Hormones and Behavior 110 (2019) 40–45

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

# Estrogen receptor-alpha knockouts and maternal memory in nulliparous rats

J.M. Gallagher[a], B.C. Nephew[a], G. Poirier[b], J.A. King[b], R.S. Bridges[a,*]

[a] Department of Biomedical Sciences, Tufts University, Cummings School of Veterinary Medicine, North Grafton, MA 01536, United States of America
[b] Center for Comparative NeuroImaging, Department of Psychiatry, University of Massachusetts Medical School, Worcester, MA 01655, United States of America

ARTICLE INFO

*Keywords:*
Estrogen receptor alpha
Esr1
Esr1 KO
Maternal behavior
Maternal memory

ABSTRACT

The current study investigated the role of estrogen receptor alpha (Esr1) in maternal memory in rats, comparing the induction and retention responses of Esr1 knockout (KO) and wild type (WT) nulliparous rats towards foster pups. Thirty days after completion of induction testing, subjects were tested for the retention of maternal care in their home cage and then for maternal behaviors in a novel cage. Both WT and Esr1 KO females displayed similar latencies to respond to foster young during the initial induction testing. Likewise, reinduction latencies to display full maternal responsiveness were similar in the Esr1 KO and WT groups during maternal memory testing in the home cage. However, in the novel cage testing WT subjects displayed modest modifications in maternal care. WT females had shorter latencies to first retrieve and mouth a test pup. These findings suggest that while Esr1 does not appear to affect the establishment of maternal care or the display of maternal memory, it may modulate aspects of pup-directed behaviors associated with the reinduction of maternal care in female rats.

## 1. Introduction

Reproductive experience includes giving birth and raising young results in neuroplastic changes that affect the female throughout her life (Fleming et al., 2008; Bridges, 2016). This experience of maternity results in long-term modifications in neuroendocrine, neuroanatomical, and behavioral processes. Many mammals show an enhancement of maternal care following a maternal experience (parturition), and multiple maternal experiences further affect these behavioral and biological changes. In sheep, multiparous ewes show a higher level of maternal care than do primiparous ewes (Poindron et al., 1988), whereas multiparous rats display heightened levels of aggression towards male intruders when compared to the responses of primiparous rats (Nephew et al., 2009). Additionally, nulliparous (virgin) female rats induced to respond maternally by pup exposure and primiparous rats given only 2 days of pup exposure, retain maternal care behaviors up to 80 days after prior pup exposure, suggesting that maternal care/memory without parturition or with minimal pup exposure is long lasting (Scanlan et al., 2006). These findings indicate that the experience of raising young creates long-term changes in the brain that result in behavioral modifications maintained throughout the female's life. The underlying neurobiological modifications produced by reproductive experience have not yet been fully elucidated.

One long-term modification that may contribute to the establishment of maternal memory is a shift in estrogen actions. Estrogens have been implicated in the onset and regulation of maternal care in rats, as female rats that were given estradiol benzoate (EB) after both a hysterectomy and ovariectomy between 10 and 19 days of pregnancy, showed shorter latencies for maternal behavior compared to rats not given EB (Rosenblatt and Siegel, 1975). In a related study it was found that ovariectomized and hysterectomized virgin rats given EB and an anti-estrogen, CI-628, had longer latencies to initiate maternal behavior than females only given EB (Siegel et al., 1978). These findings support a role for estrogens in the activation of maternal behavior. Other hormones, such as progesterone or prolactin (see Bridges, 2015) do not elicit a rapid onset of maternal behaviors by themselves. Other studies have found more Esr1 positive cells in the medial preoptic area (MPOA) of cycling primiparous females than found in age-matched, nulliparous females (Byrnes et al., 2009). This indicates that reproductive experience alters the expression of this receptor over the course of the female's lifespan. Additionally, higher quality of maternal care is associated with methylation of the Esr1 gene (Champagne et al., 2006), and dams who lick and groom their pups at higher rates (high LG) compared to dams with low LG have an increase in Esr1 expression in the MPOA (Champagne et al., 2003). Esr1 antagonist treatment with propyl-pyrazole-triol (PPT), moreover, reduced anxiety in parous female rats, but has little effect in age-matched, nulliparous female rats (Byrnes et al., 2012, 2013), suggesting that reproductive experience

* Corresponding author at: Department of Biomedical Sciences, Tufts University, Cummings School of Veterinary Medicine, 200 Westboro Road, North Graton, MA 01536-1895, United States of America.
*E-mail address:* robert.bridges@tufts.edu (R.S. Bridges).

