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Effects of Pre- and Postnatal Olfactogustatory Experience on Early Preferences at Birth and Dietary Selection at Weaning in Kittens

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Abstract

The ontogenesis of olfactogustatory preferences has been investigated in various mammals but surprisingly not in domestic cats *Felis catus.* In a first experiment, we examined how prenatal exposure (25 days prepartum) to a cheese flavor via the mother's diet can influence olfactory preferences of neonatal kittens. During 2-choice tests, 2-day-old kittens oriented first toward the cheese odor experienced in utero more frequently than toward a usual pet food odor. The choice of kittens born to mothers fed with a control diet did not differ from random. In the second experiment, we assessed the role of pre- and postnatal exposure (from 25th day before to 23rd day after birth) to cheese flavor on food preferences in weaned kittens. Forty-five-day-old cats exposed to cheese flavor during uterine and postnatal life via their mothers' diet ate higher amounts of chicken supplemented with cheese flavor than food supplemented with usual pet food flavor. On the other hand, the control group did not exhibit a preference for a specific food. Our results clearly demonstrated that pre- and postnatal olfactogustatory exposure via maternal ingestion influences later olfactory and food preferences of cats.

Key words: antenatal exposure, dietary selection, domestic cat, flavor, kitten, olfactory preference, postnatal exposure

Introduction

During uterine life, mammalian neonates have experienced a prenatal sensory environment that can profoundly modulate their postnatal behaviors. One of the major factors liable to act on prenatal environment is the mother's diet. Various flavors pass into the amniotic fluid that is regularly swallowed by the fetus (Bradley and Mistretta 1973; Schaal et al. 1995). It has been reported that repetitive exposure to various flavors can influence olfactory preferences of newborns at birth. Rabbit pups exposed to cumin flavor via mother's diet during pregnancy prefer cumin-odorized placenta than nonodorized placenta at birth (Coureaud et al. 2002). Puppies exposed to anise flavor during gestation via maternal diet orient initially toward anise odor at birth (Wells and Hepper 2006). In addition, prenatal exposure to flavor per se can have consequences on dietary selection at weaning. Twelve-day-old rat pups exposed to garlic flavor during uterine life and postnatally adopted by an alien mother orient preferentially toward garlic-flavored than toward onion-flavored food (Hepper 1988). Lambs prenatally exposed to oregano via mother's diet eat more oregano-flavored food at weaning (Simitzis et al. 2008). Furthermore, pregnant sows who ingested duck-weed give birth to piglets who prefer this food at weaning (Tien and Preston 2003).

Other work showed more generally that a combination of pre- and postnatal learning has similar impact on food preferences. Studies in rabbits (Bilkó et al. 1993), pigs (Langendijk et al. 2007), dogs (Hepper and Wells 2006), and humans (Mennella et al. 2001) demonstrated that repetitive prenatal exposure to a specific odor (via amniotic fluid), reinforced by postnatal exposure (via maternal milk), influence food selection at weaning. The relative contribution of pre- and postnatal experience has been investigated by Hepper and Wells (2006) who demonstrated in dogs that each was sufficient in itself to modulate later preferences; nevertheless, their impact was amplified by a synergic action.

In all cases, studies on chemosensory preferences acquired via maternal diet were investigated in Hominidae, Canidae, Bovidae, Muridae, and Leporidae. In this work, we examined through 2 experiments the ability of a feline, the domestic cat, to learn about olfactogustatory stimuli encountered as fetuses or as young kittens. Despite their popularity as pets, there has been no study of perinatal olfactory–gustatory learning in the cat. If newborn cats are blind, deaf, and very limited from a locomotor point of view at birth (Beaver 1980; Christiansen 1984; Mermet et al. 2008), their olfactory and gustatory senses are efficient (Levesque 1997). At 24 h after birth, kittens can discriminate between their mother's teat and an alien teat (Ewer 1961). At 8 days, kittens are able to orient themselves toward their home site on olfactory cues (Rosenblatt et al. 1969; Freeman and Rosenblatt 1978a) and to recognize their mother and siblings (Freeman and Rosenblatt 1978b). Whether cats have the ability to perceive and retain pre- or postnatally nutrient flavors remains to be investigated.

The aim of this work was 2-fold. In a first experiment, we assessed whether or not intrauterine exposure to a specific flavor via the mother's diet during the last 25 days of gestation could modulate olfactory preferences of kittens at birth. In a second experiment, we evaluated if a flavor exposure during uterine (25 days prepartum) and postnatal life (23 days postpartum) via mothers' diet could influence food selection at weaning.

