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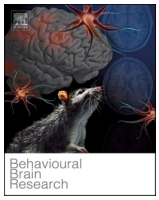

Research report

Effects of reproductive status on behavioral and neural responses to isolated pup stimuli in female California mice

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ABSTRACT

The transition to motherhood in mammals is marked by changes in females' perception of and responsiveness to sensory stimuli from infants. Our understanding of maternally induced sensory plasticity relies most heavily on studies in uniparental, promiscuous house mice and rats, which may not be representative of rodent species with different life histories. We exposed biparental, monogamous California mouse (*Peromyscus californicus*) mothers and ovariectomized virgin females to one of four acoustic and olfactory stimulus combinations (Control: clean cotton and white noise; Call: clean cotton and pup vocalizations; Odor: pup-scented cotton and white noise; Call + Odor: pup-scented cotton and pup vocalizations) and quantified females' behavior and Fos expression in select brain regions. Behavior did not differ between mothers and ovariectomized virgins. Among mothers, however, those exposed to the Control condition took the longest to sniff the odor stimulus, and mothers exposed to the Odor condition were quicker to sniff the odor ball compared to those in the Call condition. Behavior did not differ among ovariectomized virgins exposed to the different conditions. Fos expression differed across conditions only in the anterior hypothalamic nucleus (AHN), which responds to aversive stimuli: among mothers, the Control condition elicited the highest AHN Fos and Call + Odor elicited the lowest. Among ovariectomized virgin females, Call elicited the lowest Fos in the AHN. Thus, reproductive status in California mice alters females' behavioral responses to stimuli from pups, especially odors, and results in the inhibition of defense circuitry in response to pup stimuli.

1. Introduction

Around the time of parturition, female mammals exhibit a shift in behavioral responses to infants, triggered, in part, by increased attraction to sensory cues from newborns (e.g., crying, odor of amniotic fluid) [1–5]. This increased attraction is associated with changes in neural responsiveness [6–9] and can facilitate parental care and enhance survival of offspring [10]. The neural plasticity that results from the onset of motherhood is mediated by hormonal changes that accompany pregnancy, parturition and lactation [11–13].

Changes in olfactory and auditory processing of infant-related stimuli in mothers have been demonstrated in behavioral and neural studies of rodents [5]. For example, primiparous female rats (*Rattus norvegicus*) show a stronger preference for pup-soiled bedding relative to fresh bedding, whereas virgin females do not [14,15]. Similarly, primiparous female rats display more maternal behavior (e.g., pup retrieval, nest-building, and pup sniffing/licking) in the presence of pup calls than in the absence of pup calls, while virgin females show no difference [16,17]. Furthermore, primiparous house mouse (*Mus musculus*) mothers have more new neurons in their olfactory bulbs compared to age-matched virgins [18] and may be better able to distinguish pup calls from other sounds [19]. While the effects of motherhood on responses to pup stimuli have received a fair amount of attention, the relative effects of auditory and olfactory stimuli and interactions between them are not well understood.

Pup-related stimuli in different sensory modalities can have additive or synergistic effects on soliciting maternal behaviors from mothers, with most work focusing on the effects of olfactory and auditory pup stimuli [5,20]. For example, primiparous house mouse and rat mothers can locate pups or pup stimuli more quickly when both olfactory and

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acoustic stimuli are present compared to when only one of these stimuli is presented [21,22]. The neural mechanisms underlying these effects have not been well established [5], but some key brain regions for integrating multiple sensory stimuli emitted from pups have been identified. These include the medial preoptic area (MPOA), bed nucleus of the stria terminalis (BNST) and basolateral amygdala (BLA). Primiparous rat mothers and virgin female house mice allowed to interact with pups have higher expression of the immediate early gene c-fos in the MPOA, a region critical for maternal behavior, compared to females exposed to only distal pup cues [23,24]. Okabe et al. [22] found that primiparous house mouse mothers had higher Fos expression in the MPOA, BNST and BLA following exposure to both pup odors and pup vocalizations than after exposure to either stimulus alone. Other regions that may show differential Fos expression in primiparous mothers in response to individual or combined pup sensory stimuli are those central to the reward (ex. nucleus accumbens [NAcc]) and aversion circuits (anterior hypothalamic nucleus [AHN]) and structures that relay sensory information to both of these pathways (BLA and basomedial amygdala [BMA]). These regions process pup-related olfactory and auditory stimuli [25–27].

Nearly all work on sensory plasticity in rodents has focused on the house mouse and Norway rat, which are both uniparental and promiscuous and produce large litters of altricial young [13]. Since these two species do not represent the range of mammalian, or even rodent, life histories, they are not likely to represent the diversity of sensory plasticity that occurs during the transition to motherhood [5,13]. Moreover, even these two species show some disparities in sensory plasticity. For example, in house mice, primiparous mothers and virgins do not differ in their preference for pup-scented bedding compared to clean bedding [22] whereas in rats, primiparous mothers show a stronger preference than virgin females [14]. Therefore, we investigated motherhood-related sensory plasticity in a third rodent species, the California mouse (*Peromyscus californicus*). This species provides a useful model because it shows markedly different patterns of social and parental behavior compared to traditional mammalian model species: California mice are socially monogamous and biparental (i.e., both parents provide care for their offspring) and produce relatively small litters (1−4) of pups [28]. California mice, which are in the family Cricetidae, are also less closely related to house mice and rats, which are both in the family Muridae [29].

The aim of the current experiment was to determine whether motherhood and ovarian hormones alter behavioral and neural responses to olfactory and acoustic cues from pups in California mice. This study was conducted concurrently with one focused on the male partners of the females used here [30]. Behavioral and neural responses to pup odors and/or vocalizations were compared between primiparous mothers and ovariectomized (OVX) virgin adult females housed with an intact male. Because OVX females lack the necessary hormonal priming, they fail to copulate when housed with a male [31]. Thus, comparing mothers to OVX females allowed us to assess possible effects of motherhood and ovarian hormones on behavioral and neural responses to pup sensory stimuli while controlling for potential effects of cohabitating with a mate. We hypothesized that relative to OVX virgin females, mothers would display greater attraction to pup stimuli and would have stronger neural responses to these stimuli, as determined by Fos expression, in brain regions associated with maternal behavior, but might have weaker responses in brain regions associated with defense. Additionally, we hypothesized that pup olfactory and auditory stimuli would have additive or synergistic effects on maternal behavior and neural activation.

2. Methods

2.1. Animals

We used California mice that were bred at the University of California, Riverside (UCR) and that were descended from mice purchased from the Peromyscus Genetic Stock Center (University of South Carolina, Columbia, USA). At weaning age (27–31 days), prior to the birth of younger siblings, mice were removed from their parents' cage and housed in single-sex groups of 2–4 age-matched mice until they were used in this study. The initial sample included 90 females and 90 males that originated from 27 families. The design and methods mirrored those used in our previous study on males [30].

At all life stages, mice were housed in 44 × 24 x 20 cm polycarbonate cages with aspen shavings for bedding and cotton for nesting material and had ad libitum access to food (Purina 5001 Rodent Chow) and water. The lights were on a 14:10 h cycle, with lights on from 2300 h to 1300 h. The ambient temperature was kept at approximately 23 °C, and humidity was approximately 65%. All procedures were approved by UCR's Institutional Animal Care and Use Committee and were conducted in accordance with the recommendations of the *Guide for the Care and Use of Laboratory Animals*. UCR is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

2.2. Surgeries and pairing

Females with no prior exposure to unrelated males were randomly assigned to undergo ovariectomies (OVX virgin females) or sham ovariectomies (mothers) between 75 and 125 days of age. As previously described [30–32], females were anesthetized with 2.5% isoflurane vapor, and an approximately 1 cm midline incision was made. For ovariectomies only, the right and left ovaries were located and removed. The abdominal muscle was closed using absorbable sutures, and the skin was sealed using tissue glue. Following surgery, females were given 5 mg/kg carprofen (Carprieve [Norbrook Laboratories; Overland Park, KS, USA]) S.C. every 12 h for 48 h for analgesia and housed in isolation for 7 days to allow for recovery. Females were then housed with their original same-sex cage mates for 3 days before being paired with a reproductively naïve male. Pair mates were no more closely related than first cousins. After pairing, cages were checked daily for the presence of pups. Following perfusion (see Stimulus Exposure below), the reproductive tracts of all ovariectomized females were dissected to confirm that surgeries were successful.

