Supplementary Materials for

High-frequency hearing in a hummingbird

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Fig. S1
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Fig. S1. Counts of ZENK-ir cells in the brain of hummingbirds. Two blinded scorers counted ZENK-ir cells in (A) within the 3x3 inner grid on images of sections of interest. Numbered inner-grid compartments exemplify the counting procedure of a sample section. This procedure guaranteed that any cell from an adjacent area was not counted as part of a region of interest. (B) Graph showing cell counts for both scorers and the regression line in red ($R^2 = 0.9604$, $p < 0.001$).
| Table S1. Ethogram | Descriptions of behaviors that were assessed during the field playback experiments to evaluate behavioral responses to the playback of conspecific HF song compared to the playback of ambient noise. Only those responses that were produced at the onset of the sound stimulus were counted. Any behavior that was produced during intervals of silence or in response to the presence of other animals were not included in the behavioral analysis. |
|-------------------|-------------------------------------------------------------------------------------------------|
| **Aggressive display** | Males display towards the source of sound, by hovering, opening their wings and tail feathers widely. Sometimes, they will also move the tail feathers from side to side. Displays can sometimes be accompanied by chasing calls. |
| **Fly over** | Hummingbird rapidly flies over the speaker at a short distance (1 meter or less) as a sign of aggression. May be accompanied by chasing calls. |
| **Vocalizing** | Individual vocalizes in response to playback. Annotate type of vocalization: |
| | - Chasing calls (CH): long bouts of vocalizations with rapid changes in type of syllables and rich in frequency modulations. Generally produced while flying and chasing another individual. Can be produced during an aggressive display. |
| | - Contact calls (CC): single-note vocalizations at lower pitch than HFV. Generally, several renditions separated by short intervals. |
| | - Alarm calls (AC): short broadband rasping calls produced to alert of a possible threat. |
| | - High-frequency vocalizations (HFV): produced only by males. |
| **Approach** | Male or female approaches the speaker or the surrounding area but show no signs of aggression. Usually remain in the surrounding area (within a radius of 5 meters from the speaker). |
| **Avoidance** | Fly away (FA): Individual flies away from the speaker or the surrounding area after the onset of playback. |
| **Attentiveness** | The bird stays on its perch but shifts attention: |
| | - Head tilt (HT): moving the head from side to side or towards the speaker to improve sound localization in response to playback. This is accompanied by neck extensions to facilitate inspection. |
| | - Body posture (BP): adjusting body posture to improve visibility of the area where the sound is produced. |