https://doi.org/10.1016/j.yhbeh.2019.02.002
Received 5 December 2018; Received in revised form 29 January 2019; Accepted 7 February 2019
Available online 04 March 2019
0018-506X/ 

modifies the functional activation of Esr1. In female mice, null mutations of the Esr1 gene (Esr1 KO) impedes pup retrieval behavior to the nest compared to the responses of control wild type mice, and Esr1 heterozygous mice display higher incidences of infanticide (Ogawa et al., 1998). More recently, siRNA silencing of estrogen receptor-α in the medial preoptic area of female mice was found to abolish maternal care (Ribeiro et al., 2012). In a complementary study in juvenile rats, using a viral vector expressing Esr1 targeted at the medial preoptic, it was reported that overexpression of estrogen receptor-α in the medial preoptic area of juvenile female subjects reduced their latency to display maternal care to a level similar to that present in juvenile male rats (Peña and Champagne, 2014). Together these results demonstrate an important role for ER-α in maternal care and raise the possibility that estrogen receptor alpha may participate in the expression of maternal memory and the long-term enhancement of maternal care.

In the present study we examined whether the gene for estrogen receptor alpha, Esr1, plays a role in the expression of maternal memory in female rats. The approach utilized a newly developed rat Esr1 knockout (KO) rat model (Rumi et al., 2014) to evaluate whether deletion of the Esr1 alleles affected the induction of maternal care and the expression of maternal memory in pup-induced, nulliparous female rats. This KO approach is designed to help determine whether estrogens, through their actions on estrogen receptor-alpha, facilitate the activation as well as the retention of maternal care.

## 2. Methods

### 2.1. Generation of Esr1 KO rats

Two heterozygous male and two heterozygous female Holtzman Sprague Dawley Esr-1 KO (RRRC:0701 [Esr1-d482]) rats at about 50 days of age, were purchased from University of Missouri Metagenomics Center (MUMC) & Rat Resource and Research Center (RRRC) (University of Missouri). The Esr1 KO rats were initially produced by Rumi et al., 2014. Male and female heterozygous rats were bred at approximately 8 weeks of age with WT male or female rats. In addition, six WT female Sprague Dawley (SD) rats from Charles River (Kingston, MA), and six WT female Holtzman Sprague Dawley (HSD) rats from Envigo (Indianapolis, IN) were purchased to breed with the heterozygous males. All but one female became pregnant and raised litters to weaning age (21 days old).

Pups from the successful breeding pairs were kept for further breeding. Twenty Esr1 heterozygous females and 10 male rats were then bred, attempting to minimize breeding between litter mates. Fifteen of the 20 females became pregnant and gave birth. Since many of these litters were large (averaging about 16 pups) litters were culled to 10–12 pups on postpartum day two, keeping as many females as possible for subsequent behavioral testing. Animals were ear punched at weaning age (21 days old) to identify their genotype. The ear punch tissue samples were outsourced to Transnetyx (Cordova, TN) for genotyping processing (WT (+/+), heterozygous Esr1 KO (+/−) or homozygous Esr1 KO (−/−)).

### 2.2. Experimental groups

Thirty-two adult nulliparous female rats (16 WT & 16 Esr1 homozygous KO) were generated from the breeding protocol and housed in 14:10 light:dark cycle (lights on at 0500 h) and temperature-controlled (21 °C–24 °C) rooms with food and water available ad libitum. Teklad Sani-chip (Envigo, South Easton, MA) was used for bedding in all cages, and two paper wheels were added to each cage for nesting material. Fifteen additional female SD rats (200-250 g) were purchased from Charles River Laboratories (Kingston, MA) to produce donor test pups. The donor lactating rats were maintained in a separate colony room to provide test pups. All rats in this study were maintained in accordance with the guidelines of the Division of Teaching and Research Resources at Tufts University, Cummings School of Veterinary Medicine following the procedures for animal care prepared by the National Research Council Committee of the Care and Use of Laboratory Animal Resources. The research protocol was approved by Tufts IACUC (#G2016–80).

### 2.3. Maternal behavior testing

#### 2.3.1. Induction protocol

The behavioral test used to induce maternal behavior in nulliparous WT and Esr1 KO homozygous female rats was identical to that previously reported (Scanlan et al., 2006). Induction testing began between 10 and 11 weeks of age. Two days prior to the first test session, Plexiglas cage dividers were placed in the home cage (45 × 25 × 20 cm) to divide the cage into four equal quadrants and to reduce the likelihood that donor test pups would crawl to the test subject's nest.

