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Influence of morning maternal care on the behavioural responses of 8-week-old Beagle puppies to new environmental and social stimuli

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ABSTRACT

In mammals, maternal care represents a major constituent of the early-life environment and its influence on individual development has been documented in rodents, non-human primates, humans and recently in adult dogs. The quality and quantity of mother-offspring interactions exerts a multilevel regulation upon the physiological, cognitive, and behavioural development of the offspring. For example, in rats variations in maternal behaviour, such as mother-pup body contact and the amount of licking towards pups in the nest during the early days after parturition, influences the endocrine, emotional, and behavioural responses to stress in the offspring. This produces long-term consequences, which may remain into adulthood and can be transmitted to subsequent generations. Literature about maternal care in dogs and its effect on puppy behaviour is still scarce, although the topic is receiving a growing interest. The aim of the present study was to determine the effects of morning maternal care on behavioural responses of puppies to new environmental and social stimuli. In order to achieve this, maternal care (licking, ano-genital licking, nursing and mother-puppy contact) was assessed in eight litters of domestic dogs living in standard rearing conditions during the first three weeks post-partum. Puppies were subjected to two behavioural tests (arena and isolation tests) at 58–60 days of age, and their behavioural responses were video recorded and analysed. Data was analysed using multivariate analyses (PCA, PLS).

During the isolation test, a higher level of maternal care was associated with more exploration and a higher latency to emit the first yelp; on the contrary, a lower level of maternal care was associated with increased locomotion, distress vocalisations and destructive behaviours directed at the enclosure.

These results, comparable to those reported in laboratory rat models and to some extent to those recently reported in dog literature, highlight the importance of maternal care on the behavioural development of domestic dog puppies.

1. Introduction

In all mammalian species, there is an intense period of mother-infant interactions that is necessary for the survival of offspring; maternal care, especially during the early stages of postnatal life, is the main source of environmental stimuli for the progeny and a major determinant of behaviour in adulthood (Bowlby, 1988; Champagne, 2011).

Experimental evidence about the role of maternal care in mammals is commonly derived from deprivation studies. Disruption of mother-infant bonding during early lactation is known to have a great effect on the developing infant, particularly in altricial species (Mogi et al., 2011). In both primates and rodents, infants deprived of maternal care for extended periods of time exhibit dramatically increased fearfulness and anxiety, inappropriate and often excessively aggressive patterns of social behaviour, impaired cognitive development (Caldji et al., 2000b; Champagne and Curley, 2009; Levy et al., 2003; Liu et al., 2000), and enhanced neuroendocrine responses to stressors (Francis and Meaney, 1999), accompanied by epigenetic changes in the central nervous system (Weaver et al., 2004).

In *Canis familiaris* early separation from the mother at the age of 6 weeks increases disease susceptibility, weight loss and mortality in puppies (Slabbert and Rasa, 1993). Puppies prematurely

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separated from their mother between the ages of 30 and 40 days seem to be more likely to develop undesirable behaviours related to fear or anxiety than puppies that remain with their mothers until adoption at 60 days of age (Pierantoni et al., 2011). In addition to the presence of the mother, the amount of maternal care shown by the mother towards her puppies during the early period seems to be crucial in many species. Literature from animal models emphasizes the importance of the quantity of nursing, body and ano-genital licking, and mother-puppy body-contact, because these can shape the emotionality, reactivity to stress and social skills of the neonates (Caldji et al., 1998; Champagne et al., 2003; Starr-Phillips and Beery, 2013). Variation in the quantity of maternal care has been the subject of numerous investigations in several mammalian species and has been considered responsible for a range of effects on the brain development and on behaviour of the offspring (e.g. Caldji et al., 1998; Caldji et al., 2000a; Fairbanks, 1996; Foyer et al., 2016; Liu et al., 1997). For example, in rodents, naturally occurring variations in maternal behaviours during the first week of life, such as nursing, licking, and contact are associated in the offspring with the development of individual differences in the hypothalamic-pituitary-adrenal axis (HPA), brain morphology, neurotransmitters content in several brain regions and gene expressions (Caldji et al., 1998; Caldji et al., 2000a; Champagne, 2008; Francis et al., 2000; Gudsnuk and Champagne, 2011; Jensen and Champagne, 2012; Masís-Calvo et al., 2013; Meaney, 2001; Sequeira-Cordero et al., 2013; Zhang et al., 2005). Unlike rodents, scientific literature on maternal care shown by female domestic dogs during the first weeks post-partum is still scarce (Arteaga et al., 2012; Foyer et al., 2016; Guardini et al., 2015; Pal, 2005; Rheingold, 1963; Scott and Fuller, 1965) and its effects on the behaviour of adult offspring have been investigated only very recently (Foyer et al., 2016).

