Brain aromatase dynamics reflect parental experience and behaviour in male mice

Paula Duarte-Guterman[1], Dimitri A. Skandalis[2]*, Ariane Merkl*, Diana B. Geissler, Günter Ehret

Institute of Neurobiology, University of Ulm, D-89069 Ulm, Germany

* Contributed equally to this project

[1] Present address: Department of Psychology and Centre for Neuroscience, Brock University, ON, Canada.

[2] Present address: Psychological & Brain Sciences and Johns Hopkins Kavli Neuroscience Discovery Institute, Johns Hopkins University, MD, USA

Corresponding authors:

Paula Duarte-Guterman; email: pduarteg@brocku.ca

Günter Ehret; email: guenter.ehret@uni-ulm.de

Keywords: brain plasticity, estrogen, hemisphere lateralization, infanticide, limbic system, paternal care

Abbreviations: BNST, bed nucleus of the stria terminalis; CAM, cortical amygdala; LS, lateral septum; LSD, lateral septum dorsal; LDI, lateral septum intermediate; LSV, lateral septum ventral; MAM, medial amygdala; MPOA, medial preoptic area; NAC, nucleus accumbens; PIR, piriform cortex; VMH, ventromedial nucleus of the hypothalamus.

Abstract

In most mammals, providing paternal care is not automatic. In house mice, experience with pups governs the extent and quality of paternal care. First-time fathers undergo a dramatic transition from ignoring or killing pups to caring for pups. The behavioural shift occurs together with changes in brain estrogen signalling as indicated by changes in estrogen receptor presence and distribution in multiple areas regulating olfaction, emotion, and motivation. Here, we report estrogen dynamics by altered local estrogen synthesis via changes in aromatase, the enzyme converting testosterone into estrogen. The amount of paternal experience (5 or 27 days) was associated with increased numbers of immunocytochemically-identified aromatase expressing cells in the medial and cortical amygdala, piriform cortex, and ventromedial hypothalamus. In the lateral septum, and to some extent in the medial preoptic area, parental experience increased aromatase only in fathers with 27 days of experience, and only in the right hemisphere, a new case of brain-functional lateralisation with experience. Nuclei/areas associated with maternal care (medial preoptic area, bed nucleus of stria terminalis, nucleus accumbens) exhibited a left-hemisphere advantage in aromatase expressing cells. This newly found lateralisation may contribute to the left-hemisphere dominant processing and perception of pup calls to release parental behaviour.

Introduction

Mammalian paternal care is facultative, and the behavioural and neural dynamics that result in becoming a caring father are less well understood compared to those of obligatory maternal care (e.g., (Bales and Saltzman, 2016; Brunton and Russell, 2008; Dulac et al., 2014; Guoynes and Marler, 2020; Horrell et al., 2021; Lonstein and De Vries, 2000; Numan, 2020; Numan and Insel, 2003; Rilling and Mascaro, 2017; Woodroffe and Vincent, 1994)). In rodents and primates, it is hypothesised that maternal care is induced by hormonal changes during pregnancy and parturition (onset phase) whereas the continuation of pup-caring (maintenance phase) is governed by sensory stimuli from the pups (Brunton and Russell, 2008; Fahrbach and Pfaff, 1982; Rosenblatt and Siegel, 1981). In house mice, pup-naïve females, males, and even previous fathers do not experience the onset phase. Rather, they require various amounts of experience with pups (hours up to several days of contact) for the activation of caring behaviour (Akther et al., 2015; Ehret et al., 1993, 1987; Ehret and Buckenmaier, 1994; Ehret and Koch, 1989; Elwood, 1985; Koch and Ehret, 1989a; Stolzenberg and Rissman, 2011; Tachikawa et al., 2013). Nonetheless, pup-naïve females engage in consummatory (pup retrieval) and appetitive (preference for pup ultrasounds) behaviours (Koch and Ehret, 1991; Numan, 2006) after fewer days of experience with pups than either pup-naïve males or previous fathers (Ehret et al., 1993, 1987; Ehret and Buckenmaier, 1994). Pup naïve females thus appear to be more easily sensitised or motivated by pup cues than males and even fathers to provide parental care. This observation is suggestive of sex differences in the neural mechanisms underlying the activation of maternal versus paternal instincts.

Numerous studies have characterised the role of the limbic regions in the activation and regulation of parental behaviours. These regions include the medial preoptic area (MPOA), bed nucleus of the stria terminalis (BNST), lateral septum (LS), nucleus accumbens (NAC), nuclei of the amygdala, and ventromedial hypothalamus (VMH). Olfaction is thought

to provide the main modulatory sensory input, through pathways including the vomeronasal organ to the BNST and amygdala; the olfactory bulb to the amygdala; via the VMH to the LS; and via the piriform cortex (PIR) and hippocampus to the LS (e.g., (Bridges, 2015; Brunton and Russell, 2008; Dulac et al., 2014; Ehret et al., 1991; Kohl and Dulac, 2018; Numan, 2020, 2006)). Estradiol treatment can activate both maternal and paternal instincts (Ehret and Koch, 1989; Koch and Ehret, 1989a; Poindron et al., 1988; Romero-Morales et al., 2018; Rosenblatt et al., 1994; Rosenblatt and Ceus, 1998; Siegel and Rosenblatt, 1975) and changes in brain estrogen signaling (through estrogen receptors) may be part of the underlying mechanism. In house mice, expression of parental behaviours coincides with changes in the distribution of estrogen receptor (ERα) positive cells in limbic brain regions of pup-naïve and pup-experienced females (Ehret and Buckenmaier, 1994; Koch, 1990) and males (Ehret et al., 1993). For instance, in the MPOA of females and fathers, the number of ERα positive cells increased with pup contact and retrieving experience (Ehret et al., 1993; Ehret and Buckenmaier, 1994; Koch, 1990). In pup-naïve male and female voles, high levels of spontaneous pup care are correlated with higher number of ERα positive cells in the MPOA (Li et al., 2015), potentially indicating convergent circuit dynamics.

Low levels of circulating estrogens in males (Nilsson et al., 2015) imply that estrogens are primarily synthesised locally in the brain from testosterone by the enzyme aromatase (e.g., (Cornil et al., 2013, 2006; Garcia-Segura, 2008)). Locally synthesised estrogens have been implicated in the onset of paternal behaviours in California mice (Trainor et al., 2003; Trainor and Marler, 2002). Aromatase is expressed in the limbic system of male mice (LS, BNST, MPOA, nuclei of the amygdala, VMH, and PIR) (Foidart et al., 1995; Schleicher et al., 1986; Stanić et al., 2014; Wozniak et al., 1992). In the present study, we sought to examine changes in aromatase expression during the onset and maintenance of paternal behaviour of male mice (pup-naïve and fathers). We focused on LS, BNST, MPOA, nuclei of the amygdala, VMH, and PIR, which are regions in which ERα positive cells have also been located in pup-

experienced fathers (Ehret et al., 1993). Our results suggests that estrogen signalling via Erα, coupled with an aromatase-dependent estrogen supply, could be an important regulator of paternal behaviour.

Materials and methods

Animals

Adult male and female laboratory mice (outbred strain NMRI, 2-3 months old) with no previous sexual or parental experience were housed at the University of Ulm. As previously (Ehret et al., 1993), mice were randomly assigned to one of three treatment groups consisting of (1) naïve males without any experience with pups (N); (2) males co-caring with their female mate for their first litter for five days (5D); or (3) males co-caring with their female mate for their first litter until weaning and their second litter for five days. The total co-caring time of the latter males (first and second litter) was 26-27 days (27D) since after delivery of the first litter, females in post-partum estrus paired immediately and, while nursing the first litter up to weaning at 21 days, delivered the second litter after 21-22 days of gestation. Mice were housed at 22ºC and a 12 h light-dark cycle (starting at 7.00 h) in plastic cages (26.5 × 20 × 14 cm) either with other naïve males (group N; 4 brothers per cage) or with one female (groups 5D and 27D). Water and food were available *ad libitum*. The experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC) and were approved by the appropriate authority (Regierungspräsidium Tübingen, Germany).

Pup retrieval test

Testing of pup retrieval behaviours was in performed in two separate cohorts of animals (Supplemental Fig. 1) but otherwise as previously described (Ehret et al., 1993; Ehret and Koch, 1989). Male mice (alone or with litter mate and pups) were placed overnight on a running board (length 110 cm, width 8 cm) with a central nest depression (Fig. 1A) suspended

in a sound-proof and anechoic room. The running board was supplied with mouse chow and nest material from the cage and covered with a plexiglass hood having an opening for the tip of a suspended water bottle. Retrieving tests were done under dim red light. Before the start of testing, the hood, and the female with the pups (groups 5D and 27D) were removed and placed back to their home cage outside the soundproof room. Seven pups were kept in the room to serve as targets for retrieving. The males were habituated for at least 30 min to the new situation after the hood (group N) or the hood and female with pups (groups 5D and 27D) were removed. Prior to testing males of the N group, five-day old foster pups were rubbed with nest material of the respective males to ensure a familiar scent. During the test, the seven pups (always 1-3 pups at a time) were randomly placed on the running board, at least 30 cm away from the nest. A male was scored as 'retriever' if he retrieved all seven pups to the nest within 10 min. A male was scored as 'non-retriever' if he had not retrieved any pups after 10 mins. The distinction between retriever and non-retriever was clear-cut, because once a male had retrieved the first pup, he retrieved all. Retrieving tests were immediately stopped and the pups removed if a male attacked any of the pups ('aggressor').