Home cage testing began daily between 0900 and 1030 h. One healthy donor pup (ages 3–9 days old) from our donor colony was placed in each of the three non-nest quadrants of the test subject's cage. Each female was then observed continuously for 15 min and the latencies to display the following behaviors were recorded: making first contact with a pup, the retrieval of all 3 test pups to the nest, grouping all pups in the nest and crouching over them. Test subjects were then spot-checked at 30-, 45-, 60- and 120-minute time points to record the locations of the three pups and the test female. Donor pups were left in the home cage with the female until 1 hr before the test session the following day. On the next day of testing, before removing the donor pups, an overnight grouping score was recorded to note the location of the pups and test subject in the home cage. Induction testing occurred daily until the female reached full maternal behavior, or reached the maximum of 15 days. Full maternal behavior was defined as retrieving all three pups, grouping the pups and crouching over the pups within the two-hour testing period for two consecutive days. The latency of the female to respond maternally, or their maternal behavior score, was calculated as the first maternal response test day minus one. For example, if the test subject showed full maternal behavior on test days 1 and 2, its maternal behavior score would be zero. Behavioral observations were scored independent of knowledge of treatment groups.

#### 2.3.2. Retention testing

WT or Esr1 KO homozygous subjects that responded maternally during the induction test were tested for the retention of maternal behavior 30 days after their last pup exposure. The same procedures and scoring of maternal behavior were used as those employed in the induction protocol.

#### 2.3.3. Novel cage testing

Novel cage behavioral testing assessed the subject's maternal responses in a novel test setting after completion of home cage testing for the retention of maternal behavior. The novel cage was twice as large as the home test cage and measured 45 × 50 × 20 cm. Only females that responded maternally in the retention test were tested in the novel cage. Novel cage testing occurred between 0900 h–1200 h, one day after females reached full maternal responsiveness in their home cage. Females were placed in the novel cage 15 min before the start of the session. Similarly, to the home cage test, three donor pups (ages 3–9 days old) were dispersed inside the novel cage (no cage dividers present). Placement of the last donor pup in the novel cage initiated the start of the test session. The duration of the novel cage test was 30 min and was digitally recorded using a JVC HDD Everio video recorder for later analysis. Donor pups were returned to their mothers at the end of the session. Subjects were tested only once in the novel cage test.

Observations of maternal responsiveness were recorded using ODLog software (Macropod, Inc., Australia), which recorded the frequency, duration and the latency of the following behaviors: first

contact (latency only), pup contact, mouthing, retrieval (carrying) of pups to the nest, grouping (latency only) of all pups in the nest and crouching over all three pups. Mouthing is defined as, when a female rat makes physical contact with a pup with an open mouth or licks/grooms the pup. Observations of the subject's responses were scored by viewing the video recordings independent of knowledge of treatment groups.

#### 2.3.4. Statistical analyses

Data from home cage induction and retention tests and novel cage frequency results were analyzed by independent samples *t*-tests. Novel cage latency and duration data were analyzed by Mann-Whitney *U* test as these data were not normally distributed.

## 3. Results

### 3.1. Induction test

From the 16 WT and 16 Esr1 KO females tested, 12 WT and 14 Esr1 homozygous females responded maternally during the 15-day induction test period. The average response latencies were 9.9 days for WT and 9.4 for Esr1 KO females (see Fig. 1). No differences were found between Esr1 KO females and WT females in the latency to show full maternal behavior (n = 16 per group). Likewise, for all other behavior scores, no significant differences were detected between the KO and WT groups; retrieval of pup 1, 2 and 3 to the nest, grouping pups in the nest, overnight grouping and crouching over all pups in the nest (N = 16 per group for all behaviors observed).

### 3.2. Retention test

From the females tested for the retention of maternal behavior, 8 of 12 WT subjects (66.7%) and 12 of 14 Esr1 KO homozygous females (85.7%) responded maternally by day 15. Average response latencies were 9.0 days for WT females and 7.5 days for Esr1 KO females (see Fig. 1). A significant difference for the overnight grouping score (P = 0.01, $t$ = 2.5, df = 24, Cohens $d$ = 0.96; WT, N = 12; Esr1 KO, n = 14) was found between Esr1 KO homozygotes and WT subjects such that the Esr1 KO subjects were found to have the pups grouped in their nests sooner during overnight scoring (see Fig. 2). No differences were found for the behavior scores during daily home cage reinduction testing; latency for reaching full maternal behavior, retrieval latency of pups 1, 2 and 3, grouping and crouching (WT, n = 12; Esr1 KO, N = 14 for each observed behavior).

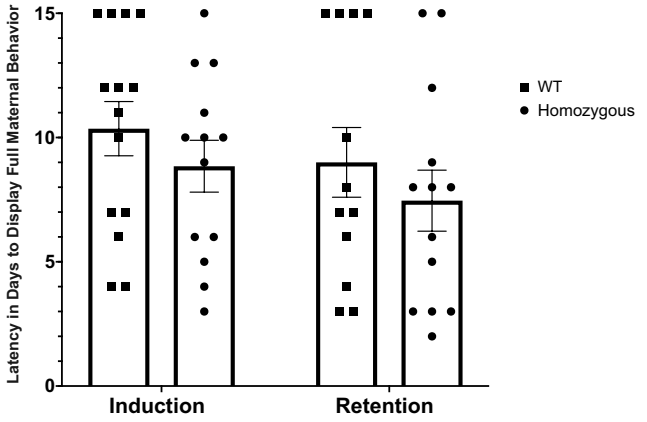


**Fig. 1.** Mean ( ± SEM) number of days to show full maternal response during initial induction and retention testing for WT and Esr1 KO homozygous females in the home cage.