2.3. Stimulus exposure

Each female was tested once in a single stimulus condition, after which it was perfused transcardially and the brain collected for immunohistochemistry (see below). Mothers were tested when their first litter of pups was between 4 and 6 days old. To control for age and the length of time since pairing, OVX virgin females were tested at a matched time point. In each test, the mouse was exposed to one olfactory stimulus (pup-scented cotton or clean cotton; see below) and one acoustic stimulus (pre-recorded pup vocalizations or white noise; see below), which produced 4 stimulus combinations: Control (clean cotton and white noise), Call (clean cotton and pup vocalizations), Odor (pup-scented cotton and white noise), and Call + Odor (pup-scented cotton and pup vocalizations). The pup olfactory stimulus was prepared by wiping the ventrum and anogenital region of an unrelated 3- to 7-day-old pup 30 times with all sides of a cotton ball. The cotton ball was then placed in a stainless-steel wire-mesh tea ball (Ø: 6 cm). Clean cotton was placed directly into the tea ball. Fresh gloves were used for each stimulus preparation. The pup call was a 25 s loop recording from an isolated 4-day-old unrelated pup held at room temperature. California mouse pup calls have been categorized as sustained sweeps that may or may not fall in the ultrasonic range [33,34]. The control auditory stimulus was a 25 s loop of 6 bursts of white noise followed by a 1 s pause, which mimicked the calling pattern of the pup-call playback. Acoustic stimuli were played through a speaker (UltraSoundGate BL Pro, Avisoft Bioacoustics, Glienecke, Germany) adjacent to the testing arena. Mothers and OVX virgins had been pair-housed with males for 50.2 ± 13.6 days and 49.9

± 10.1 days, respectively (mean ± SE; t-test: t = −0.08, df = 61, P = 0.93) and were 157.2 ± 2.5 and 152.5 ± 2.6 days of age, respectively (mean ± SE; t-test: t = −1.29, df = 61, P = 0.20), at the time of testing. Mice were assigned to stimulus conditions randomly with the exception that littermates were assigned to different conditions.

Between 0830 and 0930 h (during lights-on) on the day of testing, the female California mouse was placed individually in a 12.00 × 7.50 × 5.25 cm polycarbonate cage with aspen shavings, food and water. The cage was placed in a corner of a black acrylic open-field arena (1 ×1 x 0.5 m) in a sound-reduced environmental chamber. Stimulus exposure began 110 min after the mouse was placed in the cage, to allow for dissipation of any peaks in Fos expression in the brain related to home-cage events or handling [30,35].

The odor ball was placed in a standardized position in the front left corner of the cage, and the acoustic stimulus, played from a speaker (UltraSoundGate BL Pro, Avisoft Bioacoustics, Glienecke, Germany) located adjacent to the arena, was immediately started. The olfactory and auditory stimuli were presented to the female for 10 min and then removed from the cage and environmental chamber. To be consistent with previous studies in California mice (e.g. [30,35–38]), the mouse remained in the test cage for an additional 60 min; however, we recognize that Fos levels might have been higher had the waiting period been longer [39]. The mouse was then deeply anesthetized with an IP injection of pentobarbital (Fatal-Plus solution, Vortech Pharmaceuticals, Dearborn, MI, USA) and perfused transcardially with 0.1 M phosphate buffered saline (PBS) followed by 4% paraformaldehyde. The brain was removed rapidly and fixed in 4% paraformaldehyde for 2 days at 4 °C. It was then cryoprotected in 30% sucrose and frozen in cryoprotectant (30% sucrose, 30% ethylene glycol) at − 20 °C. Because production of fecal boli is often used as a metric of anxiety [40,41], the shavings from each female's test cage were saved, and fecal boli were collected and counted.

2.4. Immunohistochemistry

Immunohistochemistry for Fos, the protein product of the immediate early gene c-fos, was completed for 6 females per condition. Preparation of brains and immunohistochemistry were performed as previously described [30]. Three to 5 days prior to slicing, brains were thawed and transferred into 30% sucrose at 4 °C. Brains were sectioned (40 μm) using a Leica CM1950 cryostat (Leica Biosystems, Deer Park, IL, USA) set at − 20 °C. Sections were incubated overnight with polyclonal rabbit anti-cFos (1:2500; Synaptic Systems, Göttingen, Germany) followed by incubation with goat anti-rabbit IgG, Alexa Fluor 555 (Thermo Fisher Scientific, Waltham, MA, USA; 1:500 dilution) for 90 min. Procedures using Alexa Fluor and all subsequent procedures were conducted with minimal ambient light. Sections were mounted on slides with EMS Shield Mount with DABCO (Electron Microscopy Sciences, Hatfield, PA, USA) and stored covered at 4 °C. Images of the brain regions of interest were taken between 16 and 22 h after tissue was mounted using a Zeiss LSM 880 inverted microscope (Carl Zeiss Microscopy, White Plains, NY, USA). Immunohistochemistry was performed in batches containing one brain from each of the 8 reproductive status x stimulus groups.

Fos immunoreactivity was quantified in regions associated with sensory relay (MOB, BLA, BMA), parental behavior (bed nucleus of the stria terminalis medial division, ventral part [STMV], MPOA), reward (NAcc shell), and fear/anxiety (AHN) (reviewed in [42]). Brain regions were located by cross-referencing *The Mouse Brain in Stereotaxic Coordinates* [43] for *Mus musculus* and images of Nissl-stained California mouse sections ([45] http://brainmaps.org). QuPath 3.0 [44] was used to quantify the number of Fos-positive cells by outlining in each brain region of interest a 200 × 200 μm square in the area with the highest density of Fos-positive cells. Scorers were blind to reproductive status and stimulus condition during quantification of Fos immunoreactivity. Data for each region for each female were averaged from two sections from each hemisphere. Technical problems resulted in a small number of unusable images (see figures and tables for final sample sizes).

2.5. Behavior measurements

Mice were video-recorded throughout the 10-min stimulus-exposure period as well as the subsequent 60 min. Videos were scored using Behavioral Observation Research Interactive Software (BORIS53 [46]). All behaviors scored were mutually exclusive of one another. For the 10 min of stimulus exposure, we scored behavior continuously to quantify latencies to listen (ears perked in the direction of the acoustic stimulus), to sniff the odor ball (nose < 4 cm from the ball, with whiskers moving up and down), and to handle the odor ball (front paw[*s*] on ball), and the total durations of time spent listening, sniffing the ball and handling the ball. Mice that did not display these behaviors were assigned a latency of 600 s (i.e., 10 min, the length of the test). Additionally, we measured the total time spent autogrooming, other active behaviors (i.e. locomoting, drinking, eating), resting (lying down with little or no head movement), and backflipping (a stress-related behavior [47,48]). Because the exact amount of time that stimuli were presented varied slightly across tests, the time spent in each activity was normalized across all recordings by dividing the total time of the activity by the duration of stimulus exposure and multiplying by 600 s ([Σ behavior (s) / stimulus presentation (s)] * 600 s). During the hour following stimulus exposure, we performed instantaneous scans every 5 min to record the subject's behavior using the same categories listed above.

