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Social support does not require attachment: Any conspecific tranquilizes isolated guinea-pig pups

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ABSTRACT

Guinea pig pups produce typical distress whistles when isolated. Whistles' frequency is decreased or abolished when they contact with the mother and, to a lesser degree, a sibling or even an unfamiliar female, is regained. Those non-aggressive companions were considered social support providers for reducing pup physiological stress responses and whistling rate in an unfamiliar environment. However, what would happen if the isolated pup would be in contact with an adult male, normally indifferent to pups, in such distress situation? The role of attachment and familiarity to males in promoting changes in distress responses of isolated pups was verified. Tests consisted of separating three week old pups from their family, in a familiar or an unfamiliar environment, and introducing a conspecific in the cage after one minute (mother, sibling, father or a strange male). Whistling and other behaviors were compared between the alone period and the accompanied period. Main factors were *prior presence/absence of father* (pups were raised with father until testing or only for the first week after birth), *sex of pup, novelty of test environment* and *companion*. It was verified that (1) all conspecifics reduced whistling rate ($F_{4,88} = 77.89$, $p < 0.001$), but pups behaved differently with different conspecifics; (2) suppression of isolation induced behavior did not necessarily occur because of previous attachment (e.g., pups in the PAF condition spent more time pausing, $F_{1,22} = 7.68$, $p < 0.05$, less time in passive contact with companions, $F_{1,22} = 10.63$, $p < 0.01$, and ate/drank less, $F_{1,22} = 6.18$, $p < 0.05$). It was concluded that the suppression of pup's isolation induced behavior by companions must not be used alone as a measure of attachment. It must be seen in an evolutionary perspective where the presence of any conspecific represents security offering self-protective behavior cues as finding a place to hide, and providing dilution effect against predation.

1. Introduction

Mother–infant bonding is well described in guinea pigs (*Cavia porcellus*). Pups actively search and follow their mother shortly after birth and most of the time, will remain close to her until weaning, usually at the fourth week of life (Pettijohn, 1979a; Porter et al., 1973b). Such behavior has been interpreted by some authors as being dependent on a process analogous to imprinting (Shipley, 1963; Sluckin, 1968). Nevertheless, infants do not exclusively approach their mother. Pups 3 or 4 days old frequently follow adult guinea pigs other than the mother (King, 1956) and nurse from other lactating females (Fullerton et al., 1974; Takamatsu et al., 2003).

Infants frequently show signs of distress when separated from their mothers (Ainsworth et al., 1978; Harlow and Harlow, 1965) such as vocalizations, changes in locomotion and in self-directed behavior. Such reactions which diminish or stop when the contact with the mother is restored are commonly taken as indicating the existence and intensity of mother–child attachment in both humans (Ainsworth et al., 1978; Bowlby, 1984) and other mammals (Hennessy and Weinberg, 1990; Wiener et al., 1990).

Isolating guinea pig pups increase the production of high-pitched whistles and cortisol levels. These reactions are prevented

Abbreviations: Prior presence of father (PPF), the father was kept with pups and mother throughout the experiment; Prior absence of father (PAF), the father was taken to a separate cage at day 8 after the birth of the pups.

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when the mother is moved to the unfamiliar environment with them. Because of this effect, decrease in the production of isolation whistles was considered per se a measure of the pups' bonding to their mother (Berryman, 1976; Corat et al., 2012; Coulon, 1982; Hennessy et al., 1996; Hennessy and Moorman, 1989; Hennessy et al., 1995; Pettijohn, 1979b). Interestingly, a decrease in the production of isolation whistles also occurs throughout an isolation period of 30 min, together with an increase in cortisol levels (Hennessy, 1988; Hennessy and Sharp, 1991; Sachser, 1998; Hennessy et al., 2001; Ritchey and Hennessy, 1987). Corat et al. (2012) suggest that mild stress is the physiological trigger for the production of whistles vocalization and that, this kind of stress is present in a short isolation period. As the isolation period increases, cortisol levels also increase. The authors propose that this relation was probably selected in the context of naturalistic mother–pup separations that occur during foraging. Just after separation the mother probably is not far and it might be advantageous to the pup to signalize his position, maximizing the probability of regaining proximity with the mother or other conspecifics. As the isolation period increases, immobility and silence may be a protection against predators that might be attracted by conspicuous vocalizations and locomotion.

Mother effect in the isolation test is not entirely a matter of familiarity. Cortisol levels of pups isolated in an unfamiliar cage in the presence of familiar littermates were as great as those of pups tested alone (Ritchey and Hennessy, 1987). On the other hand, the ability to moderate the initial HPA response does not seem to be an exclusive domain of the mother, which is the attachment object. Distress whistling and cortisol responses may also be moderated, to a lesser degree, by the presence of other individuals, as other adult females (Hennessy, 2003; Hennessy et al., 2000). Though, the presence of a sibling was as effective as the presence of the mother in promoting a decrease in separation induced vocalization in post weaning guinea pigs (Hennessy et al., 1995).

