Journal of Neuroendocrinology

Journal of Neuroendocrinology, 2015, **27**, 245–252
© 2015 British Society for Neuroendocrinology

ORIGINAL ARTICLE

Effects of Reproductive Experience on Central Expression of Progesterone, Oestrogen α, Oxytocin and Vasopressin Receptor mRNA in Male California Mice (*Peromyscus californicus*)

J. P. Perea-Rodriguez*†, E. Y. Takahashi‡, T. M. Amador*, R. C. Hao‡, W. Saltzman*† and B. C. Trainor‡

**Department of Biology, University of California, Riverside, CA, USA.*

†*Evolution, Ecology, and Organismal Biology Graduate Program, University of California, Riverside, CA, USA.*

‡*Department of Psychology and Center for Neuroscience, University of California, Davis, CA, USA.*

Journal of Neuroendocrinology

Fatherhood in biparental mammals is accompanied by distinct neuroendocrine changes in males, involving some of the same hormones involved in maternal care. In the monogamous, biparental California mouse (*Peromyscus californicus*), paternal care has been linked to changes in the central and/or peripheral availability of oestrogen, progesterone, vasopressin and oxytocin, although it is not known whether these endocrine fluctuations are associated with changes in receptor availability in the brain. Thus, we compared mRNA expression of oestrogen receptor (ER)α, progesterone receptor (PR), vasopressin receptor (V1a) and oxytocin receptor (OTR) in brain regions implicated in paternal care [i.e. medial preoptic area (MPOA)], fear [i.e. medial amygdala (MeA)] and anxiety [i.e. bed nucleus of the stria terminalis (BNST)] between first-time fathers (n = 8) and age-matched virgin males (n = 7). Males from both reproductive conditions behaved paternally towards unrelated pups, whereas fathers showed significantly shorter latencies to behave paternally and less time investigating pups. Furthermore, fathers showed significantly lower PR, OTR and V1a receptor mRNA expression in the BNST compared to virgins. Fathers also showed a marginally significant (P = 0.07) reduction in progesterone receptor mRNA expression in the MPOA, although fatherhood was not associated with any other changes in receptor mRNA in the MPOA or MeA. The results of the present study indicate that behavioural and endocrine changes associated with the onset of fatherhood, and/or with cohabitation with a (breeding) female, are accompanied by changes in mRNA expression of hormone and neuropeptide receptors in the brain.

Correspondence to:
W. Saltzman, Department of Biology, University of California, Riverside, CA 92521, USA (e-mail: saltzman@ucr.edu).

Key words: oestrogens, oxytocin, progestogens, vasopressin, paternal care, fatherhood

doi: 10.1111/jne.12264

In most mammals, females are the sole caregivers of their young, and the onset of maternal care has been linked to the neuroendocrine changes that mothers undergo during gestation, parturition and lactation (1). In approximately 5–10% of mammalian genera, fathers also provide care for their offspring (2), and males in these biparental species typically undergo distinct hormonal changes when they become fathers or behave paternally (3). Importantly, the endocrine changes experienced by fathers involve some of the same hormones and neuropeptides that regulate maternal care (i.e. oestrogens, progestogens, prolactin, oxytocin and vasopressin, amongst others) (3,4). As in females, these steroids and peptides are presumed to act on the neural circuitry regulating parental behaviour, altering neural responses to stimuli from neonates and, as a result, influencing the probability of a male behaving paternally (1). To date no single, unifying model of the endocrine activation of male parental care in mammals has been established because a particular hormone can have contrasting effects on paternal care in different species (3).

In some biparental rodent species, males undergo changes in availability of and/or receptor densities for oestrogen (E_2) in the brain nuclei involved in paternal care [e.g. medial preoptic area of the hypothalamus (MPOA)] and anxiety [e.g. bed nucleus of the stria terminalis (BNST)] (4) at the onset of fatherhood. For example, male mandarin voles (*Microtus mandarinus*) show decreased oestrogen receptor (ER)α immunoreactivity (-IR) in the MPOA and BNST 2–3 days after the birth of their first litter compared to males with

no previous paternal experience (5). In the biparental California mouse (*Peromyscus californicus*), E_2 has been shown to increase parental care in males (6). Additionally, in this species, fathers show increased aromatase activity in the MPOA 2–3 weeks after the birth of their first litter (7). Higher aromatase activity presumably increases local E_2 levels within the brain with the onset of fatherhood (7).

Although the role of progesterone (P4) in the regulation of maternal care has been well studied (8,9), little is known about its involvement in paternal care. Work on the California mouse suggests a negative correlation between circulating P4 concentrations and the expression of paternal behaviour (7), although no experimental data are available to confirm a direct role of P4 in paternal care in any naturally biparental species. Findings from the uniparental house mouse (*Mus* sp.), however, indicate that P4 signalling can promote infanticide and inhibit paternal care. Male mice with genetic deletion of progesterone receptor (PR) show increased pup-directed care and reduced infanticide compared to wild-type males, and chronic administration of the PR antagonist RU486 to wild-type male mice increases paternal care (10). In addition, P4 administration increases pup-directed aggression (11).

Oxytocin has been implicated in a variety of social behaviours in mammals, including those involved in maternal care (4); however, as with P4, few data are available on the role of oxytocin in paternal care. Male California mice show changes in peripheral oxytocin levels with the pregnancy of their mate (12). In the Mandarin vole, both fathers and virgin males previously exposed to pups exhibit increased numbers of oxytocin-immunoreactive cells in the paraventricular nucleus of the hypothalamus and the supraoptic nucleus compared to males without previous pup experience (5).

Vasopressin (AVP) has been implicated in paternal care in both *Microtus* (13–15) and *Peromyscus* (16–19). For example, administration of AVP into the lateral ventricles or the lateral septum promotes paternal care in the prairie vole (*Microtus ochrogaster*) (15). Furthermore, meadow vole (*Microtus pennsylvanicus*) fathers, which show facultative paternal care, exhibit increased AVP receptor (V1a) densities in the anterior commissure, as well as decreased AVP receptor densities in the lateral septum, compared to nonfathers (20). Finally, male *P. californicus* and *P. leucopus* show a positive relationship between paternal care and AVP-IR in the BNST (16).

