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RESEARCH ARTICLE

Efficiency and Significance of Multiple Vocal Signals in Sibling Competition

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Abstract Animals can compete for resources by displaying various acoustic signals that may differentially affect the outcome of competition. We propose the hypothesis that the most efficient signal to deter opponents should be the one that most honestly reveals motivation to compete. We tested this hypothesis in the barn owl (*Tyto alba*) in which nestlings produce more calls of longer duration than siblings to compete for priority access to the indivisible prey item their parents will deliver next. Because nestlings increase call rate to a larger extent than call duration when they become hungrier, call rate would signal more accurately hunger level. This leads us to propose three predictions. First, a high number of calls should be more efficient in deterring siblings to compete than long calls. Second, the rate at which an individual calls should be more sensitive to variation in the intensity of the sibling vocal competition than the duration of its calls. Third, call rate should influence competitors' vocalization for a longer period of time than call duration. To test these three predictions we performed playback experiments by broadcasting to singleton nestlings calls of varying durations and at different rates. According to the first prediction, singleton nestlings became less vocal to a larger extent when we broadcasted more calls compared to longer calls. In line with the second prediction, nestlings reduced vocalization rate to a larger extent than call duration when we broadcasted more or longer calls. Finally, call rate had a longer influence on opponent's vocal behavior than call duration. Young animals thus actively and differentially use multiple signaling components to compete with their siblings over parental resources.

Keywords Begging · Call rate · Call duration · Multiple signaling · Sibling negotiation · Sibling competition · Communication

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Introduction

Multiple-components signaling across and within sensory modalities is frequent in animal courtship and territorial displays (Partan and Marler 2005; Bro-Jørgensen 2010). Because evolving several signals may seem a priori wasteful if one type of signal efficiently repels rivals and predators or attract mates, a number of hypotheses have been proposed to explain the evolution of multiple signaling (Bro-Jørgensen 2010). Multiple signals may serve to enhance message transmission in environments with visual or acoustic interferences (so-called "back-up" or "redundant" signals, Møller and Pomiankowski 1993; Johnstone 1996) or to facilitate receiver detection and discrimination (so-called "receiver psychology", Rowe 1999). Furthermore, to convey a relevant message, a combination of several signal types may be required (so-called "emergent signal", Partan and Marler 1999). Alternatively, multiple signals may convey "multiple messages" by signaling different aspects of the signaler's quality (Candolin 2003).

Several empirical papers give support to these hypotheses in various taxa, but most studies concentrated on multimodal signals, i.e. from different sensory channels such as auditory and visual (e.g. Partan et al. 2009; Smith and Evans 2009). Knowledge on the concomitant function of multiple acoustic components in vocal contests remains more elusive (but see Miller and Hauser 2004; Richardson

and Lengagne 2010; Rivera-Gutierrez et al. 2010). Also the exact role played by each vocal component and the quantification of their efficiency in the resolution of sib–sib and parent-offspring conflict remains unclear. In avian and mammalian altricial young competitive signals comprise different components, such as postures and calls. Across and within species, different behaviors and different call features (e.g. call rate, frequency or duration) encode for various aspects of individual need (Leonard and Horn 2006; Duckworth et al. 2009; Gladbach et al. 2009; Jacob et al. 2011). In altricial young, although multiple begging components can be correlated to each other (e.g. Leonard et al. 2003), they differentially influence how food is shared among the progeny (Royle et al. 2002; Tanner et al. 2008). Each single acoustic feature may not be similarly associated with an individual's need and resource holding potential (body condition (Gladbach et al. 2009), hunger level (Roulin et al. 2000; Marques et al. 2009; Reers and Jacot 2011), size (Sacchi et al. 2002; Roulin et al. 2009) and health (Saino et al. 2001). We propose the hypothesis that the signaling component most closely associated with the need for food is more efficient to win a sibling contest. Hence, it would impact sibling and parental behavior to a larger extent. Furthermore, the signaling component most closely associated with need should also be more sensitive to the prevailing social environment, that is to say the level of sibling competition.

In the present study performed in the barn owl (*Tyto alba*), we investigated and quantified the mutual roles of call rate and call duration, two widely studied acoustic features, in sib–sib vocal competition. In this species, young not only beg for food from their parents but also vocally communicate among each other in the prolonged absence of parents to resolve the contest over access to the next indivisible food item that parents will deliver (Roulin 2002). Nestling call rate and call duration in parent absence are hence essential to determine which individual will be fed at parent return (Roulin et al. 2009; Dreiss et al. 2010b). However, the relative efficiency of these two acoustic components in sib–sib vocal competition is still unknown. In this system, each nestling vocally informs its siblings about the willingness to compete over the impending indivisible food item. The hungriest individual produces more and longer calls, which deters siblings from begging conspicuously at the arrival of their parents. Owlets are thus said to vocally "negotiate" priority access to food resource before parents actually return with food, a process that reduces the level of sibling competition (Roulin 2002; Johnstone and Roulin 2003). In the present study, we experimentally examined how siblings modulate the acoustic components call rate and call duration according to their motivation to compete (i.e. hunger level) and then we investigated the response of singleton individuals to playback calls of varying durations broadcasted at different rates.