During adolescence, mothers were the most efficient to decrease stress response or as effective as an unfamiliar female in unfamiliar environments, and siblings and/or males produced no effect (Hennessy, 2003; Hennessy et al., 2002). Later, in adulthood, the presence of the bonding partner provided social support to both males and females; not only the bonding partner was able to reduce the female's stress responses, but also a familiar conspecific, though in a less effective way (Kaiser et al., 2003; Sachser et al., 1998).

Amelioration of isolation induced behaviors in guinea pig by conspecifics can also occur independently of physiological changes. Hennessy et al. (2002) showed that although unfamiliar adult males and females guinea pigs were not effective in reducing cortisol levels of isolated adults, both were effective in reducing vocalization. Hennessy et al. (2007) also demonstrated that although unfamiliar males were not effective in reducing cortisol levels of isolated adult females they courted them two times more than their partner males and showed the same amount of positive interaction with them.

These results show that domestic guinea pigs behavioral responses to isolation can be ameliorated either by familiar and unfamiliar conspecifics or preferred/non-preferred partners, indicating that the presence of a conspecific may represent a 'security-giving and arousal-reducing structure' that is independent of previous bonding.

In the present study the role of the father representing a 'security-giving and arousal-reducing structure' to the isolated guinea pig pup was examined. Although adult male domestic guinea pigs (and other species of Caviinae) may be indifferent or show simple paternal behavior (playing with offspring and grooming them; Adrian et al., 2005; Beisiegel, 1993) to pups or juveniles, they have an influence on pups' behavioral development. Males raised singly or with a female are more prone to engage in very serious, escalated fighting, with other males than colony reared individuals (Sachser, 1986). If the social learning that occurs throughout interaction between father and infants (at least, male infants) is associated to some degree of bonding, we should expect a tranquilizing influence of fathers on pups in the isolation tests. This effect should not occur in the presence of a strange male and could be much less marked if the contact between father and pups had been interrupted for some period of time before testing.

High intrasexual intolerance is well described for both domestic and wild cavies (Sachser, 1986). *Cavia aperea* adult male may even kill his own son, raised in its enclosure since birth, if he is not taken away before maturing. The avoidance/aggression starts close to the third week of life (personal observation of wild population captured and observed in captivity in the municipality of Itu, state of São Paulo, Brazil). It is then plausible to consider unfamiliar males and non-present fathers potentially aggressive conspecifics to three week old juveniles.

The experimental design included, as social stimuli present during isolation tests, the mother, father and siblings of young guinea pigs. As a control for familiarity strange males and fathers, previously separated from the pups for one week, were used as social stimuli. The novelty of the testing environment was also manipulated. Previous research has shown the importance of familiarity with environment as a factor in stress reduction: the sudden absence of the mother may not represent an imminent threat for the pup, as long as it finds itself in a familiar, protective environment (Hennessy and Sharp, 1991; Hennessy, 2003; Porter et al., 1973b).

2. Materials and methods

2.1. Animals and husbandry

Twelve males and 14 female pups from 13 families of guinea pigs (*C. porcellus*) were used. All of them were from the colony of the Departamento de Psicologia Experimental, Universidade de São Paulo. Each group was constituted by mother, father and two pups (litters were reduced to two pups to standardize procedures, surplus pups were transferred to mothers in the colony). Each family was housed in a white polypropylene cage ($60 \times 90 \times 30$ cm) with hardwood chips serving as bedding. Purina@ (Nestlé Purina Pet Care, Wilkes-Barre, PA, US) guinea pig pellets and water were continuously available, green vegetables were given once a day, after the experimental tests. Animals' health was regularly monitored by caretakers, and veterinary services were available whenever necessary. Room was kept in a 12:12 LD cycle, photoperiod 0700–1900 h, temperature $22 \pm 2\,^{\circ}$C.

Prior to testing, families were maintained in one of two conditions: *previous presence of father* (PPF, seven families, 14 pups) – the father was kept with pups and mother throughout the experiment – or *previous absence of father* (PAF, six families, 12 pups) – the father was taken to a separate cage on the 8th day after the birth of pups.

Separation tests, with male or female pups, occurred in a *familiar cage* – the pup's own home cage or an *unfamiliar cage*, identical to the home cage, which was cleaned with 92% ethanol and in which fresh bedding was placed before each test. Animals which served as companions during testing were the pup's *mother*; the pup's *father*; the pup's *sibling* or a *strange male*.

2.2. Experimental design

Our design was a 2 (prior presence/absence of father) × 2 (sex of pup) × 2 (novelty of test environment) × 4 (companion–mother, sibling, father, unfamiliar male). Each pup was subjected to a total of eight isolation tests, one to four tests a day, which were performed