Juan P. Perea-Rodriguez
Wendy Saltzman

*Department of Biology University of
California, Riverside, 3386 Spieth Hall,
Riverside, CA 92521*
E-mail: jpere035@ucr.edu

Differences in Placentophagia in Relation to Reproductive Status in the California Mouse (*Peromyscus californicus*)

ABSTRACT: *Parturient females ingest placenta in most mammalian species, whereas fathers may do so in species in which both parents provide care for their offspring. To determine if the propensity to eat placenta varies with reproductive status in the biparental California mouse, we presented placenta to virgin (housed with a same-sex pairmate), expectant (pregnant with their first litter), and multiparous adult males and females. Liver was presented identically, 3–7 days later, as a control. Multiparous females were more likely to eat placenta than expectant and virgin females (p-values <0.016), whereas both multiparous and expectant males had higher incidences of placentophagia than virgins (p-values <0.016). Liver consumption did not differ among groups within either sex. These results suggest that propensity to eat placenta increases with maternal/birthing experience in females, and with paternal experience and/or cohabitation with a pregnant female in males.* © 2013 Wiley Periodicals, Inc. Dev Psychobiol 56: 812–820, 2014.

Keywords: *California mice; biparental care; placentophagia; reproductive condition; paternal care*

INTRODUCTION

Placentophagia, or the process of ingesting placenta (and amniotic fluid) during and after parturition, is common among mammals, with only a few exceptions (humans: Young & Benyshek, 2010; semi-aquatic and aquatic mammals, camelids: Young, Benyshek, & Lienard, 2012). This behavior has been proposed to enhance maternal responsiveness, potentially by priming the mother's brain through the diverse hormonal content found in placenta (Kristal, DiPirro, & Thompson, 2012; Melo & González-Mariscal, 2003). Studies on rabbits (*Oryctolagus cuniculus* L.; González-Mariscal, Melo, Chirino, Jiménez, Beyer, & Rosenblatt, 1998), rats

(*Rattus norvegicus*; Kristal, Whitney, & Peters, 1981), sheep (*Ovis aries*; Lévy & Poindron, 1987; Lévy, Poindron, & Le Neindre, 1983), and dogs (*Canis lupus familiaris*; Abitbol & Inglis, 1997) have shown that the presence of amniotic fluid on newborns enhances mother-offspring bonding and advances the onset of maternal behaviors. In a similar manner, virgin female rats, which normally do not express maternal behavior spontaneously, show increased attraction to pups and decreased latency for maternal sensitization when presented with unrelated, placenta-smeared pups as compared to unrelated pups that were not treated with placenta (Kristal, Whitney, et al., 1981).

Females can also show physiological changes after eating placenta that can ultimately affect their behavioral responses towards their offspring. Primiparous female rats that were allowed to eat placenta while giving birth show increased plasma prolactin concentrations 1 day postpartum, as well as decreased plasma progesterone concentrations 6–8 days postpartum, as compared to primiparous mothers that were not allowed to eat placenta (Blank & Friesen, 1980). These

hormonal changes can potentially promote maternal care (e.g., lactation, licking offspring; Numan & Insel, 2003), and have been proposed to facilitate the mother's return to regular estrous cycling (Blank & Friesen, 1980). Furthermore, placenta contains high levels of endogenous opioids and opioid-enhancing factors, which increase the pain threshold of mothers during parturition by enhancing opioid-mediated analgesia (Kristal, Thompson, & Grishkat, 1985), and, as a result, might reduce the time spent in labor. These studies indicate that placenta contains active substances that can alter the physiology and behavior of individuals that ingest it.

Interestingly, females respond differently when presented with placenta depending on their reproductive state. Female rats are mostly averse to placenta when sexually inexperienced, but become attracted to it during and after pregnancy (Kristal, Whitney, et al., 1981). The highest incidence of placentophagia is seen towards the end of gestation (Kristal, Peters, et al., 1981), and in a similar fashion, female rats become placentophagous with induced pseudopregnancy (Steuer, Thompson, Doerr, Youakim, & Kristal, 1987). Additionally, lesioning of the medial preoptic area, a brain region implicated in the onset and expression of parental behaviors (paternal behavior: de Jong, Chauke, Harris, & Saltzman, 2009; Lee & Brown, 2007; maternal behavior: Olazábal, Kalinichev, Morrell, & Rosenblatt, 2002; Rosenblatt & Ceus, 1998) as well as other behaviors (partner preference: Kindon, Baum, & Paredes, 1996; Paredes, Tzschentke, & Nakach, 1998; sexual behavior: Harding & McGinnis, 2004; Markowski, Eaton, Lumley, Moses, & Hull, 1994; Powers, Newman, & Bergondy, 1987), inhibits placentophagia in parturient rats (Noonan & Kristal, 1979). These results suggest that the physiological changes that females undergo during pregnancy promote the ingestion of placenta, amniotic fluid and attached membranes. Furthermore, these findings point to similarities in the neural processes and substrates that mediate the onset of parental behavior and placentophagia. Interestingly, placentophagia can also be facilitated by social cues (i.e., the presence of a parturient or placentophagous female rat increases attraction to placenta in a female observer; Kristal & Nishita, 1981).