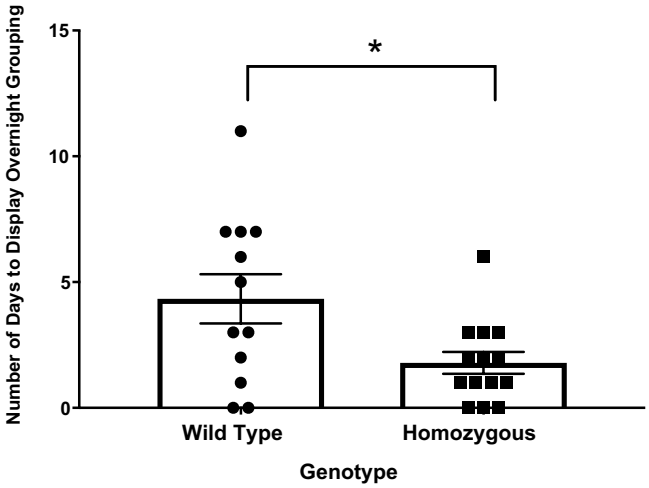


**Fig. 2.** Mean ( ± SEM) number of days to group pups in overnight grouping session during reinduction testing for WT and Esr1 KO homozygous females. Esr1 KO females responded in fewer days compared to WT females in the overnight grouping session. *P < 0.05.

### 3.3. Novel cage test

Analyses found a significant difference in carrying duration of pup 1 (P = 0.03, Mann-Whitney $U$ = 21.50) (WT, N = 8; Esr1 KO, N = 12) with WT females carrying their first pup for a longer duration of time than Esr1 KO homozygous females (see Fig. 3A). WT females also had a significantly shorter latency to retrieve a pup (P = 0.04, $U$ = 21.5) and begin mouthing a pup (P = 0.05, $U$ = 22) compared to Esr1 KO homozygous females (WT, N = 8; Esr1 KO, N = 12 (see Fig. 3B & C). No other differences were found for the other recorded behaviors; carrying duration of pup 2 and 3, pup contact duration, mouthing duration, 1st contact latency, retrieval of pup 2 and 3 (WT, N = 8; Esr1 KO, N = 12). Grouping and crouching data were not analyzed as there was only one subject per group that displayed these responses. Analysis of frequencies of the recorded behaviors revealed no significant differences between groups; carrying of pup 1, 2 and 3, pup contact and mouthing (WT, N = 8; Esr1 KO, N = 12 for each behavior). Only one WT female and one Esr1 KO homozygous female displayed full maternal responsiveness (retrieving, grouping and crouching over all three pups within the two-hour testing period for two consecutive days) during novel cage testing.

### 3.4. Body weights

As reported in Rumi et al. (2014), Esr1 KOs exhibited greater increases in body weight than the WT subjects as they aged. A two way analysis of variance was conducted to compare body weight and genotype. An interaction between body weight and genotype was found to be significant (P < 0.0001, df = 3, F = 21.91). Post hoc analysis using independent samples *t*-tests were used to analyze each age grouping. The body weights of the two groups were similar at 40 days of age, but then diverged significantly by 60 days of age (P < 0.001, df 23, $t$ = 5.89, Cohens $d$ = 0.57) with the Esr1 KO subjects weighing more than the WT animals (see Table 1). Body weights of the WT and KO subjects continued to differ significantly at the 90 and 110–121 day time points (90 days - P < 0.001, df = 23, $t$ = 8.46, Cohens $d$ = 2.38: 110–121 days - P < 0.001, df = 20, $t$ = 6.70, Cohens $d$ = 3.04) with the Esr1 KO rats weights approximately 50% greater than that of the WT females at 110–121 days of age.