In the present study, we aimed to further characterise the neuroendocrine changes associated with the onset of paternal behaviour in the monogamous, biparental California mouse. Fathers in this species engage in high levels of paternal care, performing all the same behaviours as mothers, except for nursing (21); however, sexually naïve males are quite variable in their paternal responsiveness (22). As described above, previous studies have found changes in P4, aromatase activity, oxytocin and AVP with the onset of fatherhood in this species; however, the central expression of steroid and neuropeptide hormone receptors in new fathers has not been investigated. Therefore, we aimed to characterise central levels of receptor mRNA for behaviourally relevant hormones and neuropeptides in new fathers compared to virgin males, and to relate differences in receptor expression to differences in paternal behaviour. Specifically, we aimed to determine whether ERα, PR, oxytocin receptor (OTR) and V1a mRNA expression is affected by reproductive state (breeding versus nonbreeding, sexually inexperienced males) and how these neuroendocrine parameters correlate with behavioural responsiveness toward an unfamiliar pup in male California mice. We chose to examine gene expression because this is an efficient approach for assessing both steroid and neuropeptide signalling pathways in the same animals. Although validated antibodies are available for ERα and PR, validated antibodies of OTR and V1a are not commercially available.

We focused on the MPOA, medial amygdala (MeA) and BNST to determine how fatherhood may differentially affect hormone-receptor mRNA expression in these brain regions and thus how it might influence paternal behaviour and emotional states potentially associated with it. In female rodents, the onset of motherhood is associated with reductions in fear and anxiety, which have been suggested to facilitate the onset of maternal behaviour (4). We hypothesised that fatherhood would induce changes in ERα, PR, OTR and V1a expression in brain nuclei potentially involved in parental care because fathers of this species experience central and/or peripheral changes in E_2, P4, oxytocin and AVP availability (7,12,16). Moreover, we predicted that differences between fathers and virgin males in central expression of receptor mRNA would correlate with changes in behavioural responses to pups.

Materials and methods

Animals

We used California mice that were born and reared in our breeding 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 minimise inbreeding, we routinely 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 housed in same-sex groups consisting of four age-matched individuals (littermates and/or unrelated).

Animals were housed and maintained as described previously (23). Briefly, mice were housed in polycarbonate shoebox-type cages (44 × 24 × 20 cm) with aspen shavings and cotton wool (approximately 5 g), and were provided with Purina Rodent Chow 5001 (LabDiet, Richmond, IN, USA) and water *ad lib.* Animals were kept under a 14: 10 h light/dark cycle (lights on at 05.00 h). Room temperature and humidity were maintained at approximately 18–26 °C and 60–70%, respectively. All procedures used were in accordance with the Guide for the Care and Use of Laboratory Animals and were reviewed and approved by the UCR Institutional Animal Care and Use Committee. UCR is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

Reproductive conditions

When they were at least 90 days old (mean ± SE, 118.00 ± 1.08 days), male mice were randomly assigned to one of two reproductive conditions. Virgins (n = 7) were pair-housed with an unrelated male from their original same-sex group (cage mates were tested simultaneously), had no prior sexual experience and had never been exposed to a pup (except their own littermates) prior to testing. Fathers (n = 8) were paired with an unrelated, age-matched female and, eventually, their first litter of pups. No more than one male from a particular family was used in either condition.

© 2015 British Society for Neuroendocrinology

Behavioural testing and tissue collection

Virgins were 115–178 days old (mean ± SE, 157.00 ± 3.71 days) and had been paired for 41.50 ± 0.83 days at the time of behavioural testing (see below). Fathers were tested for their paternal response when they were 142–184 days of age (164.10 ± 1.88 days) and were tested 2–4 days after the birth of their first litter (time from pair formation to first birth: 41.44 ± 0.65 days). Thus, fathers had both sexual and parental experience at the time of testing, and their mates were lactating and very likely pregnant with their second litter because this species experiences postpartum oestrus (24). The age at testing did not differ significantly between virgins and fathers ($H = 29.00$, $P = 0.95$; Mann–Whitney U-test). Behavioural testing was performed in the colony room at 19.30–20.00 h, shortly after the onset of the active (lights off) phase. Mice were isolated in a clean cage with fresh bedding, food and water for 30 min prior to being tested.

At the beginning of each test (pup-test), an unrelated pup was placed at the end of the cage opposite to the focal male's location, and the animals were videotaped under red light for 10 min. Each test was later scored for paternal behaviours [latency to approach pup, latency to behave paternally (i.e. groom and/or huddle with pup), total duration of paternal behaviour and duration of investigating (i.e. sniffing) pup] using JWATCHER event-recorder software (22,25,26). The scorer of the behaviour for each video was blind to the male's reproductive condition. Immediately after the pup-test, adult males were decapitated, brains were dissected and placed on dry ice within 1 min of decapitation, and stored at −80 °C before being shipped to the University of California, Davis for mRNA quantification (see below).

Quantification of mRNA expression

Frozen brains were cut at 500 μm on a cryostat and then transferred to RNAlater (Life Technologies, Carlsbad, CA, USA). Sections were stored overnight at 4 °C. The MPOA, BNST and MeA were microdissected using a 1-mm punch sample as described previously (27) (Fig. 1). The MPOA samples were taken from a slice at approximately 0.02 from the bregma, and each punch was taken just lateral to the third ventricle (28). Bilateral BNST samples were taken from the same slice, with each punch taken just lateral to the fornix and dorsal to the anterior commissure. This punch sample includes the sexually dimorphic oval nucleus of the BNST and lateral posterior BNST (29). The MeA samples were taken from a slice at approximately −1.7 from the bregma. Each punch sample was taken immediately lateral to the optic tract. These punch samples include both the sexually dimorphic posterodorsal MeA and posteroventral MeA (29). Punch samples were frozen on dry ice and stored at −40 °C until RNA extraction. Total RNA was extracted using Ambion RNAqueous Micro-kits (AM1931; Life Technologies) and all samples were treated with DNAse. Next, 1 μg of RNA was reverse transcribed using an iScript cDNA synthesis kit (170-8891; Bio-Rad, Hercules, CA, USA).

