation vs. communication), across frequency bands. Conventions for this figure are the same as for Fig. S5. (b) Same as a, but data shown correspond to comparisons between post-vocal periods related to echolocation and communication call production. Source data are provided as a Source Data file.



Fig. S7. Directed connectivity in the FAF-AC circuit during passive listening. (a) Frequency tuning curves (FTC) at 75 dB SPL for LFPs recorded in FAF and AC (at a representative depth of 350 μm). (b) Oscillograms (top) and spectrograms (bottom) of the sounds used for acoustic stimulation (HF-FM: high-frequency frequency-modulated). (c) Representative responses from FAF and AC (averaged across trials for one penetration pair) to the sounds presented in the passive listening experiments (HF-FM and distress). (d) Graph visualization of directed connectivity in the FAF-AC circuit during passive listening (period of 500 ms after sound onset; top: responses to HF-FM sweep, bottom: responses to distress syllables). Graph visualization

follows the conventions described for Fig. 2. (See Supplementary Notes).

passive listening (HF-FM) vs. passive listening (distress)



Fig. S8. Information flow differences in the FAF-AC circuit between passive listening and no-voc conditions. (a) Graph-based comparison of preferred directionality of information flow

across passive listening conditions (i.e. responses to HF-FM sounds vs. responses to distress sounds). Conventions and statistics in the panel are the same as those described for **Fig. S5a**. (**b**) Same as in **a**, but data show comparisons between passive listening of HF-FM sounds vs. spontaneous activity (no-voc periods). (**c**) Same as in **b**, but data correspond to comparisons between passive listening of distress syllables vs. no-voc periods. (See Supplementary Notes). Source data are provided as a Source Data file.

passive listening (HF-FM) vs. post-vocal (echolocation)



Fig. S9. Directed connectivity differences across vocalizing and passively listening conditions. (a) (*Top*) Graphs illustrating the differences in dPTE across passive listening and vocalization conditions (HF-FM sweep vs. active echolocation, post-vocal), across frequency bands. Edges shown and statistics performed following the guidelines of those in Fig. S5 (note that the basis for significant edges stem from Fig. S6). Continuous lines indicate dPTEs for passive listening of HF-FM sounds (first condition) higher than dPTEs for echolocation production (second condition). Dashed lines indicate the opposite. (*Bottom*) Net information outflow (DI_{net}) from FAF (blue bars) and AC (orange bars), in the two conditions considered. Data are shown following the conventions described in Fig. S5. (b) Same as in a, but comparing between passive listening of distress syllables vs communication production (post-vocal). (See Supplementary Notes). Source data are provided as a Source Data file.



Fig. S10. Information flow patterns during HF-communication production differ from those during echolocation production. (a) Graph visualization of directed connectivity in the FAF-AC circuit for post-vocal periods associated to the production of HF-communication calls. Edges were shown according to the conventions described in Fig. S5. (b) (*Top*) Graphs illustrating the differences in dPTE across pre-vocal and post-vocal periods related to the production of HF-communication calls. Edge comparisons and statistics were made as described for Fig. S5. (*Bottom*) Net information outflow (DI_{net}) from FAF (blue bars) and AC (orange bars), in the two conditions considered. Data are shown as described for Fig. 3. (c) Same as in b, but comparisons were made between post-vocal periods related to the utterance of HF-communication (condition 1) and echolocation (condition 2) vocalizations.

(See Supplementary Notes). Source data are provided as a Source Data file.