2.6. Statistical analyses

Final sample sizes for behavioral responses to the stimulus conditions differed because pups born to six mothers did not survive until the day of testing, four "OVX virgin" females were found to be pregnant during dissection, and technical issues arose during three stimulus exposures (two mothers, one virgin), which were not identified until videos were scored. Final sample sizes for analyses are reported in each table and figure.

Analyses were performed in STATA 15 (StataCorp LP, College Station, TX, USA). Assumptions for linear mixed-effects models (LMMs) and ANOVAs were checked through evaluation of quantile-quantile plots and Shapiro-Wilk analyses. Fos expression in MOB, NAcc, MPOA, STMV, AHN, BLA, and BMA were square-root transformed to meet assumptions for parametric tests. Significance was assessed based on $\alpha = 0.05$ (two-tailed).

Latencies and durations of each behavior during stimulus exposure and counts of behaviors in the 60 min following stimulus exposure were analyzed using non-parametric tests because measures did not meet parametric assumptions and were resistant to transformation. Mann-Whitney U tests were used to compare behavior between mothers and OVX virgin females, and Kruskal-Wallis tests were used to compare behavior among stimulus conditions. When results were significant, Dunn's pairwise comparisons were performed.

LMMs were used to assess the effects of female reproductive status (mother vs. OVX virgin), stimulus condition (Control, Call, Odor, Call + Odor), and their interaction on Fos expression in each brain region of interest (see above). Immunohistochemistry batch (the group with which each brain underwent immunohistochemistry) was included as a random variable for analyses of Fos expression. A two-way ANOVA was used to assess the effects of the same independent variables on number of fecal boli produced. Non-significant ($P > 0.05$) interactions were removed from the final models for both Fos expression and fecal bolus counts. Lastly, Pearson's correlations were used to evaluate associations between Fos expression and behavior for mothers and OVX virgin females separately. Data are available on Dryad.

3. Results

3.1. Behavior during stimulus exposure

Both mothers and OVX virgin females showed extensive inter-individual variation in behavior, both during and after stimulus exposure. Most females displayed one or more active behaviors (e.g. locomoting, backflipping), whereas some spent nearly the entire test resting in a single location, as commonly observed when California mice are in their home cages during lights-on. Additionally, most mothers (77%) and OXV virgins (75%) approached and sniffed the odor ball, and among females exposed to pup odor, all mothers and all but two OXV virgins sniffed the ball.

We first compared behavior of primiparous mothers and OVX virgin females during the stimulus exposure, collapsed across stimulus conditions, using Mann-Whitney U tests. Mothers and OXV virgins did not differ in their latencies to sniff the odor ball, handle the odor ball, or listen (Table 1). Mothers and OVX virgins also did not differ in the amount of time they spent sniffing the odor ball, handling the odor ball, or listening during the 10-minute stimulus exposure (Table 1). However, mothers spent more time grooming themselves ($P = 0.022$) and backflipping ($P = 0.047$), compared to OVX virgins (Table 1).

Effects of stimulus condition on behavior during the stimulus exposure were analyzed using Kruskal-Wallis tests for mothers and OVX virgin females separately (Table 2). Among mothers, latency to sniff the odor ball differed significantly across stimulus conditions ($P = 0.040$; Fig. 1A): mothers in the Control condition took longer to sniff the odor ball than mothers in all other stimulus conditions, and mothers in the Odor condition were quicker to sniff the odor ball compared to those in the Call condition (Dunn's post-hoc P's < 0.05). The time it took mothers in the Odor + Call condition to sniff the odor ball did not differ from those in the Odor and Call conditions. Among the four stimulus conditions, mothers showed non-significant tendencies to spend more time sniffing the odor ball when pup odor was presented compared to clean cotton ($P = 0.057$), listening when pup calls were presented compared to white noise ($P = 0.077$), and resting when no pup stimuli were presented ($P = 0.058$). Stimulus condition did not affect mothers'

Table 1
Latencies and total durations of behaviors during 10-min stimulus exposures and numbers of scan samples in which behavior was observed during the 60 min after stimulus exposure. Data are shown as median (1st and 3rd quartiles) for mothers and OVX virgins separately collapsed across all four stimulus conditions. Significant differences between mothers and virgins ($P < 0.05$, Mann-Whitney U tests) are indicated in bold.

Behavior (units)	Mothers (N = 35)	OVX virgins (N = 41)	z	*P*
During stimulus exposure (maximum = 600 s)				
Latency to sniff ball (s)	322.3 (40.8, 600.0)	413.6 (72.1, 600.0)	0.92	0.36
Latency to handle ball (s)	600.0 (295.5, 600.0)	600.0 (389.0, 600.0)	-0.18	0.85
Latency to listen (s)	600.0 (196.4, 600.0)	600.0 (445.7, 600.0)	1.24	0.22
Duration sniff ball (s)	24.2 (0.0, 74.0)	15.2 (0.0, 64.8)	-0.87	0.38
Duration handle ball (s)	0.0 (0.0, 5.5)	0.0 (0.0, 5.54)	0.49	0.62
Duration listen (s)	8.4 (0.0, 31.6)	0.0 (0.0, 20.9)	-1.51	0.13
Duration autogroom (s)	0.0 (0.0, 34.4)	0.0 (0.0, 0.0)	-2.29	**0.02**
Duration activity (s)	30.2 (0.0, 138.2)	2.5 (0.0, 54.5)	-1.41	0.16
Duration rest (s)	404.9 (174.3–600)	564.4 (247.1, 600.0)	1.34	0.18
Duration backflip (s)	0.0 (0.0, 24.9)	0.0 (0.0, 0.0)	-1.98	**0.05**
After stimuli removed (maximum = 12 instantaneous scans)				
Autogroom (count)	1 (0, 2)	1 (0, 1)	-2.18	**0.03**
Activity (count)	4 (1, 7)	2 (1, 5)	-1.64	0.10
Rest (count)	3 (1, 10)	9 (7, 11)	0.13	**0.008**
Backflip (count)	0 (0, 4)	0 (0, 1)	-3.31	**0.001**

Table 2
Latencies and total durations of behaviors during 10-min stimulus exposures and numbers of scan samples in which behavior was observed during the 60 min after stimulus exposure. Data are shown as median (1st and 3rd quartiles) for mothers and OVX virgins within each stimulus condition. Kruskal-Wallis tests among stimulus conditions: significant differences ($P < 0.05$) are indicated in bold, and non-significant trends ($0.05 < P < 0.1$) are indicated in bolded italics.