We propose the hypothesis that to be successful in sibling competition nestlings invest more effort in the vocal component that is more finely modulated in relation to variation in hunger level. Because this component best signals hunger level and hence motivation to outcompete siblings, it should have a stronger influence on the way sibling contests are resolved. In order to test this, we performed two experiments. First, we recorded vocal interactions at night in pairs of live siblings in which we manipulated hunger level by alternatively food-depriving them and offering them food ad libitum. This enabled us to investigate whether nestlings naturally increase call number to a larger extent than call duration (or the opposite) when in greater need. Second, we experimentally tested two predictions of the hypothesis that nestlings primarily compete by using the acoustic component that best signal their hunger level. To do so, we recorded the rate and duration of vocalizations of singleton nestlings responding to pre-recorded calls of varying durations broadcasted at various rates. (1) We expected that nestlings would primarily modulate the vocal component that most strongly reflects hunger level in relation to variation in the rate and duration of the broadcasted calls. Thus, when listening to more calls and calls of longer duration singleton nestlings should reduce the vocal component that is more closely associated with hunger level to a larger degree than other vocal component. (2) Variation in this playback component, rather than variation in the other component, should have a greater influence on vocal behavior of nestlings. For instance, if call rate is more sensitive to hunger level than call duration, we would expect that when we broadcasted calls at a greater frequency, nestlings should decrease the rate at which they call and the duration of their calls to a larger extent than when we broadcasted longer calls. (3) We investigated the vocal response of singleton nestlings during the playback experiments but also during 10 min of silence following each playback treatment. This procedure was useful to examine the instantaneous effects of our playbacks but also their carry-over effects. We expect that the component which is more closely associated with hunger level would influence nestling behavior longer.

Methods

Study Site and Animals

The study was performed in western Switzerland (46°49′N/06°56′E) in a population of wild barn owls breeding in nest-boxes. Parents hunt small mammals at night to feed their one to nine offspring (Roulin 2004a). Once offspring

are thermo-independent at two to 3 weeks of age, the mother begins to hunt in order to provision for the brood. We carried out the experiments after this age, when parents were naturally sleeping outside their nest-box in another barn, and before nestlings take their first flight at around 55 days.

Experiment 1: Differential Effect of Hunger on Call Rate and Call Duration

Experimental Design

To investigate whether call rate or call duration is more sensitive to variation in hunger level, we manipulated food supply in 98 nestlings, 51 males, 45 females and two individuals of unknown sex, issued from 35 broods in 2008. When aged 25–45 days (mean ± SD: 35 ± 5 days) we brought them back to the laboratory in the afternoon to be kept in a similar wooden nest-box (100 × 60 × 50 cm) as the one where they were reared in natural conditions. Nest-boxes were divided in two parts with a thin wooden wall pierced with five holes at the top so that two siblings, placed in each part of the box, could vocally communicate without interacting physically. After a first night of acclimation, we analyzed the vocal exchange of each pair of siblings from 21:00 h until 23:40 h on the second and third nights.

On one of the two nights, chosen randomly, we food-deprived the two individuals (no food given during the preceding 28 h) or food-satiated them (from midnight to 16:00 h on the recording day we offered 130 g of laboratory mice, which exceeds their daily food requirement of about 67 g Durant and Handrich 1998). Over 24 h food-deprived individuals lost on average 42 ± 1 g and when fed ad libitum they gained 16 ± 2 g. We kept nestlings in these boxes for 2 days and three nights before taking them back to their original nest in the field. To avoid superfluous disturbance, we manipulated nestlings only once per day at 16:00 h and opened nest-boxes again at midnight to add food. Nestlings were not physiologically stressed, as shown by the absence of a rise in baseline corticosterone levels compared to the situation prevailing under natural, undisturbed conditions (Dreiss et al. 2010a). Keeping owlets at the university did not negatively affect their body condition, since mean body mass and survival at fledgling did not differ between experimental nestlings and nestlings remaining in their nest during all rearing period (Dreiss et al. 2013).

We recorded calls using two microphones (MC930, Beyerdynamic GmbH & Co KG, Heilbronn, Germany) oriented in opposite directions, each facing one nestling. We could thus easily assign calls to each individual based on intensity differences between paired soundtracks. We measured call duration using a program (Dreiss et al. 2013) in Matlab v.7.7 (MathWorks, Natick, MA, USA).

Statistical Analyses

We analyzed the relative effect of food supply on call rate and call duration in 49 pairs of nestlings ($n = 98$ individuals). For each individual and food treatment we calculated the number of calls and the mean call duration (in seconds) between 21:00 and 23:40 h. We ran a generalized linear mixed model with Poisson error distribution to analyze the effect of food treatment on number of calls and a linear mixed model to analyze its effect on mean call duration, using the GLIMMIX and MIXED procedure in SAS V9.2 (SAS Institute Inc., Cary, NC, USA), respectively. Both models included nestling identity nested in the experimental nest-box and in the brood where they were raised in the field as random intercept. As independent factors, we included food treatment (starved vs. satiated), the order of the treatment across the two nights of experimentation as well as their interaction.

