Perinatal Olfactory Learning in the Domestic Dog

Peter G. Hepper and Deborah L. Wells

Canine Behaviour Centre, School of Psychology, Queens University Belfast, Belfast BT7 1NN, United Kingdom

Correspondence to be sent to: Peter G. Hepper, Canine Behaviour Centre, School of Psychology, Queens University Belfast, Belfast BT7 1NN, United Kingdom. e-mail: p.hepper@qub.ac.uk

Abstract

The ability of individuals to learn about chemosensory stimuli in the prenatal, or immediate postnatal, period may be advantageous in acquiring information about “safe” foods after weaning. In this study, we examined the influence of perinatal exposure to aniseed via the mother’s diet on a two-choice food test in the domestic dog. Pups were tested at 10 weeks of age following “prenatal” exposure to aniseed (the last 20 days of gestation), “postnatal” exposure to aniseed (the first 20 days after birth), “perinatal” exposure to aniseed (pre- and postnatal exposure combined), or no exposure to aniseed prenatally or postnatally (control). Perinatal exposure resulted in a significantly greater preference for the aniseed food than the other types of exposure. At 10 weeks, there was no evidence for the retention of any prenatal learning of the aniseed. It is suggested that exposure to a chemosensory stimulus across the perinatal period results in a greater effect than simply the sum of pre- and postnatal exposure due to priming of the chemosensory system via prenatal chemosensory experience. Such a system may confer survival advantages by promoting the acquisition of information about safe foods.

Key words: dietary preferences, dog, learning, odor, prenatal, suckling

Introduction

The role of prenatal influences on development has seen renewed interest in recent years due to the fact that they may exert a long-term influence on the individual. For example, the importance of early experience for the development and structure of the nervous system, particularly the sensory system, is well established (Berardi et al., 2000; Grubb and Thompson, 2004). Adult patterns of disease have been attributed to the individual’s environment during prenatal life (fetal origins of adult disease—Gluckman and Hanson, 2005). In recent years, the ability of individuals to learn before birth has come under scrutiny for its potential to influence behavior after birth (e.g., Hepper, 1996).

Prenatal learning of olfactory (chemosensory) stimuli has been demonstrated in all the major vertebrate groups (Brannon, 1972; Hepper, 1988a; Hepper and Waldman, 1992; Sneddon et al., 1998, 2001; Schaal et al., 2000) and invertebrates (Isingrini et al., 1985; Caubet et al., 1992). It has been suggested that this widespread occurrence of prenatal learning means that this behavior has an important functional significance for the individual and may have a key role in development (Teicher and Blass, 1977; Hepper, 1996; Hudson and Distel, 1999; Coureaud et al., 2002).

Prenatal learning of chemosensory information may play an important role for mammals in determining food preferences after birth. Flavors of the mother’s diet pass into the amniotic fluid (Mennella et al., 1995) and thus may be experienced by the fetus. This provides the individual with a mechanism that is natural (it relies on the mother’s normal behavior, eating) and safe (it relies on the mother’s knowledge of toxic and nontoxic foodstuffs) to begin its life-long acquisition of dietary preferences. A number of studies have found that following exposure to stimuli in the amniotic fluid, via the mother’s diet, individuals show a preference for these stimuli after birth (Hepper, 1988a; Bilkó et al., 1994; Mennella et al., 2001; Coureaud et al., 2002).

The mother’s milk is also “flavored” by her diet, providing another means of safe learning about potential food in the immediate postnatal period. Chemosensory stimuli experienced via the mother’s milk may result in an increased preference for food flavored with that stimulus (Bilkó et al., 1994; Mennella et al., 2001). Thus, opportunities exist in both the prenatal and immediate postnatal period to learn about food flavors.

If the learning of “food” stimuli in the prenatal or immediate postnatal period is to influence subsequent preferences after weaning, then this information must be retained for that time. Few studies have examined this. With respect to prenatal learning of auditory stimuli in the human fetus,
while prenatal exposure to a particular sound results in a preference in the newborn period (2–4 days after birth), this preference disappears by 21 days (Hepper, 1991). Bilkó et al., (1994) reported an increased preference for juniper following exposure via the mother’s milk in rabbits at 29 days of age, but this test was carried out only 24 h after being removed from the mother and her milk.

