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Prenatal and Early Sucking Influences on Dietary Preference in Newborn, Weaning, and Young Adult Cats

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Abstract

Early experiences are of potential importance in shaping long-term behavior. This study examined the relative influence of prenatal and/or early postnatal experience of chemosensory stimuli on subsequent olfactory and dietary preferences of cats as newborns, at 9–10 weeks, and at 6 months. Cats were exposed to vanillin or 4-ethylguaiacol via their mother's diet either prenatally, postnatally, perinatally (prenatal and postnatal), or experienced no exposure to the stimuli (control). Newborns were given a two-choice olfactory test between the familiar "odor" and no odor; 9–10 week olds were tested for their preference between two food treats, one flavored with the familiar stimulus and the other unflavored; at 6 months, cats were given a choice of two bowls of food, one flavored with the familiar stimulus and the other unflavored. At all ages, cats preferred the familiar, and avoided the unfamiliar, stimulus. Perinatal exposure exerted the strongest influence on preference. Prenatal exposure influenced preference at all ages and postnatal exposure exerted a stronger effect as the cat aged. We conclude that long-term chemosensory and dietary preferences of cats are influenced by prenatal and early (nursing) postnatal experience, supporting a natural and biologically relevant mechanism for the safe transmission of diet from mother to young.

Key words: cat, dietary preference, long-term preference, nursing, prenatal learning

Introduction

Early developmental experiences, either prenatal or soon after birth (e.g., nursing and sucking), may have a long-term influence on subsequent development and behavior. Experiences may shape physical and neural structure (e.g., Berardi et al. 2000; Grubb and Thompson 2004), organ function and subsequent predisposition to disease (e.g., Gluckman and Hanson 2005), behavior, and preferences (e.g., Hepper 1996).

One area where early experience may influence subsequent behavior is that of feeding. An important decision for newborn animals is their choice of diet. Newborns eventually become independent feeders, and it is vital for their survival that they feed on safe foods. For mammals, one means of acquiring this information is indirectly from the mother's diet (e.g., Hepper 1996), via flavors present in the amniotic fluid (e.g., Mennella et al. 1995) or breast milk (e.g., Mennella and Beauchamp 1991). It can reasonably be assumed that if the mother consumes a particular food then it is safe, and thus maternal transmission of dietary information provides a means for the offspring to "learn" about safe foods.

The addition of chemosensory stimuli to the maternal diet during pregnancy influences the chemosensory preferences of offspring in the newborn period. This appears to be a widespread phenomenon that has been witnessed in invertebrates (e.g., Isingrini et al. 1985; Caubet et al. 1992), fish (Brannon 1972), amphibians (e.g., Hepper and Waldman 1992), reptiles (e.g., Sneddon et al. 2001), birds (e.g., Sneddon et al. 1998; Bertin et al. 2010), and many mammalian species, including rats (Smotherman 1982), rabbits (Semke et al. 1995), sheep (Schaal et al. 1995; Simitzis et al. 2008), dogs (Wells and Hepper 2006), pigs (Oostindjer et al. 2009), and humans (Hepper 1995; Schaal et al. 2000).

The effects of prenatal exposure to chemosensory stimuli are not limited to the newborn period, but influence preferences for food or fluids later on in life, for example after weaning (e.g., dogs, Hepper and Wells 2006; rabbits, Bilkó et al. 1994; lambs, Simitzis et al. 2008; mice, Nolte and Mason 1995). Similarly, flavors present in the mother's milk experienced when sucking also influence subsequent dietary-related preferences at weaning (e.g., humans, Mennella et al. 2001; dogs, Hepper and Wells 2006; rabbits, Bilkó et al. 1994).

Although both prenatal and early postnatal (nursing) exposure have been found to contribute to subsequent preferences, most studies have only considered the influence of prenatal (e.g., Smotherman 1982; Nolte and Mason 1995; Simitzis et al. 2008; Youngetob and Glendinning 2009; Bertin et al. 2010) or postnatal (e.g., Campbell 1976; Mennella and Beauchamp 2002, Mennella et al. 2006) exposure. Some have considered both in the same study (e.g., Mennella et al. 2001), but have treated them as independent factors and any relative contribution or interaction has remained relatively unstudied. The response of rats to the odor of alcohol is enhanced following prenatal exposure if experience via social interaction with the odor of alcohol is provided, although this postnatal exposure was given after weaning, as juveniles, and not during nursing (Eade et al. 2009; Eade and Youngentob 2010). In contrast, rabbit pups show no difference in the strength of food preferences between exposure to the odor/flavor experienced prenatally or during nursing or both (Bilkó et al. 1994).

Hepper and Wells (2006) found that, in domestic dogs, combined prenatal *and* postnatal exposure to a stimulus (aniseed) led to a stronger preference for food flavored with this stimulus than either exposure to the stimulus via prenatal or postnatal means alone. Furthermore, this study examining the relative influence of pre- and postnatal exposure, found that the influence of prenatal exposure in influencing preference decreased from birth to weaning, whereas that of early postnatal exposure increased. This suggests that the relative influence of prenatal and postnatal exposure may change as an individual ages. Prenatal exposure may be more salient nearer birth, resulting in a stronger effect on dietary preference, whereas postnatal exposure may be more salient later in life. Thus, the development of the exhibition of preferences arising from prenatal and early postnatal exposure requires exploration.

The aim of the following study was to examine the impact of prenatal and/or early postnatal chemosensory exposure on the olfactory and dietary preferences of domestic cats at three developmental stages (newborn, shortly after weaning at 9–10 weeks of age, and as juveniles at 6 months). We have previously investigated the influence of early chemosensory exposure in the domestic dog (Hepper and Wells 2006; Wells and Hepper 2006); the cat was chosen deliberately as the subject species in the present investigation in order to examine the generalizability of results in carnivores, and to extend the field more generally by exploring the effect of early experience in shaping long-term dietary preferences.