Behavior	Control	Call	Odor	Call + Odor	$\chi 2$	*P*
A. Mothers	N = 6	N = 9	N = 9	N = 11		
During stimulus exposure (maximum = 600 s)						
Latency to sniff ball (s)	600.0 (600.0, 600.0)	328.2 (245.6, 600.0)	40.8 (12.1, 63.4)	180.9 (156.9, 544.2)	8.33	**0.04**
Latency to handle ball (s)	600.0 (600.0, 600.0)	600.0 (295.5, 600.0)	600.0 (217.7, 600.0)	600.0 (385.6, 600.0)	0.58	0.90
Latency to listen (s)	600.0 (524.2, 600.0)	323.3 (260.4, 600.0)	236.1 (83.2, 600.0)	600.0 (213.0, 600.0)	2.94	0.40
Duration sniff ball (s)	0.0 (0.0, 0.0)	24.2 (0.0, 72.9)	66.0 (25.6, 74.0)	56.7 (0.9, 114.7)	*7.53*	***0.06***
Handle ball (s)	0.0 (0.0, 0.0)	0.0 (0.0, 5.5)	0.0 (0.0, 19.6)	0.0 (0.0, 1.6)	0.79	0.85
Listen (s)	0.0 (0.0, 8.8)	21.5 (0.0, 121.9)	17.2 (0.0, 60.2)	0.0 (0.0, 9.6)	6.85	***0.08***
Autogroom (s)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	13.4 (0.0, 32.6)	14.4 (0.0, 45.4)	4.45	0.22
Activity (s)	0.0 (0.0, 3.5)	30.2 (0.0, 97.5)	117.7 (0, 196.0)	83.3 (0.0, 138.2)	5.12	0.16
Rest (s)	600.0 (579.0, 600.0)	456.9 (246.2, 600.0)	225.6 (0.0, 534.0)	224.8 (163.2, 600.0)	7.50	***0.06***
Backflip (s)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (44.8, 0.0)	4.79	0.19
After stimulus exposure (maximum = 12 instantaneous scans)						
Autogroom (count)	1 (0, 3)	1 (1, 2)	1 (0, 2)	2 (1, 3)	5.53	0.14
Activity (count)	2 (2, 5)	5 (1, 5)	6 (2, 8)	4 (1, 11)	0.77	0.86
Rest (count)	9 (6, 11)	2 (1, 7)	5 (3, 10)	5 (1, 9)	5.97	0.11
Backflip (count)	0 (0, 0)	4 (1, 5)	0 (0, 0)	0 (0, 5)	13.59	**0.004**
B. OVX virgin females	N = 10	N = 10	N = 9	N = 12		
During stimulus exposure (maximum = 600 s)						
Latency to sniff ball (s)	478.2 (23.7, 600.0)	487.1 (97.8, 600.0)	413.6 (23.4, 600.0)	340.0 (130.3, 600.0)	0.18	0.98
Latency to handle ball (s)	506.5 (297.0, 600)	600 (247.0, 600)	249.4 (72.8, 600)	600.0 (496.0, 600.0)	0.91	0.82
Latency to listen (s)	600.0 (212.4, 600.0)	600.0 (536.0, 600.0)	600 (14.5, 600.0)	600.0 (520.0, 600.0)	0.49	0.92
Sniff ball (s)	6.9 (0.0, 102.6)	7.6 (0.0, 22.6)	39.3 (0.0, 64.3)	35.9 (0.0, 60.7)	1.05	0.79
Handle ball (s)	0.3 (0.0, 166.3)	0.0 (0.0, 1.8)	0.0 (0.0, 230.2)	0.0 (0.0, 2.8)	1.28	0.73
Listen (s)	0.0 (0.0, 20.9)	6.2 (0.0, 28.4)	0.0 (0.0, 28.8)	0.0 (0.0, 1.9)	1.69	0.64
Autogroom (s)	0.0 (0.0, 7.2)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.9)	0.44	0.93
Activity (s)	17.9, (0.0, 42.9)	0.0 (0.0, 55.5)	0.0(0.0, 54.6)	14.6 (0.0, 115.2)	0.62	0.87

(*continued on next page*)

Table 2 (*continued*)

Behavior	Control	Call	Odor	Call + Odor	χ2	*P*
Rest (s)	562.3 (259.2, 600.0)	582.5 (102.5, 600.0)	518.7 (247.1, 600.0)	549.5 (272.0, 600.0)	0.15	0.99
Backflip (s)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	3.10	0.38
After stimulus exposure (maximum = 12 instantaneous scans)						
Autogroom (count)	1 (0, 1)	0 (0, 1)	1 (0, 1)	0.5 (0, 1)	1.47	0.69
Activity (count)	2 (1, 10)	2, (0, 4)	2(0, 5)	2.5 (1, 6)	0.72	0.87
Rest (count)	9.5 (1, 11)	9.5 (7, 12)	10 (7, 11)	8 (5.5, 12)	0.84	0.84
Backflip (count)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	1.09	0.78

latencies to handle the odor ball or listen (Table 2). Behavior of OVX virgin females during stimulus exposure did not differ among stimulus conditions (*P*'s > 0.38; Fig. 1B; Table 2).

3.2. Behavior after stimulus exposure

After olfactory and acoustic stimuli were removed, females' behavior was scored instantaneously every 5 min for one hour and analyzed using Mann-Whitney U or Kruskal-Wallis tests. Compared to OVX virgins, mothers were observed to be grooming themselves ($P = 0.029$) and backflipping ($P = 0.0009$) on more occasions, and to be resting on fewer occasions ($P = 0.008$; Table 1). Mothers' backflipping behavior after stimuli were removed was influenced by stimulus condition ($P = 0.004$; Table 2): mothers in the Call condition backflipped more than those exposed to all other stimulus conditions (Dunn's post-hoc *P*'s < 0.02). Post-exposure behavior of OVX virgin females did not differ based on stimulus condition (Kruskal-Wallis, *P*'s > 0.37; Table 2).

3.3. Production of fecal boli

Across the entire 180-minute trial, mothers produced significantly more fecal boli compared to OVX virgins ($z = 12.07$, effect $P = 0.001$, X ± SE: mothers = 25.79 ± 2.00; virgins = 15.96 ± 2.00; 2-way ANOVA, $F_{4,43} = 3.32$, $P = 0.019$). Fecal bolus production did not differ among stimulus conditions ($z = 0.49$, effect $P = 0.76$, X ± SE: Control = 21.00 ± 2.83; Call = 19.33 ± 2.83; Odor = 19.83 ± 2.83; Call + Odor = 23.33 ± 2.83). The interaction between reproductive status and stimulus condition was not significant and therefore was removed from the model.

3.4. Fos Expression

The anterior hypothalamic nucleus (AHN) was the only brain region in which we found significant differences in Fos expression. Fos in the AHN was influenced by an interaction between reproductive status and stimulus condition (LMM, model – $\chi2 = 23.42$, $P = 0.001$; interaction – $\chi2 = 12.45$, $P = 0.006$; Figs. 2, 3). Mothers exposed to the Control condition had higher AHN Fos expression compared to mothers exposed to all other stimulus conditions (*P*s < 0.04) and compared to OVX virgins exposed to the Call condition ($P < 0.001$). Mothers exposed to the Call + Odor condition had lower AHN Fos expression compared to mothers in all other conditions (*P*s < 0.05) and compared to OVX virgins exposed to all stimulus conditions except Call + Odor (*P*s < 0.03). Lastly, OVX virgin females exposed to the Call condition had lower AHN Fos expression compared to OVX virgins exposed to all other stimulus conditions (*P*s < 0.04) as well as mothers exposed to the Call condition ($P = 0.049$).

The interaction between reproductive status and stimulus condition was not significant for Fos expression in any other brain region; thus, the interaction was removed from all other models. Stimulus condition tended to affect the expression of Fos in the BLA (LMM, model – $\chi2 = 8.25$, $P = 0.08$; condition effect – $\chi2 = 8.24$, $P = 0.04$). This trend was driven by females in the Odor condition having higher BLA Fos expression than those in the Call condition.

Fos expression in the remaining brain regions of interest was not influenced by either reproductive status or stimulus condition (LMM, models – MOB: $\chi2 = 5.56$, $P = 0.23$; NAcc: $\chi2 = 3.60$, $P = 0.46$; STMV: $\chi2 = 3.25$, $P = 0.52$; MPOA: $\chi2 = 1.09$, $P = 0.75$; BMA: $\chi2 = 4.20$, $P = 0.38$; Fig. 4).