One study of the human fetus and infant demonstrated that either prenatal (last 3 weeks of gestation) or postnatal (first 2 months after birth) exposure to carrot juice via the maternal diet resulted in a changed response to the flavor of carrots at around 6 months of age compared to individuals not exposed to the flavor of carrots (Mennella et al., 2001). This study reveals that pre- or postnatal exposure may influence food preferences after weaning. This study, however, only compared prenatal exposure alone against postnatal exposure alone. In the natural setting, both the fetus and newborn will experience the food flavors continuously across the perinatal period, via the amniotic fluid before birth and in the breast milk after birth.

In this study, we examine whether chemosensory information acquired during the prenatal and/or immediate postnatal period influences feeding behavior after weaning. The study used the domestic dog. The domestic dog has been shown to learn about odors prenatally, via the maternal diet and amniotic fluid, and exhibit a preference for odors experienced prenatally up to 24 h after birth (Wells and Hepper, 2006). The duration of this memory is, however, unknown. Thus, the immediate prenatal period presents the opportunity for the fetus to learn about flavors of the maternal diet. Dogs begin to be weaned around 3 weeks when the mother begins to feed the puppies (Fox and Bekoff, 1969; Christiansen, 1984). By 4–5 weeks, mothers may begin to withdraw and prevent the pup from sucking, and milk production diminishes from this point. Pups may be on solid food from 7 weeks. There is therefore a period of approximately 3 weeks after birth when the pup is exclusively fed by sucking. This similarly presents the opportunity to learn about the maternal diet via the mother’s milk.

In this study, we examine whether prenatal (via amniotic fluid) and/or postnatal (via mother’s milk) exposure to a chemosensory stimulus influences another behavior (feeding on solid food) not exhibited by the dog during the period of exposure. Pups were examined in a two-choice feeding task at 10 weeks of age for their preference for food flavored with an odor experienced only in the perinatal period (prenatal alone, postnatal alone, or pre- and postnatal) compared to unflavored food.

**Methods**

**Subjects**

Sixteen pregnant female dogs were recruited for the study. All dogs (3 Labrador Retrievers, 3 Golden Retrievers, and 10 crossbreeds) were family owned. Four pups from each of these females served as subjects (total subject pups = 64). The dogs and their offspring were all thoroughly familiarized with the experimenter before testing took place.

**Chemosensory exposure**

Aniseed was chosen as the novel odor for this experiment. This particular spice is safe for consumption by dogs and is not known to cause any harm to pregnant bitches or developing conceptuses. Furthermore, a previous study has demonstrated that this substance passes from the maternal diet into the amniotic fluid and can be experienced by the pups (Wells and Hepper, 2006). It was presumed that a similar transport mechanism would also flavor the mother’s milk.

Dogs were randomly divided into four groups.

**Prenatal**

Four pregnant bitches (1 Labrador and 3 crossbreeds) were fed with aniseed-flavored food. To expose the fetuses to aniseed, 5 ml of liquid aniseed (Condessa, Wales, United Kingdom) was mixed with the mothers’ normal food. Toward the end of their pregnancy, the pregnant bitches’ feeding regime was changed from a single meal to two to three smaller meals (the same overall amount of food). The aniseed was mixed with the food prior to its division into smaller meals. Thus, dogs received aniseed-flavored food at each meal, and its dilution was the same whether the food was presented as one meal, early pregnancy, or two to three meals, late pregnancy. Dogs exhibited no change in their normal dietary habits with the introduction of the flavored food. Counting back from the day of whelping (day 0), mothers were fed aniseed-flavored food for an average of 20.5 days (range 19–22 days). All aniseed-flavored food was replaced with unadulterated food when the bitch began to exhibit signs of whelping.

**Postnatal**

Newborn pups were fed solely by sucking for the first 3–4 weeks after birth. Here, mothers were similarly fed their normal food flavored, as above, with 5 ml of liquid aniseed (Condessa, Wales, United Kingdom). Dogs exhibited no change in their normal dietary habits with the introduction of the flavored food. The aniseed was expected to flavor the mother’s milk, and thus newborn pups would be exposed to the aniseed during sucking. Mothers were fed the aniseed diet from the day following the birth of their litter for 20 days. Mothers were fed in a separate room from the pups. Four pregnant bitches formed the test group (1 Labrador, 1 Golden Retriever, and 2 crossbreeds).

**Perinatal**

This group of four dogs (1 Labrador, 1 Golden Retriever, and 2 crossbreeds) were fed the combined regimen of the “prenatal” and “postnatal” groups. That is, pregnant bitches...
were fed aniseed-flavored food before birth. Counting back from the day of whelping (day 0), mothers were fed aniseed flavored food for an average of 20.25 days (range 19–22 days). Aniseed-flavored food was replaced with unadulterated food when the bitch began to exhibit signs of whelping and refed to the mother the day after the birth of her litter for 20 days. Again mothers showed no change in their normal dietary habits upon introduction of the flavored food.