Immunocytochemistry and microscopy

Immediately following the pup retrieval test, males were euthanised by cervical dislocation and their brains quickly dissected and frozen over liquid nitrogen. Brain serial frontal sections (30 μm) were cut on a freezing microtome (Thermo Scientific Microm HM560), and immediately mounted on slides to keep track of the left and right sides of the brain. Sections were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH=7.4) for 60 min. Sections were rinsed three times for 5 min with 0.1 M PB in between all subsequent processing steps. Slides were incubated with 0.2% Triton X-100 (Sigma) in 0.1 M PB for 45 min to permeabilise cells and with 1% H_2O_2 (Merck) in 0.1 M PB for 20 min to block endogenous peroxidase. In a humidified chamber, the sections were treated with 2%

normal goat serum in 0.1 M PB for 60 min to block non-specific staining, then with the polyclonal rabbit anti-aromatase antibody (Abcam Cat# ab35604, RRID:AB_867729; 0.002 mg/ml in 2% normal goat serum) at 4ºC for 72h. Sections were then incubated with the secondary antibody from goat against rabbit horseradish peroxidase (Dako, Cat# PO448, 0.00125 mg/ml in 0.1 M phosphate buffer) at room temperature for 60 min, followed by staining with 0.015% diaminobenzidine tetrachloride (DAB; Sigma), 0.023% NiCl, and 0.013% H_2O_2 in 0.1 M PB for 7 min. Sections were rinsed in PB, dehydrated in a series of alcohols and xylene, and coverslipped with Entellan (Merck). At the time of purchase, the primary antibody was recommended for aromatase immunohistochemistry on mouse tissue and the supplier provided positive quality controls with mouse ovarian tissue (epitope was not described by the supplier). Dutta et al. (2014) also performed a Western blot with mouse tissue that confirmed specificity of the antibody. Previous studies have used this antibody to detect aromatase immunoreactivity in mouse ovary (Laws et al., 2014), brain stem (Ji et al., 2017) and auditory cortex (Charitidi and Canlon, 2010). We further controlled for the specificity of labelling by incubating sections with PB instead of the primary antibody. In all the control sections, non-specific immunoreactivity was completely absent.

Sections were independently analysed by two blinded experimenters. In view of varying non-specific background labelling, the experimenters started the analysis by checking many sections from all animal groups in order to agree which kind of labelling would be considered aromatase-specific. Cells were considered as expressing aromatase if they were (1) more intensely stained with shades of gray than the background, and (2) contained black granulae often grouped around the cell nucleus (Fig. 2). In cases in which a single or several black granulae without an associated cellular structure in shades of gray was seen, the structure was not counted as an aromatase-positive cell. This procedure led to (a) a high concordance of counting results of the two experimenters, (b) counts of only definite

aromatase-positive cells, and (c) relatively low numbers of aromatase-positive cells per evaluated brain area.

Sections were analysed with a light microscope (Axiophot, Zeiss Germany). Brain areas were identified with 10x objective magnification, aromatase-positive cells were identified by focussing with a 40x objective magnification. The brain areas (including plate numbers and associated Bregma coordinates) in which aromatase-positive cells were counted are listed in Table 1. These areas largely overlap with those showing ERα positive cell dynamics during the acquisition of pup-caring experience in male (Ehret et al., 1993) and female (Ehret and Buckenmaier, 1994) mice. Brain sections were matched to the mouse brain atlas plates and associated Bregma coordinates (Paxinos and Franklin, 2001) and anatomically matched across all experimental animals. Aromatase-positive cells were counted separately on the right and left hemispheres of the brain. Counts were averaged between the maximum number of consecutive sections that could be matched across all animals, i.e., two sections for LSD, LSI, LSV, BNST, NAC, and VMH or three sections for PIR, MPOA, CAM, and MAM.

Statistical Analyses

All statistical analyses were carried out using R v4.2.0 (R Core Team, 2022). Male behaviour in response to pup presentation was classified as ignoring, attacking, or retrieving a pup. The outcomes of behavioural trials were analysed as the multinomial probability of exhibiting a given response to pup presentation (aggression, ignore, retrieval) as a function of paternal experience (experience as ordered factor, N < 5D < 27D). To perform pairwise comparisons between the groups for each behaviour, we calculated the probability that the difference in the posterior predictive intervals between groups was significantly directional. We then corrected for multiple comparisons using the false discovery rate.

We combined two partially overlapping datasets of aromatase cell counts from two cohorts of animals to increase our statistical power to identify variation among cohorts and

brain regions (Supplemental Fig 1). We observed significant count variation attributable to cohort (e.g., count variation between cohorts for the PIR -1.34 data; Supplemental Fig 1). To address this issue, each dataset was standardised by the mean and variance of paternal groups (5D and 27D; the groups for which the datasets were overlapping). Counts for some brain regions were missing in some mice. Rather than rejecting these mice altogether in our statistical model (described below), we imputed the missing observations based on values inferred from the non-missing entries (R package mice, van Buuren and Groothuis-Oudshoorn, 2011). We generated five such candidate data sets and integrated over the resulting uncertainty.

Our statistical model of aromatase variation was designed to address non-independence arising from correlated variation within individuals among regions. Such non-independence could be an especially important factor when comparing identifiable subregions of an area, such as in the piriform cortex and each part of the lateral septum. We therefore examined variation in normalised cell counts among all brain regions simultaneously (multivariate ANOVA), treating mouse ID as a correlated group (random) effect. Responses were modelled as *t*-distributed to increase robustness against outlier counts. We examined the interaction of Experience x Hemisphere together.

All models were fit using the R package *brms* (Bürkner, 2017), using four chains of 2500 iterations each, and a thinning rate of 5. We assumed uniform priors throughout. Typically, model convergence would be assessed by $\hat{R}=1$ (Vehtari et al., 2021) but because each imputed data set will generate slightly different estimates at convergence, $\hat{R}$ may be misleading. We therefore assured model convergence in a separate analysis of a single data set generated by averaging over the imputed data sets. We calculated the posterior means of each group based on the fitted statistical model (*emmeans* package (Lenth R, 2022)), and used these to examine the posterior distributions of contrasts between hemispheres (left-right) and between levels of experience (5D-N, 27D-N, 27D-5D) within each hemisphere. Significance

of each contrast was judged as a Bayesian probability of direction $P>0.975$ (Makowski et al., 2019), which is an index of effect existence, i.e., our level of certainty in whether a reported change is positive or negative. This threshold closely corresponds to the frequentist two-sided *p*-value of 0.05, and so for simplicity, we report the *p*-value. Plots were generated using the *ggplot2* package (Wickham, 2016).

The residual correlations among regions represent relationships that are not explained by the other population- and group-effects, including correlated expression and unexpected technical covariation. Inspection of residual correlations can therefore inform biological hypotheses. However, direct interpretation of the residual correlation matrix is challenging because the correlation between two brain regions could be due to mutual covariation with another region. We dichotomised relationships among brain regions by studying the matrix of *partial* correlations (de la Fuente et al., 2004; Shipley, 2000). We constructed an initial graph in which edges were created between all nodes with significant ($p<0.05$) pairwise correlations. Each edge was iteratively pruned by testing whether the association remained statistically significant after testing it against all other paths between two variables, up to third order. The independence test was determined by the posterior distribution of residual correlation matrices implemented in the R package pcalg (Kalisch et al., 2012). The result is an undirected, acyclic graph representing a network of associations among brain regions. The large number of tests may be conservative and may reject true associations, and thus we also report tentative links observed when setting a less stringent threshold of $p<0.10$.

Results

Male mice retrieve pups after acquiring parental experience

Only males with paternal experience retrieved pups (Fig. 1). Naïve males with no previous sexual experience and pup contact mostly ignored the pups (n = 5/8), although some were aggressive (n = 3/8). Most fathers with 5 days (5D) and 26-27 days (27D) of experience

retrieved pups (n = 10/12 and 11/12, respectively). A small number of fathers ignored pups in the 5D (n = 2/12) and 27D groups (n = 1/12) but none were aggressive.

The probability of observing aggressive behaviour toward pups was significantly higher in naïve males compared to fathers regardless of experience (5D and 27D, Fig. 1B). The probability of other non-paternal behaviour like ignoring pups was higher in naïve males compared to 27D fathers. Parental behaviour measured as pup retrieval was significantly higher in fathers (5D and 27D) compared to naïve males. There were no significant differences in the probability of pup-retrieving between the groups of fathers, 5D and 27D (Fig. 1B).

Aromatase expression in the male mouse brain, general picture

We found aromatase-positive cells in each examined region and with each level of experience. In absolute terms, largest numbers of aromatase positive cells per section were seen in PIR, LSI, MAM, and CAM (see unstandardised data in Supplement, Fig. 1). Examples of aromatase labeling in the LSV, PIR, VMH and MAM of experimental groups N and 27D showed qualitative differences between the groups and between left and right sides of the brain (Fig. 2). Standardised cell counts together with the group means are shown in Fig 3A along with our estimates of the posterior differences in aromatase counts by hemisphere (Left – Right, Fig. 3B), and between levels of experience (Fig. 3C).

Lateralised expression of aromatase positive cells

We found evidence for significantly greater numbers of aromatase positive cells in the left hemisphere of the MPOA and BNST in all three experimental groups (Left – Right > 0, Fig. 3B). The NAC exhibited a similar trend to MPOA and BNST, but with a weakly insignificant contrast in the 27D group. We did not detect significant lateralisation of aromatase counts in any of the experimental groups in the amygdala (CAM and MAM),

VMH, or posterior PIR (Bregma -1.34) in any groups. In the other regions, the appearance of lateralisation strongly depended on the level of experience. Long-term pup-experience (27D) led to greater numbers of aromatase positive cells in the right hemispheres of anterior PIR (Bregma 0.98), the LSV, and the anterior LSI (Bregma 0.14). Posterior LSI (Bregma 0.98) exhibited a similar trend that was weakly non-significant. In the LSD, pup-experience had a short-term effect (5D) during which counts in the left hemisphere were greater. This effect was not present in the 27D group (Fig. 3B).