Only one previous study has examined the effects of early chemosensory experience on subsequent dietary preferences in the cat. Becques et al. (2010) found that newborn kittens showed a preference for pet food flavored with a stimulus (cheese odor) experienced prenatally compared to unadulterated food. This preference was maintained at 45 days, following both prenatal and postnatal exposure. However, only one condition involving both pre- and postnatal exposure was examined in relation to the kittens' preferences at weaning. Thus, it is not possible to state whether the effects were due to either the prenatal or postnatal exposure. Moreover, the study did not examine dietary preferences in older animals, a key issue examined in the present investigation.

The following study sought to examine the role of prenatal (indirectly via amniotic fluid or transfer from maternal to fetal circulation) and/or immediate postnatal (indirectly via the kitten's mother's milk) chemosensory experience on the olfactory and dietary preferences of cats in newborns, after weaning and into early adulthood, a phenomenon thus far ignored in most studies. The relative contribution of prenatal and/or postnatal exposure on subsequent preference was also examined.

General methods

Subjects

Private cat breeders were recruited for the study. One adult female was recruited from an animal rescue shelter in Dundonald, Co. Down, and her litter (1 m, 2f) was tested in the control condition at weaning. Owners were asked if they would allow their cats to participate in the study, full details of which were subsequently provided. Consent forms were completed by all participating owners. In total, 34 mixed breed cats and their litters were used. The availability of subjects for testing varied (e.g., due to time of birth for the newborn test or owner unavailability); animal numbers for each test are therefore provided below.

The research received ethical approval from the School of Psychology, Queen's University, Research Ethics Committee and was performed in accordance with the "Guidelines for Psychologists Working with Animals" produced by the Standing Advisory Committee on the Welfare of Animals in Psychology, The British Psychological Society.

Stimuli

Two stimuli were used: vanillin (vanilla odor/flavor) and 4-ethylguaiacol (smoky odor/flavor). The two stimuli were clearly distinguishable from one another by human smellers and tasters. The experimental stimuli as used in the study were manufactured and provided to the researchers in Belfast by Nestec Ltd who used the highest food grade quality vanillin and 4-ethylguaiacol provided to them by Sigma Aldrich. Flavor stock solutions in MCT (DeliosV, Cognis) were prepared by direct dilution of the flavor in the carrier. Stimuli were provided in two forms, liquid and encapsulated. The liquid stimulus concentrations were vanillin 0.5% and 4-ethylguaiacol 0.5% and were used as the stimuli in the newborn tests (see later). The encapsulated stimuli were used to mix with the cat's normal food during the experimental phase and mixed with the food used in tests after weaning and as juveniles. This form was easier to mix in with the food and provided a more even distribution of flavor throughout the animal's food. The concentrations of the encapsulated forms were vanillin 22 976 ppm (final concentration in cat food [or test stimuli]: 100 ppm) and 4-ethylguaiacol 323 ppm (final concentration in cat food: 1.5 ppm). Encapsulated versions of vanillin and 4-ethylguaicol flavors were prepared as follows. Flavor and Capsul emulsifier were dissolved at room temperature in MQ-H_2O. Maltodextrin (Glucidex DE21) was added and dissolved under magnetic stirring and with ultrasonication. The clear solution was frozen at −40 °C and freeze dried for 30 h in a Lyolab G (LSL SECFROID SA). The encapsulated product was immediately transferred into sealed aluminum sachets (1-meal doses) and shipped to Belfast for the study.

When mixed with food at the concentrations described above, stimuli smelled of equal intensity to humans. To confirm that the flavor would reach the milk of lactating cats a small pilot study was undertaken. Five lactating cats were fed their diet mixed with the flavors (vanillin or 4-EG) in the same or lower concentrations as used in this study and just the flavor system carrier mixed with their diet (control condition). Immediately after consuming a single meal (2 h in duration) approximately 1 mL of milk was obtained by manual expression of milk by the staff veterinarian. Milk samples were immediately put in a freezer for storage in individual amber glass vials covered with aluminum foils. 4-EG was analyzed by SPME/GC-MS and vanillin by HPLC-APCI-MS/MS after SPE clean up. These analyses revealed the presence of both vanillin and 4-EG in the milk in low ppb levels as estimated by external matrix matched calibration and indicate that the flavors do pass from the maternal diet to the milk.

Exposure conditions

Two exposure conditions were used: (i) prenatal exposure, in which individuals were exposed to the stimulus before birth via the mothers' diet and (ii) postnatal exposure, in which kittens were exposed to the stimulus after birth, via the mothers' diet. The stimuli were added to the mother's normal food. This differed between individual cats but inspection of the ingredients revealed that no diet contained vanillin or 4-ethylguaicaol, nor did they smell of vanilla or smoky. All individual cats were fed the same diet throughout the study.

Prenatal exposure

Pregnant queens were fed a diet "flavored" with one of the two stimuli (either vanillin or 4-ethylguaicaol). Thirty grams of encapsulated stimulus was mixed with the animal's food on a daily basis following confirmation of pregnancy. Pregnancy was confirmed by a veterinarian through palpation of the abdomen between 35 and 40 days of gestation. Once confirmed, animals were fed the diet until the onset of parturition. There was no difference in the length of prenatal exposure for animals in the prenatal and perinatal experimental conditions for all tests (see Table 1).

