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Early development of filial preferences in the rabbit: implications of nursing- and pheromone-induced odour learning?

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Newborn rabbits, *Oryctolagus cuniculus*, discriminate between different categories of adult conspecifics on the basis of their abdominal odour cues. Whether these cues can support the development of filial preferences has not been adequately tested. Using a two-choice paradigm, we assessed the ability of 3–8-day-old pups to orient selectively to the mother versus an unfamiliar female, either spontaneously or after odour conditioning. In experiment 1, nonconditioned pups roamed indifferently over the mother and an unfamiliar female. In experiment 2, pups conditioned to a neutral odorant while nursing or with the mammary pheromone became attracted by the odorant. In experiment 3, pups that had learned the odorant while nursing oriented for longer to any female carrying it, but the unscented mother and a scented unfamiliar female were equally attractive. Finally, in experiment 4, pups that had learned the odorant paired with the mammary pheromone showed a preference for their scented mother, but not systematically for a scented unfamiliar female; furthermore, they were equally attracted by the unscented mother and a scented unfamiliar female. In sum, pups did not spontaneously evince an olfactory preference for the mother when opposed to an unfamiliar female, although they seemed able to detect individual maternal odours. In fact, they appeared to react to both species-specific cues and individual cues that they had learned, and their responses depended on their degree of familiarity with the cues and on the context. The mammary pheromone by itself might act as both a releasing and a reinforcing signal in these early socially oriented behaviours.

Keywords: learning; mammary pheromone; mother–young relationship; *Oryctolagus cuniculus*; rabbit; recognition

The recognition of individuals, or categories of individuals, is a prerequisite for the selective treatment of conspecifics (Halpin 1986). Such recognition abilities are especially important between females and young, as they support the preferential investment of females in their own, or closely related, young. On the offspring's side, discriminating between conspecifics is vital for obtaining the maximal level of maternal care and resources. From birth onwards, newborns need to orient to lactating females among other neighbouring females, and usually to the mother among other related or unrelated lactating females. That newborns differentiate their mother from other females has been noted in various mammalian species (see below for examples), but the question of how such an ability initially develops is worthy of further investigation. Does it depend on postnatal learning of maternal cues (e.g. Cheslock et al. 2000; Delaunay-El Allam et al. 2006)? Does it result from prenatal learning (reviewed in Schaal 2005) or is it based on hard-wired perceptual processes (reviewed in Johnson & Bolhuis 2000)? Does it depend on a combination of such processes (e.g. Hepper & Wells 2006)? The present study is an attempt to deal with these questions.

Among other sensory features, chemical cues are strongly involved in the discrimination of adult mammal conspecifics. This has been abundantly documented in the case of kin recognition (reviewed in Fletcher & Michener 1987) and individual recognition (reviewed in

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Brennan & Kendrick 2006). In relationships involving young and adults, the ability of parents to recognize their own offspring has also been established in many mammalian groups (e.g. Poindron & Le Neindre 1980; Schaal et al. 1980). Finally, the immature organisms' capacity to recognize their biological mother based on odour cues, or to prefer maternal odorous secretions over the same secretions produced by unfamiliar lactating females, has also been well documented. For example, the precocial newborns of ungulates use olfaction to discriminate familiar from unfamiliar females, and recognize their own mother after a few postnatal hours (e.g. horse, *Equus caballus*: Wolski et al. 1980; reindeer, *Rangifer tarandus*: Källquist & Mossing 1982; sheep, *Ovis aries*: Val-Laillet et al. 2004; pig, *Sus scrofa*: Morrow-Tesch & McGlone 1990). Similar abilities have been found in altricial rodent newborns and young (rat, *Rattus norvegicus*: Polan & Hofer 1998; mouse, *Mus musculus*: Hepper 1987). In primates, squirrel monkeys, *Saimiri sciureus*, discriminate their own mother from other lactating females by her odour from 8 weeks of age (Kaplan et al. 1977), but much earlier recognition is found in human infants (e.g. McFarlane 1975; Schaal et al. 1980; Cernoch & Porter 1985). Taken together, these results establish a preference of young mammals for maternal odours that develops through perinatal and/or postnatal learning. Such learning is probably bootstrapped by the recurrent exposure of the young to maternal cues in highly reinforcing conditions.