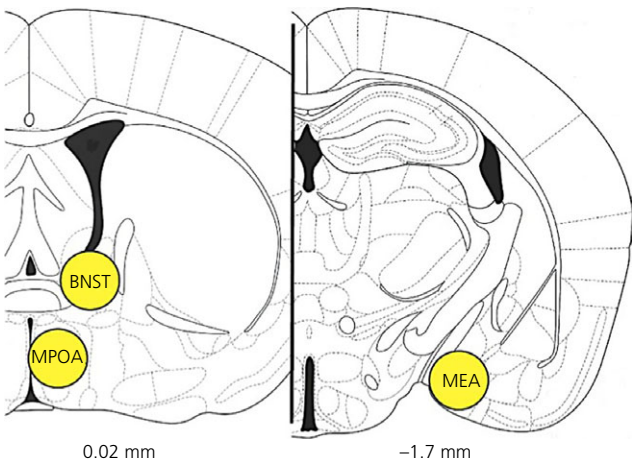

Fig. 1. Diagram indicating the location of punch samples collected for real-time polymerase chain reaction analysis. MPOA, medial preoptic area; BNST, bed nucleus of the stria terminalis; MeA, medial amygdala. Reproduced with permission from Paxinos and Franklin (46).

For the real-time polymerase chain reaction (PCR), Taqman chemistry was used to detect OTR, V1a and ERα gene expression as described previously (30). SYBR green chemistry was used to detect total PR gene expression, and a melt curve analysis indicated that a single PCR product was formed by this primer set. Sanger Sequencing (ABI Prism® 3730 Genetic Analyzer) was used to confirm product specificity. Relative gene expression was calculated by comparison with a standard curve consisting of serial dilutions of pooled California mouse hypothalamic samples (1: 2, 1: 4, 1: 5 and 1: 10) followed by normalisation to 18S gene expression (Applied Biosystems, Foster City, CA, USA). Primer and probe sequences are listed in Table 1.

Statistical analysis

Some samples were not used in some of the analyses because of complications with the dissection/extraction process. As a result, samples sizes varied across analyses (for final sample sizes, see Results).

All statistical analyses were completed using R statistical software (31). Normality was tested using Shapiro–Wilk tests. Behavioural and neuroendocrine (i.e. receptor mRNA expression) measures were compared between reproductive conditions (fathers and virgins) using t-tests for normally distributed data and Mann–Whitney U-tests for non-normal data. Spearman's rho was used to correlate receptor mRNA expression in the MPOA, BNST and MeA with males' behavioural responses to pups, as well as for correlations between behaviours. Correlations were performed using data for fathers and virgins pooled together, as well as separately. To determine whether age affects a male's behavioural response to a pup or central hormone receptor mRNA expression, we performed correlations between age and the neuroendocrine and behavioural measures recorded. One MEA sample (one father) did not yield useable RNA and during quantitative PCR analyses, two MEA samples (two virgins, one father) did not show amplification of 18S and were excluded from the analyses.

Results

Paternal behaviour

All virgin males and all fathers behaved paternally (i.e. licked and/or huddled) towards an experimentally presented pup. Fathers, however, had shorter latencies to initiate paternal behaviour compared to virgins ($H = 9.00$, $P = 0.02$; Mann–Whitney U-test) (Fig. 2). Additionally, fathers tended to have higher scores for their overall paternal response (i.e. total duration of licking + huddling; $P = 0.07$) and shorter latencies to approach pups ($P = 0.09$) compared to virgins (Fig. 2); however, these trends were not statistically significant. On the other hand, virgins spent more time investigating pups compared to fathers ($H = 8.00$, $P = 0.02$; Mann–Whitney U-test) (Fig. 2). No other differences in behavioural responses to pups were found between fathers and virgins.

Correlations between behavioural measures

Because all males in both conditions behaved paternally, Spearman correlations between behavioural measures were performed using

© 2015 British Society for Neuroendocrinology

Table 1. Primer and Probe Combinations for Quantitative Polymease Chain Reaction.

Gene	Accession number	Forward primer	Reverse primer	Probe
Oxytocin receptor	HQ651236	GCCCTTGACGCCTTTCTTCT	TTCCTTGGGCGCATTGAC	CGTGCAGATGTGGAGCGTCTGGG
V1a receptor	GU254591	CGCCTCTTGGGTGCTGAGT	CGATTTCGATCATAGAGAAGATGAAGT	CTACTGAGCACACCGCA
Oestrogen receptor α	DQ357060.1	GAACAGCCCCGCCTTGT	GCATCCAGCAAGGCACTGA	TGACAGCTGACCAGATG
Progesterone receptor	KJ364650	AGGACGCCTTCCCTCTCTAT	CCAGCGGGAAATCCGGAAAG	N/A

pooled data from all mice; results are presented in Table 2. We found a negative correlation between males' latency to approach pups and overall paternal response ($\rho = -0.84$, $P = 0.0003$, $n = 15$). Additionally, males that had longer latencies to approach pups also had longer latencies to behave paternally ($\rho = 0.91$, $P < 0.0001$, $n = 15$). Time spent investigating pups was positively correlated with both latency to approach pups ($\rho = 0.74$, $P = 0.002$, $n = 15$) and latency to behave paternally ($\rho = 0.84$, $P < 0.001$, $n = 15$). Finally, males that spent more time investigating pups showed a lower overall paternal response ($\rho = -0.90$, $P < 0.0001$, $n = 15$).