Experience-dependent expression of aromatase

In all cases in which we found significant changes of aromatase counts with experience, the number of aromatase positive cells increased (Fig. 3C). This experience-dependent increase was pervasive for the amygdala (CAM and MAM), the VMH, and the posterior PIR (Bregma -1.34). For these regions, the increase in aromatase positive cells was present in both hemispheres and with increasing experience (i.e., experience contrasts had significant positive values for both brain hemispheres and all experimental groups). In the LSD, LSI, and LSV (both Bregma coordinates), and the anterior PIR (Bregma 0.98), aromatase positive cells increased significantly only in the right hemisphere of 27D males. Experience-dependent effects were absent in the NAC, the BNST, and the MPOA, except for a significant effect in the right MPOA of 27D males (27D males had significantly more aromatase positive cells than 5D males). In fact, both BNST and MPOA exhibit trends toward increasing aromatase with experience, but this variation was not statistically significant (Fig. 3C).

Graphical analysis of covariation among brain regions

We examined the unexplained (residual) variation in aromatase cell numbers among brain regions (represented graphically in Fig. 4). Three subgroups of covariation are obvious.

First, LS is highly connected (although not in order from dorsal to ventral), and each subregion (D, I, V) is correlated across Bregma coordinates and to at least one other subregion. LS appears to form the backbone of variation, to which other nodes are connected. This includes subgroups comprised of MPOA (connected to LSV), of NAC and BNST (connected to LSI), and of CAM and VMH (connected to LSD). Second, the subgroup of MAM and anterior PIR appears to be weakly connected to other parts of the graph (MPOA and VMH), because the edges are significant only with $p<0.10$. Third, posterior PIR did not appear to be even weakly correlated with any other analysed part of the brain despite an increase in aromatase expression with experience that is similar to CAM, MAM, and VMH (see Fig. 3).

Discussion

In the present study, we found that aromatase, which catalyses the conversion of testosterone to estrogen, is expressed throughout the limbic system of all male mice. We identified important factors contributing to hierarchical variation in expression levels among individuals. First, the dramatic changes in behaviour associated with paternal experience (Fig 1) were associated with changes in aromatase expression in regions of the limbic system (Fig 3). However, not only were these changes region-specific, but also hemisphere-specific (summarised in Fig. 5). Finally, layered on these widespread patterns, we detected correlated aromatase levels among brain regions within individual mice (Fig. 4). Altogether, these factors lead to heterogeneity in aromatase expression throughout the male brain limbic system. Here, we briefly review the role of aromatase and the limbic areas to discuss how such expression patterns may influence, and be regulated by, paternal behaviours and experience.

Aromatase and the expression of paternal care

In mice, pharmacological inhibition of aromatase reduces retrieval behaviour in fathers that have been co-housed with pups for 4 days (Akther et al., 2015). Together with our study, this would suggest that the increase in brain aromatase expression is integral to the display of paternal behaviours in mice. In line with our results, Akther et al. (2015) also observed increased aromatase expression in the VMH and amygdala of males exhibiting pup retrieval. However, they also observed increases in aromatase expression in the MPOA, NAC, and LSD (Akther et al., 2015), areas where we did not observe significant changes. One difference could lie in the social context. Akther et al. (2015) housed males with their female mates in the absence of pups. We housed males with both their female mates and their pups (except during the pup retrieval test, when females were kept outside the soundproof room). The different antibodies may also have led to differences in observed spatial patterns and indeed, the difficulty of measuring aromatase protein using immunohistochemistry is well-known in laboratory rats and mice (Stanić et al., 2014). In the present study, we report aromatase expression in areas that past studies have also found to contain aromatase activity and protein, namely the LS, BNST, MPOA, NAC, PIR, VMH, MAM, and CAM (Foidart et al., 1995; Schleicher et al., 1986; Stanić et al., 2014; Wozniak et al., 1992). Studies using diverse methods of aromatase quantification will be important in the future to complement the current study.

Amygdala and ventromedial hypothalamus

Three nuclei, the MAM, CAM, and VMH were most affected by pup experience with increases in the number of aromatase positive cells from N to 5D to 27D animals (Fig. 3 A and C; Supplement Fig. 1). These regions are associated with the regulation of aggression compared to sexual and parental behaviour (e.g., (Chen et al., 2019; Dwyer et al., 2022; Hashikawa et al., 2016; Lee et al., 2014; Sheehan et al., 2001; Unger et al., 2015; Yang et al., 2017)). Important changes in aggression in the course of becoming a caring father are

mediated by olfactory signals from the vomeronasal organ via the accessory olfactory bulb to the CAM and MAM (e.g., (Hashikawa et al., 2016)) and concern the switch from aggressive to caring behaviour towards pups (Tachikawa et al., 2013). Concomitantly, aggressive motivation in fathers increases against male intruders in order to protect their own pups from potential pup killers as shown in Californian mice (Trainor et al., 2008) and prairie voles (Getz et al., 1981). This increased aggression towards intruders was not mediated by changes in ERα or ERß expression in limbic centers, but reflected in increased neuronal activation (FOS-expression) in the MPOA, MAM and, potentially the VMH (Trainor et al., 2008). We are not aware of studies in house mice investigating aggression of fathers towards intruders. Future work could examine whether the decrease in aggression towards pups likewise alters aggression toward potentially dangerous intruders. Such tests combined with aromatase measurements could clarify whether the differentiation of aggression against kin or non-kin depends on aromatase levels in the amygdala and VMH.

In contrast to aromatase expression (present study), the number of estrogen receptors (ERα) in MAM, CAM, and VMH did not change with paternal experience (5D or 27D) (Ehret et al., 1993). Together, this indicates that the presence of estrogens does not induce he expression of its own receptors, as has been shown in the VMH (Devidze et al., 2005; Malikov and Madeira, 2013), especially in combination with parental experience (Koch, 1990). We suggest that the increase in aromatase expression in the MAM, CAM, and VMH in new (5D) and highly experienced (27D) fathers (Fig. 5) supports the control of instinctive behaviour (Honda et al., 2011) including inhibition of infanticide, via increased local action of estrogens on neurons (e.g., (Cornil et al., 2013, 2006)).

Piriform cortex

In house mouse mothers, the performance of pup-retrieving does not depend on olfactory signals from the main olfactory bulb and its projections to the PIR (Koch and Ehret,

1991). Pup-naïve females, however, needed this olfactory information for learning to become as maternal as the mothers (Ehret and Buckenmaier, 1994; Koch and Ehret, 1991). While learning pup cues for becoming maternal, virgin females with maternal experience have an increase in ERα positive cells in the posterior PIR compared to females without experience (also in the entorhinal cortex; (Koch, 1990)). Similarly, as males became caring fathers, ERα positive cells become visible in the posterior PIR (also in the entorhinal cortex; (Ehret et al., 1993)). In view of the significant increase of aromatase expression in the posterior PIR with increasing paternal experience (Fig. 3C), it is possible that in PIR, unlike MAM, CAM, and VMH, estrogen may have induced its own receptors (tested for ERα) and enhanced olfactory learning of pup cues for increased paternal performance. The significant increase of aromatase positive cells only in the right hemisphere in the anterior PIR of 27D fathers does not directly correlate with any evidence known to us. In studies on ERα expression in the limbic centres of female and male mice under various conditions of the reproductive cycle, experience with pups, and intact or lesioned olfaction, no hemisphere differences have been reported in any centre (Ehret et al., 1993; Ehret and Buckenmaier, 1994; Koch, 1990; Koch and Ehret, 1991). The splitting of PIR in regions with experience-dependent lateralised (anterior PIR) and non-lateralised (posterior PIR) aromatase change shows compartmentalization of the PIR. Functional relationships need to be elucidated in further studies.

Lateral septum

The LS has a central functional role in the brain integrating cognitive and emotional information in the regulation of social relationships and instincts (Besnard and Leroy, 2022; Menon et al., 2022; Rizzi-Wise and Wang, 2021) including parental behaviour (Fleischer and Slotnick, 1978; Slotnick and Nigrosh, 1975). Our analysis of residual correlations likewise (Fig. 4) points to a central role of the LS in the variation of aromatase expression in the mouse brain. Interestingly, these partial correlations reflect main neural connections of the

subregions LSD and LSV. For example, there is input from the amygdala to the LS supporting the LSD-CAM partial correlation and there is an output from the LS to the MPOA supporting the LSV-MPOA partial correlation (Besnard and Leroy, 2022; Menon et al., 2022; Risold and Swanson, 1997; Rizzi-Wise and Wang, 2021). Thus, residual variation in aromatase expression (Fig. 4) appears to mirror functional connections and relationships of the LS.

The main effects of pup-experience on aromatase expression in the LS were changes in the left-right balance (Fig. 3B) and significant increase of aromatase-positive cells only in the right LS of highly experienced (27D) fathers (Fig. 3C). As in the PIR, ERα positive cells in the LS become visible in caring fathers compared to pup-naïve males (Ehret et al., 1993). Since hemisphere differences in ERα positive cells have not been found, we cannot directly relate increased right-hemisphere aromatase expression with non-lateralised increase of ERα expression in the LS of both hemispheres. Lateralised input to the rostral parts of the LSD and LSI comes from the hippocampal CA1 and subiculum region (Risold and Swanson, 1997; Rizzi-Wise and Wang, 2021). In rodents, the CA1 shows lateralisation in synapse morphology and receptor distribution (Shinohara et al., 2008; Xiao and Jordan, 2002) and left-right dissociation of memory processes (Shipton et al., 2014) with right-hemisphere dominance of spatial memory in mice (Shinohara et al., 2012). Also, androgen receptor levels are higher in the left hippocampus in male rats (Xiao and Jordan, 2002). It is unclear, however, whether and how lateralised hippocampal input to the LS could have caused lateralised aromatase expression in the LS of 27D fathers (Fig. 3C). Right hemisphere lateralisation is associated with the regulation of mood, affect, and stress, and with sustained and global arousal and attention (e.g., (Ehret, 2006; Palomero-Gallagher and Amunts, 2022)). Speculatively, our observed right-hemisphere LS bias in 27D fathers could be related to the stress of continuous arousal and steady involvement in a routine of pup-care over several weeks.