There are some exceptions, however, in the physical closeness and timing of mother–infant interactions in early development, some species having evolved a parsimonious strategy of mother–infant contact. One may therefore wonder whether the young of such species develop a preference for maternal odours, despite the few opportunities to interact with the mother. The European rabbit, *Oryctolagus cuniculus*, is one such species where the mother–young daily contact is restricted to less than 5 min, exclusively centred on milk transfer (Zarrow et al. 1965). This species presents two additional characteristics that encouraged us to investigate the developmental course of olfactory recognition of the mother by the young. First, newborn rabbits, being highly altricial, depend on maternal odour cues derived from prenatal learning (Bilkó et al. 1994; Coureaud et al. 2002) or from predisposed signals such as the mammary pheromone (Coureaud 2001; Schaal et al. 2003), to find their way to the nipples. Second, right after birth, pups are also very efficient at acquiring novel odorants as cues, either through associative learning while nursing (e.g. Ivanitskii 1962; Kindermann et al. 1991; Coureaud et al. 2006a) or through conditioning by the mammary pheromone (Coureaud et al. 2006a). Since all these mechanisms are simultaneously at work in the newborn rabbit, it is a useful model to investigate their respective impacts on the early recognition of the mother.

Mykytowycz & Ward (1971) suggested that 10–20-day-old rabbits (but not 4–9-day-old pups) selectively respond to the odours of their biological mother. The pups in their study indeed appeared to prefer the odour released by the mother's anal glands to the same secretions from unfamiliar females, males and juveniles. However, this observation remains to be confirmed as the data were poorly detailed and not analysed statistically. Val-Laillet & Nowak (2008) also suggested that 7-day-old pups, but not 1- and 14-day-old rabbits, prefer their mother to an unfamiliar lactating female in odour tests. However, their conclusions are also uncertain because of methodological limitations (repeated testing of the same pups with conscious and potentially stressed females and exposure to multiple odour sources on abdominal, oral and anogenital areas). Finally, in another study evaluating pup attractiveness to abdominal odours only, newborn rabbits were shown to discriminate between conspecific adults in double-choice tests, and to be more attracted by unfamiliar lactating females than by nonlactating or nulliparous females, or by males (Coureaud & Schaal 2000; Coureaud et al. 2001). However, in these latter conditions, whether pups evinced a preference for their own mother's abdominal odour was not investigated.

Our aim in the present study was therefore to evaluate the development of preferential orientation of young rabbits towards theirs mother's abdominal odour, and the role of early learning in this discrimination. We considered the abdominal odour of the mother to be a complex mixture of qualitatively and/or quantitatively distinguishable fractions, among which one fraction is likely to be individually variable as a function of dietary, metabolic, physiological and immunogenetic factors, whereas another fraction may comprise compounds (such as the mammary pheromone and other compounds from milk) that are invariant at the species or genus level. Evidence exists that rabbit pups can discriminate between odours belonging to these two kinds of fractions in conspecific milk (Coureaud et al. 2002). We investigated pups' preferences for maternal odour in a series of experiments during which they were simultaneously exposed to the abdominal odours of their own mother and those of an unfamiliar lactating female. In experiment 1, we tested whether pups are spontaneously attracted to their mother's odour, and whether their behaviour depends on their motivational (prandial) state and age. In experiments 2–4, we examined whether their preferential orientation could be modulated by odour learning that occurs during natural nursing or after induction by the mammary pheromone.

GENERAL METHODS

Animals, Breeding and Housing Conditions

We used New Zealand × Californian cross-bred rabbits (strains Grimaud GD 14 and GD 24 for females and males, respectively) housed in the breeding unit of the Etablissement National d'Enseignement Supérieur Agricole de Dijon (ENESAD). Females and males were kept in individual cages (females: 62.5 × 46.5 cm and 27.5 cm high; males: 76 × 46.5 cm and 30 cm high). For pregnant does, a nestbox (42 × 25 cm and 25 cm high) was added to the outside of the cages 2 days before parturition. The females prepared their own nest in commercial shavings (Copeaux-10, Safe, Augy, France) to which they added their own abdominal fur. On the day of birth (designated as day 0),