To investigate whether nestlings differentially increase the rate and duration of their calls with hunger level, we computed the percentage of increase in number of calls and in mean call duration per individual between a starved and satiated state (i.e. difference in call rate between the two food states divided by number of calls measured when food-satiated; similar procedure for call duration). We performed a within-individual pairwise comparison using a Wilcoxon signed rank test.

Experiment 2: Relative Role of Call Rate and Call Duration on Sibling Negotiation

Experimental Design

In 2009, we performed playback experiments to analyze the relative effect of variation in the rate and duration of broadcasted calls on the vocal behavior of singleton nestlings. We brought 19 male and 35 female nestlings issued from 15 broods to the laboratory at 16:00. They were 35 ± 4 days of age (range 25–44). We hosted them in a similar wooden nest-box as in 2008, except that an individual was placed in one side of the box, while we put a loudspeaker (near 05 experience, ESI Audiotechnik GmbH, Leonberg, Germany) in the other side behind the wooden separation. At 08:00 h on the first morning following their arrival, we provided food ad libitum and the second morning at 08:00 h we removed the remaining mice, so that nestlings were food-deprived until 21:00 h when we started the playback experiment.

We broadcasted nine playback sequences in a row, each sequence lasting 15 min, with periods of 10 min of silence

between two sequences, as described in Fig. 1. The nine sequences corresponded to the nine combinations of calls of three different durations (0.6, 0.8 and 1.0 s) broadcasted at three different rates (2, 6 and 10 calls/min). These values correspond to the mean values and to the lowest and highest 10 % of the distributions observed in the two-chick broods recorded in 2008 (Fig. 2). To avoid pseudo-replication, we built a unique soundtrack of 9 sequences for each nestling. We allocated the nine combinations in a random order, except that we limited the possibility that the nestling heard the same call rate and call duration in two consecutive sequences.

We built the nine sequences using natural calls from two randomly chosen starved nestlings (donors I1 and I2 in Fig. 1) recorded in 2008, out of 16 possible individuals (6 males and 10 females; aged 28–45 days). We built the first three and last three sequences with the calls of donor I1, and the fourth, fifth and sixth sequences with the calls of

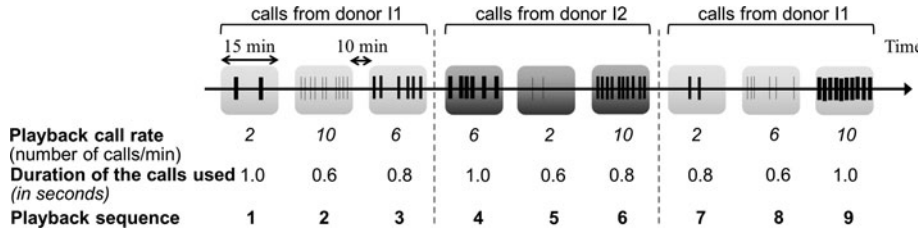

Fig. 1 Design of the playback experiment. A unique combination of nine playback sequences lasting 15 min each and separated by 10 min of silence was broadcasted to each nestling. These sequences corresponded to the nine combinations of calls of three different durations (0.6, 0.8 and 1.0 s) broadcasted at three different rates (2, 6 and 10 calls/min). We used the calls of two pre-recorded individuals: donors I1 and I2, with 10 possible distinct calls from each donor in each of the three durations. We used the calls of I1 to build the three first and three last sequences, and of I2 to build the sequences 4, 5, and 6. For a given duration, the 10 calls of I1 used in the first three sequences were different from the 10 calls of the same duration used in the last three sequences. In this example, the 10 calls lasting 1.0 s in the first sequence were different from the 10 calls of 1.0 s used to build the ninth sequence