3.5. Correlations between Fos expression and behavior

Correlations between behavior during stimulus exposure and neural responses differed between mothers and OVX virgins (Table 3). Across conditions, mothers that spent more time backflipping during the stimulus exposure had higher Fos expression in the STMV ($r = 0.443$, $P = 0.04$; Fig. 5A), and mothers that rested more had lower Fos expression in the MOB ($r = -0.441$, $P = 0.04$). OVX virgin females that spent more time sniffing the odor ball had higher Fos expression in the BMA ($r = 0.564$, $P = 0.005$; Fig. 5B) and showed non-significant tendencies to have higher expression in the NAcc ($r = 0.370$, $P = 0.08$) and BLA ($r = 0.365$, $P = 0.09$). OVX virgin females that spent more time resting had lower Fos expression in the BMA ($r = -0.417$, $P = 0.048$), and OVX virgins that spent more time resting ($r = -0.372$, $P = 0.08$)

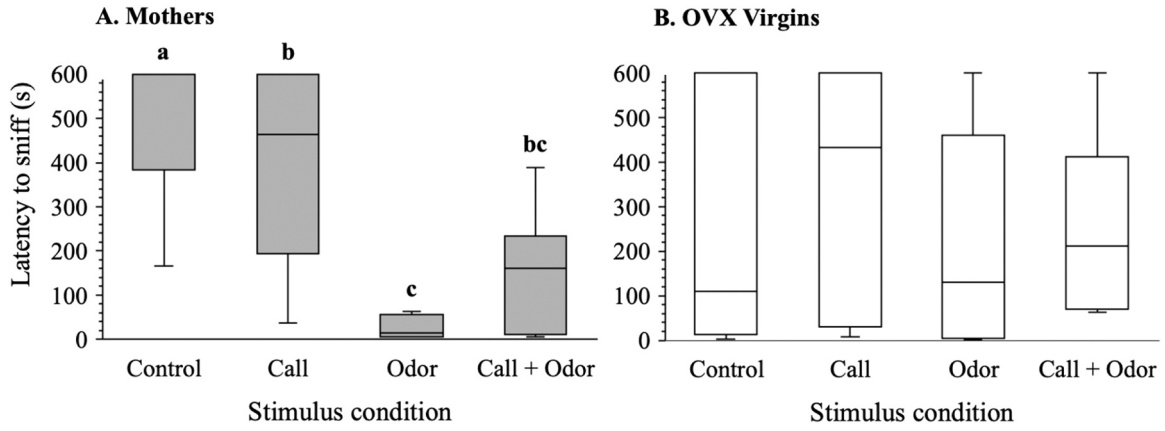

Fig. 1. Latencies to sniff the odor ball during the 10-min stimulus exposures for (A) mothers and (B) OVX virgins. Boxplots show median, 1st and 3rd quartiles. Error bars show minimum and maximum values. Letters denote significant differences among conditions based on post-hoc pairwise comparisons following Kruskal-Wallis tests: bars with the same letter do not differ significantly ($P > 0.05$). Data correspond to Table 2.

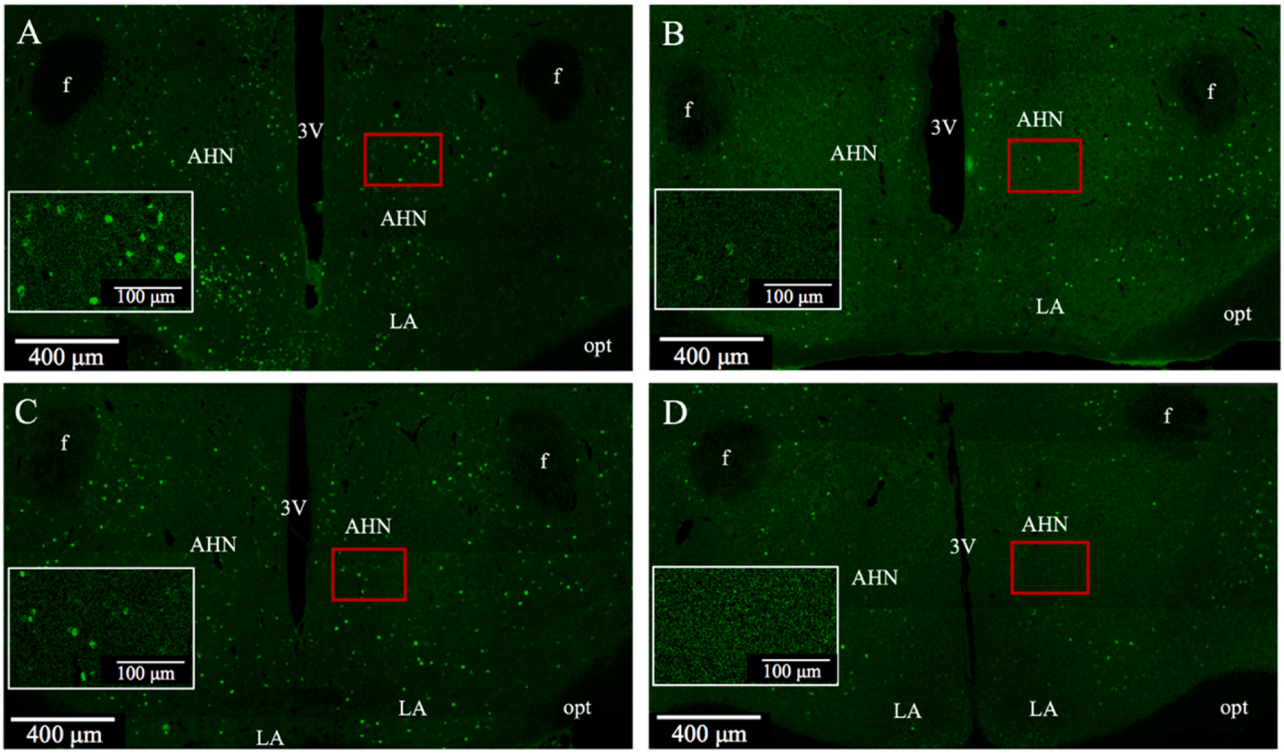

Fig. 2. Representative photomicrographs of coronal brain sections (40 µm thick) showing Fos staining in the anterior hypothalamic nucleus (AHN) of California mouse mothers exposed to (A) Control and (B) Call + Odor conditions and OVX virgin females exposed to (C) Control and (D) Call conditions. 3 V = third ventricle, f = fornix, LA = lateroanterior hypothalamic nucleus, opt = optic tract. Magnified images are of the area outlined by the red box in each micrograph.

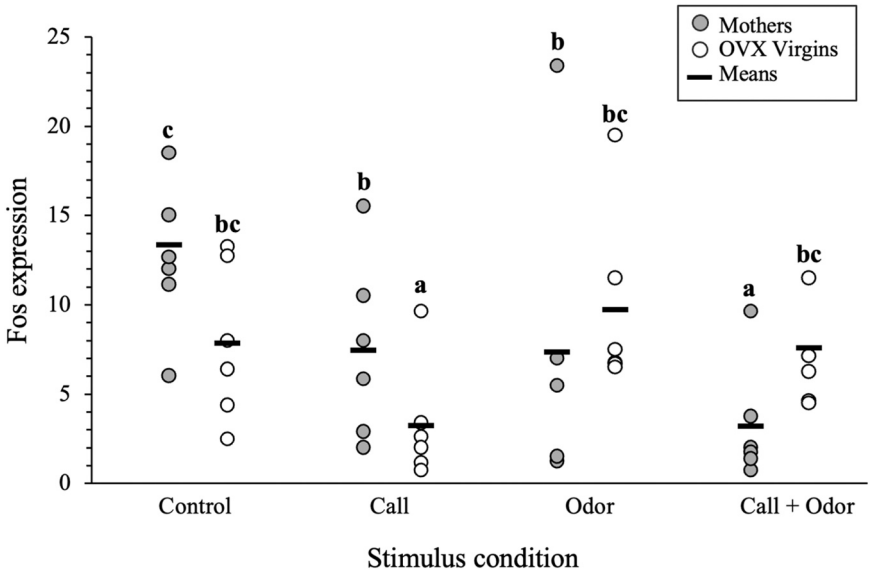

Fig. 3. Fos expression (number of Fos-positive neurons/200 ×200 µm square) in the anterior hypothalamic nucleus (AHN) across stimulus conditions for mothers and OVX virgin females. Letters denote significant differences among conditions based on post-hoc pairwise comparisons following LMM: bars with the same letter do not differ significantly ($P > 0.05$). Data shown are not transformed.

and less time in active behavior (r = 0.372, $P = 0.08$) showed non-significant tendencies to have lower Fos expression in the MOB.