C FAF and AC signal amplitude normalized commonly



Fig. S11. Electrical stimulation in FAF does not lead to consistent changes in concomitant auditory cortical LFPs. (a) Schematic representation of electrical stimulation in FAF and recording locations in AC. The illustration of auditory cortical locations follows the same guidelines than those in Fig. S1a in this document. A zoom-in into the recording locations, separated by animals, is given in the rectangle to the rightmost part of the panel. (b) Grand average (calculated over a total of 1000 trials (i.e. 50 trials in 20 penetratios)) of LFPS around the times of electrical stimulation in FAF. Signals in this panel were normalized within structure: frontal and auditory cortical LFP amplitudes are comparable within each region, but not across. For illustrative purposes, simultaneously recorded FAF and AC field potentials are shown at three representative depths per structure (50, 250, 650 μm). Note that, in the FAF, the electrical artefact produced by the electrical stimulation is dominant. In the AC, however, no effects from the electrical stimulation are evident. (c) Grand average LFPs from AC and FAF (see **b**), but signal amplitude was normalized commonly across structures. That is, amplitudes in FAF and AC are comparable to one another. All recording depths are depicted, but only LFPs around the times of the first, third, and sixth electrical pulse (in a 6-pulse train) are shown. Note that electrical artefacts in the FAF are predominant when an electrical pulse is delivered, but that the amplitude of the AC average is considerably lower in comparison. (d) Comparisons of LFP power in AC before, during, and after electrical stimulation of the FAF across frequency bands (indicated in gray, above each sub-panel; data shown as mean ± s.e.m). The LFP power was obtained per penetration, and calculated as the average power across trials (n = 50). The time windows were as follows: for pre electrical stimulation (pre eestim) segments, -2700 to -200 ms relative to first pulse onset; for segments during electrical stimulation (during e-stim), 0 to 2500 ms relative to first pulse onset; for segments post electrical stimulation (post e-stim), 2700 to 5200 ms relative first pulse onset. In other words, all segments were 2.5 seconds long, the same duration as the electrical pulse train. Band power was obtained by integrating LFP power between the given frequencies of a band (same procedure used for the data shown in **Fig. 1**). We observed no significant differences between the LFP power of pre-, during, and post- e-stim segments in AC, regardless of the frequency band considered. These results (panels b-d) indicate that the electrical stimulation of the FAF did not significantly alter auditory cortical LFPs by means of entrainment to the electrical stimulation train, or other passive propagation of electrical artefacts. Source data are provided as a Source Data file.



Fig. S12. Electrical stimulation in FAF does not elicit vocalization. (a) Probability density over the full recording period of call onset, pooled across all recording sessions (n = 3515 calls were examined). Individual call onsets are depicted with an "x" on the top of the plot; electrical stimulation trains (i.e. six pulses) are marked with grey bars. (b) Call onset times around periods of electrical stimulation (i.e. during the stimulation train, with pulses illustrated in vertical grey bars) and 2 seconds of pre- and post-time. Calls are shown for each trial of electrical stimulation, sorted from bottom to top, belonging to different recordings (n = 20), also sorted in the same manner. There were 50 trials per recording. Continuous traces illustrate a normalized density function of call onset probability (similar to the one shown in **a**), for low-and high- frequency call (in red and blue, respectively). In panels **a** and **b** no clear effect of electrical FAF stimulation can be observed on the probability of call onset across trials or penetration. (**c**) Proportion of call onset during times of electrical stimulation (black; 5.6%, 55 calls; total n = 980 calls) vs. proportion of call onset outside times of electrical stimulation (grey; 7.1%, 70 out a total of n = 980 calls). We did not see any significant differences in the proportion of call onset across periods (χ^2 -test, p = 0.17; Q = 1.9226).