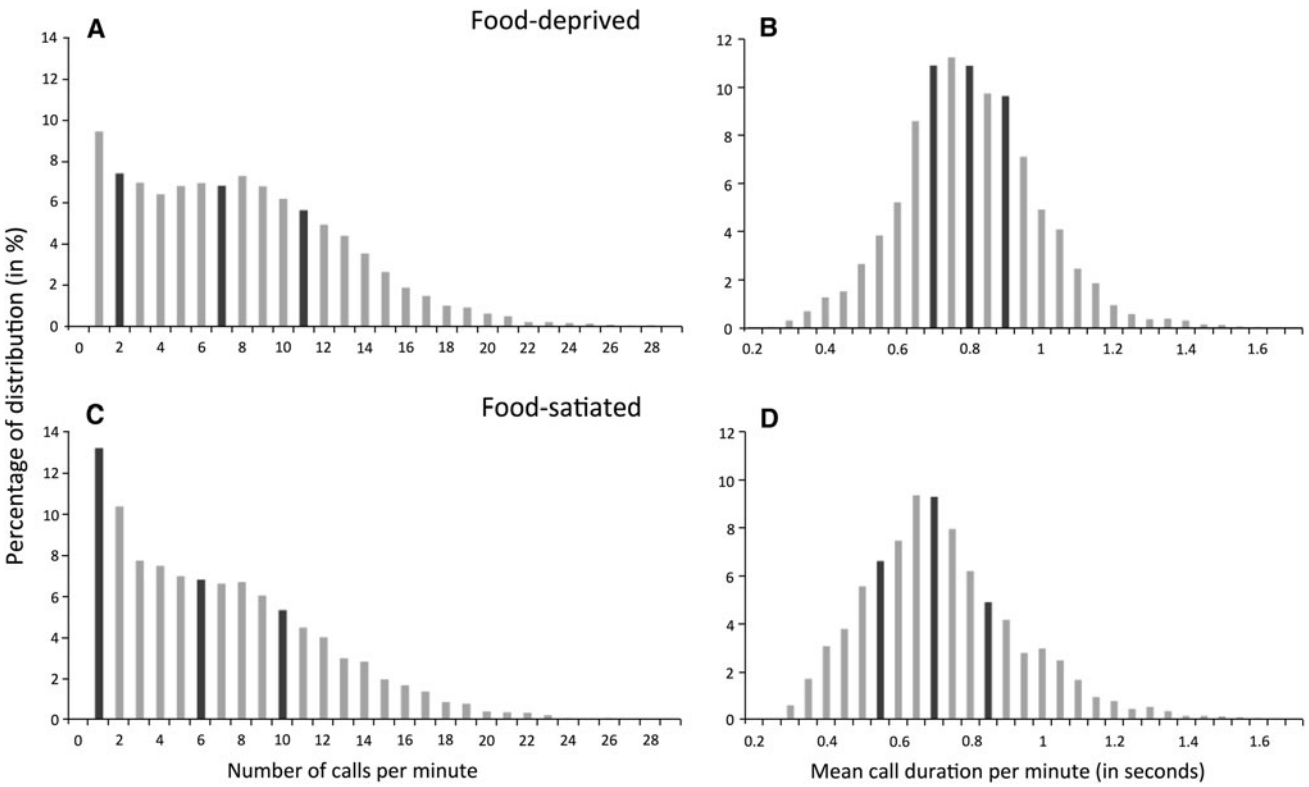

Fig. 2 Distributions of call rate (**a** and **c**) and mean call duration per minute (**b** and **d**) in barn owl siblings interacting in pairs, which were alternatively food-deprived (**a** and **b**) and satiated (**c** and **d**). Recordings were made between 21:00 and 23:40 h and the 160-min long soundtrack was divided in 1-min intervals. We then considered only those minutes during which nestlings produced at least one call. Sample size is 98 individuals from 49 pairs of siblings in each food treatment. In each *diagram darker bars* correspond, from *left* to *right*, to quartiles, i.e. values corresponding to 25, 50 and 75 % of the total distributions

donor I2. We used 10 possible distinct calls of a given duration (0.6, 0.8 or 1.0 s) of a donor to build each sequence. Each of the nine sequences was built with 10 unique calls. We randomly inserted the calls within each sequence, with the constraint that two successive calls were separated by an interval of at least one-second. In the two-chick broods recorded in 2008, a pause of one second or less between two successive calls produced by the same individual was observed in only 0.03 % of the cases (mean ± SD = 15.3 ± 106.4 s, range = 0.7–7,259.2 s). We standardized intensity of playback calls using free Audacity software v.1.3 Beta (http://audacity.sourceforge.net), a procedure that does not affect other acoustic parameters. We used Cubase software to simultaneously broadcast the playback and record the vocal response of nestlings in 2009. We measured call duration as explained above.

Statistical Analyses

For each of the 54 nestlings, we computed their number of calls and the mean call duration for each of the nine 15-min long playback soundtracks. Because in 90 recorded sequences nestlings did not call, we analyzed more call rates ($n = 486$ sequences) than mean call durations ($n = 396$ sequences). To investigate the carry-over effect of each playback sequence, we also computed the number of nestling calls and their mean call duration during the 10-min long periods of silence separating playback sequences.

To quantify the immediate and carry-over effect of the playback call rate and call duration on nestling number of calls, we ran two separate generalized mixed models with Poisson distribution and a Log link. To analyze the immediate and carry-over effect of the playback on nestling call duration, we ran two linear mixed models. In each four model, we included nestling identity nested in brood where they were reared in the field as random intercept. We also fitted the identity of the playback individuals used to generate sequences as an extra random variable. As independent variables, we included two factors (i.e. 3 levels of playback call rate and 3 levels of playback call duration) plus their interaction, and two covariates, namely the order at which each of the nine playback sequences were broadcasted, and nestling age. The age effect on vocal behavior are not discussed here as they have been described elsewhere (Roulin 2004b). We initially included as covariates nestling sex and the age and sex of the playback individuals, but since these covariates proved not significant, we removed them from the saturated models for the sake of clarity. To investigate the carry-over effect of the playback treatments on number of nestling calls during the following silence, we also included as covariate the call rate of this nestling during the previous playback. Similarly, we included nestling mean call duration during the playback as covariate in the model of the nestling's mean call duration during the following silence. For all models, we performed backward model selection; final models only contained significant effects ($P < 0.05$), and main effects involved in significant interactions. Residuals were systematically checked for normality.