Receptor gene expression

Fathers and virgin males differed significantly in expression of mRNA for several receptor types in the BNST (Fig. 3). Fathers had lower BNST mRNA expression for OTR ($t = -2.29$, d.f. = 12, $P = 0.05$), V1a ($t = 3.00$, d.f. = 14, $P = 0.02$) and PR ($t = 2.44$, d.f. = 12, $P = 0.03$) but not ERα ($H = 7.00$, $P = 0.23$; Mann–Whitney U-test) compared to virgins. In the MPOA, fathers showed a nonsignificant reduction in PR mRNA expression ($t = 6.00$, d.f. = 4.15, $P = 0.07$) compared to virgins but no difference in V1a ($t = -1.15$, d.f. = 4.81, $P = 0.24$), OTR ($t = -0.79$, d.f. = 10, $P = 0.44$) or ERα ($H = 12.0$, $P = 0.43$) mRNA expression (Fig. 3). In the MeA, fathers and virgins did not differ in mRNA expression of PR ($t = -1.93$, d.f. = 6, $P = 0.10$), ERα ($t = -1.01$, d.f. = 6, $P = 0.21$), OTR ($t = 1.69$, d.f. = 6, $P = 0.14$) or V1a ($t = 0.07$, d.f. = 1, $P = 0.94$).

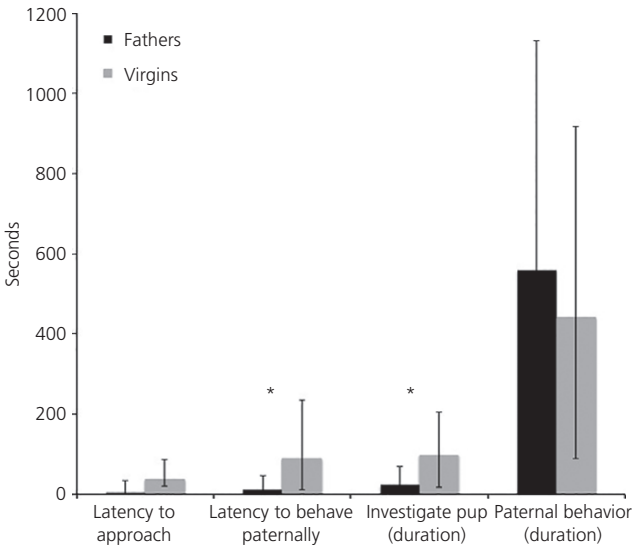

Fig. 2. Behavioural responses to an unrelated pup by fathers ($n = 8$) and virgin males ($n = 7$). Bars represent medians and error bars represent the first and third quartiles. Asterisks indicate significant differences between reproductive conditions ($P < 0.05$).

Correlations between behavioural measures and receptor mRNA expression

Correlations of behavioural and neuroendocrine data within each reproductive condition did not reveal any significant relationships between variables. For virgins, however, we found a marginally significant negative correlation between time spent investigating the experimentally presented pup and OTR mRNA expression in the BNST ($\rho = -0.82$, $P = 0.05$, $n = 7$).

When we pooled data from fathers and virgins, we found a positive relationship between V1a mRNA expression in the BNST and latency for males to behave paternally ($\rho = 0.58$, $P = 0.04$, $n = 14$) (Fig. 4). Levels of PR mRNA expression in the BNST showed a significant positive correlation with the duration of time males spent investigating the experimentally presented pup ($\rho = 0.59$, $P = 0.03$, $n = 13$) (Fig. 4). Similarly, PR mRNA expression in the MPOA was positively correlated with time spent investigating pups ($\rho = 0.54$, $P = 0.04$, $n = 12$) (Fig. 4). Finally, OTR expression in the MeA was negatively correlated with latency to behave paternally ($\rho = -0.80$, $P = 0.02$, $n = 8$).

Correlations between age of animals and the various behavioural and neuroendocrine measures did not reveal any significant relationships.

Discussion

In the present study, we aimed to characterise potential differences between first-time fathers and virgin male California mice in the central expression of PR, ERα, OTR and vasopressin (V1a) receptor gene expression. We focused on the MPOA, MeA and BNST, which are brain areas involved in paternal care, fear and anxiety. We further aimed to investigate correlations between these measures and males' behavioural responses to an unfamiliar, unrelated pup. Our results indicate that male California mice show distinct differences in receptor mRNA expression with respect to reproductive condition. Specifically, fathers had significantly lower PR, OTR and V1a mRNA expression in the BNST compared to virgins. Father and virgins had no significant differences in expression of ERα mRNA in

© 2015 British Society for Neuroendocrinology

Table 2. Spearman Correlations between Behavioural Measures, Using Pooled Data From Fathers and Virgin Males.

	Latency to behave paternally (huddle or groom pup)	Paternal behaviour (huddle + groom pup; duration)	Investigate pup (duration)
Latency to approach pup	**ρ = 0.91, P < 0.0001,** n = 15	**ρ = −0.84, P = 0.0003,** n = 15	**ρ = 0.74, P = 0.002,** n = 15
Latency to behave paternally (huddle or groom pup)		**ρ = −0.86, P < 0.001,** n = 15	**ρ = 0.84, P < 0.001,** n = 15
Paternal behaviour (huddle + groom pup; duration)			**ρ = −0.90, P < 0.0001,** n = 15

P-values ≤ 0.05 and corresponding ρ values are shown in bold.

the BNST, nor in mRNA expression for any receptors in the MeA or MPOA. Fathers did, however, tend to have lower PR mRNA expression in the MPOA (P = 0.07).

Our behavioural results confirm that captive male California mice can be paternally responsive independent of reproductive condition because both virgins and fathers huddled with and/or licked pups (22,32,33). Nonetheless, fathers and virgins differed in specific components of their behavioural response to a pup: fathers had shorter latencies to behave paternally, and spent less time investigating pups, compared to virgins. Correlations between behavioural measures revealed that latencies to approach pups and time spent investigating pups were negatively correlated with a male's parental response (duration of huddling and licking). These findings suggest that the investigation of pups is distinct from affiliative components of paternal behaviour and in fact shows a negative relationship with paternal responsiveness.