Medial preoptic area, bed nucleus stria terminalis, and nucleus accumbens

The MPOA together with the BNST and their output via the NAC are essential for controlling parental behaviour in rodents (e.g., (Bridges, 2015; Numan, 2006; Wu et al., 2014)). Significant increases have been observed in the MPOA both of the number of and area occupied by ERα positive cells in one-day pup-experienced virgin female mice (Ehret and Buckenmaier, 1994) and in 5D and 27D pup-experienced father mice (Ehret et al., 1993). The number of ERα positive cells increase in the BNST of one-day pup-experienced virgin females (Ehret and Buckenmaier, 1994) and, although absent in pup-naïve males, ERα-positive cells are present in the BNST of 27D fathers (Ehret et al., 1993; Koch and Ehret, 1989b). We did not observe a statistically significant change in the number of aromatase positive cells in the BNST and NAC and found only a small increase in the MPOA in just the 27D males relative to 5D. This suggests that changes in sensory processing and behavioural expression with experience in MPOA, BNST, and NAC may be more dependent on regulation of estrogen signalling through binding to receptors rather than local estrogen action after synthesis via aromatase. Brain regions that regulate social behaviours, including parental care, are conserved in vertebrates (O'Connell and Hofmann, 2012). However, neuroendocrine ligand spatial profiles are more variable than receptor distributions within the social decision-making network among vertebrates, although there are regional differences (O'Connell and Hofmann, 2012). Our work supports experience-dependent regional differences, and specifically that MPOA, BNST, and NAC may depend on changes in ERα distribution, whereas VMH and amygdala may depend on changes in the synthesis of the ligand. Overall, our results point to a region-specific regulation of receptor and ligand with parental experience.

The brain regions MPOA, BNST, and NAC exhibited substantial lateralization of aromatase, biased toward the left hemisphere (Fig. 3B). Left-hemisphere dominance has been noted in a number of contexts, including maternal perception and processing of pup-ultrasounds, in the latter case, through cFOS expression (Ehret, 1987; Geissler et al., 2016).

Maternal pup retrieval behaviour likewise is associated with left- but not right-hemisphere oxytocin receptor expression and action (Marlin et al., 2015). To what extent can these sources of left-hemisphere bias in females (primarily auditory cortex) inform our findings? The left-hemisphere bias in females has primarily been observed in the auditory cortex (Ehret, 1987; Geissler et al., 2016; Marlin et al., 2015). Lateralisation of cFOS expression has not been observed in the neural responses to pup presence in the limbic system of female mice, MPOA and BNST included (Geissler et al., 2013). Nonetheless, neural activation characterized by cFOS labelling concerns the input side of neurons (Sheng et al., 1993, 1990). Currently, it is not known whether males also display hemisphere differences in neural activation and if so, how those may be related to hemisphere differences in aromatase expression (the output side of neurons) and behaviour.

Correlated individual variation in aromatase-positive cells between brain regions

The brain's connectivity suggests that cell counts within individuals may be correlated across regions. For example, aromatase could be uniformly lower or higher in some individuals compared to others, or could vary highly non-uniformly across brain regions. We expect that such variation will contribute to individual differences in behaviour, such as some fathers continuing to ignore pups (Fig. 1). Our study found significant residual correlated variation among brain regions (Fig. 4), generally not depicted in the data shown in Fig. 3. It is noteworthy that the posterior PIR and, to a lesser degree, the anterior PIR and the MAM remained outside this connectivity. Indeed, Supplementary Fig. 1 shows that posterior PIR (Bregma -1.34) was the sole case in which we observed substantial divergence in overall cell counts between the two cohorts of animals. Together, these observations suggest to us that there are additional sensory and genetic factors that modulate the numbers of aromatase-positive cells in PIR and MAM, which may integrate other information as brain pathways reorganise to accommodate paternal care, such as stress and arousal status. Our work

therefore suggests that going forward, it will be fruitful to consider both the overall regulation of the entire network, along with relative and absolute differences in specific brain regions.

Limitations

We measured pup retrieval, aggression, and ignoring pups as behavioural measures of paternal care. We did not observe differences in behaviours between 5D and 27D males (Fig. 1), however there is evidence that further experience with pups results in behavioural differences between 5D and 27D males. For example, 27D males, but not 5D males, can discriminate ultrasounds (i.e., indicated by a preference for 50 kHz tone over 20 kHz sounds; (Ehret and Koch, 1989). From our work, it is possible that further increases in aromatase levels with experience (CAM, MAM, VMH, and PIR) could be involved in regulating differences in 5D and 27D fathers.

We chose to house naïve males in groups as we have done in the past (Ehret et al., 1993, 1987; Ehret and Koch, 1989). This allows us to compare current results with previous work and to control for sexual experience and female cues. The aim of our study was to examine the effect of paternal experience as a whole, this includes sexual experience and pup exposure. In terms of behaviour, males of the NMRI strain require five days of pup exposure to retrieve pups but with the current results, we do not know how much of the aromatase expression is contributed by sexual experience or other cues by the female. Further work could aim to disentangle sexual experience and pup care experience (e.g., by removing pups when they are born).

Conclusion

Aromatase expression in the male limbic brain is regulated with parental experience in a region-, hemisphere-, and experience-specific manner. Acquiring paternal pup-caring experience enhanced aromatase expression (number of aromatase positive cells) in brain

centres processing olfactory information (PIR, CAM, MAM) and regulating aggression, sexual behaviour and other social relations (MAM, VMH, LS). In centers essential for controlling parental behaviour (MPOA, BNST, NAC), aromatase expression was instead present with left-hemisphere dominance. In general, the aromatase expression dynamics we observed did not mirror the previously reported patterns and dynamics in estrogen receptors (ERα). This suggests that levels of estrogen receptors are not simply dependent on levels of locally produced aromatase-dependent estrogen through an autoregulatory feedback loop. Rather, paternal behaviour may depend on differential regulation of estrogen signalling through receptor or synthesis in a region-dependent way.

Acknowledgements

We thank H. Sabine Schmidt with assistance with animal care, immunocytochemistry, and microscopy. This study was supported by the Deutsche Forschungsgemeinschaft, EH53/19 to GE. PDG was funded by a postdoctoral fellowship from the Fonds Quebecois Recherche Nature et Technologie. PDG is currently supported by the Canada Research Chair Program. DAS was partially funded by a postdoctoral fellowship from the Kavli Neuroscience Discovery Institute.

Table 1. Brain regions analysed for aromatase expression, their Bregma coordinates and plate numbers from the mouse brain atlas (Paxinos and Franklin, 2001), and sample sizes per group per brain region.

Brain region		Bregma Coordinates	Plate number	Sample Size
Lateral septum dorsal (LSD)	Anterior and posterior	0.98 mm and 0.14 mm	23 and 30	n=7 (N) n=10 (5D) n=11 (27D)
Lateral septum intermediate (LSI)	Anterior and posterior	0.98 mm and 0.14 mm	23 and 30	n=7 (N) n=10 (5D) n=11 (27D)
Lateral septum ventral (LSV)	Anterior and posterior	0.98 mm and 0.14 mm	23 and 30	n=7 (N) n=10 (5D) n=11 (27D)
Piriform cortex (PIR)	Anterior and posterior	0.98 mm and -1.34 mm	23 and 42	n=7 (N) n=8 (5D) n=9 (27D)
Nucleus accumbens (NAC)		0.98 mm	23	n=7 (N) n=10 (5D) n=11 (27D)
Bed nucleus stria terminalis (BNST)		0.14 mm	30	n=7 (N) n=10 (5D) n=11 (27D)
Medial preoptic area (MPOA)		0.14 mm	30	n=7 (N) n=10 (5D) n=11 (27D)
Cortical amygdala (CAM)		-1.34 mm	42	n=7 (N) n=9 (5D) n=8 (27D)
Medial amygdala (MAM)		-1.34 mm	42	n=7 (N) n=9 (5D) n=8 (27D)
Ventromedial hypothalamus (VMH)		-1.34 mm	42	n=7 (N) n=9 (5D) n=8 (27D)

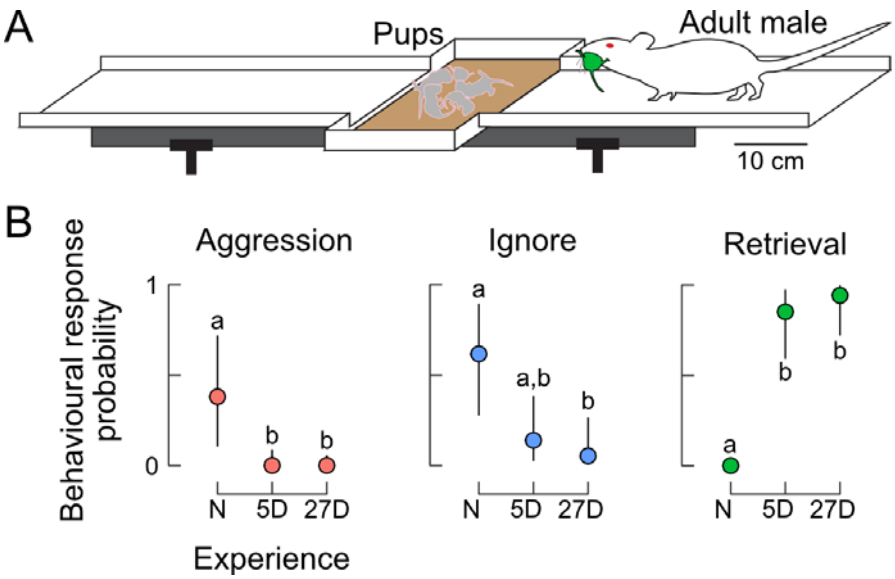

Figure 1. Pup-caring behaviour (retrieving) is determined by paternal experience. **A.** Experimental setting for the pup-retrieving tests on a running board with a central nest depression. Responses were scored as aggression (attacking pups), ignoring, or retrieving (returning pups to the nest). **B.** Probabilities of behavioural expression in naïve (N) males and in fathers with five (5D) or 27 days of experience (27D). Aggression declined immediately from N to 5D fathers. Although fathers occasionally still ignored pups, the probability of retrieval was very high. Different letters indicate statistical differences between the groups of males. Sample size was n=8 for N, n=12 for 5D, and n=12 for 27D.

