Behavioural Brain Research 437 (2023) 114153

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Neural activation associated with maternal and aversive interactions with pups in the Mongolian gerbil (*Meriones unguiculatus*)

Luis Romero-Morales [a], Brenda García-Saucedo [a], Martín Martínez-Torres [a], Mario Cárdenas [b], René Cárdenas-Vázquez [c], Juana Luis [a,*]

[a] Laboratorio de Biología de la Reproducción, UMF, FES Iztacala, Universidad Nacional Autónoma de México, Av. de los Barrios No. 1, Tlalnepantla, Edo. de México, Mexico
[b] Laboratorio de Biología de la Reproducción, Instituto Nacional de Ciencias Médicas y Nutrición, Salvador Zubirán, Ciudad de México, Mexico
[c] Laboratorio de Biología Animal Experimental, Depto. de Biología Celular, Facultad de Ciencias, Universidad Nacional Autónoma de México, Mexico

ARTICLE INFO

Keywords:
Maternal interactions
Aggressive interactions
Mongolian gerbil
Neural activation
Estradiol

ABSTRACT

According to approach-avoidance model, virgin female laboratory rats display maternal behaviour when the tendency to approach and interact with the pup is stronger than avoiding it. A positive neural mechanism that includes the medial preoptic area (mPOA)/bed nucleus of the stria terminalis (BNST) and a negative mechanism that involves the anterior hypothalamic nucleus (AHN)/ventromedial nucleus (VMN)/ periaqueductal grey (PAG) underlie to these behaviours. Unlike virgin rats, which avoid the pups, virgin females Mongolian gerbils (*Meriones unguiculatus*) can be immediately either maternal or aggressive with the pups. Furthermore, the Mongolian gerbil is monogamous and biparental species. Despite these difference, we hypothesised that maternal and aggressive interaction with the pups could activate mPOA/BNST and AHN/VMH/PAG, respectively, and that maternal response could be associated with high concentrations of estradiol (E_2). Twenty virgin maternal females and 20 aggressive toward the pups were selected. Ten maternal females interacted with the pups (MAT-pups) and 10 with candy (MAT-candy). Of the 20 aggressive females, 10 interacted with the pups (AGG-pups) and 10 with candy (AGG-candy). Immediately after the test, blood samples were taken to quantify E_2. The brains were dissected for c-Fos immunohistochemistry. MAT-pups females had significantly higher activation in mPOA/BNST than MAT-candy females, while AGG-pups showed significant activation in AHN/VMH/PAG compared with AGG-candy females. The maternal response was associated with high concentrations of E_2. These results suggested a positive and a negative mechanism in the regulation of maternal behaviour in the Mongolian gerbil, and that the immediate maternal response could be due to high E_2 concentrations.

1. Introduction

In mammals, maternal behaviour consists of a set of activities carried out by a mother to ensure the survival and successful development of the offspring [1]. Laboratory rats (*Rattus norvegicus*) have been the model of choice in studies of the biological bases of maternal behaviour [1,2]. Virgin female rats have been used to elucidate the neural mechanisms underlying maternal behaviour resulting from the approach-avoidance model. According to the model, approach-avoidance maternal behaviour is displayed when the tendency to approach and interact with pup stimuli is greater than the tendency to avoid them. There is a positive mechanism descending from the medial preoptic area (mPOA) through the lateral hypothalamus to the ventral mesencephalon, including the bed nucleus of the stria terminalis (BNST). Furthermore, there is a negative mechanism that descends from the medial hypothalamus through the anterior hypothalamic nucleus (AHN)/ventromedial nucleus (VMN) to the periaqueductal grey (PAG) [1,3–6]. Both neural regions are involved in the facilitation and inhibition of maternal behaviour and have multiple connections with the medial amygdala (MeA), which receives projections from the olfactory bulb (OB) [3–8].

Estradiol (E_2) plays an essential role in the regulation of maternal behaviour. In rats, the onset of maternal behaviour depends on changes in the concentrations of progesterone and E_2 (a decrease in progesterone concentration and an increase in E_2 in last third of pregnancy) [1]. In

* Correspondence to: Av. de los Barrios No. 1, Col. Los Reyes Iztacala, Tlalnepantla, Edo. México c.p. 05490, Mexico.
E-mail addresses: luis_8r@comunidad.unam.mx (L. Romero-Morales), brenmgsaucedo@yahoo.com.mx (B. García-Saucedo), marcar@unam.mx (M. Cárdenas), luisdc@unam.mx (J. Luis).

https://doi.org/10.1016/j.bbr.2022.114153
Received 22 November 2021; Received in revised form 26 September 2022; Accepted 7 October 2022
Available online 8 October 2022

this rodent, virgin ovariectomized females exposed to small E_2 implants of 1 or 2 mm for two weeks do not display maternal behaviour, whereas large E_2 implants of 10 mm stimulate maternal responsiveness [9]. Similarly, a dose of 100 μg/kg of estradiol benzoate triggers maternal behaviour in virgin rats, but a dose of 20 μg/kg is ineffective [10].