Methods

Experimental subjects

A total of 30 kittens (15 males and 15 females) were used in the experiments. They were issued from 14 pregnant female cats. All mother–litter groups (11 Skogkatt cats and 3 Maine Coons) were family owned (8 different breeders) and familiarized with the experimenter to prevent fear reactions at the time of testing. These breeds have been chosen for their prolificacy (the average size of the litters was 4.38 ± 1.45 kittens) and for their tolerance for manipulation by humans, especially during parturition. Due to the reduced number of kittens, we sometimes used more than 1 kitten per litter in a specific experiment (3 kittens in 2 cases and 2 kittens in 6 cases).

Flavors used for chemosensory exposure and during choice tests

The 2 flavors (usual pet food flavor vs. cheese flavor) used during the choice tests were made by SPF Diana, the leading supplier of palatability solutions to the global pet food industry. Their exact composition cannot be revealed because it is protected by a confidential clause. The usual pet food flavor was a common constituent of industrial food ("commercial cat food") widely used in the pet food industry (and incorporated to the kibbles during industrial manufacture). It was mainly composed of poultry. Tests realized by SPF Diana in adult cats showed that the usual pet food flavor is neither attractive nor aversive (Claire Larose, unpublished data). "Commercial cat food" (Babycat 34 Royal Canin, Kitten Hill's) delivered to mothers of control kittens was thus already poultry flavored. In contrast, the flavor chosen for mothers of the experimental group was a cheese flavor that was added to the commercial cat food previously described. Cheese concentrate was mixed with commercial cat food by using a mixer (FORBERG International AS), a sophisticated system that allows coating of the kibbles. The liquid concentrate (cheese flavor) was applied to dry food at 3% and mixed for 2 min. About 5 min later, the food was put in hermetically sealed plastic bags by a specific machine and kept at 20 °C for 2 weeks before consumption.

Procedure

Experiment 1: role of antenatal and perinatal exposure on newborn olfactory preferences

To evaluate the influence of antenatal and perinatal exposure to the cheese flavor, 2 groups were constituted. The experimental group was exposed in utero to the cheese flavor during the last 25 ± 7 days (mean ± SD) of gestation and the 2 days after birth. The control group was not exposed.

Kittens of control group were tested at the age of 45.4 ± 5 h (N = 9; 7 Skogkatt cats and 2 Maine Coons); kittens of experimental group were tested at 31.5 ± 11.2 h (N = 6; 4 Skogkatt cats and 2 Maine Coons). There was no difference between the ages of tests in the 2 groups (permutation test, P = 0.820). Freeman and Rosenblatt (1978b) demonstrated that temperature and odors of the home site are very attractive for 1- to 15-day-old kittens. Therefore, kittens were tested a few meters away from the home site in a separated room. They were removed from the home box only for a few minutes. During a 2-choice test, each kitten was placed in a clean Plexiglas box (28 × 10 × 15 cm), with its nose placed at the centre point between the 2 stimuli, 10 cm from each stimulus. Two cotton swabs were held by self-adhesive tape and set up 4 cm above the floor surface and 20 cm apart. We applied 0.4 ml of cheese flavor to one cotton swab and 0.4 ml of usual pet food flavor to the other swab. For half of the tests, the cheese-flavored solution was on the left swab, and for the other half of the tests, it was on the right swab. Each test lasted for 5 min and was video recorded. After each test, the box was washed with pure water and an antiseptic (chlorhexidine), then water again, and finally dried. Kittens were gently cleaned with water and cotton to avoid odor contamination of the litter and then returned back into their litter. All mothers accepted their kittens after the test and nursed them.

Experiment 2: role of antenatal and postnatal exposure on dietary selection in weaning kittens

To evaluate the influence of antenatal and postnatal exposure to the cheese flavor, 2 groups were constituted. One was exposed to the cheese flavor during 25 ± 7 days before parturition and 23 ± 5 days after parturition; it was constituted of kittens from the litter that were used in Experiment 1 that had not been tested before and were specifically kept for Experiment 2. A control group was not exposed.