Fig. S13. Referencing schemes account for dissimilarities between the current study and that of Weineck et al. (2020). (a) LFPs in the bat FAF during vocalization (echolocation and communication) across various depths. The data is the same as that presented in Fig. 1d; however, the top channel in the FAF was used as a reference for LFPs in that structure (yielding a flat, zero-line at the topmost trace). The referencing was done offline (i.e. in the post-processing stage) by simply subtracting the LFP of each FAF channel from the first channel in the same structure. This referencing scheme mostly affects low frequency oscillations, which are highly correlated across electrodes in FAF (e.g. see Fig. 1d). (b) As a consequence, differences between pre-vocal power changes related to the utterance of echolocation and communication calls in low frequencies (e.g. δ , θ , or α) are strongly affected (c.f. **Fig. 1f**). For γ_2 frequencies, however, pre-vocal power differences are still evident. Power changes preceding the vocalization of an echolocation or a communication call are shown across all channels in FAF (n = 138 echolocation, blue; n = 734 communication, orange; data as mean ± sem). (c) When using the top channel in the FAF as reference, low-frequency (δ - α) pre-vocal power changes in FAF lose their predictive power to a great extent (c.f. Fig. 1g). Nevertheless, LFPs in the γ_2 -band continue to predict whether animals produce echolocation or communication utterances. (d) Analysis of the data from Weineck et al. (2020), where the top FAF channel was used as a reference online (i.e. determined by hardware in the setup), with the same GLM paradigm used in the current study. Note the similarities between the predictive power of the pre-vocal LFP at various frequencies between Weineck et al.'s dataset and that of our current dataset with altered referencing (panel c). Thus, by accounting for the differences in reference used, we can fully replicate results from Weineck et al. Low frequency effects may have been overlooked in the latter study as a consequence of using the top FAF channel as reference, on account of the well-correlated low-frequency activity across electrodes in the frontal cortex. (e) In terms of the functional connectivity measured with the dPTE metric, we still observe information flowing top-down (FAF \rightarrow AC direction) during prevocal periods for δ -frequencies of the LFP. Information flow reverses after echolocation in this frequency band, but not after communication vocalization. Thus, imposing an artificial top-channel referencing scheme does not drastically affect our original observations, namely an echolocation-related reversal of information flow in the FAF-AC network. Data in the dPTE matrices are shown following the conventions of **Fig. S4** (note the legend in the panel). Source data are provided as a Source Data file.

Supplementary tables

Table S1. N-way ANOVA examining effects of AC depth and sound latency on response strength changes between Estim and no-Estim conditions (animals listening to an echolocation pulse).

Source	Sum Sq.	d.f.	Mean Sq.	F	Prob > F
channel	97.461	15	6.4974	1776.29	< 0.000001
latency	354.526	7	50.6466	13845.97	< 0.000001
channel*latency	147.385	105	1.4037	383.74	< 0.000001
Error	233.634	63872	0.0037		
Total	833.006	63999			

Table S2. N-way ANOVA examining effects of AC depth and sound latency on response strength changes between Estim and no-Estim conditions (animals listening to distress syllable).

Source	Sum Sq.	d.f.	Mean Sq.	F	Prob > F
channel	196.832	15	13.1221	4375.63	< 0.000001
latency	83.861	7	11.9801	3994.81	< 0.000001
channel*latency	60.399	105	0.5752	191.81	< 0.000001
Error	191.547	63872	0.003		
Total	532.638	63999			

Supplementary Note 1

FAF-AC connectivity patterns in pre- and post-vocal periods depend on whether animals vocalize echolocation or communication calls

Connectivity patterns during pre-vocal periods

To quantitatively address the differences in preferential information flow shown in **Fig. 2**, we compared connectivity dynamics in the FAF-AC network across vocal conditions (i.e. pre-voc and post-voc; no-voc comparisons shown in Fig. S5). The top row of Fig. S6a summarizes the outcomes of such comparisons during pre-vocal periods across frequency bands, for the echolocation vs. communication case. Edges in the graphs show significant differences (Wilcoxon rank sum tests, significance when $p < 10^{-4}$) with large effect sizes (|d| > 0.8) in the directionality of information flow between two given nodes. Edges were weighted according to the effect size (d) of the corresponding comparisons. Thus, graphs in Fig. S6a (top) show that significant differences (with large effect sizes) between the cases of pre-vocal echolocation and pre-vocal communication, in terms of $FAF \rightarrow AC$ connectivity, occurred only in the γ_2 -band. Within FAF, significant differences in dPTE between were strongest in the δ and α ranges, although sparse significant differences occurred also in the θ and β_1 bands. The strength of preferred FAF \rightarrow AC directionality of information flow was significantly weaker for pre-vocal echolocation than for no-voc periods in δ frequencies (**Fig. S5**). However, in the γ_2 band, FAF \rightarrow AC connectivity was significantly stronger during pre-vocal echolocation periods. Significant differences in the directionality of information flow between communication and no-voc conditions were rare (Fig. S5).