## 4. Discussion

The results of this study provide support for the argument that the

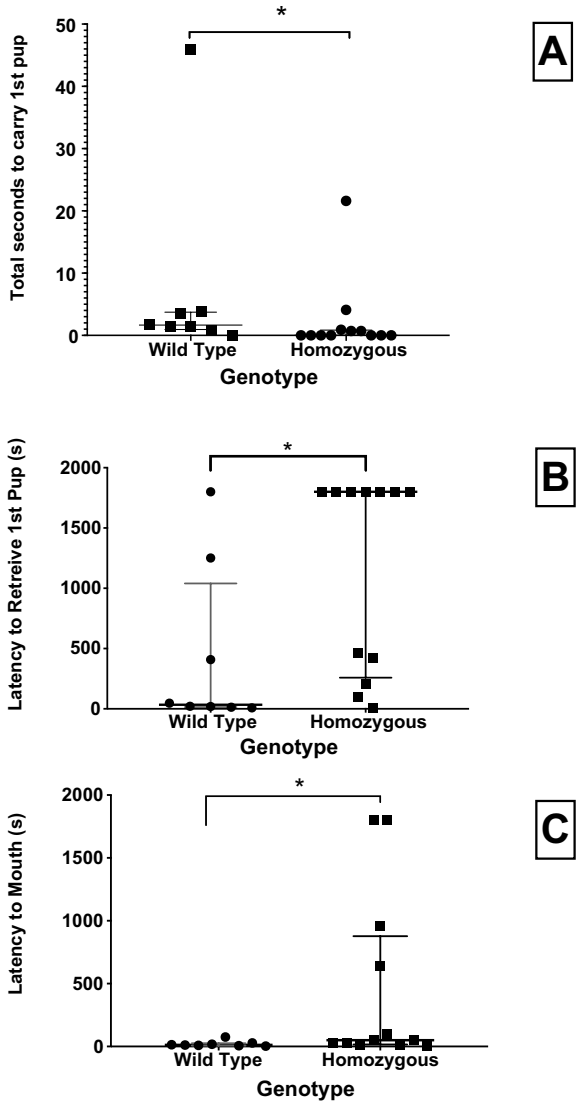


**Fig. 3.** (A) Median with interquartile range in seconds for carrying duration of pup 1 in the novel cage test. Esr1 KO homozygous females carried pup 1 for a shorter duration compared to WT females. (B) Median with interquartile range in seconds for the latency to retrieve the 1st pup in the novel cage. Esr1 KO homozygous females had longer latency times to retrieve the 1st pup compared to WT females. (C) Median with interquartile range in seconds for the latency to begin mouthing a pup in the novel cage. Esr1 KO homozygous females had longer latency times to begin mouthing a pup compared to WT females. *Ps $< 0.05$.

**Table 1**
Effects of Esr1 gene deletion on body weights from 40 to 120 days of age in wildtype and Esr1 knockout (KO) nulliparous, female rats. Values are expressed as mean grams ± SEM. Numbers in parentheses are sample sizes for each time point.

| Treatment groups | Age in days | | | |
|---|---|---|---|---|
| | 40 | 60 | 90 | 110–121 |
| Wildtype | 190.5 ± 3.4 (12) | 270 ± 5.7 (12) | 319.3 ± 9.6 (11) | 362.4 ± 14.0 (9) |
| Esr1KO | 201.8 ± 6.8 (14) | 333.9 ± 8.8* (13) | 471.1 ± 13.9* (14) | 533.2 ± 19.9* (13) |

* $P < 0.001$ versus same age wildtype subjects.

estrogen receptor alpha gene does not play a critical role in the expression of maternal memory in the rat. Deletion of the Esr1 gene in female rats did not delay the expression of maternal behaviors during reinduction testing nor did it affect the initial rate of induction of home cage maternal care. This model provides evidence that maternal memory and the major components of maternal care are not dependent upon the presence of a functional Esr1 gene.

In this knockout study, latencies to initially induce home cage maternal responsiveness in the Esr1 KO rats were comparable to that found in the WT subjects. Both groups required approximately 10 days of daily pup exposure before full maternal care was established. The similarity in responsiveness differs from that reported in Esr1 KO mice (Ogawa et al., 1998) in which mice lacking the estrogen receptor alpha alleles displayed deficits in maternal as well as paternal care. It is unknown what contributes to this species difference in the role of the Esr1 gene in maternal care. It is possible that the underlying nonhormonal basis for maternal care differs between rats and mice contributed to each species response. It is established that the pituitary gland and its regulatory factors are not essential for the expression of maternal behavior in the rat (Rosenblatt, 1967). Consistent with this idea, the present study demonstrates that Esr1 including receptors located throughout the body, including the brain, are also non-essential for the display of the basic capacity to exhibit maternal care, supporting previous work by Stolzenberg and Rissman.

When the subjects that responded maternally in the induction testing were tested for the retention of maternal care after 30 days of separation from foster young, both groups responded with similar reinduction latencies in home cage testing. It was somewhat surprising, however, that neither group displayed shortened latencies to respond upon reinduction testing. Latencies again averaged about 9–10 days. In fact, a small percentage of subjects failed to re-induce over the 15-day test period, results that differ from previous reports (Cohen and Bridges, 1981; Scanlan et al., 2006). When comparing responses during overnight observations during retention testing, it was noted that the Esr1 KOs had grouped and crouched over their test pups in fewer days than did the WT subjects. This, however, did not translate into shorter latencies during the 2-hour home cage test. This response may reflect a difference in motivation or perhaps activity between the pretest period and the test sessions in the Esr1 knockout animals.