In rodents, the MPOA is essential for the expression of both maternal and paternal behaviour (4). Studies in California mice show that lesions of the MPOA cause deficits in parental care in both sexes (34,35), and parentally experienced males show increased Fos-IR in the MPOA after being exposed to an unrelated pup compared to virgin males or males whose mates were pregnant with their first litter (19). Our results suggest that changes in PR mRNA in the MPOA might influence paternal care in the California mouse because males with paternal experience (fathers) tended (P = 0.07) to have a lower expression of PR mRNA in the MPOA, compared to virgin males. Circulating concentrations of P4, which might be negatively correlated with PR density (36), are lower in California mouse fathers 2–3 weeks after the birth of their first litter compared to nonfathers (7); however, this may not reflect P4 signalling during the early postpartum period (e.g. 2–4 days postpartum), which is the time frame used in the present study. Alter-

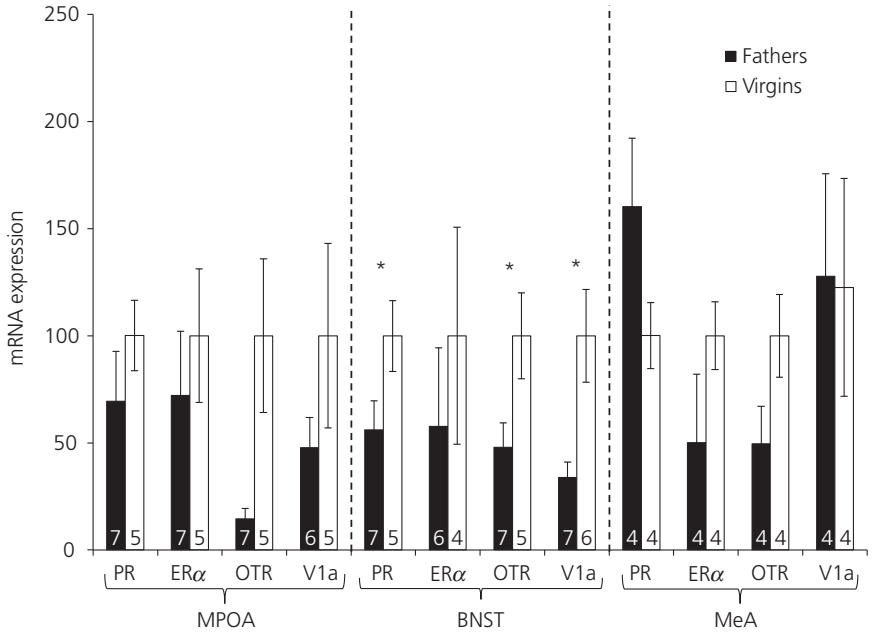

Fig. 3. mRNA expression for hormone and neuropeptide receptors in the medial preoptic area (MPOA) (left), bed nucleus of the stria terminalis (BNST) (centre) and medial amygdala (MeA) (right) of fathers (black bars) and virgin males (white bars). Bars represent means and error bars represent the SEM, and sample sizes for each brain nucleus and receptor type are shown within each bar. Asterisks indicate significant differences between reproductive conditions (P < 0.05). PR, progesterone receptor; ER, oestrogen receptor; OTR, oxytocin receptor; V1a, vasopressin receptor.