We compared the magnitude to which nestlings modulated number of calls and call duration in response to variations in playback call rate and call duration. We only considered the extreme playback values, i.e. when we broadcasted 2 and 10 calls/min and when we broadcasted calls lasting 0.6 and 1.0, leaving out the playbacks of 6 calls/min and 0.8 s. We adopted this procedure because the change in nestling vocal response was linear across the three-levels for both call rate and mean call duration. We thus computed the percentage of change in nestling response, i.e. call rate or mean call duration, as the average call rate (or average call duration) for the playback at 2 calls/min minus the average call rate (or average call duration) for the playback at 10 calls/min divided by the average call rate (or average call duration) for the playback 10 calls/min. In each case, nestling average call rate was computed over all the three possible playback call durations, since interaction between both the playback call rate and the playback call duration proved non-significant in linear mixed models. A similar procedure was applied for playback call durations of 0.6 and 1.0 s. Then, across all individuals we compared the within-individual percentage of change in average call rate and in average call duration according to varying playback call rates and according to varying playback call durations using a Wilcoxon Signed Rank test. Similarly, we compared the percentages of change in nestling average call rate and in its average call duration according to playback call rates and then according to playback call durations.

Results

Experiment 1: Differential Effect of Hunger onCall Rate and Call Duration

Owlets produced more calls in a food-deprived compared to food-satiated state (mean ± SE = 4.24 ± 0.35 vs. 7.54 ± 0.05 calls per minute; linear mixed models on number of calls, food treatment: $F_{1,96} = 747.04$, $P < 0.0001$; order of food treatment: $F_{1,96} = 2163.7$, $P < 0.0001$; interaction: $F_{1,96} = 0.10$, $P = 0.74$; Fig. 2). Owlets also produced longer calls when food-deprived than food-satiated (0.778 ± 0.018 s vs. 0.700 ± 0.018 s; food treatment: $F_{1,96} = 50.11$, $P < 0.0001$; order of food treatment: $F_{1,96} = 6.33$, $P = 0.014$, interaction: $F_{1,96} = 0.03$, $P = 0.85$; Fig. 2). The within-individual percentage of

increase in call rate from a food-satiated to a food-deprived state was proportionally greater than the percentage of increase in call duration (242 ± 65 vs. 13 ± 2 %; Wilcoxon signed rank test, $S = 997$, $P = 0.0003$, $n = 98$ nestlings). Nestlings produced more and longer calls during the second than first food treatment (estimate ± SE = 0.168 ± 0.006 and 0.029 ± 0.012, respectively).

Experiment 2: Relative Role of Call Rate and Call Duration on Sibling Negotiation

Immediate Vocal Adjustment by Nestlings During the 15-Min Long Playbacks

During the playback both the duration of the broadcasted calls and the rate at which they were broadcasted exerted a significant effect on the nestling's call rate and on its call duration (Table 1). When we broadcasted calls at a higher rate and for a longer duration, we observed a linear reduction in the rate at which nestlings vocalized and in the duration of their calls (Table 1; Fig. 3). The effects of the rate at which calls were broadcasted and of the duration of playback calls on the nestlings' response were independent from each other, as shown by the absence of significant interaction between these two factors (Table 1, both $P > 0.09$). The sequence order covariate indicates that with time nestlings increased both the rate at which they called and the duration of their vocalizations (Table 1, both $P \leq 0.007$, estimate ± SE = 0.1 ± 0.03 calls/min, 0.01 ± 0.02 s).

To deter a sibling to negotiate an individual could therefore produce many long calls. However, a high call rate appears to be more efficient than long calls as suggested by the following two arguments. First, variation in the rate at which calls were broadcasted exerted a stronger effect on nestling vocal behavior than variation in the duration of broadcasted calls. Accordingly, owlets were more dissuaded to vocalize when hearing higher call rates than longer calls (within-individual pairwise comparison between the percentage of decrease in nestling mean call rate while hearing calls broadcasted at 2 and 10 calls/min (25 ± 11 %) and the decrease while hearing broadcasted calls of 0.6 and 1.0 s (18 ± 0.09 %), Wilcoxon Signed Rank test, $V = 800$, $P = 0.03$, $n = 48$ nestlings). Similarly, they shortened their calls much more when hearing calls broadcasted at a higher rate than longer calls (similar comparison of the percentage of decrease in nestling mean call duration while hearing calls broadcasted at 2 and 10 calls/min (6 ± 1 %) and while hearing broadcasted calls of 0.6 and 1.0 s (2 ± 2 %): $V = 786$, $P = 0.04$).