We use the DI_{net} metric (see main text, **Fig.3**) to compare information outflow from each cortical region when animals vocalized either echolocation or communication calls. Significant differences in DI_{net} values across conditions (echolocation vs. communication; **Fig. S6a**, bottom) occurred with large effect sizes (|d| > 0.8) only in the γ_2 band, considering information flowing from the FAF. Specifically, FAF-related DI_{net} in the γ_2 band was significantly (FDR-corrected Wilcoxon rank sum tests, $p_{corr} < 0.05$)

higher when animals vocalized echolocation calls as compared to when communication calls (**Fig. S6a**; $p_{corr} = 4.27 \times 10^{-105}$, d = 1.83) or no call whatsoever (**Fig. S5a**; $p_{corr} = 2.24 \times 10^{-84}$, d = 1.51). Conversely, δ -band net information outflow was significantly higher during no-voc periods as compared to the pre-vocal echolocation (**Fig. S5a,b**; $p_{corr} = 5.15 \times 10^{-30}$, d = -0.78) and the pre-vocal communication (**Fig. S5c**; $p_{corr} = 1.08 \times 10^{-29}$, d = -0.78) conditions, although with no large effect sizes in either case.

Connectivity patterns during post-vocal periods

There were major differences in connectivity during post-vocal periods between vocalization conditions (Fig. S6b). Preferential top-down information flow was significantly lower for echolocation calls than for communication vocalizations in δ and β_1 frequencies, but significantly higher in the γ_2 band (**Fig. S6b**, top; $p < 10^{-4}$, |d| > 0.8). Remarkably, post-vocal preferred directionality of information flow in the δ and β_1 bands was strongest in the bottom-up direction (AC \rightarrow FAF) for the echolocation condition. Similar effects were seen when comparing connectivity patterns obtained from post-vocal echolocation and no-voc periods (Fig. S5c, top). In other words, the post-vocal echolocation condition exhibited the weakest top-down information transfer and the strongest bottom up-information flow in bands δ and β_1 . Top-down γ_2 causal influences remained strongest when animals vocalized an echolocation pulse, as compared to communication call production or no-voc periods. Within-area changes were observed in the α -band in FAF, where preferential superficial-to-deep information transfer was significantly higher for echolocation vocalizations (Fig. S6b, top), while deep-tosuperficial information flow was strongest in post-vocal communication and no-voc related periods (Fig. S6b, S5). Finally, significant differences between post-vocal communication and spontaneous activity were limited to δ frequencies, and strongest for no-voc LFPs.

We compared the net information outflow across conditions in each structure for postvocal periods (**Fig. S6b**, bottom). In the δ -band, preferred information outflow from the FAF was weakest (with large effect sizes) when animals vocalized echolocation calls (FDR-corrected Wilcoxon rank sum tests; echolocation vs. communication: **Fig. S6b**, $p_{corr} = 1.32 \times 10^{-112}$, d = -1.66; echolocation vs. no-voc: **Fig. S5**, $p_{corr} = 1.57 \times 10^{-171}$, d = -

21

4.27). A similar effect was observed when comparing communication DI_{net} values with no-voc ones: preferential post-vocal net information outflow from FAF was significantly lower for vocalization-related LFPs (**Fig. S5**, $p_{corr} = 2.58 \times 10^{-125}$, d = -2.22). Similarly, post-vocal DI_{net} values for the β_1 -band in the FAF were significantly stronger during communication than during echolocation production, reaching large effect sizes (Fig. **S6b**, $p_{corr} = 1.71 \times 10^{-38}$, d = -0.85). γ_2 -related net information outflow from FAF was always strongest in the case of echolocation (echolocation vs. communication: Fig. S6b, $p_{corr} = 3.8 \times 10^{-144}$, d = 2.69; echolocation vs. no-voc: Fig. S5, $p_{corr} = 2.54 \times 10^{-128}$, d = 2.22). In the δ -band, net information outflow from AC was significantly stronger, with large effect sizes, during echolocation production than for post-vocal communication or no-voc periods (echolocation vs. communication: **Fig. S6b** bottom, $p_{corr} = 1.45 \times 10^{-91}$, d = 1.26; echolocation vs. no-voc: Fig. S5, $p_{corr} = 2.84 \times 10^{-127}$, d = 1.5). Also, in the β_1 -band, net information outflow from AC was strongest for post-vocal echolocation than communication periods although without large effect sizes (Fig. S6b; $p_{corr} = 2.55 \times 10^{-34}$, d = 0.72). Significant changes between echolocation and no-voc cases in the same frequency band did not occur with large effect size (**Fig. S5**; $p_{corr} = 1.84 \times 10^{-12}$, d = 0.37). Differences in other frequency bands, or other across-condition comparisons (e.g. communication vs. no-voc), were either not reflected in the differential connectivity graphs, or did not have large effect sizes.

