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Social motivation does not go hand in hand with social bonding between two familiar Japanese quail chicks, *Coturnix japonica*

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Social bonds between conspecifics (sexual pairmates, parent–infant or siblings) and social motivation can influence the way birds interact. Social bonds between familiar conspecifics may also be formed, especially in chicks from precocial species in which behaviour is characterized by a strong social attraction. We investigated the establishment of such a bond and the effect of social motivation using a separation/reunion test in 1-week-old Japanese quail. In experiment 1, the behaviour of paired quail selected for high (HSR) or low (LSR) social reinstatement behaviour was recorded during three successive 10 min situations: baseline, separation and return of the cagemate. Both lines showed behavioural changes during social separation but the distress response was higher in the HSR line. Return of the familiar cagemate induced a return to baseline activity in both lines. The specificity of this response was tested in experiment 2 in which paired quail of both lines were separated, and their behavioural and vocal responses to the introduction of the familiar or an unfamiliar bird were compared. LSR chicks responded differently both behaviourally and vocally according to the familiarity of the introduced bird, whereas the response of HSR chicks differed only for aggressive pecks. HSR quail chicks thus revealed a nonspecific social attraction for conspecifics, whereas LSR chicks showed a bond with the cagemate. Our results highlight the importance of vocal communication as an indicator of the strength of the bond. Our findings show that high social motivation in quail chicks is not related to the development of a social bond.

The formation and maintenance of social bonds among conspecifics play a fundamental role in social group cohesion, modulating social relationships and consequently social organization within species (Curley & Keverne 2005; Broad et al. 2006; Emery et al. 2007). A wide variety of social, sexual and maternal bonding has been described across mammalian species, including humans. Notably, these types of bonding comprise affiliation defined as preferential positive interactions between two related or unrelated individuals, sexual pairing for one breeding season or in a permanent way, and mother–infant attachment defined as an affectional relationship between two individuals that is specific in its focus and endures over time (Gubernick 1981; Hennessy 1997). There is also evidence that social bonds exist in birds, especially between parents and offspring, between siblings, and between sexual partners (Waldman & Bateson 1989; Bolhuis 1991; Lefevre et al. 1998; Cézilly et al. 2000; Charrier et al. 2001; Nakagawa & Waas 2004), but little is known about social bonding between conspecifics related only by familiarity, that is, conspecifics become familiar with individuals with which they have direct social contact (Ligout & Porter 2003).

A number of studies have shown the influence of social motivation, defined as the motivation to seek close proximity with conspecifics, on several aspects of the social interaction of birds (Jones & Mills 1999; Väisänen & Jensen 2003). Some of these studies have been performed using two lines of quail divergently selected for their high (HSR) or low (LSR) social reinstatement behaviour (Mills & Faure 1991). Under various experimental conditions, it was found that quail with high social motivation preferentially approached conspecifics and spent more time close to them than quail with low social motivation (Launay et al. 1991; Carmichael et al. 1998; François et al. 1998, 1999; Formanek et al. 2008b). These results support the idea that social motivation is likely to determine how conspecifics interact with each other and consequently might

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influence the formation of social bonds between unrelated familiar conspecifics. Investigating how social motivation influences the formation of social bonds between familiar quail would provide a better understanding of social relationships in quail chicks. Under natural conditions, precocial chicks remain around the mother, close to each other in a compact group, and although they quickly express interest in their environment and exploratory behaviours, they show a great affinity for maternal proximity (Guyomarc'h 1975; Mills et al. 1997). In domestic quail, where chicks are bred without their mother, they also express a strong social attraction and consequently social bonds might be formed between familiar individuals.

The separation/reunion paradigm is commonly used to demonstrate the existence of a social bond between two individuals by assessing the distress caused by disruption of the bond and the return to baseline activity after reunion (Cirulli et al. 1996; Boissy & Le Neindre 1997; Boivin et al. 2000; Remage-Healey et al. 2003; Bardet et al. 2007). However, distress caused by separation from a familiar individual either indicates a nonspecific reaction caused by social isolation itself or a social bond between familiar individuals. The most important requisite for differentiating between these two cases and to satisfy the definition of a social bond is to demonstrate different behavioural responses during the introduction of familiar and unfamiliar conspecifics following social separation, especially preferential positive interactions towards a familiar individual (Gubernick 1981; Bardet et al. 2007). Both behavioural interactions and communication between individuals can reveal differential responses to familiar and unfamiliar birds. Vocalizations are a major cue to recognition in birds (Phillmore et al. 2003; Nakagawa & Waas 2004) and are recognized as an emotional indicator of disruption of social contact (Guyomarc'h 1974b). Distress calls are commonly used to assess the effect of social separation in domestic chicks, *Gallus gallus domesticus* (Rajecki et al. 1977; Marx et al. 2001; Feltenstein et al. 2002) and Japanese quail (Bertin & Richard-Yris 2005; Formanek et al. 2008b). Other types of vocalization are rarely used to demonstrate preferential social relationships between conspecifics, although a rich vocal repertoire has been reported in quail (Guyomarc'h 1974a, b). Thus, an emotional response expressed by vocalizations associated with a behavioural response could provide a more precise view of social bonding between quail chicks.