Knowledge of neural mechanisms that regulate maternal behaviour has been obtained using electrolytic lesions, excitotoxic lesions, and magnetic resonance imaging. Furthermore, the functional anatomy of the neuroendocrine system is mapped using neuronal markers, such as the products of early gene expression, including the Fos family [3,7,11–17]. Recently, studies on laboratory rats and mice have focused on more precisely determining the subsets of neurons that regulate maternal behaviour within some neural nuclei, such as mPOA [18–20]. However, aside from laboratory mice [21,22], no studies have been conducted on other rodent species regarding the neural circuits of maternal behaviour. Initially, virgin rats actively avoid pups and display maternal behaviour only after 6–12 consecutive days of pup exposure [23]. In contrast, virgin female Mongolian gerbils (*Meriones unguiculatus*) can be immediately maternal or aggressive with pups of the same species [24]. Furthermore, the Mongolian gerbil is a monogamous and biparental rodent [24]. Despite these differences from laboratory rats, we hypothesised that maternal and aggressive interaction with pups could activate the medial preoptic area (mPOA)/bed nucleus of the stria terminalis (BNST) and the anterior hypothalamic nucleus (AHN)/ventromedial nucleus (VMN)/periaqueductal grey (PAG), respectively. We also hypothesized that the maternal response could be associated with hormonal status, particularly the concentration of E_2. The activation of MeA and OB was also determined in maternal and aggressive interactions with pups because both areas are part of the neural circuit of maternal behaviour in laboratory rats [1].

2. Materials and methods

2.1. Animals

In this study, we used virgin female Mongolian gerbils between 180 and 210 days old. Gerbils were weaned at the age of 25–28 days. The animals came from a breeding colony kept at Facultad de Estudios Superiores Iztacala. All gerbils were kept with an inverted photoperiod of 12:12 h (light:dark cycle; onset of light at 18:00) at ambient temperature between 17 and 21 °C. The animals were fed with Lab Chow 5001 pellets (Nutrimentos Purina, México) and tap water ad libitum. A carrot was also provided once a week. Three or four females were housed in a polycarbonate cage (37 × 27 × 15 cm) with sawdust bedding from weaning until they were subject to screen tests for maternal behaviour. Twenty maternal females and 20 aggressive females toward pups were selected through those tests. After maternal behaviour tests, maternal females and aggressive females were separated remaining three or four of them per cage until each female was placed individually in a cage, as part of the experimental procedure. Maternal behaviour tests (second exposure to the pups) were performed 15–20 days after the screening tests. At the end of these tests, blood samples were taken to quantify E_2 concentrations, and then the brains were dissected for c-Fos immunohistochemistry. All experiments were carried out according to the ethical guidelines of the National Institutes of Health for the Care and Use of Laboratory Animals (NIH Publication no. 8023) and the Mexican Official Norm for the Production, Care, and Use of Laboratory Animals [25].

2.2. Screening and maternal behaviour tests

For the screening test, each female was placed in a cage with the same characteristics as the housing cage with clean sawdust bedding. After 10 min of acclimatisation, two pups aged 1–3 days were introduced, and the female's behaviour toward the pups was recorded. When the females displayed maternal behaviour, the screening test was terminated after five minutes, but if they attacked the pups, the test was terminated immediately. All behaviours toward the pups were recorded but not quantified. The criteria for aggressive females included strong shaking (shaking the pups with their snouts) or biting the pups if they are not removed quickly. Maternal females touch pups with their noses, crouch over them, and groom them [21,22]. After 15 and 20 days of screening tests, 10 maternal females interacted with pups (MAT-pups), and 10 interacted with candy (MAT-candy). Of the 20 aggressive females, 10 interacted with pups (AGG-pups), and 10 interacted with candy (AGG-candy). The candy was a gummy bear, which was used as a control stimulus due to its softness and similar size to Mongolian gerbil pups. Candy has also been used as a control stimulus in male-pup interactions among prairie voles [23,24] and male Mongolian gerbils [26]. Maternal and aggressive interactions were examined using the method described for screening tests except for the use of candy for some tests (control groups). Before interactions with the pups or candy, maternal and aggressive females remained in individual cages for 24 h and were not disturbed so that they could be isolated from any stimuli that could cause neural activation. At the end of the isolation period, the interactions with the pups or candy were performed. In maternal interactions, the time spent crouching over pups, grooming, and sniffing was quantified. In aggressive interactions with the pups, the behaviours were not quantified because the pups were immediately removed from the cage when they were strongly shaken or bitten. The pups were used only once. Three pups were slightly bitten, and their wounds were treated with gentian violet (1 %), after which they were returned to their parents. The test lasted 80 min because c-Fos expression reaches its maximum at 70–90 min [13,27]. Although in females' AGG-pups interactions, the pups were quickly removed from the female's cage when they were attacked, the experimental procedure was continued to 80 min. The same was performed with the control group (AGG-candy). All behavioural tests were carried out for between 11:00 and 14:00 during the dark period under red light illumination. Sessions were videotaped with a high-definition infra-red camera (IR Bullet camera, 2.1 megapixels).