Second, call rate of singleton nestlings was more sensitive to variations in our playbacks than was their call duration. Owlets reduced the rate of their vocalizations to a higher magnitude than their duration in response to an increase in both playback call rate and call duration (within-individual pairwise comparison between the percentage of decrease in nestling mean call rate (25 ± 11 %) and mean call duration (6 ± 1 %), while hearing calls broadcasted at 2 and 10 calls/min, $V = 987$, $P < 0.0001$; between the percentage of decrease in nestling mean call rate (18 ± 0.09 %) and mean call duration (2 ± 2 %) while hearing calls of 0.6 and 1.0 s: $V = 932$, $P = 0.0004$).

Carry-Over Effect of the Playback on Nestling Vocal Behavior During the 10-Min Long Periods of Silence

During the silence following the playback, nestlings still significantly modulated their call rate, but not their call duration, as a function of the rate and the duration of broadcasted calls (Table 1, effect of "PB call rate x PB call duration"). Nestling number of calls during silence was affected by former playback call duration, but only when playback call rate was 10 calls/min ($F_{2,104} = 3.2$, $P = 0.045$). At this playback rate, the duration of the broadcasted calls positively affected nestling number of calls (estimates 0.6 vs. 0.8 s: −0.10 ± 0.04, $P = 0.03$; 0.8 vs. 1.0 s: −0.09 ± 0.04, $P = 0.03$). At lower playback call rates, playback call duration did not significantly affect number of calls produced by singleton nestlings during the following silence period ($P > 0.1$). Whatever the playback call duration, number of calls produced during the silence was positively related to former playback call rate (Fig. 3). On average, after having heard 10 calls/min, nestlings emitted 8 ± 2 ($P = 0.0002$) and 15 ± 02 ($P < 0.0001$) more calls than after having heard 6 and 2 calls/min respectively. Interestingly, the effect of variation in playback call rates and durations on nestling call rate during the silence was hence the reverse compared to when calls were being broadcasted.

Nestlings that produced many calls of longer duration during playback also emitted longer calls at higher frequency during the following silence period (Table 1; effect of nestling call rate during playback on the call rate during silence: 0.56 ± 0.04 call/min; effect of call duration during playback on the call duration during silence: 0.34 ± 0.04 s, both $P < 0.0001$). As can be seen in Fig. 3, nestlings increased their call rate and their mean call duration after the playback ended, except when the playback calls had been broadcasted at 2 calls/min, where they maintained a similar call rate and duration during the following silence (Wilcoxon Signed Rank tests for within-individual pairwise comparison of nestling's call rate and mean duration during and after a playback sequence: for playback call rate > 2 calls/min: all $P \leq 0.002$; for playback call rate of 2 calls/min: all $P > 0.14$).

Table 1 Linear mixed models on call rate and mean call duration of nestling barn owl nestlings during the 15-min long playbacks and the 10-min long period of silence following the playback

Fixed effects	Nestling number of calls (calls/min)		Nestling call duration (sec)	
	F_{df}	*P* value	F_{df}	*P* value
During playback				
Nestling age	$F_{1,427} = 3.22$	.07	$F_{1,325} = 1.9$	.17
Sequence order	$F_{1,427} = 17.7$	<.0001	$F_{1,325} = 43.5$	<.0001
Playback (PB) call rate	$F_{2,427} = 23.2$	<.0001	$F_{2,325} = 6.7$	.001
2 versus 6	*23 ± 3*	*<.0001*	*0.4 ± 0.1*	*.0004*
6 versus 10	*10 ± 3*	*.002*	*0.1 ± 0.1*	*.24*
PB call duration	$F_{2,427} = 10.4$	<.0001	$F_{2,325} = 3.4$	.04
0.6 versus 0.8 s	*13 ± 3*	*<.0001*	*0.03 ± 0.1*	*.01*
0.8 versus 1.0 s	*5 ± 3*	*.09*	*0.02 ± 0.1*	*.07*
PB call rate × PB call duration	$F_{4,423} = 1.5$	.22	$F_{4,321} = 2.0$	.09
During the following silence				
Call rate/duration during playback	$F_{1,422} = 36.9$	<.0001	$F_{1,304} = 56.7$	<.0001
Nestling age	$F_{1,422} = 1.7$	.19	$F_{1,302} = 1.8$	.18
Sequence order	$F_{1,422} = 11.4$	.0008	$F_{1,304} = 12.0$	.0006
PB call rate	$F_{2,422} = 140.2$	<.0001	$F_{2,302} = 1.4$	.26
PB call duration	$F_{2,422} = 6.4$	.002	$F_{2,300} = 0.1$	.89
PB call rate × PB call duration	$F_{4,422} = 5.1$	.0005	$F_{4,296} = 0.8$	.56

Models are based on a total of 486 observations for call rate, 396 and 385 for mean call duration during playback and the following silence, respectively. The 54 nestlings were issued from 15 nests. Nestling identity nested in brood where they were raised in the field was fitted as a random intercept, as well as the identity of the playback individual from which we used the calls. Model selection was based on a stepwise elimination of non-significant effects, beginning with interactions. Estimates are indicated in italics for significant playback effects ($P < 0.05$)

Discussion

We investigated the role of multiple components in vocal signaling, i.e. number and duration of calls, in the resolution of sibling competition over the share of parental food resource in the barn owl. Nestlings vocally compete with each other in the absence of parents over the next indivisible food item to be delivered (Roulin 2002). As predicted from the observation that nestling call rate is more closely related to hunger level than call duration, we found that call rate was more efficient in repelling competitor siblings than call duration. Furthermore, singleton nestlings adjusted their call rate more extensively than their call duration to variation in our playbacks. Finally, call rate had a longer influence on opponent's vocal behavior than call duration.

Previously published correlative data revealed that by producing more calls of longer duration hungrier owlets deter their less needy siblings from begging food from parents and hence from obtaining the prey (Roulin 2001; Roulin et al. 2009; Dreiss et al. 2010b). Accordingly, pairs of siblings increased both the rate and duration of their vocalizations when they were food-deprived compared to when they were food-satiated, showing that both components reflect nestling need and motivation to compete over food resources. Furthermore, individuals reduced both the rate and duration of their calls when responding to playbacks of longer calls broadcasted at higher rates. To our knowledge, only the playback study by Marques et al. (2011) in tree swallow chicks (*Tachycineta bicolor*) experimentally demonstrated that young birds actively adjust their signaling level to their siblings' begging calls, yet the authors did not identity which vocal component of siblings influences nestlings' response. Here, our findings clearly show that young may actively use multiple acoustic components to compete with each other over the share of parental resources. Our results also indicate that the acoustic component that best reflects variations in the current need (call rate) is more finely adjusted than the other vocal component (call duration).

When experimentally food-deprived, barn owl nestlings increased call rate to a larger extent than call duration. This indicates that the number of calls may be a more reliable signal of food requirements than the duration of calls and thus indicate a greater motivation to outcompete siblings. As a consequence, we predicted that call rate should play a more important role in the resolution of sibling competition than call duration. Accordingly, our playback experiment revealed that nestlings modulated the number of their vocalizations as a function of the rate and the duration of

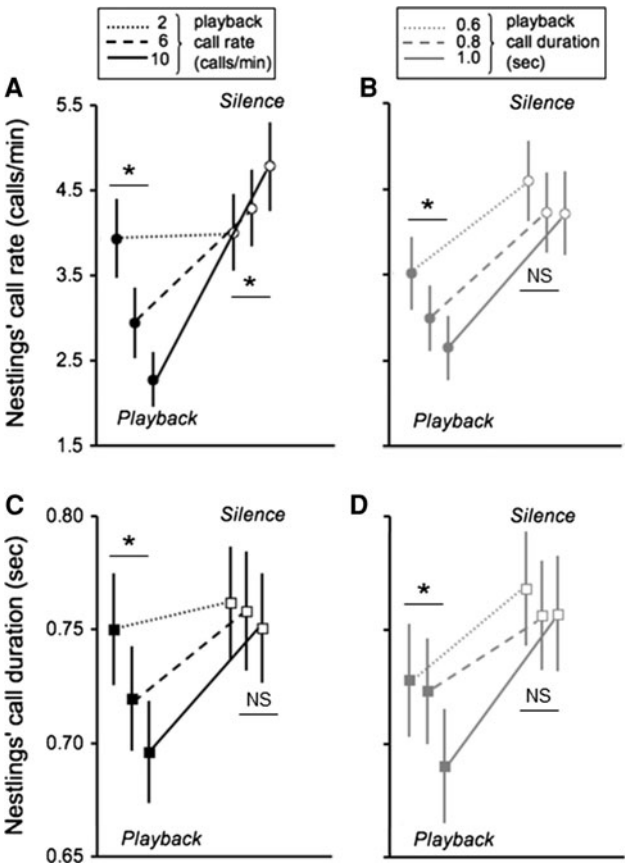

Fig. 3 Vocal response of singleton barn owl nestlings exposed to playbacks of pre-recorded nestlings. **a**, **b** Mean call rate during the 15-min long playback (*filled circles*) and the 10-min long period of silence just after the playback was stopped (*open circles*) and **c**, **d** mean call duration (*filled* and *open squares*), according to the three broadcasted call rates (*black*, **a** and **c**) and call durations (*grey*, **b** and **d**). Interactions in final linear mixed models between playback call rates and durations being non-significant (Table 1), the average for each broadcasted call rate was computed over the corresponding three call durations for each individual pooled together. A similar procedure was applied for call duration over the corresponding three call rates. Means are given ± SE and were computed over 54 nestlings issued from 15 nests

broadcasted calls to a larger degree than the duration of their vocalizations. Complementarily, the rate at which we broadcasted pre-recorded calls exerted a stronger influence on how nestlings vocalized than variation in the duration of the broadcasted calls. Our study therefore suggests that in the barn owl, the most important vocal component to outcompete siblings is call rate followed by call duration. This conclusion is consistent with a previously published correlative study showing that producing longer calls deters siblings from vocally negotiating in the absence of parents (Dreiss et al. 2010b). In contrast, producing more calls in the absence of parents more directly affects the outcome of sibling rivalry, since it induces siblings to refrain from begging food from parents, which directly influences which offspring is fed first (Dreiss et al. 2010b). Studies performed in other species have shown that young nestlings adjust multiple begging components in relation to hunger level, but the exact function of each single component is usually not entirely clear (e.g. Iacovides and Evans 1998; Villasenor and Drummond 2007; Roulin et al. 2009). Our experimental study thus adds new information in this context. Although several components of begging behavior may redundantly signal food needs honestly, they may not necessarily be used interchangeably given that they differentially affect the outcome of sibling competition. This is likely to be the case in most animals, since offspring concurrently adjust vocal and physical behaviors, such as their position relative to the location where parents predictably deliver food in the nest and the intensity of postural and vocal begging (e.g. Kacelnik et al. 1995; Leonard et al. 2003). Research should thus focus on the interplay between the multiple components of begging within and across sensory modalities to understand their relative function.

Assuming that the number of vocalizations suffices to deter siblings, why do barn owl nestlings modulate call duration even if it is a priori a redundant and apparently weaker signal of need than call rate? This issue is particularly interesting given that we did not detect any interactive effect of variations in playback call rate and call duration on the vocal response of nestlings, but rather the effect of these two components was additive. This potentially explains why nestlings increase both the rate at which they vocalize and the duration of their calls when they become hungrier. Call duration might act as a "backup" signal allowing nestlings to "spread the load" over these two signal types (Johnstone 1996). Since producing many long calls is likely to be costly (e.g. Clutton-brock and Albon 1979; Vannoni and McElligott 2009), another possibility is that call duration may act as a "reinforcement" signal of endurance (Payne and Pagel 1996; Rowe and Guilford 1999). Owlets may jointly adjust call rate and call duration to further signal their motivation to outcompete siblings. Because siblings challenge each other for hours, this joint modulation may vary through time depending on cues that remain to be identified. We believe that siblings first challenge each other by increasing the duration of their calls explaining why the duration of their calls is correlated (Roulin et al. 2009). If this contest over call duration does not allow siblings to establish who will have priority access to food resources, they would start to escalate the rate at which they call. This explains why call frequency more finely signals hunger level than call duration, since call frequency is the ultimate signal component to decide which nestling "wins" the vocal contest and in turn obtain the incoming food item. Furthermore, investing in both call rate and call duration may be the only possibility for

individuals with a low resource holding potential to compete with their stronger siblings. This proposition is consistent with the observation that the smallest individuals of a brood emit more calls of a longer duration than their older siblings, probably in an attempt to compensate for their lower physical ability to monopolize food resources (Roulin 2004b; Roulin et al. 2009).

The present study reveals that nestlings adjusted their vocal behavior to variations in broadcasted call rates not only while hearing the playback, but also after the playback ended. Since this was independent of their own call rate during playback, this indicates that barn owls memorize the different features of their siblings' calls to optimally modulate their own vocal behavior at least a couple of minutes later. Our findings are in line with predictions derived from the "sibling negotiation hypothesis", namely that a nestling will refrain from vocally negotiating to a larger extent if its siblings invest more effort in vocal sibling negotiation in order to save energy to be invested once the sibling has been fed and hence is less vocal (Roulin et al. 2000; Roulin 2002). Indeed, when listening to more intense playbacks nestlings refrained from vocalizing to a larger degree. Conversely, as soon as the playback ceased, the increase in vocal behavior by these nestlings was positively related to the playback call rate, and to a lower extent to call duration. This suggests that in front of a highly competitive sibling, barn owl nestlings reduce their investment in vocal negotiation to be invested once the competitive sibling has eaten and hence momentarily withdraws from the competition (see Roulin 2002 for empirical demonstration). Furthermore, when a sibling momentarily vocalizes less intensely in the prolonged absence of parents, a focal individual increases its investment in vocalizations in an attempt to dominate the vocal interaction (unpubl. data). Previous studies in other systems also suggest that memory may be at work to optimize the energetic budget allocated to sibling competition. For instance, nestling birds are able to memorize the highest profitability zones where parents allocate food (Kölliker et al. 1998) or the competitive level they experience within a brood (Lotem 1998). Here, our playback experiments reveal that nestling birds can selectively memorize the most important acoustic components previously displayed by siblings, since we found that mainly the broadcasted call rate still influenced the nestlings' vocal response after the playback ceased.

To conclude, our study demonstrates that sibling barn owls use multiple vocal signaling in competition. The interesting issue here is that these signals are differentially related to food requirements, which raises a number of questions regarding their exact functional value. We focused on two components within the same sensory modality, i.e. vocal signaling, but it would be worth analyzing other sensory modalities (i.e. non-vocal signals). The finding that playback influences nestling behavior after it ended suggests that nestling barn owls take into account previously experienced sibling vocalizations to adjust their own vocal behavior. The carry-over effect of signaling is an original and neglected aspect of interactions taking place between family members. In particular, research should focus more deeply on how offspring encode information about their need and resource holding potential through both vocalizations and other non-vocal behaviors and actually use these signals to outcompete siblings.

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