It is not clear why neither group displayed shortened reinduction latencies. One possibility is that the process of breeding the knockout animals using a combination of Sprague -Dawley rats from two sources may have altered the responsiveness of subjects such that neither group displayed reduced reinduction latencies indicative of maternal memory. Regardless of this finding, it is clear that the lack of the Esr1 gene did not change the responses of the KO subjects when compared with the responses of the wildtype controls during either during the initial maternal induction process or during retention testing. Although the responses of the groups were somewhat anomalous, comparisons between the KO and WT subjects appear valid.

Slight differences did emerge between the re-induced maternal KO and WT groups in the novel cage testing following home cage testing. The maternal WT subjects appeared to display a more intense response in the novel cage testing. Specifically, WT subjects displayed a shorter latency to retrieve/carry a pup, exhibited enhanced carrying of the first pup, and had a shorter latency to engage in oral contact (mouthing) than did the Esr1 KO subjects. One interpretation of these responses is that maternal WT rats display a higher level of motivation than Esr1 KO maternal rats to engage in pup-directed behaviors. Nevertheless, comparison between the WT and Esr1 KO revealed more similarities than differences in pup-induced maternal responses. It could be that the Esr1 gene may modulate the maternal response of the subject in more challenging environmental conditions but that the basic capacity to display both the induction and re-induction of maternal care in the home cage is quite comparable. Novel environment maternal responding is mediated by both the aversiveness of the environment and

pup salience, and estrogen receptors are implicated in the neuroendocrine and behavioral responses to stress (Handa et al., 2012). Perhaps test conditions that induce a more robust stress response and demand a greater level of motivation are required to delineate potential differences in the stress response and/or maternal care between animals lacking the Esr1 gene and those having the normal complement of alleles. It is possible that the current novel cage differences were mediated by a more substantial stress response in the Esr1 KO females.

It is of interest that an initial maternal experience in virgin mice produces both changes in subsequent maternal behavior as well as possible changes in neural gene expression. Stolzenberg et al. (2012) proposed that experience-based changes in maternal responsiveness may be mediated by chromatin modifications which may then promote changes in gene expression. They found that treatment of virgin female mice with the histone deacetylase inhibitor, sodium butyrate, both stimulated maternal care and the expression of several genes, including estrogen receptor-β and oxytocin, but not estrogen receptor-α. It would be of interest to determine whether prior maternal experience in parous rats would result in similar increases in gene expression, and most notably, whether Esr1 expression is modified by prior parity in specific brain regions. Based upon the results of the present study, however, one would predict that long-term changes in Esr1 expression would not be found in previously parous rats.

In a related study in mice that examined the involvement of estrogen in experience-induced maternal behavior, it was shown that in the absence of estrogen exposure, maternal experience produced long-lasting modifications in maternal responsiveness (Stolzenberg and Rissman, 2011). Hence, it appears that in mice and rats the effects of maternal experience on subsequent maternal care may not be dependent upon an involvement of estrogens and estrogen receptor alpha. A recent review of the effects of epigenetic mechanisms and experience in the hormonal and non-hormonal regulation of maternal behavior provides a substantive analysis of these processes and relationships (Stolzenberg and Champagne, 2016).

Dramatic differences in body weight were found across development in the Esr1 KOs and WT rats. The increased body weight in the KO females both supports a role for Esr1 in weight regulation and validates the accuracy of genetic screening during the postnatal period in our study. Rumi et al. (2014), earlier reported that Esr1 KO female rats were statistically heavier at about 10 weeks of age, whereas knockout males were lighter. We found that by 60 days of age the Esr1 KO females weighed significantly more than WT subjects and that at 120 days of age that Esr1 KO rats weighed > 500 g, approximately 50% more than WT subjects. It is well established that estrogens help to maintain lower weights by increasing metabolic rates and decreasing food consumption (Drewett, 1973; Asarian and Geary, 2002). While we did not measure food intake or metabolic rate, it seems likely that these factors were altered in the Esr1 KO subjects. Another study found that white adipose tissue in Esr1 KO male and female mice increased with age more than that found in WT male and female mice (Heine et al., 2000). Heine et al. (2000) reported that Esr1 KO male mice did not display increased energy intake, but had a decrease in energy expenditure compared to that of WT males, indicating that lower activity in Esr1 KO mice might contribute to their obesity. In another study in mice, the decrease in food intake normally observed in cycling females was prevented with an Esr1 antagonist in ovariectomized female rats given estradiol or in cycling female rats, but no effect was found on food intake following treatment with an estrogen receptor beta antagonist (Santollo et al., 2010). It is possible, although not established, that Esr1 KO female rats may have lower activity levels that could inhibit their motivation to engage in pup-directed behaviors.