Postnatal exposure

Following the birth of their litter, queens were fed a diet "flavored" with one of the two stimuli: 30 g of encapsulated stimulus was mixed with the animal's food on a daily basis, starting after the kittens had undertaken the newborn

Table 1 The number of individual subjects and number of litters in each experimental condition examined in newborn, 9–10 weeks, and 6 months tests

Condition	No. of subjects	No. of litters	No. of males and females		Number of subjects exposed to vanillin	Number of subjects exposed to 4-Ethyl.	Av. duration of prenatal exposure (days ± SD)	Mean age at testing (days ± SD)
			M	F				
Newborn								
Prenatal	14	4	6	8	10	4	26.25 ± 2.1	1.25 ± 0.5
Control	13	6	5	8	0	0		1.33 ± 0.5
9–10 Weeks								
Prenatal	24	6	13	11	13	11	25.5 ± 2.9	68.7 ± 8.8
Perinatal	25	8	12	13	13	12	25.37 ± 2.8	66.6 ± 11.0
Postnatal	24	7	11	13	12	12	0	64.1 ± 8.0
Control	25	7	12	13	0	0	0	65.1 ± 11.0
6 Months								
Prenatal	15	4	9	6	7	8	24.75 ± 3.3	175.5 ± 12.7
Perinatal	13	6	7	6	5	8	25.50 ± 3.0	179.8 ± 7.0
Postnatal	18	5	10	8	11	7	0	179.2 ± 0.4
Control	17	5	8	9	0	0	0	178.6 ± 6.7

The number of male and female subjects is included. The number of subjects exposed to vanillin and 4-ethylguaiacol and the average duration of exposure prenatally and their mean age at testing is presented.

test (see later). This ensured that the stimuli tested on the newborns were not transmitted postnatally via the mother's diet (although see Discussion). All animals began exposure on day 2 after their kittens were born. Kittens often show an interest in their mother's food around 4 weeks of age (Mermet et al. 2008), and may try to eat it. Therefore, kittens' exposure to the stimulus stopped at 28 days of age to prevent any additional exposure via the maternal diet. Kittens thus experienced 26 days of postnatal exposure. Cats were fed their food in a different room from their litter to prevent any direct exposure by the kittens to the flavored food and access to the food bowl was restricted to the mother only.

All owners were fully briefed on the study before it commenced. Instructions on how to mix the food with the stimuli were provided and demonstrated to the owners. Owners were visited twice a week during the exposure phase(s) of the study to: check on progress; provide fresh supplies of the stimulus; ensure there were no difficulties in the preparation of the food and its delivery to the females; and, to assist owners if necessary. None of the owners had any difficulty in following the procedure. Between visits, owners were called by telephone every day, to check on progress and, if necessary to provide any assistance that was required. None of the owners reported any difficulties.

Experimental conditions

Kittens were placed into one of the four following conditions depending on their exposure to the stimulus:

1. *Prenatal exposure*. Kittens were exposed to the stimulus via their mothers' diet before birth. There was no exposure to the stimulus after birth.
2. *Perinatal exposure*. Kittens were exposed to the stimulus via their mothers' diet both before *and* after birth.
3. *Postnatal exposure*. Kittens were exposed to the stimulus via their mothers' diet after their birth. There was no exposure to the stimulus before birth.
4. *Control*. Kittens were not exposed to the experimental stimulus before *or* after birth.

All cats fed the flavored "experimental" diets ate the food and there was no reported drop in the amount of food the cats ate from before being fed the flavored food and when being fed the flavored food. No differences were observed in the general development, including growth, of kittens between the four study conditions.

Tests

Animals were tested at three different ages: (i) 1–2 days old (newborn); (ii) 9–10 weeks (after weaning had finished); and (iii) 6 months (juvenile). All of the subjects were tested singly in their own home, separated from all other animals. For ease of reference, the chemosensory stimulus the animals had been exposed to experimentally is termed the *familiar* stimulus and the stimulus to which they had not been exposed is referred to as the *unfamiliar* stimulus.

Newborn olfactory preferences

Subjects

Twenty-seven kittens took part in the newborn tests, see Table 1.

Procedure

Newborn kittens were tested within 48 h of birth in a two-choice test. For a given trial, kittens were presented with a choice between a swab impregnated with an odor and an "un-odorized" swab. Animals were given two trials: on one trial, the odor was the familiar stimulus; the other trial used the unfamiliar stimulus as the odor.

Two cotton swabs were held by a clamp arrangement 4 cm above the floor surface and 20 cm apart. Two drops of the relevant chemosensory stimulus were applied to one of the swabs; two drops of distilled water were applied to the other swab as a control. Each kitten was briefly removed from its mother and placed on a heated pad covered with flannelling with its nose at the center point between the two swabs. The kitten was left in this position until its head made contact with one of the swabs; it was then recorded as having a preference for that side. The position of the odor was randomized between left and right across all kittens and odor conditions (familiar/unfamiliar).

Dietary preference at 9–10 weeks

Subjects

Ninety-eight kittens took part in the tests at this stage (see Table 1). There was no difference in the age of the kittens in different conditions at the time of testing (see Table 1).

Procedure

Trials began approximately 3 h after kittens were last fed. For each trial, the animal was given a choice of two food treats (minced chicken). One treat was flavored with the experimental stimulus, the other unflavored. For the flavored treat, 30 g of the encapsulated stimulus (vanillin or 4-ethylguaiacol) was thoroughly mixed with 100 g of the chicken and then divided into 2.5 g portions. Control treats constituted 2.5 g of unflavored minced chicken. Kittens were given six trials with the familiar flavored treat versus the control treat and six trials with the unfamiliar flavored treat versus the control treat. For each trial, treats were placed approximately 20 cm apart on the floor in the middle of the room. The kitten was placed in the middle of the treats and the treat first consumed by the kitten recorded. For half the trials, the flavored treat was on the kitten's left hand side, and for the other half on the kitten's right hand side. The order of flavored food presentation (familiar or unfamiliar) was randomized for each cat. A minimum of 15 min was left between trials.

Food preference at 6 months

Subjects

Sixty-three cats took part in the tests at 6 months and there was no difference in age of the animals in different conditions at the time of testing (see Table 1).