2.3. Hormone assay

Immediately after the interactions with the pups or the candy, blood samples (250 μL) were collected from the retro-orbital sinus of all females in the four groups. Five females were chosen randomly from each group and anaesthetised with a mild dose (5 mg/kg xylazine and 60 mg/kg ketamine) to obtain blood samples. The remaining five females were anaesthetised with a heavy dose (10 mg/kg xylazine and 90 mg/kg ketamine i.p.) and then perfused as described in the next section. In these females, blood samples were taken before perfusion following the same collection procedure described. The blood samples were collected in heparinised capillary tubes. All blood samples were taken between 13:00 and 14:00 once the animals had fallen asleep. Due to the anaesthesia's effects, each sample was taken in less than one minute. Plasma was separated by centrifugation and stored at − 7 °C. ELISA was done for hormone analysis, and E_2 was measured with a DRG commercial kit (DRG Diagnostics, Marburg, Germany), which has a sensitivity of 0.083 ng/ml. Each serum sample was analysed at four concentrations: whole serum and three dilutions. To establish a correlation between E_2 concentrations and the standard curve, only the lower part was considered since 88 % of the results fell in this part. There was good correlation ($r = 0.99$). The intra-assay and inter-assay coefficients of variation were 3.9 % and 4.6 %, respectively. The recovery rate for E_2 was 95.0 % ($r = 1.0$). The plate was read using a plate reader (model Multiskan Ascent V1.25, with a filter of 450-nm wavelength; Thermo Electron Corporation).

2.4. c-Fos immunohistochemistry

Females were perfused through the heart with physiological saline, followed by 4 % paraformaldehyde in sodium phosphate buffer (0.1 M;

pH 7.6). The brain was removed and postfixed for 18 h in the same solution. This tissue was processed and cut into 7-μm-thick coronal sections with a microtome. The neural areas were located using the stereotaxic atlas of the Mongolian gerbil [30]. The location of OB was + 1.7 mm, image 300; the mPOA and the BNST were located at − 0.1 mm, image 520; the MeA and the AHN were located at − 0.888 mm, image 590; the VMH was located at − 1.3 mm, image 640; and the PAG was located at − 3.5 mm, image 860. After obtaining sections of the different neural areas, the samples were placed on gelatinised slides (Nutrient Gelatin, 70151-500G-F; Sigma-Aldrich, CA, USA). Each of the following steps was followed by rinsing in phosphate-buffered saline (PBS) for 5 min: (1) 10 min of incubation in 3 % H_2O_2 in PBS, (2) 20 min of incubation in 5 % normal goat serum (Vectastain ABC kit, PK-4000; Vector Laboratories) in PBS, and (3) 16 h of incubation at 25 °C with 1:50 dilution of rabbit c-Fos antibody epitope located in the N-terminus of human origin (sc-52; Santa Cruz Biotechnology, Inc.) in PBS. After two 5-min rinses in PBS, the sections were incubated with biotinylated goat antirabbit antibody in PBS for 90 min and rinsed twice in PBS (Vectastain ABC kit, PK-6102; Vector Laboratories). The sections were then incubated with an avidin-biotin complex (Vectastain ABC kit, PK-6100; Vector Laboratories) for 30 min, followed by two additional rinses with PBS. Finally, binding was visualised using 3,3′-diaminobenzidine as the chromogen (DAB Peroxidase Substrate, SK-4100; Vector Laboratories). The sections were dehydrated and then cover-slipped. For negative control sections, the incubation with the primary antibody was omitted. All neural tissues were processed within the same assay. The c-Fos antibody has been utilised in other rodents, such as *Microtus ochrogaster* [28,29] and male Mongolian gerbils [26].