 © 2015 British Society for Neuroendocrinology

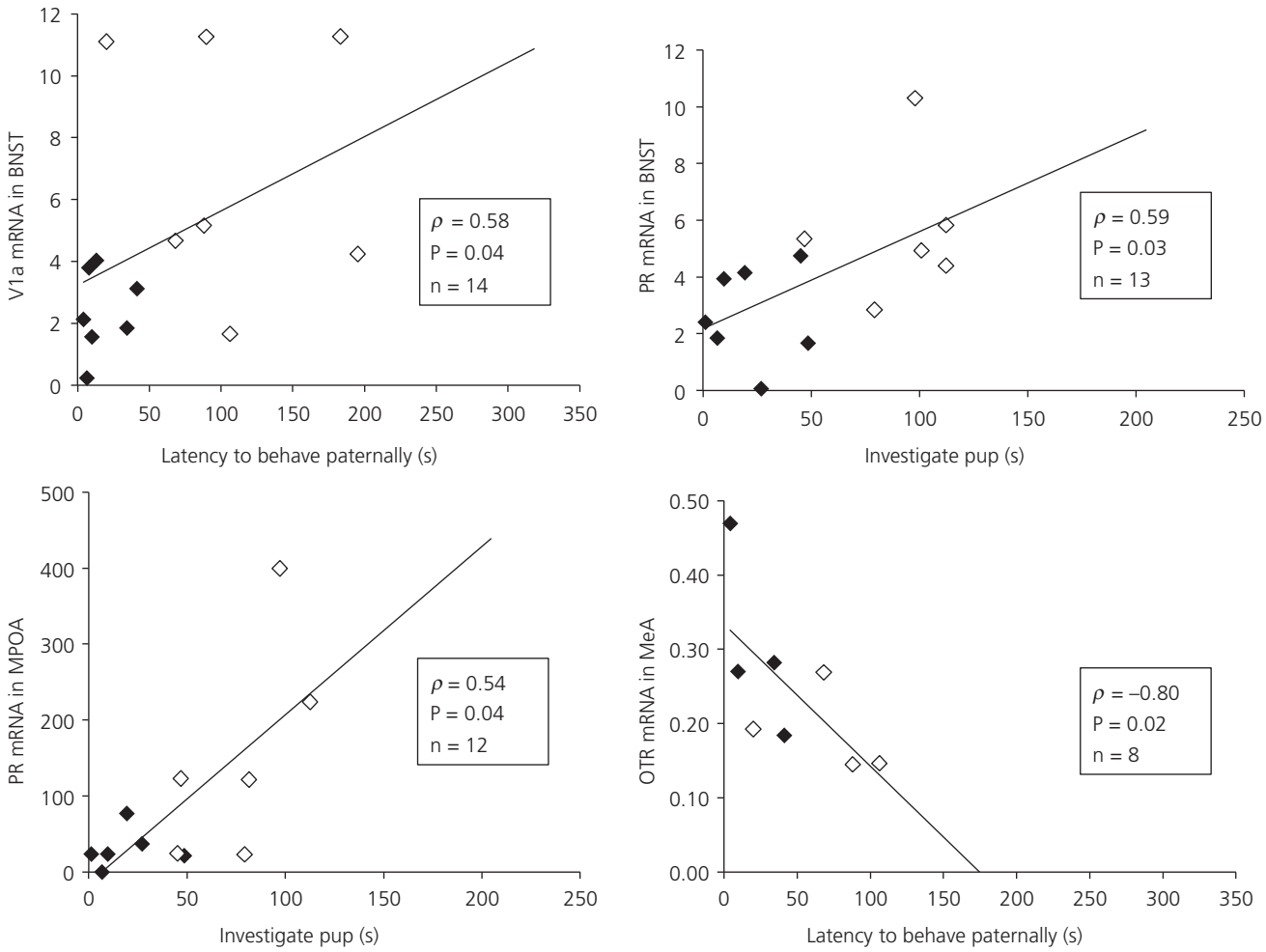

Fig. 4. Spearman correlations between pooled behavioural and mRNA data from fathers (black) and virgins (white).

natively, PR expression in fathers could have been influenced by other endocrine changes, such as possible changes in E_2 availability within the brain (37). Independent of the mechanism, males might experience reduced expression of PR mRNA during the early postpartum period, when altricial pups are in most need of parental care (38). Because P4 signalling has been shown to inhibit paternal care and increase rates of infanticide in uniparental house mice (10,11), it may be beneficial for males to become less responsive to P4 when they become fathers. Indeed, we found a positive correlation between PR mRNA expression in the MPOA and time spent investigating pups.

Fathers showed a lower expression of mRNA for PR, OTR and V1a receptors in the BNST, suggesting that the BNST potentially becomes less responsive to these hormones and neuropeptides before or during the early postpartum period. In rodents, the BNST is strongly implicated in the behavioural response to aversive, unpredictable stimuli (i.e. anxiety) (39), although it has also been implicated in paternal care and aggression. For example, California mouse fathers show an increase in Fos-IR in the BNST after exposure to a mesh ball containing an unrelated pup compared to exposure to an empty mesh ball, as well as compared to virgin males similarly exposed to a pup (19). Additionally, among California mice housed under a short-day length regime, which increases aggressive behaviour, males tested in a resident–intruder aggression test have elevated levels of cellular activity (as estimated by phosphorylated extracellular signal regulated kinase) in the posterior BNST compared to males under the same day-length regime not exposed to an intruder (40).

As discussed above, P4 has been shown to negatively influence males' parental response (3). We found that PR mRNA expression in the BNST was positively correlated with the time spent investigating pups, and males that spent more time investigating showed an overall lower paternal response. Thus, our correlational data suggest that a reduced expression of PR mRNA in the BNST might increase a male's paternal responsiveness.

In contrast to P4, both OT and AVP have been shown to enhance paternal care in biparental rodents (41,42). Consistent with these findings, males with higher levels of OTR expression in the MeA were faster to display paternal behaviour than males with lower levels of OTR. By contrast, males with more V1a mRNA expression in the BNST were slower to behave paternally, suggesting that a reduced expression of V1a mRNA in the BNST might

© 2015 British Society for Neuroendocrinology

reduce paternal motivation in California mice. Typically, stimulatory effects of AVP on paternal behaviour are considered to be mediated by projections from the BNST to the lateral septum (15), rather than the actions of AVP within the BNST. On the other hand, the effects of OT on parental behaviour could be mediated by the local effects of OT in the BNST and paraventricular nucleus of the hypothalamus (42,43). The mechanism that results in decreased OTR and V1a mRNA expression in the BNST in fathers compared to virgin males is unknown. At this point, it is not clear why, at a functional level, the expression of mRNA for OTR and V1a receptors in the BNST would decrease in fathers. One possibility is that these neuroendocrine changes might not affect paternal care directly but might regulate paternal aggression in this species because California mouse fathers are significantly more aggressive than virgins (44).

E_2 is important for the maintenance of paternal care in the California mouse. Castration reduces paternal responsiveness in experienced fathers 2–3 weeks after birth of a litter, whereas E_2 treatment restores paternal care (6). During this same period, new fathers have higher aromatase activity in the MPOA compared to males without paternal experience (7). In the present study, we found no significant difference 2–4 days postpartum in ERα mRNA expression in either the MPOA or the BNST of fathers compared to virgins. Moreover, ERα mRNA expression was not correlated with any aspect of males' behavioural responses to a pup. It is possible that local increases in E_2 within the MPOA in fathers are not associated with changes in receptor mRNA expression in the same region. Alternatively, increased local aromatase activity might be accompanied by an increase in ERα expression in the MPOA, although these effects might develop later in the postpartum period compared to the time period used in the present study.

Several limitations of the present study should be taken into consideration when interpreting our results. First, because males that become fathers experience several behavioural and physiological changes during pair-bonding (4), we cannot determine whether the behavioural and neuroendocrine changes that we found in fathers result from pair-bonding or from becoming a father, engaging in paternal care and/or cohabiting with a (pregnant) female. A second limitation is that punch-sampling techniques have a coarser level of anatomical detail than immunohistochemistry or *in situ* hybridisation. For example, it is not possible to determine whether proteins are expressed in the cell bodies, dendrites or nuclei based on quantitative PCR. However, some of our observations are consistent with previous immunostaining studies. Our observation of no difference in ERα mRNA conforms with the results reported in a previous study showing no difference in ERα immunoreactivity in the MPOA or BNST between virgins and fathers (45). Third, it is possible that changes in PR and neuropeptide receptor mRNA might not be reflected at the protein level. This can be addressed in future studies, which could also test whether these receptors directly regulate paternal behaviour. Fourth, because the BNST is involved in several physiological and/or behavioural functions, including anxiety, aggression and stress, changes in receptor mRNA expression in this region might not relate directly to changes in paternal responsiveness or anxiety. Finally, because we used relatively small sample sizes, our analyses had low statistical power; however, our results suggest that site-specific manipulations of PR in the MPOA and BNST, as well as neuropeptide receptors in the BNST, are excellent candidates for further study.