3.6. Correlations between fos expression and social variables

We also found significant correlations between Fos expression and social variables in mothers and in OVX virgin females. Across conditions, mothers that had been housed with their pair mates for longer had higher Fos expression in the NAcc (r = 0.520, $P = 0.01$) and tended to have higher Fos expression in the MOB (r = 0.380, $P = 0.07$; Table 3). The amount of time OVX virgins were housed with their male pair mates did not correlate with Fos expression in any brain region. Additionally, mothers that were exposed to odor from older stimulus pups (overall range of 3–7 days old) had higher Fos expression in the MPOA (r = 0.665, $P = 0.018$). Similarly, OVX virgins that were exposed to odor from older stimulus pups tended to have higher Fos expression in

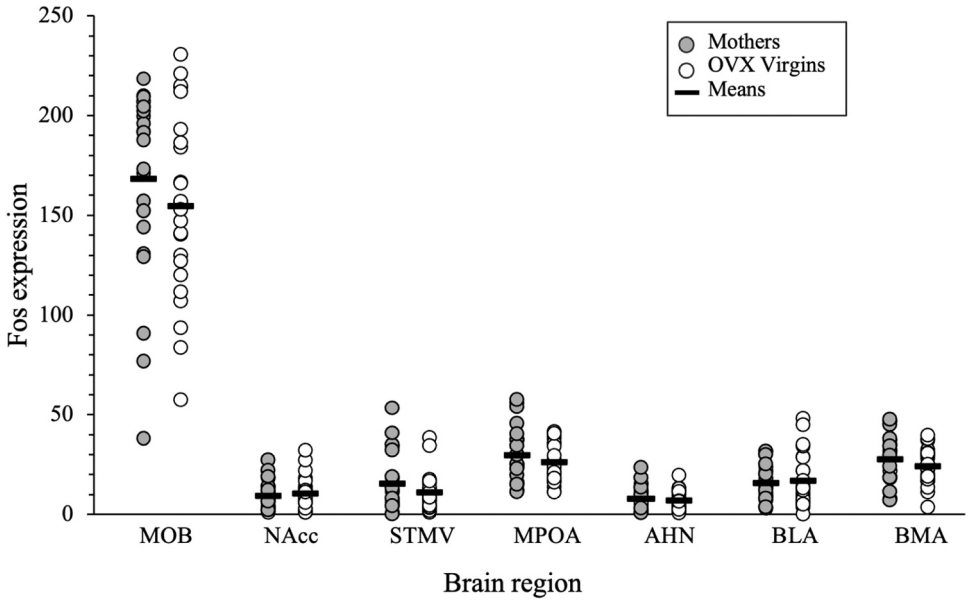

Fig. 4. Fos expression (number of Fos-positive neurons/200 ×200 μm square) in mothers and OVX virgin females collapsed across all four stimulus conditions. MOB[a] = main olfactory bulbs, NAcc = nucleus accumbens, STMV = bed nucleus of the stria terminalis medial division, ventral part, MPOA[a] = medial preoptic area, AHN = anterior hypothalamic nucleus, BLA = basolateral amygdala, BMA = basomedial amygdala. LMMs evaluating effects of reproductive status, condition, and their interactions were significant only for AHN (see text for details). Data shown are not transformed. [a]Immunohistochemistry batch contributed significantly to the model.

the STMV (r = 0.576, $P = 0.050$).

4. Discussion

In this study we tested the hypothesis that new California mouse mothers, compared to ovariectomized (OVX) virgin females, would display greater attraction to pup stimuli and have stronger neural responses to these stimuli in brain regions associated with maternal behavior, but potentially weaker responses in brain regions associated with defense. We also addressed the hypothesis that these effects in mothers would be enhanced by simultaneous exposure to olfactory and auditory stimuli from pups. Few differences in Fos expression were found between mothers and OVX virgin females. This was particularly surprising because the two groups differed not only in reproductive experience but also in the presence of ovaries, and ovarian hormones are important for mediating maternally induced neural plasticity [12]. Auditory pathways, olfactory pathways, the medial preoptic area (MPOA) and the bed nucleus of the stria terminalis medial division, ventral part (STMV) of primiparous house mouse mothers contain receptors for estrogen [12,49–51]. Specifically, estrogen is thought to play a dominant role in changing the valence of pup sensory stimuli from aversive before pregnancy to attractive after parturition, as demonstrated in rats and house mice [5,52]. Additionally, estradiol treatment may alter auditory processing in ovariectomized mice [49].

4.1. Neuronal activation

The only brain region considered in this study in which Fos activation differed among groups was the anterior hypothalamic nucleus (AHN). In rodents, AHN activity is associated with defensive behavior [53], and the transition to motherhood results in the inhibition of defense circuitry in response to pups [27]. Consistent with this pattern, we found that in both mothers and OVX virgins, AHN Fos activation differed among stimulus conditions. Mothers had significantly lower AHN Fos expression when exposed to pup stimuli (Call, Odor, and Call + Odor) compared to the control condition. Additionally, Fos expression was significantly lower in mothers tested in the Call + Odor condition compared to mothers in both the Call and Odor conditions, suggesting that auditory and olfactory cues from pups had an additive effect on inhibiting activity in the AHN. Interestingly, OVX virgin females had the lowest AHN Fos expression when exposed to pup calls, suggesting that virgin female California mice may not perceive pup calls as aversive. The few studies that have quantified behavior of reproductively naive female rodents in response to pup calls support this finding. For example, virgin female rats are not as attracted to vocalizing pups as are new mothers, but they do not differ from new mothers in their attraction to playbacks of pup calls [21,54]. On the other hand, pup odors are aversive to virgin female rats [4], and we found support for this pattern in California mice, in which OVX virgin females exposed to pup odors (Odor and Call+Odor) had higher Fos expression in the AHN relative to the Call condition.

No differences between mothers and OVX virgin females or across stimulus conditions were found for Fos expression in the main olfactory bulbs (MOB), which was high in females in all treatment conditions compared to other brain regions (Fig. 3). Consistent levels of MOB Fos expression suggest that the transition to motherhood does not alter MOB neuronal activation in response to pup stimuli and that other forms of neural plasticity likely underlie behavioral differences between OVX virgin females and mothers exposed to pup odors. Few studies have focused specifically on changes in the MOB soon after parturition (reviewed in [5]), but those studies that did report neural changes in the olfactory bulb or MOB as a result of the transition to motherhood. Compared to virgins, primiparous house mouse mothers had higher dendritic spine stability in the olfactory bulbs but lower spine density at 4 days post-partum [55] and stronger olfactory bulb mitral cell GCaMP responses to pup and nest odors at 3–5 days post-partum [56]. Additionally, neurogenesis in the subventricular zone, which supplies new neurons to the MOB, peaks 7 days before and 7 days after parturition in CD1 mice [18]. Interestingly, olfactory bulb Fos activation in our study was negatively correlated with the amount of time females rested during the stimulus exposure: females that rested more might have engaged with their olfactory environment less than active females. Thus, Fos activation quantified here may not be specific to smelling pup odors, but may be more closely related to general olfactory activity and exploration.