In a handful of biparental species (i.e., species in which both parents provide care for their offspring), males, in addition to females, readily ingest placenta during the female's parturition (dwarf hamster, *Phodopus campbelli*: Jones & Wynne-Edwards, 2000; California mouse, *Peromyscus californicus*: Lee & Brown, 2002; prairie vole, *Microtus ochrogaster*: K. L. Bales, pers. comm.; common marmoset, *Callithrix jacchus*, and cotton-top tamarin, *Saguinus oedipus*: T.

E. Ziegler, pers. comm.; silvery marmoset, *C. argentata*: J. A. French, pers. comm.). In the two species in which placentophagia by males has been best characterized, the dwarf hamster and the California mouse, fathers lick their parturient mate's anogenital region, clean and pull neonates as they are expelled, and ingest amniotic fluid and placenta in the process. In dwarf hamsters, the frequency of placentophagia does not change with age in sexually inexperienced males, but increases in expectant fathers on the day before their mate gives birth (Gregg & Wynne-Edwards, 2005). These changes in propensity to eat placenta by males mirror those seen in females; however, the mechanisms that enable males to become placentophagous are unknown. Increased incidence of placentophagia in reproductive males might be due to cohabitation with a female, mating, and/or exposure to changing chemical cues produced by females throughout their pregnancy (Jemiolo, Gubernick, Yoder, & Novotny, 1994).

In this study, we investigated the factors influencing the propensity for placentophagia in male and female California mice. This species is socially and genetically monogamous, and both males and females invest heavily in their offspring (Gubernick & Alberts, 1989; Ribble, 1991). Data on the frequency of placentophagia throughout an individual's life history are lacking. Such data may be important for elucidating the factors influencing the expression of placentophagia as well as the potential role of placentophagia in the onset of parental behavior in this species (Jones & Wynne-Edwards, 2000). In the present study, therefore, we aimed to characterize the frequency of placentophagia in male and female California mice in different reproductive conditions. Specifically, we aimed to determine how social housing condition (same-sex groups vs. heterosexual pairs), parental experience, and pregnancy may affect an individual's propensity to eat placenta.

Each mouse was presented with freshly extracted, full-term placenta from an unrelated female on a single occasion. To determine if mice show changes in their attraction to placenta specifically or to highly vascularized tissues in general, we also presented animals with liver in a similar manner (Gregg & Wynne-Edwards, 2005, 2006; Melo & González-Mariscal, 2003). Consistent with the hypothesis that placentophagia facilitates the onset of parental behavior in both sexes (Gregg & Wynne-Edwards, 2005, 2006; Jones & Wynne-Edwards, 2000), we predicted that both males and females would show increased likelihood of ingesting placenta with pregnancy (of their mates in the case of males), and would show further increases in placentophagia with birthing and/or parental experience. Additionally, we predicted that the prevalence of liver

ingestion would not differ between the sexes or among reproductive conditions; we argue that although California mice mostly eat seeds (Merritt, 1974; Meserve, 1976), they are potentially likely to eat meat if the opportunity arises (pers. obs.).

METHODS

Animals

California mice are medium-sized rodents (40–70 g) found throughout most of coastal California, from San Francisco to the Baja Peninsula (Gubernick & Alberts, 1987). As mentioned above, they are genetically monogamous (Ribble, 1991) and breed throughout the year in the lab and in the wild, with gestation periods ranging from 29 to 34 days (average gestation length is 31.6 days (Gubernick, 1988)). California mice produce small litters containing 1–4 pups (average litter size: 2; Gubernick & Alberts, 1987). Their life expectancy in the wild is 9–18 months (Gubernick & Alberts, 1987), and they can live up to 2–4 years in captivity (C. A. Marler, pers. comm.; unpub. data). In our lab, their maximum recorded reproductive lifespan is 16–18 months (unpub. data).

We used mice that were born in our colony at the University of California, Riverside (UCR) and descended from animals purchased from the *Peromyscus* Genetic Stock Center (University of South Carolina, Columbia, SC, USA). To minimize inbreeding, we avoid pairing males and females that are more closely related than second cousins. Mice were weaned at 27–32 days of age, prior to the birth of siblings. At weaning, animals were ear-punched for identification and housed in same-sex groups consisting of four age-matched individuals (littermates and/or unrelated). Some animals remained in these groups throughout the experiment (see below); others were placed in male-female pairs when they were at least 90 days old.

Mice were maintained as described previously (Chauke, Malisch, Robinson, de Jong, & Saltzman, 2011; Harris, Perea-Rodriguez, & Saltzman, 2011). Briefly, mice were housed in 44 cm × 24 cm × 20 cm polycarbonate shoebox-type cages with aspen shavings and cotton wool (~5 g), and were provided with Purina Rodent Chow 5001 (LabDiet, Richmond, IN) and water ad libitum. Animals were kept on a 14:10 light/dark cycle with lights on at 0500 h and lights off at 1900 h. Room temperature and humidity were maintained at approximately 18–26°C and 60–70%, respectively. All of the procedures used were in accordance with the *Guide for the Care and Use of Laboratory Animals* and were reviewed and approved by the UCR IACUC. UCR is fully accredited by AAALAC.

Reproductive Conditions

Animals from each sex were grouped into the following three reproductive conditions:

Virgins. Virgin males (V-Males, $n = 11$) and females (V-Females, $n = 10$) had no prior sexual experience and had never been housed with a pup (except their own littermates) prior to testing. These animals were housed in groups of four age-matched, same-sex mice per cage. Virgins were 110–412 days old (V-Males: 259.4 ± 124.7, V-Females: 147.2 ± 32.1, mean ± SE) at the time of placenta testing. Males in this group included two littermates, whereas females included two mice from each of two litters from different lineages.