In cats, the weaning period ranges from the sixth to the eighth week (Christiansen 1984; Mermet et al. 2008). For this experiment, 9 control kittens were tested at the age of 45 ± 1.7 days (4 Skogkatts and 5 Maine Coons) and 6 experimental kittens were tested at 45.7 ± 1.5 days (3 Skogkatt cats and 3 Maine Coons). There was no difference between the ages of tests in the 2 groups (permutation test, $P = 0.071$). At this stage, all sensory systems are fully developed, and kittens are in an intensive exploratory phase. To limit exploratory behavior during trials, subjects were tested in their familiar environment (the room in which they spend most of the time) 3 ± 0.42 h after their last feed.

Kittens were given a choice between 2 containers, one containing 20 g of minced chicken (chicken made by "Douce France") flavored with 0.6 ml of cheese solution and the other contained 20 g of the minced chicken flavored with 0.6 ml of usual pet food solution. The odors of the 2 containers were easily distinguishable by a human nose and were 20 cm apart. They were placed on the floor, and their position (right or left) was reversed at the end of each test. Each test lasted for 10 min and was video recorded. We analyzed the first orientation of each kitten toward 1 of the 2 bowls, and the amount of ingested food was measured (Bioblock Scientific balance, precision ± 0.1 g).

Statistical analysis

For Experiment 1, according to Hepper and Wells (2006) and because it was the most relevant variable, we compared the first orientation toward the cheese-flavored swab or toward the usual pet food–flavored swab in each group (i.e., the experimental and the control groups) using binomial tests. All the statistical analyses were performed using StatXact-3 (Cytel Software Corporation).

For Experiment 2, in each group, we compared the first orientation toward a specific container (cheese vs. usual pet food flavor) using binomial tests. We also compared the amount of food of each type eaten by the kittens in each group using a permutation test for paired samples. In order to facilitate the visual comparison between the groups, we calculated a preference index for the cheese-flavored minced chicken based on the quantities of food ingested by each kitten as follows:

$$\text{preference index} = (Q_c - Q_n)/(Q_c + Q_n),$$

where Q_c was the quantity of cheese-flavored minced chicken ingested and Q_n the quantity of chicken flavored by the usual pet food solution ingested.

Results

Experiment 1: role of antenatal and perinatal exposure on newborn olfactory preferences

Five out the 9 control kittens oriented toward the cheese-flavored cotton swab first. This distribution did not differ from random (binomial test: $P > 0.99$). All the 6 kittens of the experimental group oriented first toward the cheese odor (binomial test: $P = 0.031$).

Experiment 2: role of antenatal and postnatal exposure on dietary selection in weaning kittens

Four out the 9 control kittens oriented toward the cheese-odorized container. This distribution did not differ from random (binomial test: $P > 0.99$). In all the 6 kittens of the experimental group, the first orientation was always toward the cheese-flavored chicken (binomial test: $P = 0.031$).

Kittens from the experimental group tended to eat more cheese-flavored chicken than chicken flavored with a solution of their usual pet food (permutation test: $P = 0.063$) as shown by the positive preference index (Figure 1). In fact, 5 out of 6 kittens of the experimental group did so, whereas the proportion was of 4 out of 9 kittens in the control group. Due to the small size of the samples, the difference did not reach significance ($\chi^2 = 5$, $P = 0.089$). There was no significant difference between the consumption of each type of food in the control group (permutation test: $P = 0.551$).

Discussion

Two main findings resulted from the present study. Firstly, exposure to cheese flavor via maternal diet during pregnancy influenced postnatal olfactory preferences in 2-day-old kittens. Experiment 1 showed that kittens exposed prenatally

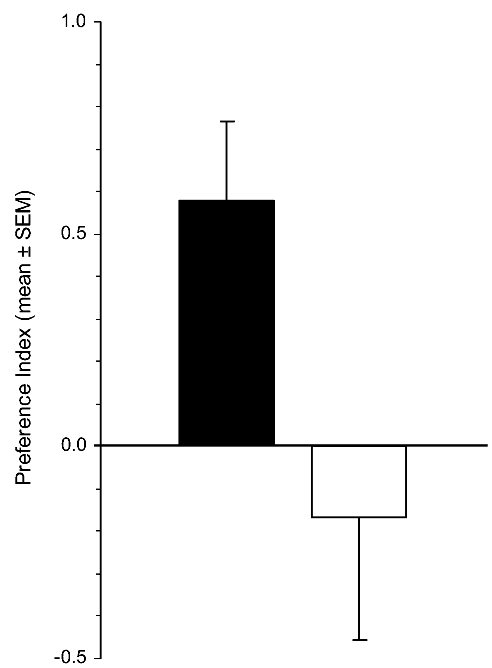

Figure 1 Preference index (mean ± standard error of the mean [SEM]) for cheese-flavored chicken ingested by 45-day-old kittens in experimental (N = 6, black bar) and control groups (N = 9, empty bar). The experimental group was exposed in utero and postnatally to the cheese flavor during 25 ± 7 days before parturition and 23 ± 5 days after parturition through maternal diet; the control group was not exposed to cheese flavor.