Altogether, these results indicate that pre-vocal and post-vocal directional information flow in the FAF-AC network occurs mostly in low and high-frequency bands. The patterns and strength of preferred directionality depend on whether a vocalization is produced and on the type of vocal output. When animals produced echolocation calls, post-vocal bottom-up influences dominated in δ frequencies, while top-down influences weakened in post-vocal periods compared to spontaneous activity. These results could reflect both a waning of top-down control from the FAF, and an increase in bottom-up transfer in δ and β_1 frequencies. These two possible explanations are not mutually exclusive.

Supplementary Note 2

<u>Passive listening of high- or low-frequency natural sounds does not explain</u> <u>information flow patterns of active vocalization</u>

Recordings for this study were made mostly from an area of the *C. perspicillata*'s AC whose neurons are specialized for processing echolocation sounds. Therefore, it is sensible to assume that the reversal of preferred directionality of information flow from pre- to post-vocal periods during vocalization could be attributed to strong acoustic feedback originating from an echolocation call, interacting with the tuning of the cortical areas recorded. In the following, we present evidence demonstrating that mere auditory feedback is not sufficient to explain our main results.

In a first step, we quantified frequency tuning in the AC (and FAF, see Methods) and observed the tuning of recorded LFPs did not favour the frequency range of echolocation calls (i.e. > 60 kHz), as it peaked at 20-40 kHz for most recording sites (**Fig. S7** shows LFP frequency tuning curves measured with 75 dB SPL, 10 ms tones, across penetrations). Thus, LFP responses in the AC, at least based on frequency tuning alone, would not elicit on average a stronger response and therefore a stronger bottom-up transfer towards the FAF. Note that many recordings were responsive at high frequencies, often exhibiting double-peaked tuning curves (e.g. red trace in **Fig. S7a**). This type of tuning is common in the auditory systems of *C. perspicillata* and other bats ^{1, 2, 3}, potentially facilitating neurons to respond to both echolocation and communication sounds. In the FAF, we did not observe clear frequency tuning based on LFPs.

In a second step, we quantified information transfer dynamics in the FAF-AC network in response to acoustic stimulation. Sounds were a high-frequency frequency-modulated sweep ("HF-FM"; intended to mimic an echolocation pulse) and a natural distress syllable ("distress"; as a representative of a communication utterance). These sounds are illustrated in **Fig. S7b**, together with simultaneous cortical responses to each from FAF and AC in **Fig. S7c**. When considering LFPs taken in the period from 0-500 ms after stimulus onset, we observed that information flowed predominantly in the FAF \rightarrow AC direction for bands δ , θ , and γ_2 regardless of the sound considered (**Fig S7d**). The patterns

of information flow were very similar across the types of acoustic stimuli used, although with some significant differences in the α and γ_2 bands (quantified in **Fig. S8**). Overall, preferential information flow dynamics in these and other frequencies were reminiscent of those observed for no-voc periods, as confirmed by scarce differences between information flow patterns associated to passive stimulation and spontaneous activity (**Fig. S8**).