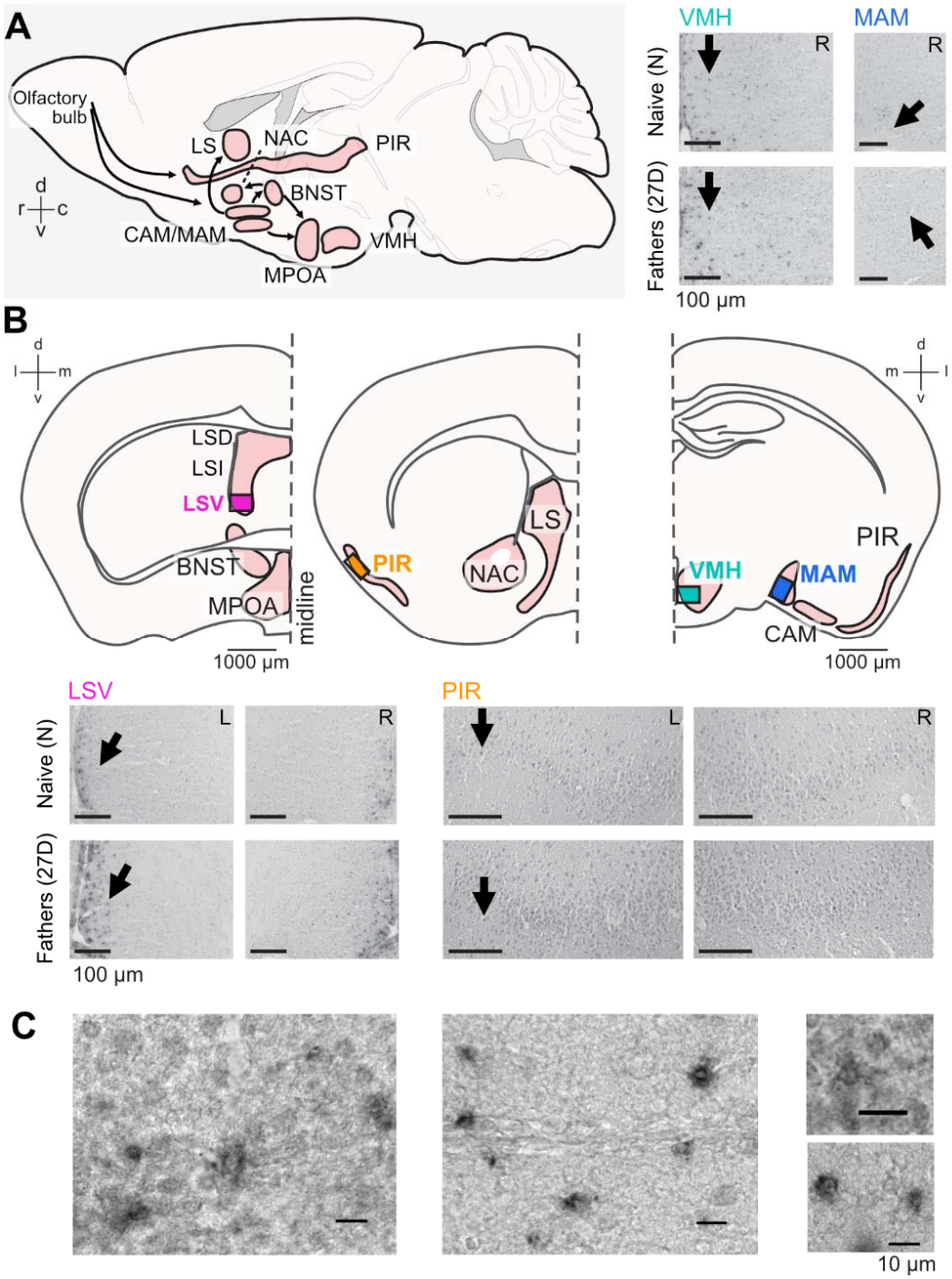

Figure 2. Aromatase expression in the paternal brain. Sagittal view (**A**) and cross sections (**B**) of the brain regions analysed here (Bregma coordinates: 0.98 mm; 0.14 mm; and -1.34 mm). Positions and extensions of nuclei/areas are illustrative only and do not indicate anatomical relationships. The connectivity denoted in (A) shown by the arrows are exemplars

for main information pathways (Kohl et al., 2017; Numan, 2020). Representative images from regions highlighted in (B), showing aromatase immunostaining (arrows) in naïve and experienced fathers (27D) in the left (L) and right (R) hemisphere. High magnification (C) images of examples of aromatase expressing cells in the MPOA and VMH showing cytoplasmic expression and black granulae around the cell nucleus. Scale bars indicate 100 µm for (A) and (B) and 10µm for (C).

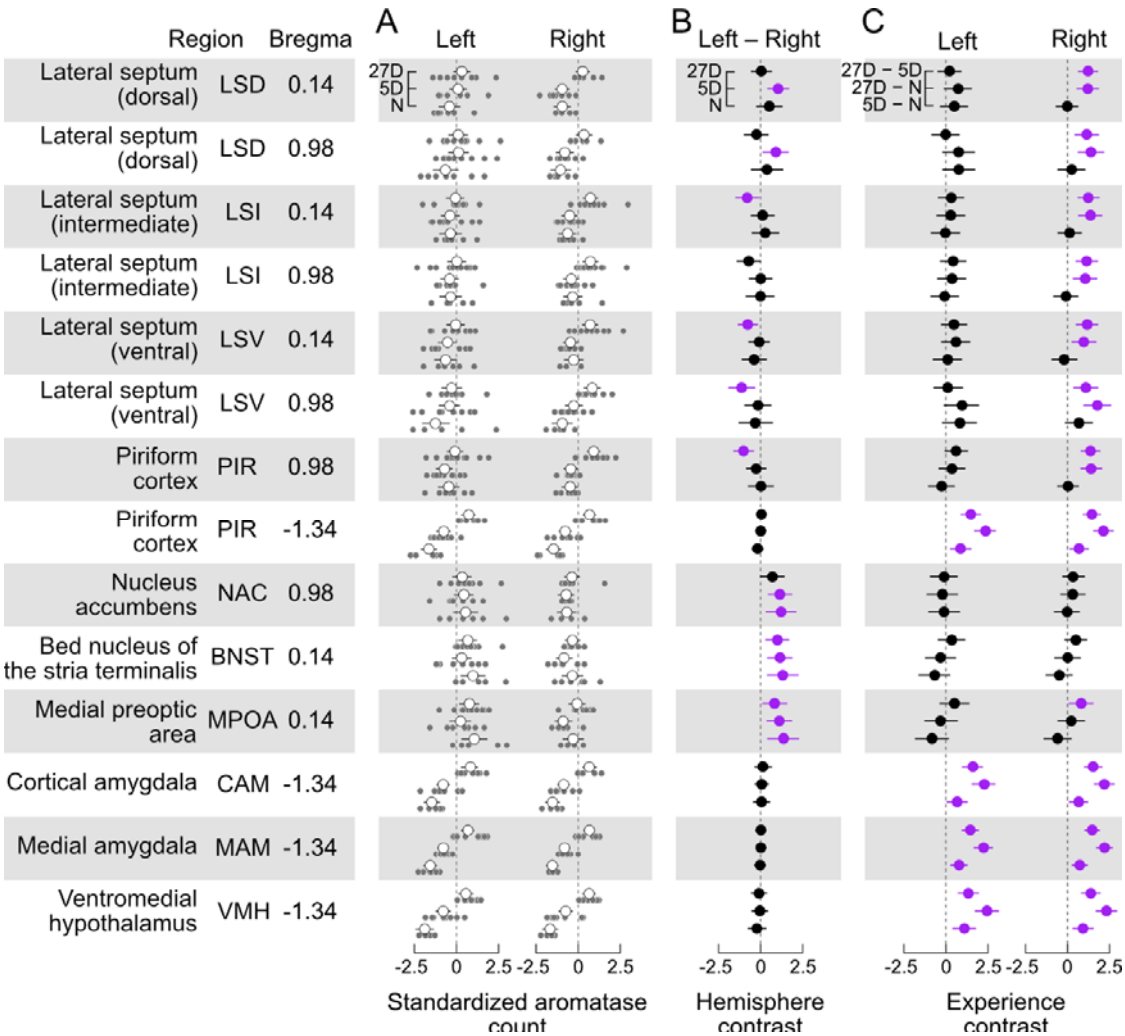

Figure 3. Aromatase expression differentially depends on paternal experience and brain hemisphere. **A.** Standardised cell counts (light grey circles) and posterior means (± 95% confidence interval, white circles) for each investigated nucleus/area separately for the left and right sides of the brain and levels of pup-experience (N, 5D, 27D). **B.** Hemisphere contrast (left – right, with 95% confidence intervals) of the standardised counts of aromatase positive cells for each brain nucleus/area and experimental group. Contrasts with intervals that exclude zero are highlighted in purple ($p<0.05$, see text for description). We found evidence for lateralisation of aromatase cell counts both independent of (BNST, MPOA, and most likely NAC) and dependent on experience (most LS and anterior PIR (Bregma 0.98)). **C.** Experience contrast (5D–N; 27D–N, 27D–5D, with 95% confidence intervals) for the standardised counts of aromatase positive cells, separately for each hemisphere and brain nucleus/area. We found that aromatase positive cells in posterior PIR (Bregma -1.34), CAM, MAM, and VMH significantly increased with experience level. Expression of aromatase in the LS and anterior PIR significantly increased only in the right hemisphere of the 27D group. No changes were observed in the NAC and BNST. Contrasts with intervals that exclude zero are highlighted in purple ($p<0.05$, see text for description). Sample sizes are described in Table 1.