In domestic dogs, several authors have examined how some characteristics of the mother, of the puppies or of the early environment may affect the personality and temperament of young and adult dogs. For instance, previous studies have focused on the effects of: parity of mother (Foyer et al., 2016; Foyer et al., 2013; Wilsson and Sundgren, 1998a), litter size (Foyer et al., 2016; Foyer et al., 2013; van der Waaij et al., 2008; Wilsson and Sundgren, 1998a), material used in the whelping box (Wilsson and Sundgren, 1998a), gender of the puppies (Beerda et al., 1999a,b; Courreau and Langlois, 2005; Foyer et al., 2016; Foyer et al., 2013; Svartberg, 2002; van der Waaij et al., 2008; Wilsson and Sundgren, 1998a,b), weight of the puppies (Foyer et al., 2013; Wilsson and Sundgren, 1998a), and temperature and season of birth (Foyer et al., 2016; Foyer et al., 2013; van der Waaij et al., 2008; Welker, 1959; Wilsson and Sundgren, 1998a). In the above-mentioned studies, only that of Foyer et al. (2016) explored the mother-pup interactions (mother in the box, lying in contact, nursing, licking, sniff/poke) as influential factors for the dog adult behaviour. They analysed maternal care at four time points during the first three weeks after birth (1st, 7th, 14th 21st day post-partum) and they found that maternal care is correlated with the behaviour of the adult offspring, mainly with respect to behaviours classified as physical and social engagement, as well as aggression.

With regard to the effects of the quantity of maternal care received in early life on the behaviour and stress responses of young puppies, literature is still limited.

The aim of the current study was to evaluate the impact of maternal care on the behaviour of puppies. We hypothesize in our study, as already demonstrated in rodents' literature, that Beagle puppies that receive more maternal care during the first weeks after the birth, will be able to better cope with stressful situations and will shown better responses towards new environmental and social stimuli.

In order to achieve this, we analysed the behaviour of eight Beagle mothers towards their puppies every day for the first three weeks after birth, differently from the methodology used by Foyer et al. (2016). Then, we analysed the behaviour towards new environmental and social stimuli of their puppies at eight weeks of age, in two potentially stressful situations (arena and isolation tests).

2. Materials and methods

2.1. Subjects

Eight litters of Beagle dogs belonging to a professional canine reproduction centre and living in standardised rearing conditions were recruited, giving a total of 54 puppies.

Each mother was kept with her own litter in an individual enclosure (3.20 m × 1.80 m), which was contiguous with other identical enclosures in the maternity area of the establishment.

Details of the eight litters (characteristics of the mothers and puppies) included in the study are reported in Table 1.

2.2. Protocol of the analysis of maternal care: mother-litter interactions

Every day, from day 1 to 21 after birth, a 15 min video of each mother with her puppies was recorded. Being an observational study and not an experimental one, it only needed to be approved by the Ethical Committee of the facility where the study was carried out.

Videos were made in the morning, when the mother returned to the whelping box after having freely walked into the corridor of the maternity area in the presence of a caregiver for approximately ten minutes. All puppies were identified in two ways; using different coloured satin ribbons and shaving different small areas of fur. This means that every puppy wore a coloured satin ribbon and had a small area shaved, the area being different one from the other. This double identification allowed us to always be able to recognize each puppy during the recording process, even when the neck or other body areas were not visible. Since all the puppies underwent the same treatment, there was no difference in the quantity and quality of handling they received.

To assess maternal care given to each puppy, a list of behaviours from Guardini et al. (2015) was used. Behaviours observed included: mother-puppy physical contact (later referred as contact), licking, licking the ano-genital area (later referred as licking and licking-ag, respectively), and nursing. For each behaviour included in the list, the interaction within a specific mother-puppy dyad was analysed.

2.3. Behavioural tests for puppies

At 58–60 days of age, each puppy was subjected to two behavioural tests on the same day; first the arena test (Gazzano et al., 2008), and then, after 1–3 h, the isolation test (Gazzano et al., 2008).

2.3.1. Arena test

The arena test aimed at evaluating the behaviour of the puppies in a novel environment in the presence of an unknown human being and a variety of objects (a puppy Kong®, a puppy plaited rope of Trixie®, a plastic disk of Trixie®, a small ball of Trixie®).

The arena was similar to that used by Gazzano et al. (2008): 3.6 m × 2.2 m, and divided into twenty-four 55 cm × 60 cm rectangles with a central circle of 1.60 m in diameter. The arena itself was located in a room that was unfamiliar to the puppies.

The arena was enclosed on three sides by the walls of the test room and for the remaining short side by a 91 cm high metal fence covered externally with a dark cloth.

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