to the cheese flavor responded differently to the stimulus experienced in utero, whereas the control group did not. This result is the first evidence of early olfactogustatory learning in the domestic cat and corroborates various studies in other mammals, notably in rabbit pups (Coureaud et al. 2002) and in human babies (Schaal et al. 2000). Only one study has investigated such mechanism in carnivores. Wells and Hepper (2006) demonstrated that antenatal exposure to anise flavor during the last 20 days of pregnancy positively affected the preference toward the aniseed flavor in 10-min-old as well as in 23-h-old puppies. Under our conditions, kittens were tested at 39 h of age. In both cases, we cannot exclude the possibility that newborn kittens may have been in contact with the experimental flavor postnatally from odors conveyed by their mother's body. Neonates may have perceived the flavor not only via inhalation and ingestion of amniotic fluid but also through extracorporeal secretions such as saliva, body odors, feces, or urine. In our experiment, maternal milk could also have conveyed the cheese flavor. Regardless of the way of transmission, our study clearly demonstrated that perception of a flavor through the mother's diet during pregnancy and within a few hours after birth can influence olfactory preferences of 2-day-old kittens.

Experiment 2, where test subjects were exposed to the flavor both prenatally (25 days prepartum) and postnatally (23 days postpartum), found that this exposure influenced the kittens' subsequent dietary preference. Kittens exposed to the cheese flavor during pregnancy and the first weeks after birth oriented preferentially toward the cheese-flavored chicken, whereas control group did not express this preference. Moreover, 5 out of 6 kittens from the experimental group ate more chicken mixed with cheese flavor than chicken flavored with the usual pet food, whereas only 4 out of 9 kittens from the control group did so. Supplementation of the diet of the mother during prenatal and early postnatal life clearly altered food choice of kittens at weaning. Similar results have been described in other mammals. Rabbit pups raised by mothers who were fed with aromatic juniper berries during pregnancy and lactation preferred the diet of their mother at weaning (Bilkó et al. 1993). Piglets born to a mother who had consumed garlic and aniseed diet during antenatal and postnatal exposure had a higher food intake of the odorized food than of the neutral food (Langendijk et al. 2007). Prenatal and early postnatal exposure to the carrot flavor via the mother's diet enhanced the carrot-flavored food intake at weaning in human babies (Mennella et al. 2001). Last but not least, 10-day-old puppies preferentially ate more aniseed food at weaning than neutral food after a pre- and postnatal exposure via the mother's aniseed diet (Hepper and Wells 2006).

It is interesting to notice in Experiment 1 that control kittens did not prefer usual pet food–flavored swab to cheese-flavored swab and that in Experiment 2 the same phenomenon was observed with a lack of preference for chicken flavored with a solution of usual pet food compared with cheese-flavored chicken. As control kittens were exposed pre- or postnatally to the usual pet food flavor through maternal diet, we can wonder why their usual pet food flavor was not attractive after early exposure. The most convincing hypothesis is that the usual pet food flavor fundamentally differs from cheese flavor in its composition. Usual pet food flavor is a complex blend of poultry molecules, whereas cheese flavor is reduced to a few molecules and may be easier to retain by fetuses and neonates.

In all cases, we may conclude that amniotic fluid, milk, and other odors conveyed by the mother's body are potent vectors liable to modulate preferences of very young cats. It would be interesting to investigate if this early "passive" learning induced by the diet of the mother could be reinforced by a more "active" social learning (observation of mother's behavior and imitation). One study demonstrated that kittens learned to eat banana by observing their mother who felt positive sensation via intracerebral stimulation during banana consumption (Wyrwicka 1993). Moreover, Galef and Clark (1971) showed that the presence of an adult at a feeding site can influence food choice in young rats who preferred food consumed by adults. In our work, the hypothesis of learning by observation should be excluded because 45-day-old kittens were tested on their own and had the choice between 2 new substrates, cheese-flavored chicken and usual pet food–flavored chicken, whereas mothers were fed only with kibbles and never experienced the 2 flavored chickens. It is therefore quite likely that social learning interacts with passive odor learning and enhances the establishment of later food preferences.

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