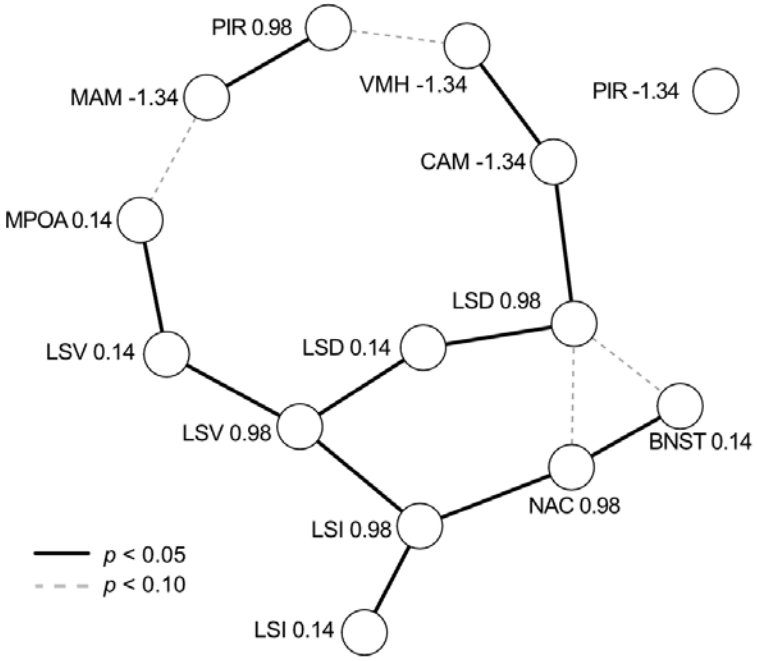

Figure 4. Residual correlations among brain nuclei/areas viewed as a network. Each nucleus/area at each Bregma coordinate (see Table 1) is represented by a node, with edges representing significant partial correlations (solid for $p < 0.05$, dashed for $p < 0.10$).

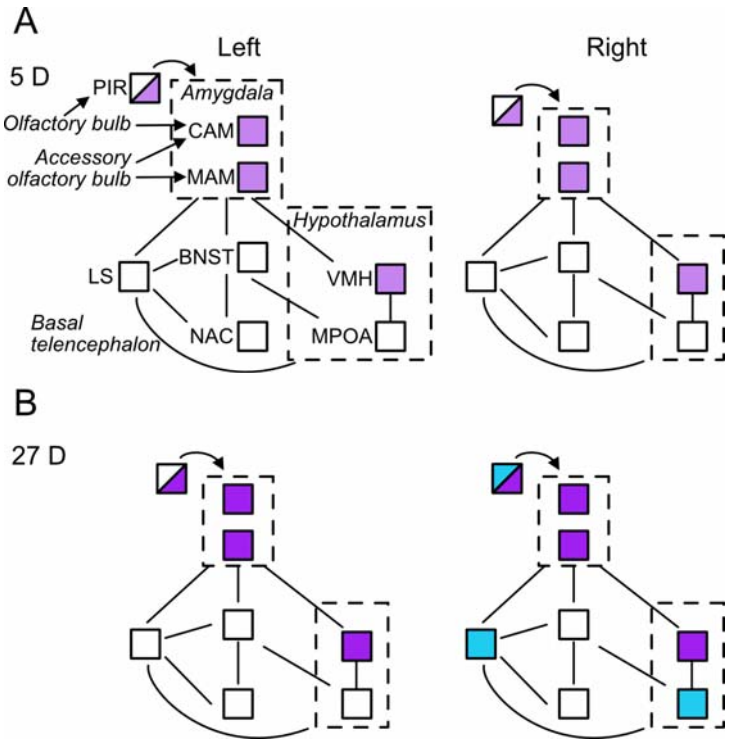

Figure 5. Effects of paternal experience on aromatase expression in areas of the limbic system. Summary of the changes in aromatase expression in 5D **(A)** and 27D **(B)** males in the left and right hemispheres of the brain nuclei/areas analysed in the current study. **A.** Pup experience in first-litter fathers (5D group) increased the numbers of aromatase positive cells (indicated by light purple) relative to naïve males in the amygdala (CAM and MAM), hypothalamus (VMH), and in posterior piriform cortex (PIR, half shading indicates these partial changes). **B.** Second-litter fathers (27D group) had further increased aromatase expression in the same areas as 5D males (indicated as darker purple), i.e., the amygdala (CAM and MAM), hypothalamus (VMH), and posterior piriform cortex (PIR) in both sides of the brain. In the right hemisphere, the number of aromatase positive cells increased (indicated by blue) in the anterior PIR, lateral septum (LS) relative to naïve and 5D males, and potentially in the MPOA relative to 5D males only.

References

Akther, S., Huang, Z., Liang, M., Zhong, J., Fakhrul, A.A.K.M., Yuhi, T., Lopatina, O., Salmina, A.B., Yokoyama, S., Higashida, C., Tsuji, T., Matsuo, M., Higashida, H., 2015. Paternal retrieval behavior regulated by brain estrogen synthetase (aromatase) in mouse sires that engage in communicative interactions with pairmates. Front. Neurosci. 9. https://doi.org/10.3389/fnins.2015.00450

Bales, K.L., Saltzman, W., 2016. Fathering in rodents: Neurobiological substrates and consequences for offspring. Hormones and Behavior 77, 249–259. https://doi.org/10.1016/j.yhbeh.2015.05.021

Besnard, A., Leroy, F., 2022. Top-down regulation of motivated behaviors via lateral septum sub-circuits. Mol Psychiatry 27, 3119–3128. https://doi.org/10.1038/s41380-022-01599-3

Bridges, R.S., 2015. Neuroendocrine regulation of maternal behavior. Front Neuroendocrinol 36, 178–196. https://doi.org/10.1016/j.yfrne.2014.11.007

Brunton, P.J., Russell, J.A., 2008. The expectant brain: adapting for motherhood. Nat Rev Neurosci 9, 11–25. https://doi.org/10.1038/nrn2280

Bürkner, P.-C., 2017. brms: An R package for Bayesian multilevel models using Stan. Journal of Statistical Software 80, 1–28. https://doi.org/10.18637/jss.v080.i01

Charitidi, K., Canlon, B., 2010. Estrogen receptors in the central auditory system of male and female mice. Neuroscience 165, 923–933. https://doi.org/10.1016/j.neuroscience.2009.11.020

Chen, P.B., Hu, R.K., Wu, Y.E., Pan, L., Huang, S., Micevych, P.E., Hong, W., 2019. Sexually dimorphic control of parenting behavior by the medial amygdala. Cell 176, 1206-1221.e18. https://doi.org/10.1016/j.cell.2019.01.024

Cornil, C.A., Ball, G.F., Balthazart, J., 2006. Functional significance of the rapid regulation of brain estrogens: Where do the estrogens come from? Brain Res 1126, 2–26. https://doi.org/10.1016/j.brainres.2006.07.098

Cornil, C.A., Seredynski, A.L., de Bournonville, C., Dickens, M.J., Charlier, T.D., Ball, G.F., Balthazart, J., 2013. Rapid control of reproductive behaviour by locally synthesised oestrogens: focus on aromatase. J Neuroendocrinol 25, 1070–1078. https://doi.org/10.1111/jne.12062

de la Fuente, A., Bing, N., Hoeschele, I., Mendes, P., 2004. Discovery of meaningful associations in genomic data using partial correlation coefficients. Bioinformatics 20, 3565–3574. https://doi.org/10.1093/bioinformatics/bth445

Devidze, N., Mong, J.A., Jasnow, A.M., Kow, L.-M., Pfaff, D.W., 2005. Sex and estrogenic effects on coexpression of mRNAs in single ventromedial hypothalamic neurons. Proceedings of the National Academy of Sciences 102, 14446–14451. https://doi.org/10.1073/pnas.0507144102

Dulac, C., O'Connell, L.A., Wu, Z., 2014. Neural control of maternal and paternal behaviors. Science 345, 765–770. https://doi.org/10.1126/science.1253291

Dutta, S., Mark-Kappeler, C.J., Hoyer, P.B., Pepling, M.E., 2014. The steroid hormone environment during primordial follicle formation in perinatal mouse ovaries. Biology of Reproduction 91, 68, 1–12. https://doi.org/10.1095/biolreprod.114.119214