Overall, the results demonstrate that the estrogen receptor alpha gene is not critical for the expression of maternal behavior in nulliparous female rats, but may be involved in other behavioral processes, such as motivation and food regulation. Since the subjects in this study were born with their KO genotype, it would be interesting to examine a conditional KO that could be induced at different developmental time points and then compare maternal memory in both WT and conditional KO rats. Furthermore, a conditional region-specific Esr1 KO, such as one directed at the MPOA in mice using siRNA to silence ER-α (Ribeiro et al., 2012), an area vital for the expression of maternal behavior (Numan et al., 1977; Numan and Stolzenberg, 2009) would help clarify a possible involvement of Esr1 in this brain region in rats and its effects on maternal responsiveness and maternal memory. It is also important to employ these and other models to address these issues. One other approach, for example, would be to interfere with maternal memory by administering previous parous rats with a selective estrogen receptor-alpha antagonist. Preliminary results from our laboratory using this approach failed to block maternal memory with the ER-α antagonist methyl-piperidino-pyrazole hydrate (MPP; Zhou et al., 2009), thus providing support that the ER-α receptor may not be critical for the expression of maternal memory (Gallagher & Bridges, unpublished findings). Finally, it is noted that the present study is unique in that it utilized the Esr1 KO to investigate the expression of maternal behavior in rats. Use of this KO model provides the opportunity to explore the role of Esr1 in this and related physiological and behavioral processes.

## Acknowledgements

This research was supported by Public Health Service Grant HD086381 from the National Institutes of Health awarded to RSB. We thank Dr. Michael Soares for developing the Esr1 knockout rats and serving as a consultant on this project.

## Conflict of interest

The authors of this research have no conflicts of interest.

## Funding

This work was supported by Public Health Service Grant HD086361 from the National Institutes of Health awarded to RSB.

## References

Asarian, L., Geary, N., 2002. Cyclic estradiol treatment normalizes body weight and restores physiological patterns of spontaneous feeding and sexual receptivity in ovariectomized rats. Horm. Behav. 42, 461–471. https://doi.org/10.1006/hbeh.2002.1835.

Bridges, R.S., 2015. Neuroendocrine regulation of maternal behavior. Front. Neuroendocrinol. 36, 178–196.

Bridges, R.S., 2016. Long-term alterations in neural and endocrine processes induced by motherhood in mammals. Horm. Behav. 77, 193–203. https://doi.org/10.1016/j.yhbeh.2015.09.001.

Byrnes, E.M., Babb, J.A., Bridges, R.S., 2009. Differential expression of oestrogen receptor alpha following reproductive experience in young and middle-aged female rats. J. Neuroendocrinol. 21, 550–557. https://doi.org/10.1111/j.1365-2826.2009.01874.x.

Byrnes, E.M., Casey, K., Bridges, R.S., 2012. Reproductive experience modifies the effects of estrogen receptor alpha activity on anxiety-like behavior and corticotropin releasing hormone mRNA expression. J. Neuroendocrinol. 61, 44–49. https://doi.org/10.1016/j.yhbeh.2011.10.001.

Byrnes, E.M., Casey, K., Carini, L.M., Bridges, R., 2013. Reproductive experience alters neural and behavioural responses to acute oestrogen receptor α activation. J. Neuroendocrinol. 25, 1280–1289. https://doi.org/10.1111/jne.12113.Reproductive.

Champagne, F.A., Weaver, I.C.G., Diorio, J., Sharma, S., Meaney, M.J., 2003. Natural variations in maternal care are associated with estrogen receptor alpha expression and estrogen sensitivity in the medial preoptic area. Endocrinology. 144, 4720–4724. https://doi.org/10.1210/en.2003-0564.

Champagne, F.A., Weaver, I.C.G., Diorio, J., Dymov, S., Szyf, M., Meaney, M.J., 2006. Maternal care associated with methylation of the estrogen receptor-α1b promoter and estrogen receptor-α expression in the medial preoptic area of female offspring. Endocrinology. 147, 2909–2915. https://doi.org/10.1210/en.2005-1119.

Cohen, J., Bridges, R., 1981. Retention of maternal behavior in nulliparous and primiparous rats: effects of duration of previous maternal experience. J. Comp. Physiol. Psychol. 95, 450–459.

Drewett, R., 1973. Oestrous and dioestrous components of the ovarian inhibition on hunger in the rat. Anim. Behav. 21, 772–780.

Fleming, A.S., Gonzalez, A., Afonso, V.M., Lovic, V., 2008. Plasticity in the maternal neural circuit: experience, dopamine, and mothering. In: Bridges, R.S. (Ed.), Neurobiology of the Parental Brain. Academic Press, San Diego, pp. 519–535.