In summary, the present is the first to demonstrate that California mouse fathers experience changes in P4, OT and AVP (V1a) receptor gene expression in brain nuclei important in paternal care and anxiety, and that these changes correlate with aspects of behavioural responsiveness to pups. In some rodent species, the onset of both motherhood and fatherhood is associated with changes in parents' levels of anxiety and behavioural responses to stressors (3,4), and the BNST is heavily involved in regulating anxiety-like and/or aggressive behaviours (39,40). It is possible, therefore, that changes in hormone and neuropeptide receptor expression in the BNST associated with fatherhood may lead to changes in males' behavioural responses to a pup (or to novel stimuli in general) through changes in their state of anxiety (or aggression), facilitating contact between fathers and their offspring. Further work should investigate the mechanisms underlying these changes, as well as their causal role, if any, in the onset of paternal care.

Acknowledgements

We would like to thank Dr. Akiko Sato, Linda McCloud, and Johnny Phan for assistance with animal care and maintenance; Pauline Nguyen, Mindy Hernandez, Aaron Stamp, Ashwin Sharma, and Eric Kung for assistance with experimental procedures; and three anonymous reviewers for very helpful comments on an earlier version of the manuscript. This study was supported by NIH grants R21 MH087806 to WS and R01 MH085069 to BCT, and by funds provided by the University of California, Riverside.

Received 28 April 2014,
revised 3 February 2015,
accepted 4 February 2015

References

1 Brunton PJ, Russell JA. The expectant brain: adapting for motherhood. *Nat Rev Neurosci* 2008; **9**: 11–25.
2 Kleiman DG, Malcolm JR. The evolution of male parental investment in mammals. In: Gubernick DJ, Klopfer PH, eds. *Parental Care in Mammals*. New York, NY: Plenum Press, 1981: 347–387.
3 Saltzman W, Ziegler TE. Functional significance of hormonal changes in mammalian fathers. *J Neuroendocrinol* 2014; **26**: 685–696.
4 Numan M, Insel TR. *The Neurobiology of Parental Behavior*. New York, NY: Springer, 2003.
5 Song Z, Tai F, Yu C, Wu R, Zhang X, Broders H, He F, Guo R. Sexual or paternal experiences alter alloparental behavior and the central expression of ERα and OT in male mandarin voles (*Microtus mandarinus*). *Behav Brain Res* 2010; **214**: 290–300.
6 Trainor BC, Marler CA. Testosterone promotes paternal behaviour in a monogamous mammal via conversion to oestrogen. *Proc R Soc Lond* 2002; **269**: 823–829.
7 Trainor BC, Bird IM, Alday NA, Schlinger BA, Marler CA. Variation in aromatase activity in the medial preoptic area and plasma progesterone is associated with the onset of paternal behavior. *Neuroendocrinology* 2003; **78**: 36–44.

 © 2015 British Society for Neuroendocrinology

8 Siegel HI, Rosenblatt JS. Progesterone inhibition of estrogen-induced maternal behavior in hysterectomized-ovariectomized virgin rats. *Horm Behav* 1975; **6**: 223–230.

9 Siegel HI, Rosenblatt JS. Duration of estrogen stimulation and progesterone inhibition of maternal behavior in pregnancy-terminated rats. *Horm Behav* 1978; **11**: 12–19.

10 Schneider JS, Stone MK, Wynne-Edwards KE, Horton TH, Lydon J, O'Malley B, Levine JE. Progesterone receptors mediate male aggression toward infants. *Proc Natl Acad Sci USA* 2003; **100**: 2951–2956.

11 Schneider JS, Burgess C, Horton TH, Levine JE. Effects of progesterone on male-mediated infant-directed aggression. *Behav Brain Res* 2009; **199**: 340–344.

12 Gubernick DJ, Winslow JT, Jensen P, Jeanotte L, Bowen J. Oxytocin changes in males over the reproductive cycle in the monogamous, biparental California mouse *Peromyscus californicus*. *Horm Behav* 1995; **29**: 59–73.

13 Insel TR, Wang ZX, Ferris CF. Patterns of brain vasopressin receptor distribution associated with social organization in Microtine rodents. *J Neurosci* 1994; **14**: 5381–5392.

14 Parker KJ, Lee TM. Central vasopressin administration regulates the onset of facultative paternal behavior in *Microtus pennsylvanicus* (meadow voles). *Horm Behav* 2001; **39**: 285–294.

15 Wang Z, Ferris CF, De Vries GJ. Role of septal vasopressin innervation in paternal behavior in prairie voles (*Microtus ochrogaster*). *Proc Natl Acad Sci USA* 1994; **91**: 400–404.

16 Bester-Meredith JK, Marler CA. Vasopressin and the transmission of paternal behavior across generations in mated, cross-fostered *Peromyscus* mice. *Behav Neurosci* 2003; **117**: 455–463.

17 Frazier CRM, Trainor BC, Cravens CJ, Whitney TK, Marler CA. Paternal behavior influences development of aggression and vasopressin expression in male California mouse offspring. *Horm Behav* 2006; **50**: 699–707.

18 Lambert KG, Franssen CL, Bardi M, Hampton JE, Hainley L, Karsner S, Tu EB, Hyer MM, Crockett C, Baranova A, Ferguson T, Ferguson T, Kinsley CH. Characteristic neurobiological patterns differentiate paternal responsiveness in two *Peromyscus* species. *Brain Behav Evol* 2011; **77**: 159–175.