Control

The group of four dogs (1 Golden Retriever and 3 cross-breeds) were fed their normal food unadulterated with any aniseed.

All dogs remained in their owners’ home for the duration of the project, and weaning of the pups to solid food began around 4–5 weeks of age. At the time of testing, 10 weeks, pups were eating solid food. All pups had remained with their littermates and mother until testing. No pup had been exposed experimentally to aniseed outside of the exposure reported above.

Procedure

Pups were tested singly in a familiar room in their own home approximately 3 h after their last feed. Pups were brought to the task by their owners and the responses of the pup recorded by the experimenters. Four pups from each litter were tested.

Dogs were given a choice of two dog “treats,” one flavored with one drop of liquid aniseed and the other with distilled water. The treats were 2.5 g of cooked, minced chicken. To the experimenters the flavored treat smelled of aniseed, but this smell was not overpowering. The flavored and unflavored treats were clearly distinguishable by odor. The two treats were placed on the floor in the middle of the room by the experimenter and this was observed by the dog being held by its owner approximately 6 ft away. The treats were placed approximately 10 cm apart. The owner was unaware of which treat was flavored with aniseed and which was not. The dog was then let go by the owner and the treat eaten first by the dog (aniseed flavored or “control”) recorded. Dogs were given five trials a day over a consecutive 2-day period. For half the trials the aniseed-flavored treat was on the dog’s left and for half on the dog’s right. The number of times the dog ate the aniseed treat first on each of the 10 trials was recorded. Approximately 20 min was left between consecutive trials on each day.

Analysis

The number of times each pup ate the aniseed-flavored treat first was analyzed by a $2 \times 2$ analysis of variance (ANOVA) for factors of prenatal exposure (yes or no) and postnatal exposure (yes or no). A second analysis was undertaken in order to take account of possible litter effects. For this, the number of times pups in each litter ate the aniseed treat first was averaged to produce a mean score for each litter. This was also analyzed by a $2 \times 2$ ANOVA for factors of prenatal exposure (yes or no) and postnatal exposure (yes or no).

To determine whether the individual dogs exhibited a simple overall preference for the aniseed-flavored treat, they were classified according to their behavior on each of the 10 trials. If they ate the aniseed-flavored treat first on six or more trials, they were regarded as showing a preference for the aniseed treat. If they ate the aniseed-flavored treat first in four or less trials, they were regarded as showing a preference for the control, unflavored treat. Pups that ate the aniseed-flavored treat first on five trials were recorded as showing no preference. The results were analyzed by a chi-squared test for group (control, prenatal, postnatal, and perinatal) and preference (aniseed or unflavored). Two analyses were run. The first included only those dogs that exhibited a preference, that is, dogs that exhibited no preference were excluded, and, second, dogs exhibiting no preference were included in the analysis, conservatively classified as preferring the unflavored treat.

Litters were also classified as whether the dogs in each litter showed an overall preference for the unflavored treat (one dog or less showing an overall preference for the aniseed treat), no preference (two dogs showing an overall preference for the aniseed treat), or an overall preference for the aniseed treat (three dogs or more showing an overall preference for the aniseed treat). As overall numbers were small, no analysis was able to be performed on this data, but they are presented for information.

Results

There was no significant difference in litter size between the four groups: prenatal, 6.25 (range 4–8); postnatal, 6.00 (range 4–8); perinatal, 6.25 (range 4–8); and control, 5.75 (range 4–8). The results did not differ between breeds or sex of pups, so the data from all dogs were grouped for statistical analysis.

The ANOVA examining the number of times individual pups ate the aniseed-flavored treat first revealed a significant effect of postnatal exposure ($F = 13.012$, df = 1,60, $P < 0.001$) but not prenatal exposure ($F = 3.253$, df = 1,60, $P = 0.076$). This was further explained by a significant interaction between prenatal and postnatal exposure ($F = 5.783$, df = 1,60, $P = 0.019$). Figure 1 indicates that pups in the perinatal group, exposed to aniseed prenatally and postnatally, showed the greatest preference for the aniseed treat, and there was little difference between the preference exhibited by pups in the control, prenatal, and postnatal groups. A one-way ANOVA, computed to analyze the difference between groups, revealed that there was a significant difference between groups ($F = 7.349$, df = 3,60, $P < 0.001$), and post hoc Sheffé test revealed that the preference for the aniseed treat was significantly greater for the perinatal group than
all the other groups \((P < 0.05)\) but there were no other differences between the groups.

