

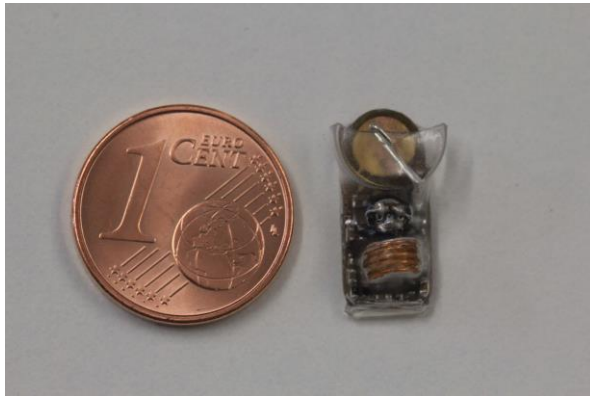
## Miniature wireless microphones reveal vocal relationships in a group of small laboratory animals

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We have developed lightweight wireless microphones that can be carried by small animals. The microphones weigh 0.6 g including battery, run for two weeks and have a range of over 5 m, sufficient to be used in an indoor aviary (Figure 1). We operate the device without an external antenna in order to minimize interference with behavior.

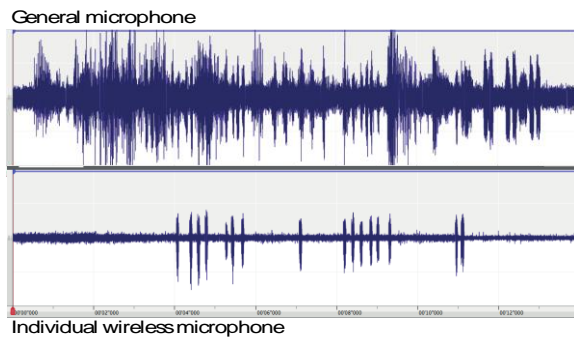


**Figure 1.** Wireless microphone used to record individual vocalizations.

The spread in transmitting frequency is used to enable the simultaneous recording of up to 12 animals. For each animal one needs a separate receiver. Transmitting frequency is about 300 MHz. All experiments were done indoors. Crossed yagi antennae (Winkler Spezialantennen, Kreuzdipol 300, directional antenna tuned at 300 MHz) were connected to AOR 8600 receivers (AOR, Ltd., Japan) that were modified to an audio bandwidth of 12 KHz. Signals are received as FM, at an intermediate frequency bandwidth of 100 KHz. The antennae were less sensitive to changes in orientation of the transmitters relative to the antennae, for instance when the animal moved. To compensate for frequency drift due to a change in battery voltage or temperature fluctuations, the frequencies of the transmitters were tracked by the receivers using a custom-built software based tracking system.

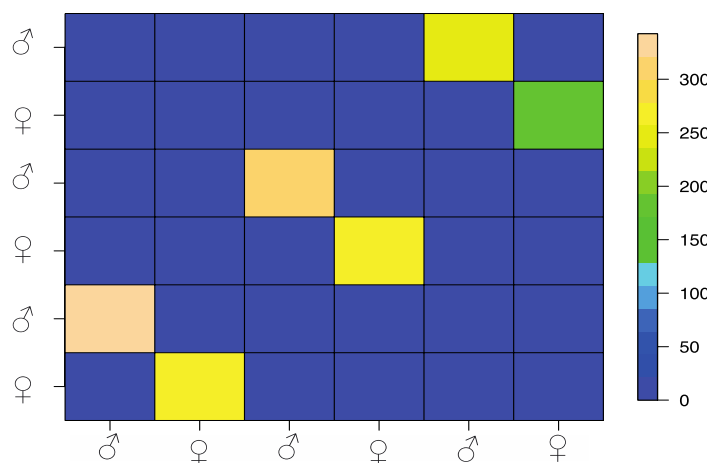
The device is used to obtain recordings from each individual in a group. By mounting the device such that the (actual) microphone component faces towards the animals' bodies, a recording is obtained that selectively records the vocalizations of each bird (Figure 2).

Vocal interactions were analyzed as follows. The output of the receivers was stored as wave files. The vocalizations were identified based on acoustical parameters using custom software written in Delphi Pascal and C++ ((Jansen et al., 2005). Different types of vocalization were sorted and assigned cluster numbers that were time-stamped. Based on this information the temporal relationships between the sounds could be established using peristimulus time histograms that essentially measure the probability of one call occurring before or after the call of another animal (Figure 3;[1]).



**Figure 2.** Selectivity of the wireless microphone. Upper trace: recording of 6 animals in an aviary using a standard microphone. The wireless microphone (lower trace) records the vocalizations of one animal only. This allows the correlation of individual vocalization patterns to

As an example of the uses to which the wireless microphones can be put we report on the vocal communication of a highly social songbird, the zebra finch. In the zebra finch, song is essentially a one-way vocal communication pathway that is shaped by sexual selection [2]. Zebra finches also produce large numbers of soft, unlearned calls, among which “stack” calls are uttered frequently [3]. The social function of these calls is, however, poorly understood. To chart the vocal interactions between the individuals in a group, it is critical to be able to determine unequivocally the calls produced by every animal. We achieved this by mounting miniature wireless microphones on all individuals (Figure 2). This revealed that group living males and females communicate using bilateral stack calling. This vocal pattern occurred predominantly between bonded partners.



**Figure 3.** Mapping out social interactions based on peristimulus time histograms. Here, the strength of the interaction, disregarding direction, is shown for three bonded pairs in an aviary.

Using quantified measures of direction and strength of the vocal interactions a model of the social structure can be developed. We quantified the intensity of the vocal interactions in groups of 2 to 4 pairs kept in an aviary (Figure 3). Response strength calculation is based on a PSTH consisting of 2 x 80 bins of 50 msec. General response strength:

$$RS = \frac{(N_{before} + N_{after}) - (N_{basebefore} + N_{baseafter})}{(N_{before} + N_{after}) + (N_{basebefore} + N_{baseafter})}$$

where  $N_{before}$  and  $N_{after}$  are the counts in the 9 bins before and after the start of the source event (=call) and  $N_{basebefore}$  and  $N_{baseafter}$  are the first and last 9 bins in the PSTH.

The strength and direction of the interaction were calculated on the basis of the cross-correlations between the calls of the individual birds. In this example we saw interactions only among bonded partners, which is the predominant pattern.

To our knowledge, we are the first to conduct a detailed study of the intricate vocal interactions in social groups of small songbirds.

## Ethics statement

Carrying the transmitter as a backpack and keeping the birds in aviaries was approved by the government of Upper Bavaria, "Sachgebiet 54 – Verbraucherschutz, Veterinärwesen, 80538 München" with the record number: Az. 55.2-1-54-231-25-09. All further animal husbandry or handling was conducted according to the directives 2010/63/EU of the European parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes.

## References

1. Abeles M (1982) Quantification, smoothing, and confidence limits for single-units' histograms. *J Neurosci Methods* 5: 317-325.
2. Gahr M (2007) Sexual Differentiation of the Vocal Control System of Birds. *Genetics of Sexual Differentiation and Sexually Dimorphic Behaviors* 59: 67-105.
3. Zann RA (1996) *The Zebra Finch*; M. PC, editor. Oxford: Oxford University Press.