Dwyer, J., Kelly, D.A., Bergan, J., 2022. Brain-wide synaptic inputs to aromatase-expressing neurons in the medial amygdala suggest complex circuitry for modulating social behavior. eNeuro 9. https://doi.org/10.1523/ENEURO.0329-21.2021

Ehret, G., 2006. Hemisphere dominance of brain function--which functions are lateralized and why?, in: 23 Problems in Systems Neuroscience, Computational Neuroscience. Oxford University Press, New York, NY, US, pp. 44–61. https://doi.org/10.1093/acprof:oso/9780195148220.003.0003

Ehret, G., 1987. Left hemisphere advantage in the mouse brain for recognizing ultrasonic communication calls. Nature 325, 249–251. https://doi.org/10.1038/325249a0

Ehret, G., Buckenmaier, J., 1994. Estrogen-receptor occurrence in the female mouse brain: Effects of maternal experience, ovariectomy, estrogen and anosmia. Journal of Physiology-Paris 88, 315–329. https://doi.org/10.1016/0928-4257(94)90012-4

Ehret, G., Jürgens, A., Koch, M., 1993. Oestrogen receptor occurrence in the male mouse brain: modulation by paternal experience. Neuroreport 4, 1247–1250. https://doi.org/10.1097/00001756-199309000-00009

Ehret, G., Koch, M., 1989. Ultrasound-induced parental behaviour in house mice is controlled by female sex hormones and parental experience. Ethology 80, 81–93. https://doi.org/10.1111/j.1439-0310.1989.tb00731.x

Ehret, G., Koch, M., Haack, B., Markl, H., 1987. Sex and parental experience determine the onset of an instinctive behavior in mice. Naturwissenschaften 74, 47–47. https://doi.org/10.1007/BF00367047

Ehret, G., Koch, M., Jirikowski, G.F., 1991. Hormone, ZNS und Fortpflanzungsverhalten. Allergologie 14, 383–394. https://doi.org/10.5414/ALP14383

Elwood, R.W., 1985. Inhibition of infanticide and onset of paternal care in male mice (Mus musculus). Journal of Comparative Psychology 99, 457–467. https://doi.org/10.1037/0735-7036.99.4.457

Fahrbach, S.E., Pfaff, D.W., 1982. Hormonal and neural mechanisms underlying maternal behavior in the rat, in: Pfaff, D.W. (Ed.), The Physiological Mechanisms of Motivation. Springer, New York, NY, pp. 253–285. https://doi.org/10.1007/978-1-4612-5692-2_9

Fleischer, S., Slotnick, B.M., 1978. Disruption of maternal behavior in rats with lesions of the septal area. Physiology & Behavior 21, 189–200. https://doi.org/10.1016/0031-9384(78)90041-0

Foidart, A., Harada, N., Balthazart, J., 1995. Aromatase-immunoreactive cells are present in mouse brain areas that are known to express high levels of aromatase activity. Cell Tissue Res 280, 561–574. https://doi.org/10.1007/BF00318360

Garcia-Segura, L.M., 2008. Aromatase in the brain: not just for reproduction anymore. Journal of Neuroendocrinology 20, 705–712. https://doi.org/10.1111/j.1365-2826.2008.01713.x

Geissler, D.B., Sabine Schmidt, H., Ehret, G., 2013. Limbic brain activation for maternal acoustic perception and responding is different in mothers and virgin female mice. Journal of Physiology-Paris 107, 62–71. https://doi.org/10.1016/j.jphysparis.2012.05.006

Geissler, D.B., Schmidt, H.S., Ehret, G., 2016. Knowledge about sounds—context-specific meaning differently activates cortical hemispheres, auditory cortical fields, and layers in house mice. Frontiers in Neuroscience 10.

Getz, L.L., Carter, C.S., Gavish, L., 1981. The mating system of the prairie vole, Microtus ochrogaster: Field and laboratory evidence for pair-bonding. Behav Ecol Sociobiol 8, 189–194. https://doi.org/10.1007/BF00299829

Guoynes, C., Marler, C., 2020. Paternal behavior from a neuroendocrine perspective, in: Oxford Research Encyclopedia of Neuroscience. https://doi.org/10.1093/acrefore/9780190264086.013.9

Hashikawa, K., Hashikawa, Y., Falkner, A., Lin, D., 2016. The neural circuits of mating and fighting in male mice. Curr Opin Neurobiol 38, 27–37. https://doi.org/10.1016/j.conb.2016.01.006

Honda, S.-I., Wakatsuki, T., Harada, N., 2011. Behavioral analysis of genetically modified mice indicates essential roles of neurosteroidal estrogen. Front Endocrinol (Lausanne) 2, 40. https://doi.org/10.3389/fendo.2011.00040

Horrell, N.D., Acosta, M.C., Saltzman, W., 2021. Plasticity of the paternal brain: Effects of fatherhood on neural structure and function. Dev Psychobiol dev.22097. https://doi.org/10.1002/dev.22097

Ji, Y., Zhao, M., Liu, Y., Chen, L., Hao, P., Sun, C., 2017. Expression of aromatase and estrogen receptors in lumbar motoneurons of mice. Neuroscience Letters 653, 7–11. https://doi.org/10.1016/j.neulet.2017.05.017

Kalisch, M., Mächler, M., Colombo, D., Maathuis, M.H., Bühlmann, P., 2012. Causal inference using graphical models with the R package pcalg. Journal of Statistical Software 47, 1–26. https://doi.org/10.18637/jss.v047.i11

Koch, M., 1990. Effects of treatment with estradiol and parental experience on the number and distribution of estrogen-binding neurons in the ovariectomized mouse brain. Neuroendocrinology 51, 505–514. https://doi.org/10.1159/000125384

Koch, M., Ehret, G., 1991. Parental behavior in the mouse: effects of lesions in the entorhinal/piriform cortex. Behavioural Brain Research 42, 99–105. https://doi.org/10.1016/S0166-4328(05)80044-0

Koch, M., Ehret, G., 1989a. Estradiol and parental experience, but not prolactin are necessary for ultrasound recognition and pup-retrieving in the mouse. Physiol Behav 45, 771–776. https://doi.org/10.1016/0031-9384(89)90293-x

Koch, M., Ehret, G., 1989b. Immunocytochemical localization and quantitation of estrogen-binding cells in the male and female (virgin, pregnant, lactating) mouse brain. Brain Res 489, 101–112. https://doi.org/10.1016/0006-8993(89)90012-7

Kohl, J., Autry, A.E., Dulac, C., 2017. The neurobiology of parenting: A neural circuit perspective. Bioessays 39, 1–11. https://doi.org/10.1002/bies.201600159

Kohl, J., Dulac, C., 2018. Neural control of parental behaviors. Curr Opin Neurobiol 49, 116–122. https://doi.org/10.1016/j.conb.2018.02.002

Laws, M.J., Kannan, A., Pawar, S., Haschek, W.M., Bagchi, M.K., Bagchi, I.C., 2014. Dysregulated estrogen receptor signaling in the hypothalamic-pituitary-ovarian axis leads to ovarian epithelial tumorigenesis in mice. PLOS Genetics 10, e1004230. https://doi.org/10.1371/journal.pgen.1004230

Lee, H., Kim, D.-W., Remedios, R., Anthony, T.E., Chang, A., Madisen, L., Zeng, H., Anderson, D.J., 2014. Scalable control of mounting and attack by ESR1+ neurons in the ventromedial hypothalamus. Nature 509, 627–632. https://doi.org/10.1038/nature13169

Lenth R, 2022. emmeans: estimated marginal means, aka least-squares means. R package version 1.8.0, <https://CRAN.R-project.org/package=emmeans>.

Li, Y., Lian, Z., Wang, B., Tai, F., Wu, R., Hao, P., Qiao, X., 2015. Natural variation in paternal behavior is associated with central estrogen receptor alpha and oxytocin levels. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 201, 285–293. https://doi.org/10.1007/s00359-015-0979-6

Lonstein, J.S., De Vries, G.J., 2000. Sex differences in the parental behavior of rodents. Neurosci Biobehav Rev 24, 669–686. https://doi.org/10.1016/s0149-7634(00)00036-1

Makowski, D., Ben-Shachar, M.S., Lüdecke, D., 2019. bayestestR: Describing effects and their uncertainty, existence and significance within the Bayesian framework. Journal of Open Source Software 4, 1541. https://doi.org/10.21105/joss.01541

Malikov, V., Madeira, M.D., 2013. Regulation of ERα protein expression by 17β-estradiol in cultured neurons of hypothalamic ventromedial nucleus. Neurochem Res 38, 82–89. https://doi.org/10.1007/s11064-012-0891-1

Marlin, B.J., Mitre, M., D'amour, J.A., Chao, M.V., Froemke, R.C., 2015. Oxytocin enables maternal behaviour by balancing cortical inhibition. Nature 520, 499–504. https://doi.org/10.1038/nature14402

Menon, R., Süß, T., Oliveira, V.E. de M., Neumann, I.D., Bludau, A., 2022. Neurobiology of the lateral septum: regulation of social behavior. Trends in Neurosciences 45, 27–40. https://doi.org/10.1016/j.tins.2021.10.010

Nilsson, M.E., Vandenput, L., Tivesten, Å., Norlén, A.-K., Lagerquist, M.K., Windahl, S.H., Börjesson, A.E., Farman, H.H., Poutanen, M., Benrick, A., Maliqueo, M., Stener-Victorin, E., Ryberg, H., Ohlsson, C., 2015. Measurement of a Comprehensive Sex Steroid Profile in Rodent Serum by High-Sensitive Gas Chromatography-Tandem Mass Spectrometry. Endocrinology 156, 2492–2502. https://doi.org/10.1210/en.2014-1890

Numan, M., 2020. The Parental Brain: Mechanisms, Development, and Evolution. Oxford University Press.