The MPOA in rodents is rich in estrogen receptors [12,57], and these

Table 3
Correlations between Fos expression (number of Fos-positive neurons per 200 ×200 μm square) and behavior durations (seconds) or social variables observed during stimulus exposure. For each correlation, Pearson's *r* (top line) and *P*-value (bottom line) are shown. Significant correlations ($P < 0.05$) are indicated in bold, and non-significant trends ($0.05 < P < 0.1$) are indicated in bolded italics.

Fos-IR/ Behavior	MOB	NAcc	STMV	MPOA	AHN	BLA	BMA
A. Mothers	N = 22	N = 22	N = 22	N = 22	N = 22	N = 22	N = 22
Sniff odor ball (s)	0.120	-0.112	0.293	-0.197	-0.288	-0.209	-0.190
	0.38	0.62	0.19	0.38	0.20	0.30	0.40
Handle odor ball (s)	0.262	0.058	-0.260	-0.058	-0.269	0.016	0.317
	0.24	0.80	0.24	0.80	0.23	0.95	0.15
Listen (s)	0.093	-0.012	0.123	0.073	-0.213	-0.019	-0.092
	0.68	0.956	0.59	0.75	0.34	0.93	0.69
Autogroom (s)	0.027	-0.301	0.001	-0.237	-0.163	0.156	-0.111
	0.91	0.17	0.99	0.29	0.47	0.49	0.62
Activity (s)	0.303	0.091	0.354	0.073	0.004	0.105	0.039
	0.17	0.69	0.11	0.75	0.99	0.64	0.86
Rest (s)	**-0.441**	-0.064	-0.270	-0.017	0.248	-0.021	-0.143
	0.04	0.78	0.22	0.94	0.27	0.93	0.53
Backflip (s)	0.317	0.253	**0.443**	0.233	0.129	0.228	0.051
	0.15	0.26	**0.04**	0.30	0.57	0.31	0.82
Time since paired	***0.380***	**0.520**	-0.060	0.214	0.045	0.158	0.287
	0.07	**0.01**	0.78	0.32	0.84	0.46	0.17
Number of pups	0.075	-0.0396	0.172	0.112	-0.088	0.071	-0.094
	0.73	0.85	0.42	0.60	0.68	0.74	0.66
Age of odor- stimulus pup[a]	0.179	0.424	0.295	**0.665**	-0.152	0.341	-0.043
	0.58	0.17	0.35	**0.02**	0.64	0.28	0.90
B. OVX virgin females	N = 23	N = 23	N = 23	N = 23	N = 23	N = 23	N = 23
Sniff odor ball (s)	0.186	***0.370***	0.196	-0.081	0.076	***0.365***	**0.564**
	0.40	***0.08***	0.37	0.71	0.73	***0.09***	**0.01**
Handle odor ball (s)	0.182	0.278	-0.145	-0.096	-0.072	0.053	0.221
	0.41	0.20	0.51	0.67	0.74	0.81	0.31
Listen (s)	0.113	0.147	-0.002	-0.243	0.002	0.276	0.310
	0.61	0.50	0.99	0.27	0.99	0.20	0.15
Autogroom (s)	0.057	0.331	0.213	-0.001	-0.098	-0.053	0.061
	0.80	0.12	0.33	0.99	0.66	0.81	0.78
Activity (s)	***0.372***	-0.044	0.009	0.174	0.184	0.022	0.138
	0.08	0.84	0.97	0.42	0.40	0.92	0.53
Rest (s)	***-0.372***	-0.300	0.020	0.025	-0.064	-0.182	**-0.417**
	0.08	0.17	0.93	0.91	0.77	0.41	**0.05**
Backflip (s)	0.247	-0.043	0.054	0.172	0.238	-0.075	-0.028
	0.26	0.84	0.81	0.43	0.27	0.73	0.90
Time since paired	0.274	0.160	-0.11	-0.058	0.093	0.177	0.116
	0.20	0.46	0.59	0.79	0.67	0.41	0.59
Age of odor- stimulus pup[a]	-0.141	-0.365	**0.576**	0.359	0.060	0.238	0.061
	0.66	0.24	**0.05**	0.25	0.85	0.46	0.85

[a] $N = 12$

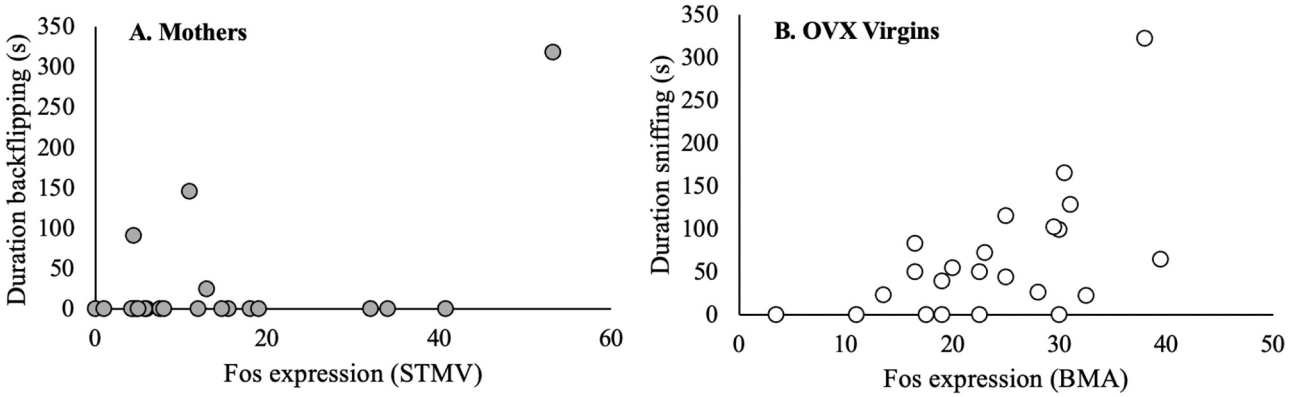

Fig. 5. Correlations between Fos expression (number of Fos-positive neurons/200x 200 μm square) and durations of select behaviors during stimulus exposure. (A) Fos expression in the STMV (bed nucleus of the stria terminalis medial division, ventral part) and time spent backflipping in mothers (Pearson's r = 0.443). (B) Fos expression in the BMA (basomedial amygdala) and time spent sniffing the odor ball in OVX virgins (Pearson's r = 0.564). Pearson's correlations *P*'s < 0.05 for both comparisons. Data correspond to Table 3.

receptors play a role in MPOA activation [58]. Therefore, we predicted that OVX virgin females would have lower Fos expression in the MPOA when exposed to pup stimuli, compared to mothers. However, we found no differences in MPOA Fos immunoreactivity between mothers and OVX virgins or among groups exposed to different stimulus conditions. Because the MPOA is involved in appetitive behaviors [59], it is possible that pup stimuli in the absence of a physical pup with which to interact are not sufficient to induce Fos expression in the MPOA of mothers.

Consistent with this possibility, Numan and Numan [24] showed that in primiparous female rats, MPOA Fos expression was higher in mothers that were able to fully interact with pups compared to mothers exposed to pups enclosed in mesh and unable to interact. Furthermore, Calamandrei and Keverne [23] found that MPOA Fos expression was higher for both intact and ovariectomized virgin female mice that interacted with pups compared to those exposed to only pup auditory and olfactory stimuli. Thus, increased MPOA activation may occur in mothers only in the presence of pups rather than isolated pup stimuli.

4.2. Behavior and behavior-Fos correlations

The transition to motherhood alters female rodents' responses to pup odors [4,5]. Rat mothers, for example, are attracted to pup odors in late pregnancy whereas virgins show no preference between pup odors and clean bedding [14]. We found that female California mice follow this pattern, since mothers showed a preference for pup odors while OVX virgins did not: mothers exposed to pup odors had shorter latencies to approach the stimulus ball compared to mothers exposed to clean cotton, whereas OVX virgin females showed no differences between the two stimulus conditions.