Procedure

Animals were presented simultaneously with two identical bowls containing food, approximately 10 cm apart. Both bowls contained the animals' normal food. However, one was flavored with the experimental stimulus and the other not flavored (control). To flavor the food, 30 g of encapsulated stimulus was added, and thoroughly mixed with 100 g of food. Both bowls contained identical weights of food. The bowls were placed on the floor at the cat's normal feeding time and left for 24 h. The bowl the cat first fed from, and the total amount of food consumed (in grams) from each bowl, were both recorded at the end of the 24-h period. In total, cats received six trials on consecutive days (one trial per day). For three trials, cats were presented with the familiar flavored food and unflavored food; for the other three trials, the cats were given a choice between unfamiliar flavored food and unflavored food. The position of the flavored bowl was counterbalanced such that for three trials it was on the left hand side and for three on the right hand side. The order of flavored food presentation (familiar or unfamiliar) was randomized for each cat.

Analysis: General considerations

The first series of analyses were undertaken to examine whether previous exposure to a stimulus affected subsequent preference and whether there was any difference in the distribution of preference between the different exposure conditions. Both litters and individual data were examined. Care was taken in the analyses to avoid potential spurious significant results that may have arisen from inappropriate consideration of individual data, since, in this study, the litter is the appropriate experimental unit. Given that individuals in the same litter may be more alike than individuals in different litters, there is a lack of independence between observations obtained from littermates which may increase Type I errors (Rao and Scott 1972). However, useful information may be obtained from individual data. To overcome the problems of within-litter correlations, an adjusted chi-squared (Donner and Donald 1988) was used to analyze the data from individuals; this took account of the within-litter correlation (Reed 2004). Analyses at the level of the litter, where the problem of within litter correlations is absent, were undertaken with chi-squared and Fisher tests (Siegel 1956).

To examine the relative contribution of pre- and/or postnatal exposure upon subsequent preferences, a 2×2 between subjects ANOVA for factors of exposure (prenatal:postnatal) and preference (odor:no odor) was undertaken. For these analyses, litter means were calculated by first determining the average of all male kittens in any specific litter and then separately the mean for female kittens in that litter. The mean litter score used in the analysis was therefore the average of the means derived from male and female subjects in that litter.

Two sets of identical analyses were undertaken: First, for the tests in which animals were given a choice between the familiar stimulus and the unadulterated stimulus, and second, for the tests in which animals were given a choice between the unfamiliar stimulus and the unadulterated stimulus.

Analysis: Effect of sex, stimulus, and repeat testing

At each testing age, analysis was undertaken to examine whether there was any differential effect of subject sex on the results across all four experimental conditions and whether there was any differential effect of the stimulus being exposed to across the three experimental conditions involving stimulus exposure.

Adjusted chi-squared analyses (Donner and Donald 1988) were used to assess whether males and females responded differently within each test condition (i.e., sex [male vs. female] and preference [odor vs. no odor] for prenatal and separately for other groups) for both the familiar and the unfamiliar odor/flavor. Additionally, for animals tested just after weaning and as juveniles, a between subjects ANOVA for exposure condition (prenatal, perinatal, postnatal, and control) and sex (male, female) was performed on litter scores (using mean male and female scores for each litter) separately for both response to the familiar and unfamiliar flavored food. No differential effect of animal sex was found in either analysis at any age. The factor of sex is thus not considered further.

This set of analyses was repeated, replacing the factor of sex with stimulus of exposure (vanillin or 4-ethylguaiacol) and using mean litter scores for the ANOVA. Since control animals were not exposed to either stimulus, they were excluded from the analysis. There was no differential effect of the stimulus of exposure in any test, or at any age, and as a consequence this factor is not considered further. The stimulus the animal was exposed to is termed the "familiar" stimulus and that the animal was not exposed to the "unfamiliar" stimulus.

A set of analyses were performed examining the responses of the control animals to the stimuli used in the study. A 2×2 adjusted chi-squared analysis was performed at each age for factors of test stimulus (vanillin:4-ethylguaiacol) and preference (odor:no odor). Since animals at the weaning stage could be classified as having "no preference," two analyses were run: (a) using only those animals who exhibited a preference and (b) assigning no preference sequentially to either the flavored or unflavored side, alternating the first placement between flavored and unflavored. These analyses determined whether the two stimuli were responded to differently by naïve animals. Importantly, there was no difference in the response of naïve animals to the vanillin or 4-ethylguaiacol at any age. Newborns appeared to exhibit an aversion to the novel odor, while at 9–10 weeks and 6 months of age, animals exhibited no preference (see Table 2).

One final analysis was undertaken. Some of the animals tested at weaning had been previously tested as newborns. To ensure that this experience did not affect subsequent preference, a 2×2 adjusted chi-squared analysis was performed examining, within each exposure condition, the preference of kittens at weaning (odor:no odor) and previous test experience (tested as newborns:not tested as newborns). No significant difference in the distribution of preferences was observed. A similar test was undertaken for animals tested as juveniles who had previously been tested as newborns. Again no differences in the response of animals as a result of newborn testing or not were observed. All animals tested as juveniles were tested at weaning.

Newborn

Analysis

Kittens were divided into two groups at this age, since they were tested before the start of any postnatal exposure. Kittens were divided into two groups depending on their previous exposure: prenatal—the kitten's mother had been exposed to the stimulus whilst pregnant; control - the kitten's mother received no exposure to the stimulus. The number of kittens (prenatal and control separately) who chose the "odorized" swab in each test was calculated. An adjusted chi-squared analysis (Donner and Donald 1988) was performed on each test condition comparing the number of kittens who preferred the odorized swab and the "unodorized" swab.

Although numbers are small, each litter was categorized as either showing an overall preference for the odorized swab or the "unodorized" swab. A Fisher Exact Probability test (Siegel 1956) was performed on litter preferences.

Results

The kittens exposed prenatally to a chemosensory stimulus preferred the odor of this stimulus after birth significantly more than the "unodorized" swab (see Table 3). Control newborns and all newborns when tested with the unfamiliar stimulus, exhibited an aversion to the unfamiliar odor (see Table 3).

Table 2 The number of kittens in the control group exhibiting a preference for odorized test stimulus and the control (no odor) stimulus when either vanillin or 4-ethylguaiacol was the test stimulus at each testing age

	Newborn		9–10 Weeks			6 Months	
	Odor	No odor	Odor	No preference	No odor	Odor	No odor
Vanillin	3	10	4	15	6	8	9
4-Ethyl.	3	10	3	16	6	9	8

Weaning

Analysis

To examine whether individual kittens exhibited a preference for the flavored treat, an adjusted chi-squared analysis (Donner and Donald 1988) was undertaken. For this, animals were classified as either showing a preference for the flavored treat (preferring that treat in four to six trials), no preference (preferring the flavored treat in three out of six trials), or a preference for the control (no stimulus) treat (preferring the flavored treat in zero to two trials). Two analyses were run. The first used only those animals showing a preference, whilst the second used all animals, assigning those showing no preference sequentially to either the flavored or unflavored side, always beginning with the unflavored side. An adjusted 4×2 chi-squared test was performed to explore the association between exposure (*prenatal*, *perinatal*, *postnatal*, and *control*) and the animals' preference (*flavored, unflavored*).

Table 3 The number of newborn cats who chose the "odorized" swab (Odor) and 'non-odorized' swab (No odor) when tested in Test 1—prenatal familiar (the odor was that experienced by newborns in prenatal condition) and Test 2—prenatal unfamiliar (the odor was not experienced previously) and the number of litters exhibiting an overall preference for the "odorized" swab and "non-odorized" swab in each trial

	Individuals			Litters		
	Odor	No odor	χ_A^2	Odor	No odor	Fisher Exact test
Test 1: Prenatal familiar						
Prenatal	11	3	8.808 $P < 0.001$	4	0	$P = 0.048$
Control	2	11		1[a]	4	
Test 2: Prenatal unfamiliar						
Prenatal	2	12	0.623 $P > 0.7$	1	3	$P = 1.00$
Control	4	9		2[b]	3	

[a] = 1 litter and [b] = 2 litters, respectively exhibited no preference and were categorized conservatively against the hypothesis for the Fisher test.

Each litter was classified as to whether it exhibited a preference for the familiar flavored treat (i.e., the majority of kittens showed a preference for this treat), no preference (i.e., the majority of kittens showed no preference), or showed a preference for the control treat (i.e., the majority of kittens showed a preference for the control treat). A 4×3 chi-squared test (Siegel 1956) was performed examining the association between exposure (*prenatal*, *perinatal*, *postnatal*, and *control*) and preference (flavored, no preference, unflavored). However, numbers are low and so caution must be made in interpreting the results from these chi-squared tests.

To examine the relative effects of pre- and postnatal exposure, the mean number of times that kittens ate the flavored treat first was calculated for each litter as described above. This was analyzed by a 2×2 ANOVA for factors of prenatal exposure (yes/no) and postnatal exposure (yes/no).

Results

Familiar flavored stimulus test

The chi-squared test revealed a significant difference in the distribution of preferences for both individual kittens (all animals: $\chi_A^2 = 30.991$, df = 4, $P < 0.005$:those showing a preference $\chi_A^2 = 15.803$, df = 4, $P < 0.005$) and litters ($\chi^2 = 19.111$, df = 6, $P < 0.005$). Nearly all kittens, and all litters, in the perinatal condition exhibited a preference for the familiar flavored treat (see Table 4). Prenatal exposure resulted in a strong preference at kitten and litter level, whereas both postnatal exposure and the control group showed no overall preference.

Analysis of the mean litter scores revealed a similar pattern of results. There was a significant effect of prenatal exposure ($F[1,24] = 31.798$, $P < 0.001$), postnatal exposure ($F[1,24] = 6.302$, $P = 0.019$), and a significant interaction between both factors ($F[1,24] = 4.625$, $P = 0.042$) on kittens' preference for the familiar treat. This interaction was explored further by a one-way ANOVA which revealed a highly significant effect of exposure condition on the kittens' preference ($F[3,24] = 4.308$, $P < 0.001$). Scheffé post hoc tests revealed that *perinatal* (prenatal *and* postnatal) exposure (mean preference ± SD = 4.64 ± 0.62) resulted in a significantly stronger preference for the flavored treat than *prenatal* ($P = 0.029$) (mean preference 3.70 ± 0.51), *postnatal* ($P < 0.001$) (mean preference 3.08 ± 0.59) and no exposure, *control* ($P < 0.001$) (mean preference 3.01 ± 0.85) (Figure 1).

Unfamiliar flavored test

There were no significant effects at individual (all animals—$\chi_A^2 = 2.750$, df = 4, $P > 0.500$:those showing a preference $\chi_A^2 = 2.214$, df = 4, $P > 0.500$) or litters ($\chi^2 = 3.244$, df = 6, $P = 0.355$) level (see Table 4). Similarly, no significant effects were found for litter preferences (*prenatal*: $F[1,24] = 1.750$, $P = 0.198$; *postnatal*: $F[1,24] = 1.411$, $P = 0.247$; interaction: $F[1,24] = 0.001$, $P = 0.971$).

Summary

The kittens' preferences for a flavored food treat at weaning were influenced by previous exposure. A combination of prenatal and postnatal exposure (perinatal condition) led to the strongest preference. Prenatal exposure alone resulted in a stronger preference than postnatal exposure. The preference was specific to the chemosensory stimulus previously experienced.

Table 4 The number of kittens at 9–10 weeks who exhibited an overall preference for the flavored stimulus (Flavor), the unflavored stimulus (Unflav) or no preference (No pref), on the familiar and unfamiliar stimulus tests, and similarly for the number of litters

	Individuals				Litters			
	Flavor	No pref.	Unflav	χ_A^2	Flavor	None	Unflav	χ^2
Familiar								
Prenatal	15	8	1	$P < 0.005$	5	1	0	$P < 0.005$
Perinatal	23	2	0		8	0	0	
Postnatal	7	12	5		2	4	1	
Control	5	15	5		0	6	1	
Unfamiliar								
Prenatal	0	13	11	$P > 0.500$	0	4	2	$P > 0.500$
Perinatal	2	11	12		0	4	4	
Postnatal	3	12	9		0	3	4	
Control	2	16	7		0	6	1	

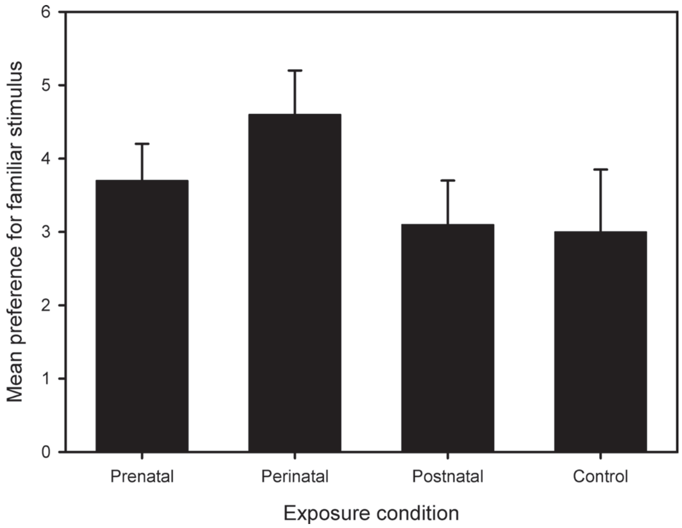

Figure 1 Mean (± SD) number of times kittens in each condition choose the familiarly flavored treat when tested at 9–10 weeks of age (3 = no preference).

Six-month tests analysis

Food eaten

The task employed to test the dietary preferences of 6-month-old cats involved ad-lib feeding over a 24-h period. Unfortunately, the cats ate all of the food, and thus data from this part of the study were unusable. Increasing the amount of food simply resulted in increased consumption and there was concern regarding weight gain and possible illness.

Side of first choice

The analysis was identical to that undertaken for the 9–10 week tests.

Results

Familiar flavored test

A chi-squared test revealed a significant difference in the distribution of preferences for both individual cats ($\chi_A^2 = 9.480$, df = 3, $P < 0.025$) and litters ($\chi^2 = 10.588$, df = 3, $P < 0.25$). In both cases, animals in the three experimental conditions exhibited significantly more of a preference for the familiar flavored food than the control cats (see Table 5).

The ANOVA examining the number of times individual cats ate from the familiar flavored food bowl first revealed a significant effect of *prenatal* exposure ($F[1,15] = 19.255$, $P < 0.001$) and *postnatal* exposure ($F[1,15] = 5.204$, $P = 0.038$), but a nonsignificant interaction ($F[1,15] = 3.553$, $P = 0.09$) (see Figure 2). A one-way ANOVA revealed a highly significant effect of exposure condition on the kittens' preference ($F[3,15] = 10.239$, $P = 0.001$). Scheffé post hoc tests revealed that *control* cats (mean preference 1.25 ± 0.40) exhibited a significantly smaller preference for the familiar flavored food than cats in the *perinatal* ($P = 0.001$, mean preference ± SD = 2.66 ± 0.41) and *prenatal* ($P = 0.006$, mean preference 2.58 ± 0.68) and just not significant difference from cats in the *postnatal* condition ($P = 0.077$, mean preference 2.13 ± 0.31).

Unfamiliar flavored test

There were no significant differences revealed by the chi-squared tests for individual cats ($\chi_A^2 = 4.333$, df = 3, $P > 0.25$) or litters ($\chi^2 = 0.293$, df = 3, $P > 0.995$).

When the analysis was performed on the tests with the unfamiliar flavored treat, there was a significant effect of *prenatal* exposure ($F[1,159] = 7.834$, $P = 0.013$) on cats' preferences. Cats exposed prenatally to a chemosensory stimulus exhibited significantly less of a preference for an unfamiliar flavored food when tested against unadulterated food, than animals not given any prenatal exposure (mean preference of: cats exposed prenatally to a chemosensory stimulus 1.167 (±0.40); cats having no prenatal exposure 1.7222 (±0.455)). There was no significant effect of *postnatal* exposure ($F[1,15] = 0.685$, $P = 0.421$), nor an interaction ($F[1,15] = 0.108$, $P = 0.747$).

Summary

Prenatal and/or postnatal exposure to a chemosensory stimulus altered the cats' preference for food flavored with that stimulus later in life, with individuals exhibiting a preference for food flavored with the previously encountered stimulus. Post hoc tests suggest that perinatal exposure exerted the greatest effect on cats' subsequent food preferences, and that prenatal exposure may have a greater effect than postnatal

Table 5 The number of cats at 6 months who exhibited an overall preference for the flavored stimulus (Flavor) or the unflavored stimulus (Unflav), on the familiar and unfamiliar stimulus tests, and similarly for the number of litters

	Individuals			Litters		
	Flavor	Unflav	χ_A^2	Flavor	Unflav	χ^2
Familiar						
Prenatal	13	2	$P < 0.025$	4	0	$P < 0.025$
Perinatal	13	0		6	0	
Postnatal	17	1		5	0	
Control	9	8		2	3	
Unfamiliar						
Prenatal	5	10	$P > 0.25$	0	4	$P > 0.995$
Perinatal	3	10		0	4	
Postnatal	11	7		0	3	
Control	8	9		0	6	

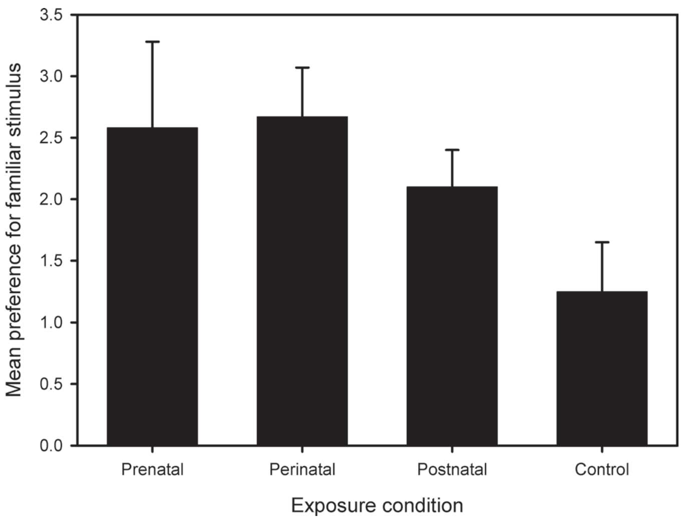

Figure 2 Mean (± SD) number of times cats in each condition choose the familiarly flavored food when tested at 6 months (1.5 = no preference).

exposure. Moreover, prenatal exposure may exert an effect on the cats' responses to familiar and unfamiliar odor/flavor stimuli.

Discussion

The findings from this study indicate that the olfactory/flavor preferences of cats are influenced by early exposure to a chemosensory stimulus in the immediate and long term.

One aim of this study was to examine the relative influence of prenatal and early postnatal (during nursing) exposure on the subsequent dietary preferences of cats. The results demonstrate that combined pre- and postnatal exposure (perinatal exposure) leads to the strongest influence on dietary preferences, supporting results observed in dogs (Hepper and Wells 2006). This is perhaps not surprising and could be based purely on the greater duration of exposure (although see later). Perhaps more surprising was the result that prenatal exposure alone exerted a stronger effect than postnatal exposure alone. This may reflect the increased salience of exposure to the stimulus in the prenatal period, or a different underlying mechanism mediating the exposure effects.

Prenatal exposure exerted an effect on the olfactory/dietary preferences of newborn, 9–10-week-old, and 6-month-old cats. The newborns preferred the chemosensory stimulus experienced prenatally, supporting results from other studies (e.g., Hepper 1995, 1996; Schaal et al. 1995, 2000; Coureaud et al. 2002; Wells and Hepper 2006; Becques et al. 2010). It should be noted that newborn cats reveal a strong aversion to unfamiliar odors, thus the period of prenatal exposure moves the newborn's response from aversion to a preference. In contrast to the previous study involving the cat, in which there was some experimental postnatal exposure via the mother's diet for newborns (Becques et al. 2010), the present investigation did not begin postnatal exposure until after the newborns had been tested. Thus, exposure to the stimulus as part of the experimental protocol in the postnatal period was absent. However, the possibility remains that there could have been some indirect exposure arising from amniotic fluid/breast milk containing the stimulus during prenatal exposure. The time course for the clearance of the stimulus experienced prenatally is unknown in the cat and therefore, the possibility remains that the kittens may have experienced the stimuli after birth, even though maternal exposure was confined to the prenatal period. Although the duration of experience of the stimulus for the kittens in the prenatal group may have persisted postnatally, this is as a result of only prenatal maternal ingestion. The results of tests at 9–10 weeks and 6 months were unlikely to be influenced by any residual chemosensory exposure as all exposure to the stimuli ceased at 28 days after birth. Becques et al. (2010) demonstrated a preference for the odor of cheese in cats following early exposure at 45 days, but their cats experienced both prenatal and postnatal exposure. This is therefore the first study to demonstrate a longer term effect of prenatal exposure in the cat. Interestingly, prenatal exposure still exerted an effect on dietary preferences at 6 months of age. Simitzis et al. (2008) reported a preference for oregano in lambs at 7.5 months after being exposed to this herb via their mother's diet in the womb. It would appear that prenatal exposure can therefore exert a long-term effect on choice of diet.

The effects of early postnatal chemosensory exposure were stronger at 6 months compared to 9–10 weeks. Exposure via breast or bottle milk has been shown to result in preferences after the exposure period, for example, in dogs (Hepper and Wells 2006), rabbits (Bilkó et al. 1994), and in the case of humans some 4–5 years after exposure (Mennella and Beauchamp 2002) and into later adulthood (Haller et al. 1999). The reason behind the strengthening of the effect of early postnatal exposure is unclear. In some studies when tested soon after exposure, individuals do not exhibit a preference for the stimulus due to sensory specific satiety (Rolls 2000; Mennella et al. 2006), but do when tested sometime after exposure (cf., Mennella and Beauchamp 1999; Mennella et al. 2001). However, the test in this study was undertaken 4–5 weeks after the last exposure to the stimulus and this may be unlikely. It may be the case that the familiar stimulus, having been associated with sucking only, assumed a positive valence in the context in which it was experienced, that is sucking. Some time may be required to pass before it acquires its positive valence in wider contexts, especially if associated with the negative process of weaning. All cats were tested after being fully weaned and whether the weaning process influenced the perception of stimuli associated with nursing is unknown.

Comparisons between different studies in this area are often difficult due to differences in methodology which may exert an effect on both the individuals' acquisition of the stimuli and exhibition of any preference (Bertin et al.

2010). Stimulus intensity during exposure or testing, duration of exposure, and indeed the stimuli themselves all differ between studies and may influence results. Previously, we found that prenatal exposure alone had no influence on the dietary choice of dogs at 9–10 weeks when tested in a similar task as used here for cats (Hepper and Wells 2006). Whether this reflects a difference in some aspect of methodology, or a fundamental difference in the biology or behavior between cats and dogs, is unknown and warrants further study.

Unlike the previous study in cats (Becques et al. 2010), the present investigation found that newborns avoided the unfamiliar odor. Indeed, at all ages, cats avoided the unfamiliar odor. This is similar to results observed in dogs (Hepper and Wells 2006; Wells and Hepper 2006), rabbits (Coureaud et al. 2002), and humans (Hepper 1995; Schaal et al. 2000), suggesting a generalizable neophobic response across species. Interestingly, when tested as adults, the neophobic response of animals exposed prenatally appeared to have increased, with these animals being more likely to avoid the unfamiliar odor than cats that experienced the stimulus postnatally. The reasons for this are unclear.