19 de Jong TR, Chauke M, Harris BN, Saltzman W. From here to paternity: neural correlates of the onset of paternal behavior in California mice (*Peromyscus californicus*). *Horm Behav* 2009; **56**: 220–231.

20 Parker KJ, Kinney LF, Phillips KM, Lee TM. Paternal behavior is associated with central neurohormone receptor binding patterns in meadow voles (*Microtus pennsylvanicus*). *Behav Neurosci* 2001; **115**: 1341–1348.

21 Gubernick DJ, Alberts JR. Postpartum maintenance of paternal behaviour in the biparental California mouse *Peromyscus californicus*. *Anim Behav* 1989; **37**: 656–664.

22 de Jong TR, Korosi A, Harris BN, Perea-Rodriguez JP, Saltzman W. Individual variation in paternal responses of virgin male California mice (*Peromyscus californicus*): behavioral and physiological correlates. *Physiol Biochem Zool* 2012; **85**: 740–751.

23 Harris BN, Perea-Rodriguez JP, Saltzman W. Acute effects of corticosterone injection on paternal behavior in California mouse (*Peromyscus* californicus) fathers. *Horm Behav* 2011; **60**: 666–675.

24 Gubernick DJ. Reproduction in the California mouse *Peromyscus californicus*. *J Mammal* 1988; **69**: 857–860.

25 Blumstein D, Daniel JC. *Quantifying Behavior the JWatcher Way*. Sunderland, MA: Sinauer Associates, 2007.

26 de Jong TR, Measor KR, Chauke M, Harris BN, Saltzman W. Brief pup exposure induces Fos expression in the lateral habenula and serotonergic caudal dorsal raphe nucleus of paternally experienced male California mice (*Peromyscus californicus*). *Neuroscience* 2010; **169**: 1094–1104.

27 Greenberg GD, Laman-Maharg A, Campi KL, Voigt H, Orr VN, Trainor BC. Sex differences in stress-induced social withdrawal: role of brain derived neurotrophic factor in the bed nucleus of the stria terminalis. *Front Behav Neurosci* 2014; **7**: 1–14.

28 Trainor BC, Rowland MR, Nelson RJ. Photoperiod affects estrogen receptor α, estrogen receptor β and aggressive behavior. *Eur J Neurosci* 2007; **26**: 207–218.

29 Campi KL, Jameson CE, Trainor BC. Sexual dimorphism in the brain of the monogamous California mouse (*Peromyscus californicus*). *Brain Behav Evol* 2013; **81**: 236–249.

30 Laredo SA, Orr VN, McMackin MZ, Trainor BC. The effects of exogenous melatonin and melatonin receptor blockade on aggression and estrogen-dependent gene expression in the California mouse (*Peromyscus californicus*). *Physiol Behav* 2014; **128**: 86–91.

31 R Core Team. *R: A language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna. URL: http://www.R-project.org/.

32 Gubernick DJ, Schneider KA, Jeannotte LA. Individual differences in the mechanisms underlying the onset and maintenance of paternal behavior and the inhibition of infanticide in the monogamous biparental California mouse *Peromyscus californicus*. *Behav Ecol Sociobiol* 1994; **34**: 225–231.

33 Lambert KG, Franssen CL, Hampton JE, Rzucidlo AM, Hyer MM, True M, Kaufman C, Bardi M. Modeling paternal attentiveness: distressed pups evoke differential neurobiological and behavioral responses in paternal and nonpaternal mice. *Neuroscience* 2013; **234**: 1–12.

34 Lee AW, Brown RE. Medial preoptic lesions disrupt parental behavior in both male and female California mice (*Peromyscus californicus*). *Behav Neurosci* 2002; **116**: 968–975.

35 Lee AW, Brown RE. Comparison of medial preoptic, amygdala, and nucleus accumbens lesions on parental behavior in California mice (*Peromyscus californicus*). *Physiol Behav* 2007; **92**: 617–628.

36 Bouchard P. Progesterone and the progesterone receptor. *J Reprod Med* 1999; **44**: 153–157.

37 Sa SI, Pereira PA, Malikov V, Madeira MD. Role of estrogen receptor alpha and beta in the induction of progesterone receptors in hypothalamic ventromedial neurons. *Neuroscience* 2013; **238**: 159–167.

38 Gubernick DJ, Alberts JR. 'Resource' exchange in the biparental California mouse (*Peromyscus californicus*): water transfer from pups to parents. *J Comp Psychol* 1987; **101**: 328–334.

39 Davis M. Neural systems involved in fear and anxiety measured with fear-potentiated startle. *Am Psychol* 2006; **61**: 741–756.

40 Trainor BC, Crean KK, Fry WH, Sweeney C. Activation of extracellular signal-regulated kinases in social behavior circuits during resident-intruder aggression tests. *Neuroscience* 2010; **165**: 325–336.

41 Wynne-Edwards KE, Timonin ME. Paternal care in rodents: weakening support for hormonal regulation of the transition to behavioral fatherhood in rodent animal models of biparental care. *Horm Behav* 2007; **52**: 114–121.

42 Bosch OJ, Neumann ID. Both oxytocin and vasopressin are mediators of maternal care and aggression in rodents: From central release to sites of action. *Horm Behav* 2012; **61**: 293–303.

43 Neumann ID. Brain oxytocin: a key regulator of emotional and social behaviours in both females and males. *J Neuroendocrinol* 2008; **20**: 858–865.

44 Trainor BC, Sima Finy M, Nelson RJ. Rapid effects of estradiol on male aggression depend on photoperiod in reproductively non-responsive mice. *Horm Behav* 2008; **53**: 192–199.

45 Trainor BC, Finy MS, Nelson RJ. Paternal aggression in a biparental mouse: parallels with maternal aggression. *Horm Behav* 2008; **53**: 200–207.

46 Paxinos G, Franklin KBJ. *The Mouse Brain in Stereotaxic Coordinates*. New York, NY: Academic Press, 2001.

© 2015 British Society for Neuroendocrinology