The aim of this study was to determine whether Japanese quail chicks establish a social bond with familiar conspecifics and how social bonding is affected by social motivation. Our first experiment investigated social bonding in pairs of 1-week-old HSR and LSR quail chicks using a separation/reunion test. We hypothesized that the disturbance induced by separation would be higher in HSR than LSR quail chicks, as observed by Mills et al. (1993). Since a return to the baseline level has been found in young domestic fowl (Feltenstein et al. 2002) and zebra finches, *Taeniopygia guttata* (Remage-Healey et al. 2003) when social contact is restored, we hypothesized that the return of the cagemate would induce a calming effect in both lines. To assess the specificity of the bond with the cagemate, our second experiment compared the behavioural responses of 1-week-old HSR and LSR quail chicks to the return of the familiar cagemate and the introduction of an unfamiliar bird after a separation period. Indeed, as mentioned previously, the existence of a social bond is defined by distress caused by separation and a calming effect following the return of the conspecific, but the most important requisite to demonstrate the specificity of the social bond between two familiar conspecifics is to observe different behavioural responses to familiar and unfamiliar conspecifics (Bardet et al. 2007). Note that this paradigm allows one to reveal a social discrimination based on familiarity but does not address individual recognition. The emotional response of HSR and LSR quail chicks was assessed by measuring their different vocalizations. We hypothesized that the social bond between familiar cagemates would be stronger in HSR than LSR quail chicks. We therefore predicted more disturbance behaviour following social separation, and more variation in the behavioural and vocal responses between familiar and unfamiliar stimulus birds in HSR quail chicks than in LSR ones. This prediction is supported by the observation that interindividual distances are smaller in pairs of HSR than LSR quail (François et al. 1999), suggesting that HSR quail chicks interact and consequently form social bonds more easily.

METHODS

Animals and Treatments

We used 43rd-generation chicks of two lines of Japanese quail selected for their high (HSR) or low (LSR) social reinstatement behaviour (Mills & Faure 1991). Quail were selected and maintained at the UEPEAT experimental unit of INRA, Nouzilly, France, where the experiment was carried out. On the day of hatching, unsexed Japanese quail chicks were housed in pairs of the same line in wooden cages (50.5×43.0 cm and 31.0 cm high) with a floor covered with wood shavings and a wire-mesh cover. To obtain birds that had never been in contact with each other before we formed the pairs and introduced an unfamiliar conspecific, we separated the eggs of each line into two groups at incubation, so that the birds from one group served as the unfamiliar quails for the birds of the other group. Consistent with standard recommendations for quail husbandry (Kerharo 1987), chicks were housed under continuous lighting. Temperature was maintained at approximately 38 °C for the first 3 days and then progressively reduced to 32 °C by 1 week. Temperature was regulated using 100 W incandescent lights (one bulb suspended above two cages). A commercial quail starter diet (Gibelit démarrage, DP Nutrition, Loches, France) and water were provided ad libitum.

Experiment 1: Separation and Reunion with Familiar Cagemate

Test procedures

We used 23 pairs of HSR and LSR quail chicks and tested them at 1 week of age. Twenty-four hours before testing, a focal bird was chosen at random from each of the 23 pairs and was marked on its back with a small white paint spot. The marking paint did not contain solvent and was neither toxic nor an irritant (CRC Industries, F-95102 Argenteuil, France). Other chicks did not particularly peck at the paint mark. Moreover, the paint mark did not induce abnormal behaviour in the tested quail, which only preened it a little more than other parts of the plumage. On the test day, we observed the behavioural response of each focal chick during three successive 10 min periods. They were first observed for 10 min in their home cage (baseline condition). The cagemate was then removed for 10 min and placed in a separate room. After this separation period, they were reunited for 10 min. Quail chicks from the HSR and LSR groups were tested alternately. Three categories of behaviour were recorded using digital camcorders suspended above the cages. Maintenance behaviours included the time spent feeding and in exploratory behaviour (scratching and pecking at the litter or the walls). We assessed disturbance behaviours by measuring the frequency of jumps and distress calls and the time spent pacing and in high-posture observations. This posture is typical of alertness, animals standing on 'tiptoe' with their bodies and neck fully erected. Jumps, pacing and high posture are observed during social separation, and distress calls are generally produced at the same time (Guyomarc'h 1974b; Formanek et al. 2008a). We also recorded three types of social interaction for the