Analysis of the mean scores for each litter revealed an identical pattern of results. There was a significant effect of postnatal exposure \((F = 14.764, df = 1.12, P = 0.002)\) but no effect of prenatal exposure \((F = 2.858, df = 1.12, P = 0.117)\). This was further explained by a significant interaction between prenatal and postnatal exposure \((F = 5.315, df = 1.12, P = 0.040)\). Again, the combination of prenatal and postnatal exposure to aniseed resulted in the greatest preference with no effect of prenatal exposure alone on preference \([\text{mean} \pm \text{SD}]\) for control, prenatal, postnatal, and perinatal—5.25 (0.45), 5.00 (0.61), 5.87 (1.1), and 7.5 (0.79), respectively. A one-way ANOVA revealed a significant difference between the groups \((F = 7.646, df = 3.12, P = 0.004)\), and post hoc Sheffe test revealed that the preference for the aniseed treat was significantly greater for the perinatal group than all the other groups \((P < 0.05)\) but there were no other differences between the groups.

The chi-squared test on the number of pups showing a preference for the aniseed treat was significant whether the pups exhibiting no preference were excluded \((\chi^2 = 10.338, df = 3, P = 0.016)\) or included \((\chi^2 = 14.400, df = 3, P = 0.002)\). All the dogs in the perinatal group and all the litters in this group showed an overall preference for the aniseed-flavored treat (Table 1).

**Discussion**

The findings from this study indicate that a combination of prenatal and postnatal exposure to aniseed results in a preference for a chemosensory stimulus experienced only in the perinatal period later in development. Moreover, this preference was observed in a novel behavior that was not exhibited by the dog during exposure. There was little evidence that dogs only exposed to the aniseed prenatally alone or postnatally alone exhibited a preference for the aniseed at the time of testing. The results indicate that perinatal exposure to a chemosensory stimulus results in a “long-term” change in preferences that can influence subsequent behavior.

A previous study (Wells and Hepper, 2006) reported that dogs exposed prenatally to aniseed exhibited a preference for this stimulus after birth. In that study, a maximum of 24 h had passed between their prenatal experience and subsequent testing. The exposure regimen adopted in this study was identical to that adopted previously, so there is no reason to suppose that fetuses were not exposed to the aniseed via the prenatal exposure. This is confirmed by the fact that there was a difference in the response between the postnatal and perinatal groups. For the pups in the prenatal group, this study took place 10 weeks after the last exposure. Thus, during this time any memory trace had waned and did not exert an influence on the dogs’ behavior. The preference of control dogs was similar to those in the prenatal group, again suggesting that any effects of prenatal exposure had disappeared. Thus, this study indicates that in the absence of any postnatal exposure, this memory is lost by 10 weeks. Previous studies have reported longer term retention from prenatal exposure alone, for example, Bilkó et al. (1994)—29 days after birth in the rabbit, Smotherman (1982)—60 days after birth in the rat, and Mennella et al. (2001)—6 months after birth in the human. This may reflect some yet unknown difference between the different species studied. A number of procedural differences may also account for these results, for example, duration and intensity of prenatal exposure. Alternatively, the results may be explained by reference to the time of testing and developmental stage of test subject. Dogs are usually completely weaned by around 7 weeks, and the test undertaken here was some 3 weeks after this. Bilkó et al. (1994) tested their rabbit pups 24 h after weaning from the mother. In the study of Mennella et al. (2001), 39 of 46 mothers were still nursing at the time of testing. It may
well be that preferences acquired prenatally persist while sucking and immediately thereafter but disappear after the end of sucking. Possibly, a continuum of sucking amniotic fluid and then breast milk maintains this preference. How long the memory of prenatal exposure lasts is unknown and is deserving of further exploration. However, in this study 10 weeks after the final prenatal exposure and 3 weeks after weaning, dog pups showed no indication of a chemosensory preference arising from prenatal exposure alone.

