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Original investigation

Do whiskers contribute to nipple-search and suckling behavior in newborn rabbits (*Oryctolagus cuniculus*)?

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Abstract

Rabbit pups are only nursed for about 3 min once a day. They depend on a pheromone on the mother's ventrum to locate nipples and on tactile stimulation of the muzzle to grasp them. In a continuing study of the sensory input guiding suckling behavior we investigated the whisker array in newborn pups and the possible contribution of the whiskers to suckling. Rabbits are born with approximately 76 whiskers arranged in seven to nine rows and increasing in length from rostral to caudal. No significant difference was found between pups with whiskers cut and intact controls in latency to perform the stereotyped nipple-search behavior, latency to attach to nipples, time spent on nipples, milk ingested, or in the strength of conditioning to a novel odor paired with suckling. Thus, the whiskers do not seem important for suckling in newborn rabbits.

Key words: *Oryctolagus cuniculus*, suckling, vibrissae, altricial mammals

Introduction

For newborn mammals the task of finding, attaching to and successfully sucking a nipple or teat is one of the principle challenges of postnatal life. The European rabbit (*Oryctolagus cuniculus*) is a notable example. Immediately after giving birth, female rabbits leave their altricial young and only return to nurse them for about 3–4 min every 24 h (reviewed in Hudson and Distel 1982, 1989). The young, in strong competition with littermates (Drummond et al. 2000), drink up to 25% of their body weight in this short time (Lincoln 1974; Hudson et al. 1996). A pheromone on the mother's ventrum is essential for the release of the stereotyped nipple-search behavior and to guide pups to nipples (Hudson and Distel 1983, 1995). In addition, at the nipples somatosensory input to the muzzle is then essential for nipple attachment (Distel and Hudson 1985).

Perioral stimulation is also important for learning novel odors associated with suckling. If the mother's ventrum is scented with an artificial odorant, pups learn to associate the novel odor with suckling in just one 3-min nursing episode. When tested 24 h later on an animal or a fur scented with the odorant, conditioned but not control pups show the stereotyped nipple-search response (Hudson 1985; Kindermann et al. 1991, 1994; Allingham et al. 1999). Not milk but rather perioral stimulation from sucking is the main reinforcer of such learning (Hudson et al. 2002).

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In the present study, we asked whether in rabbits the whiskers play a role in suckling-associated behaviors since the pups are born with a conspicuous array of these. In contrast to adult mammals (Brecht et al. 1997; Landers and Sullivan 1999a; Cardenas et al. 2001; Krupa et al. 2001), there have been few behavioral studies of whisker function in newborns (but see Landers and Sullivan 1999a, b), and to our knowledge, none in a natural biological context. It was therefore our aim (1) to provide a description of the whisker array in the newborn rabbit, (2) to investigate the effect of removing the whiskers on nipple-search behavior and the learning of olfactory cues associated with suckling, and (3) to investigate the effect of removing the whiskers on suckling performance under natural conditions in the nest.

Material and methods

The following three experimental sets were performed:

The whiskers

Four two-day-old chinchilla-breed pups (day 0 = day of birth) from four litters, weighing 69.4–96 g, and killed with an i.p. overdose of pentobarbital (Sedal-Vet®, Ttokkyo Laboratories) were used. Under a magnifying lamp each whisker was plucked and using fine calipers and a Vernier scale, its length was recorded and entered onto the whisker map for that particular pup. In five cases whiskers broke during plucking and were excluded from the analysis. To estimate the length of the subcutaneous portion, in two additional pups the whiskers were dyed, plucked, and the undyed section measured. Depilatory cream was then applied for 3 min and the skin wiped clean and rubbed with graphite to enhance visibility of the vibrissal pores. Under the magnifying lamp the pores on both sides of the muzzle were mapped onto a schematic representation of the mystacial area.

Nipple-search behavior and olfactory learning

Thirty-two two-day-old chinchilla-breed pups from 11 litters were used. On day 1 they were separated from the mother to ensure high motivation in the nipple-search task the following day, and color-marked in the ears for individual identification. At 10:00 h on the morning of day 2 they were weighed and assigned to one of two groups ($n = 16$/group) in such a way as to balance weights between treatments; experimental pups whose whiskers were cut 1 h before testing (group W−; mean weight 62.8 g, SE 3.0) and control pups with whiskers intact (group W+; mean weight 60.4 g, SE 2.4; two-tailed t-test for difference in weight between groups: $t = 0.65$, df 30, $P > 0.62$). Whiskers were cut rather than plucked, cauterized or removed with depilatory cream to avoid damage to the fine mystacial skin. Removing whiskers by cutting is sufficient to disrupt a variety of whisker-mediated behaviors in adult rats (Brecht et al. 1997; Cardenas et al. 2001; Krupa et al. 2001).

At 11:00 h the pups were exposed individually to a standard olfactory conditioning procedure (Hudson et al. 2002). Experimental and control pups were placed alternately on the ventrum of an anesthetized lactating doe restrained on her back in a Plexiglas trough forming an arena enclosing the six rear nipples (Hudson 1985; Hudson et al. 2002). The doe's ventral fur and area around the nipples had been lightly perfumed with the cologne Chanel No. 5 (Hudson 1985; Hudson and Distel 1986, 1987; Kindermann et al. 1991, 1994; Hudson et al. 2002). Pups were allowed to move freely and to attach to and suck 10 nipples for a maximum of 10 s each. Their behavior was recorded using a video camera mounted above the arena, and latencies to search, to attach to nipples and the duration of attachments were scored.

The next day, pups were given a recall test in which they were placed individually for 5 min on a tanned rabbit fur warmed to 30 °C enclosed by an acrylic arena ($13 \times 18 \times$ height 13 cm^3), and perfumed with three spots of Chanel placed 2 cm apart along the central long axis (Kindermann et al. 1991, 1994; Allingham et al. 1999; Hudson et al. 2002; Fig. 3). Latency to start the stereotyped nipple-search behavior, time taken to initiate search bouts, total search time, and the total time searching was performed within the stimulus area defined as an ellipse enclosing the Chanel spots (Hudson et al. 2002; Fig. 3) were scored.

Suckling performance

Six chinchilla-breed litters standardized to 10 pups each were used. Ten pups represents the upper end of the range in litter size for rabbits and was chosen to ensure competition among pups during suckling (Drummond et al. 2000). On the day of birth (day 0) pups were separated from their mother, color-marked in the ears for individual identification,