Handa, R.J., Mani, S.K., Uht, R.M., 2012. Estrogen receptors and the regulation of neural stress responses. Neuroendocrinology 96, 111–118.
Heine, P.A., Taylor, J.A., Iwamoto, G.A., Lubahn, D.B., Cooke, P.S., 2000. Increased adipose tissue in male and female estrogen receptor-alpha knockout mice. Proc. Natl. Acad. Sci. 97, 12729–12734. https://doi.org/10.1073/pnas.97.23.12729.
Nephew, B.C., Bridges, R.S., Lovelock, D.F., Byrnes, E.M., 2009. Enhanced maternal aggression and associated changes in neuropeptide gene expression in multiparous rats. Behav. Neurosci. 123, 949–957. https://doi.org/10.1037/a0016734.Enhanced.
Numan, M., Stolzenberg, D.S., 2009. Medial preoptic area interactions with dopamine neural systems in the control of the onset and maintenance of maternal behavior in rats. Front. Neuroendocrinol. 30, 46–64. https://doi.org/10.1016/j.yfrne.2008.10.002.
Numan, M., Rosenblatt, J.S., Komisaruk, B.R., 1977. Medial preoptic area and onset of maternal behavior in the rat. J. Comp. Physiol. Psychol. 91, 146–164.
Ogawa, S., Eng, V., Taylor, J., Lubahn, D.B., Korach, K.S., Pfaff, D.W., 1998. Roles of estrogen receptor-α gene expression in reproduction- related behaviors in female mice. Endocrinology. 139, 5070–5081. https://doi.org/10.1210/endo.139.12.6357.
Peña, C.J., Champagne, F.A., 2014. Neonatal overexpression of estrogen receptor-α alters midbrain dopamine neuron development and reverses the effects of low maternal care in female offspring. Devel. Neurobiol. 75, 1114–1124.
Poindron, P., Ric, F.-D.I., Vy, L.I., Krehbiel, D., 1988. Genital, olfactory, and endocrine interactions in the development of maternal behaviour in the parturient ewe. Psychoneuroendocrinology. 134, 99–125.
Ribeiro, A.C., Musatov, S., Shteyler, A., Simanduyev, S., Arrieta-Cruz, I., Ogawa, S., Pfaff, D.W., 2012. siRNA silencing of estrogen receptor-α expression specifically in medial preoptic area neurons abolishes maternal care in female mice. Proc. Natl. Acad. Sci. U. S. A. 109, 16324–16329.
Rosenblatt, J., 1967. Nonhormonal basis of maternal behavior in the rat. Science. 156, 1512–1514. https://doi.org/10.1126/science.156.3781.1512.
Rosenblatt, J.S., Siegel, H.I., 1975. Hysterectomy-induced maternal behavior during pregnancy in the rat. J. Comp. Physiol. Psychol. 89, 685–700. https://doi.org/10.1037/h0077052.
Rumi, M.A.K., Dhakal, P., Kubota, K., Chakraborty, D., Lei, T., Larson, M.A., Wolfe, M.W., Roby, K.F., Vivian, J.L., Soares, M.J., 2014. Generation of esr1-knockout rats using zinc finger nuclease-mediated genome editing. Endocrinology. 155, 1991–1999. https://doi.org/10.1210/en.2013-2150.
Santollo, J., Katzenellenbogen, B.S., Katzenellenbogen, J.A., Eckel, L., 2010. Activation of ERα is necessary for estradiol's anorexigenic effect in female rats. Horm. Behav. 58, 872–877. https://doi.org/10.1016/j.steroids.2010.06.016.
Scanlan, V.F., Byrnes, E.M., Bridges, R.S., 2006. Reproductive experience and activation of maternal memory. Behav. Neurosci. 120, 676–686. https://doi.org/10.1037/0735-7044.120.3.676.
Siegel, H.I., Doerr, H.K., Rosenblatt, J., 1978. Further studies on estrogen-induced maternal behavior in hysterectomized-ovariectomized virgin rats. Physiol. Behav. 21, 99–103.
Stolzenberg, D.S., Champagne, F.A., 2016. Hormonal and non-hormonal bases of maternal behavior: the role of experience and epigenetic mechanisms. Horm. Behav. 77, 2014–2210.
Stolzenberg, D.S., Rissman, E.F., 2011. Oestrogen-independent, experience-induced maternal behaviour in female mice. J. Neuroendocrinol. 23, 345–354.
Stolzenberg, D.S., Stevens, J.S., Rissman, E.F., 2012. Horm. Behav. 62, 128–135.
Zhou, H.B., Carlson, K.E., Stossi, F., Katzenellenbogen, B.S., Katzenellenbogen, J., 2009. Analogs of methyl-piperidinopyrazole (MPP): antiestrogens with estrogen recetpor alpha selective activity. Bioorg. Med. Chem. Lett. 19, 389–399. https://doi.org/10.1016/j.asieco.2008.09.006.