Numan, M., 2006. Hypothalamic neural circuits regulating maternal responsiveness toward infants. Behav Cogn Neurosci Rev 5, 163–190. https://doi.org/10.1177/1534582306288790

Numan, M., Insel, T.R., 2003. The neurobiology of parental behavior, Hormones, brain, and behavior. Springer, New York.

O'Connell, L.A., Hofmann, H.A., 2012. Evolution of a vertebrate social decision-making network. Science 336, 1154–1157. https://doi.org/10.1126/science.1218889

Palomero-Gallagher, N., Amunts, K., 2022. A short review on emotion processing: a lateralized network of neuronal networks. Brain Struct Funct 227, 673–684. https://doi.org/10.1007/s00429-021-02331-7

Paxinos, G., Franklin, K., 2001. The mouse brain in stereotaxic coordinates, 2nd ed. Academic Press, San Diego.

Poindron, P., Lévy, F., Krehbiel, D., 1988. Genital, olfactory, and endocrine interactions in the development of maternal behaviour in the parturient ewe. Psychoneuroendocrinology 13, 99–125. https://doi.org/10.1016/0306-4530(88)90009-1

R Core Team, 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rilling, J.K., Mascaro, J.S., 2017. The neurobiology of fatherhood. Curr Opin Psychol 15, 26–32. https://doi.org/10.1016/j.copsyc.2017.02.013

Risold, P.Y., Swanson, L.W., 1997. Connections of the rat lateral septal complex. Brain Res Brain Res Rev 24, 115–195. https://doi.org/10.1016/s0165-0173(97)00009-x

Rizzi-Wise, C.A., Wang, D.V., 2021. Putting together pieces of the lateral septum: multifaceted functions and its neural pathways. eNeuro 8, ENEURO.0315-21.2021. https://doi.org/10.1523/ENEURO.0315-21.2021

Romero-Morales, L., Martínez-Torres, M., Cárdenas, M., Álvarez, C., Carmona, A., Cedillo, B., Loya-Zurita, E., Luis, J., 2018. An increase in estradiol facilitates the onset of paternal behavior in the dwarf hamster (Phodopus campbelli). Hormones and Behavior 99, 35–40. https://doi.org/10.1016/j.yhbeh.2018.02.002

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. https://doi.org/10.1006/hbeh.1997.1430

Rosenblatt, J.S., Siegel, H.I., 1981. Factors governing the onset and maintenance of maternal behavior among nonprimate mammals, in: Gubernick, D.J., Klopfer, P.H. (Eds.), Parental Care in Mammals. Springer US, Boston, MA, pp. 13–76. https://doi.org/10.1007/978-1-4613-3150-6_2

Rosenblatt, J.S., Wagner, C.K., Morrell, J.I., 1994. Hormonal priming and triggering of maternal behavior in the rat with special reference to the relations between estrogen receptor binding and ER mRNA in specific brain regions. Psychoneuroendocrinology 19, 543–552. https://doi.org/10.1016/0306-4530(94)90039-6

Schleicher, G., Stumpf, W.E., Morin, J.K., Drews, U., 1986. Sites of aromatization of [3H]testosterone in forebrain of male, female and androgen receptor-deficient Tfm mice: an autoradiographic study. Brain Res 397, 290–296. https://doi.org/10.1016/0006-8993(86)90630-x

Sheehan, T., Paul, M., Amaral, E., Numan, M.J., Numan, M., 2001. Evidence that the medial amygdala projects to the anterior/ventromedial hypothalamic nuclei to inhibit maternal behavior in rats. Neuroscience 106, 341–356. https://doi.org/10.1016/s0306-4522(01)00286-x

Sheng, H.Z., Fields, R.D., Nelson, P.G., 1993. Specific regulation of immediate early genes by patterned neuronal activity. J Neurosci Res 35, 459–467. https://doi.org/10.1002/jnr.490350502

Sheng, M., McFadden, G., Greenberg, M.E., 1990. Membrane depolarization and calcium induce c-fos transcription via phosphorylation of transcription factor CREB. Neuron 4, 571–582. https://doi.org/10.1016/0896-6273(90)90115-v

Shinohara, Y., Hirase, H., Watanabe, M., Itakura, M., Takahashi, M., Shigemoto, R., 2008. Left-right asymmetry of the hippocampal synapses with differential subunit allocation of glutamate receptors. Proceedings of the National Academy of Sciences 105, 19498–19503. https://doi.org/10.1073/pnas.0807461105

Shinohara, Y., Hosoya, A., Yamasaki, N., Ahmed, H., Hattori, S., Eguchi, M., Yamaguchi, S., Miyakawa, T., Hirase, H., Shigemoto, R., 2012. Right-hemispheric dominance of spatial memory in split-brain mice. Hippocampus 22, 117–121. https://doi.org/10.1002/hipo.20886

Shipley, B., 2000. Cause and correlation in biology: a user's guide to path analysis, structural equations, and causal inference in R. Cambridge University Press, Cambridge, UK.

Shipton, O.A., El-Gaby, M., Apergis-Schoute, J., Deisseroth, K., Bannerman, D.M., Paulsen, O., Kohl, M.M., 2014. Left–right dissociation of hippocampal memory processes in mice. Proc Natl Acad Sci USA 111, 15238–15243. https://doi.org/10.1073/pnas.1405648111

Siegel, H.I., Rosenblatt, J.S., 1975. Estrogen-induced maternal behavior in hysterectomized-ovariectomized virgin rats. Physiology & Behavior 14, 465–471. https://doi.org/10.1016/0031-9384(75)90012-8

Slotnick, B.M., Nigrosh, B.J., 1975. Maternal behavior of mice with cingulate cortical, amygdala, or septal lesions. Journal of Comparative and Physiological Psychology 88, 118. https://doi.org/10.1037/h0076200

Stanić, D., Dubois, S., Chua, H.K., Tonge, B., Rinehart, N., Horne, M.K., Boon, W.C., 2014. Characterization of aromatase expression in the adult male and female mouse brain. I. Coexistence with oestrogen receptors α and β, and androgen receptors. PLoS One 9, e90451. https://doi.org/10.1371/journal.pone.0090451

Stolzenberg, D.S., Rissman, E.F., 2011. Oestrogen-independent, experience-induced maternal behaviour in female mice. J Neuroendocrinol 23, 345–354. https://doi.org/10.1111/j.1365-2826.2011.02112.x

Tachikawa, K.S., Yoshihara, Y., Kuroda, K.O., 2013. Behavioral transition from attack to parenting in male mice: a crucial role of the vomeronasal system. Journal of Neuroscience 33, 5120–5126. https://doi.org/10.1523/JNEUROSCI.2364-12.2013

Trainor, B.C., Bird, I.M., Alday, N.A., Schlinger, B.A., Marler, C.A., 2003. Variation in aromatase activity in the medial preoptic area and plasma progesterone is associated with the onset of paternal behavior. Neuroendocrinology 78, 36–44. https://doi.org/10.1159/000071704

Trainor, B.C., Finy, M.S., Nelson, R.J., 2008. Paternal aggression in a biparental mouse: parallels with maternal aggression. Horm Behav 53, 200–207. https://doi.org/10.1016/j.yhbeh.2007.09.017

Trainor, B.C., Marler, C.A., 2002. Testosterone promotes paternal behaviour in a monogamous mammal via conversion to oestrogen. Proc. R. Soc. Lond. B 269, 823–829. https://doi.org/10.1098/rspb.2001.1954

Unger, E.K., Burke, K.J., Yang, C.F., Bender, K.J., Fuller, P.M., Shah, N.M., 2015. Medial amygdalar aromatase neurons regulate aggression in both sexes. Cell Reports 10, 453–462. https://doi.org/10.1016/j.celrep.2014.12.040

van Buuren, S., Groothuis-Oudshoorn, K., 2011. mice: multivariate imputation by chained equations in R. Journal of Statistical Software 45, 1–67. https://doi.org/10.18637/jss.v045.i03

Vehtari, A., Gelman, A., Simpson, D., Carpenter, B., Bürkner, P.-C., 2021. Rank-normalization, folding, and localization: an improved Rˆ for assessing convergence of MCMC (with discussion). Bayesian Analysis 16, 667–718. https://doi.org/10.1214/20-BA1221

Wickham, H., 2016. ggplot2: elegant graphics for data analysis. Springer-Verlag, New York.

Woodroffe, R., Vincent, A., 1994. Mother's little helpers: Patterns of male care in mammals. Trends in Ecology & Evolution 9, 294–297. https://doi.org/10.1016/0169-5347(94)90033-7

Wozniak, A., Hutchison, R.E., Hutchison, J.B., 1992. Localisation of aromatase activity in androgen target areas of the mouse brain. Neuroscience Letters 146, 191–194. https://doi.org/10.1016/0304-3940(92)90075-I

Wu, Z., Autry, A.E., Bergan, J.F., Watabe-Uchida, M., Dulac, C.G., 2014. Galanin neurons in the medial preoptic area govern parental behaviour. Nature 509, 325–330. https://doi.org/10.1038/nature13307

Xiao, L., Jordan, C.L., 2002. Sex Differences, Laterality, and Hormonal Regulation of Androgen Receptor Immunoreactivity in Rat Hippocampus. Hormones and Behavior 42, 327–336. https://doi.org/10.1006/hbeh.2002.1822

Yang, T., Yang, C.F., Chizari, M.D., Maheswaranathan, N., Burke, K.J., Borius, M., Inoue, S., Chiang, M.C., Bender, K.J., Ganguli, S., Shah, N.M., 2017. Social Control of Hypothalamus-Mediated Male Aggression. Neuron 95, 955-970.e4. https://doi.org/10.1016/j.neuron.2017.06.046