Both during and after exposure to stimuli, mothers groomed themselves and backflipped more, as well as produced more fecal boli, than virgin females. Backflipping, autogrooming, and fecal bolus production may all be markers of stress or anxiety. Backflipping is more common in female California mice housed in smaller cages [60] and may be indicative of neophobia [61]. Additionally, under stressful conditions, California mouse mothers autogroom more than virgin females [62]. Zhao et al. [63] also reported higher fecal bolus counts for California mouse mothers compared to (ovary-intact) virgin females, which could indicate higher levels of anxiety [40,64–66]. However, it is important to note that greater production of fecal boli can also relate to digestive tract changes that occur during lactation [67]. Overall, these data that suggests that new California mouse mothers likely experience the same or higher levels of stress and anxiety compared to OVX virgin females [62,63]. In contrast, in rats, mice and several other species, stress reactivity and anxiety are blunted in mothers compared to virgins [68–71]. Interestingly, we did not find a significant main effect of reproductive status on Fos expression in the AHN, which, as described above, is activated when pup stimuli are perceived as aversive [26,27,72]. This disparity may be explained by the differences in AHN Fos levels across stimulus conditions within each group.

Behaviors exhibited by mothers and OVX virgins during stimulus presentation correlated with neuronal activation in brain regions associated with maternal behavior in mothers (STMV) and involved in the reward pathway (BMA) in OVX virgins. The STMV receives information from both olfactory and auditory pathways and can activate reward and stress responses. On one hand, it projects to the ventral tegmental area, which activates the nucleus accumbens (NAcc) in response to rewarding stimuli (reviewed in [42,73]. On the other hand, the STMV also projects to the paraventricular nucleus of the hypothalamus and is activated in response to stressful stimuli [74,75]. In mothers in our study, higher STMV Fos activation was associated with greater durations of backflipping, which was driven by only a small number of animals that displayed backflipping behavior during stimulus exposure (Fig. 5A), and many mothers with high STMV activity did not backflip. Thus, while STMV activation could indicate higher levels of stress for some females, this association cannot necessarily be extended to all mothers.

Fos expression in the BMA was higher in OVX virgin females that spent more time sniffing the odor ball and lower in those that rested more. BMA activation can occur in rodent mothers following exposure to pup stimuli [12] but can also occur in response to novel environments [76]. Since the BMA plays a key role in relaying sensory information from sensory cortices to the reward circuitry [12], it is not surprising that BMA Fos expression was lower in OVX virgin females that rested more, since these mice likely had lower exposure to sensory stimuli from pups. The positive association between BMA Fos expression and duration of sniffing the pup ball is more likely to be associated with the novelty of the female's environment rather than activation of the maternal reward system, since pup olfactory stimuli may be aversive to virgin female California mice, as with virgin females of other species [4]. Thus, both of these correlations are likely attributable to variation among OVX virgin females in sensory engagement during stimulus exposure as opposed to variation in pup-specific stimulus engagement. It is important to note, however, that the relationships between females' behavior during stimulus exposure and Fos expression are correlations only, and we cannot assume that behavior is driving differences in neural activation. It is also possible that differences in affective state (e.g., stress/anxiety, reward), as indexed by Fos expression, might have influenced the animals' behavior. For example, is it possible that some virgins spent more time sniffing the ball because it was more rewarding to these females (if higher BMA Fos led to higher reward-pathway activation), rather than that these females had higher BMA Fos because they spent more time sniffing the odor ball.

4.3. Correlations between Fos and social variables

Social variables were assessed primarily to control for possible sources of variation within the study design. We had no a priori expectations of how pairing length or the age of the stimulus pup (within the narrow age range of the pups in our study, i.e., 3–7 days) used to collect the odor stimulus might influence neuronal activation since, to our knowledge, these variables have not been considered previously. Fos activation in the MPOA of mothers and the STMV of OVX virgin females was higher when the odor stimulus was collected from an older pup. Studies quantifying changes in pup odor soon after birth have not, to our knowledge, been conducted in rodents. During the early postnatal period, however, pup odor is likely to transition from being dominated by amniotic fluid to urine. Alternatively, these correlations could be related to differences in the amount of pup odor collected from pups of different ages, as California mouse pups grow quickly after birth (3-day mass $\bar{x} \sim 5$ g; 7-day mass $\bar{x} \sim 7$ g; [47]).

Lastly, Fos activation in the NAcc of mothers was higher when females had been paired with their mate for longer. Mothers were paired with their male mates between 39 and 93 days before testing, which could have led to differences in the timing of neural plasticity that occurs in relation to pair bonding and the transition to motherhood. Plasticity in the NAcc plays a central role in the formation and maintenance of pair bonds in monogamous mammals, which is mediated by dopamine, oxytocin and vasopressin [77–79]. Dynamics of pairing length may influence NAcc activity through variations in receptor density, which could result in the NAcc being more active for longer-paired mothers. Alternatively, the positive correlation between NAcc Fos expression and time since pairing in our study could be related to breeding latency following pair formation, as the time of testing was based on the birth of the first litter.

4.4. Comparison to fathers

Comparison of the present study with our previous study on male California mice [30] provides an opportunity to evaluate potential sex differences in parenthood-related sensory plasticity. The two experiments used the same pairs of animals tested under identical conditions but yielded different findings on both behavioral and neural responses to pup stimuli. An important difference between these studies is that female virgins, but not male virgins, were gonadectomized, which might have contributed to differences between males and females. Fathers and intact virgin males did not differ in behavior during or after stimulus exposure, but did differ in Fos expression in several brain regions (MOB, STMV and MPOA; [30]. In contrast, mothers and OVX virgin females in the present study showed differences in behavior (autogrooming and backflipping) both during and after stimulus exposure, but neural

responses to pup stimuli differed only in the anterior hypothalamic nucleus (AHN), an effect that depended on stimulus condition. These findings suggest that the neural basis of sensory plasticity may differ between mothers and fathers in a biparental species, which presents a promising direction for future studies.

4.5. Future directions

The results of this study reveal several areas of potential investigation to disentangle the effects of ovarian hormones from parental experience on the effects of sensory perception. First, we found that mothers and OVX virgin females differed in the ways that neural activation related to behavioral responses to pup stimuli with no overlap in correlations between neural (Fos) activation and behavior during stimulus exposure. This finding, as well as the differences in neural activation in response to pup stimuli could be related to reduced estrogen and progesterone rather than plasticity mediated by the transition to motherhood per se. For example, ovariectomized virgin rats implanted with estradiol and progesterone capsules showed a greater preference for nesting material from a lactating dam's nest compared to ovariectomized virgins without hormonal implants [80]. Additionally, ovariectomized CBA/Ca female mice with implanted 17β -estradiol capsules had lower expression of α estrogen receptors along their auditory tracks compared to ovariectomized females [49].

While OVX virgin females were expected to have significantly lower levels of circulating estrogen and progesterone, central synthesis of these hormones could have also contributed to patterns of observed neural activation. Studies quantifying central levels of estrogen and progesterone as well as the density of their respective receptors in mothers and OVX virgin females would improve our understanding of the role of gonadal hormones in maternally-induced neural plasticity.

CRediT authorship contribution statement

WS supervised the study. KMW and WS were responsible for conceptualization and methodology. KMW, AMA, MH and KMR-T conducted the experiment (investigation). KMW, MH, KMR-T, and MGC analyzed data. KMW and WS wrote the original draft of the manuscript, and KMW, WS and AMA edited the manuscript.

Data Availability

Data is available on dryad: https://doi.org/10.5061/dryad.8gtht76vt

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