2.5. Image analysis

The number of cells that showed c-Fos immunoreactivity (ir) were quantified bilaterally in three cuts per female in five females per group. Nuclei counts were performed in similar areas of 180 μm^2. Microphotographs were taken with a Motic camera (10 megapixels) attached to a Leica microscope.

2.6. Statistical analysis

The numbers of c-Fos-ir-cells in the mPOA, BNST, AHN, VMH, MeA, and OB between females of the MAT-pup, MAT-candy, AGG-pups, and AGG-candy groups were compared using a nonparametric Kruskal-Wallis test due to the non-normality of the data (Anderson-Darling test, $P > 0.05$). Pairwise comparisons were performed using the Mann-Whitney U test and Bonferroni correction. E_2 concentrations in plasma were also compared using a nonparametric Kruskal-Wallis test since they showed a non-normal distribution (Anderson–Darling test, $P > 0.05$). The Mann-Whitney U test and Bonferroni correction for multiple testing were also applied for pairwise comparisons. Correlations between E_2 concentrations and the numbers of c-Fos-ir cells in the mPOA, BNST, AHN, VMH, and PAG were performed using Spearman correlational analyses. Statistical analyses were performed using SPSS version 21.0 (IBM SPSS, Armonk, NY).

3. Results

3.1. Behaviours

All females that were maternal during the screening test and subsequently tested with the pups (n = 10) continued to provide maternal care in maternal behaviour tests. Likewise, all aggressive females with the pups also displayed aggression in the second exposure to the pups (n = 10). Table 1 shows the behaviours recorded and their quantification in maternal behaviour tests.

3.2. c-Fos immunoreactivity

The number of c-Fos-ir cells varied significantly between the MAT-pups, MAT-candy, AGG-pups, and AGG-candy in the mPOA ($H = 10.99$, $df = 3$, $P < 0.05$, Figs. 1 and 8) and BNST ($H = 11.64$, $df = 3$, $P < 0.05$, Figs. 2 and 8). Post hoc analyses revealed that the MAT-pups group had significantly more c-Fos-ir cells in the mPOA and BNST than the MAT-candy ($U = 40$, $P = 0.007$; $U = 40$, $P = 0.001$, respectively), AGG-pups ($U = 40$, $P = 0.007$; $U = 40$, $P = 0.001$; respectively), and AGG-candy groups ($U = 40$, $P = 0.007$; $U = 40$, $P = 0.001$; respectively) (Bonferroni adjusted $P = 0.008$). Maternal females that interacted with the pups displayed crouching over pups, grooming, and sniffing.

The number of c-Fos-ir cells between the AGG-pups, AGG-candy, MAT-pups, and MAT-candy groups was significantly different in the AHN ($H = 12.30$, $df = 3$, $P < 0.05$, Figs. 3 and 8), VMH ($H = 11.45$,

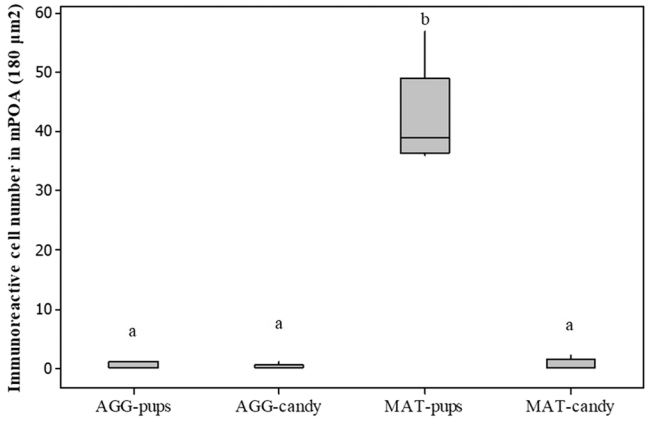

Fig. 1. Maternal females that interacted with pups (MAT-pups) have a significantly higher number of c-Fos-ir cells in mPOA than maternal females that interacted with candy (MAT-candy) and aggressive females (AGG-pups and AGG-candy). Data are presented as the median. Letters indicate significant differences.

Table 1
Behaviours recorded in maternal and aggressive females of the Mongolian gerbil during the interactions with unfamiliar pups of the species.

Maternal females (n = 10)			Aggressive females (n = 10)		
Behaviour	Number of females that performed this behaviour	$\bar{x} \pm$ ES (s)	Behaviour	Number of females that performed this behaviour	$\bar{x} \pm$ ES (s)
Onset latency of maternal behaviour	10	13.0 ± 8.6	Attack latency	10	5.2 ± 2.0
Crouching over pups	10	219.8 ± 54.5	Strongly shaken	7	7 times
Grooming	10	221.3 ± 78.6	Bitens	3	3 times
Sniffing	10	40.1 ± 15.6			

Behaviours recorded and quantified in maternal behaviours tests of the Mongolian gerbil.