How pups “solved” the task is unknown. Observation of the pups in action did little to belie the belief that they are “eating machines.” There was little appearance of careful investigation of the food treats prior to consumption. In most cases, the food was immediately consumed by the pups. The acuity of the dogs’ nose is well renowned (Hepper, 1988b; Hepper and Wells, 2005), and upon approach to the treats their different smells may have been readily apparent to the pup. Pups did not pick up one treat and then drop it and go for the other, which suggests that the initial decision to eat a particular treat was based on odor cues rather than taste. Their exposure in both prenatal and postnatal exposures was by ingestion of the amniotic fluid and colostrum/milk, respectively, providing both olfactory and gustatory stimulation. Importantly, the results indicate a qualitative similarity between the aniseed as exposed via the mother’s milk and that present in the food.

The most interesting aspect of the study was the strong preference established by prenatal and postnatal exposure combined. Rabbit pups exhibited no difference in their preference for juniper whether it was exposed either prenatally or postnatally alone or in combination (Bilkó et al., 1994). Rats, however, only attached to a citral-scented nipple if they had experienced this odor both prenatally and postnatally (Pedersen and Blass, 1982). The greater effect observed here due to perinatal exposure may reflect simply the increased amount of exposure to the aniseed, approximately double that of the prenatal and postnatal groups (40 days as compared to approximately 20 days, respectively). However, the strength of the preference suggests that the combination is greater than the sum of its parts.

Acquiring information about chemosensory stimuli early in the perinatal period may play a role in the development of food preferences. In the dog, independent feeding follows after a period of time when the pup is exclusively fed by its mother’s milk. It has been proposed that flavors acquired during sucking may influence subsequent dietary habits (Mennella and Beauchamp, 1991; Hudson and Distel, 1999; Schaal et al., 2004). Indeed, such an approach imparts safety and security to the newborn’s learning. Its mother eating appropriate and safe foods can pass on this information through transmission of flavors, tastes, and smells from her milk thus ensuring her offspring are exposed to appropriate food flavors. The same processes that flavor her breast milk also flavor her amniotic fluid and thus enable the individual in the womb to experience these flavors (e.g., Schaal et al., 2004). Such learning has been argued to be important in the establishment of sucking (Teicher and Blass, 1977; Hepper, 1996). With both prenatal and postnatal exposure, a longer period of experience of safe stimuli may be had. The question remains of whether pre- and postnatal exposure combine in more than a simple additive way.

One possibility is that prenatal exposure primes the chemosensory system toward certain stimuli. Studies on the visual system clearly demonstrate the role of experience in shaping sensory receptors and receptivity (Hubel and Weisel, 1963; Grubb and Thompson, 2004). Similarly, studies of the olfactory system have revealed that early exposure to odors influences subsequent physiological responses of the olfactory system as measured by 2-deoxy-D-glucose uptake (Coopersmith and Leon, 1986) and electro-olfactogram (Semke et al., 1995). Furthermore, early exposure to odors increases cell numbers in both the accessory and main olfactory bulb (Rosselli-Austin and Williams, 1990). It may be that prenatal experience of chemosensory stimuli influences elements of the chemosensory system, changing their receptivity, increasing sensitivity, or altering receptor populations, thus increasing the saliency of the stimulus when experienced after birth.

Given the potential transnatal continuity of chemosensory stimuli across the perinatal period (Coureaud et al., 2002; Schaal et al., 2004), the developing individual would expect to experience similar chemosensory stimuli before and after birth. Prenatal experience of such stimuli may influence the olfactory system to ensure greater salience of these stimuli after birth. This increased saliency is advantageous in the acquisition of chemosensory stimuli relating to subsequent diet. Further work is needed to examine this possibility.

Such a priming effect would not be expected to be a permanent or imprinting effect as if the mother’s diet changed then the individual’s olfactory system may be locked onto stimuli no longer present. Thus, in the absence of any postnatal exposure, the changes in the olfactory system induced by prenatal exposure are lost or overwritten by the postnatal exposure. This would account for the different responses exhibited by the prenatal and perinatal groups.

Overall, this study demonstrates that perinatal experience of a chemosensory stimulus is retained by the dog for at least 5 weeks following the last exposure to the stimulus and can influence novel behavior, that is, behavior that was not exhibited during the exposure period. Perinatal exposure encompassing the prenatal and early postnatal period appears to result in a stronger preference than the simple addition of pre- and postnatal exposure, and it is suggested that prenatal exposure may prime the chemosensory system to the prenatally experienced stimulus changing receptivity and increasing the salience of this important stimulus after birth. Such a mechanism would have an evolutionary advantage, promoting survival by increasing the probability of learning about safe foods and potentially changing chemosensory system sensitivity to be more responsive to these same items.
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References


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