There are a number of methodological points to be considered with respect to this study. First, some individuals were tested as newborns and then again at 9–10 weeks and at 6 months of age. However, a comparison of the performance of individuals tested and not tested as newborns revealed no differences in performance; hence, it is unlikely that the testing procedure and re-exposure to the stimulus itself influenced the results.

Second, the study used two chemosensory stimuli, each with different olfactory properties. Vanillin is a unimodal odorant that primarily stimulates the olfactory neurons (Savic, et al. 2002), whereas 4-ethylguaiacol is a bimodal odorant that stimulates both olfactory and trigeminal pathways (Tucker 1971). There was no significant difference in the preferences of cats exposed to vanillin and those exposed to 4-ethylguaiacol, nor a difference in the response of naïve cats to the stimuli. In both exposure and test phases, it appears that the mechanism of action and mediation of responses is uninfluenced by mode of stimulation.

Third, the study confirms the continuity of stimulus perception across prenatal and postnatal exposure periods and subsequent testing. This indicates that the stimuli are passed from the mother's diet to both the fetus and the newborn. Fetal experience may arise from the presence of the stimulus or via transfer from the mother's blood system to that of the fetus (Hepper 1990). Postnatally, the most obvious means of stimulus experience is via the mother's milk postnatally, although it is also possible that the kittens could experience the stimulus after feeding on the mother's breath or body. In both cases, transfer of the stimulus from mother's diet to her offspring is achieved in a manner that preserves sufficient quality to make the stimuli recognizable when subsequently tested.

Fourth, with regard to testing, the newborn test was primarily an olfactory one and observations of the kittens at 9–10 weeks and at 6 months suggests the decision of which treat, and which bowl, respectively to eat from first was made on an olfactory basis. Kittens and cats sniffed the treats, or bowl, first before consuming it, or eating from it. This does not preclude some sensation being received via the gustatory route, but it would appear that the olfactory route was the primary sense involved in making the initial decision.

One key finding of interest is the dietary preference for the familiar stimulus observed at 6 months of age. Although animals had experienced the flavor of the control food daily for over 5 months, they exhibited a preference for a stimulus experienced *in utero*, or immediately after birth, over that presented by their normal food. Six-month-old cats prefer a chemosensory stimulus experienced for approximately 26 days before and/or after birth to one experienced for around 140 days preceding testing. It must be noted that this response was not based on the presence of a different odor as the familiar odor was preferred but the unfamiliar odor avoided. This suggests that chemosensory exposure during very early development may establish long-term food preferences for the individual (cf., Mennella and Ventura 2010). Further work is required to explore this.

In this study perinatal exposure, combining both prenatal and early postnatal exposure through nursing resulted in the strongest olfactory/dietary preferences. This was also found when examining the dietary preferences of dogs (Hepper and Wells 2006) and behavior of pigs (Oostindjer et al. 2009). This is perhaps to be expected since this reflects best the natural situation; that is, animals will be exposed to the odors and tastes of safe foods consumed by the mother both during gestation and nursing.

The mode(s) of action of prenatal and postnatal exposure may be different (Hepper and Wells 2006). Exposure to a chemosensory stimulus in the prenatal period alters the structure and functioning of the olfactory system (Coopersmith and Leon 1986; Rosselli-Austin and Williams 1990; Semke et al. 1995; Todrank et al. 2011). It has been suggested (e.g., Hudson 1999; Todrank et al. 2011) that the structure and responsiveness of the olfactory system becomes tailored to the individual's prenatal chemosensory environment, enabling stimuli of significance to have enhanced access to the olfactory system. Postnatal chemosensory exposure, as well as enhancing the olfactory/gustatory system responsiveness, may also alter preferences through association with nursing. The receipt of milk and the context of nursing are highly rewarding situations and this positive reinforcement may enhance the significance of the stimulus associated with sucking (Delaunay-El Allam et al. 2010).

Perinatal experience of chemosensory stimuli has advantages for young, naïve animals by enabling them to acquire information about their future diet safely from their mother (Hepper 1996). Although the exact mechanism is unknown, a combination of passive chemosensory exposure that shapes and tailors the responsiveness of the olfactory and gustatory system to "safe" stimuli with more active association through sucking reinforcing the stimuli may combine to form the underlying basis for the effects observed. Moreover, continued exposure would further strengthen any associations and hence maintain the preference. As such, combined exposure may exert a stronger effect than simply addition of prenatal and postnatal exposure (Hepper and Wells 2006).

It is important to consider how such a system, evolved in the wild animal as opposed to the domesticated pet animals as used in this, and other studies, could operate. In carnivores, the mother plays a crucial role in the development of feeding behavior by bringing back prey for the young to eat and practice their hunting skills (Leyhausen 1979). Whereas this develops their ability to capture and kill prey, they still have to consume this. Given the mother's prey will flavor her diet, it is likely that naturally the unborn and newborn kittens will experience the flavor of this prey through the amniotic fluid and milk, respectively as the mother eats this. It may be that perinatal learning primes animals for the safe choice on their initial independent consumption of food. Tests of animals in their more natural environment would be required to examine this. Alternatively, other functions, such as social attachments and recognition, may be promoted by such learning (Hepper 1996).

In summary, long-term chemosensory and dietary preferences of cats are influenced by prenatal and early (nursing) postnatal experience, supporting a natural and biologically relevant mechanism for the safe transmission of diet from mother to her young. Perinatal exposure leads to the strongest preference. We suggest perinatal experience may structure and shape the chemosensory system to respond to certain stimuli, which become further reinforced through nursing and sucking to equip newly weaned kittens with the "knowledge" to consume safe food, thereby ensuring survival. The impact of this early experience continues into early adulthood, overcoming far longer experiences with food outside of this period. This suggests that flavor exposure in early development may permanently influence long-term dietary preferences, promoting "safe" feeding habits. The study indicates that early experiences, prenatal and during nursing, are of vital importance in shaping our long-term behavior and preferences.

Disclosures

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