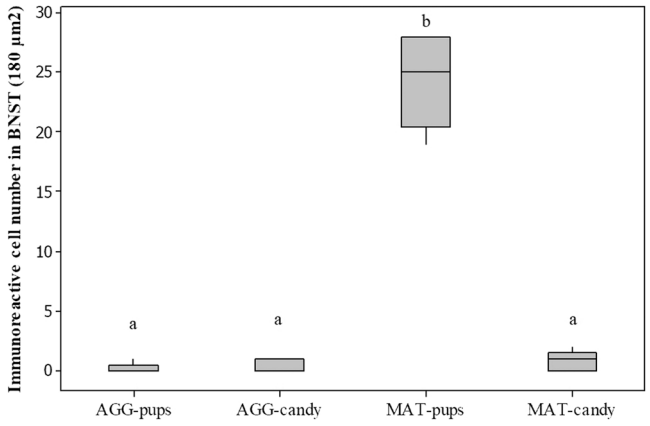

Fig. 2. Maternal females that interacted with pups (MAT-pups) had higher c-Fos-ir cells in BNST than maternal females that interacted with candy (MAT-candy) and aggressive females (AGG-pups and AGG-candy). Data are presented as the median. Letters indicate significant differences.

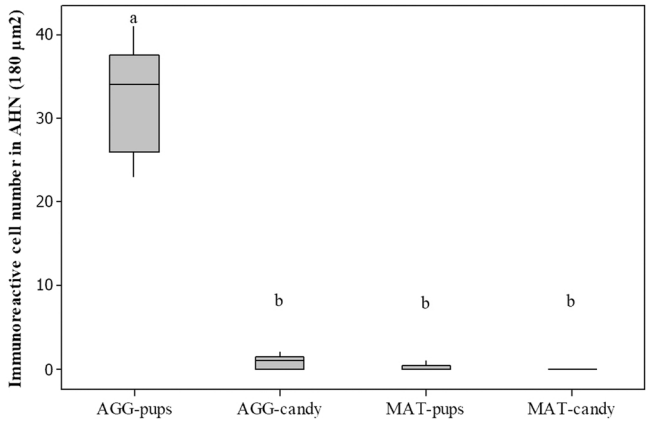

Fig. 3. Aggressive females that interacted with pups (AGG-pups) presented significantly higher c-Fos-ir cells in AHN than aggressive females that interacted with candy (AGG-candy) and maternal females (MAT-pups and MAT-candy). Data are presented as the median. Letters indicate significant differences.

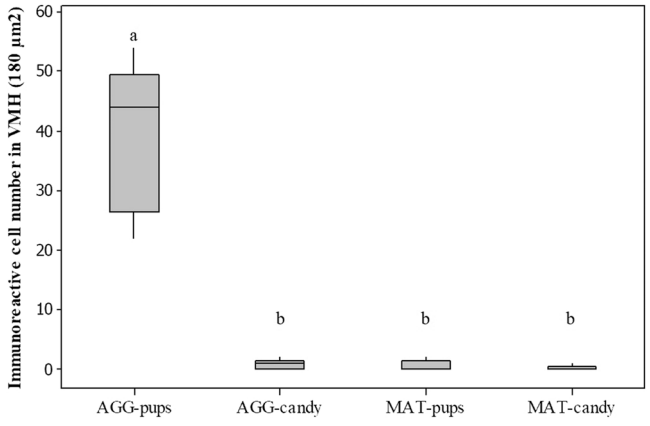

Fig. 4. In VMH c-Fos-ir cells number was significantly higher in aggressive females that interacted with pups (AGG-pups) than aggressive females that interacted with candy (AGG-candy) and maternal females (MAT-pups and MAT-candy). Data are presented as the median. Letters indicate significant differences.

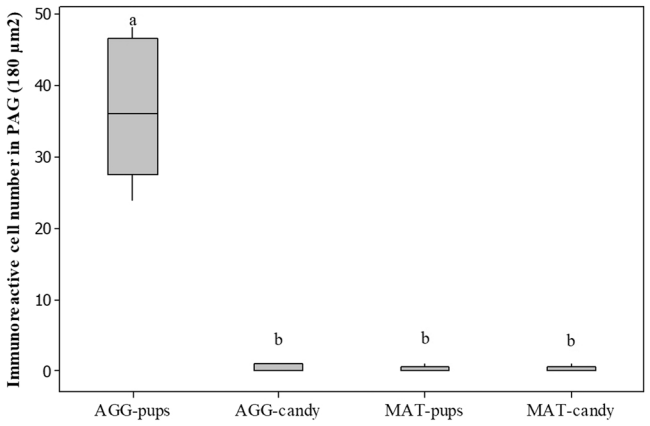

Fig. 5. Aggressive females that interacted with pups (AGG-pups) have significantly higher c-Fos-ir cells in PAG than aggressive females that interacted with candy (AGG-candy) and maternal females (MAT-pups and MAT-candy). Data are presented as the median. Letters indicate significant differences.

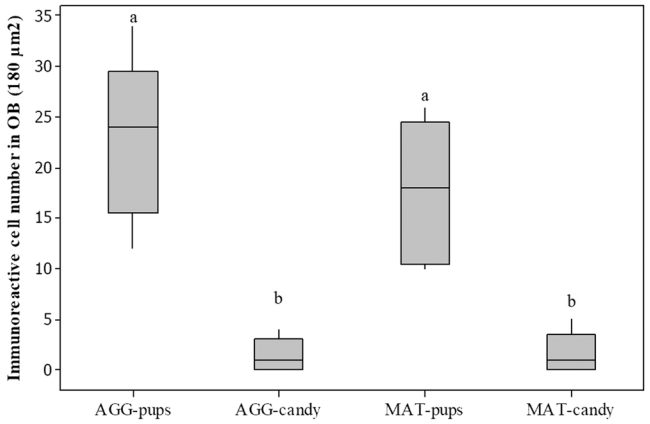

Fig. 6. Both maternal and aggressive interactions with pups had greater c-Fos-ir cells in OB than those that interacted with candy. Data are presented as the median. Letters indicate significant differences.

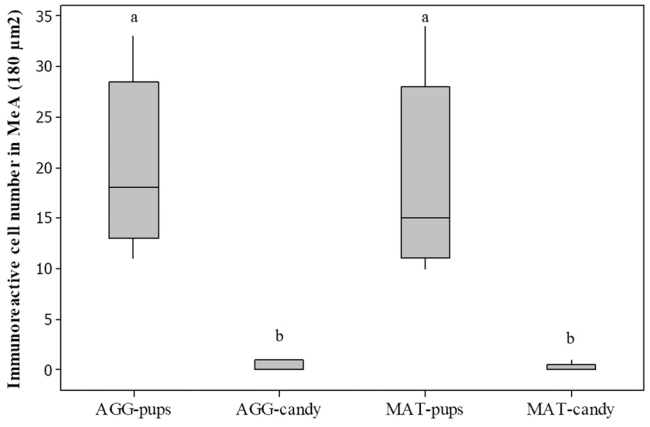

Fig. 7. Both maternal and aggressive interactions with pups have significantly higher c-Fos-ir cells in MeA than those that interacted with candy. Data are presented as the median. Letters indicate significant differences.

df = 3, $P < 0.05$, Figs. 4 and 8), and PAG ($H = 10.93$, df = 3, $P < 0.05$, Figs. 5 and 8). The pairwise comparison revealed that the AGG-pups group had significantly higher c-Fos-ir cells in the AHN, VMH, and PAG than the AGG-candy ($U = 40$, $P = 0.005$; $U = 40$, $P = 0.007$;

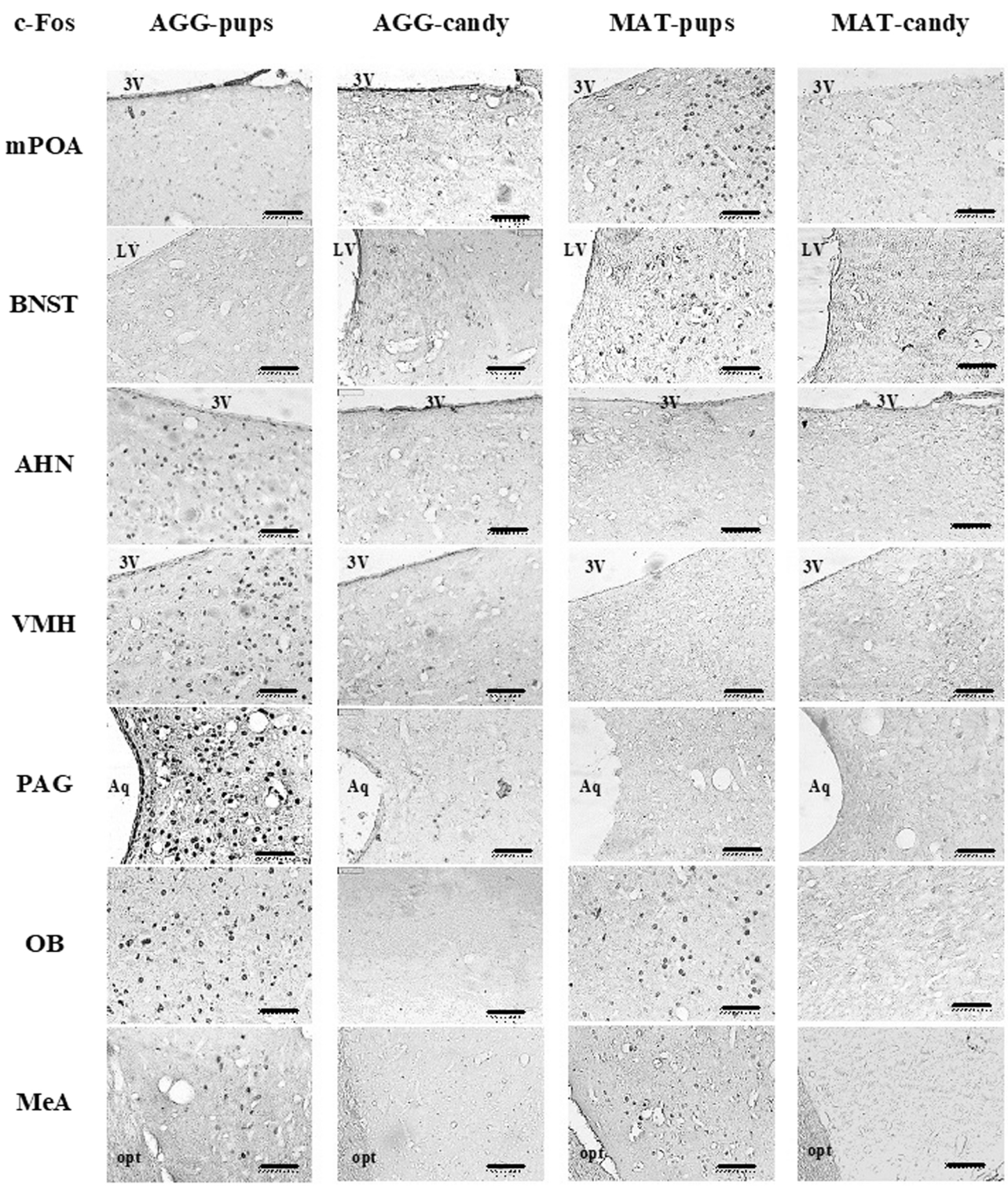

Fig. 8. Representative photomicrographs showing c-Fos-ir cells in the mPOA, BNST, AHN, VMH, PAG, MeA, and OB of the females of different groups. 3 V = third ventricle, LV = lateral ventricle, Aq = aqueduct, opt = optic tract. Coronal sections, scale bars = 100 μm.

U = 40, P = 0.007; respectively), MAT-pups (U = 40, P = 0.005; U = 40, P = 0.007; U = 40, P = 0.007; respectively), and MAT-candy groups (U = 40, P = 0.005; U = 40, P = 0.007; U = 40, P = 0.007; respectively) (Bonferroni adjusted P = 0.008).

The number of c-Fos-ir cells in the MeA (H = 14.40, df = 3, P < 0.05, Figs. 6 and 8) and OB (H = 14.63, df = 3, P < 0.05, Figs. 7 and 8) in the MAT-pups, MAT-candy, AGG-pups, and AGG-candy groups was significantly different. Pairwise comparison revealed that the MAT-pups group had significantly more c-Fos-ir cells in the MeA and OB than the AGG-candy (U = 40, P = 0.007; U = 40, P = 0.001, respectively) and MAT-candy groups (U = 40, P = 0.007; U = 40, P = 0.001, respectively), but there were no significant differences between the MAT-pups and AGG-pups groups in these same neural areas (U = 25.5, P = 0.7533; U = 22, P = 0.2963, respectively) (Bonferroni adjusted P = 0.008).