Expectant Parents. Expectant males (E-Males, $n = 10$) and females (E-Females, $n = 11$) had been paired for at least 21 days at the time of the placenta test, and the female was pregnant with the pair's first litter (i.e., primiparous). Thus, these individuals were sexually experienced, had no parental experience at the time of testing, and had never been housed with a pup other than their own littermates. Expectant males and females were tested 2–28 days prepartum (9.5 ± 8.4 days; average gestation length for California mice is 31.6 days (Gubernick, 1988)). Pregnancy was monitored on the basis of typical weight increases seen in pregnant females in our colony (see below) and confirmed by subsequent parturition. Expectant parents were tested with placenta when they were 125–305 days of age (E-Males: 202.2 ± 64.2, E-Females: 202.2 ± 61.2) and with liver 3–7 days later. Two of the females and none of the males in this group were littermates.

Multiparous Parents. Multiparous males (M-Males, $n = 13$) and females (M-Females, $n = 10$) had produced multiple litters (range: 3–13 litters; 7.6 ± 6.7) and thus were both sexually and parentally experienced. These animals had 1- to 10-day-old pups living with them at the time of placenta testing, and females were likely to be pregnant, as this species undergoes postpartum estrus and copulates on the day of parturition (Gubernick, 1988; pers. obs.). M-Males and M-Females were 179–632 days old (M-Males: 406.6 ± 117.9, M-Females: 362.3 ± 129.9) when tested with placenta. This group contained no same-sex littermates.

Each animal was tested under only a single reproductive condition. We determined pregnancies (or the lack thereof) by weighing females twice weekly and monitoring them for sustained weight gain after pair formation or after they gave birth to a previous litter. Data from each animal were inspected for a gradual and sustained increase in weight, as well as a rapid weight increase during the last week of pregnancy (unpub. data).

Within each sex, age at the time of testing differed significantly among mice in the three reproductive conditions (females, $\chi^2 = 8.72$, $p < 0.0001$: Kruskal–Wallis test; males, $F = 11.26$, df = 2, $p = 0.002$; one-way ANOVA). Appropriate post hoc pair-wise comparisons showed that M-Females were significantly older than E-Females and V-Females (p-values <0.05), whereas M-Males were older than E-Males and V-Males (p-values <0.05). No differences in ages were found between expectant and virgin animals of either sex (p-values >0.05).

Behavioral Tests

Each mouse underwent a single placenta test, followed 3–7 days later by a liver test. Approximately 30 min before

each test, the animal's cagemate(s) were removed, while the test animal remained in the home cage. At the outset of the test, placenta or liver (~0.2 g; see below) in a small, hexagonal, plastic weigh boat (2.5 cm diameter × 0.95 cm deep) was placed in one end of the cage. The animal was videotaped for 10 min or until it consumed all of the tissue, whichever came first. The weigh boat and any remaining tissue were then removed from the cage, the tissue was reweighed, and the animal's cagemate(s) were returned. Behavior was later scored from videotapes using the JWatcher event-recorder program (Blumstein & Daniel, 2007). Tests were conducted in the colony-housing room during lights-on, between 1100 and 1800 h. The animals were not food-deprived prior to or during behavioral testing. When two or three mice from the same cage were tested on the same day, both/all cagemates were reunited in the home cage for at least 30 minutes following one animal's test, before the next focal animal was isolated in the home cage prior to testing. No more than three cagemates were tested in a single day.

Animals were considered placentophagous if they ate all or some of the experimentally presented placenta during the test, as determined visually (see below). The same criterion was used for liver tests. In many instances dehydration or contact with the bedding dramatically changed the weight of the experimentally presented tissues. As a result, the post-test tissue weights were not reliable and thus were not used when categorizing animals as placentophagous or not. The proportion of individuals that ate each tissue (liver or placenta) as well as the latency to approach the tissue was determined.

A total of 8 videotapes (2 from placenta tests, 6 from liver tests) were lost due to a camera malfunction. As a result, the final sample sizes used for quantitative behavioral analyses for placenta tests or liver tests (i.e., latency to approach tissue) ranged from 8 to 12.

Tissue Procurement

Placenta. Placentas were harvested from pregnant females from our breeding colony (417.7 ± 39.3 days old) that had given birth previously to 1–13 litters. The test animals and donors were no more closely related than second cousins. All extracted placentas were close to full term (30–33 days after the previous birth). Pregnancies were monitored by weighing the females twice per week as described above. Donors were euthanized by CO_2 inhalation, and uterine horns were extracted immediately. Each amniotic sac was dissected individually, and fetuses were euthanized with sodium pentobarbital (Fatal Plus, Vortech Pharmaceuticals, Dearborn, MI; ~0.2 ml, i.p.). We lightly dried individual placentas and the adhering membranes by pressing them briefly onto paper towel, and then placed the tissues in plastic weighing boats with 1.5 ml of saline. Placentas were subsequently blotted lightly on a paper towel, cut in half into two ~0.2 g sections (~1.0 × 0.5 × 0.25 cm [length × width × depth]; the average weight of a single placenta is ~0.4 g [unpub. data]), and transferred to a clean, dry weighing boat immediately before being used in a behavioral test, which commenced 10–30 minutes after harvesting of the tissues.

