over the bird's beak. In a sound attenuation chamber (model chamber AC2; IAC, New York, NY, USA), the bird was immobilized in a custom-made stereotaxic holder that allowed the head to be tilted at 45°. Lidocaine cream was applied to the skin overlying the skull before incision and a small hole was made in the inner skull layer. After making an incision in the dura, a single custom-made nichrome electrode (0.4–0.5 MΩ impedance) attached to the microconnector was positioned 0.3–0.5 mm lateral and 0.7–0.9 mm rostral to the bifurcation of the central sinus, into either the left or the right hemisphere, using a micromanipulator. The reference electrode was inserted between the outer and the inner skull layers. After lowering the recording electrode into the brain, when the signal-to-noise ratio was relatively high we briefly presented call stimuli to ensure that the recorded neurons showed an auditory response. The final placement depth of the electrode ranged from 1200 to 1900 μm below the brain surface. Many females received two fixed electrodes. The microconnectors were fixed with dental acrylic cement.

After a week-long recovery period, the electrodes were connected to the FM transmitter device that was placed on the bird's back, and the bird transferred to a 60 × 30 × 40 cm plastic mesh cage (the use of metal parts was avoided to prevent interference with the reception of radio waves). The bird was then placed in a sound attenuation chamber containing a speaker through which the call stimuli were presented. The chamber was illuminated and a microphone was used to remotely monitor calling behaviour. Although the telemetric device was relatively heavy in comparison with the bird's weight, it did not prevent the bird from freely moving because it was placed on the bird's back (supplementary movie at http://www.cnps.u-psud.fr/up/menardy_telemetric_system_zf4.mov). Awake freely moving birds exhibited all normal behaviours, including perching and calling. In particular, as previously described (Vignal *et al.*, 2004, 2008), the presentation of the mate's call generally evoked a stereotypic behaviour in which they approached the source of the sound and they responded by emitting a call. Females that were implanted with two electrodes underwent one electrophysiological recording session per electrode, each conducted on a single day, with at least 1 week separating the two recording sessions. A typical experiment lasted a maximum of 2 h and took place between either 09:00 and 12:00 or 14:00 and 17:00. Calls were presented when the female was silent.

Each call of the set of auditory stimuli was presented successively 50 times to each site, in blocks of 10 calls with an interstimulus interval of 1 s within a block and of 5 s between two blocks. A silence of 30s separated the playback of two different call stimuli. All stimuli were normalized to achieve a maximum amplitude of 65–70 dB (Avisoft software) at the level of the bird. They were presented in random order and we ensured that the same call stimulus did not appear twice at the same position in three successive birds.

Acute experiments

Females (12 mated and five control) were anesthetized and prepared for neural recordings as described above. After stereotaxic location of the left or right NCM, a relatively high impedance tungsten microelectrode (10–12MΩ; FHC, Inc. Bowdoin, ME, USA) was lowered into the brain with a microdrive to a depth of ~2 mm below the brain surface. The coordinates used were in most cases 0.8 mm anterior (range, 0.5–0.9) and 0.4 mm lateral (range, 0.2–0.5 mm) to the bifurcation of the sagittal sinus and 1.4 mm deep (range, 1.0–1.9 mm). Recording sites were at least 100 μm apart to guarantee that the neural activity recorded from two successive sites originated from different units. Once the last neural recording was achieved, an electrolytic lesion was made by passing current (10 μA for 10 s) through the recording electrode.

The neural signal was amplified (gain, 5000; bandpass, 0.3–10 kHz), monitored on-line via an oscilloscope and sent in parallel to an audio monitor. When the neural trace was dominated by one individual neuron, sound stimuli were delivered. The neural signal was digitized by a data acquisition system (CED Power 1401 interface; Cambridge Electronic Design) and stored on a personal computer. In parallel, as an additional channel, call stimuli were concomitantly recorded using a microphone and digitized by the CED system. This enabled us to precisely determine the onset of the sound stimulus with respect to the auditory response.

As described above, a set of three call stimuli was played while recording from each single unit. Once the set was completed we searched for a new recording site. We played call stimuli in a random order while recording from different sites. When we recorded the neural activity of a single unit in a control female, we presented the same set of three call stimuli used to examine auditory responses in a selected anesthetized mated female. The three call stimuli had never been heard previously by the control female. The stimulus sets of two different mated females were successively presented to each control female. Single units in both mated and control females were tested with the calls of two different familiar males (Fam1, 64% and Fam2, 36%) and two different unfamiliar males (Unfam1, 64% and Unfam2, 36%).

Data processing and analysis

Analysis of responses to call stimulus presentation

In anesthetized females, neural traces dominated by the activity of a single neuron were subject to template-based spike detection and sorting (Spike2 software, version 5; CED, Cambridge, UK). In chronically implanted birds, neural traces of multiunit activity (AMU) were subjected to threshold spike detection. All spike event times were binned at 10-ms intervals for analysis. For all repetitions of a given call stimulus at a single recording site, a peristimulus histogram (10 ms per bin) was built. Spiking activity was first analysed by calculating spontaneous activity (defined as the mean frequency of spikes generated in the last 500 ms preceding call presentation). We also measured spike frequency during and for 50 ms following call stimulus presentation.

To evaluate the degree of excitation driven by a given call stimulus, we quantified the length of the call stimulus which resulted in a significant increase in the spike rate over baseline levels. To this end, we calculated the number of 10-ms bins during which spiking activity exceeded the mean spontaneous firing rate by at least 4 SD and divided this by the total number of bins covered by the call stimulus.

Spike rates can vary broadly across a population of single units. To estimate the strength of the response evoked by a sound stimulus, it was important to control for differences in the level of spontaneous activity between units and to limit the influence of one or a few very active units. To this end, we normalized each unit's stimulus response as reported in Stripling *et al.*, 1997, 2001. The response strength (RS) index was calculated by subtracting the spontaneous activity rate ($S_{baseline}$) from the activity rate generated during the stimulus presentation (S_{during}), and then dividing this value by their sum:

$$RS = (S_{during} - S_{baseline})/(S_{during} + S_{baseline})$$

RS values fall between +1 and −1, where values > 0 indicate an excitatory response and values < 0 indicate an inhibitory response.

To quantify each neuron's call selectivity, the psychophysical metric d', which estimates the discriminability between two stimuli, was used (Green & Swets, 1966; Theunissen & Doupe, 1998). The d' value for the response to a given stimulus A relative to a different stimulus B is calculated as follows: