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CONFLICT RESOLUTION THROUGH STRATEGIC VOCAL NEGOTIATION AND COOPERATION IN NESTLING BARN OWLS (TYTOALBA)

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Ducouret Pauline, 2019, CONFLICT RESOLUTION THROUGH STRATEGIC VOCAL NEGOTIATION AND COOPERATION IN NESTLING BARN OWLS (TYTOALBA)

Originally published at : Thesis, University of Lausanne

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Document URN : urn:nbn:ch:serval-BIB_1FCB6DC32ADB8

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Faculté de biologie
et de médecine

Département d'écologie et d'évolution

CONFLICT RESOLUTION THROUGH STRATEGIC VOCAL NEGOTIATION AND COOPERATION IN NESTLING BARN OWLS (*TYTO ALBA*)

Thèse de doctorat ès sciences de la vie (PhD)

présentée à la

Faculté de biologie et de médecine
de l'Université de Lausanne

par

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Lausanne 2019

UNIL | Université de Lausanne
Faculté de biologie
et de médecine

Ecole Doctorale
Doctorat ès sciences de la vie

Imprimatur

Vu le rapport présenté par le jury d'examen, composé de

Président·e	Monsieur	Prof.	Pierre **Goloubinoff**
Directeur·rice de thèse	Monsieur	Prof.	Alexandre **Roulin**
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le Conseil de Faculté autorise l'impression de la thèse de

Madame Pauline Ducouret

Ingénieure en Qualité de l'Environnement et Gestion des Ressources* / Master Ecologie Biodiversité et Evolution** ENSAT, Toulouse, France* / AgroParisTech, Paris, France**

intitulée

Conflict resolution through strategic vocal negotiation and cooperation in nestling barn owls (*Tyto Alba*)

Lausanne, le 4 février 2019

pour le Doyen
de la Faculté de biologie et de médecine

Prof. Pierre Goloubinoff

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ACKNOWLEDGMENT

Je tiens tout d'abord à remercier mon directeur de thèse, Alexandre Roulin, pour m'avoir donné l'opportunité de faire cette thèse au sein du département d'Ecologie et d'Evolution et sur cette espèce incroyable. Je te remercie pour ta disponibilité, ce qui est vraiment appréciable, et ton soutien. Je te remercie également pour la confiance et l'autonomie que tu m'as accordées dans le développement de mes projets. J'aimerais ensuite remercier tous les membres de mon jury de demi-thèse ainsi que de thèse, à savoir mon président Pierre Golloubinoff et mes experts, Michel Chapuisat, Marta Manser et Redouan Bshary. Un grand merci à vous pour avoir pris le temps d'évaluer et commenter mes travaux de recherches.

Egalement un très grand merci à Amélie et Andrea, pour votre écoute, vos conseils et commentaires qui m'ont permis de me construire et de grandir scientifiquement. Merci à nos collaborateurs acousticiens : Xavier et Patrick, et roboticiens : Francesco et Ronald, pour le développement et le suivi de nos outils acoustiques indispensables pour nos expériences. Merci aux étudiantes qui ont travaillé sur mes projets: Vanessa, Karine, Charlène, Jessica, Auréline et Manon ; à Franck Chalard, pour sa créativité et sa disponibilité pour la mise en place des expériences sur le terrain et enfin, à Jason Buser, pour avoir fourni autant de souris que nécessaires pour nourrir les poussins.

J'aimerais continuer en remerciant toute l'équipe Roulin avec qui j'ai passé des moments inoubliables et qui m'ont permis de me sentir vraiment bien au sein du DEE. Notamment Polo, pour m'avoir formé à manipuler la filoche et mes chers poussins ; Baudouin, Robinoush et Kimounette pour les journées (et nuits) mémorables sur le terrain ; Ana et Vera, pour avoir corrigé si gentiment mes immondes fautes d'orthographe; Valouche, Nono, Luis, Pauline, Céline, Anne-Lyse, Guillaume, Steeve, Diana : pour avoir fait, pour faire, et qui feront j'en suis sûre, de ce groupe un environnement de travail génialissime. Merci d'avoir partagé mes joies, mes complaintes et mes déprimes sans sourciller.

Enfin, un énormissime merci à ma famille, les personnes qui comptent le plus à mes yeux. Malgré leur questionnement sur la raison de mon enthousiasme dans mon travail, ils n'ont jamais cessé de me soutenir et de m'encourager dans ce travail de thèse. Leur compréhension de mon indisponibilité et de mes sauts d'humeurs a été un pilier pour moi. Merci à ma merveilleuse nièce, Julia et mon merveilleux neveu, Oscar qui ont sût, sans même savoir parler, me garder les pieds sur terre et la tête sur les épaules. L'innocence est le meilleur remède aux doutes. Le meilleur pour la fin mais surtout le plus impacté, un merci particulier à Olcay pour sa compréhension, son réconfort et sa capacité à me remotiver dans les moments les plus durs. Merci d'avoir supporté mes absences prolongées pendant la saison de terrain et en cette fin de thèse.

RÉSUMÉ

La communication est un aspect fondamental de la vie animale car elle permet la médiation des conflits d'intérêts entre individus, que ce soit pour les ressources limitantes (e.g. nourriture, territoire) ou pour des tâches collectives au sein de groupes sociaux (e.g. recherche de nourriture, défense d'un territoire). L'échange d'informations entre les parties quant à leur capacité, leur besoin et leur motivation à rivaliser permet aux compétiteurs de décider d'entrer dans la compétition, de continuer à s'y investir ou bien de s'y retirer en fonction de leur chance de succès.

Au cours de cette thèse, j'ai exploré différents aspects de la communication vocale, dans le contexte de la compétition pour les proies amenées par les parents, chez les jeunes Chouettes effraies (*Tyto alba*). Dans ce contexte familial, les poussins ont un intérêt commun pour leurs survies mutuelles puisqu'ils bénéficient de la propagation de leurs gènes en commun (théorie de sélection de parentèle). En l'absence des parents, jusqu'à neufs poussins négocient vocalement entre eux pour l'obtention de la prochaine proie rapportée au nid. Cette négociation a pour but d'informer la fratrie de son niveau de faim, de dissuader les moins affamés de négocier en retour et, par la suite, de quémander la nourriture aux parents à leur retour. Pour cela, les poussins émettent un cri qu'ils font varier en nombre et en durée : un poussin affamé émettant plus, et de plus longs cris qu'un non affamé.

La négociation est un processus dynamique dont chaque étape influence la suivante. Afin d'améliorer l'issue de la négociation, chaque individu peut stratégiquement ajuster sa communication, en augmentant ou diminuant l'intensité de sa vocalisation en fonction de celle de son rival. Dans un premier temps, nous avons testé, à l'aide de playback interactif, l'efficacité de ces stratégies d'ajustements. Nous avons démontré que pour induire le retrait d'un/e frère/sœur de la compétition, le plus efficace était d'ajuster positivement sa durée de cri à celle de son/sa frère/sœur, tout en ajustant par contre négativement le nombre de cris émis. En d'autres termes, pour dominer une négociation, il est plus efficace de défier son/sa frère/sœur en augmentant simultanément la durée de ses cris. De plus, il est plus efficace de lui laisser l'opportunité de communiquer et d'attendre que celui-ci diminue son nombre de cris pour augmenter le sien. Nous avons également pu démontrer que ces stratégies conduisent le poussin (ici le playback) à émettre plus de cris et de plus longue durée. Démontrer sa motivation requiert donc un investissement plus important, ce qui pourrait aider au maintien de l'honnêteté de cette communication. Les autres stratégies étant moins efficaces, les interactions répétées entre poussins pourraient permettre de renforcer l'aspect coopératif de la négociation. De manière similaire, nous avons montré que les poussins corrigeaient leur frère/sœur lorsque celui/celle-ci ne respectait pas la règle sociale d'alterner ses cris avec ceux de son rival. Corriger ses frères et sœurs pourrait renforcer la stabilité évolutive de la négociation vocale au sein de la fratrie comme un moyen non agressif de se répartir la nourriture.

Dans un second temps nous nous sommes intéressés au rôle que pouvait jouer la négociation pour décider à qui prodiguer un service tel que l'épouillage ou le don de proie. La sélection de parentèle et la réciprocité des services biologiques sont des explications distinctes pour l'origine et le maintien évolutif des comportements coopératifs et de l'altruisme. Bien que considérées comme compatibles, l'interaction entre ces théories et les conditions favorisant la coopération a rarement été testée. Nous avons montré que 1) les poussins les plus âgés et en meilleur condition sont plus enclins à partager la nourriture avec leurs frères et sœurs; 2) le don de proie se produit en particulier lorsque les poussins reçoivent un surplus de nourriture de la part des parents et lorsque la quantité totale de nourriture stockée dans le nid est abondante, c'est donc lorsque le coût de la renonciation à cette proie est faible qu'un poussin la donnera ; 3) le receveur du don de proie est de préférence un frère ou une sœur dans le besoin (le poussin qui négocie le plus avant le don) ou celui qui a le plus coopéré avec le donneur au préalable (le poussin qui a le plus épouillé le donneur).

Cette thèse souligne donc l'importance de considérer la résolution de conflit comme interactive avec des stratégies d'ajustements, de la coopération et des règles sociales. Enfin, la chouette effraie se révèle être une espèce modèle pour l'étude de la communication animale et la résolution des conflits.

SUMMARY

Communication is a fundamental aspect of animal existence as it mediates survival and reproduction when conflicts of interest occur (over territory, mate or food) or for social integration (acceptation in a group, cooperative behaviour). Assessing a contestant's relative resource holding potential and motivation to compete through signalling allows individuals to decide whether to engage, to keep competing or to retreat from a contest according to their chances of success.

During this thesis, I investigated various aspects of vocal communication in the context of competition over prey brought by parents in broods of barn owl (*Tyto alba*). In this family context, nestlings have a common interest in their mutual survival and reproduction since they benefit from the propagation of their shared genes (kin selection theory). In the absence of parents, up to nine nestlings vocally negotiate to get the priority access to the next prey brought by parents. The purpose of this negotiation is to inform siblings about hunger level, dissuade the less hungry siblings from negotiating in return and afterwards from begging when parents arrive. To negotiate, the nestlings emit one type of call that vary in number and duration: a hungry nestling emitting more and longer call than a non-hungry.

Negotiation is a dynamic process with each step of the process affecting the next. To improve its negotiation outcome, each individual can strategically adjust its communication to the contestant's communication; that is to say, when and how to increase/decrease its vocal intensity according to the contestant's intensity. We first tested, using interactive playback, the efficiency of these adjustment strategies to the contestant's signalling. We demonstrated that the most efficient strategy to deter a sibling from the competition is to match the call duration (increasing and decreasing its call duration simultaneously with the sibling), and to mismatch the number of calls (increasing when the sibling decreases its call rate and reversely). In other words, to dominate a negotiation, it is more efficient to challenge a sibling by simultaneously increasing the call duration. However, it is more efficient to give a sibling the opportunity to communicate by waiting for it to decrease its call rate before increasing its number of calls. We were also able to demonstrate that these strategies lead the nestling (here the playback) to emit more and longer calls. Transmitting motivation to a sibling requires therefore a higher investment which could help maintain this communication honest. Because following other strategies is less efficient to deter a sibling from competing, the repeated interactions between nestlings may reinforce the cooperative aspect of the negotiation. Similarly, we showed that nestlings gave social feedback when a nestling did not respect the social rule of alternating its calls with those of its rival. This social feedback could enhance the evolutionary stability of vocal negotiation within siblings as a non-aggressive way of sharing food.

In a second time, we investigated the role that negotiation could play in deciding with whom to share a prey. Kin selection and the reciprocation of biological services are separate explanations for the origin and evolutionary maintenance of cooperative behaviours and altruism. Although considered as non-mutually exclusive, the cost-to-benefit balance to behave altruistically or reciprocally cooperate, and the conditions promoting a switch between such different strategies have rarely been tested. We showed that 1) older individuals in better conditions are more likely to share food with their siblings than nestlings in poor conditions; 2) the share of prey occurs when the nestlings receive extra food from the parents and the food is abundant, thus especially when the cost to renounce a prey is low; 3) the receiver of the prey donation is preferentially a sibling in need (the nestling who negotiates the most before the donation) or the one who has cooperated the most with the donor beforehand (the nestling that has allopreened the donor the most).

This thesis therefore emphasizes the importance of considering the conflict resolution as an interactive process with strategical adjustment, cooperation and social rules. Finally, barn owls appear to be a key model species for the study of animal communication and conflict resolution.

GENERAL INTRODUCTION

In nature, animal conspecifics are often in conflict over the share of limited resources such as territories, mating sites, mates or food (McGregor, 2005). To maximise their Darwinian fitness (i.e. survival and reproduction), the evolutionary theory predicts that individuals should compete to have access to these limited resources to derive the greatest benefits at the lowest cost (Maynard Smith, 1976). Animal communication is a fundamental aspect of animal existence as it functions in mediating these widespread conflicts. Indeed, rather than physically competing for resource access, which may lead to serious or lethal injuries, individuals commonly signal their 'resource holding potential' (i.e. fighting ability) and motivation to compete (Parker, 1974). Assessing a contestant's relative resource holding potential and motivation through signalling allows individuals to decide whether to engage, to keep competing or to retreat from a contest according to their chances of success.

A signal can be defined as "any act or structure that: (i) affects the behaviour of other organisms; (ii) evolves (or is maintained) because of those effects; and (iii) is effective because it transfers functional information to receivers" (Carazo & Font, 2010). To transmit information, a signal can use multiple sensory channels such as acoustic (e.g. vocalizations: Schwartz & Freeberg, 2008; Todt & Naguib, 2000), chemical (e.g. pheromones: Paquet & Fuller, 1990; Rich & Hurst, 1998) and visual (e.g. behavioural displays: Hofmann & Schildberger, 2001; Mercier & Dejean, 1996; or body coloration: Senar, 2006). Some signals become fixed at a given developmental or ontological stage, implying that they do not fluctuate quickly, as for instance some morphological signals such as plumage coloration in birds. Contrarily, many signals remain flexible through life (e.g. vocalisation, most behavioural displays) and signalling intensity can fluctuate over short periods of time independently of the sender's condition. These quick fluctuations raise the question of how individuals adjust their level of investment in signalling at each time point, given their own condition and that of their potential audience (whether or not it is involved in the signal exchange). For instance, quick fluctuations can be required to avoid interferences (Brumm & Slabbekoorn, 2005; Ficken, Ficken, & Hailman, 1974), to adapt to the presence of an audience (Munn, 1986) or to the resource holding potential, motivation and condition of contestants (Bell, 2008; Godfray, 1995; Oliveira, McGregor, & Latruffe, 1998).

Signal adjustment to a contestant's signalling rules

Game theories have studied these signal fluctuations and several models predict different signalling structures and dynamics (i.e. intensity escalation, contestants' intensity adjustment) depending on whether the weakest contestant bases its decision of giving up the contest on: i) its own motivation; ii) the contestant's motivation; or iii) the relative motivation between both contestants (Table 1). The '*sequential assessment model*' is based on the assumption that the contestants assess each other's quality and compare it to its own (i.e. relative quality), in order to allow the weakest one to give up (Enquist & Leimar, 1983). In this model, the signal intensity for each contestant is predicted to remain constant as the assessment is based on the average intensity. The signal repetition might be required simply to maximize the assessment accuracy in a noisy environment with assessment error. There is therefore no display adjustment between contestants. However, the contest can have multiple phases with an escalation between them if the preceding phase does not allow to distinguish the dominant individual. The '*war of attrition models*' are based on the assumption that an individual signals to advertise endurance to a contestant, and gives up the contest when the accumulated cost of its own signalling process exceeds an intrinsic threshold (Bishop & Cannings, 1978; Hammerstein & Parker, 1982; MestertonGibbons, Marden, & Dugatkin, 1996; Payne & Pagel, 1996). In these models, contestants are expected to match their display intensities at any time to avoid cheating. Indeed, if they do not match display intensity, an individual could cheat by delaying its display and take advantage through the exhaustion of its contestant. Finally, the '*cumulative assessment game model*' is based on the assumption that an individual gives up the contest when the accumulated cost inflicted by its contestant exceeds an intrinsic threshold cost (Payne, 1998). This inflicted cost does not necessarily refer to direct physical damage, but to any type of fitness cost (e.g. time loss or increased predation risk). In this latter model, there is no prediction concerning the display adjustment between contestants, and it can be used to explain more complex display adjustments.

Table 1 – Assumptions and predictions of key theoretical models of contest behaviour (based on: Briffa & Sneddon, 2010)

Model	Sequential Assessment	War of Attrition	Cumulative Assessment
Assumption			
Decision rule based on:	Relative motivation	Own motivation	Other's motivation
Function of repeated display	Reduce sampling error	Advertise endurance	Inflict costs directly on contestant
Predictions			
Matching between contestants?	No	Yes	No
Contest structure	Escalates between phases but constant within phases	Escalates within phase; Energetic War of Attrition: constant, escalates or de-escalates	Constant, escalates or de-escalates within non-injurious phases; escalates within injurious phases

While game theory predicts that such social interactions are interactive with tactical adjustments to the contestant's behaviour (McNamara, Gasson, & Houston, 1999), very few empirical studies have investigated the importance of such short-term adjustment tactics for conflict resolution through signalling. Short-term adjustment refers to the intensity at which an individual should signal, according to the current contestant's signal and intensity. In other words, when an individual can escalate or reduce display intensity, depending on its contestant's behaviour. This adjustment might actually be as important as the global intensity of the contest itself. For instance, in satin bowerbirds (*Ptilonorhynchus violaceus*), the male ability to adjust the intensity of sexual displays to the female's response explains the success of a male courtship to the same extent (around 30%) than the average display intensity (Patricelli, Uy, Walsh, & Borgia, 2002). Therefore, by examining the average signal intensity only, researchers risk to overlook a major aspect of information transfer and adaptive decision-making (Briffa, Elwood, & Dick, 1998; Patricelli, Krakauer, & McElreath, 2011; Van Dyk, Taylor, & Evans, 2007).

The assessment models presented above have been mainly empirically tested in one-off contests among non-familiar adults, which have fully incompatible interests, besides the common interest of signalling instead of relying on harmful aggression in order to settle a contest (e.g. Keil & Watson, 2010; Reddon et al., 2011) . Nevertheless, conflicts over resources also occur among individuals that partially share interest in each other's fitness (Roberts, 2005). Indeed, when contestants are genetically related, losing the contest and authorising their kin access to the resources still rewards the looser individual through indirect fitness benefit such as increasing the survival and reproduction of its kin (Hamilton, 1964). Similarly, when regrouped in stable social groups, individuals repeatedly interact and benefit from groupmates' survival. When interest overlaps, less aggressive contests are expected, and more 'cooperative' signal adjustments instead of 'matching intensity escalation' are likely to evolve (Cant & Young, 2013, "play aggressive away, not at home" citation from: Foley, Forber, Smead, & Riedl, 2018). For instance, offspring of altricial birds can modulate their begging intensity (i.e. signals conveyed to attract parental care) according to their siblings' hunger level, by reducing signal investment if facing a highly vocal sibling (Marques, Leonard, Horn, & Contasti, 2011; Price, 1996; Romano, Caprioli, Boncoraglio, Saino, & Rubolini, 2012; Romano et al., 2015; Roulin, Kolliker, & Richner, 2000; H. G. Smith & Montgomerie, 1991). In this case, the adjustment of the signals intensities of the two siblings mismatches: increasing in one while decreasing in the other. Different mechanisms can favour this kind of cooperative behaviour by lowering the risk of cheating and being exploited, such as reciprocation (Trivers, 1971), threat of punishment (Clutton-Brock & Parker, 1995) and social feedback (Vitousek, Zonana, & Safran, 2014). These social mechanisms are part of the social environment that shapes the development and the maintenance of signal behaviour.

The social environment has been considered to play a major role in the learning and maintenance of vocal behaviour in vertebrates (Doya & Sejnowski, 1998; Takahashi, Liao, & Ghazanfar, 2017; Tschida & Mooney, 2012). Vocal behaviour refers to different aspects of communication skills. For instance, the presence of conspecifics during early ontogeny influences the acquisition of call/song structure (Adret, 2004; Eales, 1989) and the accurate use of call for the good function. Thanks to early social interactions, individuals acquire better performance skills in socially competitive situations, such as mating success (A. P. King, White, & West, 2003; White, Gersick, Freed-Brown, & Snyder-Mackler, 2010), dominance hierarchy formation (Branchi, D'Andrea, Gracci, Santucci, & Alleva, 2009), brood care (Margulis, Nabong, Alaks, Walsh, & Lacy, 2005), resource competition (Arnold & Taborsky, 2010). Finally, the social environment enables individuals to learn temporal coordination skills, such as call overlap avoidance (Henry, Craig, Lemasson, & Hausberger, 2015; Takahashi, Fenley, & Ghazanfar, 2016). Historically considered to be required only during development stage, auditory feedback is actually still required after the learning stage to maintain song structure (Leonardo & Konishi, 1999) and social skills (Gersick, Snyder-Mackler, & White, 2012).

Signalling in cooperation

Among animal interactions that require signal exchange, cooperation is often disregarded (Noe, 2006). Although individuals compete for the access to limited resources, altruistic and cooperative behaviours such as allogrooming/allopreening and food sharing are widespread in nature. Kinship is considered to be the main mechanism that explains the evolutionary stability of adopting costly behaviours in favour of another individual without receiving an immediate benefit. Altruist behaviour can provide indirect fitness benefit through promoting related individuals, whenever the indirect fitness benefits of helping relatives outweigh the cost of performing altruistic behaviours (Hamilton, 1964). Cooperative behaviours can also provide direct benefit if reciprocated by counterparts (Nowak, 2006; Trivers, 1971; West, Griffin, & Gardner, 2007). Reciprocity can be achieved by exchanging the same social service, like mutual grooming/preening in mammals and birds (Adiseshan, Adiseshan, & Isbell, 2011; Gill, 2012; Radford & Du Plessis, 2006; Roulin et al., 2016a), or food sharing (Carter & Wilkinson, 2013; de Waal, 2000), but also by trading different services (de Waal, 1997; Fruteau, Voelkl, van Damme, & Noe, 2009; Kern & Radford, 2018; Noe & Hammerstein, 1995; Roulin et al., 2016a). Kin selection and reciprocation are non-mutually exclusive mechanisms and the interplay between them might be condition-dependent (Lehmann & Keller, 2006; Taborsky, Frommen, & Riehl, 2016). Indeed, in the presence of a highly needy relative, it may be more beneficial to perform an altruistic behaviour without receiving any direct benefit, whereas in other conditions it may be more rewarding to exchange commodities. Signalling is expected to help mediate and optimize the decision of whether to provide help to relatives or to the neediest individual, and at which rate. For instance in Vervet monkeys *(Chlorocebus pygerythrus)*, adults display different signals (i.e. lip smacking and body part presentations) that induce longer grooming bouts (van de Waal, Spinelli, Bshary, Ros, & Noe, 2013). Being informed about other's grooming necessity, the partner can optimally decides its investment in the cooperative behaviour without being exploited.

GOAL AND OUTLINE OF MY THESIS

In the present PhD thesis, I will examine two complementing aspects of communication: i) temporal dynamics of communication among several individuals and the role of social feedback to reinforce communication rules and ii) the role of communication in cooperative behaviour. These are two key aspects to tackle in order to understand how communication mediates conflicts over limited resources in socially grouped animals, even if it entails the inherent difficulty of studying interactive communication among several individuals. Direct resolution over limited resources has been understudied perhaps because it is neither practical nor ethical to study contest behaviour that may end with physical aggression (Briffa & Sneddon, 2010; Elwood & Parmigiani, 1992). Offspring of altricial birds offer a good opportunity to investigate these aspects because they are dependent on the parental provisioning which can be experimentally manipulated. Furthermore, the membership of the brood is fixed with no alternative option (i.e. a nestling cannot change brood) and groupmates identity are fixed over time (i.e. a stranger nestling cannot invade a nest).

The topic of communication within family is interesting because each member competes at several levels: sex conflict between parents, where parents compete over the parental care; parent-offspring conflict, where parents and offspring compete over the amount of parental care and; siblings competition, where siblings compete over the sharing of parental care (Mock & Parker, 1998; Morales & Velando, 2013; Trivers, 1974). In order to resolve these conflicts, the transfer of information between counterparts is inevitable, in which case a family can be viewed as a communication network (Kilner & Johnstone, 1997; McGregor, 2005). In birds, nestling begging behaviour in the presence of parents is particularly conspicuous and was comprehensively studied (Wright & Leonard, 2002). The individual showing the most ostentatious begging behaviour induces its parents to feed it in priority, because begging conveys honest information about the need for food (Godfray, 1995; Kilner & Johnstone, 1997; Smiseth & Amundsen, 2002). However, nestlings may also partly control food share by vocally and physically competing between each other (scramble competition: Kilner & Johnstone, 1997; Roulin, 2004a). Kin selection is expected to play a key role in the evolution of begging strategies by reducing individual selfishness (Godfray, 1995; Hamilton, 1964; Mock & Parker, 1997; Moreno-Rueda, 2007; Wright & Leonard, 2002). When the indirect fitness benefits exceed the direct fitness costs, offspring may allow for a larger share of the food to be allocated to their related hungry competitors (Forbes, 2007; Godfray, 1995; Wright & Leonard, 2002). Indeed, young individuals can modulate the intensity of solicitations to parents according to their siblings' motivation by reducing begging investment if facing a highly-motivated sibling (Marques et al., 2011; Price, 1996; Romano et al., 2012; Romano et al., 2015; Roulin et al., 2000; H. G. Smith & Montgomerie, 1991). This suggests that offspring use vocal, postural and physical displays not only to solicit food from parents, but also to compete with siblings for the priority access to food. The 'sibling negotiation hypothesis' predicts that offspring communicate to induce their less needy siblings to withdraw from competition over parental food (Roulin, 2002a; Roulin et al., 2000). Nestlings that negotiate would optimize their energy investment by communicating according to their own motivation to compete but also according to their sibling's motivation. The least hungry individuals would retreat from competition. They would therefore decrease their likelihood of winning the food contest, but would save energy that they could reallocate when the likelihood of winning is higher (Dreiss, Lahlah, & Roulin, 2010; Johnstone & Roulin, 2003; Roulin, 2002a; Roulin et al., 2000). The negotiation can thus be considered as a form of altruism as its main function is to reduce conflict between genetically related siblings. Negotiation occurs only when sibling competition is costly because food is difficult to monopolize (e.g. when asynchronous brood leads to size hierarchy) and when there is an asymmetry in food requirement (e.g. when food resources brought by parents are indivisible, Johnstone & Roulin, 2003). To date, the sibling negotiation hypothesis has been demonstrated in the barn owl, *Tyto alba*, (Roulin et al., 2000), in the spotless starling, *Sturnus unicolor* (Bulmer, Celis, & Gil, 2007) and has been suggested in the barn swallow, *Hirundo rustica* (Romano, Boncoraglio, Rubolini, & Saino, 2013; Romano et al., 2015), but it remains poorly studied in other bird and mammal species.

My thesis follows on from two decades of studies on vocal negotiation and more generally on social interactions in nestling barn owls. In order to properly appreciate the goal of my PhD, it is important to highlight the particularity and the complexity of social interactions in nestling barn owls.

SIBLING INTERACTIONS IN BARN OWL NESTLINGS: THE STATE OF THE ART

The barn owl is an ideal model to study signalling interactions among siblings as a social network. Indeed, broods can comprise up to nine nestlings that interact during a relatively long rearing period, i.e. 56 days. Nestlings markedly differ in age and body size because the hatching is asynchronous, siblings hatching every 2.5 days (Roulin, 2002b). Nestlings are usually full siblings, as extra-pair copulations are very rare in this species (Roulin et al., 2004). Therefore, they have a common inclusive fitness interest in avoiding costly competition and promoting a large share of resources to hungry siblings. When the younger nestling has around 15 days, parents no longer stay in the nest and come only briefly to bring food at night. Each nestling eats on average 3 to 4 prey items (small mammals) per night that parents deliver (Durant & Handrich, 1998). Although parental visits are unpredictable, with two consecutive visits being spaced from a few minutes to hours (pers. obs.), the majority of prey items is brought at the beginning of the night (Roulin & Bersier, 2007). Often parents bring food faster than nestlings can eat it, which leads to a stock of uneaten food in the nest (Roulin, 2004b). Finally, parents distribute the food to the bill of a particular nestling, but because they do not dissect rodents, food consumption takes a long time increasing the risk of prey being stolen by siblings (Roulin, Colliard, Russier, Fleury, & Grandjean, 2008).

1. The sibling negotiation

Nestlings most often swallow the entire prey, thus only one nestling is fed per parental visit. This sporadic feeding leads to a high asymmetry in food requirement between siblings. The outcome of competition is then predictable with the neediest (i.e. hungriest) nestling being more motivated to compete. When parents are hunting, nestlings vocally negotiate for priority access to the indivisible prey item next delivered and each one can call up to thousands of times per night. The nestling that invests the most during the vocal negotiation phase ultimately begs the most when a parent arrives and thus has a higher probability to receive the prey item (Dreiss et al., 2010; Roulin, 2001; Roulin et al., 2000). This vocal negotiation is directed toward sibling and not toward parents, who forage too far from the nest to be able to hear nestlings and do not adjust provisioning rate to the number of negotiation calls nestlings emit (Roulin et al., 2000).

a. Information content of a nestling's negotiation call

Although nestling barn owls emit a single call type, they convey multiple information through different call features (for more details see Box 1). A hungry nestling reliably informs its sibling about its need by emitting many long calls (i.e. through call rate and duration; Dreiss et al., 2010; Dreiss, Ruppli, & Roulin, 2014; Roulin, Dreiss, Fioravanti, & Bize, 2009; Roulin et al., 2000; Ruppli, Dreiss, & Roulin, 2013) and by rapidly emitting a call after a sibling's call ended (i.e. latency time; Dreiss et al., 2010; Dreiss, Ruppli, Faller, & Roulin, 2015). Age differences are pronounced between barn owl siblings which may favour older and hence larger nestlings to be better able to monopolize food by physically outcompeting their siblings (e.g. T. E. Smith, Leonard, & Smith, 2005). Probably in order to compensate their submissiveness, junior barn owl nestlings emit more negotiation calls and longer calls than seniors, independently of hunger level, as already observed in other species (Cotton, Wright, & Kacelnik, 1999; Dreiss et al., 2010; Ruppli et al., 2013; Smiseth & Amundsen, 2002). Finally, nestlings might vocally transmit their identity, since call structure is similar within-individual and statistically distinguishable between individuals (Dreiss, Ruppli, & Roulin, 2014). Nestlings have therefore the opportunity to distinguish, identify and characterize their siblings by age hierarchy and hunger level, thanks to the acoustic parameters of their negotiation calls.

Box 1 – Barn owl negotiation call structure and information content

(Dreiss et al., 2010; Dreiss, Ruppli, & Roulin, 2014; Roulin, Dreiss, Fioravanti, & Bize, 2009; Roulin et al., 2000; Ruppli, Dreiss, & Roulin, 2013)

Nestlings emit a single call type that they can modulate in multiple call parameters (Fig. 1).

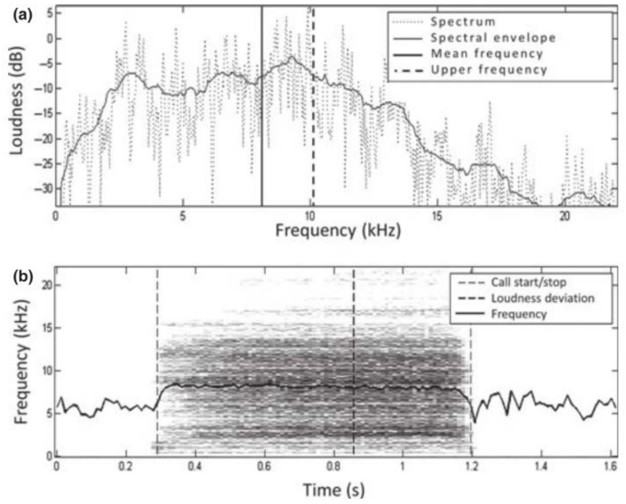

Figure 1: Frequency distribution (a) and sonogram (b) of a negotiation call (taken from Dreiss et al. 2014)

- Call rate (calls/min)
- Call duration (sec)
- Loudness (dB)
- Loudness deviation (0-1): when above 0.5, calls are louder at the end than at the beginning of the call.
- Mean frequency (kHz)
- Frequency variation (kHz): Vibrato of the calls
- Upper frequency (kHz): Pitch of the call

Information available:

	Hungry level **(change percentage)**		**Age hierarchy**	
	Food deprived	Food satiated	Junior	Senior
Call rate	▶	(12%)	▶	
Call duration	▶	(11%)	▶	
Loudness	▶	(4%)	▶	
Frequency variation	◀	(3%)	◀	
Loudness deviation	▶	(2%)	▬	
Upper frequency	◀	(1%)	◀	
Mean frequency	▬		▬	

Nestling identity

Statistical discrimination between siblings occurred in 62% of the cases for 6 nestling broods, and in 90% of the cases for 2 nestling broods. Independently of hunger state, individual nestling barn owls consistently emitted negotiation calls with similar structure (within-individual repeatability values ranged from 0.42-0.71).

b. Signalling adjustment to sibling vocalization

The sibling negotiation hypothesis predicts that nestlings should reduce their investment in negotiation if their chance to get the next prey is low due to the presence of a hungrier, and thus more motivated to compete sibling. Accordingly, nestlings adjust their vocal investment to their chance of winning the contest. They refrain from calling (i.e. emit fewer and shorter calls) if facing a sibling that emits many long calls and that responds quickly after its own call (Dreiss et al., 2010; Roulin et al., 2000; Ruppli et al., 2013), but not if facing a sibling that emits loud calls (Appendix 1, Dreiss et al., 2017). Nestling barn owls also adjust their vocalizations according to the number of competitors that are calling, investing in vocal negotiation preferentially if facing a lower number of nestlings regardless of the global call rate. However, they preferentially invest when facing two contestants that emit fewer calls rather than one contestant that emits many calls (Ruppli et al., 2013). Nestlings modulate their call rate to a larger extent than their call duration, and the variation of an experimental playback call rate has a higher effect on nestling vocalization than the variation of call duration (Ruppli et al., 2013). The most important vocal parameter to outcompete siblings seems therefore to be the call rate followed by the call duration. Call rate is also the vocal parameter that is the most influenced by hunger level (Dreiss, Ruppli, & Roulin, 2014).

In social networks, individuals interact repeatedly with each other and can eavesdrop other's social interactions to glean information. A bystander can extract information by observing the signalling interaction of social groupmates (e.g. resource holding potential, courtship vigour; reviewed in Earley, 2010) and fuse this gleaned information with their personal information gained through direct interaction, in order to optimize their social decisions (Peake, Terry, McGregor, & Dabelsteen, 2002). Accordingly, nestlings adjust their vocalization to the motivation and the age hierarchy of competitors gleaned from a previous vocal exchange between competitors, investing in vocal negotiation preferentially if facing a younger and less motivated competitor (Dreiss, Ruppli, Faller, & Roulin, 2012).

Evolutionary theories predict that an individual should rely on memory as long as the behavioural decision taken based on the past interaction information derived enough benefits to outweigh the cognitive costs of retaining information (Dukas, 1999; Mery & Kawecki, 2005). Then, if the information is not relevant anymore because of internal or external change (e.g. food supply or individual condition), memorised information should be updated or ignored in the decision process (Dunlap, McLinn, MacCormick, Scott, & Kerr, 2009; McNamara & Houston, 1987). Parental visits in barn owl are unpredictable (Roulin & Bersier, 2007) with two visits potentially spaced by several hours. Although retaining information on sibling motivation could be of high interest to limit the cost of vocal negotiation, nestlings rely on memory for only a few minutes (Dreiss et al., 2012). They therefore need to continuously display, in order to efficiently trigger the withdrawal of a sibling. Multiple hypotheses can be proposed: first, because of assessment error, repetition is required to enhance the estimation accuracy of the competitors' motivation (Payne & Pagel, 1996, 1997). Second, but not mutually exclusive, nestling motivation can change over a too short period of time because of a parental visit or because of the consumption of a prey item stored in the nest. In such cases, the time at which information is accurate is unpredictable, meaning that it might rather be safer to rely on short-term vocal negotiation rather than on relative long-term memory.

c. Coordination rules in communication: turn-taking

When the decision of giving up a contest relies on information exchanged through signals, the accuracy of the information assessment is crucial. The avoidance of signal interference is therefore an important component of animal communication (Brumm & Slabbekoorn, 2005), and temporal coordination rules might be required. The turn-taking rules firstly described by Sacks et al. (Sacks, Schegloff, & Jefferson, 1978) can be considered as a universal feature within human cultural differences (Stivers et al., 2009) and is acquired during development even before the gestural and linguistic skills (Bateson, 1975). Turn-taking rules apply to dyadic interactions as well as group interactions with two main temporal rules: alternation of utterance between parties and avoidance of simultaneous speakers. In order to facilitate overlap avoidance, interlocutors often change multiple vocal parameters (e.g. frequency decreases, gazing changes) and perform particular gestures at the end of their turn so that counterparts can anticipate when resuming (Chen, 1970). Although originally thought to have evolved only in human species, it appears to be increasingly clear that such temporal coordination is shared across taxa (reviewed by Pika, Wilkinson, Kendrick, & Vernes, 2018).

Overlap avoidance (antiphony) is common in the context of social call exchanges (e.g., Carter, Skowronski, Faure, & Fenton, 2008; Ghazanfar, Smith-Rohrberg, Pollen, & Hauser, 2002; Soltis, Leong, & Savage, 2005; Versace, Endress, & Hauser, 2008), but also in the context of competitive call exchanges (Popp, 1989; Stokes & Williams, 1968; Wilson, Ratcliffe, &

Mennill, 2016; Yang, Ma, & Slabbekoorn, 2014). Barn owl nestlings overlapped calls five times less often than expected by chance, which represents only 2% of the emitted calls. The call overlap occurrence was also not correlated with the within-brood age hierarchy, neither to the hunger level, which suggests that overlapping is not used by nestlings to demonstrate a physical nor a motivation dominance (Dreiss et al., 2013).

Recently, Takahashi et al. confirmed similarity between turn-taking dynamic in humans and in marmoset monkeys (*Callithrix jacchus*) using a coupled oscillator model (Takahashi, Narayanan, & Ghazanfar, 2013). Moreover, in Japanese monkeys (*Macaca fuscata*) and vervet monkeys (*Chlorocebus pygerythrus*), individuals decrease the fundamental frequency at the end of a call bout so that their counterpart can anticipate when they will call and avoid overlap (Hauser & Fowler, 1992). The sibling negotiation in barn owls can be separated in two sub-phases according to the temporal coordination of utterance. Siblings communicate either in 'solo' during which only one nestling calls for at least 10 consecutive calls which represents 67% of the calls emitted, or through frequent vocal alternation during which individuals exchange turns rapidly (Dreiss et al., 2015). A nestling's solo can be composed by 10 calls or by a thousand calls which raises the question of how a sibling decides to resume. When communicating in solo, a nestling does not keep its call rate and call duration constant but progressively decreases its call rate and increases its call duration. The probability that a sibling interrupts a nestling's solo is higher when a sibling or the playback starts to decrease its call duration in addition to the decreasing call rate. These coordination rules were found irrespectively of the within-brood age hierarchy and hunger level, suggesting that these temporal adjustments can be considered as social convention (Dreiss et al., 2015).

d. Real-time adjustment rule

Although the main goal of vocal negotiation is to emit more and longer calls than a sibling, when focusing on the short-term adjustment between contestants (i.e. last 10 calls emitted), call duration of sibling pairs appears to be positively correlated (i.e. the duration of both contestant calls increases and decreases simultaneously) in natural vocal exchanges (Dreiss et al., 2015; Roulin et al., 2009). Conversely, the call rate between contestants is negatively correlated (Dreiss et al., 2015). Because call duration and rate intermittently increase and decrease with time, these positive and negative correlations are not due to an increasing hunger level.

2. Cooperative behaviour

Allopreening and food sharing are two cooperative behaviours that are widely performed by nestling barn owls (Roulin, Da Silva, & Ruppli, 2012; Roulin et al., 2016b). Allopreening behaviour is considered to have a hygienic function as it consists of removing parasites from a sibling's body. This is especially important if it is directed towards body parts that are not easily accessible by the receiver nestling (i.e. preenee, Gill, 2012; Villa, Goodman, Ruff, & Clayton, 2016). Allopreening may also have a function of appeasement as it may induce a reduction of stress level in both the preener and the preenee (Fraser & Bugnyar, 2010; Gill, 2012; Radford & Du Plessis, 2006). In the latter case, both individuals benefit from performing allopreening. The hygienic and appeasement functions are non-mutually exclusive (e.g. Radford & Du Plessis, 2006). As mentioned earlier in this introduction, providing benefit to a conspecific with a costly behaviour is evolutionary stable as long as the donor received indirect benefit by promoting kin rather than non-kin and/or received direct benefit through reciprocation of this costly behaviour. Roulin et al. (2016b) showed that nestling barn owls reciprocate these cooperative behaviours with a nestling being more inclined to allopreen (allofeed) a sibling if in turn this sibling allopreens (allofeeds respectively) it too. Nestlings not only trade the same commodities, but will also trade different commodities with a nestling being more inclined to allofeed a sibling by whom the said nestling has been allopreened. In barn owl nestlings, allofeeding is mainly performed by older nestlings, while allopreening is mainly performed by younger ones (Roulin et al., 2012; Roulin et al., 2016b). Older nestlings are stronger than younger ones, and might be better in monopolizing food items. Allofeeding a sibling may be thus less costly to perform by older than by younger nestlings, which could explain this difference of strategy between younger and older siblings. In turn, and potentially as a means of compensation, younger siblings allopreen more often than older ones.

PART 1: SIGNAL TEMPORAL AND INTENSITY ADJUSTMENT RULES TO CONTESTANT'S SIGNALLING

The first goal of my PhD was to study dynamic aspects of communication among several nestlings by experimentally testing the temporal and intensity adjustment rules to contestant‘s signalling observed in barn owl nestlings [Chap 1 and 2]. I designed two studies to tackle this question. The advances in computer science now enable the development of a *new generation* of interactive playback that improves accuracy in testing hypotheses on the temporal dynamics of communication. Playback is considered as "interactive" as soon as one parameter is interactive, for instance, when the starting time of the playback depends on the focal individual or when the vocal exchange, broadcast by the playback, represents an interactive vocal exchange (S. L. King, 2015). By *new generation* of interactive playback, I mean an automated routine that continually takes decisions on the type and timing of calls to broadcast based on the animal's currently changing vocal behaviour. The main limitation of this tool is that one needs a good background knowledge on the study model to develop a meaningful routine. For barn owls, such a background knowledge is available.

In natural vocal exchanges between two live siblings, nestlings positively adjust call durations to their contestant's call duration (Dreiss, Ruppli, Antille, & Roulin, 2014) while nestlings negatively adjust call rate to their contestant's call rate (Dreiss et al., 2015). First, we investigated the impact of the moment that a nestling decides to escalate vocal competition (increases its call duration and its call rate) according to its contestant vocal behaviour [Chapter 1]. We designed two different interactive playback experiments, one for testing the call duration and another one for testing the call rate (the two main call parameters). The playback follows two different strategies: the "matching" strategy in which playback escalates when nestling escalates and the "mismatch" strategy in which playback escalates when nestling reduces. We hence predicted that a playback following the matching strategy for the call duration and the mismatching strategy for the call rate would better succeed in deterring the sibling from competing. Because the playback broadcast depends on the vocalization of a live nestling, the design enabled us to investigate the impact of these strategies on the playback broadcast. To prevent dishonesty, signalling should entail costs, either realization costs (the handicap principle: Grafen, 1990; Zahavi, 1974), performance costs or potential costs of cheating (Lachmann, Szamado, & Bergstrom, 2001). We hence predicted that if matching duration and mismatching call rate is more efficient to deter a sibling from competition, following these strategies should be costlier than not following them in terms of number of calls and call duration emitted.

In a second study [Chapter 2], we investigated the role of social feedback in maintaining social coordination rules. Social control through auditory feedback might play a role in the low occurrence of call overlap observed during sibling negotiation in barn owl (2%) (Dreiss et al., 2013). The aim of my second chapter was to investigate the existence of social control if a nestling overlaps the call of a sibling by using an interactive playback that either overlaps or does not overlap the call of a nestling, keeping other call parameters constant. It has been repeatedly demonstrated that by increasing call rate and duration, nestlings deter siblings from competing, thus getting access to the next food item. We therefore predicted that when overlapped by a playback call, a nestling should increase its call duration and rate in order to give a social feedback.

PART 2: DOES A NESTLING RELY ON VOCAL NEGOTIATION TO DECIDE TO WHOM IT WILL SHARE A PREY?

Kin selection and reciprocation are non-mutually exclusive mechanisms that explain the evolution and maintenance of cooperative behaviours. A previous study showed that nestling barn owls trade food sharing with allopreening, a nestling being more inclined to give a prey to the sibling by whom it was allopreened the most (Roulin et al., 2016a). However, the occurrence of allopreening and food sharing has been studied only under an experimentally reduced brood (i.e. two or three nestlings) that was fed *ad libitum*, and did not take into account the level of vocal negotiation. Two aspects have not been investigated in this design: the market quality in which the trade is made (i.e. food abundance) and the role of the negotiation in the decision process to favour indirect benefit only (feed the neediest sibling) over additional direct benefit (feed the sibling that allopreened you). The goal of my third chapter was therefore to investigate the cost-to-benefit balance of allofeeding behaviour considering prey abundance, vocal negotiation and allopreening behaviour. To this end, I monitored these social behaviours (i.e. allopreening, allofeeding and vocal negotiation) during two consecutive nights, in a complete brood *in natura* under two different feeding conditions (food supplemented, or naturally fed by parents). By doing this experiment *in natura* we succeeded in monitoring parental food provisioning, which might also play an important role. We predicted that a nestling will be more inclined to allofeed a sibling if the food is abundant (food supplemented condition) and/or if parents unequally distribute food by favouring this nestling. Second, when a nestling is facing the choice of to whom it will provide food, a nestling should favour the sibling that preened it the most except if there is a highly needy sibling (highly vocal).

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CHAPTER 1

Test for the optimal strategy of vocal adjustment during sibling competition. Real time adjustment with an automated interactive playback.

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Keywords: Communication, Interactive Playback, Sibling Negotiation, Temporal Dynamics, Matching, Mismatching, *Tyto alba*

ABSTRACT

During animal conflict, the role of signal performance to outcompete a contestant is well known, however dynamic adjustments to the contestant's signalization can be as important, but remains understudied. Here we use an automatic interactive playback experiment to test the efficiency of different adjustment strategies in the barn owl (*Tyto alba*) nestlings that vocally negotiate for food when the parents are absent. We found that to induce the withdrawal of a focal nestling from the competition, it is more efficient to match its call duration (i.e. mimicking the live nestling's change) and to mismatch its call rate (i.e. doing the opposite change than the live nestling's change). By analysing the effect of the adjustment strategies on the interactive playback broadcast, we found that these most efficient strategies require a higher investment of the playback (more and longer calls) than the less efficient strategy. Although the most efficient strategy is to give one's sibling the opportunity to call by waiting for a relaxation of its call rate before increasing one's own number of calls, in the end it is actually costlier than escalating over the contestant's vocalization. Our result highlight the importance of signalling adjustment which might be species and context dependent.

INTRODUCTION

To resolve conflicts over limited resources or collaborative tasks, animals communicate to assess their relative motivation to compete, to decide to retreat or keep investing in the contest (Maynard Smith, 1982; Parker, 1974). Although some signals are fixed, such as colouration, transient signals can vary quickly independently of changes in internal condition (Briffa, Elwood, & Dick, 1998; Greenfield, Tourtellot, & Snedden, 1997). Such communication is well-known to be an interactive process (Briffa et al., 1998; Enquist & Leimar, 1983; Enquist, Leimar, Ljungberg, Mallner, & Segerdahl, 1990; Payne & Pagel, 1996). However, the adjustment strategies at a small time scale and their impacts are yet understudied, despite having a potentially large impact on conflict outcome (Briffa et al., 1998; Dreiss, Ruppli, Faller, & Roulin, 2015; Patricelli, Uy, Walsh, & Borgia, 2002; Van Dyk, Taylor, & Evans, 2007). To outcompete conspecifics, the adjustment strategy – that is to say, when to escalate and when to de-escalate according to the contestant's communication – may be as crucial as the average signal strength (Patricelli et al., 2002).

In the context of conflict over territory, for which the role of communication has been described in depth, signalling has been mainly studied through the use of aggressive signals in order to demonstrate the contestants' dominance and/or motivation to compete (Searcy & Beecher, 2009). A signal is considered as aggressive when it signals a willingness to escalate the conflict. The use of aggressive signals leads therefore to an escalation over signalization until one of the contestants retreats or physical fight arises (Enquist & Leimar, 1983; Enquist et al., 1990). A fine scale adjustment has been reported during territorial conflict where competitive males match the contestant's song type (Akcay, Tom, Campbell, & Beecher, 2013; Beecher, Stoddard, Campbell, & Horning, 1996; Burt, Campbell, & Beecher, 2001; Krebs, Ashcroft, & Vanorsdol, 1981) or acoustic cues like call frequency (Otter, Ratcliffe, Njegovan, & Fotheringham, 2002) which is considered to be an aggressive signal too in such context. In the context of conflict over food, passerine nestlings mainly escalate begging behavior toward each other's begging (Leonard & Horn, 1998; Price & Ydenberg, 1995; Smith & Montgomerie, 1991). However, in passerines this adjustment appears to be inevitable because parents deliver food during a very short period of time, implying that nestlings have to beg rapidly at a precise time point. However, when conflict of interest occurs in social group context and time is not limited, the use of escalation strategy could be counterproductive because it is likely that future interactions will occur between the same individuals. The evolution of communication strategies that do not lead to physical aggression and in which individuals are keener to withdraw in front of motivated competitors could emerge (Crowley, 2001; Franz, van der Post, Schulke, & Ostner, 2011). Studying the short-term adjustment should hence provide key elements to understanding the interactive dynamics that lead to the resolution of a conflict in a non-aggressively social context.

In the present study, we investigated the impact of adjustment strategies of vocal communication in the context of family conflict among barn owl siblings (*Tyto alba*). While waiting for the food item brought by a parent, barn owl siblings "negotiate" to set up the priority access to it. The nestling that succeeds in silencing its siblings would most likely obtain the prey item. To do so, nestlings use one call type that they modulate in rate and duration (Johnstone & Roulin, 2003; Roulin, Kolliker, & Richner, 2000). Negotiation occurs for each prey item, which correspond to 2-4 preys per nestling and per night on average (Taylor, 2004). Therefore, the context of negotiation is a long-term interaction during which multiple negotiation sessions occur with the same participants that are kin, hence sharing indirect genetic benefits. The use of aggressive signals should therefore be avoided. Accordingly, two main rules are used. First, the hungrier a nestling is, the more and longer calls it emits. Second, nestlings call less and emit shorter calls when facing a sibling emitting long calls at a high rate on average (Dreiss, Lahlah, & Roulin, 2010; Roulin, Dreiss, Fioravanti, & Bize, 2009; Ruppli, Dreiss, & Roulin, 2013). Hence, the sibling negotiation process does not escalate, with nestlings tending to withdraw when confronted with vocal siblings, if the likelihood of receiving the next indivisible prey is low. However, on a fine scale, the process is more complex and it appears that nestlings assess a contestant‘s acoustic change (an increase or decrease) and use it to decide when they engage in a negotiation session (Dreiss et al., 2015). Nestlings are more likely to start vocalizing when the contestant decreases its call rate and call duration. Furthermore, siblings can adjust to each other during rapid vocal exchange. When two live sibling are exchanging calls, they globally adjust their call duration positively to that of their contestants (Dreiss, Ruppli, Antille, & Roulin, 2014) while negatively adjusting call rate (Dreiss et al., 2015). In other words, each duration of call is positively correlated to the duration of the contestant's previous calls while the call rate at small time scale (10th previous calls) is negatively correlated to contestant's call rate during the same time lapse. These suggest that nestlings match others’ change for the call duration and mismatch others’ change for the call rate. Although these adjustment strategies have been observed in vocal exchanges between two nestlings and between a nestling and a playback broadcast, no experiment has been developed to test whether these vocal adjustment strategies were optimal to silence a contestant. We hence predicted that following these adjustment strategies would increase the chances of reaching an agreement, resulting in the retreat of the less hungry nestling.

To test these predictions, we designed two automated interactive playback experiments, which varied in the way the playback adjusted its broadcasted calls to the nestlings’ vocal behaviour. The first experiment tested the effect of call duration adjustments and the second tested call rate adjustments. A playback is considered as “interactive” if one parameter is interactive, for instance, when the starting time of the playback depends on the focal individual or, when the vocal exchange broadcasted by the playback represents an interactive vocal exchange (King, 2015). By automated interactive playback, we mean an automated routine that continually makes decisions on the type and timing of calls to broadcast based on the animal’s changing vocal behaviour. In our design, playback calls varied depending on proximal changes of the focal nestling’s calls parameters. The playback follows two adjustment strategies: the “matching” adjustment in which the playback matches the contestant’s vocalization (i.e. increases when nestling increases vocal investment and reversely) and the “mismatching” adjustment in which the playback mismatches the contestant’s vocalization (increases when nestling decreases vocal investment and reversely). Throughout these experiments, we were able to analyse the direct impact of defined adjustment strategies on a focal nestling’s vocalization, for the mean value and the change of its acoustic cues. On the other hand, we were able to analyse the direct impact on the playback vocalization following the defined adjustment.

METHODS

General procedures

This study was performed on a wild barn owl population in western Switzerland (46°4’N, 6°5’E). Between April 30th and September 2nd 2015, 114 nestlings were brought to the laboratory for 3 days and 2 nights (age: 34±0.5 days; range 22-41 days; 52 males, 57 females and 5 of unknown sex). The first night was an acclimation period during which siblings stayed together in a wood nest-box (similar to the one where they were raised). They were fed *ad libitum* (67g of mice per nestling, (Durant & Handrich, 1998)). At 08:00 the following morning, the remaining food was removed. Then, at 12:00, all nestlings were weighed and isolated in separated experimental wood nest-boxes to allow for habituation to the new environment before 22:00, when the experiment started. Between 22:00 and 01:00, the "call duration" interactive playback was performed and between 1:30 and 4:30, the "call rate" interactive playback was performed. On the following morning, nestlings were fed and returned to their original nest.

The experimental nest-box was similar to the one in which the nestlings were raised but was divided in two equal parts by a thin wooden wall pierced with five holes. A loudspeaker (near05 experience, ESI Audiotechnik GmbH, Leonberg, Germany) was placed in one of the partitions while the nestling occupied the second. Two microphones (MC930, Beyeradjustment GmbH & Co KG, Heilbronn, Germany) per nest-box were fixed on the roof and were oriented toward the nestling. One microphone was used to record the nestling all night long and the other was used for the interactive playback programs developed in Matlab R2012b 8.0.0.783 (MathWorks. Natick, MA, U.S.A.).

Playback experiment design

The program detected in real time the calls emitted by the nestling and determined their duration (for detail on acoustic criteria used see Supplementary S1 and Ducouret, Dreiss, Marmaroli, Falourd, & Roulin, 2016).

a. Playback adjustment in Call Duration.

In order to assess the global variation of the call duration of focal nestlings, at the end of each 10s lapse, the computer program compared the mean nestling call duration with the mean duration during the previous 10s lapse. In this experiment, the playback broadcasted one call every 10s so the playback call rate was 6 calls/min, corresponding to the mean call rate observed in two-nestling broods when food deprived (Ruppli et al., 2013). According to this comparison, the playback modified its call duration, following two different inter-individual adjustments (Figure 1, for an example of calculation see Supplementary S2). (1) In the matching adjustment (termed "Match-Call Duration"), the playback modified its call duration in the same manner as the sibling. If the nestling increased (or decreased) its call duration between two successive 10s lapses, the playback broadcasted a call with the same extent of increase (or decrease) in duration. (2) In the mismatching adjustment (termed “Mismatch-Call Duration”), the playback modified its call duration in the opposite direction as the nestling. If the nestling increased (or decreased) its call duration between two successive 10s lapses, the playback broadcasted a call with a decreased (or increased) call duration within the same extent. In the two above adjustments, playback’s call duration remained constant when no call was emitted by the nestling during a 10s lapse. The playback followed a unique adjustment during 15min, before changing to another adjustment (randomly selected). At the end of the first 10s lapse of each 15min period, the playback emitted a call with the same duration as the nestling’s mean call duration.

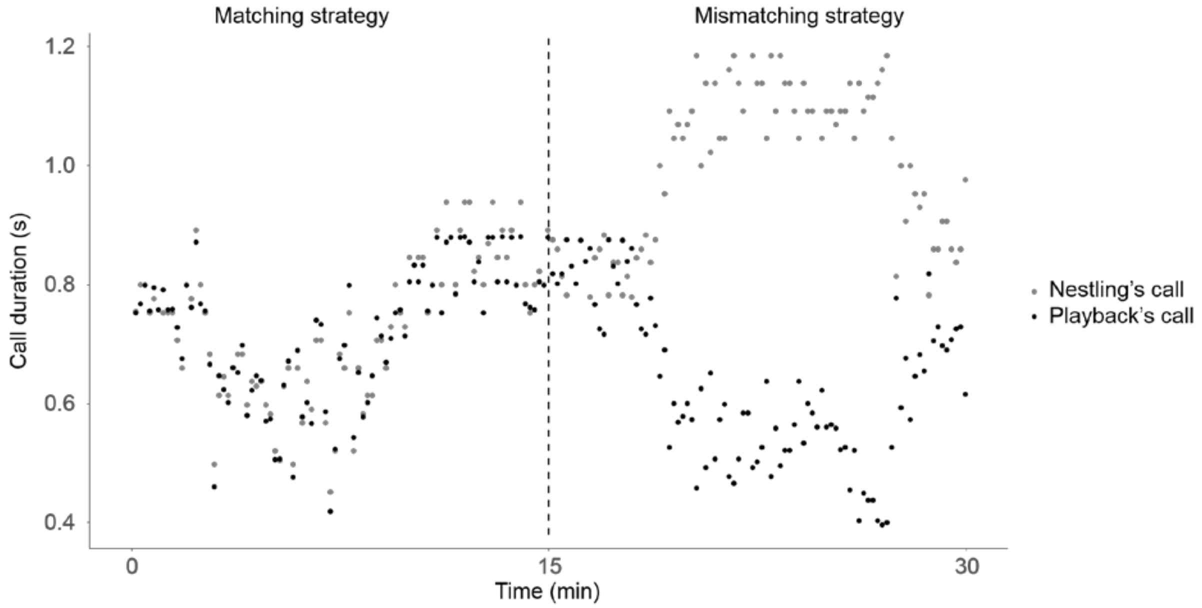

Figure 1 – Two periods illustration of interactive playback experiment on call duration. The computer program automatically detects the calls emitted by a focal nestling and measures its duration. Then, the program compares this duration to the previous one and, according to this duration change, chooses a call to broadcast of a duration based on a pre-programmed calling strategy. Two strategies were developed: under matching strategy, left part of the graphic, the playback changes its call duration similarly to the nestling and under the mismatching strategy, right part of the graphic, changes its call duration reversely to the nestling. Each treatment lasted 15 minutes and were randomly ordered across the 4 hour experiment.

b. Playback adjustment in Call Rate.

The second interactive playback was developed with the same approach as the above experiment but focusing on the number of calls emitted by the nestling and not on the call duration (for an example see Supplementary S3). As such, (1) in the matching adjustment (termed “Match-Call Rate”), if the nestling emitted x more (or less) calls than during the previous 10s lapse, the playback broadcasted x more (or less) calls than during the previous 10s lapse. (2) In the mismatching adjustment (termed “Mismatch-Call Rate”), if the nestling emitted x more (or less) calls than during the previous 10s lapse, the playback broadcasted x less (or more respectively) calls than during the previous 10s lapse. In the two adjustments above, the playback’s calls were distributed during the 10s lapse. We fixed the broadcast call duration at 800ms on average (*SE* = 50 ms), the average value obtained from interacting pairs of nestlings (Ruppli et al., 2013). The playback followed a unique adjustment during 15min, before changing to another adjustment (randomly selected). At the second 10s lapse of each 15min period, the playback emitted the same number of call as the nestling during the first 10s lapse.

Construction of playback soundtracks

Calls broadcasted by the playback were isolated from four different individuals (age *mean ± SE*: 32.5 ± 2.25 days, two males and two females) recorded in a three-nestling brood experiment conducted in 2011 (for experimental setup details see Dreiss et al., 2017). At the beginning of the experiment, a unique individual was selected randomly by the computer to be broadcasted to one focal nestling. Therefore, a focal nestling faced a unique playback individual. In total, 120 calls were isolated, 15 in each of the eight call-duration groups: 300-400ms, 400-500ms, 500-600ms, 600-700ms, 700-800ms, 750-850ms, 800-900ms, 900-1000ms and 1000-1100ms. The computer randomly picked up one call within the relevant group. For the call rate experiment, only calls from the 750-850ms group were picked up by the playback. Each call was first normalized to have the same loudness using Matlab R2012b.

Acoustic analyses

In order to detect a call emitted by the nestling in “pseudo” real time, the audio record was analysed each 46ms time windows to determine if there is an acoustic event or not with the sound pressure level. Then, to determine if this call is a negotiation call, we use 2 frequency descriptors and a temporal descriptor. For further technical details see Ducouret et al. (2016) and supplementary S1. We tested the accuracy of this detection in pseudo real time by comparing manually 180.5 hours of a recording including 55247 negotiation calls of an interacting pair of nestlings made in similar conditions with the same acoustic material. We achieved an accuracy of 97% of true detection (97% of the negotiation call were correctly detected) and 3% of wrong detection (the software detect another type of call or noise as a negotiation call).

Statistical analyses

Overall, 55 nestlings either did not emit any call, called less than five calls per period or only called during one period in the call duration experiment. These individuals were removed from the subsequent analyses. The absence of calls may be explained by a playback call rate representing a more competitive individual than the tested nestling. For the call rate experiment, a total of three individuals were removed from the analyses due to their absence of calls during the 3-hour experiment.

All the statistical analyses were done with the R software (2.15.2 version, The R Project for Statistical Computing). The package used for performing linear mixed models was “lme4” (Linear mixed-effects models using Eigen and s4). Residuals were checked for homoscedasticity for each model.

In each model presented below, the order in which the adjustment strategies appeared as well as the nestling’s age and sex were included as covariates and cofactor. Nestling identity, nested within its brood identity, was included as a random factor to control for pseudoreplication.

Impact of playback adjustments on the absolute value of nestling’s and playback’s call parameters

We investigated the global effect of the adjustment strategies (Match- and Mismatch-Call Duration and Rate) by considering the total number of calls and the mean call duration emitted by the nestling and by the playback in each 15-min period for both experiments. We then tested the effect of the two adjustments strategies on the focal parameter (i.e. the call rate for the call rate experiment and the call duration for the call duration experiment) and the cross effect on the other call parameter (i.e. the call rate for the call duration experiment and reversely). We ran two independent but similar linear mixed models for the focal parameter, one to investigate the effect on the nestling's call parameters and one on the playback's call parameters. Concerning the cross effect on the other call parameters, we ran two independent but similar linear mixed models for the call duration and the call rate. Call rate was boxcox transformed in order to analyse it as Gaussian variable.

Impact of playback adjustments on the trend of nestling's call parameters

We investigated the change of nestling's focal call parameters in function of time using a cubic linear mixed model for the call duration experiment and a generalized cubic mixed model with "Poisson" distribution for the call rate experiment. We included a comparison between adjustment strategies, incorporating the factor treatment in interaction with each time parameter. We centred each time variable to correct for collinearity and we controlled for the overdispersion of the call rate analyses by incorporating a observation variable as random factor (Zuur, Ieno, Walker, Saveliev, & Smith, 2009). We used the call rate per minute to assess the change in function of time.

Impact of playback dynamics on the negotiation outcome: focal nestling withdrawal

In order to assess the efficiency of an adjustment strategy in inducing the focal nestling to retreat, we calculated the duration of the final phase during which the focal nestling didn't emit calls. We considered that a nestling had retired when it stopped emitting calls for more than one minute before the end of each 15-min periods (thresholds of 30 s and 2 min were also tested and lead to similar results). Because of the high number of zeros, which correspond to individuals that did not stop vocalising, we investigated if the probability that a nestling retired was higher when playback used one or another adjustment strategy using a generalized mixed model with binomial distribution.

Ethical notes

Experiments were done at a rearing stage (on average mean ± SD: 34 ± 0.5 days) at which it does not disturb parental care as parents stay outside the nest and only enter briefly to bring the hunted prey. At least two nestlings were left at the natural nest to continue stimulating the parental care and prevent abandon which was not observed during the experiment. It was already shown that this type of experiment does not stress nestlings and does not lead to a worse condition at the fledgling stage. Experiments were carried out within University of

Lausanne's facilities, in hold of all required permits from the veterinary services (authorization 2109.2).

RESULTS

Impact of playback adjustment strategies on nestling vocalization

a- Playback adjustment in Call Rate

The best playback strategy to induce a nestling to emit fewer calls was to mismatch its call rate, i.e. stay almost silent when the nestling is calling but increase call rate when the contestant decreased the number of emitted calls (Table 1a, Figure 2a). When facing a playback that mismatched their call rate, nestlings reduced call rate more rapidly than when facing a playback that matched their call rate, as shown by the cubic equation of the number of nestling calls in function of time (Table 2, Figure 2c). The mismatching strategy also led the nestlings to total silence more ($X^2 = 89.98$, $P < 0.001$, mean probability of becoming silent was 0.65 and 0.13 for the mismatching and matching strategy, respectively; Table 4). Nestlings that listened to a playback that mismatched the call rate also emitted shorter calls than when they listened to a playback that matched call rate (estimate ± SD: -0.018 ± 0.0086 s, Table 3).

b- Playback adjustment in Call Duration

The best negotiation strategy for adjusting call duration was exactly the opposite of the one for call rate. To induce nestlings to emit shorter calls, playbacks had to emit calls that matched rather than mismatched the duration of the nestlings' calls (Table 1a, Figure 2b). Nestlings delayed the moment when they started to increase call duration and this increase was less pronounced when listening to a playback that matched call duration, as confirmed by the cubic equation of call duration in function of time (Table 2, Figure 2d). Nestlings that listened to match and mismatch strategies called at a similar rate and were as likely to stop vocalizing (probability is 0.17, $X^2 = 0.30$, $P = 0.58$, Tables 3 and 4), indicating that when the playback modulates call duration this affects nestling call duration but not call rate.

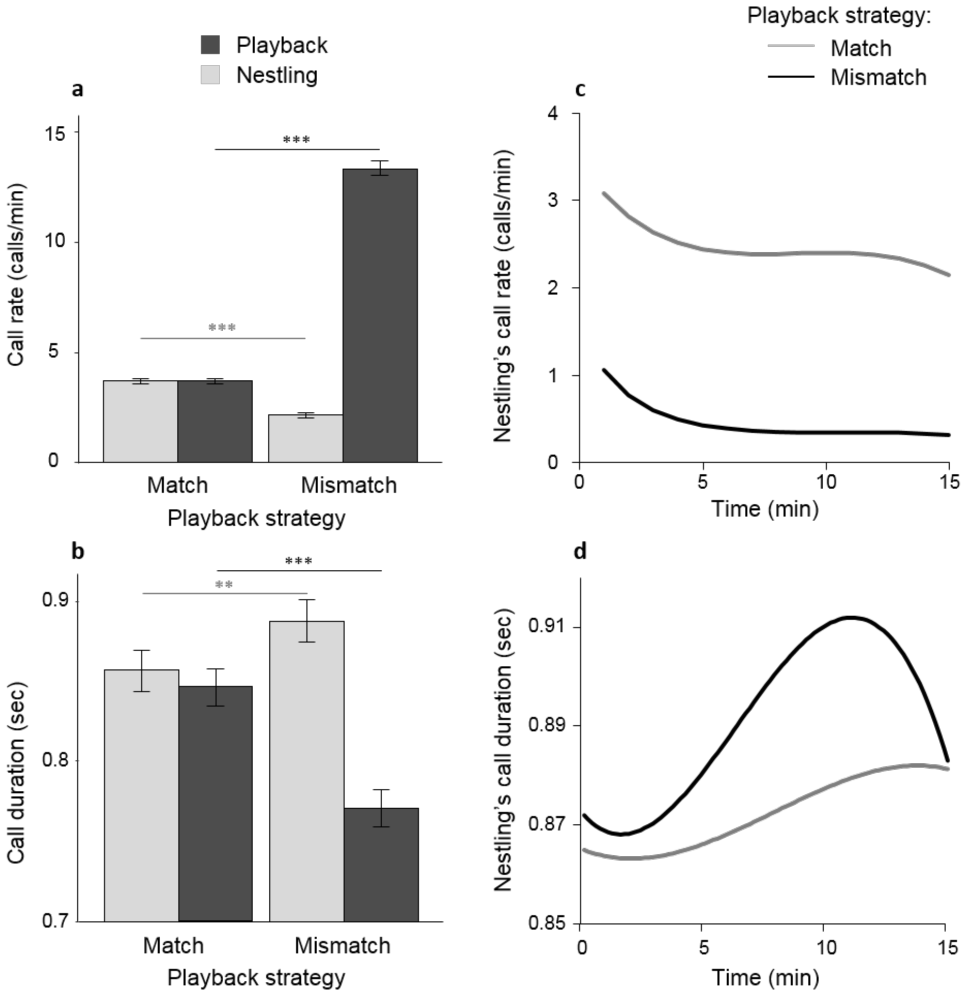

Figure 2 – Mean call rate (a) and mean call duration (b) of live nestlings and of the playback matching and mismatching strategies. P-values (*** is for P < 0.001 and ** for P < 0.01) are derived from the linear mixed model presented in Table 1. Fitted number of calls (c) and call duration (d) emitted by nestling barn owls in relation to time (an experiment lasted 15 minutes), based on the cubic models presented in Table 2. All other numeric covariates of the final model were set at their means.

Impact of playback adjustment strategies on playback call parameters

Mismatch-Call Rate strategy led the playback to broadcast more calls than Match-Call Rate (Table 1, Figure 2a). Match-Call Duration strategy led the playback to broadcast longer calls than Mismatch-Call Duration (Table 1b, Figure 2b). By following the adjustment strategies that are the most efficient to restrain a nestling from vocalising (i.e. Match-Call Duration and Mismatch-Call Rate), the playback vocalized more intensely, emitting more calls during the call rate experiment and longer calls during the call duration experiment.

Table 1 – Effect of the playback negotiation strategies on the vocal behaviour of nestling barn owls (a) and of the playback itself (b). (a) Linear mixed models testing whether the duration of nestling negotiation calls and the rate at which they were emitted are related to the matching vs. mismatching playback strategies. (b) Similar models testing whether the playback emits more or shorter calls when its strategy is to match the nestling behaviour rather than when mismatching the nestling behaviour. Nestling identity nested in brood identity was included as random factor to correct for pseudoreplication.

(a)	**Nestling call rate**			**Nestling mean call duration**		
Fixed effects	**Estimate (SE)**	**F**	**P-value**	**Estimate (SE)**	**F**	**P-value**
Playback strategy	-0.77(0.043)	$F_{1,517} = 315.03$	<0.001	15.47(6.98)	$F_{1,262} = 4.91$	0.028
Nestling age	-0.034(0.014)	$F_{1,86} = 5.74$	0.019	-0.22(5.81)	$F_{1,47} = 0.001$	0.97
Nestling sex	-0.17(0.10)	$F_{1,80} = 2.67$	0.11	-81.58(46.39)	$F_{1,48} = 3.09$	0.085
Order of playback strategies	-0.013(0.007)	$F_{1,516} = 3.29$	0.070	7.07(1.21)	$F_{1,263} = 34.20$	<0.001

(b)	**Playback call rate**			**Playback mean call duration**		
Fixed effects	**Estimate (SE)**	**F**	**P-value**	**Estimate (SE)**	**F**	**P-value**
Playback strategy	1.30(0.036)	$F_{1,540} = 1300.1$	<0.001	-79.27(14.77)	$F_{1,295} = 28.80$	<0.001
Order of playback strategies	-0.0038(0.006)	$F_{1,539} = 0.42$	0.52	3.94(2.52)	$F_{1,312} = 2.44$	0.12

Table 2 – Temporal effect of the playback negotiation strategy on the vocal behaviour of nestling barn owls. A cubic linear mixed model tested the temporal change in the duration of nestling calls and a cubic generalized linear mixed model tested the temporal change in the rate at which nestlings called. The time, time^2 and time^3 were standardized to correct for collinearity and nestling identity nested in brood identity was included as random factor to correct for pseudoreplication.

	Nestling call rate			**Nestling mean call duration**		
Fixed effects	**Estimate (SE)**	**Chisq**	**P-value**	**Estimate (SE)**	**F**	**P-value**
Playback strategy	-0.79(0.021)	1218.3	<0.001	18.36(1.94)	$F_{1,13651} = 89.16$	<0.001
Time	-0.13(0.032)	89.05	<0.001	-8.98(13.28)	$F_{1,13648} = 3.48$	0.062
Time^2	0.015(0.0046)	41.15	<0.001	36.75(31.74)	$F_{1,13647} = 14.18$	<0.001
Time^3	-0.00057(0.00019)	26.18	<0.001	-21.5(19.85)	$F_{1,13647} = 17.47$	<0.001
Nestling age	-0.035(0.013)	7.31	0.0068	-0.75(5.69)	$F_{1,46} = 0.02$	0.90
Nestling sex	-0.19(0.096)	3.9	0.048	-89.64(45.51)	$F_{1,48} = 3.88$	0.055
Order of playback strategies	-0.0070(0.0033)	4.51	0.034	8.13(0.33)	$F_{1,13658} = 585.78$	<0.001
Time x Playback strategies	-0.31(0.053)	33.12	<0.001	-16.56(18.44)	$F_{1,13647} = 0.81$	0.37
Time^2 x Playback strategies	0.026(0.0079)	10.64	0.0011	93.02(44.05)	$F_{1,13647} = 4.46$	0.035
Time^3 x Playback strategies	-0.00071(0.00034)	4.41	0.036	-72.56(27.53)	$F_{1,13647} = 6.95$	0.0084

Table 3 – Cross-effects of the playback strategies on the vocal behaviour of nestling barn owls. Linear mixed models tested whether the two call duration playback strategies (matching vs. mismatching) affected the rate at which nestling vocalized and whether the two call rate playback strategies affected the duration of nestling calls. Nestling identity nested in brood identity was included as random factor to correct for pseudoreplication.

	Nestling call duration (Call rate experiment)			**Nestling call rate (Call duration experiment)**		
Fixed effects	**Estimate (SE)**	**F**	**P-value**	**Estimate (SE)**	**F**	**P-value**
Playback strategy	-0.018(0.0086)	$F_{1,313} = 4.56$	0.033	0.023(0.071)	$F_{1,264} = 0.11$	0.74
Nestling age	-0.0088(0.006)	$F_{1,67} = 2.15$	0.15	-0.061(0.027)	$F_{1,38} = 4.98$	0.031
Nestling sex	-0.12(0.044)	$F_{1,60} = 7.55$	0.0079	-0.034(0.22)	$F_{1,37} = 0.024$	0.88
Order of playback strategies	0.00099(0.0014)	$F_{1,314} = 0.48$	0.49	0.0017(0.012)	$F_{1,268} = 0.018$	0.89

Table 4 – Effect of the playback negotiation strategies on the probability that nestling barn owls became silent at least 1 minute before the end of the 15-min experimental period. Generalized linear mixed models (binomial) testing whether the probability that barn owl nestlings retreat from vocally negotiating is related to the playback matching vs. mismatching strategies for call rate and call duration. Nestling identity nested in brood identity was included as random factor to correct for pseudoreplication.

	Probability that nestling became silent					
	Call rate experiment			**Call duration experiment**		
Fixed effects	**Estimate (SE)**	**Chisq**	**P-value**	**Estimate (SE)**	**Chisq**	**P-value**
Playback strategy	2.50(0.26)	89.98	<0.001	0.17(0.32)	0.30	0.58
Nestling age	0.039(0.041)	0.89	0.34	0.0023(0.059)	0.0015	0.97
Nestling sex	0.46(0.31)	2.25	0.13	0.59(0.46)	1.62	0.20
Order of playback strategies	0.037(0.035)	1.11	0.29	0.00049(0.055)	0.0001	0.99

DISCUSSION

Using automated interactive playback, experiments that modify in real time the broadcasted calls' parameters according to nestling's call parameters, we have pinpointed the importance of short time scale adjustments during the resolution of food conflict in barn owl nestlings. We demonstrated that Match-Call Duration strategy (i.e. increasing and decreasing call duration at the same time as the contestant) and Mismatch-Call Rate strategy (i.e. increasing the call rate when the contestant decreases its call rate and reversely) lead to a de-escalation in the contestant's vocalization (shorter and fewer calls respectively). By naturally following these adjustment strategies during the sibling negotiation process, nestlings establish dominance without inducing vocal escalation from their contestant (Dreiss et al., 2010; Roulin et al., 2009; Ruppli et al., 2013). In the present study, we also obtained clear evidence that by following these strategies, the playback gradually intensifies its signal (more and longer calls). It is, to the best of our knowledge, the first implementation of such interactive playback design that allowed to investigate at the same time the receiver and the sender perspective. We believe that this design will be very fruitful in future studies of complex animal signalling systems.

In barn owl nestling negotiation, although call duration and call rate are signals used to resolve the same conflict over food, adjustment strategies have opposite effects on the resolution. Indeed, Match-Call Rate induces an increase of contestant's call rate whereas Match-Call Duration induces a decrease of contestant's call duration. This suggests that these two signals may have two different functions within the same conflict resolution. During territorial conflict, male song sparrows (*Melospiza melodia*) also use different threatening signals with a hierarchical temporality, song type matching being an early threatening signal, followed by soft-song emission and finally wing waves (Akcay et al., 2013). By gradually emitting different signals, an individual gives the contestant the possibility to abandon the contest at an early stage without having invested too much in competition. Barn owl nestlings emit only one type of call but can follow different adjustment strategies. Call duration appears to be used as a challenging parameter, as the most efficient adjustment is to escalate and deescalate at the same time as the contestant, probably to test how far the contestant is willing to vocally compete. Contrarily, call rate seems to be used as a deterring parameter, as the most efficient dynamic is to escalate only when the contestant relaxes. It is also during the Mismatch-Call Rate treatment that the probability that nestlings retreated (i.e. stopped calling) before the end of the 15 min period is clearly the highest (i.e. 0.53 vs 0.20 for Constant Rate and 0.09 for Match-Call Rate and 0.17 for each adjustments strategies during the call duration experiment). Mismatch-Call Rate seems to be the ultimate signal that deters contestants from the contest. We hypothesize that vocal negotiation in nestling barn owl is a hierarchical signalling system. A nestling could challenge a sibling first by emitting a low rate of calls but matching sibling's call duration in order to assess the other's motivation. Next, when a dominance appears because a sibling relaxes its call rate, the nestling could increase its call rate to clearly demonstrate its dominancy and momentarily deter its sibling from calling. The negotiation call is also used as an alarm call by increasing its duration up to 3s.

During negotiation, nestlings emit calls at a duration far from their maximum (less than 1.5s) which suggests that such small increases are less costly than an increase of the number of calls. By doing so, an individual would only gradually engage during the negotiation to invest just enough to take the lead on the conflict. Future studies should take account such temporality in vocal competition strategies during vocal negotiation.

We have also shown that Match-Call Duration and Mismatch-Call Rate, the most efficient strategies, require a higher investment in terms of number of calls and call duration emitted by the sender, here the playback. Signalling its resource holding potential, motivation or quality when a conflict of interest occurs only is evolutionary stable as long as signals contain reliable information about sender (Maynard Smith & Harper, 2003; Searcy & Nowicki, 2005). To prevent dishonesty, signalling should entail costs (handicap principle: Grafen, 1990; Zahavi, 1974). In barn owl nestlings, displaying a high motivation by matching the contestant's call duration and mismatching the contestant's call rate requires a higher investment in vocalization with more and longer calls. Even if emitting calls might not be primarily costly (i.e. metabolic cost), demonstrating a high motivation by following the most efficient strategy requires not only a higher investment with more and longer calls but also a particular attention to the contestant's vocalization that could be costly because of processing of received signals.

This result concerning the sender vocalization (here the playback) is inherent to the species model as the playback broadcast depends on the live individual. We however could imagine that the result observed is also inherent to the adjustment rule defined. We chose to keep the playback's call parameter constant when the nestling did not emit any call during the previous 10s lapse. This choice could be questionable. Indeed in natural vocal exchange, nestlings decrease their call duration and rate after having deterred a sibling and such decrease is used as a signal to re-enter into the vocal negotiation (Dreiss et al., 2015). By not decreasing the playback's call parameter after having silenced the nestling could explain why the playback emitted so many calls during the Mismatch-Call Rate treatment for example. However, we did the same analysis by limiting the data to the ones where the nestlings kept calling and found similar results to those presented here. We can therefore consider fairly that our results are not artefacts of algorithm rules.

By waiting until their contestant relaxes before increasing their call rate, nestlings favour the exchange of information and give the opportunity for a sibling to vocalize. This finding confirms what previous studies demonstrated that barn owl nestlings avoid overlapping sibling’s call to limit signal interference (Dreiss et al., 2013) and favour alternation of monologue (i.e. only one nestling emits a series of calls during a period of time) to rapid alternation of senders (Dreiss et al., 2015). However, it is difficult to understand why an individual would wait to demonstrate its motivation knowing the risk that a parent could arrive at any moment. Two important aspects in barn owl nestlings could explain it: temporal and context. First, in barn owl, parental absence can be up to multiple hours and thus the negotiation process is a long-lasting one. This contrasts with many other birds where offspring signal only during the parents visits which are quick. In such species, offspring adjust also their level of begging to each other’s (Blanc, Ogier, Roux, Denizeau, & Mathevon, 2010; Leonard & Horn, 2001), however this usually induces offspring to escalate their begging behaviour in an attempt to be louder than their siblings quickly. Second, barn owl nestling negotiate with full siblings. The contested resource is thus consumed by a genetically related individual, providing inclusive fitness benefits to the individual that gave up (Hamilton, 1964). Moreover, this negotiation process occurs for each prey brought back by the parents every night during the rearing period. Nestlings can thus be considered as a social group within which multiple negotiation processes occur between the same participants. Giving the opportunity to a sibling to communicate without involving an escalation could be beneficial as it could appease vocal exchange and increase the trust between negotiators for future negotiation which could counter-balance the risk that a parent arrives.

Thanks to automated interactive playback we succeed in testing the impact of specific adjustment strategies, not only on the receiver response but also on the sender vocalization. However, three aspects have to be highlighted. First, since the playback analysed individual behaviour in pseudo-real time, the detection has to be reliable to be automatized. Second, a good knowledge of the species model is required to cover all the possible scenarios. Third, the parameter of interest has to be well defined in order to fix as much as possible the remaining parameters. Nevertheless, we believe that this design will be very rewarding in future studies of complex animal signalling systems because it refines the analyses of a dynamic process to a reduced time scale. It would be interesting to apply such design in different contexts of competition and species model to understand how such adjustments could be differently costly and have different signification.

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SUPPLEMENTARY MATERIAL

S1. Detection of nestlings' calls on pseudo-real time

The nestlings' calls have been recorded through one channel of a Presonus Firestudio sound card of 8 channels (i.e. maximum 8 parallel experiments were possible), sampled at 44100 Hz. The detection consists in considering recordings as a succession of small frames of N = 2048 samples which correspond approximatively to 46 ms. For each frame the algorithm answers two questions:

- Does an acoustic event occur?
- Does this acoustic event is a negotiation call or something else?

The first question is simply answered by comparing the noise level to a threshold (-10dB). The noise level is calculated through the sound pressure level L_p (in dB) by using the equation (1):

$$L_p[q] = 20\log_{10}\left(\sqrt{\frac{1}{N}\sum_{k=1}^{N} y^q[k]^2} \Big/ 2e^{-5}\right) \qquad (1)$$

where y^q is the sound pressure amplitude (in Pascal) of the current frame q with a size of Nx1

The answer of the second question is more complex and is based on three audio descriptors detailed below.

First descriptor: the spectral variance

Figure s1.a represent waveforms and spectrograms of a negotiation call and a chirp call emitted by the same nestling. The distribution of the average sound level of each frequency bin is also represented. A clear difference of the harmonicity of the calls emitted can be observed. Chirps have a marked harmonicity (a fundamental frequency and its integer multiples) which contrasts to the negotiation calls that cover the entire bandwidth uniformly.

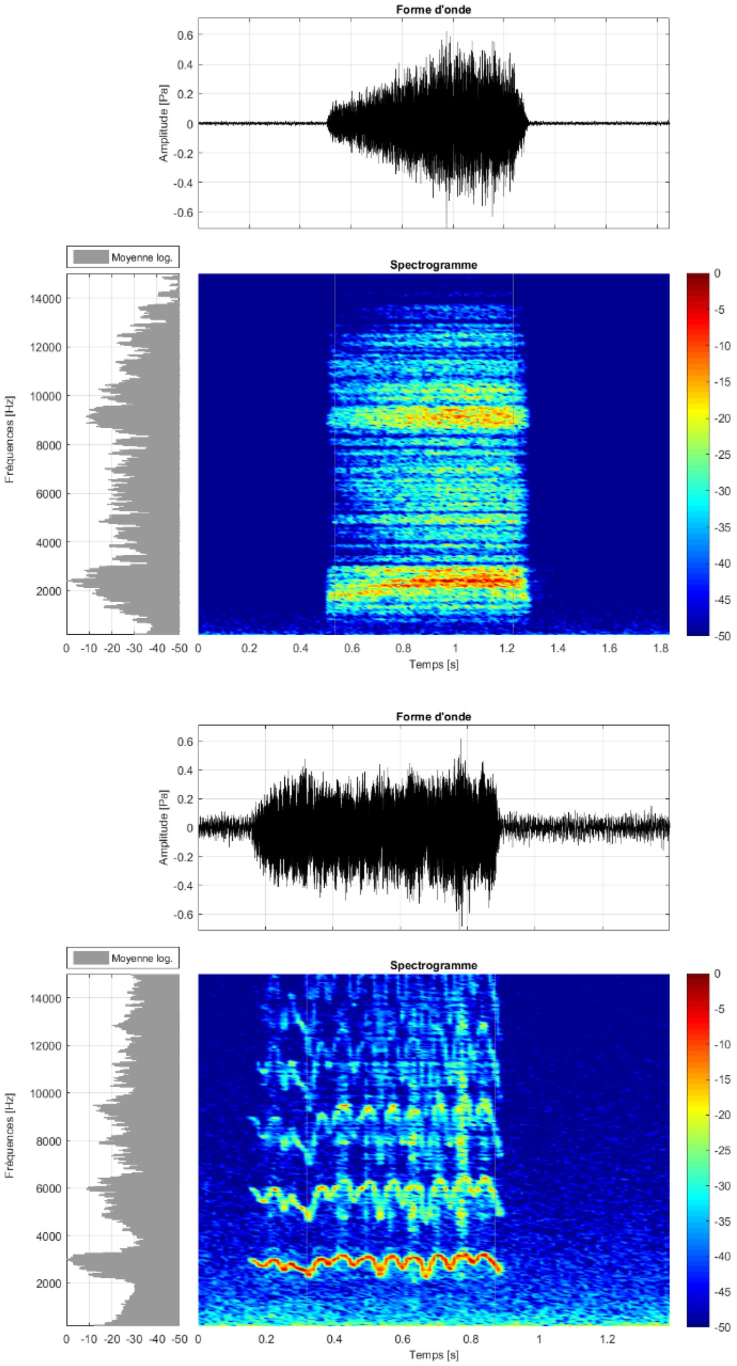


Figure s1.a – Waveform and spectrogram of a negotiation call on the top and of a chirp on the bottom of a barn owl nestling. Color scale is in dB.

Evaluating the more or less harmonic nature of a signal is a time-consuming process. We are therefore interested in observing an indirect consequence of this presence or not of harmonicity, using the variance of the spectrum, with the underlying idea that a negotiation call has a wider spectral range than chirps. We define this descriptor D_1 according to equation (2):

$$\mathrm{D_1[q]} = \log_{10}(\mathrm{Var}(|\mathbf{Y^q}|)) \qquad (2)$$

where $\mathbf{Y^q}$ is the Short Time Fourier Transform (STFT) of the qth frame

Second descriptor: minimal value of cepstral coefficients

Figure s1.b shows the evolution of cepstral coefficients (MFCC) of the same two calls than in Figure s1.a. We notice a fall of the third cepstral band value for the negotiation call, which does not appear for the chirp call. It was therefore decided to establish a descriptor D_2 to return the minimum value of the cepstral coefficient, as defined by equation (3):

$$\mathrm{D_2[q]} = min(\mathbf{MFCC^q}) \qquad (3)$$

where $\mathbf{MFCC^q}$ are the cepstral coefficient of the qth frame

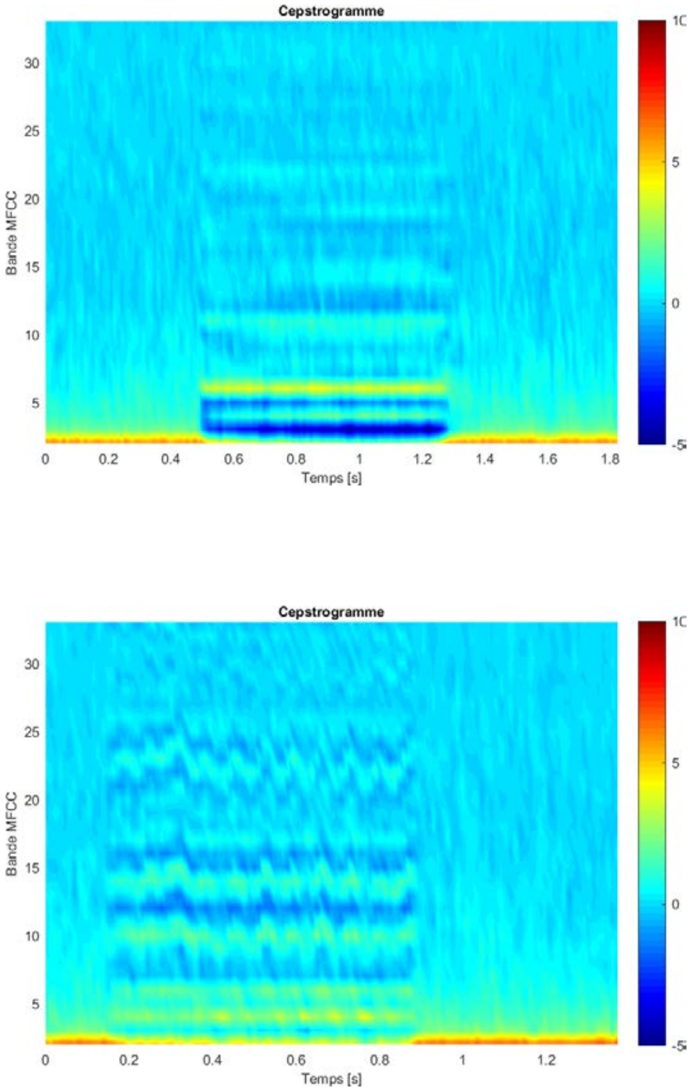


Figure s1.b – Cepstrogram of a negotiation call on the top with a fall in the third cepstral band value and of a chirp call on the bottom. Color scale is in dB.

To be considered as a negotiation call the current frame has to respect the condition (4), which correspond to a high variance and a minimum value of weak MFCC.

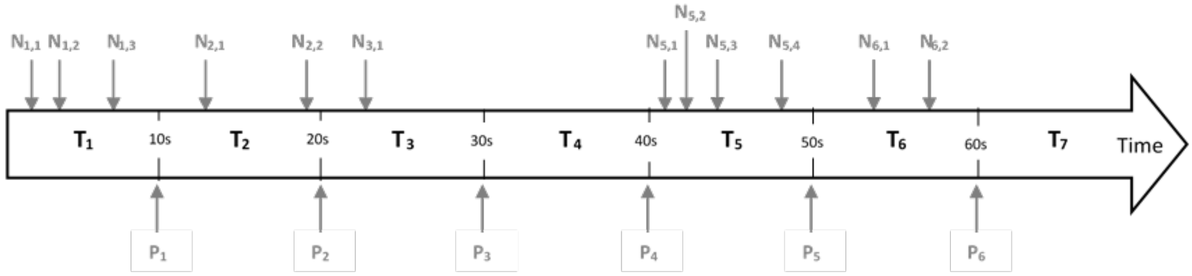
$$\mathrm{D}_1[\mathrm{q}] > \lambda_1 \ and \ \mathrm{D}_2[\mathrm{q}] < \lambda_2 \tag{4}$$

where λ_1 et λ_2 are empirically defined thresholds. $\lambda_1 = -6$; $\lambda_2 = -0.5$

Third descriptor: temporal consistency

A third descriptor, called temporal consistency, consists in considering the acoustic event as a negotiation call if it last at least 322ms which correspond to seven consecutive frames. In other words, seven frames must consecutively verify the condition (4) to be effectively considered as a negotiation call.

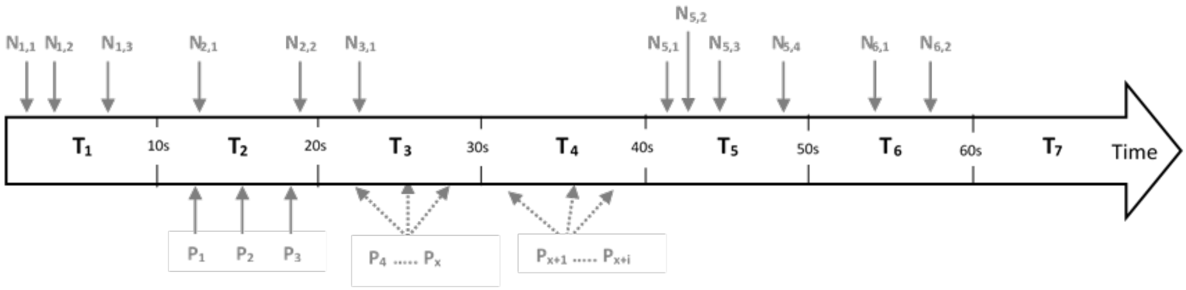
S2. Interactive playback experiment design for call duration: a one minute example



10s time lapse	Nestling call duration (ms)	Playback soundtrack call duration (ms)	
		Matching	Mismatching
T1	$D_{T1} = MEAN(D_{N1,y})$	$D_{P1} = D_{T1}$	$D_{P1} = D_{T1}$
T2	$D_{T2} = MEAN(D_{N2,y})$	$D_{P2} = D_{T2}$	$D_{P2} = D_{P1} - (D_{T2} - D_{T1})$
T3	$D_{T3} = D_{N3,1}$	$D_{P3} = D_{T3}$	$D_{P3} = D_{P2} - (D_{T3} - D_{T2})$
T4	$D_{T4} = NA$	$D_{P4} = D_{P3}$	$D_{P4} = D_{P3}$
T5	$D_{T5} = MEAN(D_{N5,y})$	$D_{P5} = D_{T5}$	$D_{P5} = D_{P4} - (D_{T5} - D_{T3})$
T6	$D_{T6} = MEAN(D_{N6,y})$	$D_{P6} = D_{T6}$	$D_{P6} = D_{P5} - (D_{T6} - D_{T5})$

Figure s2 – Example of one-minute interactive playback on the call duration. T_x: xth time lapse of 10s, $N_{x,y}$: yth call emitted by the nestling during xth time lapse, P_x: call broadcasted by the loudspeaker at the end of the xth time lapse of 10s, $D_{Nx,y}$: duration of the call $N_{x,y}$, D_{Tx}: average duration of the call emitted by the nestling during the xth time lapse of 10s, D_{Px}: duration of the call emitted by the playback at the end of the xth time lapse.

S3. Interactive playback experiment design for call rate: a one minute example



10s time lapse	Nestling number of call (/10s)	Playback soundtrack call rate (/10s)	
		Matching	Mismatching
T1	$R_{T1} = 3$	$R_{P1} = R_{T1} \rightarrow 3$	$R_{P1} = R_{T1} \rightarrow 3$
T2	$R_{T2} = 2$	$R_{P2} = R_{T2} \rightarrow 2$	$R_{P2} = R_{T1} - (R_{T2} - R_{T1})$ $\rightarrow 4$
T3	$R_{T3} = 1$	$R_{P3} = R_{T3} \rightarrow 1$	$R_{P3} = R_{T2} - (R_{T3} - R_{T2})$ $\rightarrow 5$
T4	$R_{T4} = 0$	$R_{P4} = R_{T4} \rightarrow 0$	$R_{P4} = R_{T3} - (R_{T4} - R_{T3})$ $\rightarrow 6$
T5	$R_{T5} = 4$	$R_{P5} = R_{T5} \rightarrow 4$	$R_{P5} = R_{T4} - (R_{T5} - R_{T4})$ $\rightarrow 2$
T6	$R_{T6} = 2$	$R_{P6} = R_{T6} \rightarrow 2$	$R_{P6} = R_{T5} - (R_{T6} - R_{T5})$ $\rightarrow 4$

Figure s3 – Example of a one-minute interactive playback on the call rate. T_x: xth time lapse of 10s, $N_{x,y}$: yth call emitted by the nestling during the xth time lapse of 10s, P_x: call broadcasted by the loudspeaker at the end of the xth time lapse of 10s, R_{Tx}: nestling's call rate during the xth time lapse of 10s, R_{Px}: playback call rate during the x+1th time lapse.

CHAPTER 2

Barn owl nestlings vocally escalate when interrupted by a sibling: evidence from an interactive playback experiment.

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This chapter is published in **Animal Behaviour (2018) 51-57**

Animal Behaviour 145 (2018) 51–57

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Barn owl nestlings vocally escalate when interrupted by a sibling: evidence from an interactive playback experiment

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ARTICLE INFO

Article history:
Received 7 June 2018
Initial acceptance 20 July 2018
Final acceptance 31 August 2018

MS. number: 18-00383R

Keywords:
auditory feedback
communication
interactive playback
overlapping
sibling negotiation
temporal dynamics
Tyto alba

To resolve conflicts of interest, animals can vocally signal their resource-holding potential and motivation to compete. This allows conspecifics to adjust their behaviour to each other without fighting physically. Making sure that competitors correctly assess each other's vocal information requires mechanisms to prevent signal interference. Alternating calls with those of an opponent (i.e. waiting until the opponent's call has ended before starting to vocalize) is widely observed in animals and could be partly acquired through learning. Regardless of whether competitors interrupt conspecifics as a signal of dominance or by accident, the information transferred by the interrupted individual is likely to be partly blurred. Interrupted individuals would hence benefit from counterattacking by calling more intensely, indicating to their competitors that calling simultaneously is counterproductive. We tested this 'social feedback' hypothesis in the barn owl, *Tyto alba*, in which young siblings negotiate vocally over which individual will have priority access to the next food item delivered. It has already been shown that nestlings actively avoid interrupting each other, but it remains untested whether nestlings give social feedback when interrupted. To test this, we developed an 'automated interactive playback' which broadcast calls that either interrupted or did not interrupt the calls of a singleton nestling. When a playback call interrupted a nestling, this individual immediately intensified vocal communication by quickly producing a long call and by producing more calls. As previously shown, this reaction tends to silence competitors and thereby increases the individual's likelihood of obtaining the next food item. Such social feedback could reinforce the evolutionary stability of vocal sibling negotiation as a nonaggressive way to share food.

To reduce the cost of conflict in animals, natural selection has favoured the evolution of communication about resource-holding potential and motivation to compete (Maynard Smith, 1982; Parker, 1974). The individuals that are unlikely to win a contest should momentarily refrain from competing until the likelihood of winning increases (Parker, 1974). Refraining from competing can be beneficial to save energy that can be invested later, once the probability of monopolizing resources increases (Johnstone & Roulin, 2003). Transmitting information about each other's motivation to compete and assessing the opponents' motivation is therefore crucial to determine the likelihood of monopolizing a resource and in turn how much energy should be allocated in a specific contest. Not being accurately informed about each other's motivation to compete due to signal interference should be detrimental for competitors (McGregor & Peake, 2000; Todt & Naguib, 2000) and thus should be avoided.

Call alternation (i.e. when an opponent starts a call after its counterpart has terminated its call) is widely accepted as a strategy to avoid communicative interference. The avoidance of call overlap has been well studied in the context of noncompetitive (e.g. Carter, Skowronski, Faure, & Fenton, 2008; Ghazanfar, Smith-Rohrberg, Pollen, & Hauser, 2002; McCauley & Cato, 2000; Miller, Iguina, & Hauser, 2005; Schulz, Whitehead, Gero, & Rendell, 2008; Soltis, Leong, & Savage, 2005; Versace, Endress, & Hauser, 2008) and competitive vocal interactions (e.g. Dreiss et al., 2013; Popp, 1989; Stokes & Williams, 1968; Wasserman, 1977; Wilson, Ratcliffe, & Mennill, 2016; Yang, Ma, & Slabbekoorn, 2014). When two calls overlap, the transfer of information through vocal signals of both

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https://doi.org/10.1016/j.anbehav.2018.09.003
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the interrupted and the interrupting individuals are likely to be blurred, while when the calls are produced one after the other, the transfer of information is optimized. However, in some contexts call overlap cannot be avoided. In altricial species, parents often allocate food to their offspring according to ostentatious signals produced by their progeny referred to as ‘begging’ (Godfray, 1995; Kilner & Johnstone, 1997; Wright & Leonard, 2002). In species in which parents stay a limited amount of time with their offspring to decide to which individual(s) to allocate food, nestmates call simultaneously to attract the attention of the feeding parent. In situations where young nestmates vocalize for much longer to decide which one will be prioritized during feeding, they tend to call one after the other by carefully avoiding overlapping nestmates' calls. For instance, in meerkats, *Suricata suricatta*, parents and helpers forage close to their offspring and hence can hear their begging calls. In this system, the pups are constantly begging without interrupting each other, which increases total feeding rate (Madden, Kunc, English, Manser, & Clutton-Brock, 2009). In species such as the European starling, *Sturnus vulgaris* (Chaiken, 1990) and the barn owl, *Tyto alba* (Roulin, Kolliker, & Richner, 2000), offspring also vocalize in the long absence of the parents which forage far from the nest. In this case, the vocalizations, produced at a relatively low rate, are directed to siblings rather than to parents and tend not to be produced simultaneously (Dreiss et al., 2013). Although call overlap is avoided and not used as a signal of dominance, it can still occur accidentally when individuals call during the same period of time (Helfer & Osiejuk, 2015).

To avoid being interrupted, individuals should leave enough time between two calls to give conspecifics the opportunity to vocalize (Camacho-Schlenker, Courvoisier, & Aubin, 2011) and, to avoid interrupting conspecifics, individuals should wait until an opponent has finished its call before starting to vocalize (Versace et al., 2008). Adhering to these specific alternation rules might not be innate but learned through ‘social feedback’ from conspecifics. Typically, human children speak simultaneously more often if they are neglected by their parents (Black & Logan, 1995). Similarly, in the European starling, when nestlings are raised in captivity without any contact with adults, they sing continuously without giving conspecifics the opportunity to call without being interrupted. This suggests that starlings learn to avoid interrupting conspecifics through social feedback from adults (Henry, Craig, Lemasson, & Hausberger, 2015). In line with this idea, common marmoset parents, *Callithrix jacchus*, stop vocalizing when an offspring interrupts their calls. By doing so, parents inform their offspring not to interrupt them again (Chow, Mitchell, & Miller, 2015). Once the rules to avoid vocalizing simultaneously to conspecifics are learned, social feedback should still be used if an individual persists in interrupting conspecifics.

In the present study, we examined the hypothesis that interrupting conspecifics is socially controlled with ‘social feedback’. To this end, we considered the barn owl where young siblings vocally negotiate priority of access to the next prey item delivered by a parent (Roulin et al., 2000). Each nestling produces thousands of calls per night during the prolonged absence of the parents while these are foraging. In our population, each nestling consumes three to four prey items per night, usually voles. Since at each parental visit, occurring every 45 min on average, the delivered indivisible prey item is consumed by a single nestling, siblings differ strongly in food need. For this reason, the outcome of sibling competition is predictable, with the hungriest individual being most likely to monopolize the next food item, given its high motivation to compete (Johnstone & Roulin, 2003; Roulin, 2002). Nestlings therefore vocalize in the absence of parents to inform their siblings about their relative motivation to compete with the most vocal individual indicating to its siblings its intention to compete fiercely for access to the next food item. As a response, its less hungry siblings momentarily refrain from competing until the likelihood of successfully monopolizing a food item increases (Dreiss, Lahlah, & Roulin, 2010; Roulin, Dreiss, Fioravanti, & Bize, 2009; Ruppli, Dreiss, & Roulin, 2013). This communication system, referred to as ‘sibling negotiation’, is directed to siblings rather than to parents which hunt too far from the nest to hear their offspring (Roulin et al., 2000).

It has already been shown that siblings interrupt each other five times less often than expected by chance, which corresponds to only 2% of the calls produced by an individual in nonexperimental conditions. Moreover, nestlings actively avoid interrupting playback calls (Dreiss et al., 2013). The tendency to interrupt does not vary with nestling age, position in the within-brood age hierarchy (in this species there is a pronounced age hierarchy resulting from staggered hatching) and hunger level, suggesting that this behaviour is used neither as a signal to dominate siblings nor to transmit information about the motivation to compete. We thus believe that interrupting siblings is not done on purpose but occurs by chance (Dreiss et al., 2013). Interestingly, bystander nestlings engage in more intense vocal negotiation when listening to a playback in which they could hear a nestling interrupted by another individual (Dreiss et al., 2013). This reaction to interrupted calls could be social feedback to inform siblings to avoid calling simultaneously. Such social feedback could help maintain the very low rate of overlapping calls observed by Dreiss et al. (2013).

The response to being interrupted has not yet been tested because it requires a highly sensitive interactive playback that would be able to interrupt very short calls (owlet calls last 0.8 s on average). To this end, we developed an ‘automated interactive playback’ that detects negotiation calls produced by a nestling in real time. The playback then broadcasts a prerecorded call that either does or does not overlap the nestling's call. We predicted that when a nestling is interrupted by the interactive playback, it should vocalize more intensely than when not interrupted, to send social feedback. Intensifying vocal behaviour after having been interrupted would reduce the likelihood of the interrupter getting the next food item. The interrupter would thus be motivated to take more care not to interrupt its siblings again. This interpretation is based on the fact that by vocalizing more intensely, an individual induces its siblings to withdraw from a contest (Dreiss et al., 2010; Roulin et al., 2009; Ruppli et al., 2013). The production of social feedback could therefore reinforce the evolutionary stability of vocal sibling negotiation by reducing interference while communicating which would improve signal transmission (McGregor & Peake, 2000; Todt, 1981; Todt & Naguib, 2000).

METHODS

Experimental Procedure

The study was performed on 10 free-living broods of barn owls in western Switzerland (46°4′N, 6°5′E). Between 8 August and 9 September 2014, 23 male and 15 female nestlings aged 41 days on average (range 30–48 days) were brought to the laboratory for 3 full days (and hence 2 nights). During the first night of acclimation, siblings were placed together in a wooden nestbox similar to the one where they were raised. Upon arrival at the laboratory in the morning, they were fed ad libitum (67 g of mice per nestling; Durant & Handrich, 1998). At 08:00 the next morning, the uneaten food was removed and at 12:00, all nestlings were weighed and isolated in experimental wooden nestboxes to allow them to get used to their new environment and to avoid disturbance prior to the experiment. The interactive playback was performed from

22:00 to 02:00. In the morning, nestlings were offered food and brought back to their original nest.

The experimental wooden nestbox was divided in half by a thin wooden wall pierced with five holes. A loudspeaker (near 05 experience, ESI Audiotechnik GmbH, Leonberg, Germany) was placed in one of the partitions and the nestling in the other. In each nestbox, we fixed two microphones (MC930, Beyerdynamic GmbH & Co KG, Heilbronn, Germany) on the roof oriented towards the nestling. One microphone was connected to a preamplifier PreSonusDigimax FS and a computer with the interactive playback algorithm developed in Matlab R2012b 8.0.0.783 (MathWorks, Natick, MA, U.S.A.). To increase the computing capacity, the recorded calls were deleted as soon as they were analysed. For this reason, we used a second microphone connected to a preamplifier Steinberg UR44 and to a second computer to record all calls produced during the 4 h experiment. Nestboxes were acoustically isolated with fibreglass so that external noises were not audible to the nestling and not recordable by the microphone.

Interactive Playback

The computer algorithm automatically detected the nestlings' calls and measured their duration (the script is provided as Supplementary Material). The detection consists in considering recordings as a succession of small frames of approximately 48 ms. For each frame, the algorithm detects through the noise level whether there is an acoustic event or not. Then, three different acoustic descriptors (i.e. the spectral variance, the skewness and the dominant spectral component) are calculated based on the spectrogram to distinguish negotiation calls from other acoustic events (i.e. other calls or noise).

To keep the call rate as constant as possible, we split time into periods of 10 s with the computer playing a call only after the first call produced by the nestling during the 10 s period (Fig. 1). If the individual did not vocalize during this time, the computer did not play any call. The rate at which playback calls were produced thus varied between 0 and 6 calls/min, with 6 calls/min corresponding to the mean call rate of food-deprived nestlings (Ruppli et al., 2013). The playback algorithm randomly selected a call from 36 different calls of a mean duration of 800 ms (SD 50 ms), which corresponds to the mean call duration of food-deprived nestlings (Ruppli et al., 2013). Computing constraints meant we could isolate calls from only one nestling recorded in 2008. However, we did a second interactive playback the following year with four different individuals and no effect of the broadcast individuals was detected.

A prerecorded call was played 0.5, 1 or 1.5 s after the beginning of a call produced by a nestling (Fig. 1). In the present study, the mean duration of nestling calls was 0.89 ± 0.23 s (SD, minimum 0.20 s and maximum 2.13 s). Therefore, with a latency of 0.5 s the computer played a call before the nestling call ended in 99.6% of cases (i.e. the nestling calls were overlapped). With a latency of 1 s there was almost no silence interval between the call of the nestling and the playback (silence duration 0.18 ± 0.08 s, only 0.47% of nestlings' calls were overlapped). Finally, with a latency of 1.5 s, the silence interval between the nestling and playback calls corresponded approximately to the mean call duration (silence duration 0.70 ± 0.095 s and only 0.1% of nestlings' calls were overlapped). The small proportion of overlapped calls during the 1 s and 1.5 s treatments did not modify the results and were therefore kept for the analyses. The experiment lasted 4 h and was divided into 16 periods of 15 min. Every 15 min, the computer randomly chose a new latency time.

Statistics

To investigate whether being overlapped affected nestling vocal response, we analysed (1) the duration of the nestlings' calls (i.e. mean duration calculated for each 15 min period) (2) the call rate (i.e. number of calls in each 15 min period), and (3) the response time (i.e. time between the beginning of a playback call and the next nestling call). We investigated whether the call that was overlapped by the playback ('overlapped call'), the subsequent call ('first following call') and the next calls ('other calls') differed in duration (Fig. 1). In each statistical model, the treatment, treatment order and individual sex and age were included as independent variables. Nestling identity, nested in brood identity, was included as a random factor to control for repeated measures. Call rate and the response time were log-transformed to obtain normally distributed data to use in linear mixed models. Model assumptions were verified (normal distribution of residuals and homoscedasticity) and P values smaller than 0.05 were considered significant. Statistics were conducted with R software (2.15.2 version, The R

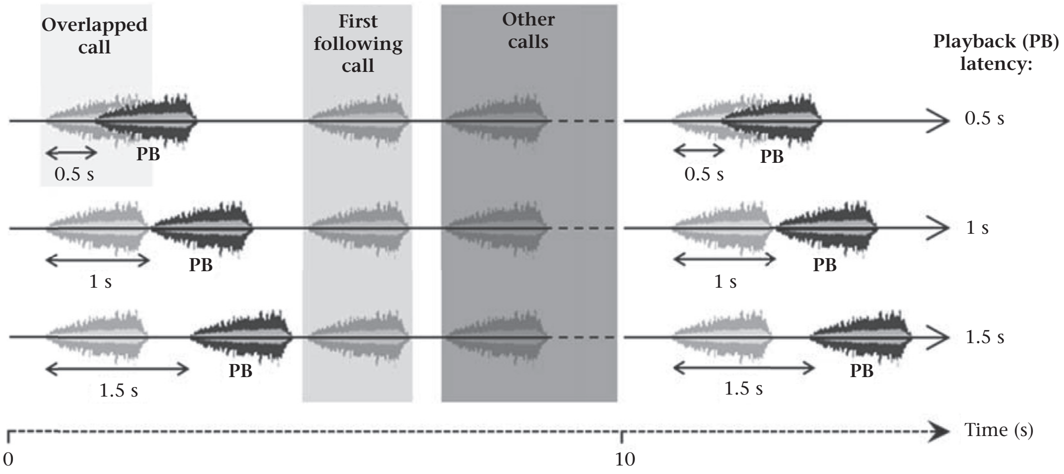

Figure 1. Playback experimental design. In each treatment a call was played only after the first nestling's call within a 10 s period and not after the following calls ('First following call' and 'Other calls'). The call was played at three different latencies (i.e. the time between the start of the nestling call and the start of the playback call). With a latency of 0.5 s, the nestling was interrupted, whereas with a latency of 1 s and 1.5 s the nestling was not interrupted. Treatments lasted 15 min and were randomly ordered across the 4 h of the experiment.

Project for Statistical Computing, Vienna, Austria), package ‘lme4’ (linear mixed-effects models using ‘Eigen’ and S4; version 1.1–15).

Of the 38 nestlings, five did not call and one produced fewer than 10 calls during the 4 h experiment. We therefore removed these six individuals from the analyses. In one further case, the computer did not play any calls because of an unknown detection problem. Therefore, the final sample size was 31 nestlings. A similar number of calls were played during each experimental treatment (ANOVA: $F_{2,365} = 0.97$, $P = 0.15$).

Ethical Note

After hatching, the mother stays inside the nestbox to feed and keep the offspring warm. At the age of 30 days, nestlings are thermo-independent and can swallow an entire prey item by themselves. From this stage onwards, the parents visit the nest only to bring prey items at night (Taylor, 2004). To reduce disturbance as much as possible, we performed the experiments only once the nestlings were at least 30 days of age, with age being estimated shortly after hatching by measuring the length of the left wing from the bird's wrist to the tip of the longest primary (Roulin, 2004). As the parents roost during the daylight hours outside the nestbox, we could manipulate the offspring without disturbing them. We brought two to six chicks per nest to the laboratory and always left at least two nestlings inside the nestbox to ensure that parents did not abandon the nest, which they never did. The 10 manipulated broods had a slightly higher fledging rate (0.72) than the 88 unmanipulated broods (0.64) monitored from the same population (Student's *t* test: $t = 2.05$, $P = 0.048$). Nestlings brought to the laboratory and those that remained in their nest had a similar body condition measured as the weight divided by the length of wing when fledged (Wilcoxon signed-rank tests: $W = 137$, $N = 9$, $P = 0.13$). Experiments were carried out under the legal authorization of the veterinary service of the ‘canton de Vaud’ (authorization 2109.2).

RESULTS

When calls were overlapped by the playback (0.5 s treatment), the nestlings responded quicker to the playback and produced more calls than when the nestling calls were not overlapped (1 and 1.5 s treatments, Table 1, Fig. 2). During the 0.5 s treatment, the call produced just after the overlapped call (‘first following call’) was particularly long, whereas the subsequent calls (‘other calls’) had a similar duration as the overlapped call (Table 2, Fig. 3). Regardless of the order of the calls, nestlings produced longer calls with the playback latency of 1 s than with the latency of 1.5 s after a nestling call (Table 2, Fig. 3). Finally, during the 0.5 s treatment, the call produced just after the overlapped call (‘first following call’) was on average longer than the first following call during the 1 s treatment and any calls during the 1.5 s treatment, regardless of the order of the calls (Table 2, Fig. 3).

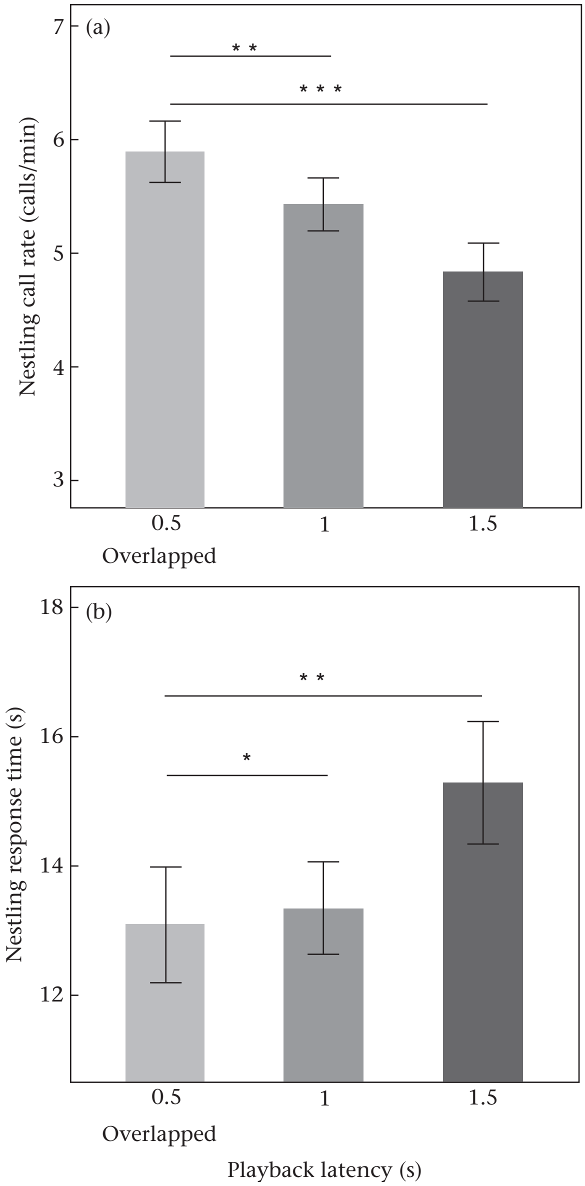

Figure 2. Mean (a) call rate ±SE and (b) response time ±SE of barn owl nestlings hearing playback calls at three latencies (i.e. the time between the start of the nestling call and the start of the playback call): 0.5, 1 or 1.5 s. Levels of significance (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$) are derived from mixed models presented in Table 1. Nestling sex, age and the order in which treatments appeared were included as independent variables. Nestling identity, nested in brood identity, was included as a random factor to control for repeated measures.

Table 1
Linear mixed models testing whether call rate and call response time of barn owl nestlings are related to the timing of a playback call after a nestling starts a call (0.5, 1.0 or 1.5 s)

Fixed effect	Call rate			Response time		
	F	*t*	*P*	*F*	*t*	*P*
Treatment order	$F_{1,343.6} = 6.93$		**0.009**	$F_{1,344.1} = 9.04$		**0.003**
Sex	$F_{1,28.6} = 2.58$		0.12	$F_{1,28.6} = 1.46$		0.24
Age	$F_{1,25.5} = 0.32$		0.58	$F_{1,26.0} = 0.88$		0.36
Playback response time	$F_{2,341.2} = 10.25$		**<0.001**	$F_{2,341.7} = 5.23$		**0.006**
0.5 s vs 1 s		−3.17	**0.002**		2.32	**0.021**
0.5 s vs 1.5 s		−4.42	**<0.001**		3.14	**0.002**
1 s vs 1.5 s		−1.40	0.16		0.92	0.36

Nestling call response time corresponds to the time between the beginning of a playback call and the next call produced by the nestling. We used backward model selection; final models only contained significant effects ($P < 0.05$). The significant effect of the factor ‘Treatment order’ indicates that as time passed, nestlings increased call rate and decreased their response time. Significant *P* values are shown in bold.

DISCUSSION

We have shown that when a barn owl nestling is vocally interrupted by a nestmate, it responds faster with a long call and increases the call rate. As previously shown, the propensity to

Table 2
Linear mixed models testing whether call duration of barn owl nestlings is related to the timing of a playback call after a nestling starts a call (0.5, 1.0 or 1.5 s)

		F	*t*	*P*
Treatment order		$F_{1,784.7} = 32.69$		**<0.001**
Sex		$F_{1,25.5} = 4.05$		0.055
Age		$F_{1,26.7} = 0.70$		0.41
Playback response time		$F_{3,782.9} = 4.39$		**<0.001**
Overlapped call 0.5 s	vs first following call 0.5 s		2.27	**0.023**
	vs other calls 0.5 s		−1.28	0.20
	vs first following call 1 s		0.91	0.36
	vs other calls 1 s		0.06	0.95
	vs first following call 1.5 s		−0.66	0.51
	vs other calls 1.5 s		−2.38	**0.017**
First following call 0.5 s	vs. other calls 0.5 s		−3.40	**<0.001**
	vs first following call 1 s		−1.35	0.18
	vs other call 1 s		−2.19	**0.028**
	vs first following call 1.5 s		−2.87	**0.004**
	vs other call 1.5 s		−4.55	**<0.001**
Other call 0.5 s	vs first following call 1 s		2.14	**0.033**
	vs other call 1 s		1.34	0.18
	vs first following call 1.5 s		0.64	0.52
	vs other call 1.5 s		−0.99	0.32
First following call 1 s	vs other call 1 s		−0.89	0.37
	vs first following call 1.5 s		−1.60	0.11
	vs other call 1.5 s		−3.36	**<0.001**
Other call 1 s	vs first following call 1.5 s		−0.74	0.46
	vs other call 1.5 s		−2.51	**0.012**
First following call 1.5 s	vs other call 1.5 s		−1.78	0.075

We distinguished between calls that were overlapped (‘Overlapped call’), the subsequent call (‘First following call’) and calls produced afterwards (‘Other calls’). We used backward model selection; final models only contained significant effects ($P < 0.05$). The significant effect of the factor ‘Treatment order’ indicates that as time passed, nestlings increased the duration of their calls. Significant *P* values are shown in bold.

interrupt siblings is not related to either the position in the within-brood age hierarchy or to hunger level indicating that interrupting siblings is not a strategy to compete for food but rather occurs by chance (Dreiss et al., 2013). Since nestlings retreat from a contest when facing a voluble sibling (Dreiss et al., 2010; Roulin et al., 2009; Ruppli et al., 2013), we suggest that by intensifying vocal communication, the interrupted individual decreases the likelihood that the interrupter gets the next prey item rendering call overlap counterproductive. This would dissuade siblings from interrupting again. Even if calling one after the other is beneficial by limiting interference, social feedback is crucial to learn when to call, as shown in European starlings, common marmosets and humans (Black & Logan, 1995; Chow et al., 2015; Henry et al., 2015). In the barn owl, social feedback might be useful when siblings persist in interrupting conspecifics on purpose or by chance, a social control that may limit the occurrence of call overlap.

Increasing call rate after having been interrupted can also be a way to repeat the blurred message to make sure that the information about current need for food is well understood. If this interpretation is correct, social feedback may be limited to the production of a single call very quickly after having been interrupted to indicate that calling simultaneously should be avoided. Such social feedback could therefore not be very costly and it now remains to demonstrate that it is efficient and provides an adaptive benefit to the interrupted individual. A potential experiment would be to broadcast long calls more rapidly after a target nestling has overlapped a sibling to test whether this individual is less likely to interrupt siblings again compared to when shorter calls are broadcast long after a target nestling has overlapped a sibling.

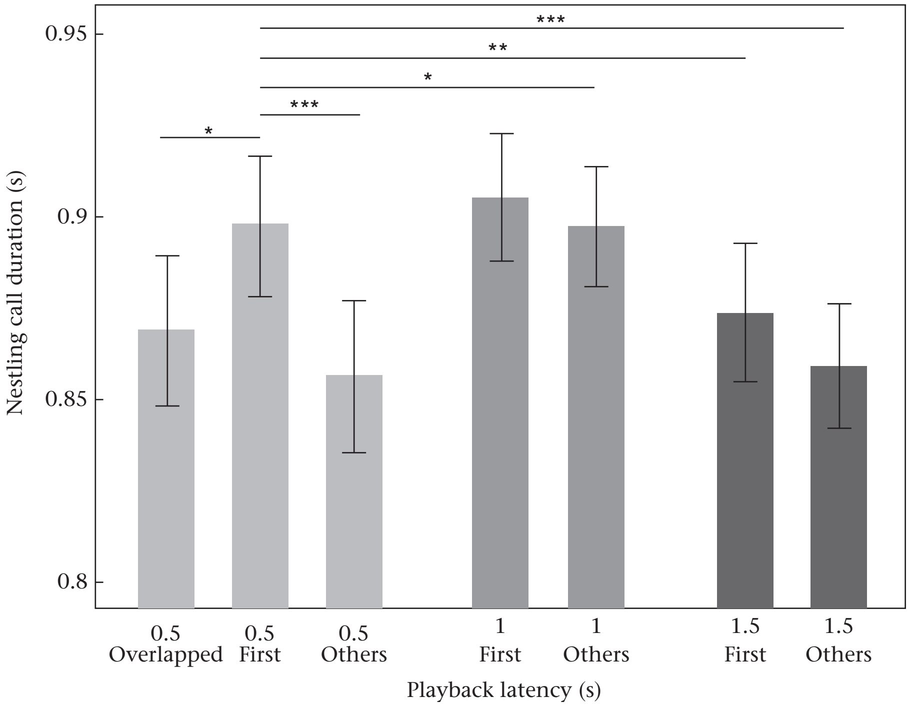

Figure 3. Mean duration of calls ±SE of barn owl nestlings hearing playback calls at three latencies (i.e. the time between the start of the nestling call and the start of the playback call): 0.5, 1 or 1.5 s. We investigated whether the call that was overlapped by the playback (‘Overlapped’), the subsequent call (‘First’) and the next calls (‘Others’) differed in duration. Levels of significance ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$) are derived from mixed models presented in Table 2. Nestling sex, age and the order in which treatments appeared were included as independent variables. Nestling identity, nested in brood identity, was included as a random factor to control for repeated measures.

We performed our study in the context of sibling negotiation between young animals that are still dependent upon parental feeding and in which call interruption does not seem to be used as a signal. Similar reactions to vocal interruption have been repeatedly found in the context of sexual selection. Nightingale, *Luscinia megarhynchos*, males increased call rate after having listened to a playback of two males that overlapped their songs compared to a playback in which two males sang one after the other (Naguib, Fichtel, & Todt, 1999). In black-capped chickadees, *Poecile atricapillus*, and great tits, *Parus major*, males modified the structure of their song when interrupted by a conspecific (Dabelsteen, McGregor, Shepherd, Whittaker, & Pedersen, 1996; Mennill & Ratcliffe, 2004). This suggests that this is a general response to vocal interruption regardless of the competitive context. There might still be situations where interrupting an opponent is done on purpose as an aggressive or threatening signal. Although this has been the prevailing hypothesis in the past (Naguib & Mennill, 2010), it is currently debated (Searcy & Beecher, 2009, 2011) because the probability that an individual interrupts the calls of conspecifics does not usually exceed the random expectation.

The interactive playback experiment allowed us to control when a nestling was interrupted regardless of other parameters, such as call duration, call rate or any other behaviour. Although we could not disentangle whether the vocal response of the interrupted nestlings was specific to being overlapped by a sibling's negotiation call rather than by any noise, we are confident that this response was directed to the interrupting nestling. Indeed, nestlings can discriminate noise from negotiation calls with nestlings producing hissing calls in response to unknown noises (van den Brink, Dolivo, Falourd, Dreiss, & Roulin, 2012), something that did not occur during our experiment. We can therefore objectively argue that a nestling is able to detect and recognize calls of opponents even if they overlap its call.

Our study highlights that experiments based on automated interactive playbacks are ideal to evaluate the adaptive function of specific turn-taking rules in animal communication and could hence be expanded to other biological systems. A major problem in animal communication is that researchers cannot ‘force’ animals to behave in a specific way, which prevents a formal test of the function of specific behaviours (King, 2015). An automated interactive playback mimics an individual that adopts certain behavioural conventions. This method allows researchers to observe the reaction in controlled conditions. However, to perform biologically relevant experiments it is vital to know the species well. It is also important to have a back-up record to manually verify that the automatic detection of calls is correct.

To conclude, our study suggests the existence of social feedback in owlets, which could help explain the low occurrence of nestlings calling simultaneously and improve information transmission. Such social feedback can be adaptive by improving the efficiency of sibling negotiation and may promote the evolution of specific turn-taking rules. Automated interactive playback could be used to test the existence of such social feedbacks in other biological systems.

Acknowledgments

We thank Karine Vincent, Vanessa Formenti and Robin Séchaud for their assistance in the field, and the Swiss National Science Foundation (grant n° 31003A_173178) for financial support. We are grateful to two anonymous referees who gave useful comments on the manuscript.

Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.anbehav.2018.09.003.

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CHAPTER 3

Elder barn owl nestlings flexibly redistribute parental food according to siblings' need or in return to allopreening.

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This chapter is in revision in **Proceedings of the Royal Society B-Biological Sciences**

Keywords: biological market; conflict resolution; direct cooperation; kin selection; sibling cooperation; sibling negotiation

ABSTRACT

Kin selection and reciprocation of biological services are distinct explanations invoked for the origin and evolutionary maintenance of altruistic and cooperative behaviours. Although these behaviours are considered as non-mutually exclusive, the cost-to-benefit balance to behave altruistically or reciprocally cooperate, and the conditions promoting a switch between such different strategies have rarely been tested. Here we examined the association between food gift, allopreening and vocal solicitation in wild broods of the barn owl (*Tyto alba*) under different food abundance conditions: natural food provisioning and after an experimental food supplementation. Allofeeding was mainly performed by elder nestlings (hatching is asynchronous) in prime condition, especially when the cost to renounce to a prey was small (when parents allocated more preys to the food donor and after food supplementation). Nestlings preferentially shared food with the sibling that vocally solicited food the most, thus maximizing indirect fitness benefits, or the one that provided more allopreening to the donor, thus promoting direct benefits from reciprocation. Finally, allopreening was mainly directed towards older siblings, perhaps in the hope to be fed in return. Helping behaviour among relatives can therefore be driven by both kin selection and direct cooperation, although it is dependent on the contingent environmental conditions.

INTRODUCTION

Ever since Darwin (Darwin, 1859), the presence of altruistic and cooperative behaviours in non-human species has fascinated biologists (Axelrod & Hamilton, 1981; Hamilton, 1964; Hammerstein & Noe, 2016; Lehmann & Keller, 2006; Nowak, 2006; Trivers, 1971; West, Griffin, & Gardner, 2007). Theoretical explanations for the origin and evolutionary maintenance of cooperation are classified into two broad categories, depending on whether the actor receives indirect (Breed, 2014; Hamilton, 1964) or direct (Leimar & Hammerstein, 2010; Trivers, 1971) fitness benefits as a consequence of adopting a costly action in favour of another individual. Kinship is considered a major promoter for the occurrence of cooperation, because the indirect fitness benefits of positively influencing reproduction and/or survival of relatives can outweigh the cost of performing altruistic behaviours (Hamilton, 1964). Kin selection has in fact been invoked to be the primary driver for the emergence of (pro)social behaviours in animal societies (Hamilton, 1964). However, cooperation also occurs among non-relative individuals, and even between members of different species (Clutton-Brock, 2009), thus leading to the identification of other mechanisms, based on direct fitness returns, that can explain its evolutionary stability (Lehmann & Keller, 2006; Noe, 2006; Nowak, 2006; Queller, 2011; West et al., 2007). A cooperative behaviour yields direct fitness benefits when the fitness of the individual performing the action is increased through reciprocation, thus resulting in a mutual advantage for both the counterparts (Nowak, 2006; Trivers, 1971; West et al., 2007). Reciprocity can be realized by exchanging the same social service, like mutual grooming/preening in mammals and birds respectively (Adiseshan, Adiseshan, & Isbell, 2011; Gill, 2012; Radford & Du Plessis, 2006; Roulin et al., 2016), or food sharing (Carter & Wilkinson, 2013; de Waal, 2000), but also trading different commodities (de Waal, 1997; Fruteau, Voelkl, van Damme, & Noe, 2009; Kern & Radford, 2018; Noe & Hammerstein, 1995; Roulin et al., 2016). Empirical evidence is accumulating for immediate commodity trades as a key element of intra- and inter-specific cooperation in non-primate species (Hammerstein & Noe, 2016), but there is a dearth of studies about interactions involving a temporal delay between the behaviours that are exchanged (but see Kern & Radford, 2018).

The tendency to cooperate and reciprocate is expected to vary according to the cost-to-benefit balance to be competitive, cooperative or altruist, which is expected to depend on the contingent environment to which both the donor and the receptor are exposed, as well as to their condition (e.g. Dolivo & Taborsky, 2015; Fruteau et al., 2009). Therefore species and phenotypes, but also single individuals across their life, can vary in their cooperation level. Both mechanisms can simultaneously act in the same social context, and a given individual is expected to switch between different strategies depending on the consequent putative fitness payoffs (Lehmann & Keller, 2006). A pure altruistic behaviour without receiving any direct advantage should be favoured in the presence of a needy relative, whereas in other conditions it can be more convenient to act cooperatively with others in order to exchange reciprocal services. Unfortunately, to the best of our knowledge, the fitness payoffs between direct and indirect benefits and costs of behaving altruistically vs. directly cooperating has never been tested in any species the wild.

A natural setting where direct cooperation and kin selected altruism can evolve and coexist is the brood of young animals, which rely on parental care, because offspring of altricial species are constrained in a small space where they compete for a long time for the same resources. This should be especially the case for populations/species where the extra-pair paternity is negligible, and broods are composed by full siblings. However, sibling interactions have been always considered as more competitive than harmonious (Mock & Parker, 1997; Trivers, 1974), and prosocial and altruistic behaviours in young animals have been examined only rarely (Forbes, 2007; Romano, Caprioli, Boncoraglio, Saino, & Rubolini, 2012; Roulin, Da Silva, & Ruppli, 2012; Roulin et al., 2016; Smale, Holekamp, Weldele, Frank, & Glickman, 1995).

In this study, we investigated food sharing and allopreening behaviours in entire broods of the barn owl (*Tyto alba*) recorded in natural conditions. Allofeeding and allopreening, where individuals feed and preen each other, are appropriate behaviours to investigate the fitness payoffs of complex social interactions, also because they are repeated events that involve two or more individuals, which can reciprocate the service (Gill, 2012; Radford & Du Plessis, 2006; Roulin et al., 2016). Allofeeding consists in the donation of a food item from one individual to another, and it is usually interpreted as a mechanism that reinforces social bonds (Smith, 1980). Indeed, it has been reported mostly in adult birds during courtship and in cooperative breeders (Kalishov, Zahavi, & Zahavi, 2005; Smith, 1980), but very rarely in nestlings (Roulin et al., 2016; Stamps, Clark, Arrowood, & Kus, 1985). Allopreening is considered as a prosocial behaviour because it confers protection against ectoparasites, particularly when it is directed to body parts that cannot be self-reached (e.g. typically neck and head; Radford & Du Plessis, 2006; Villa, Goodman, Ruff, & Clayton, 2016). It can also have a role in social interactions by reducing conflicts and stress (Fraser & Bugnyar, 2010; Gill, 2012; Radford, 2008; Radford & Du Plessis, 2006), and may thus provide mutual benefits to the donor and the recipient when it is reciprocated. Hygienic and social bond functions are not mutually exclusive, and have been proven to occur in the same avian systems (e.g. Radford & Du Plessis, 2006).

The barn owl is an ideal model organism to examine the payoff between competition and cooperation among nestlings because they live in a narrow space where they share the same parental food for a prolonged period. Albeit a large hatching asynchrony generating a considerable size hierarchy among chicks, which in most bird species cause a food monopolization by the oldest, biggest siblings (Mock & Parker, 1997), different cooperative behaviours have been observed in barn owl nestlings. They have been generally interpreted as a common strategy to reduce the costs associated to sib-sib competition (Roulin et al., 2012; Roulin et al., 2016; Roulin, Kolliker, & Richner, 2000). In particular, when parents are absent from the nest, siblings vocally negotiate which individual will have priority access to the next prey delivered by parents (Roulin et al., 2000). When an individual negotiates the most, its siblings refrain to compete for the next indivisible food item delivered by parents. In addition, owlets can exchange allopreening among each other, share food with siblings, and can also trade food sharing with allopreening (Roulin et al., 2016). However, the occurrence of allopreening and food sharing has been studied only under laboratory experimental conditions involving two or three nestlings, a number significantly lower than the average brood size (4-5 chicks), which can reach up to 9 chicks.

Here, we expanded the scope of previous studies on complete brood (Roulin et al., 2012; Roulin et al., 2016) by investigating the occurrence of allofeeding and its trade in exchange with allopreening by also taking into account the level of vocal negotiation and the food abundance level. We tested the following straightforward predictions: 1) individuals in better condition (i.e. oldest chicks) should be more inclined to share food with siblings than the chicks in poor conditions; 2) food share should especially occur when the cost to renounce to an indivisible food item is small (i.e. when chicks received a surplus of food by parents and/or when the amount of food stored in the nest is abundant); 3) the receptor of the prey donation should preferentially be either a very needy sibling (i.e. a chick that negotiates intensely prior to the food share event) or the sibling that previously cooperated the most with the donor (i.e. the chick that provided the donor with the largest allopreening); 4) if allofeeding is traded against allopreening and is performed mostly by oldest chicks, allopreening should be mainly directed towards high-ranking dominant siblings. To these aims, we recorded the aforementioned behaviours (allofeeding, allopreening and negotiation) in entire broods during two consecutive nights under different conditions of food abundance: one night under the natural food provisioning regimes provided by parents and a second one after an experimental food supplementation. Notably, by using new developed miniaturized microphones, we were able for the first time to assign each negotiation call to its emitter in natural condition.

MATERIALS AND METHODS

Study population and field procedures

The study was performed in a population of barn owls breeding in Western Switzerland (46°49′N/06°56′E) from April to September 2016. Adults breed in wood nest boxes installed in private barns (size of nest boxes is 65.2 × 35.9 × 46 cm) and composed by a main room, where eggs are laid, and the nestlings are reared and an entrance hall. The hatching is asynchronous with 2.5 days difference between each chick, which leads to a large within-brood size-hierarchy. Parents hunt small mammals at night to feed their offspring (Roulin, 2004). At two to three weeks of age, offspring are thermo-independent and can swallow entire prey item by themselves. Once this age is reached, parents enter the nest only to bring preys at night. The experiment was performed when the oldest nestling was 40 days old to avoid disturbing parents, and before nestlings take their first flight when they are ca. 55 days old. The sex of each nestling was determined using molecular markers (Py, Ducrest, Duvoisin, Fumagalli, & Roulin, 2006) and age by measuring the left wing from the bird's wrist to the tip of the longest primary (Roulin, 2004).

Behaviours of 127 nestlings (56 males, 70 females, 1 of unknown sex; age range 20-42 days) were monitored from 27 broods (brood size: mean = 4.7 ± 0.8 SD chicks; range = 3-6 chicks) during two consecutive days and nights. In one of the two experimental nights, randomly chosen, a food supplementation treatment was applied to each brood. The treatment consisted on adding two laboratory white mice *Mus musculus* per nestling which corresponds to half of their daily diet (Durant & Handrich, 1998) around 30 minutes before the sunset (e.g. in a 4-chicks brood 8 mice were added). The remaining night no experimental food supplementation was applied to the broods, so nestlings received only parental food. At the same time, microphones were turned on and nestlings were weighed. Every morning, at around 9 am, the remaining food on the nest was removed in order to homogenize the hunger level of each nestling before experimental observations. Experiments were carried out under the legal authorization of the veterinary service of the "Canton de Vaud" (authorization 2109.2).

Experimental set up

Four infrared miniature cameras (Active Media Concept – Technologie & Systèmes, CCTV miniature camera 520 lines with invisible infrared) were fixed on the nest box ceiling to video record the nest content. Two cameras were disposed at the entrance hall and two in the main room. Each nestling of the brood was equipped with a microphone recorder to identify which nestling emitted a call (see S1 for details). Six out of the 127 chicks could not be equipped with microphone because they were too small to hold it (threshold: wing <10 cm). Finally, a marker representing a geometric shape was added to the microphone to individually identify nestlings on video footages. The microphone had an autonomy of 10 hours, which allowed us to record complete nights. All the materials were installed (but turned off) 24h before the beginning of the experiment in order to allow chicks and parents to get used to recording equipment.

Behavioural data

Information about parental feeding behaviour and nestling social behaviours (allofeeding, allopreening and negotiation) was collected by watching video footages. Each parental visit was monitored and the identity of the nestling receiving the prey recorded. Thanks to these observations, we could estimate if parents equally distributed food among their offspring or if some of them were over selected. To this aim, we calculated an 'index of parental food distribution inequity' as the total number of prey items received by a nestling during each night minus the expected number of preys received per nestling if the distribution would be equitable (i.e. total number of preys brought divided by the brood size). In addition, we collected information about the allofeeding between chicks (i.e. who gave the prey and who received it) and the allopreening bout between chicks (i.e. who preened whom). An allofeeding event is defined as a behaviour through which a nestling gave a prey to a sibling directly to its beak or deposited it at its feet. This behaviour is easy to be identified because the donor steps back immediately after giving the prey. During this experiment, allofeeding events happened only at night as we removed the remaining food in the morning. The allopreening bout is defined as a nestling preening a sibling with its beak. Data about allopreening behaviour were collected only during the 6h preceding the sunset because previous studies documented a peak of allopreening during this period (Roulin et al., 2016; Scriba et al., 2017). This peak was confirmed on 10 broods for which we watched the video footage of the entire experiment (34% of allopreening bouts were given during these 6h, which is significantly larger than expected: t = 2.92, P = 0.01). Moreover, the number of allopreening bouts given per nestling during these 6h was highly correlated with the number observed during 24h (Pearson correlation of paired samples: t = 11.13, P <0.001, estimate = 0.77 ± 0.088 (SE)). To be considered as two allopreening bouts, a pause of at least 2s was required (see also (Roulin et al., 2016)). We calculated the number of allofeeding events and allopreening bouts per donor and receptor per night. The number of allopreening bouts is a good proxy of the total allopreening given and received (correlation between number of allopreening bouts and total allopreening duration: Spearman's ρ = 0.92), which also accounts for the number of direct interactions among siblings.

A program on Matlab R2012b 8.0.0.783 (MathWorks, Natick, MA, U.S.A.) was developed to post-process the vocal soundtracks to determine which nestlings emitted each call (details in S1). Afterward, we calculated the call rate of each nestling during each negotiation session, which corresponds to the period between two parental visits.

Statistical analyses

Statistical analyses were performed with the software R.3.4.0. All generalized linear mixed models (GLMM) included individual identity nested within brood identity as random effect to correct for pseudoreplication. Non-significant interaction terms were removed from final models. Homoscedasticity has been checked for each model.

What predicts allofeeding occurrence?

To investigate whether the number of allofeeding events per night varied with nestling condition (hypothesis 1) and with the food abundance (hypothesis 2), we used a GLMM with Poisson error distribution. We included the position in the within-brood age hierarchy based on hatching order (hereafter "rank hierarchy") and body mass corrected by age (i.e. residuals of the regression of body mass on age) to test whether nestlings in better condition were more inclined to allofeed a sibling. We included the food supplementation treatment (i.e. food supplemented or control night) and the index of parental food distribution inequity to test whether food supply induces generosity. Interaction between the food supplementation treatment and the index of parental food distribution inequity was also included. To properly compare the rank hierarchy of nestlings across broods that vary in size (brood sizes varied between 3 and 6 chicks), we assigned the category "junior", "middle-born" or "senior" to each nestling depending on hatching order (see S2). Qualitatively similar results were obtained when 'hatching order' was set as a continuous covariate (results not shown). We also included the nestling's sex and the brood size as independent variables. Each continuous variable was standardized.

Who receives allofeeding?

To test whether social behaviours (vocal negotiation and allopreening) encourage an individual to give a prey to a particular sibling (hypothesis 3), we focused our analyses on the 15 broods where allofeeding event occurred and on the siblings' behaviour toward the food donor (each sibling except the donor). As dependent variable we used a dichotomous variable indicating whether a given individual received a prey or not from siblings in a GLMM with binomial distribution. For each individual, we included as predictors the number of allopreening bouts given to the food donor and the call rate emitted during the negotiation phase preceding each allofeeding event. The rank hierarchy (junior, middle-born or senior) and the sex of each potential receptor were also included, as well as the interaction between the call rate and the number of allopreening bouts given. Due to technical issues, 4 broods had to be removed from this analysis because of missing negotiation (e.g. the microphone applied to a nestling did not work) or allopreening (e.g. the cameras did not record the entire nest box content) data.

Who receives allopreening?

To test whether the frequency of allopreening was related to the rank hierarchy and the sex of the donor and receptor of allopreening (hypothesis 4), we ran a GLMM with Poisson error distribution with the number of allopreening bouts as the dependent variable. As independent variables we included hierarchy rank of the donor and of the receptor as well as their interaction. We also included the sex of the donor and of the receptor as well as their interaction. Because of the large number of comparisons between different levels of the interaction between donor and receptor ranks, we corrected the p-value for multiple tests with the false discovery rate method (Nakagawa, 2004).

RESULTS

We observed 45 allofeeding events (60% directly to the sibling's beak and 40% to its feet) performed by 28 out the 127 nestlings in 15 out of the 27 experimental broods. Nestlings performed on average 1.95 allopreening bouts (SD = 1.93) per hour (mean bout length = 16.57 ± 19.78 s), which corresponds to a total duration of 32.39 s per hour on average (SD = 36.93).

Table 1 – Generalized linear mixed model testing whether the number of allofeeding events is related to individual condition (weight and rank hierarchy category) and to food abundance (food supplementation treatment and index of parental food distribution inequity). Sex and brood size were also included as predictors. Nestling identity nested within brood identity was included as random factor. Sample size was 127 nestlings from 27 broods.

Predictors	*Estimate (SE)*	χ^2	*z*	*P*
Rank category		**13.43**		**0.0012**
Junior vs. Middle-born	-0.53 (0.68)		-0.78	0.44
Junior vs. Senior	-1.87 (0.59)		-3.18	0.0014
Middle-born vs. Senior	-1.34 (0.52)		-2.55	0.011
Sex	-0.26 (0.42)	0.37		0.54
Body mass	**0.020 (0.0091)**	**4.79**		**0.028**
Food supplementation treatment	**-0.77 (0.36)**	**4.56**		**0.033**
Index of food distribution inequity	**0.51 (0.15)**	**11.62**		**0.00065**
Brood size	0.16 (0.25)	0.41		0.52

What predicts allofeeding occurrence?

The occurrence of allofeeding depended on the rank of the donors (Table 1) with senior nestlings sharing more food items with siblings than junior and middle-born individuals, while no difference was observed between junior and middle-born chicks (Table 1, Figure 1a). Nestlings allofed more often in nights when we experimentally added food compared to control nights (Table 1, Figure 1b). A nestling was more inclined to allofeed when it received the main share of parental feedings (i.e. index of parental food distribution inequity; Table 1, Figure 1c). Finally, nestlings with larger body mass relative to their age allofed more often than lighter nestlings (Table 1). Brood size, nestling sex and the interactions between food supplementation and the index of parental food distribution inequity did not significantly predict the number of allofeeding events neither the interaction between food supplementation and the index of parental food distribution inequity (Table 1).

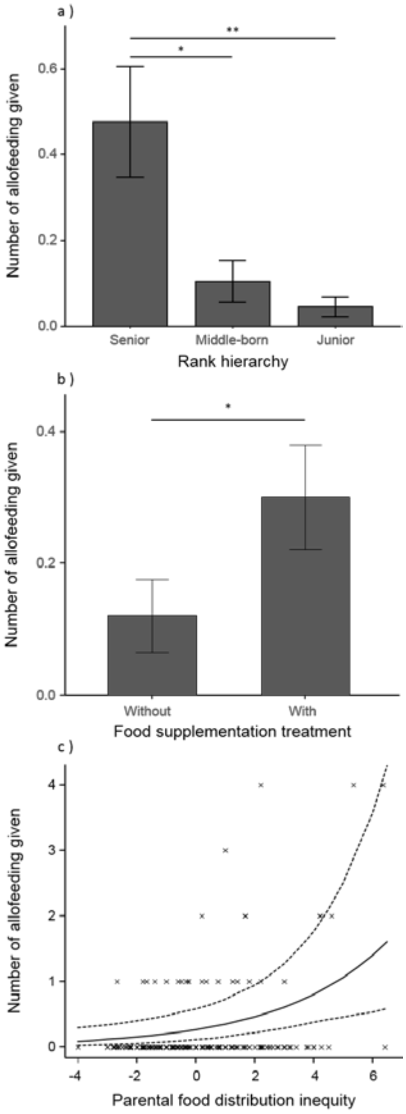

Figure 1 – Relationship between the number of allofeeding events given and a) position in the within-brood age hierarchy of the food donor (i.e. rank), b) the food supplementation treatment and c) the index of parental food distribution inequity (a positive value indicates that a nestling was fed by parents more often than expected if food was allocated equally among the progeny). Predicted value (continuous line) and 95% confidence interval (dotted lines) are based on the model in Table 1. Asterisks indicate significant differences between groups (**: P<0.01 and *: P<0.05).

Who receives allofeeding?

The probability of being fed by a sibling was significantly related to the interaction between the call rate emitted during the preceding negotiation phase and the allopreening given to the food donor ($\chi^2 = 4.24$, $P = 0.039$; Figure 2). In particular, prey donation was mainly directed towards either the sibling that emitted very intense negotiation calls prior to the food share, irrespective of its allopreening behaviour, or under low-to-medium level of negotiation to the one that provided the donor with the largest allopreening (Figure 2). The probability of receiving a prey was not related to nestling's sex ($\chi^2 = 0.25$, $P = 0.61$) and rank hierarchy ($\chi^2 = 0.60$, $P = 0.74$).

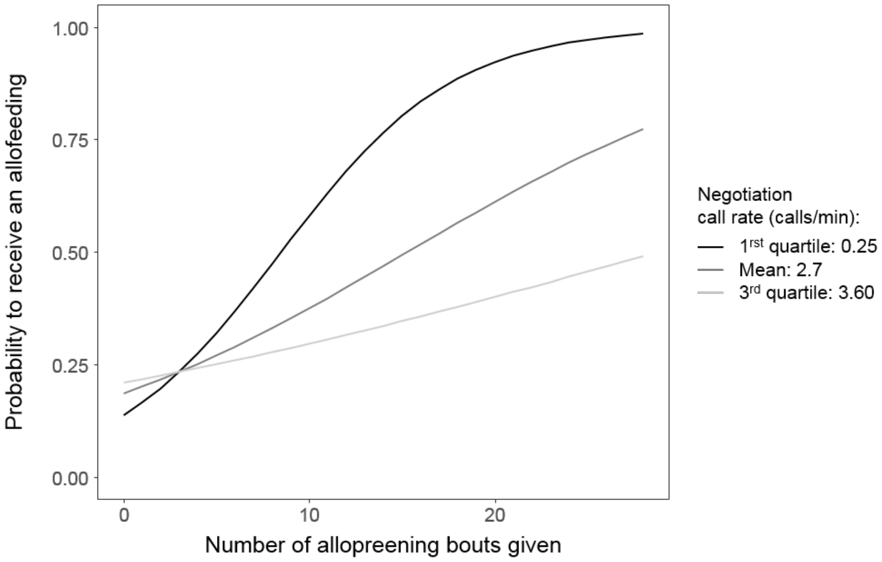

Figure 2 – Predicted probability to receive a prey item from a sibling according to the number of allopreening bouts given to the food donor when vocal negotiation was at the first quartile value (black); at the mean value (dark grey) and at the third quartile value (light grey). These values have been chosen to illustrate the interaction between two continuous variables. The predicted lines are based on the model presented in Table 2. Each other predictor was set at their mean values. Note that the light grey curve (third quartile of vocal negotiation) is not significant.

Who receives allopreening?

A nestling allopreened a sibling differently according to its rank and the rank of the receptor (interaction between the donor's and the receptor's ranks: $\chi^2 = 47.66$, $P < 0.001$, Table S3a, Figure 3). Whatever the rank of the donor, nestlings allopreened higher ranked siblings. In other words, middle-born nestlings allopreened more their senior siblings compared to middle-born and junior ones (Table S3b, Figure 3), and junior nestlings allopreened more both middle-born and senior siblings than junior ones (Table S3b, Figure 3). Finally, senior nestlings allopreened more other senior than middle-born and junior siblings (Table S3b, Figure 3). However, a nestling allopreened a sibling regardless its sex and the sex of the receptor (Table in S3a).

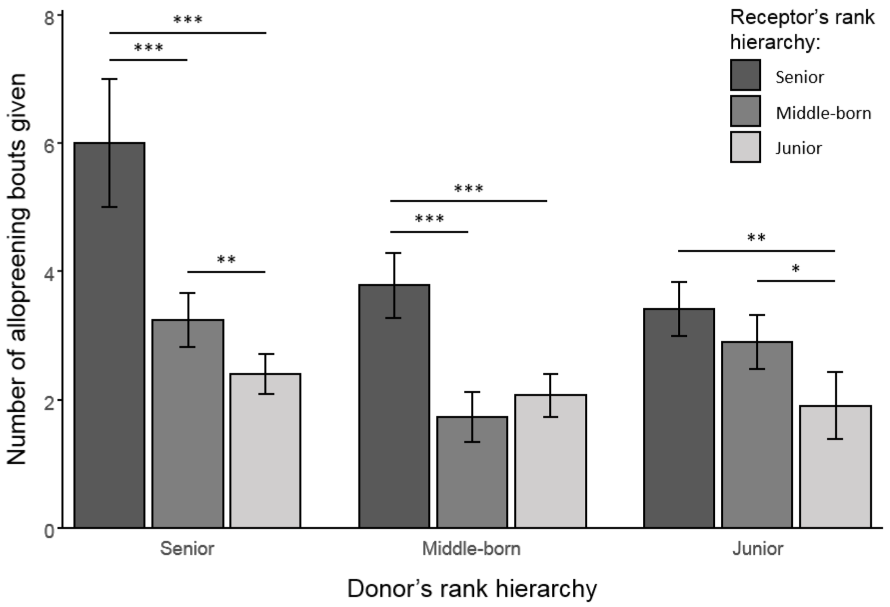

Figure 3 – Number of allopreening bouts given according to the donor's and the receptor's rank hierarchy. Only statistical significant comparisons within each donor's rank hierarchy block are presented and are based on the model in Table S3. P-values have been corrected for multiple testing with the false discovery rate method. Asterisks indicate significant differences between groups after correction (***: P<0.001; **: P<0.01; *: P<0.05).

DISCUSSION

One of our main findings was that within a brood the barn owl chicks in better condition (oldest and heaviest relatively to their age) were more prone to give an indivisible prey item to siblings than younger nestling. This result is coherent with previous studies performed on experimentally reduced broods where nestlings were selected to maximize their rank difference (Roulin et al., 2012; Roulin et al., 2016). The observed pattern of food sharing was expected because this behaviour is predicted to entail some direct fitness costs that only dominant chicks can sustain because they potentially have a privileged access to parental resources, and because they are more responsive to modulate their behaviour according to siblings' need (Dreiss, Ruppli, Delarbre, Faller, & Roulin, 2017). Indeed, it is not surprising that this was especially the case when the nestling naturally received a higher number of parental food and when the number of preys stored in the nest was experimentally enhanced, thus suggesting that this helping behaviour is condition-dependent, being favoured when the cost to renounce to food is lowered.

The costs of allofeeding behaviour should be overcompensated by some fitness advantages to the donor to be maintained (Hammerstein & Noe, 2016; Lehmann & Keller, 2006; Nowak, 2006; West et al., 2007). In the barn owl, extra-pair paternity is almost nihil (ca. 1% in our study population; Henry et al., 2013), and nest mates are full siblings. Under such circumstances, allofeeding may confer indirect benefits through kin selection processes by enhancing the condition of a needy sibling, but also direct benefits if this behaviour is reciprocated through mutual exchange of services or if it appeases siblings and social interactions. These two processes, which have been often considered independent to explain the origin and the evolutionary stability of cooperation, can instead synergistically interact (Lehmann & Keller, 2006; Van Cleve & Akcay, 2014), but unfortunately no study had investigated their interplay in determining individual behavioural strategies in young animals. Another main finding of our study seems, at least partly, to fill this gap because we showed that chicks can optimally modulate food sharing according to the balance between direct and indirect fitness benefits and costs of behaving altruistically vs. directly cooperating. Indeed, food share mainly occurred with the neediest sibling (i.e. the one emitting intense negotiation calls) irrespective of its allopreening behaviour, or with the most cooperative sibling (i.e. the one providing the largest allopreening) even if it did not negotiate. On the one hand, this choice is expected to have a positive impact on the indirect fitness of the donor because it should enhance the pre-fledging survival prospects of a kin (see below), especially when the kin is particularly hungry and therefore when the value of a food item is high. On the other hand, in the absence of very needy siblings, food donation was directed towards the nestlings that had previously provided the largest allopreening to the donor, suggesting that siblings exchange commodities (food against allopreening), as previously demonstrated in experimentally reduced brood (Roulin et al., 2012; Roulin et al., 2016).

Allopreening is mainly directed to body regions that are difficult to self-preen, such as head, neck and back, thus potentially conferring direct advantages to the receptor in terms of ectoparasite removal (Radford & Du Plessis, 2006; Villa et al., 2016), other than through a decrease in social stress (Fraser & Bugnyar, 2010; Gill, 2012; Radford, 2008; Radford & Du Plessis, 2006; Stowe et al., 2008). Unfortunately, with the present data we are not in the position to disentangle which one of these mechanisms (or even both simultaneously) is responsible for the observed exchange of commodities among owlets. Nevertheless, allopreening is preferentially directed to chicks of higher rank than the donor. This is not surprising because it can be subsequently rewarded by a food donation, while the opposite is not the case (i.e. food donation to later obtain allopreening), as suggested by the lack of an effect of food received during a night on allopreening given the subsequent day (see S4 for details). Irrespective of the proximate benefits from receiving allopreening, barn owl nestlings seem to optimally trade different services with the siblings and to modulate this reciprocation according to the contingent context, as predicted by the ‘biological market’ theory (Hammerstein & Noe, 2016; Noe & Hammerstein, 1995). The dynamics of reciprocity in cooperative behaviour is indeed expected to vary according to the conditions of each participant and to the value that both the donor and the receptor attribute to the traded commodities (i.e. demand and supply of each commodity), which, in turn, depends on their availability on the market (Hammerstein & Noe, 2016; Noe & Hammerstein, 1995). To this complex puzzle for evolution of cooperation, we add a further level of complexity by suggesting that, among relatives, a commodity in the biological market can also be traded against an indirect fitness return (i.e. food consumption by very needy sibling) in case it is larger than a direct benefit (i.e. allopreening received). Interestingly, a preliminary analysis on the same sample of broods showed that the fledging rate (i.e. proportion of chicks that fledged) was significantly larger in the broods where allofeeding was observed comparing to those where it was not (ANOVA: $F_{1,25} = 5.93$, $P = 0.022$). This was especially the case for middle-born and junior chicks (ANOVA: $F_{1,25} = 8.42$, $P = 0.0076$). Although this is a partial analysis including only two days of allofeeding data, potentially not entirely reflecting the total allofeeding propensity during the entire rearing period, this finding suggests that food sharing may have an effective positive role to promote sibling survival, and thus to increase the inclusive fitness of the donors. It therefore has the potential to be evolutionary maintained.

Interestingly, allopreening is mostly performed during daytime while allofeeding exclusively during the night (Roulin et al., 2016; present study), thus indicating that sibling cooperation involves a temporal delay between the traded behaviours. The existence of delayed cooperation in non-primate animals has been questioned (Barrett, Henzi, & Rendall, 2007; Stevens & Hauser, 2004). However, convincing evidence has been provided that this type of cooperation can also occur in species other than primates (vampire bats: Carter & Wilkinson, 2013; mongooses: Kern & Radford, 2018; birds: Krams, Krama, Igaune, & Mand, 2008). Here we provided the first documented evidence of delayed cooperation in young animals. This observation implies the ability by barn owl chicks to recognize the nest mates individually, as previously suggested because of the presence of individual-specific vocal signatures (Dreiss et al., 2013; Dreiss, Ruppli, & Roulin, 2014), but also to memorize the actors of past behaviours and act accordingly.

We showed that individuals were more inclined to share a prey if they received more food from parents. An open question remains about why such unfair parental distribution was observed only in a part of the studied broods. One scenario envisages the possibility that in some families, parents might 'delegate' the oldest chicks to distribute food to their siblings. Such a helping behaviour by 'responsible' chicks may allow parents to limit the time spent in the nest and therefore to increase their hunting activities, with a common benefit for the entire brood. This form of parent-juvenile cooperation (see Kramer, 2011) might especially occur in broods reared by only one parent, because of the death or the abandon of the other one (especially the female) to do a second brood, as it is common in our population (Beziers & Roulin, 2016). In addition, this behaviour can be beneficial also because the 'responsible' chick should discriminate better than parents which is the neediest nest mate to be fed because of direct and prolonged comparison between the degree of negotiation among all the siblings. Alternatively, the oldest chicks could easily monopolize parental resources by, for instance, occupying the most favourable position in the nest to receive food (e.g. close to the nest-box entrance), and then redistributed it according to its own advantage. However, the observation that chicks provided food to siblings when they negotiate conspicuously also the receiver did not directly cooperate with the donor makes this scenario as an unlikely one.

In conclusion, we showed a complex interplay between allofeeding, allopreening and negotiation among barn owl siblings, corroborating the hypotheses that helping behaviour among relative individuals can be driven by both kin selection and direct cooperation, as well as that it varies depending on the food availability. Future studies in other non-primate species combining information on individual relatedness, reciprocal exchange of different social services and variation in their value on the biological market will allow to test for the generality of the patterns documented here.

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SUPPLEMENTARY MATERIAL

S1. Individual microphone recorder description

We modified a pre-existing microphone recorder (Spy USB microphone recorder). We externalized the microphone in order to place it as close as possible to the nestling's bill with a steep pole. We added a resistor between the microphone and the record system in order to decrease the sensitivity. The microphone was fixed on a piece of leather (2mm thick) before fixing it as a bag back on the nestling. The microphone recorder measured 3 cm width, 9 cm length, and weighed 15.7g, which represents between 3.8% and 7.5% of the nestling body mass at the age of 25 to 42 days.

Identifying the individual that emitted the calls was based on the comparison of sound intensity between each microphone. In order to correct for potential differences between microphones, a white noise and a sweep noise (sound which increases in frequency from 60 Hz to 10 kHz) was recorded before each experiment. A program on the software Matlab R2012b 8.0.0.783 (MathWorks, Natick, MA, U.S.A.) was developed to post-process the vocal soundtracks by 1) correcting for potential microphone differences using the sweep and white noise records, 2) detecting the calls, and 3) performing the multi-intensity comparison of all microphone records to determine which individual emitted each call. Before this procedure had been applied, the recordings of each microphone were synchronized on time thanks to a "clap" that we made at the beginning of each recording. The "clap" consisted of a noise made by two wood pieces close enough to the nestling to be present on each microphone.

S2. Rank hierarchy categorization

Table representing the classification of the hatching order within a brood into a three level rank hierarchy category: i.e. "senior", "middle-born" and "junior"

	Rank hierarchy category		
Brood size	*Senior*	*Middle-born*	*Junior*
3 chicks	1st	2nd	3rd
4 chicks	1st	2nd and 3rd	4th
5 chicks	1st and 2nd	3rd	4th 5th
6 chicks	1st and 2nd	3rd and 4th	5th and 6th

S3. Relationship between nestling rank and allopreening

(a) Generalized linear mixed model testing whether nestlings allopreened sibling according to their own rank in the within-brood age hierarchy and sex and according to the receptor’s rank and sex. Nestling identity nested in brood identity was included as random factor. (b) Post hoc multiple comparisons for the interaction between donor and receptor rank. *P*-values have been corrected for multiple comparisons with the false discovery rate method (see Fig. 3). Sample size is 110 nestlings from 23 broods.

(a)

Predictors	X^2	***P***
Donor’s sex	0.14	0.71
Receptor’s sex	0.0038	0.95
Donor rank	**10.99**	**0.0041**
Receptor rank	**157.72**	**<0.001**
Donor sex × Receptor sex	0.065	0.80
Donor rank × Receptor rank	**38.02**	**<0.001**

(b)

Donor / Receptor Rank	***Donor / Receptor Rank***	***Estimate (SE)***	***z***	***P***	***Adjusted P***
Senior / Senior vs.	**Senior / Middle-born**	-0.71 (0.09)	-7.91	<0.001	<0.001
	Senior / Junior	-0.97 (0.088)	-11.03	<0.001	<0.001
	Middle-born / Senior	-0.66 (0.17)	-3.95	<0.001	<0.001
	Middle-born / Middle-born	-1.32 (0.20)	-6.60	<0.001	<0.001
	Middle-born / Junior	-1.24 (0.17)	-7.13	<0.001	<0.001
	Junior / Senior	-0.77 (0.16)	-4.74	<0.001	<0.001
	Junior / Middle-born	-0.87 (0.17)	-5.25	<0.001	<0.001
	Junior / Junior	-1.17 (0.19)	-6.28	<0.001	<0.001
Senior / Middle-born vs.	**Senior / Junior**	-0.26 (0.084)	-3.09	0.0020	0.0055
	Middle-born / Senior	0.055 (0.16)	0.34	0.73	0.73
	Middle-born / Middle-born	-0.61 (0.20)	-3.07	0.0021	0.0055
	Middle-born / Junior	-0.52 (0.17)	-3.07	0.0021	0.0055
	Junior / Senior	-0.056 (0.16)	-0.35	0.72	0.73
	Junior / Middle-born	-0.16 (0.16)	-0.95	0.34	0.42
	Junior / Junior	-0.45 (0.18)	-2.47	0.013	0.027
Senior / Junior vs.	Middle-born / Senior	0.32 (0.16)	1.92	0.054	0.089
	Middle-born / Middle-born	-0.35 (0.20)	-1.74	0.081	0.13
	Middle-born / Junior	-0.26 (0.17)	-1.53	0.12	0.19
	Junior / Senior	0.20 (0.16)	1.26	0.21	0.28
	Junior / Middle-born	0.10 (0.16)	0.63	0.53	0.59
	Junior / Junior	-0.19 (0.18)	-1.05	0.29	0.37
Middle-born / Senior vs.	**Middle-born / Middle-born**	-0.66 (0.14)	-4.83	<0.001	<0.001
	Middle-born / Junior	-0.58 (0.087)	-6.68	<0.001	<0.001
	Junior / Senior	-0.11 (0.16)	-0.69	0.49	0.57
	Junior / Middle-born	-0.21 (0.17)	-1.27	0.20	0.28
	Junior / Junior	-0.51 (0.19)	-2.72	0.0064	0.014
Middle-born / Middle-born vs	Middle-born / Junior	0.085 (0.14)	0.59	0.56	0.61
	Junior / Senior	0.55 (0.20)	2.77	0.0057	0.014
	Junior / Middle-born	0.45 (0.20)	2.24	0.025	0.045
	Junior / Junior	0.15 (0.22)	0.71	0.48	0.57
Middle-born / Junior vs.	**Junior / Senior**	0.47 (0.17)	2.74	0.0061	0.014
	Junior / Middle-born	0.37 (0.17)	2.13	0.033	0.057
	Junior / Junior	0.072 (0.19)	0.37	0.71	0.73
Junior / Senior vs.	Junior / Middle-born	-0.10 (0.079)	-1.25	0.21	0.28
	Junior / Junior	-0.40 (0.11)	-3.50	<0.001	0.0015
Junior / Middle-born vs.	**Junior / Junior**	-0.30 (0.12)	-2.45	0.014	0.027

S4. Allopreening given according to allofeeding received during the previous night

To examine if the relationship between allopreening and allofeeding is uni- or bidirectional, we tested if the total number of allopreening bouts received in a day was predicted by the allofeeding given during the night before. To this purpose we ran a GLMM assuming a Poisson error distribution, including brood identity as a random factor. This model included as predictors a dichotomous factor indicating whether an individual gave a prey or not, as well as the rank hierarchy and the sex of the chicks receiving allopreening. Unfortunately, this analysis could be performed only for one night (the first experimental night and the subsequent day) because we did not collect any data before the first experimental day and after the second experimental night.

The analysis showed that nestlings did not receive more allopreening bouts from a sibling if it shared a prey the night before compared to if it did not share a prey the night before (Table S4).

Table S4 – Generalized linear mixed model testing whether the number of allopreening bouts received by a nestling is related to the fact that the same nestling shared food the night before. We included the rank hierarchy, the food supplementation treatment and the sex of this nestling. Brood identity was included as random factor. Sample size was 65 nestlings from 13 broods.

Predictors	***Estimate (SE)***	***X^2***	***z***	***P***
Rank category		**26.45**		**<0.001**
Junior vs. Middle-born	-0.45 (0.11)		-3.90	<0.001
Junior vs. Senior	-0.64 (0.13)		-5.04	<0.001
Middle-born vs. Senior	-0.19 (0.11)		-1.81	0.07
Give a prey Yes vs. No	-0.21 (0.16)	1.81		0.18
Food supplementation treatment	0.66 (0.38)	2.99		0.083
Sex	0.13 (0.10)	1.47		0.22

GENERAL DISCUSSION

During my PhD, I went a step further in the understanding of vocal communication in the context of food competition in nestling barn owls. Since genetically related siblings present a high asymmetry in food requirement (i.e. food items are indivisible and there is an important age and size hierarchy), a negotiation process has evolved between the two to nine siblings of barn owl broods. By transmitting information about its hunger level in the absence of the parents, a nestling deters the less needy siblings from competing and thereby from getting priority access to the next prey. In this species, sibling negotiation can therefore be considered as a 'cooperative' behaviour that promotes food access to the neediest individual. However, despite lowering their chance to get the next prey when withdrawing from the negotiation, nestlings also save energy that will be available later when their chance to obtain the prey is higher. The resolution of conflict over parental food includes therefore interconnected negotiation phases in which the same individuals are involved. Because these repeated negotiation phases take place between genetically related individuals, 'cooperative' communication strategies may have evolved. By cooperative communication strategies I refer to strategies that lead to reduce the overall investment in the communication of each contestant and to increase the transmission efficiency (e.g. call overlap avoidance). Indeed, I highlighted in Chapter 1 and 2, using innovative interactive playback experiments, the importance of short-term adjustment of vocal communication between contestants (i.e. temporal and intensity adjustment) in order to dominate the negotiation process. In these two chapters I also highlighted the potential role of contestant in reinforcing communication strategies by rendering other strategies inefficient and sending social feedback to correct a sibling when the latter does not follow a communication rule (Chapter 2, call overlap avoidance). I also uncovered in Chapter 3 the importance of vocal negotiation in the decision process of whom to share a prey with, and the environmental condition that favours food sharing. I will first briefly discuss these results in the context of the barn owl as well as in the broader context of communication, and then I will present those aspects that need to be explored in future studies. This latter perspective part includes, among others, studies that will be conducted soon using the behavioural data collected in 2016 and that were partially used in Chapter 3.

PART 1: SIBLING NEGOTIATION IN NESTLING BARN OWLS: A DYNAMIC AND INTERACTIVE PROCESS

To dominate a negotiation process, nestlings have to succeed in producing more and longer calls than their siblings (Dreiss, Lahlah, & Roulin, 2010; Roulin, Dreiss, Fioravanti, & Bize, 2009; Roulin, Kolliker, & Richner, 2000). It has already been shown that nestlings assess the temporal variation of the sibling's call parameters (i.e. an increase or decrease of call duration and rate) and use it to decide when resuming the negotiation (Dreiss, Ruppli, Faller, & Roulin, 2015). Nestlings also use these temporal variations to adjust their call parameters at a fine-time scale to those of their siblings, by matching call duration (i.e. increasing and decreasing call duration at the same time as the contestant) and mismatching call rate (i.e. increasing the call rate when the contestant decreases its call rate and reversely; Dreiss, Lahlah, et al., 2010; Dreiss et al., 2015; Roulin et al., 2009; Ruppli, Dreiss, & Roulin, 2013). In Chapter 1, we experimentally showed that these strategies, even if reversed (i.e. positive or negative adjustment), are the most efficient to induce the withdrawal (fewer and shorter calls) of a sibling from the vocal negotiation. Call rate has been repeatedly demonstrated to be the most important call parameter within the negotiation process, i.e. a parameter that is modulated to a larger extent according to hunger level and has the most pronounced influence on a sibling's withdrawal (Dreiss, Lahlah, et al., 2010; Roulin et al., 2000; Ruppli et al., 2013). This was also confirmed in Chapter 1 as the probability that nestlings retreated (i.e. stopped calling) was clearly the highest during Mismatch-Call Rate.

The call duration is used in a competitive manner by increasing and decreasing at the same time as the contestant's (i.e. matching strategy) probably to test how far the contestant is willing to vocally compete. According to the prediction of the different game theories presented in introduction, it seems that the call duration is used to advertise endurance (i.e. the war of attrition models: Bishop & Cannings, 1978). By contrast, the call rate is used in a more 'cooperative' manner by waiting until the sibling's decrease in call rate before increasing its own call rate, and reversely. This strategy might be counterintuitive as it seems risky to wait to signal its hunger level when a parent might arrive at any moment. However, by doing so, nestlings give a sibling the opportunity to communicate without having to both escalate at the same time. This 'gentle' strategy could ultimately be beneficial to appease vocal exchange and hence reduce the cost (i.e. overall emission rate) of a long-lasting negotiation process. Indeed, barn owl nestlings negotiate during the absence of the parents that can last more than an hour, as well as for all the prey brought across the night and rearing period. Exchanging long utterance (i.e. monologue) rather than frequently changing the calling turn might therefore have common benefits for both contestants by lowering the overall number of calls and increasing the information transmission. This observation could explain the high proportion of 'solo' during the negotiation process (67%: Dreiss et al., 2015). This high percentage of monologue suggests that the number of calls emitted during such a monologue is responsible at a large extent for the overall call rate of a complete negotiation phase. It would be interesting to investigate whether a monologue is a temporary success or a part of a longer process. Indeed, succeeding to call alone in a solo may confer the nestling with the advantage that if a parent arrives at this moment, this nestling will get priority access to the prey. It may also be a part of a longer process of long utterance exchanges and if the nestling is not the one that called the most in general, it will not get the prey no matter if it is the one that calls in the last monologue. In other words, is the nestling that get access to the prey the one that calls in the monologue preceding parental arrival or is it the one that calls the most during the entire negotiation phase? In the first case (the last 'monologuer', the winner), the risk of allowing a sibling to call alone by mismatching the call rate is more risk than in the second case (the most frequent monologuer, the winner). This is however a tricky question because the nestling that is hungriest is expected to call the most and thus to be the one that calls the most in monologue. Therefore, the probability that a parent arrives when this hungrier nestling is calling is higher than when a less hungry sibling is calling and thus both cases are confounded. It would therefore be necessary to manipulate the moment when a parent arrives according to the sibling vocal negotiation.

When nestlings do not follow these strategies (i.e. Match-Call Duration and Mismatch-Call Rate) they are less efficient in deterring siblings from vocalizing. This observation suggests that the social environment and experience through repeated interactions might play a reinforcement role of these communication strategies (i.e. social skill). This might explain why the responsiveness (i.e. the extent to which a nestling adjusts its vocal behaviour to a sibling's behaviour) of barn owl nestlings increases with age (Dreiss, Ruppli, Delarbre, Faller, & Roulin, 2017). In accordance, we also showed in Chapter 2 that social environment might reinforce the temporal coordination rule (turn-taking rule). Indeed, when interrupted by the playback, nestlings immediately intensified vocal communication by quickly producing a long call and more calls. This response renders the call overlap inefficient in deterring siblings from competition and thus from obtaining the next food item. This social feedback might explain the very few occurrences of call overlap during sibling negotiation (2%: Dreiss, Ruppli, et al., 2013) and could be a mechanism that reinforces the evolutionary maintenance of vocal sibling negotiation as a nonaggressive way to share food. The role of social feedback in learning conversational turn-taking rules has already been demonstrated in the European starling (*Sturnus vulgaris*, Henry, Craig, Lemasson, & Hausberger, 2015) and in the common marmoset (*Callithrix jacchus*, Chow, Mitchell, & Miller, 2015). However, to the best of our knowledge, no study has investigated its role in the maintenance of turn-taking rules (i.e. after the learning stage), although it has been demonstrated that such social feedback is essential to maintain song structure (Leonardo & Konishi, 1999) and social skills required during mating competition (Gersick, Snyder-Mackler, & White, 2012). Here we showed that social feedback is still used after the turn-taking rule was acquired although we do not know if call overlap avoidance is acquired earlier during development or if it is innate in nestling barn owls.

We demonstrated that these social adjustment strategies (i.e. Match-Call Duration and Mismatch-Call Rate) are more efficient in deterring siblings from vocalizing and therefore could be considered as a signal by itself. As for any signals, mechanism(s) to prevent individuals from dishonestly following these strategies are required. Indeed, one of the most puzzling aspects of the evolutionary stability of signalling under conflict of interest is that the signal must honestly reflect the non-observable quality (i.e. resource holding potential, motivation or condition) and not be exaggerated or misused by a contestant. To prevent dishonesty, signalling should entail costs (Enquist, Hurd, & Ghirlanda, 2010; Grafen, 1990; Searcy & Nowicki, 2005; Vehrencamp, 2000; Zahavi, 1974) so that it is too costly to cheat (i.e. the handicap principle: Grafen, 1990) or might be too costly in case of potential social punishment (i.e. inflicted cost: Lachmann, Szamado, & Bergstrom, 2001). Interestingly, we have also shown that Match-Call Duration and Mismatch-Call Rate, the most efficient strategies in deterring sibling from competing, require a higher investment in terms of number of calls and call duration emitted by the sender, here the playback. Although producing calls may not be primarily costly (Bachman & Chappell, 1998; McCarty, 1996), demonstrating a high motivation by following the most efficient strategies requires at least a higher investment with more and longer calls (i.e. marginal cost). It requires also a particular vigilance to the contestant's vocalization which could be costly due to attention requirement and received signals processing (Szamado, 2011).

Communication during conflicts of interest has mainly been studied through the duration of the contest and the contest outcome rather than as an interactive process with response rules (Arnott & Elwood, 2009; Payne, 1998). Although these different rules might have evolved due to kin selection in our case, other mechanisms that lead to a common interest in contestants' fitness (e.g. interdependency) might be responsible for them too. Investigating these strategical adjustments across the continuum of overlap interest level between contestants would be highly valuable to better understand their roles in the decision-making process (Fleck, Volkema, & Pereira, 2016). To the best of our knowledge, similar adjustment strategies have been studied only in the context of males that seek reproduction with females (Patricelli, Uy, Walsh, & Borgia, 2002). Another interesting case to study would be, for instance, siblings that seek to receive food in the presence of parents (when parents stay long enough to enable an adjustment between siblings' signalling). Indeed in other altricial species, siblings may also cooperatively beg in the presence of parents to keep a constant global intensity and elicit care provisioning by parents (Bell, 2007; Forbes, 2007; Johnstone, 2004; Mathevon & Charrier, 2004). A nestling's begging intensity is thus negatively related to the overall begging intensity of the brood. However, in these cases, communication influences parental provisioning and is thus considered to be directed toward the parents. Therefore, no study has investigated in what way siblings decide how the global intensity is shared among them. It would be interesting to replicate similar interactive playback experiments in such species to test how nestlings decide the intensity at which they beg according to others' begging intensity and condition. Other interesting cases to study would be neighbours that seek to settle territorial boundaries (e.g. Vehrencamp, Ellis, Cropp, & Koltz, 2014) or groupmates that seek to coordinate cooperative behaviours (e.g. Bell, Radford, Smith, Thompson, & Ridley, 2010; Palagi, 2008). Using interactive playback is also a promising tool because it allows biologists to investigate not only the impact on the focal individuals of particular strategical adjustment (i.e. receiver point of view) but also the impact on individuals that follow them (i.e. sender point of view). This is particularly interesting because such impact of adjustment strategy on the individual that follows them is species and condition dependent. Therefore, a same adjustment strategy might have different impacts according to the context and the level of common interest between contestants.

PART 2: DO NESTLINGS RELY ON VOCAL NEGOTIATION TO DECIDE WITH WHOM TO SHARE A PREY?

Being integrated in a social group forces individuals to repeatedly interact with each other to resolve conflicts (e.g. limited resources and collaborative tasks). The 'market' quality, i.e. the supply (e.g. food abundance) and the demand (e.g. the brood size) to which the individuals are exposed, as well as their personal conditions, may promote intense agonistic, cooperative or altruistic behaviour (e.g. Dolivo & Taborsky, 2015; Fruteau, Voelkl, van Damme, & Noe, 2009). In accordance, we showed in Chapter 3 that nestlings were more prone to share a prey with siblings when the food was abundant (i.e. during the food supplemented nights of the experiment) and when they received more prey items from the parents than they should have received in case of equal food distribution. Nestlings were also more prone to share a prey if they were in better condition (i.e. older and heavier), confirming the results from previous studies (Roulin, Da Silva, & Ruppli, 2012; Roulin et al., 2016). This results were expected as sharing a prey rather than eating it entails a direct cost that only individuals in better condition can handle especially when the value of a prey is low given its abundance.

Although cooperative behaviours entail costs, they may confer indirect benefits through kin selection mechanisms when helping a genetically related sibling (Hamilton, 1964), and/or direct benefits if this behaviour is reciprocated through the exchange of the same or other commodities (Nowak, 2006; Trivers, 1971; West, Griffin, & Gardner, 2007). These two processes can synergistically interact (Lehmann & Keller, 2006; Van Cleve & Akcay, 2014), but studies investigating their interplay are still scarce. We showed in Chapter 3 that nestlings can optimally modulate their decision of whom to share a prey with using vocal negotiation. Indeed, when all the conditions that favour the share of prey are met, nestling barn owls have to choose to either receive indirect fitness benefits only, as extra paternity is rare in this species, or receiving additional direct benefits through reciprocation. We showed that a nestling is more inclined to share food with a sibling that has previously provided him allopreening service (i.e. direct benefice), as has been previously showed (Roulin et al., 2016), but only if there is not a highly vocal and thus, highly needy sibling around. In this case, a nestling favours the highly needy sibling regardless of the amount of allopreening received from this needy sibling. Thanks to the vocal negotiation, nestlings are better informed of their siblings' current needs and are thus better able to accurately decide towards whom displaying a cooperative behaviour to potentially gain the highest benefit. Our findings therefore highlight the importance of investigating cooperative behaviour taking into account each potential cost and benefit and the interplay between them, in order to fully tackle the mechanisms responsible for the emergence and maintenance of cooperative behaviour.

PERSPECTIVES

Ontogeny of the sibling negotiation behaviour

It remains unclear how and when the social rules examined here appear during development. Three hypotheses can be proposed: first, the negotiation rules are innate and the presence of conspecifics is not required to develop them; second, the negotiation rules are learned by "trial-error" and might not require the presence of adults, but can be learnt within successive sibling interactions (i.e. social reinforcement); finally, negotiation rules are learned through active guidance by parents (Henry et al., 2015; Hudson & Trillmich, 2007). In order to experimentally test these three hypotheses, nestlings from the same brood should be raised in different conspecific presence conditions, i.e. with parents, with siblings only and alone, and then test whether negotiation rules are followed by the nestlings in the different settings. This experimental design would be possible only with captive individuals. It has been shown that older nestlings are more responsive to sibling vocal communication than younger ones (Dreiss et al., 2017). Two hypotheses have been proposed, either because cognitive capacities increase with age or because nestlings adopt different strategies according to their within-brood age hierarchy. In Chapter 1, we highlighted a third hypothesis that individuals improve their skill due to repeated social interaction.

Prey theft to punish cheater

As mentioned earlier in the discussion, theory predicts that signals have to be costly to be honest if there is conflict of interest between individuals (handicap principle: Grafen, 1990; Zahavi, 1974). However, many signals have been demonstrated to be relatively cheap rather than costly and more recent theories state that honest signalling without realized cost paid by the signallers can be maintained if the potential cost of cheating is sufficiently high (Hurd, 1995; Lachmann et al., 2001). Szamado (2011) reviewed different mechanisms that can be responsible for potentially enhancing cheating costs. Among those mechanisms, two can primarily act within grouped animals: the inclusive fitness mechanism if groupmates are genetically related (Johnstone, 1999) and the social cost mechanism by punishment/retaliation (Lachmann et al., 2001). In the first mechanism, an individual that obtained the resource by exaggerating its signal would directly increase its own fitness, but, at the same time, would reduce its inclusive fitness, as the resource would no longer be available for its kin (Moreno-Rueda, 2007). In the second case, cheating can be assessed by a groupmate, which will punish the cheater. Therefore, the risk of being caught and punished should be sufficient to discourage dishonest signalling.

Thanks to an individual recognition mechanism presented in the introduction (Dreiss, Ruppli, & Roulin, 2014), nestling barn owls have the opportunity to verify that a sibling does not cheat by monopolizing a prey without having negotiated beforehand. In case of cheating, nestlings may punish the cheater in different ways. For instance, a nestling may physically harass the cheater (Clutton-Brock & Parker, 1995). This is unlikely to be the case because aggressions are very rarely observed in barn owl nestlings (Roulin et al., 2012, pers. obs.). A second option, one that is frequently observed, is the theft of prey (Roulin, Colliard, Russier, Fleury, & Grandjean, 2008; Roulin et al., 2012). This hypothesis will be investigated thanks to the data collected for Chapter 3, in which 101 food stealing events were observed. Therefore, we are able to investigate whether the individual from whom the prey was stolen had ‘cheated’ according to its previous vocal negotiation and whether the stealer is the one that negotiated the most beforehand, and thus the one that should have received the prey.

Plasticity and interplay between competitive, cooperative and altruistic behaviour

The quality of the environment in which individuals interact, as well as each individual’s condition, can promote intense agonistic, cooperative or altruistic behaviours (e.g. Dolivo & Taborsky, 2015; Fruteau et al., 2009). Offspring of several altricial species are well known not to only use vocal signals but also physical behaviours (e.g. push to access the closest positions to where parents predictably deliver food in the nest) to resolve the conflict over food (Wright & Leonard, 2002). When the resources are scarce, more competitive behaviour is predicted than if they are abundant, when more cooperative and altruistic behaviour is then expected. This was confirmed in Chapter 3 with the probability to observe food sharing being higher when the brood was food supplemented. The rearing environment quality (i.e. that relies on parental hunting efficiency and habitat quality in the case of altricial nestlings) is to some extent stochastic, fluctuating quickly. Individuals should therefore efficiently choose between competitive, cooperative and altruistic behaviour according to this change. Future studies should thus investigate the interplay between the multiple components of begging within and across sensory modalities to understand their relative function taking into account the environment quality. In the barn owl, nestlings can vocally negotiate in the main part of the nest box and only some nestlings briefly approach a parent that enters the nest box to deliver a prey. In contrast, all nestlings can vocally negotiate and push each other to get the place closest to the nest box entrance (Dreiss, Calcagno, et al., 2013, pers. obs.). In the latter case, each nestling is amassed together in the nest box corridor. In the 2016 experiment, every morning we removed the prey that had not been eaten during the night in order to standardize the hunger level of each nestling (i.e. each nestling did not eat during at least 12 hours). We will thus be able to investigate whether nestlings choose between competitive or cooperative strategy according to their short-term conditions (i.e. beginning vs. end of the night), the recent quality of the environment (i.e. feeding rate during the night before) or long-term quality of the environment (i.e. growth rate and body condition). Concerning the long-term quality, we will be able to calculate an index that reflects the life history of a nestling thanks to repeated measures of body condition done during the rearing period (25 days, 35 days and 40 days). We predict that nestlings should rely on long-term quality to decide on whether to act competitively. Indeed, pushing their way closest to the entrance largely increases the risk of falling down the nest and die, something that we have observed.

Cooperation between parent(s) and older nestlings

We showed in Chapter 3 that individuals were more inclined to share a prey if they received a disproportionately larger amount of food from the parents. We articulated 2 hypotheses to explain why unequal parental distribution of prey was observed only in a part of the studied broods depending on whether it is due to parents or to the older nestlings. Parents may purposely over-select older nestlings to ‘delegate’ the food distribution. By doing so, a parent would limit the time spent in the nest and reallocate this time to hunting. This could be seen as a parent-older nestling cooperation (see Kramer, 2011) and might especially occur in single parental broods to increase feeding efficiency. Mono paternal care is widespread in the barn owl either because of the death of the female or her abandonment to do a second brood (Beziers & Roulin, 2016). This behaviour would be beneficial also because the 'responsible' nestling would discriminate better than the parents which nest mate most needs to be fed due to the vocal negotiation. Alternatively, the oldest nestlings could monopolize parental resources thanks to their size advantage and then redistribute it according to their own benefit. To do that however, the distinction between male and female provisioning rate is required to fully tackle this issue which we don't have from experiment. In addition, older positioning in the nest would be a good proxy to evaluate its role in this unfair distribution.

GENERAL CONCLUSION

To conclude, social behaviours that act to resolve conflicts of interest emerge as highly dynamic through the different chapters of my thesis. This highlights the importance of investigating behaviour in an interactive manner both within a particular behaviour (e.g. vocal negotiation) and between behaviours (e.g. interplay between vocal negotiation, allofeeding and allopreening). The different social skills studied here appear in early life, in a species with no particular social organization at the adult stage. This suggests that it might not require a high cognitive complexity (Barrett, Henzi, & Rendall, 2007). Moreover, the signal rate and the signal length are commonly used by vertebrates and invertebrates to compete and communicate as shown in non-human primates (Palagi, 2008), birds (Todt & Naguib, 2000), frogs (Reichert, 2014) and insects (Takeuchi & Imafuku, 2005). Hence, the dynamic rules observed in barn owls might be broadly shared. Finally, barn owl nestlings appear to be a powerful model to study conflict over resources and animal communication. Because parents quit the nest early in the rearing process, visiting only briefly to provide prey, behaviour can be studied in the wild as well as in laboratory conditions without impacting fledgling success and with minimal stress (Chapter 2; Dreiss, Henry, Ruppli, Almasi, & Roulin, 2010).

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CFA/VISHNO 2016

Quelle est la stratégie optimale pour remporter la négociation ? Expériences de playbacks interactifs avec les poussins de Chouette effraies

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Afin de résoudre la compétition liée aux ressources, les animaux peuvent échanger des informations quant à leur capacité et leur motivation à rivaliser. En se basant sur ces informations, les compétiteurs peuvent décider de continuer d'investir dans la compétition ou bien de se retirer. En l'absence des parents, les membres d'une même fratrie de chouettes effraies (*Tyto alba*) négocient vocalement pour se défier les uns les autres et gagner la priorité sur la prochaine proie amenée par les parents. Celui qui fait taire ses frères et sœurs, dominant ainsi cette phase de négociation, aura de grande chance d'obtenir la proie au retour des parents. L'investissement vocal fourni dans la négociation ne dépend pas seulement de la motivation personnelle à obtenir une proie mais également de celle de ses frères et sœurs. Les poussins suivent des règles sociales pour prendre la parole lors de cette interaction vocale. Ces règles ne se basent pas uniquement sur la valeur absolue des paramètres vocaux du rival (nombre de cris et durée du cri) mais aussi sur l'évolution dans le temps de ces paramètres, d'où l'importance d'étudier cette communication de manière interactive. Quelle est la meilleure stratégie d'interaction vocale pour dissuader un rival et prendre la place de dominant lors de l'interaction ? Pour répondre à cette question, deux expériences de playback interactif ont été réalisées afin de comparer l'efficacité de différentes stratégies vocales. Trois stratégies centrées sur l'évolution des cris du poussin ont été définies : i) ajustement positif : l'investissement du playback augmente si celui du poussin augmente et inversement, ii) ajustement négatif : l'investissement du playback diminue si celui du poussin augmente et inversement, iii) contrôle : investissement du playback reste constant quel que soit celui du poussin. Cette technique de playback nous permet de déterminer expérimentalement si le poussin suit la stratégie la plus efficace pour écarter ses rivaux.

1 Introduction

La communication est un aspect fondamental de la vie animale car elle permet la médiation des conflits sur les ressources limitantes telles que la nourriture, le partenaire ou encore le territoire. Un individu qui exhibe des signaux (ornementaux, de couleur ou bien acoustique) plus ostentatoires que son rival aura une plus grande probabilité de recevoir la ressource [1-3]. Même si certains signaux sont fixes à court terme tel que la coloration, la plupart d'entre eux peuvent fluctuer rapidement tel que les signaux acoustiques et la plupart des comportements. Ainsi, le niveau des signaux envoyés peut fluctuer rapidement afin d'éviter les interférences [4], de s'adapter à la présence d'une audience [5] ou à son rival [6, 7]. L'issue du conflit est donc le résultat d'un processus interactif. Ces fluctuations de signaux lors de ces interactions soulèvent la question de comment un individu décide d'entrer ou de sortir de la compétition pour la ressource mais surtout comment un individu ajuste son investissement dans la communication à chaque instant en fonction de sa propre condition et de celle de son rival.

Ces rapides fluctuations du signal acoustique lors d'interactions compétitives soulèvent la possibilité que les individus analysent la dynamique temporelle du signal d'un rival et non sa valeur absolue [8]. Les interactions sociales ont majoritairement été étudiées dans le cadre de la théorie des jeux. Cependant, la dynamique temporelle reste encore peu explorée expérimentalement. Ces études nous permettraient pourtant d'augmenter notre compréhension des règles de décisions sociales qui régissent ces interactions.

Une raison pour laquelle cette dynamique reste peu étudiée vient probablement du fait de la difficulté à mettre en place des protocoles expérimentaux permettant de l'étudier. En effet, la majorité des études concernant la communication vocale sont soit corrélatives soit expérimentales, utilisant alors des playbacks non interactifs. De telles expériences de playback permettent de comparer la réponse d'un animal à l'écoute de différents sons.

Les moyens informatiques actuels nous permettent désormais de développer une « nouvelle génération » de playback interactif, permettant d'évaluer l'impact de la dynamique temporelle du signal. Un playback est considéré comme interactif dès lors qu'au moins un paramètre de l'expérience est fonction du comportement de l'individu focal. Par exemple, l'instant de diffusion d'un enregistrement ou encore le type de son émis par le playback. Cependant, la majorité des playback interactifs existants se base sur le type de cri émis et non sur l'évolution de ce cri dans le temps [9]. Nous entendons par « nouvelle génération » de playback interactif, une routine automatique qui prend continuellement des décisions quant au paramètre du cri émis. Ces décisions sont basées sur l'évolution en temps réel des signaux émis par l'individu focal.

Dans cette étude, nous utilisons cette nouvelle génération de playback interactif afin de tester expérimentalement l'importance du moment auquel un individu décide d'escalader dans la compétition vocale. Nous nous intéressons pour cela au comportement de négociation vocal au sein de la fratrie qui a lieu chez la chouette effraie (*Tyto alba*) [10, 11]. Lorsque les parents sont en train de chasser et donc absents du nid, les poussins négocient vocalement l'obtention de la prochaine proie indivisible apportée par les parents. Durant cette phase de négociation, l'individu qui vocalise le plus (i.e. émet plus de cris et plus longs) aura une plus grande probabilité de recevoir la proie au retour des parents que l'individu qui vocalise moins. Chaque individu investit dans cette communication en fonction de son niveau de faim – l'individu le plus affamé produit plus de cris et de plus longue durée – mais également en fonction de la communication de ses frères et sœurs – un individu face à un frère émettant de longs et nombreux cris aura tendance à se retirer de la compétition [12, 13]. Ce comportement permettrait d'optimiser l'énergie investie dans la compétition en ne défiant ses frères et sœurs que lorsque la probabilité d'obtenir une proie est forte.

Durant cette phase de négociation, chaque poussin devrait avoir pour but de dissuader ses rivaux afin de prendre la place de dominant dans la compétition (être celui qui émet le plus de cris). Un but sous-jacent est d'obtenir l'information sur le niveau de faim de la fratrie afin de ne pas gâcher de l'énergie dans la compétition quand la probabilité de gagner est faible. En effet, l'asymétrie du niveau de faim entre les jeunes et le fait que la proie est indivisible (seul un jeune sera nourri) rend la compétition relativement prévisible, l'individu le plus affamé ayant une plus forte motivation à entrer en compétition vocalement et physiquement [10]. Une précédente étude corrélative a montré que les individus suivent des règles sociales pour prendre la parole lors de l'interaction vocale. Ces règles ne se basent pas sur la valeur absolue des paramètres (nombre de cris et durée du cri) mais sur l'évolution dans le temps de ces paramètres. Le poussin semble ajuster positivement la durée de ses cris à celle des cris de son rival et semble ajuster négativement son taux de cris à celui de son rival [14, 15].

Nous présentons ici deux expériences de playbacks interactifs indépendantes mimant plusieurs stratégies de dynamique temporelle des paramètres acoustiques pour l'obtention de la proie. Notre postulat de départ est que les stratégies observées dans la nature chez les jeunes chouettes devraient être les plus efficaces pour dissuader les frères et sœurs de rester dans la compétition. Les expériences de playback interactif nous permettront d'évaluer quels paramètre et stratégie sont les plus efficaces.

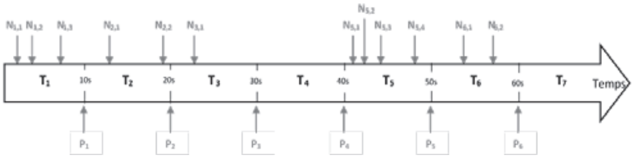
2 Méthodes générale

Les expériences ont eu lieu du 30 avril au 2 septembre 2015 au laboratoire de l'Université de Lausanne. Chaque poussin de chouette effraie a été amené et placé dans un nichoir similaire au nichoir naturel. Au total, 114 poussins âgés de 34 ± 0.5 jour (de 22 à 41 jours) ont été étudiés dont 54 mâles, 56 femelles et 5 de sexe inconnu. Chaque nichoir est composé de deux compartiments : un pour le poussin, l'autre pour un haut-parleur simulant son rival (near05 experience, ESI Audiotechnik). Deux microphones (MC930, Beyerdynamic GmbH & Co KG, Heilbronn, Germany) sont fixés au couvercle et dirigés vers le poussin. Un des microphones est connecté à une carte son type Steinberg UR44 afin d'enregistrer la totalité de la nuit. L'autre microphone, respectivement le haut-parleur, est connecté à une entrée, respectivement une sortie, d'une carte son type PreSonusDigimax FS afin de réaliser le playback interactif. L'acquisition, le traitement des signaux audio et le pilotage des haut-parleurs est gérée par un script Matlab et l'utilitaire Playrec. L'expérience sur la durée des cris a eu lieu de 22h00 à 01h00 et celle sur le taux de cris de 01h30 à 04h30.

3 Règle de décision du playback

3.1 Playback interactif : durée des cris

Le programme Matlab détecte (Cf chapitre 4) en pseudo temps réel les cris émis par le poussin et en détermine la durée. Toutes les 10 secondes, les durées collectées sont comparées à la durée moyenne des cris obtenue durant les 10 secondes précédentes. D'après cette comparaison, trois stratégies ont été développés : la stratégie de « l'ajustement positif », la stratégie de « l'ajustement négatif » et la stratégie de « contrôle ». Dans la stratégie d'ajustement positif : le playback escalade vocalement (ou désescalade) en même temps et dans les mêmes proportions que le poussin (augmentation de la durée des cris émis par le haut-parleur si augmentation de la durée des cris émis par le poussin et inversement). Dans la stratégie d'ajustement négatif : le playback escalade vocalement quand le poussin désescalade (augmentation de la durée des cris émis par le haut-parleur si diminution de la durée des cris émis par le poussin et inversement). Dans la stratégie de contrôle : le playback émet des cris d'une durée constante (Figure 1). Les stratégies sont alternées toutes les 15 minutes et chaque stratégie est répétée quatre fois durant l'expérience de 3 heures. L'ordre d'apparition des stratégies est aléatoire et la succession de deux stratégies identiques est évitée.



Laps de 10s	Durée des cris du poussin (ms)	Durée du cri du playback (ms)		
		Similaire	Inverse	Constante
T1	$D_{T1} = \text{Moy}(D_{N1,y})$	$D_{P1} = D_{T1}$	$D_{P1} = D_{T1}$	$D_{P1} = 800$
T2	$D_{T2} = \text{Moy}(D_{N2,y})$	$D_{P2} = D_{T2}$	$D_{P2} = D_{P1} - (D_{T2} - D_{T1})$	$D_{P2} = 800$
T3	$D_{T3} = D_{N3,1}$	$D_{P3} = D_{T3}$	$D_{P3} = D_{P2} – (D_{T3} – D_{T2})$	$D_{P3} = 800$
T4	$D_{T4} = \text{NA}$	$D_{P4} = D_{P3}$	$D_{P4} = D_{P3}$	$D_{P4} = 800$
T5	$D_{T5} = \text{Moy}(D_{N5,y})$	$D_{P5} = D_{T5}$	$D_{P5} = D_{P4} – (D_{T5} – D_{T3})$	$D_{P5} = 800$
T6	$D_{T6} = \text{Moy}(D_{N6,y})$	$D_{P6} = D_{T6}$	$D_{P6} = D_{P5} – (D_{T6} – D_{T5})$	$D_{P6} = 800$

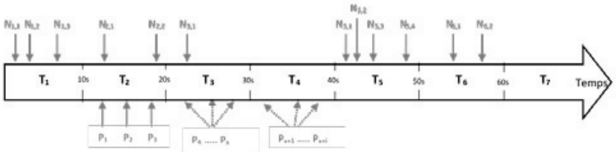
Figure 1 : Exemple de playback interactif sur une minute faisant varier la durée des cris. Tx: $x^{ème}$ laps de temps de 10s, $N_{x,y}$: $y^{ème}$ cri émis par le poussin pendant le $x^{ème}$ laps de temps de 10s, P_x: cri émis par le haut-parleur à la fin du $x^{ème}$ laps de temps de 10s, $D_{Nx,y}$: durée du cri $N_{x,y}$, D_{Tx}: durée moyenne des cris émis par le poussin pendant le $x^{ème}$ laps de temps de 10s, D_{Px}: durée du cri émis par le playback à la fin du $x^{ème}$ laps de temps de 10s.

Le playback émet un cri toute les 10 secondes afin de garder son taux de cris constant (6 cris/min : valeur moyenne du taux de cris observé lors de l'expérience de 2008 durant laquelle deux poussins affamés vocalisaient librement). Les cris émis par le playback proviennent de 4 individus (âge moyen ± *SE*: 32.5 ± 2.3 jours, deux mâles et deux femelles) d'une précédente expérience en 2011 durant laquelle trois poussins interagissaient vocalement librement. Nous avons isolés 120 cris au total par poussin répartis dans 8 groupes de durée : 300-400 ms, 400-500 ms, 500-600 ms, 600-700 ms, 700-800 ms, 800-900 ms, 900-1000 ms et 100-1100 ms. Le programme sélectionne aléatoirement le cri à diffuser parmi le groupe de cris désiré. Chaque cri a été normalisé en amplitude pour minimiser les différences d'intensités entre les cris.

En cas d'absence de cri du poussin lors d'un laps de temps de 10 secondes, le playback considère la valeur de durée moyenne obtenue lors du précédent laps de temps. Le playback garde donc constant la durée de son cri jusqu'à ce que le poussin émette un cri.

3.2 Playback interactif : taux de cris

Cette seconde expérience ressemble en tout point à la précédente (laps de temps de 10s, stratégies) mis à part que l'on s'intéresse cette fois à l'évolution du taux de cris du poussin et non plus à l'évolution de la durée de ses cris. Nous avons donc fixé la durée des cris à 800 ± 50ms (durée moyenne des cris observée lors de l'expérience de 2008 durant laquelle deux poussins affamés vocalisaient librement). Le playback émet les cris répartis de manière régulière pendant le laps de temps de 10 secondes suivant.



Laps de 10s	Nombre de cris du poussin (/10s)	Nombre de cri du playback (/10s)		
		Similaire	Inverse	Constante
T1	$R_{T1} = 3$	$R_{P1} = R_{T1}$ → 3	$R_{P1} = R_{T1}$ → 3	$R_{P1} = 1$
T2	$R_{T2} = 2$	$R_{P2} = R_{T2}$ → 2	$R_{P2} = R_{T1} - (R_{T2} - R_{T1})$ → 4	$R_{P2} = 1$
T3	$R_{T3} = 1$	$R_{P3} = R_{T3}$ → 1	$R_{P3} = R_{T2} - (R_{T3} - R_{T2})$ → 5	$R_{P3} = 1$
T4	$R_{T4} = 0$	$R_{P4} = R_{T4}$ → 0	$R_{P4} = R_{T3} - (R_{T4} - R_{T3})$ → 6	$R_{P4} = 1$
T5	$R_{T5} = 4$	$R_{P5} = R_{T5}$ → 4	$R_{P5} = R_{T4} - (R_{T5} - R_{T4})$ → 2	$R_{P5} = 1$
T6	$R_{T6} = 2$	$R_{P6} = R_{T6}$ → 2	$R_{P6} = R_{T5} - (R_{T6} - R_{T5})$ → 4	$R_{P6} = 1$

Figure 2 : Exemple de playback interactif sur une minute faisant varier le taux de cris. Tx: $x^{ème}$ laps de temps de 10s, $N_{x,y}$: $y^{ème}$ cri émis par le poussin pendant le $x^{ème}$ laps de temps de 10s, P_x: cri émis par le hautparleur à la fin du $x^{ème}$ laps de temps de 10s, R_{Tx}: taux de cris du poussin pendant le $x^{ème}$ laps de temps de 10s, R_{Px}: taux de cris du playback pendant le $x^{ème}$ laps de temps de 10s.

4 Détection d'un évènement

Les algorithmes de playback présentés plus haut (sections 3.1 et 3.2) supposent une détection pseudo temps réelle des cris de négociation. La fréquence d'échantillonnage des signaux audio enregistrés est de 44.1 kHz. Les signaux audio sont traités par trames de 2048 échantillons, soit environ 46 ms. Pour chacune de ces trames, l'algorithme de détection doit répondre aux questions suivantes :

- Cette trame contient-t-elle un évènement sonore ?
- Si oui, est-ce un cri de négociation ou bien un autre bruit ?

La réponse à la première question s'obtient aisément, par exemple, en appliquant un seuil empirique sur le niveau sonore de la trame courante. Ce niveau sonore peut s'exprimer sous la forme d'un niveau de pression L_p, en dB, suivant l'équation (1) :

$$L_p[q] = 20\log_{10}\left(\sqrt{\frac{1}{N}\sum_{k=1}^{N} y^q[k]^2} \Big/ 2e^{-5}\right) \qquad (1)$$

Où y^q est la $q^{ème}$ trame de signal (convertie en Pascal) de taille Nx1, et k représente l'indice de l'échantillon considéré, $k \in [\![1, N]\!]$

La réponse à la deuxième question est plus complexe et nous décrivons ci-après la stratégie déployée pour identifier si un évènement sonore correspond ou pas à un cri de négociation. Cette détection repose sur l'implémentation de deux descripteurs fréquentiels et d'un descripteur temporel.

4.1 Premier descripteur fréquentiel : la variance spectrale

La figure 3 et la figure 4 représentent respectivement les formes d'ondes et les spectrogrammes d'un cri de négociation et de gazouillis produits par le même poussin. La distribution du niveau sonore moyen de chaque bin fréquentiel est également représentée. On constate immédiatement une différence relative à l'harmonicité des cris produits. Les gazouillis présentent une harmonicité marquée (une fréquence fondamentale et ses multiples entiers) contrairement aux cris de négociation qui couvrent l'ensemble de leur bande passante de manière plus uniforme. Ces deux cris peuvent être écoutés en cliquant sur le lien [16].

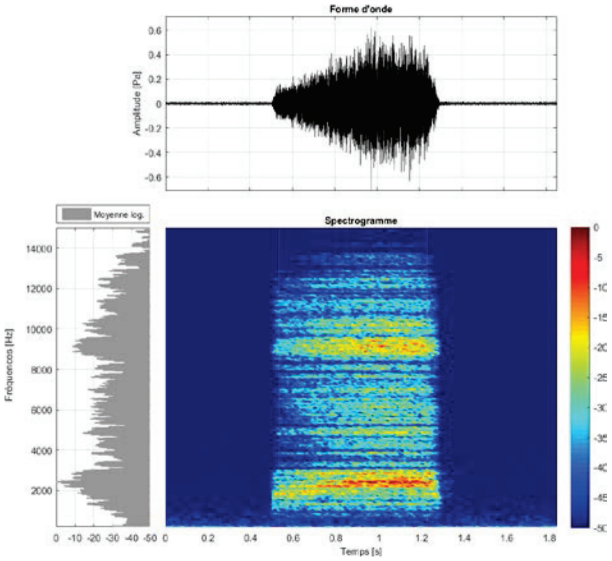


Figure 3 : Forme d'onde et spectrogramme d'un cri de négociation de chouette effraie. L'échelle de couleurs est exprimée en dB (relatif).

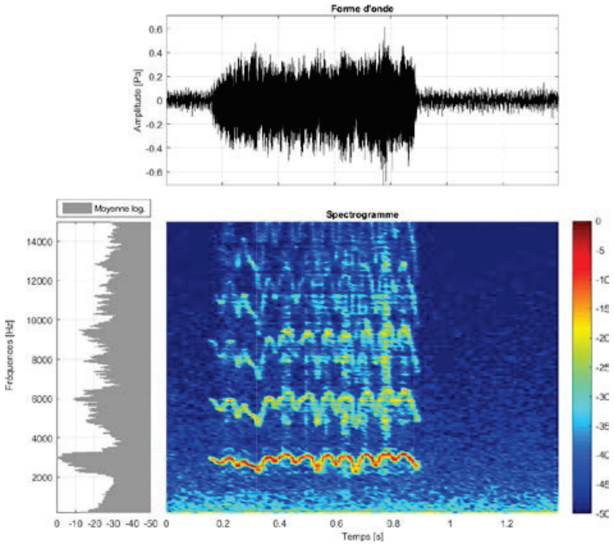


Figure 4 : Forme d'onde et spectrogramme de gazouillis de chouette effraie. L'échelle de couleurs est exprimée en dB (relatif).

L'évaluation du caractère plus ou moins harmonique d'un signal est un processus coûteux en temps de calcul. Nous nous sommes donc intéressé à observer une conséquence indirecte de cette présence ou non d'harmonicité, à savoir la variance du spectre, avec l'idée sous-jacente qu'un cri de négociation a une étendue spectrale plus large que des gazouillis. Nous définissions ce descripteur D_1 selon l'équation (2) :

$$D_1[q] = \log_{10}(\mathrm{Var}(|\mathbf{Y}^q|)) \qquad (2)$$

Où $\mathbf{Y}^q$ est la transformée de Fourier discrète de la $q^{\text{ème}}$ trame de signal, pondérée par une fenêtre de Hann.

L'analyse ROC de ce descripteur appliqué sur une base de données de 500 cris de négociation et 500 gazouillis retourne un taux de détection vraie de 80% et un taux de fausse alarme de 16%. La plage de fréquence pour l'application de ce descripteur a été judicieusement choisie pour optimiser ces scores.

4.2 Deuxième descripteur fréquentiel : valeur minimale des coefficients cepstraux

La figure 5 et la figure 6 montrent l'évolution des coefficients cepstraux (MFCC) des deux mêmes cris. On remarque pour le cri de négociation une chute de la valeur de la troisième bande cepstrale, laquelle n'apparait pas sur les gazouillis. Il a donc été décidé d'établir un descripteur D_2 visant à renvoyer la valeur minimale des coefficients cepstraux, comme défini par l'équation (3) :

$$D_2[q] = min(\mathbf{MFCC}^q) \qquad (3)$$

Où $\mathbf{MFCC}^q$ sont les coefficients cepstraux de la $q^{\text{ème}}$ trame de signal.

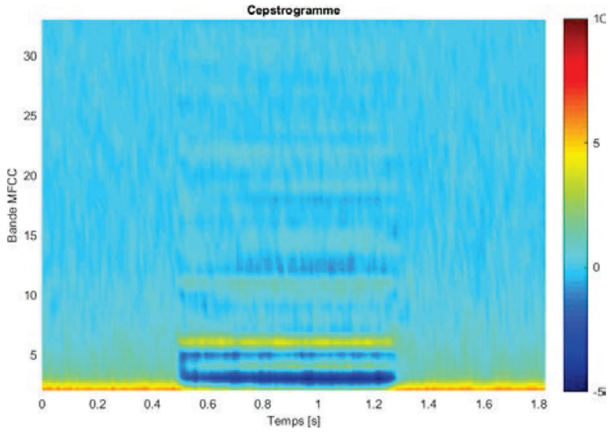


Figure 5 : Cepstrogramme d'un cri de négociation (chute de la 3$^{\text{e}}$ bande MFCC). L'échelle de couleurs est exprimée en dB (relatif).

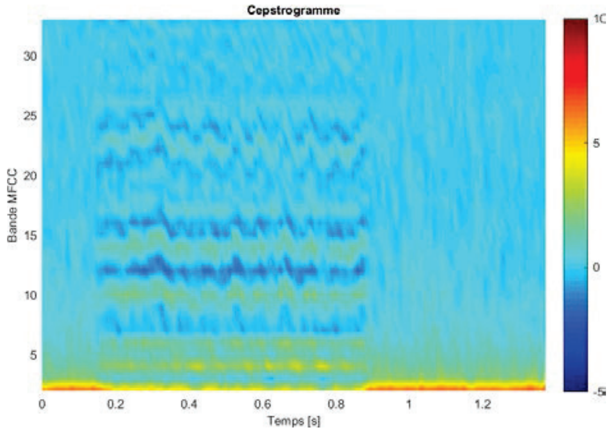


Figure 6 : Cepstrogramme d'un gazouillis (pas de chute de valeurs). L'échelle de couleurs est exprimée en dB (relatif).

L'analyse ROC de ce descripteur appliqué sur une base de données de 500 cris de négociation et 500 gazouillis retourne un taux de détection vraie de 75% et un taux de fausse alarme de 13%.

La validité d'une trame sur le plan fréquentiel est ainsi jugée sur le respect de la condition (4), à savoir, une simultanéité entre une variance élevée et une valeur minimale de MFCC faible :

$$D_1[q] > \lambda_1 \ \text{ et } \ D_2[q] < \lambda_2 \qquad (4)$$

où λ_1 et λ_2 sont des seuils définis empiriquement d'après les analyses ROC précédentes.

4.3 Troisième descripteur : consistance temporelle

La distinction entre cris de négociation, gazouillis et autre bruits s'opère également par l'étude de leur durée. Un troisième descripteur, dit de consistance temporelle, consiste à ne pas considérer les signaux constitués d'un nombre insuffisant de trames valides au sens de la condition (4), parce que trop courts. En effet, les cris de négociations ont une durée minimale d'environ 300 ms. La prise en compte de cette information améliore la robustesse de l'algorithme de détection. Dans notre algorithme, sept trames (soit 322 ms) doivent consécutivement vérifier la condition (4) pour être effectivement considérée comme un cri de négociation.

4.4 Score

Une comparaison manuelle opérée sur 180.5 heures d'enregistrements, comprenant 55247 cris, montre que l'algorithme de détection pseudo temps réel basé sur les trois descripteurs décrit précédemment atteint un taux de détection vraie de 97% et un taux de fausse alarme de 3%. Ces scores assurent une excellente fiabilité pour le dimensionnement d'expériences de playback automatique de longue durée et sur 8 individus en parallèle.

5 Conclusion

Ce papier décrit le protocole expérimental du playback interactif mis au point pour étudier la dynamique temporelle de communication vocale chez la chouette effraie. Ce protocole est basé sur la connaissance du modèle biologique et le développement d'une routine automatique qui prend continuellement des décisions quant au paramètre du cri émis (durée ou nombre). Les résultats sont en cours d'analyse et devraient confirmer l'importance de considérer la communication de manière interactive et non pas statique. En effet, les poussins de chouette effraie décident d'escalader dans la communication (augmenter le nombre et la durée des cris) de manière non aléatoire mais dépendamment de la communication de leur rival. Cet ajustement permet d'améliorer l'efficacité de la négociation et donc d'améliorer la résolution du conflit. Le playback interactif nous permet également d'identifier la stratégie optimale concernant la dynamique de communication. Il faut néanmoins avoir conscience des implications lié à cette méthode. Premièrement, étant interactif, chaque paramètre est susceptible d'être modifié au cours de l'expérience. Deuxièmement, la prise de décision se faisant en fonction de l'animal en temps réel, l'anticipation de tous les scénarios possibles est primordiale, d'où l'importance d'avoir au préalable une bonne connaissance du modèle biologique. Troisièmement, la détection et l'analyse en temps réel doit être fiable pour automatiser les expériences, d'où la nécessité de développer un logiciel dédié.

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Received: 30 September 2016 | Revised: 19 December 2016 | Accepted: 16 March 2017
DOI: 10.1111/eth.12612

RESEARCH PAPER

WILEY ethology

No need to shout: Effect of signal loudness on sibling communication in barn owls *Tyto alba*

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Funding information
Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung, Grant/Award Number: 31003A-120517

Editor: L. Fusani

Abstract

In animal communication, signal loudness is often ignored and seldom measured. We used a playback experiment to examine the role of vocal loudness (i.e., sound pressure level) in sibling to sibling communication of nestling barn owls *Tyto alba*. In this species, siblings vocally negotiate among each other for priority access to parental food resources. Call rate and call duration play key roles in this vocal communication system, with the most vocal nestlings deterring their siblings from competing for access to the food item next delivered by parents. Here, we broadcast calls at different loudness levels and call rate to live nestlings. The loudness of playback calls did not affect owlets' investment in call rate, call duration or call loudness. The rate at which playback calls were broadcast affected owlets' call rate but did not influence their response in terms of loudness. This suggests that selection for producing loud signals may be weak in this species, as loud calls may attract predators. Moreover, given that owlets do not overlap their calls and that they communicate to nearby siblings in the silence of the night, loud signals may not be necessary to convey reliable information about food need.

KEYWORDS
acoustics, amplitude, begging, communication, competition, multiple signals, negotiation, playback

1 | INTRODUCTION

Loudness has been largely understudied in animal acoustic communication. Researchers usually focus on the frequency spectrum of signals, their duration and the rate at which they are emitted. However, loudness can encode important information that is relevant to assess quality or motivation of the signaller. For instance, eastern screech-owls *Megascops asio* that beg louder are fed first by parents (Hofstetter & Ritchison, 1998). The begging loudness usually decreases with the relatedness level within broods, suggesting that high competition levels favours loud calls (Briskie, Naugler, & Leech, 1994). Some female birds prefer to mate with males producing loud rather than weak songs (red-winged blackbirds *Agelaius phoeniceus*, Searcy, 1996; zebra finches *Taeniopygia guttata*, Ritschard, Riebel, & Brumm, 2010). Producing louder signals may not have strong metabolic costs (Zollinger, Goller, & Brumm, 2011), although it may require a slightly higher oxygen consumption (Oberweger & Goller, 2001) and stronger respiratory muscle activity and hence be condition dependent (Goller & Cooper, 2004). The main cost may be a stronger social aggression from competitors or higher detectability by predators (reviewed in Zollinger & Brumm, 2015).

One of the reasons why signal loudness is often overlooked in animal communication studies is the difficulty in accurately measuring loudness, especially in the field. The difficulty in estimating sound loudness rises in the necessity to account for signaller's orientation, sound propagation in the environment and distance to the microphone. In the laboratory, by measuring from a fixed distance and directly from above the animal, these problems can be solved (Brumm & Todt, 2002). Here, we report an experiment performed in the laboratory to examine the effect of loudness variation in sibling vocal communication of barn owl *Tyto alba* nestlings. When owlets are alone in the nest waiting for a parent to come back with a prey item, they continuously exchange hissing calls to advertise their hunger levels to siblings. Hungry individuals produce more calls and longer calls, which progressively deters their siblings from calling and competing for the food item next delivered by the parent (Dreiss, Ruppli, Faller, & Roulin, 2015; Ruppli, Dreiss, & Roulin, 2013). This communication system reduces the cost of sibling competition between related individuals (Johnstone & Roulin, 2003). Although hungry owlets produce louder calls (Dreiss, Ruppli, & Roulin, 2014), the effect of call loudness on sibling propensity to compete or to retreat from sibling competition is yet unknown. This aspect has to be tackled experimentally because call loudness is correlated with call rate and call duration (Dreiss et al., 2014).

Identifying the full range of signalling components used by young animals is important to understand how they resolve competition over shared resources. So far we have demonstrated that call rate, call duration and the rapidity at which nestlings call after a sibling play some role in sibling negotiation (Dreiss, Ruppli, Faller, & Roulin, 2013; Ruppli et al., 2013). Multiple-component signalling across or within sensory modalities is frequent in animal displays (Bro-Jørgensen, 2010). Because displaying several signals may seem wasteful if one type of signal efficiently repels rivals and predators or attracts mates, hypotheses have been proposed to explain the maintenance of multiple signalling. Using multiple "redundant" signals may be a way to reinforce communication efficiency. For instance, eastern grey squirrels *Sciurus carolinensis* showed enhanced responses to multisensory alarm signals compared to single-sensory signals (Partan, Larco, & Owens, 2009). Additionally, redundant signals enable signaller to increase a modality if another modality is restrained. For instance, tree swallow *Tachycineta bicolor* nestlings reinforce their call rate when they are physically constrained and cannot approach the nest entrance where parents deliver food (Leonard, Horn, & Parks, 2003). The use of multiple parameters can alternatively allow for encoding of different information. For instance, different song traits, consistency and repertoire size, of the great tit *Parus major* are related to different male attributes, age and survival (Rivera-Gutierrez, Pinxten, & Eens, 2010).

A major function of loud signals is to ensure that an individual is heard over long distances (Klump, 1996) or detected in an environment with a lot of background noise (Brumm, 2004; Leonard, Horn, Oswald, & McIntyre, 2015). It may also help being heard when several individuals are calling simultaneously, as in begging litters of broods (Brumm & Todt, 2004). In the barn owl, selection may, however, not favour the evolution of loud calls for several reasons. First, nestlings are aggregated in narrow nests and hence siblings can easily hear each other. The need to call loudly may be less pronounced than in diurnal species because at night background noise is usually less pronounced than during the day. Second, calling loudly may attract predators in young birds (McDonald, Wilson, & Evans, 2009; Roulin, 2001), an issue that may be particularly relevant in the barn owl because nestlings produce thousands of calls in a single night, a behaviour that is already enough conspicuous to predators. To reduce predation risk, nestlings may therefore be selected to produce informative vocal signals without having to call very loudly (Briskie, Martin, & Martin, 1999). More specifically, nestling barn owls follow specific turn-taking rules (Dreiss, Ruppli, Faller, et al., 2015) allowing them to avoid calling simultaneously (Dreiss, Ruppli, Oberli, et al., 2013). Hence, they would not need to call very loudly to overcome siblings. The above arguments support the expectation of a minor role of call loudness in the barn owl sibling negotiation process. On the other hand, because hungrier nestlings produce louder calls in this species (Dreiss et al., 2014), we would expect siblings to vocally withdraw in front of a loud nestmate.

To investigate the potential role of loudness in sibling negotiation, we played-back calls at three loudness levels and three call rates to experimental three-chick broods. Age hierarchy among siblings affects call production, younger siblings producing more, louder and longer calls (Dreiss, Lahlah, & Roulin, 2010; Dreiss et al., 2014), probably as a way to compensate their competitive inferiority. We thus analysed owlets' response according to their position in the age hierarchy. Similar playback settings were successful in confirming the role of call rate and duration in sibling negotiation (Ruppli et al., 2013). We hence examined the effect of playback call rates on nestling calls' loudness. If nestlings adjusted loudness in a similar way as call rate and duration, we expected that they would produce louder calls when facing a playback nestmate displaying a low motivation (low-call rate) rather than a high motivation to compete (high-call rate). We then examined the effect of playback loudness on nestling vocal production. If call loudness is perceived as a competitive signal, we expected that nestlings would withdraw from the vocal contest when hearing loud calls; hence loud playback calls would trigger less numerous and shorter calls in owlets than quiet playback calls.

2 | METHODS

2.1 | Experimental procedure

The study was performed in western Switzerland (46°4′N, 6°5′E) on a population of wild barn owls breeding in nest boxes located in barns. From 2nd June to 22nd September 2011, three siblings from 28 broods (42 female and 42 male nestlings) were brought to the laboratory at 12:00. Nestlings (age 32.5 ± 0.5 days; range 21–40) were placed in a circular box (diameter 100 cm, height 51 cm, Supplementary Material S.1) divided into three equal parts with wire mesh, each individual in a separated part. In each part, we placed on the floor 51 ± 1 g of mice (*Mus musculus*). In a central cylinder (diameter 15 cm), we placed a loudspeaker (W2-800SL; Tangband, Taipei, Taiwan, R.O.C.) connected to a monitor (6301B; Fostex, Tokyo, Japan). Due to staggered hatching, siblings differed in age, and each experiment comprised a junior (21–36 day-old), middle-born (27–38 day-old) and senior nestling (31–40 day-old).

We broadcast nine playback sequences in a row from 00:30, each sequence lasting 4 min, with periods of 10 min of silence between two sequences. The nine sequences corresponded to the nine combinations of three call loudness relative levels (−64, −56 and −48 dB, from the softest to the loudest) and three call rates (2, 6 and 10 calls/min). We allocated the nine sequences in a random order across the broods. The call rate 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 (Ruppli et al., 2013) and the call loudness levels were chosen by ear by A.D. so that they matched the natural range of loudness heard in natural conditions. Recordings of the present experiment confirmed that playback loudness was within natural range, as nestling call loudness ranged from −88 to −19 dB (lowest and highest 10% of the values: −59 and −27 dB). The loudness is negative because it was calculated as the logarithm of the ratio between the amplitude (Pa) of the detected call and a reference value, empirically chosen to improve the detection rate during the automated call detection phase (Supplementary S.2, Leq_m). The loudness level of the median treatment corresponded to the absolute levels of 51 dB SPL, measured with a sonometer (Nor118; Norsonic AS, Lierskogen, Norway) placed at 1 m of the loudspeaker; the background noise level was estimated at 4.8 dB. Call loudness of broadcast calls was manipulated using normalisation procedure of Audacity v.1.3 Beta (http://audacityteam.org). To avoid pseudo-replication, each brood heard a unique combination of calls, built with a set of 24 different calls (0.8 s each, corresponding to average value of call duration) emitted by a single young recorded in the laboratory in 2008 (Ruppli et al., 2013). We used in total seven sets of 24 calls, providing from seven young (four males and three females, aged 29 ± 2 days).

2.2 | Acoustic analyses

Eight microphones (Perception 170; AKG Acoustics, Northridge, CA 91329, USA) were fixed on the box roof constituted of rigid wire mesh to record nestlings' response. During the 4 min playback sequences nestling calls were detected automatically (see Supplementary Material S.2) using a post-processing Matlab script (version R2012b, MathWorks, Natick, MA, USA) and measured for duration (s) and mean loudness (i.e., sound pressure level in dB, Supplementary Material S.2). By comparing the recordings at the eight microphones, the localisation of the sound was estimated with the SRP-PHAT method (DiBiase, 2000), and the identity of the caller was determined (junior, middle-born or senior). For further technical details, see Supplementary Material (S.3). Approximately 5.7% of calls (241 of 4230) could not be assigned to a specific nestling, because they were emitted too close from the wire mesh separating two nestlings, and were discarded.

2.3 | Statistical analyses

To determine whether playback call loudness and playback call rate affected nestling vocal response, we analysed nestling vocalisations within each of the nine playback sequences of 4 min for the 28 three-chick broods. For each statistical model, we set as independent factors the playback call rate and loudness, the order at which the playback sequence was broadcast (1st to 9th), the age rank of the experimental nestling (junior, middle-born or senior), its sex, and the interaction between playback call rate and loudness. The interactions were removed from the final models when non-significant (p-values smaller than .05). As a random factor, we set nestling identity nested in the brood. To determine whether playback loudness affects nestling probability to vocally respond, we used a generalised mixed model with binomial distribution, with as dependant variable the probability of emitting a call during a playback sequence. For the nestlings producing at least one call and which vocalised within at least three 4-min playback sequences, we then examined whether the call duration (with a linear mixed model), the number of calls emitted (with generalised mixed model with Poisson distribution) and the call loudness (Box-Cox transformed, with a linear mixed model) were affected by playback treatments. Model assumptions were checked (departure of the scaled Pearson statistic, normal distribution of residuals and homoscedasticity). All statistical analyses were performed with the software SAS v.9.3 (SAS Institute Inc., Cary, NC, USA).

2.4 | Ethical notes

At the time of the experiment, nestlings were old enough to thermoregulate and feed on dead rodents without parental help. Moreover, they are used to parental absence, as parents rest outside the nest and only visit the nest during the night to bring prey items. The laboratory mice *Mus musculus* were euthanised by CO_2 (bought frozen from an animal house, Reptiles Farm, Servion, Switzerland). Nestlings returned to their natural nest box after two nights at the laboratory. We always left one or two nestlings in the natural nest and removing several nestlings from a nest during two nights never induced parents to abandon their brood. Nestlings were transported in opaque aerated plastic boxes, with a foam floor. In the laboratory, nestlings were not physiologically stressed, as shown by the absence of a rise in baseline corticosterone level compared to the situation prevailing under natural, undisturbed conditions (Dreiss, Henry, Ruppli, Almasi, & Roulin, 2010) or behavioural signs of stress. The stay at the laboratory did not negatively affect their body condition and fledging success (Dreiss, Ruppli, Antille, & Roulin, 2015). The experiment was carried out under the legal authorisation of the Veterinary Service of Vaud canton (N°2109.1).

3 | RESULTS

Owlets emitting louder calls produced longer and more calls on average (Spearman correlation: $r_{72} = .32$, $p = .006$; $r_{72} = .46$, $p < .0001$; respectively), as shown previously in another set of individuals (Dreiss et al., 2014). Nestlings produced louder, longer and more calls later in the night (playback order effect, Table 1).

The probability of a nestling emitting at least one call was not related to playback call loudness or call rate (Table 1a). Playback loudness did not affect any call feature, that is, nestlings' call loudness, number of calls and call duration (Table 1, Figure 1). By contrast and

TABLE 1 Barn owl nestlings' vocalisation according to playback broadcast. Results of mixed models

	a. Probability to call (*df* = 663)			b. Number of calls (*df* = 152)			c. Call duration (*df* = 148)			d. Call loudness (*df* = 148)		
	Estimate ± *SE*	*F*	*p*	Estimate ± *SE*	*F*	*p*	Estimate ± *SE*	*F*	*p*	Estimate ± *SE*	*F*	*p*
Playback call rate		0.06	.937		3.83	.024		2.93	.057		2.11	.13
(2 vs.10)	0.08 ± 0.21			0.42 ± 0.15			0.02 ± 0.02			0.05 ± 0.04		
(6 vs.10)	0.04 ± 0.22			0.21 ± 0.15			0.05 ± 0.02			0.08 ± 0.04		
Playback loudness		0.85	.43		0.19	.83		0.48	.62		1.11	.33
(−64 vs. −48)	−0.27 ± 0.22			0.09 ± 0.15			−0.01 ± 0.02			−0.04 ± 0.04		
(−56 vs. −48)	−0.06 ± 0.21			0.05 ± 0.15			0.01 ± 0.02			0.03 ± 0.04		
Playback order	0.08 ± 0.03	5.50	.019	0.08 ± 0.02	10.62	.001	0.01 ± 0	5.84	.017	0.01 ± 0.01	4.02	.047
Nestling age rank		4.16	.016		0.59	.55		0.82	.44		0.25	.78
(Junior vs. Senior)	1.18 ± 0.41			−0.28 ± 0.33			−0.08 ± 0.07			−0.17 ± 0.25		
(Middle-born vs. Senior)	0.72 ± 0.42			−0.28 ± 0.26			−0.08 ± 0.07			−0.16 ± 0.27		
Nestling Sex (*F* vs. *M*)	0.13 ± 0.34	0.15	.69	−0.51 ± 0.24	4.96	.027	0.01 ± 0.05	0.06	.81	0.01 ± 0.19	0.01	.96
Playback call rate x Playback loudness		0.81	.52		0.68	.61		0.35	.84		0.62	.65

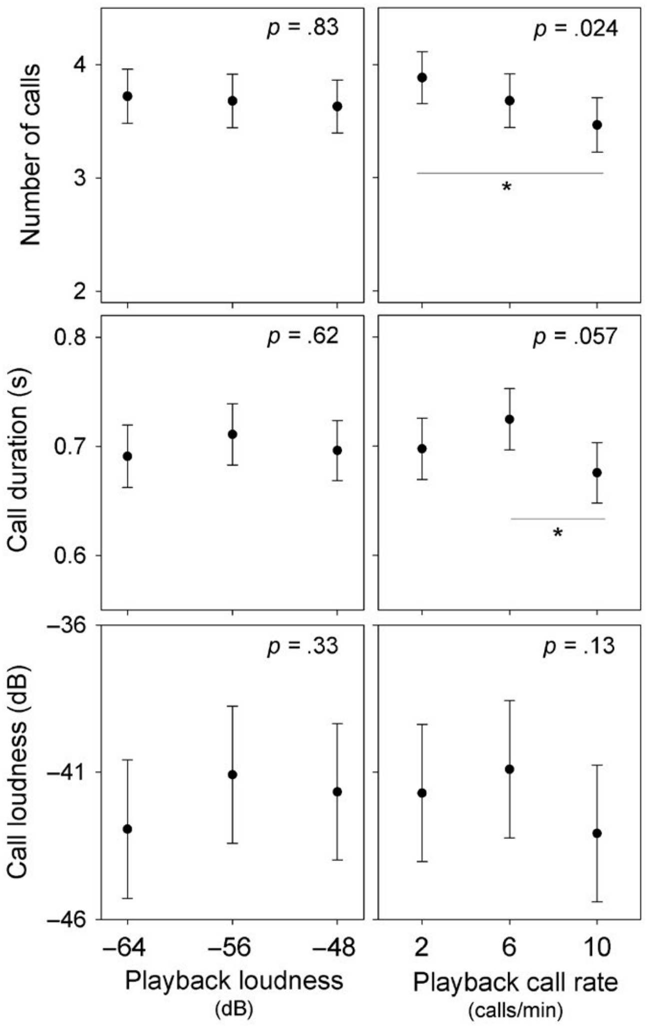

FIGURE 1 Effect of playback call loudness and call rate on barn owlets' number of calls, call duration (s) and loudness (dB) during a 4-min playback sequence. Means are represented with *SE*

as already demonstrated before (Ruppli et al., 2013), higher playback call rates induced nestlings to produce fewer calls and to some extent shorter calls (Table 1bc, Figure 1). However, it did not significantly affect nestlings' call loudness (Table 1d). Playback call rate affected nestmate call production whatever the loudness at which these calls were broadcast, as shown by the lack of statistical interaction between call rate and loudness (Table 1).

Younger owlets were more likely to call than their older siblings (Table 1a), similarly to what was found in previous studies (Dreiss, Lahlah, et al., 2010; Dreiss et al., 2014). Contrary to previous studies, age rank was not significantly related to call duration and loudness (Dreiss, Lahlah, et al., 2010; Dreiss et al., 2014). Vocal females produced slightly more calls than males (Table 1b).

4 | DISCUSSION

Extensive variation in playback loudness did not significantly influence the investment of barn owl nestlings in sibling competition, contrary to variation in call rate and duration (present study and Ruppli et al., 2013; Dreiss, Ruppli, Faller, et al., 2015). Call loudness had weak or no influence on the negotiation process, as the playback loudness treatment did not affect the key acoustic parameters (call rate and duration) used in sibling negotiation. Moreover, the call rate, the most efficient parameter of sibling competition (Ruppli et al., 2013), did not influence the loudness of nestling calls. Call loudness is hence not likely to be used as a primary signal of motivation to compete for food. Because altricial barn owl nestlings are in close proximity to one another and because altricial nestlings are easy targets for predators, this result may not be surprising. Sibling barn owls would hence little rely on variations of loudness to communicate.

However, nestlings modulated their call loudness, producing louder calls when hungrier as shown experimentally by Dreiss et al. (2014). Calls became louder with time in the present study, which could also reflect an effect of increased hunger. Younger nestlings were shown to produce louder calls, as well as more calls and longer calls (Dreiss et al., 2014), but this result was not confirmed in the present study, younger siblings only producing more calls. Call loudness was also positively correlated with call duration and call rate (Dreiss et al., 2014 and present results), two parameters that determine the negotiation outcome (Dreiss, Lahlah, et al., 2010) and are finely adjusted to sibling vocalisation (Dreiss, Ruppli, Faller, et al., 2015; Ruppli et al., 2013). However, owlets modify their call loudness to a lower extent than their number of calls and the duration of their calls (Dreiss et al., 2014).

Several hypotheses could account for the observed modulation of call loudness. Producing louder calls may be a way to be identified more easily by competing siblings when hungry and when in low-ranking position. Hungry nestlings would indeed benefit from being identified when they call, in order to be given the priority access to the impending food item. Hungry nestlings were indeed more easily distinguishable from one another than satiated nestlings (Dreiss et al., 2014), and they may use a loud signal to favour this recognition. Alternatively, loudness may be a “backup” or “redundant” signal (Johnstone, 1996), which has low effect but may enhance message transmission and detection (Rowe & Guilford, 1999). Loud calls would hence be used by lower competitive younger nestlings, in combination with more calls and longer calls, maybe as a mean to counterbalance their low-ranking position.

We can also hypothesise that loudness is used in some particular contexts. For instance, if nestlings are limited in the number of calls they can emit, they may compensate by producing loud calls, when in presence of highly vocal siblings (Dreiss, Ruppli, Faller, et al., 2015) or in noisy environments (Johnstone, 1996). This could be tested by broadcasting longer playback sequences than the 4-min sequences used in the present experiment, by broadcasting much higher playback call rate (call rate could be up to 45 calls/min), or by manipulating the background noise. We have shown here that playback loudness did not affect nestling vocal production, but we could also speculate that this lack of effect was due to nestlings only adjusting here to the most relevant signals (call rate) of the two playback acoustic signals (Rubi & Stephens, 2016). To test this hypothesis, we would need to broadcast a playback varying only in call loudness and examine whether it triggers a differential response from nestlings. Further studies are hence needed to test these alternative hypotheses and to deepen our understanding of use of loudness in various contexts.

ACKNOWLEDGEMENTS

We thank two anonymous referees for comments on a previous version of the manuscript. The study was financed by the Swiss National Science Foundation (grant n° 31003A-120517).

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Dreiss AN, Ducouret P, Ruppli CA, et al. No need to shout: Effect of signal loudness on sibling communication in barn owls *Tyto alba*. *Ethology*. 2017;123: 419–424. https://doi.org/10.1111/eth.12612

J Ornithol (2017) 158:1001–1012
DOI 10.1007/s10336-017-1458-3

ORIGINAL ARTICLE

Nocturnal, diurnal and bimodal patterns of locomotion, sibling interactions and sleep in nestling Barn Owls

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Received: 17 January 2017 / Revised: 20 March 2017 / Accepted: 18 April 2017 / Published online: 8 May 2017

Abstract Temporal variation in physical activity is mainly determined by the day–night cycle. While this may be true for diurnal species whose vision at night is often poor, the situation might be more complex in nocturnal animals as many such species can see both in the dark and in the daylight. We examined in Barn Owl (*Tyto alba*) nestlings whether temporal variation of behavioural activities and sleep is shaped by parental feeding visits occurring during the first part of the night and the extent to which they also occur during daylight hours. We measured several behaviours in 280 individuals from 90 broods recorded in 4 years. Parental feeding visits progressively declined in frequency from the beginning to the end of the night, and a number of offspring behaviours followed the same pattern of activity (feeding, vocalization and self-preening). Surprisingly, nestlings were awake not only at sunset, but also at sunrise. Several behaviours (locomotion, wing flapping and sibling interactions, such as pecking and allopreening among nestlings) showed peaks of activity at sunset and sunrise, suggesting that they were performed for other reasons than to interact with parents. Allopreening was performed more often during the day than at night. We conclude that although adult Barn Owls are nocturnal, nestlings display a complex temporal pattern of activity that is governed not only by feeding but also by other unknown factors.

Keywords Barn Owl · Behaviour · Bimodality · Diel organization · Ontogeny · Sleep

Zusammenfassung

Nächtliche, tägliche und bimodale Muster der Lokomotion, der Interaktionen mit Geschwistern und des Schlafs bei Schleiereulennestlingen

Der zeitliche Verlauf der körperlichen Aktivität wird hauptsächlich vom Tag-Nacht-Rhythmus bestimmt. Besonders tagaktive Arten sind oft nicht in der Lage nachts zu sehen, während nachtaktive Arten sowohl am Tag als auch nachts sehen können. Wir untersuchten an Schleiereulennestlingen (*Tyto alba*), ob zeitliche Variation von Verhalten und Gehirnaktivität von den elterlichen Besuchen in der ersten Hälfte der Nacht abhängen und ob Aktivitäten während des Tages ausgeführt werden. Wir bestimmten Verhaltensweisen von 280 Individuen aus 90 Bruten über vier Jahre. Elterliche Fütterungsbesuche nahmen über die Nacht stetig in der Frequenz ab, und einige der Verhaltensweisen der Nestlinge folgten diesem Muster (Fressen, Rufen und Putzen). Erstaunlicherweise waren Nestlinge nicht nur bei Sonnenuntergang wach, sondern auch bei Sonnenaufgang. Einige Verhaltensweisen (Lokomotion, Flügel schlagen und Interaktionen mit

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Communicated by F. Bairlein.

Electronic supplementary material The online version of this article (doi:10.1007/s10336-017-1458-3) contains supplementary material, which is available to authorized users.

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Geschwistern wie Picken und gegenseitiges Putzen) zeigten hohe Aktivität bei Sonnenuntergang und –aufgang, was darauf hindeutet, dass sie aus anderen Gründen ausgeführt wurden als mit den Eltern zu interagieren. Gegenseitiges Putzen wurde tagsüber öfter durchgeführt als nachts. Zusammenfassend ist zu sagen, dass Schleiereulen zwar nachtaktiv sind, Nestlinge aber ein komplexes Aktivitätsmuster zeigen, das durch die elterliche Fütterung vorgegeben ist, aber auch durch weitere, noch unbekannte Faktoren bestimmt ist.

Introduction

Specific behaviours should be expressed at the appropriate time of the day or night to maximize the associated benefits while minimizing the costs. An animal's activity pattern can be shaped by environmental factors, such as food availability, temperature and light intensity, or by the social environment (Vasquez 1996; Elvert et al. 1999; Mrosovsky 1999; Boydston et al. 2001). For example, in teleost fishes, rain improves nocturnal foraging efficiency, which in turn induces them to switch their activity period from diurnal to nocturnal (Payne et al. 2012). Predation risk can also limit the activity of prey species to the periods with the lowest risk of predation. Three-toed sloths (*Bradypus variegatus*) exposed to nocturnal predators preferentially sleep at night, whereas the pygmy sloth (*Bradypus pygmaeus*) living on an island without any predator shows no preference for sleeping during the night or day (Voirin et al. 2014).

Although numerous studies demonstrate the influence of environmental and species-specific adaptation on activity pattern in adults (Hut and Beersma 2011), few studies have examined the activity and sleep–wakefulness patterns during development. Studies from a wide range of taxa are required to determine the major underlying factors that influence temporal variation in sleep–wakefulness states and locomotor activity (e.g. Scriba et al. 2013b). Nocturnal species are interesting because their visual systems allow for active behaviour during both the day and night (Hall and Ross 2007; Hall 2008). Consequently, nocturnal animals may be more flexible in their timing of activity than diurnal species (Hoogenboom et al. 1984; Levy et al. 2007; Daan et al. 2011).

We have been studying whether Barn Owl (*Tyto alba*) nestlings are primarily active at night, like their parents (Erkert 1969; Scriba et al. 2013a), or throughout both the day and night. Although nocturnal provisioning by the parents might favour nocturnal activity in nestlings, the storage of food in the nest may free nestlings to also feed during the daylight hours (Roulin 2004). Furthermore, nestlings experience various environmental and social influences, such as environmental disturbances during the day and interactions with siblings.

We thus designed a study in which we recorded a number of behaviours in different individuals monitored in 2007, 2011, 2012 and 2014. These behaviours included the timing of nestling cerebral wakefulness and sleep, parental food provisioning, nestling feeding, preening of themselves and of their siblings (i.e. allopreening), locomotion, wing flapping, vocalization and competitive behaviour (pecking). We assessed these activities in the field and also in the laboratory in the absence of the parents. We predicted that some activities, such as feeding and vocal competition for food, would follow the nocturnal timing of the parental visits. This nocturnal pattern may however shift to bimodality if there is some benefit in performing activities at different times of day (e.g., exposure to different cues, for improved sensory development or having many bouts of sleep for cognitive development). As a consequence, nestlings might be highly active shortly after sunset and around sunrise. In rats, the rhythmic clock gene expression important for a circadian rhythm develops gradually during the first weeks after birth (Vallone et al. 2007). In young quail, a circadian rhythm for feeding activity first occurs with 4–6 weeks of age (Formanek et al. 2011). These findings raise the interesting possibility that nestlings might not yet display a circadian rhythm in various behaviours at this stage of their development as they live in a non-rhythmic or slightly rhythmic environment (e.g. Kouba et al. 2014). Digestive constraints might also lead to cyclic behaviour. When the owlet's stomach is full, it may be best to invest in sleep-related functions. In particular, sleep is an important state for learning, memory, the immune system, energy homeostasis and brain maintenance (Vyazovskiy et al. 2008; Markwald et al. 2013; Rasch and Born 2013; Xie et al. 2013; Opp and Krueger 2015), and sleep deprivation negatively impacts physiology and cognition (Van Dongen et al. 2003). Hence, nestlings are likely to be awake only during some parts of the nights. Superimposed on this expected nocturnal or bimodal activity pattern, nestlings may also have an endogenous need for sleep at regular short intervals, since developing animals spend more time asleep than adults (e.g. Roffwarg et al. 1966). Additionally, interactions between siblings are important before the night, as nestlings show reciprocation between allopreening taking place during the day and food-sharing at night (Roulin et al. 2016). Nestling Barn Owls vocally negotiate the priority of access to the impending food, a process that takes time and hence is already expressed during the day (Dreiss et al. 2016a). The use of prosocial behaviour allows animals to reduce the cost of competition, but this activity is commonly time consuming (Lewis et al. 2007), implying that such behaviours may have to be expressed not only at night but also during the day.

Furthermore, nestlings can feed on stored food at any time of the day or night to optimize energy intake (Roulin 2004). Hence, some behaviour directed towards siblings, such as allopreening and pecking, may show a more even temporal distribution. Answering all of these questions is a huge undertaking, and a first approach to tackle this research agenda is to perform a descriptive study that describes the temporal pattern of activity in many behaviours. This was the goal of the study reported here.

Methods

Study system

We studied a population of Barn Owls breeding in nest boxes (62 × 56 × 37 cm) fixed to the external wall of barns in western Switzerland (46°49′N, 06°56′E). Hence, nestlings were exposed to the light–dark cycle through the nest box entrance hole (13 × 20 cm), and they could hear sounds in the environment from a variety of external sources, including humans. We also studied nestlings in the laboratory in similarly sized, ventilated nest boxes with a weak light source (LEDs, 1 W), switched on during natural daytime. Nestlings in the laboratory were therefore also exposed to the light–dark cycle, as in nature. We recorded behaviour and electroencephalograms (EEG) in nestlings at about 4 weeks of age when they were able to thermoregulate and eat without maternal help (we regularly visited nests to determine hatching dates of all siblings after measuring wing length a few days after hatching; Roulin 2004). At this time, the parents were not sleeping in the nest box, but somewhere close by during the daytime. For each behaviour, we calculated the mean hourly values 4 h before and after sunset and sunrise, respectively; specific details including sample sizes and behavioural definitions are provided in the following sections for each of the different aspects of this study. We recorded nestlings between May and October, during which time day length varies by about 4 h. Therefore, we examined locomotion, sleep and behavioural pattern in relation to sunset and sunrise rather than the exact time of the day or night. For each 1-h interval around sunset and sunrise (up to 4 h before and after these specific time points), we recorded behaviours and cerebral activity to evaluate temporal variation in relation to natural light conditions to accommodate seasonal changes in night length. We chose this methodology, because we predicted that nestling behaviour would be sensitive to the day–night cycle. Indeed, Barn Owl parents bring the first prey item of the night at around 2230 hours in June but at 1830 hours in October (personal observation).

Sleep and locomotor activity in the field in 2011

The Barn Owl, like other birds and mammals, exhibits two sleep states, namely REM (rapid eye movement) and non-REM sleep, which alternate in short intervals and can be recorded with EEG (Rattenborg et al. 2011). Between May and October 2011 we recorded the brain activity of nestlings continuously for about 5 days using minimally invasive subcutaneous electrodes. This was done in the field in 29 broods involving 31 male and 35 female nestlings, aged 27–48 days [mean ± standard deviation (SD) 38.2 ± 0.6 days]. Using Somnologica software (Medcare, Embla Systems Europe, Amsterdam, The Netherlands), a single person analysed the last 24-h period of the recordings. Only one 24 h-period was analysed in order to exclude the periods during which owlets habituated to the recording device. The EEG signals were scored for wakefulness, REM and non-REM sleep in 4-s epochs, and epochs containing more than one state were scored according to the predominant state. Wakefulness was characterized by low-amplitude, high-frequency EEG activity, but it was often also accompanied by movement artefacts characterized by high frequency and amplitude activity when the birds were engaged in active behaviour. Non-REM sleep consisted of low-frequency, high-amplitude EEG activity. REM sleep was characterized by low-amplitude, fast-frequency activity, accompanied by head dropping or swaying of the body detected via accelerometer recordings (for more details, see Scriba et al. 2013a, b; 2014). We calculated the fraction of time per hour spent awake, in non-REM and REM sleep, respectively. The recording equipment, which weighed at most 2.1% of the bird's body weight, did not seem to have an adverse effect on the nestlings, as all of the birds fledged, and recruitment into the breeding population in the following year was actually higher in those Barn Owl nestlings which had their sleep recorded (26.3%) than in those that had not (19.0%; Scriba et al. 2013a, b; 2014). Furthermore, we installed the device on the nestlings during daylight hours, and they usually went asleep a few minutes after we completed the procedure, demonstrating that our method allowed us to record brain activities without artefacts. We found that the temporal variation in REM and non-REM sleep was very similar (Electronic Supplementary Material Fig. 1), because REM sleep mostly takes place after a bout of non-REM sleep; hence we report here only temporal variation in wakefulness (we nevertheless separately examined whether REM and non-REM sleep are more often performed at day than night). We did not examine how the number and duration of wakefulness–sleep bouts vary over a 24-h period, because this is closely associated with the percentage of time spent asleep. However, we did examine whether the number and duration of wakefulness and REM and non-REM sleep bouts differ between the light and dark phase.

To record locomotor activity, a neurologger (Neurologger 2A; Vyssotski et al. 2009, http://www.vyssotski.ch/neurologger2) equipped with an accelerometer to monitor locomotor activity at 200 Hz was placed on the head of the nestling (Scriba et al. 2013a; Anisimov et al. 2014). The logger electronics was complemented with a board carrying a three-dimensional (3D) accelerometer chip (LIS302DLH; STMicroelectronics Inc., Calamba, Laguna, Philippines) and communication microcontroller PIC18LF13K22 (Microchip Technology Inc., Chandler, AZ). Acceleration in each direction was measured in the range ±2 g and stored as an 8-bit value. Acceleration data were analysed in R (version 2.15.2; R Core Team 2012) using custom written scripts. To estimate the amount of body movement, the absolute value of the 3D acceleration vector was computed, and the variance of this vector was taken as an estimate of locomotor activity. We obtained activity data for 64 of the 66 nestlings for the same period of time during which we recorded their EEG.

Feeding, allopreening, pecking and wing flapping in the field in 2007

Between May and September 2007, we artificially reduced 21 broods to three nestlings to better score nestling behaviour of 49 individuals for which we successfully obtained data from the video footage. The other nestlings were kept in ventilated boxes (with food) at some distance from the nests. The older nestlings of these three-chick broods were on average 42 days old (range 35–50 days), the middle-born individuals 38 (range 28–47) days and the juniors 34 (range 21–44) days. We used two infrared-sensitive video cameras per nest box to film under dark conditions from 1900 to 0700 hours. All of the video recordings were analysed by the same observer, who was blind to the study question. For each hour, this observer counted the number of times nestlings were pecking and allopreening each other. Pecking is an agonistic behaviour characterized by one individual hitting another one with its beak, while allopreening involves one individual using its beak to preen its sibling. The observer also scored for each hour the number of times nestlings were flapping their wings. Finally, the observer recorded the time when parents were bringing food to the nest and when nestlings were eating.

Vocal negotiation in the field in 2014

Barn Owl nestlings communicate vocally during the night while parents are foraging with the purpose to negotiate which individual will have priority access to the next delivered prey item (Roulin 2001). Between June and August 2014 we installed microphones inside of ten nest boxes containing on average 5.7 (range 4–9) nestlings with a mean age of 36.9 (range 20–45) days. We counted the so-called “negotiation calls” of the entire broods (calls could not be assigned to specific individuals) using Matlab (R2012b 8.0.0.783; MathWorks, Natick, MA). For each 1-h interval around sunset and sunrise, we divided the total number of calls by the number of nestlings. Calls were recorded from 2000 or 2200 hours to 0400 or 0700 hours during the dark period, explaining why we could not always count calls up to 4 h before sunset and 4 h after sunrise.

Locomotor activity, self-preening, allopreening, vocal negotiation and feeding in the laboratory in 2012

Between May and August 2012, we brought 116 nestlings (average age 30.6 days; range 16–39 days) from 30 broods to nest boxes in the laboratory. These boxes were soundproofed so that nestlings placed in one box could not hear other individuals placed in neighbouring boxes. From 1400 hours to 1300 hours the following day, we recorded their behaviour using video cameras (TVCCD-150SET; Monacor International GmbH and Co. KG, Bremen, Germany) and microphones (MC930; Beyerdynamic GmbH and Co. KG, Heilbronn, Germany). Each nestling was recorded alone for one 23-h period and with another sibling during the next 23-h period (or the other way round, with the order randomized). Nestlings are known to produce negotiation calls also when they are alone in the nest box.

We recorded the number of negotiation calls per nestling in the same way as described in section “Vocal negotiation in the field in 2014”. Locomotor and feeding behaviours were assessed in a randomly chosen subsample of 44 nestlings from 13 broods, and self-preening and allopreening were assessed in 79 randomly chosen nestlings from 20 broods and placed alone (to measure self-preening) or in pairs (to measure self-preening and allopreening). We considered a subsample because measuring these behaviours during a 23-h period cannot be done automatically but manually, which is very time-intense. Nestlings were fed ad libitum at 1400 hours so that we could monitor the time when they consumed food. Video images of boxes were divided in 5 × 4 squares to measure locomotor activity. The video recording was split in 1-min episodes, and nestlings were considered to be active if they moved from one square to another. Allopreening was measured as explained in section “Feeding, allopreening, pecking and wing flapping in the field in 2007”. An individual was considered to be self-preening when it touched its feathers with its bill or scratched its body with its feet. We measured the amount of time nestlings were self-preening and, for each hour, we calculated the proportion of time nestlings were self-preening.

Temporal variation in the frequency of allopreening was very similar in 2012 in the laboratory as in 2007 in the wild (data not shown). Therefore, for each year we standardized the data [(value − mean)/SD] and combined the 2 years of data to calculate the mean standardized frequency of allopreening for each hour in relation to sunset and sunrise.

Statistical analyses

The time of sunset and sunrise had been obtained from the Astronomical Almanac by the H.M. Nautical Almanac Office in the UK and the U.S. Naval Observatory. We calculated the frequency of each behaviour in relation to time to sunset (i.e. 4, 3, 2 or 1 h before sunset; during the hour comprising sunset; and 1, 2, 3 and 4 h after sunset). A similar procedure was applied to sunrise. We used this approach (i.e. calculate mean hourly values) and standardized the data [i.e. (value − mean)/SD] to compare all behaviours between each other and in relation to sunset and sunrise. The data were normally distributed, and hence we used parametric paired *t* tests to determine if behaviours differed between light and dark periods, using average values per brood. Note that, with the exception for sleep and locomotor activity in nature, we did not record behaviours during the entire dark–light period. We therefore compared the mean values of light and dark periods during the studied period only. We performed non-parametric Spearman's correlations when samples sizes were small. All *P* values are two-tailed, and $P < 0.05$ was considered to be significant. Statistical analyses were performed with the JMP software version 11.0. Using the Hartigan's dip test statistic for unimodality (package 'diptest'; Maechler 2012; version 0.75-74, in R), we tested if the distribution of each behaviour (using the recorded 16-h periods) departs from unimodality.

Results

Comparison between diurnal and nocturnal behaviour

Parental feeding visits, nestling feeding, calling, locomotion and self-preening behaviours were significantly more often performed at night than during the day. Cerebral wakefulness, wing flapping, pecking and REM sleep were observed as often at night as during the daylight hours. Only allopreening in the field and non-REM sleep were significantly more frequent during the day than at night (Fig. 1).

Temporal variation in nocturnal behaviours

In the field, feeding activities were synchronized with the parental visits (parents brought food to the nest). There was a strong correlation between the mean hourly parental feeding visits and the number of prey items offspring consumed at night (Spearman's correlation $r_s = 0.88$, $n = 9$ night periods of 1 h, $P = 0.002$; Fig. 2). Parental feeding visits and offspring feeding mainly started 1 hour after sunset (Fig. 2) and progressively declined throughout the night (parents: $r_s = -0.69$, $n = 9$, $P = 0.038$; offspring: $r_s = -0.83$, $n = 9$, $P = 0.005$). A similar pattern of nestling feeding activity was observed in the laboratory, although it was significant only in the situation when nestlings were placed alone in a nest box for 23 h ($r_s = -0.67$, $n = 9$, $P = 0.049$; Fig. 2) but not when placed in a nest box in pairs ($r_s = -0.12$, $n = 9$, $P = 0.77$). Not surprisingly, the temporal pattern of nestling vocal negotiation followed the same trend, with high vocal activity at the beginning of the night followed by a progressive decline over the night ($r_s = -0.83$, $n = 9$, $P = 0.005$; Fig. 3). Finally, self-preening activities followed a similar temporal pattern at night ($r_s = -0.93$, $n = 9$, $P = 0.002$; Fig. 4).

Do behaviours show a bimodal pattern of nocturnal activity?

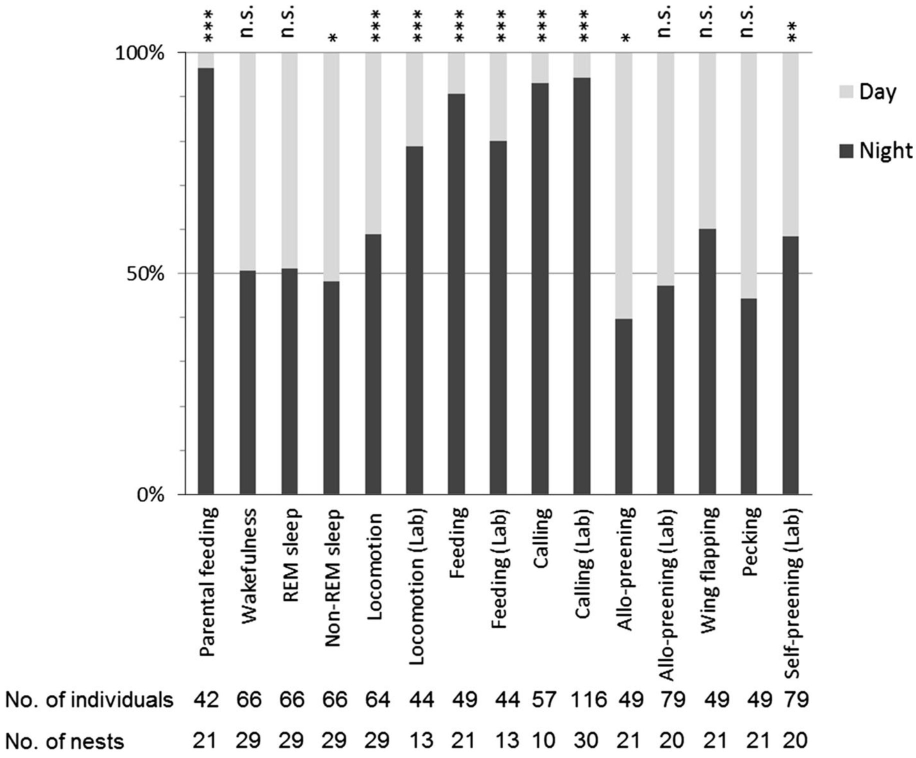
According to the Hartigan's dip test statistic, allopreening, pecking, wing flapping, feeding, self-preening, activity and wakefulness were weakly bimodally distributed (HDS, *P* values between 0.06 and 0.09), whereas parental visits and calls followed an unimodal distribution ($P > 0.13$).

In the field, nestlings slept mainly during the daylight hours and in the middle of the night until sunrise when there was a sudden and strong peak of cerebral wakefulness (filled symbols in Fig. 5a). Another, less pronounced peak of wakefulness took place during the first 3 h after sunset (Fig. 5a). Locomotor activity showed a similar temporal pattern when measured in the same individuals (Fig. 5a) and in another set of individuals in the laboratory (Fig. 5b). Although the mean hourly values of the frequency of nestlings flapping their wings, pecking each other or allopreening were not significantly different from each other (paired *t* tests, $P > 0.05$), there were two peaks of maximal activity at sunset and sunrise, respectively (Figs. 6, 7).

Discussion

Studying temporal variation in locomotor activity and sleep in young birds is particularly interesting in the context of examining whether activity is mainly governed by feeding

Fig. 1 Extent to which behaviour and cerebral activity are nocturnal vs. diurnal in Barn Owl (*Tyto alba*) nestlings. *Bars* represent brood average values for the daylight and dark periods divided by the daylight and dark average, respectively, to obtain a percentage. *, **, *** Difference (according to paired *t* test on mean values per brood) is significant at $P < 0.05$, $P < 0.001$ and $P < 0.0001$, respectively. *n.s.* Non-significant



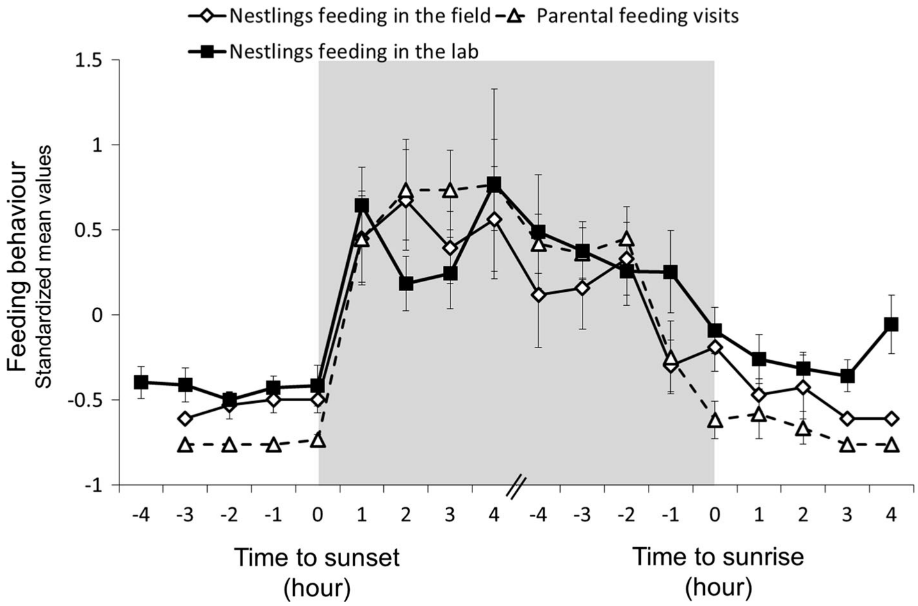


Fig. 2 Parental feeding visits and nestling feeding activities in the Barn Owl. *Data points* are mean standardized hourly values [*whiskers* standard error (SE)] according to time to sunset and time after sunrise as indicated by *0* (i.e. up to 4 h before and 4 h after each of these time points, respectively). *Shaded area* dark period. Data on feeding in nestlings were collected in 21 three-chick broods in the field in 2007 and in the laboratory in 44 nestlings from 13 broods in 2012. The data were standardized for each year, and mean values are presented. Data on parental food provisioning in the field were collected in the same 21 three-chick broods in 2007 (note that we fed the nestlings in the laboratory therefore cannot provide these data for the laboratory)

or whether it is also sensitive to social interactions among family members. When sibling competition is mainly dictated by size-related dominance, social interactions may be relatively simple and take place mainly when parents bring food to the nest. In systems where young siblings can behave altruistically or cooperatively to obtain food, interactions may be more complex because prosocial interactions may take time and involve multiple

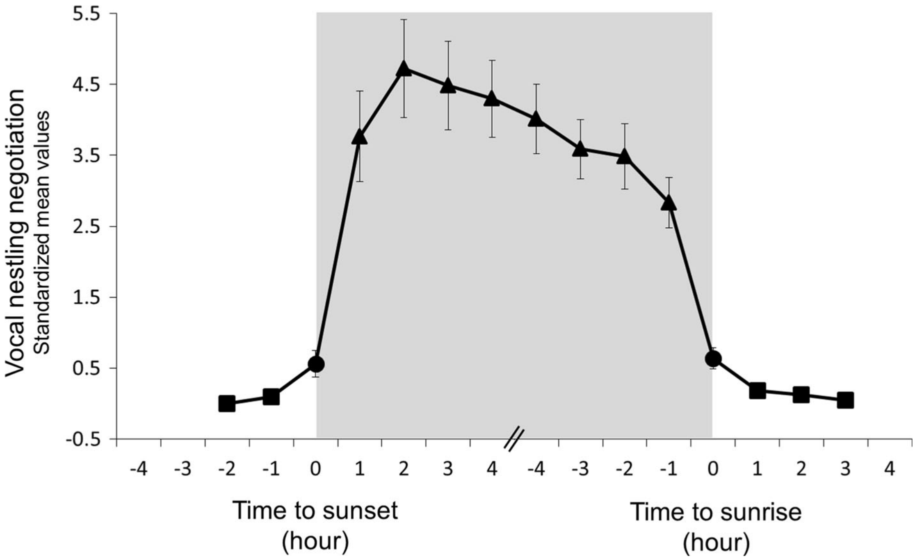
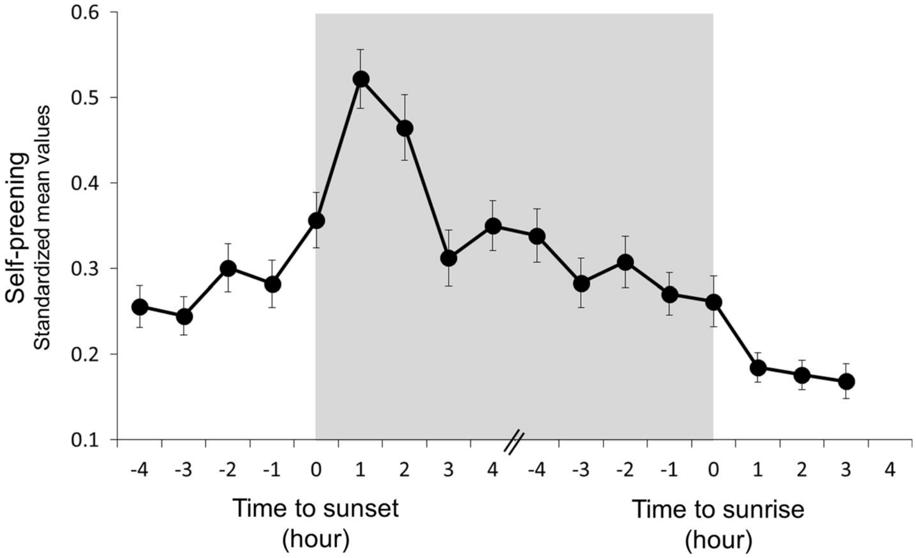


Fig. 3 Vocal negotiation in Barn Owl nestlings. *Data points* are mean standardized hourly number of calls per nestling (*whiskers* SE) according to sunrise and sunset as indicated by *0* (i.e. up to 4 h before and 4 h after each of these time points, respectively). *Shaded area* dark period. Negotiation calls were recorded in the field in 2014 in 10 broods and in the laboratory in 2012 in 116 nestlings from 30 broods placed alone ('solo') or in pairs ('duo') in a nest box during a 23-h period (for each individual a mean value of the two recorded values—solo and duo—was calculated). Values obtained in the field and in the laboratory were separately standardized before being averaged and shown in this figure. *Different symbols* (*squares*, *circle*, *triangles*) indicate that values are significantly different from each other using paired *t* test analyses

Fig. 4 Self-preening in Barn Owl nestlings in the laboratory. *Data points* are mean hourly values (*whiskers* SE) of self-preening events according to sunrise and sunset as indicated by *0* (i.e. up to 4 h before and 4 h after each of these time points, respectively). *Shaded area* dark period. Self-preening was recorded in the laboratory in 2012 in 79 nestlings placed in pairs or alone in the nest box. For each individual, a mean value was calculated



behaviours, such as allopreening, allofeeding, pecking and vocal negotiation. For these reasons, the temporal pattern of behaviours may not always follow the pattern of feeding activities, and hence individuals may not show circadian rhythms, but rather complex temporal patterns of activity. This is an interesting aspect to study, but, to the best of our knowledge, we are not aware of any other similar study performed in other animals, thus preventing us from comparing our findings with other systems.

The adult Barn Owl is mainly nocturnal (Scriba et al. 2013b), and in our study parents were not sleeping in their nest during daylight hours but at some distance from the nest. However, our results show that the temporal variation in nestling behaviour is more complex. Nestlings are not strictly nocturnal, with some behaviour being displayed as often during the daylight hours as at night (wing flapping, pecking; Fig. 1) and some behaviour (locomotor activity, wing flapping, pecking and

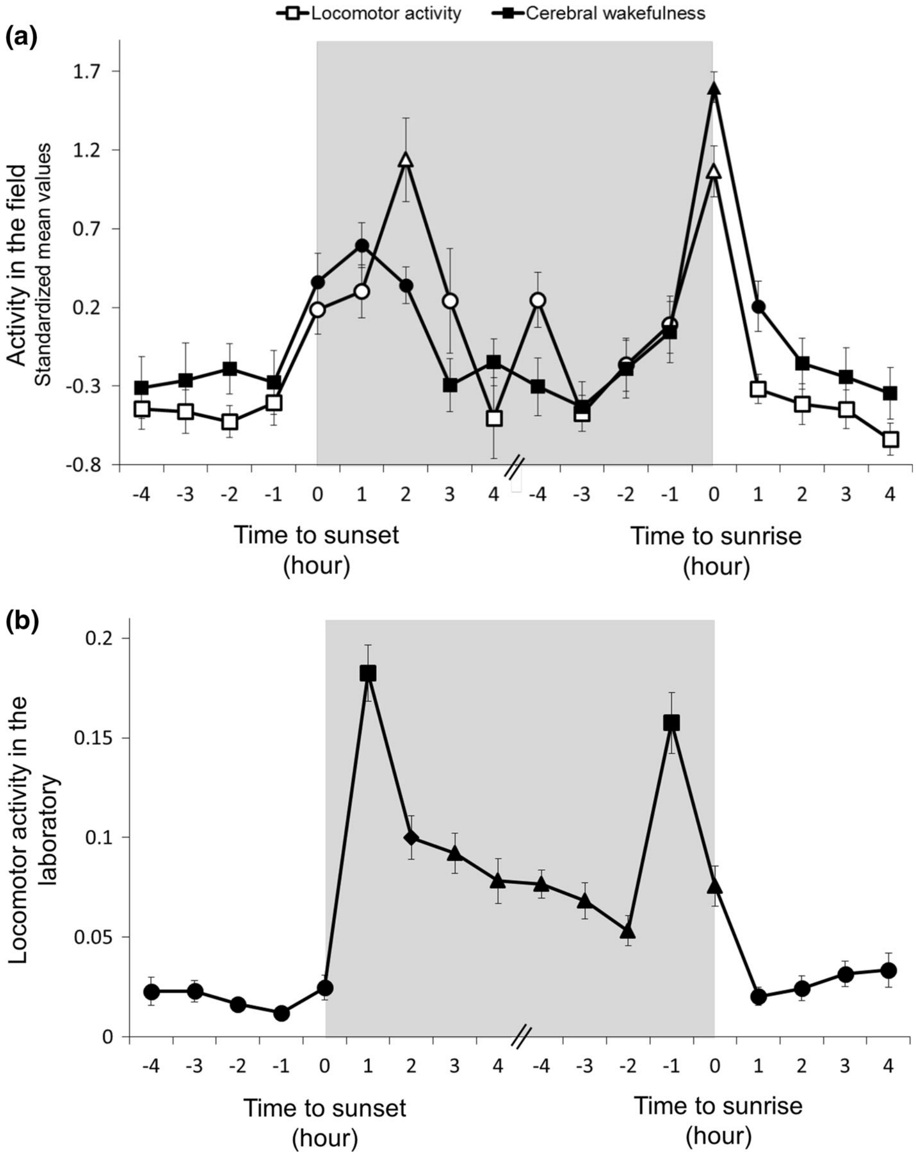


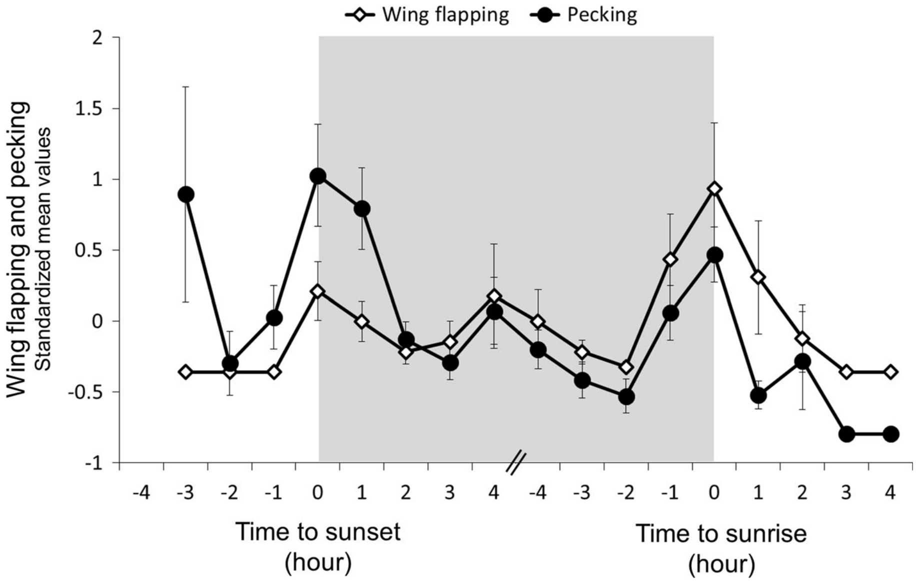
Fig. 5 Sleep-wakefulness and locomotor activity in Barn Owl nestlings in nature recorded in 2011 (**a**) and in the laboratory recorded in 2012 (**b**). *Data points* are mean hourly standardized values (*whiskers* SE) according to sunrise and sunset as indicated by *0* (i.e. up to 4 h before and 4 h after each of these time points, respectively). *Shaded area* dark period. **a** Data were collected on 66 nestlings from 29 broods in the field in 2011. **b** Locomotor activity was recorded in the laboratory in 44 nestlings in 2012. *Different symbols* (*triangles*, *diamonds*, *squares* and *circles*) indicate whether within individuals the amount of time spent awake or physically active was sequentially significant according to paired *t* test analyses

allopreening; Figs. 5, 6 and 7) showing a bimodal pattern of activity with a first peak at sunset and a second peak at sunrise. Our recordings revealed that allopreening was slightly more frequent during the day (Fig. 1), at least when measured in the field. The activity patterns show a second peak in nestling activity at sunrise that did not coincide with feeding activities, which were low at this time (Fig. 2).

Bimodal pattern

Nestlings behaved differently at different times of the 24-h period. As expected, nestlings were physically active around sunset—but, rather surprisingly, they were as active around sunrise. These two peaks in activity apply, for example, to locomotion, wing flapping and to sibling interactions (pecking and allopreening). We can only speculate on the potential reasons why nestlings are active around sunrise. Bimodal activity patterns are common in animals and can be a strategy to reduce daily energy expenditure (Erkert and Kappeler 2004). Alternatively, nestlings may move and flap their wings to warm up in the cold early morning. Movements performed at sunset may also correspond to maintenance behaviours, which are less important at night when the owlets compete for food. Wing flapping is an important maintenance behaviour as nestlings need to adjust their body mass to reach aerodynamically appropriate wing loadings at fledging (Wright et al. 2006). Accordingly, in our study the propensity to flap wings increased with age, as also observed in Tengmalm's Owls (*Aegolius funereus*) (Kouba et al. 2014). Additionally, nestlings might be active at sunrise to find a place in

Fig. 6 Wing flapping and pecking in Barn Owl nestlings in nature in relation to sunrise and sunset. *Data points* on wing flapping (*open diamonds*) and pecking (*filled circles*) are given as mean standardized hourly values (*whiskers* SE) according to sunrise and sunset as indicated by *0* (i.e. up to 4 h before and 4 h after each of these time points, respectively). *Shaded area* dark period. Mean hourly values were not significantly different from each other according to the paired *t* test. Data are from 21 three-chick broods in 2007



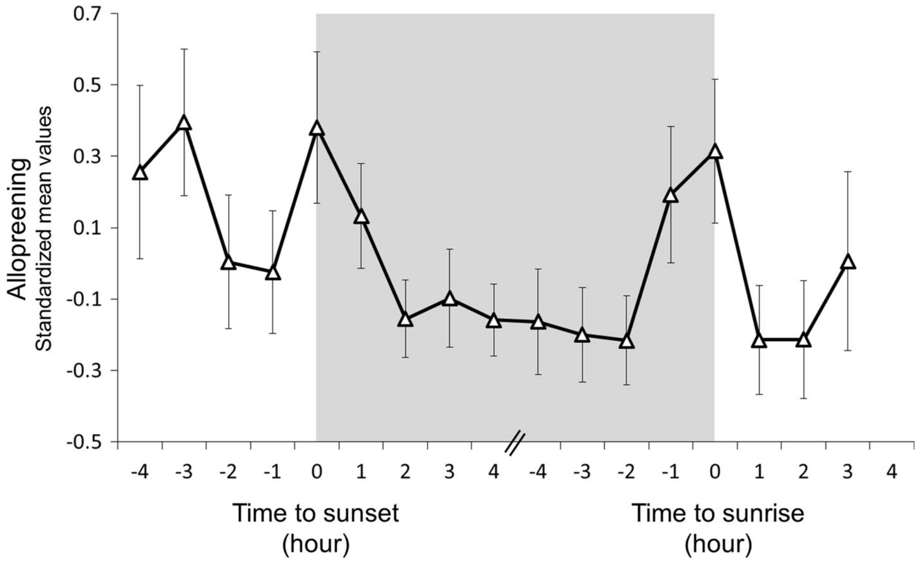


Fig. 7 Allopreening in Barn Owl nestlings. *Data points* (*whiskers* SE) are given as mean hourly values according to sunrise and sunset as indicated by *0* (i.e. up to 4 h before and 4 h after each of these time points, respectively). *Shaded area* dark period. Data were collected in the field in 21 three-chick broods in 2007 and in the laboratory in 20 two-chick broods in 2012. Because the pattern of temporal variation was globally similar in 2007 as in 2012, we standardized the data collected in each year and then calculated a mean overall value. Mean hourly values were not significantly different from each other according to the paired *t* tests

their nest where they will rest and huddle during the day (Dreiss et al. 2016b). Because huddling implies close contact between siblings, they may engage in diverse social interactions, including pecking and allopreening, the latter behaviour being known to reduce social stress and conflicts (Lewis et al. 2007; Fraser et al. 2008; Fraser and Bugnyar 2011).

These temporal patterns of activity indicate that in nestlings, at least in the range of age considered in the present study (16–50 days), nocturnality has not yet been established. A similar result was found in Tengmalm's Owl nestlings which were spending time at the nest box entrance not only during night when the parents came with food, but also during daylight (Kouba et al. 2014). This daytime behaviour might occur as a preparation for fledging, as we found that older nestlings spent more time at the entrance or in the alert state if a predator was trying to enter the nest. Additionally, the cost for being active at any time of day or night might be low for the nestlings. From the data obtained in this study, we cannot draw any conclusion on circadian rhythmicity; for this, recordings of the behavioral pattern are needed over several 24-h periods.

Daylight activities

During the daylight hours (excluding the period around sunset) nestlings were mostly physically inactive and engaged in more and longer sleep bouts. Nonetheless, nestlings did not sleep continuously during the light phase, and the duration of time spent awake was the same during the day and night. It is possible that the nestlings are disturbed by human activity during the day since Barn Owls breed in nest boxes placed in barns. This interpretation is consistent with the finding that nestlings are physiologically more stressed, as measured by blood circulating corticosterone, when located closer to inhabited houses and livestock (Almasi et al. 2015). Alternatively, some behaviours may be realized during the day for adaptive reasons, which would explain the time spent awake in the day. During the light period nestlings sometimes eat prey remains (Roulin 2004), as also observed in the present study (Fig. 2). The duration of digestion might prevent individuals from ingesting the daily food intake within a short time interval at night, since the egestion of a pellet takes about 6.5–10 h after feeding (Smith and Richmond 1972), which might explain why nestlings sometimes feed during the day. Furthermore, social interactions, such as allopreening and pecking, occurred during the light period, especially before sunset. Nestlings may anticipate parental visits with prosocial (allopreening) and competitive (pecking) behaviours, to induce siblings to later share food. As we recently showed, reciprocation does occur between food-sharing and allopreening in owl nestlings. An individual that preens its sibling during daylight hours is more likely to be later fed by this sibling. These behaviours may reduce the level of sibling competition (Roulin et al. 2016).

Locomotor and vocal activities resume around sunset, before the first parental feeding visit. Nestlings may be active before the sun goes down to position themselves closer to the nest box entrance where the likelihood of being fed by parents is higher (Dreiss et al. 2013a, b; Kouba et al. 2014). Siblings vocally negotiate priority access to the impending food items. In the Barn Owl, parents bring a single non-divisible food item per visit that is consumed by a single offspring. Each individual eats three to four items per night, and there is a pronounced asymmetry in food need between the differently aged siblings. Indeed, as soon as one individual has consumed a food item, it is much less hungry than its siblings. To reduce the level of competition, siblings communicate vocally, a process referred to as “sibling negotiation”. Because such a process takes a long time (Dreiss et al. 2015), nestlings start to vocally negotiate long before the first parental feeding visit explaining, in part, why they can be active during the daylight hours.

Ethics statement

All experiments were performed under the legal authorization of the Veterinary Office (Vaud Canton, Switzerland). Nestlings recorded in the laboratory 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. 2010). Keeping owlets at the university for a few days 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. 2013a, b). The minimally invasive EEG recording method did not appear to have any long-term adverse effects on the Barn Owl nestlings, as all nestlings in which the brain activity had been recorded fledged, and the number of owls coming back for breeding in the following year was even higher in owls in which we recorded sleep (26.3%), than in those without sleep recording (19.0%). Also the owls habituated to the electrodes and data logger quickly within a few hours and behaved normally afterwards (as confirmed by video recordings), and nestlings were sleeping as early as 5–10 min after being placed back into the nest box after electrode placement.

Acknowledgements The research was supported by the Swiss National Science Foundation, the Société Académique Vaudoise and the Max Planck Society. The experiments comply with the current laws of Switzerland.

Author contributions MFS, AD, IH, PB, CR, PD collected data. ALV contributed the neurologger. EI, ADS, BDM analysed video recordings. MFS, AD, NCR, AR analyzed data. MFS, NCR, AR wrote the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All experiments were performed under the legal authorization of the Veterinary Office (Vaud Canton, Switzerland). All procedures performed in the studies were in accordance with the ethical standards of the institution at which these studies were conducted.

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