Liver. Livers were harvested from adult virgin females (135 ± 54.5 days old) that were no more closely related to the test animals than second cousins. Donors were euthanized by CO_2 inhalation, and their livers were extracted, divided into ~0.2 g sections (~1.0 cm × 0.5 cm × 0.25 cm [length × width × depth]), lightly blotted, placed in plastic weighing boats with 1.5 mL saline, transferred to dry weighing boats, and presented to the test animal following the same procedures used for placenta. Again, behavioral tests commenced within 10–30 minutes following harvesting of livers.

Statistical Analyses

Data were analyzed using R version 15.0 (Vienna, Austria). To characterize differences in the prevalence of placentophagia or liver consumption among reproductive states and between sexes, pairwise comparisons were made using Fisher's Exact-Boschloo tests; the alpha values of these pairwise comparisons were Bonferroni-corrected to 0.016 ($\leq \alpha/n$, where n = number of pairwise comparisons; 0.05/3 = 0.016). The remaining analyses were evaluated using a critical p-value of 0.05 (two-tailed).

One-way ANOVAs (for normally distributed data) or Kruskal–Wallis tests (for non-normally distributed data) and appropriate pairwise post hoc tests (Tukey HSD or Dunn tests, respectively) were used to compare latencies to approach tissues. McNemar tests were used to determine differences in the propensity for individual mice within each reproductive condition to eat liver and to eat placenta. Mann–Whitney U tests were used to determine if age or number of days prepartum (expectant females and males only) differed among animals within each sex that did and did not consume placenta.

RESULTS

Females

The prevalence of placentophagia differed markedly among females in the three reproductive conditions (Fig. 1). Multiparous females (M-Females, 8 of 10) had the highest incidence of placentophagia, followed by expectant females (E-Females, 5 of 11) and virgin females (V-Females, 2 of 10). M-Females were significantly more likely to eat placenta than were V-Females ($p = 0.012$; Fisher's Exact-Boschloo test). No significant differences in the prevalence of placentophagia were found between M-Females and E-Females ($p = 0.18$; Fisher's Exact-Boschloo test) or between E-Females and V-Females ($p = 0.29$; Fisher's Exact-Boschloo test, Fig. 1).

In contrast to placentophagia, females' propensity to eat liver showed no significant pairwise differences among the three reproductive groups when we employed a Bonferroni-adjusted critical p-value of 0.016 (M-Females vs. E-Females, $p = 0.52$; M-Females vs. V-Females, $p = 0.042$; E-Females vs. V-Females,
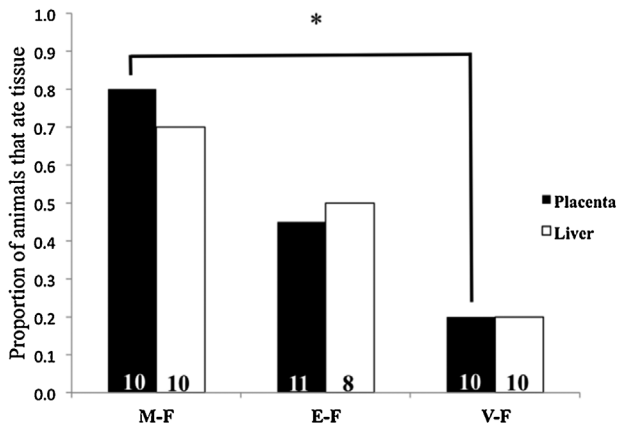
FIGURE 1 Proportion of female California mice that ingested placenta (black bars) and liver (white bars) among multiparous (M-F), expectant (E-F), and virgin females (V-F). Numbers within bars represent sample sizes. *$p < 0.016$ (Fisher's Exact-Boschloo test).

$p = 0.29$; Fisher's Exact-Boschloo tests; Fig. 1). Further analyses revealed that within each of the three reproductive conditions, individual females were equally likely to eat liver and placenta (all p-values >0.05; McNemar tests).

The latency to approach placenta did not differ among females from the three reproductive conditions ($\chi^2 = 0.34$, df $= 2$, $p = 0.84$; Kruskal–Wallis test; Table 1). In contrast, latencies to approach liver differed significantly among reproductive conditions ($\chi^2 = 11.0$, df $= 2$, $p = 0.012$; Kruskal–Wallis test; Table 1). Specifically, M-Females and E-Females approached the liver more quickly than V-Females (p-values < 0.05; Dunn's tests).

As described above (see Methods Section), females in the three reproductive conditions differed significantly in age. Therefore, to determine whether age might influence the propensity of female California mice to eat placenta, we performed a Mann–Whitney U test comparing ages of all placentophagous E-Females and V-Females with those of all non-placentophagous E-Females and V-Females; we excluded M-Females

since they both were significantly older than the other groups and had maternal experience. This analysis revealed that age did not differ significantly between females that ate placenta ($n = 7$; 193.0 ± 22.5 days old) and those that did not ($n = 14$, 168.0 ± 4.6 days old; $U(20) = 27$, $p = 0.10$; Mann–Whitney U test). Similarly, the number of days prepartum did not differ between E-Females that did ($n = 5$; 10.2 ± 3.3 days prepartum) and did not ($n = 6$; 10.0 ± 4.9 days prepartum) eat placenta ($U(11) = 13.0$, $p = 0.78$; Mann–Whitney U test).