Preferential information flow patterns associated to post-vocal echolocation periods were significantly different than those reported for the passive listening of HF-FM sounds (Fig. S9a). Specifically, for δ frequencies, information flow in the FAF \rightarrow AC direction was stronger in the passive listening condition, but stronger in the AC \rightarrow FAF direction for post-vocal echolocation periods. Differences were significant with large effect sizes also when considering the net information outflow across structures in this band (p <= 1.12×10^{-127} , $|d| \ge 1.5$; Fig S9a, bottom). In the θ band, predominant information flow was strongest in the FAF \rightarrow AC direction when animals listened to the HF-FM stimulus (p = 3.45×10^{-58} , d = 1.17). However, information transfer in the FAF \rightarrow AC direction was strongest for post-vocal echolocation periods in the γ_2 band (comparison of DI_{net} values: p $= 6.98 \times 10^{-100}$, d = -1.72). Passive listening of distress syllables yielded stronger information flow in the FAF \rightarrow AC direction when compared to post-vocal communication periods in the δ and θ frequency bands (comparison of DI_{net} values: p <= 5.29x10⁻³⁷, d >= 0.88; **Fig. S9b**, bottom). For β_1 frequencies, information flowed more strongly in the AC \rightarrow FAF direction considering post-vocal communication periods, but the differences in DI_{net} values did not occur with large effect sizes (p = 1.83×10^{-18} , d = 0.52).

Altogether, these results demonstrate that acoustic input (as may occur from feedback after call utterance) does not account for the reversal of information transfer in δ frequencies when animals produce echolocation calls. Our results also provide evidence for a highly dynamic network, in which information reverses in different manners during vocalization and passive listening.

Supplementary Note 3

<u>Information transfer patterns related to HF-communication calling differ to those</u> <u>associated with echolocation</u>

The passive listening of high frequency acoustic stimuli does not explain the information transfer patterns associated to echolocation production (**Fig. S7-9**), but it is possible that the utterance of high frequency sounds in general (irrespective of whether echolocation or communication) suffices to account for such dynamics. To explore this possibility, we quantified information transfer in the FAF-AC network when animals produced HF-communication sounds.

Post-vocal information transfer dynamics associated to the production of HFcommunication vocalizations are shown in Fig. S10a. Mostly for frequency bands θ and α (and more weakly, β_1), it is apparent that information flows predominantly in the AC \rightarrow FAF direction. When compared to pre-vocal periods (Fig. S10b, top), we observed pre-vocal to post-vocal switch in directionality across FAF and AC for θ -band LFPs, but not for δ -band ones (i.e. the band where the reversal of information flow occurred when producing echolocation pulses). The described effect in θ was complemented by differences in DI_{net} values ($p = 1.25 \times 10^{-45}$, d = -0.99; Fig. S10b, bottom). Indeed, a comparison between patterns of predominant information transfer associated to HFcommunication and echolocation production (Fig. S10c), revealed that information flowed more strongly for echolocation production than for HF-communication utterance in δ frequencies (also complemented by DI_{net} values: p = 1.66x10⁻⁷⁰, d = -1.12). Moreover, small differences were found in the case of AC \rightarrow FAF dPTE values in the θ band, indicating that bottom-up information transfer for post-vocal echolocation and HFcommunication periods were not considerably different from one another. Additional differences between the conditions of echolocation and HF-communication production were observed in other frequency bands (see α , β_1 , and γ_2 in **Fig. S10c**), although for these frequencies there were no significant differences between pre- and post-vocal periods when animals vocalized HF-communication sounds (Fig. S10b). Taken together, these results indicate that the information transfer dynamics in the FAF-AC circuit

25

observed when animals vocalize echolocation pulses are not accounted for solely by the frequency content of the calls produced by the bats.

Supplementary references

- 1. Lopez-Jury L, Garcia-Rosales F, Gonzalez-Palomares E, Kossl M, Hechavarria JC. Acoustic Context Modulates Natural Sound Discrimination in Auditory Cortex through Frequency-Specific Adaptation. *J Neurosci* **41**, 10261-10277 (2021).
- 2. Kanwal JS, Fitzpatrick DC, Suga N. Facilitatory and inhibitory frequency tuning of combination-sensitive neurons in the primary auditory cortex of mustached bats. *J Neurophysiol* **82**, 2327-2345 (1999).
- 3. Radtke-Schuller S, Schuller G. Auditory cortex of the rufous horseshoe bat: 1. Physiological response properties to acoustic stimuli and vocalizations and the topographical distribution of neurons. *Eur J Neurosci* **7**, 570-591 (1995).