Males

Similar to females, males in the three reproductive conditions differed significantly in their propensity to eat placenta. Both M-Males (11 of 13) and E-Males (7 of 10) were significantly more likely to ingest placenta than V-Males (2 of 11; M-Males vs. V-Males, $p = 0.002$; E-Males vs. V-Males, $p = 0.002$; Fisher's Exact-Boschloo tests; Fig. 2). The incidence of placentophagia did not differ significantly between M-Males and E-Males ($p = 0.51$; Fisher's Exact-Boschloo test).

In contrast to placentophagia, the incidence of liver ingestion did not differ reliably among males in the three reproductive groups when we utilized the Bonferroni-corrected p-value ($p = 0.016$) (M-Males vs. E-Males, $p = 1.0$; M-Males vs. V-Males, $p = 0.046$, E-Males vs. V-Males, $p = 0.07$; Fisher's Exact-Boschloo tests; Fig. 2). Further analysis revealed that M-Males tended to have higher rates of placentophagia than liver ingestion, but this trend was not significant ($p = 0.06$; McNemar test). Males in each of the remaining two conditions were equally likely to eat placenta and liver (E-Males, $p = 0.25$; V-Males, $p = 1.0$; McNemar tests). Furthermore, males' latencies to approach placenta and liver did not differ among the three reproductive conditions (placenta: $\chi^2 = 1.06$, $p = 0.59$; liver: $\chi^2 = 0.34$, $p = 0.84$; Kruskal–Wallis tests; Table 1).

As with females, we compared age at the time of placenta tests between E-Males and V-Males that did and did not eat placenta; again, we excluded M-Males

Table 1. Latencies (in Seconds; mean $\pm$ SE) of Multiparous (M-), Expectant (E-), and Virgin (V-) Female and Male California Mice to Approach Experimentally Presented Placenta and Liver

	M-Females	E-Females	V-Females	M-Males	E-Males	V-Males
Placenta	74.1 ± 12.0 ($n = 9$)	69.5 ± 12.6 ($n = 10$)	56.1 ± 9.8 ($n = 10$)	37.9 ± 4.2 ($n = 12$)	36.1 ± 3.6 ($n = 10$)	91.8 ± 15.6 ($n = 11$)
Liver	12.3 ± 0.99 ($n = 9$)	15.7 ± 1.5 ($n = 10$)	125.6 ± 13.7 ($n = 9$)	18.5 ± 1.9 ($n = 11$)	34.4 ± 8.2 ($n = 8$)	25.9 ± 1.8 ($n = 11$)

Samples sizes are shown in parentheses. M-Females and E-Females approached the liver more quickly than V-Females (p-values < 0.05; Dunn's tests).
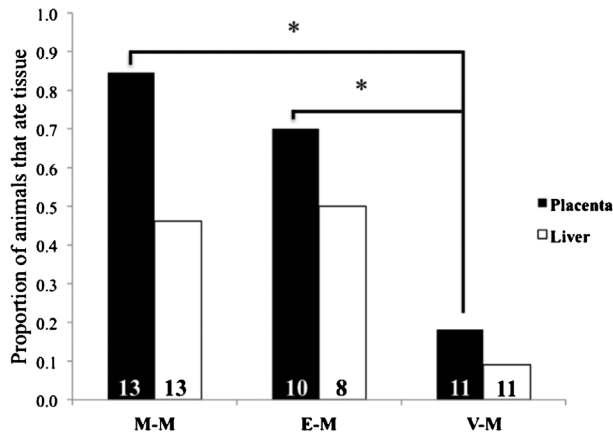
FIGURE 2 Proportion of male California mice that ingested placenta (black bars) and liver (white bars) among multiparous (M-M), expectant (E-M), and virgin males (V-M). Numbers within bars represent sample sizes. *$p < 0.016$ (Fisher's Exact-Boschloo tests).

because they were both significantly older than E-Males and V-Males and parentally experienced. In contrast to females, placentophagous males ($n = 9$; 224.0 ± 13.4 days old) were slightly but significantly younger than non-placentophagous males ($n = 12$ 232.9 ± 1.5 days old; $U(20) = 20.0$, $p = 0.047$; Mann–Whitney U test). The number of days prepartum did not differ between placentophagous ($n = 7$; 8.0 ± 1.0 days prepartum) and non-placentophagous E-Males ($n = 3$; 13.0 ± 4.3 days prepartum; $U(9) = 8.0$, $p = 0.66$; Mann–Whitney U test).

Comparisons between males and females from the same reproductive condition showed no differences between the sexes in the propensity to ingest either placenta or liver (all p-values >0.1; Fisher's Exact-Boschloo tests).

DISCUSSION

In this study we sought to characterize the incidence of placentophagia in male and female California mice in three different reproductive conditions (multiparous parents, expectant first-time parents, and virgins), and to determine how parental and/or sexual experience might influence this behavior. Our results indicate that both males and females differ in their propensity to eat placenta depending on their reproductive condition. In contrast, the incidence of liver ingestion was not affected by an animal's reproductive condition, suggesting that effects of reproductive condition are specific to placentophagia and not to ingestion of any highly vascularized tissue. Additionally, we found similar

patterns of placentophagia and liver ingestion in males and females from the same reproductive condition.

Among females, virgins showed the lowest incidence (20%) of placentophagia, whereas multiparous females showed the highest, with 80% of experienced breeding females eating some or all of the presented placenta. These results suggest that female California mice increase their attraction to placenta as a result of parenting experience and/or parturition, including previous exposure to placenta. Moreover, the finding that the prevalence of placentophagia did not differ between expectant and virgin females suggests that neither sexual experience nor pregnancy increases the propensity to ingest placenta in female California mice. Age also did not appear to be an important determinant of placentophagia, as age did not differ reliably between expectant and virgin females that did and did not eat placenta. This finding differs from results in female dwarf hamsters, in which rates of placentophagia decreased with age (Gregg & Wynne-Edwards, 2005).

Although pregnancy did not appear to affect the incidence of placentophagia in females, expectant females and multiparous females had significantly lower latencies to approach liver than virgin females. This finding suggests that pregnancy and/or lactation may decrease neophobia and increase exploratory behavior in females, which could be motivated by an increased need for food (Bartness, 1997; Johnstone & Higuchi, 2001). Neophobia and exploratory behavior may be influenced by the neuroendocrine changes that females undergo during gestation, parturition and lactation, as demonstrated in rats and mice (*Mus* spp.) (Numan & Insel, 2003).

For male California mice, parental experience and their mate's pregnancy seemed to increase attraction to placenta, as both multiparous males and expectant males showed significantly higher rates of placentophagia (84% and 70%, respectively) when compared to virgin males (9%). It is unclear if these results reflect the effect of copulation, cohabitation with a female, or sensory cues specifically from a pregnant female. Interestingly, however, we have found that males pair-housed with an unrelated female that, for unknown reasons, failed to become pregnant showed low prevalence of placentophagia (5 out of 10; unpub. data), suggesting that cohabitation with a female is not sufficient to induce placentophagia.

In contrast to females, latency to approach either placenta or liver did not differ among males in the three reproductive conditions, suggesting that neophobia did not differ among male reproductive conditions (see also Chauke, de Jong, Garland, & Saltzman, 2012) and did not contribute to differences in placentophagia. Moreover, since males from the three reproductive

conditions were equally likely to eat liver, the high incidence of placentophagia in multiparous males and expectant males did not result from an increased attraction to vascularized tissues in general. Although California mice mainly eat seeds (Merritt, 1974; Meserve, 1976), they will eat meat opportunistically (pers. obs.), and this may explain why males (and females) from different reproductive conditions did not differ in the tendency to ingest liver. Furthermore, lesions of the lateral hypothalamus, which negatively affect ingestive behaviors, do not affect placentophagia in parturient female rats, suggesting that placentophagia is regulated by different mechanisms from those that control hunger (Kristal, 1973). Additionally, in contrast to females, younger expectant and virgin males were more likely to ingest placenta than older expectant and virgin males. Importantly, however, 7 out of 9 placentophagous males from these two groups were expectant fathers, which tended to be younger than virgin males; thus, reproductive condition might play a larger role than age in influencing placentophagia. In dwarf hamsters, placentophagia is not affected by age in sexually inexperienced males (Gregg & Wynne-Edwards, 2005).

Several caveats should be kept in mind when interpreting the results of this study. First, because all animals were tested first with placenta and then with liver 3–7 days later, it is possible that the response to liver was influence by previous exposure to placenta. Second, we cannot rule out the possibility that the age differences found among groups within each sex might have contributed to the differences among groups in the propensity to ingest placenta, as suggested by the results of the age comparison between placentophagous and non-placentophagous males. Nonetheless, as described above, we believe that age made little, if any, contribution to the observed differences in placentophagia. Third, all animals were separated from their cagemates prior to behavioral testing, and this procedure might have affected behavior differently in the different groups. In particular, if a strong pair bond exists between opposite-sexed pairmates, it might be expected that even brief disruption of the pair bond could alter the animals' performance in behavioral tests. In a previous study, we compared putative anxiety-related behavior and neophobia between breeding, expectant, and virgin male California mice (Chauke et al., 2012). As in the present study, males were removed from their cagemates shortly before testing. Very few behavioral differences were found among males that were pair-housed with either a postpartum female (and pups), a pregnant female, or a male; however, numerous differences were found between these animals and singly housed virgin males. These findings suggest that short-term separation from a male or female pairmate may not differentially alter behavior in male California mice. Unfortunately, we do not have comparable data for females. Finally, in contrast to multiparous and expectant parents, virgin males and females in the present study were not housed in pairs but in groups of four animals, which might have affected their responses to the behavioral tests.

In summary, our results show that the pattern of placentophagia in female California mice is similar to that in female rats (Kristal, 1980) and rabbits (Melo & González-Mariscal, 2003) in that females tended to increase their attraction to placenta with maternal experience. In contrast to findings in rats (Kristal, Peters, et al. (1981)), rabbits (Melo & González-Mariscal, 2003), dwarf hamsters (Gregg & Wynne-Edwards, 2005) and Djungarian hamsters (Gregg & Wynne-Edwards, 2006), however, our results indicate that pregnancy alone does not increase placentophagia in female California mice. Among males, the prevalence of placentophagia was higher in expectant first-time fathers and in experienced fathers than in virgins, similar to findings in the biparental dwarf hamster (Gregg & Wynne-Edwards, 2005). Importantly, we found that high levels of placentophagia in males emerge with pregnancy of their mate (and potentially with sexual experience) and persist for at least several days postpartum.

Placentophagia has been shown to facilitate the onset of maternal behavior in some female mammals (Kristal, 1980, 2009). Placenta contains a variety of hormones that can potentially affect neuroendocrine activity in individuals that consume it and, as a result, might alter their behavior towards neonates (Kristal, 1980, 2009; Kristal et al., 2012). Although the specific hormones and relative amounts present in placenta vary among species, placenta has been reported to contain progestagens, estrogens, oxytocin, lactogens, corticotropin-releasing hormone, and opioids (Petraglia, Florio, Nappi, & Gennazzani, 1996), all of which have been shown to influence maternal behavior in several mammalian species (Numan & Insel, 2003). The presence of maternally derived estrogens in placenta is of particular relevance, as estradiol has been shown to activate paternal behavior in California mice (Trainor & Marler, 2002). Further investigation is needed into the potential physiological and behavioral consequences of placentophagia in males to determine its possible role in the onset of paternal behaviors.

NOTES

REFERENCES

Abitbol M. L., & Inglis, S. R. (1997). Role of amniotic fluid in newborn acceptance and bonding in canines. Journal of Maternal-Fetal and Neonatal Medicine, 6, 49–52.

Bartness, T. J. (1997). Food hoarding is increased by pregnancy, lactation, and food deprivation in Siberian hamsters. American Journal of Physiology—Regulatory Integrative Comparative Physiology, 272, 118–125.

Blank M. S., & Friesen, H. G. (1980). Effects of placentophagy on serum prolactin and progesterone concentrations in rats after parturition or superovulation. Journal of Reproduction and Fertility, 60, 273–278.

Blumstein D. T., & Daniel, J. C. (2007). Quantifying behavior the JWatcher Way. Sinauer Associates, Inc. Sunderland, MA.

Chauke, M., de Jong, T. R., Garland, T. Jr, & Saltzman, W. (2012). Paternal responsiveness is associated with, but not mediated by reduced neophobia in male California mice (Peromyscus californicus). Physiology & Behavior, 107, 65–75.

Chauke, M., Malisch, J. L., Robinson, C., de Jong, T. R., & Saltzman, W. (2011). Effects of reproductive status on behavioral and endocrine responses to acute stress in a biparental rodent, the California mouse (Peromyscus californicus). Hormones and Behavior, 60, 128–138.

de Jong, T. R., Chauke, M., Harris, B. N., & Saltzman, W. (2009). From here to paternity: Neural correlates of the onset of paternal behavior in California mice (Peromyscus californicus). Hormones and Behavior, 56, 220–231.

González-Mariscal, G., Melo, A. I., Chirino, R., Jiménez, P., Beyer, C., & Rosenblatt, J. S. (1998). Importance of mother/young contact at parturition and across lactation for the expression of maternal behavior in rabbits. Developmental Psychobiology, 32, 101–111.

Gregg J. K., & Wynne-Edwards, K. E. (2005). Placentophagia in naïve adults, new fathers, and new mothers in the biparental dwarf hamster Phodopus campbelli. Developmental Psychobiology, 47, 179–188.

Gregg J. K., & Wynne-Edwards, K. E. (2006). In uniparental Phodopus sungorus, new mothers, and fathers present during the birth of their offspring, are the only hamsters that readily consume fresh placenta. Developmental Psychobiology, 48, 528–536.

Gubernick, D. J. (1988). Reproduction in the California mouse, Peromyscus californicus. Journal of Mammalogy, 69, 857–860.

Gubernick, D. J., & Alberts, J. R. (1987). The biparental care system of the California mouse, Peromyscus californicus. Journal of Comparative Psychology, 101, 169–177.

Gubernick D. J., & Alberts, J. R. (1989). Postpartum maintenance of paternal behaviour in the biparental California mouse Peromyscus californicus. Animal Behaviour, 37, 656–664.

Harding S. M., & McGinnis, M. Y. (2004). Androgen receptor blockade in the MPOA or VMN: Effects on male sociosexual behaviors. Physiology & Behavior, 81, 671–680.

Harris, B. N., Perea-Rodriguez, J. P., & Saltzman, W. (2011). Acute effects of corticosterone injection on paternal behavior in California mouse (Peromyscus californicus) fathers. Hormones and Behavior, 60, 666–675.

Jemiolo, B., Gubernick, D. J., Yoder, M. C., & Novotny, M. (1994). Chemical characterization of urinary volatile compounds of Peromyscus californicus, a monogamous biparental rodent. Journal of Chemical Ecology, 20, 2489–2500.

Johnstone L. E., & Higuchi, T. (2001). Food intake and leptin during pregnancy. Progress in Brain Research, 33, 215–227.

Jones J. S., & Wynne-Edwards, K. E. (2000). Paternal hamsters mechanically assist the delivery, consume amniotic fluid and placenta, remove fetal membranes, and provide parental care during the birth process. Hormones and Behavior, 37, 116–125.

Kindon, H. A., Baum, M. J., & Paredes, R. J. (1996). Medial preoptic/anterior hypothalamic lesions induce a female-typical profile of sexual partner preference in male ferrets. Hormones and Behavior, 30, 514–527.

Kristal, M. B. (1973). Effects of lateral hypothalamic lesions on placentophagia in virgin, primiparous, and multiparous rats. Journal of Comparative and Physiological Psychology, 84, 53–62.

Kristal, M. B. (1980). Placentophagia: A biobehavioral enigma (or De gustibus non disputandum est). Neuroscience & Biobehavioral Reviews, 4, 141–150.

Kristal, M. B. (2009). The biopsychology of maternal behavior in nonhuman mammals. ILAR Journal/National Research Council, Institute of Laboratory Animal Resources, 50, 51–63.

Kristal, M. B., DiPirro, J. M., & Thompson, A. C. (2012). Placentophagia in humans and nonhuman mammals: Causes and consequences. Ecology of Food and Nutrition, 51, 177–197.

Kristal, M. B., Whitney, J. F., & Peters, L. C. (1981). Placenta on pups' skin accelerates onset of maternal behaviour in non-pregnant rats. Animal Behaviour, 29, 81–85.

Kristal, M. B., Peters, L. C., Franz, J. R., Whitney, J. F., Nishita, J. K., & Steuer, M. A. (1981). The effect of pregnancy and stress on the onset of placentophagia in Long-Evans rats. Physiology & Behavior, 27, 591–595.

Kristal M. B., & Nishita, J. K. (1981). Observing birth and placentophagia affects placentophagia but not maternal behavior of virgin rats. Animal Learning & Behavior, 9, 545–550.

Kristal, M. B., Thompson, A. C., & Grishkat, H. L. (1985). Placenta ingestion enhances opiate analgesia in rats. Physiology & Behavior, 35, 481–486.

Lee A. W., & Brown, R. E. (2002). The presence of the male facilitates parturition in California mice (*Peromyscus californicus*). Canadian Journal of Zoology, 80, 926–933.

Lee A. W., & Brown, R. E. (2007). Comparison of medial preoptic, amygdala, and nucleus accumbens lesions on parental behavior in California mice (*Peromyscus californicus*). Physiology & Behavior, 92, 617–628.

Lévy F., & Poindron, P. (1987). The importance of amniotic fluids for the establishment of maternal behaviour in experienced and inexperienced ewes. Animal Behaviour, 35, 1188–1892.

Lévy, F., Poindron, P., & Le Neidre, P. (1983). Attraction and repulsion by amniotic fluids and their olfactory control in the ewe around parturition. Physiology & Behavior, 31, 687–692.

Markowski, V. P., Eaton, R. C., Lumley, L. A., Moses, J., & Hull, E. M. (1994). AD 1 agonist in the MPOA facilitates copulation in male rats. Pharmacology, Biochemistry and Behavior, 47, 483–486.

Melo A. I., & González-Mariscal, G. (2003). Placentophagia in rabbits: Incidence across the reproductive cycle. Developmental Psychobiology, 43, 37–43.

Merritt, J. F. (1974). Factors influencing the local distribution of *Peromyscus californicus* in northern California. Journal of Mammalogy, 55, 102–114.

Meserve, P. L. (1976). Food relationships of a rodent fauna in a California coastal sage scrub community. Journal of Mammalogy, 57, 300–319.

Numan M., & Insel, T. R. (2003). The neurobiology of parental behavior. Springer-Verlag. New York.

Noonan M., & Kristal, M. B. (1979). Effects of medial preoptic lesions on placentophagia and on the onset of maternal behavior in the rat. Physiology & Behavior, 22, 1197–1202.

Olazábal, D. E., Kalinichev, M., Morrell, J. I., & Rosenblatt, J. S. (2002). MPOA cytotoxic lesions and maternal behavior in the rat: Effects of midpubertal lesions on maternal behavior and the role of ovarian hormones in maturation of MPOA control of maternal behavior. Hormones and Behavior, 41, 126–138.

Paredes, R. G., Tzschentke, T., & Nakach, N. (1998). Lesions of the medial preoptic area/anterior hypothalamus (MPOA/AH) modify partner preference in male rats. Brain Research, 813, 1–8.

Petraglia, F., Florio, P., Nappi, C., & Gennazzani, A. R. (1996). Peptide signaling in human placenta and membranes: Autocrine, paracrine, and endocrine mechanisms. Endocrine Reviews, 17, 156–186.

Powers, J. B., Newman, S. W., & Bergondy, M. L. (1987). MPOA and BNST lesions in male Syrian hamsters: Differential effects on copulatory and chemoinvestigatory behaviors. Behavioural Brain Research, 23, 181–195.

R Development Core Team. (2008). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. ISBN 3-900051-07-0, URL http://www.R-project.org

Ribble, D. O. (1991). The monogamous mating system of *Peromyscus californicus* as revealed by DNA fingerprinting. Behavioral Ecology and Sociobiology, 29, 161–166.

Rosenblatt J. S., & Ceus, K. (1998). Estrogen implants in the medial preoptic area stimulate maternal behavior in male rats. Hormones and Behavior, 33, 23–30.

Steuer, M. A., Thompson, A. C., Doerr, J. C., Youakim, M., & Kristal, M. B. (1987). Induction of maternal behavior in rats: Effects of pseudopregnancy termination and placenta-smeared pups. Behavioral Neuroscience, 101, 219–227.

Trainor B. C., & Marler, C. A. (2002). Testosterone promotes paternal behaviour in a monogamous mammal via conversion to oestrogen. Proceedings of the Royal Society B: Biological Sciences, 269, 823–829.

Young S. M., & Benyshek, D. C. (2010). In search of human placentophagy: A cross-cultural survey of human placenta consumption, disposal practices, and cultural beliefs. Ecology of Food and Nutrition, 49, 467–484.

Young, S. M., Benyshek, D. C., & Lienard, P. (2012). The conspicuous absence of placenta consumption in human postpartum females: The fire hypothesis. Ecology of Food and Nutrition, 51, 198–217.