3.3. Estradiol levels

The concentrations of E_2 in plasma from the MAT-pups, MAT-candy, AGG-pups, and AGG-candy groups were significantly different ($H = 31.63$, $df = 3$, $P < 0.05$, Fig. 9). The pairwise comparison revealed that the MAT-pups group had significantly higher E_2 concentrations in plasma than the MAT-candy ($U = 155$, $P = 0.003$), AGG-pups ($U = 187$, $P = 0.005$), and AGG-candy groups ($U = 176$, $P = 0.005$). The MAT-candy group did not show significant differences in E_2 concentrations in plasma compared with the AGG-candy group ($U = 99.5$, $P = 0.8965$). Interestingly, the AGG-pups group had lower E_2 concentrations than the AGG-candy group ($U = 55$, $P = 0.002$) (Bonferroni adjusted $P = 0.008$). In the MAT-pups group, E_2 concentrations in the plasma and the number of c-Fos-ir cells were significantly correlated in the mPOA ($r = 0.93$, $P < 0.05$) and BNST ($r = 0.95$, $P < 0.05$). In the AGG-pups group, E_2 concentrations in the plasma and the number of c-Fos cell in the AHN ($r = -0.95$, $P > 0.05$), VMH ($r = -0.96$, $P > 0.05$), and PAG ($r = -0.92$, $P > 0.05$) were not significantly correlated. In addition, the correlations were negative.

4. Discussion

Virgin maternal females displayed crouching, grooming, and sniffing behaviours toward the pups. These behaviours have been reported as components of maternal conduct of the Mongolian gerbil. It should be noted that gerbil mothers, in addition to the behaviours already mentioned, display nest building, suckling, and retrieval of the young [24]. This difference between virgins and mothers is due to the exposure of mothers to pregnancy and parturition [1]. Furthermore, the time that virgin females of this rodent spent with pups in this study was relatively short, so other maternal behaviours, such as nest building, may have been missed. Aggressive behaviours such as shaking, and biting exhibited by aggressive virgin females with pups are also displayed by sexually inexperienced aggressive male gerbils [31]. Elwood mentions that aggressive virgin female Mongolian gerbils bite and eat pups [32].

In accordance with our hypothesis, virgin maternal female Mongolian gerbils that interacted with the pups had significantly more c-Fos-ir cells in the mPOA and BNST than virgin maternal females that interacted with candy. These results show that these neural regions are strongly activated when the females of this rodent display maternal behaviour, which suggests that the mPOA and the BNST are part of the positive mechanism that regulates maternal behaviour in the Mongolian gerbil. Many studies have shown that mPOA and BNST are activated in both virgin and lactating female laboratory rats when they display maternal care [3,33,34]. For example, when female rats interact with pups, they have a high degree of c-Fos activation in mPOA and BNST, but not when they are exposed to only stimuli from pups [35]. In this rodent, the use of electrolytic and excitotoxic lesions reaffirm that mPOA has a crucial function in the display of maternal behaviour, and when neurons in this nucleus are injured, maternal behaviour is severely affected [36–40]. Likewise, several investigations support that BNST plays an important role in regulating maternal behaviour, particularly the ventral region [3,41,42].

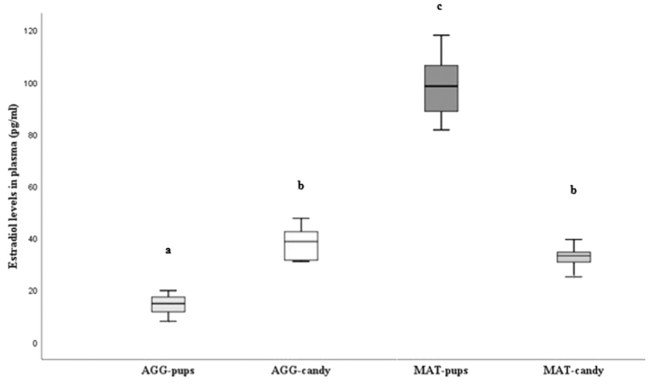

Fig. 9. Maternal females that interacted with pups (MAT-pups) had significantly higher E_2 levels than maternal females that interacted with candy (MAT-candy) and aggressive females (AGG-pups and AGG-candy). Data are presented as the median. Letters indicate significant differences.

Female Mongolian gerbils that were aggressive toward pups had a significantly higher number of c-Fos-ir cells in the AHN, VMH, and PAG than aggressive females that interacted with candy. These results suggest that these neural regions are involved in the negative mechanism regulating aggressive interactions with the pups in females of this rodent. The AHN, VMH, and PAG are components of the neural circuit that mediates aversion toward pups and defensive behaviours in female laboratory rats [3,42–44]. Supporting this view, studies causing excitotoxic lesions of the AHN and VMH stimulated a rapid onset of maternal behaviour by oestrogen-treated nulliparous rats [45]. In rats, Rizvi et al. [46] suggested that PAG and mPOA have reciprocal connections that may affect maternal behaviour, among other functions. In male gerbils, mPOA/BNST and AHN/VMH/PAG are activated in paternal and aggressive interactions with pups, respectively [47].

The number of c-Fos-ir cells in the MeA and OB was significantly higher in MAT-pups females than MAT-candy females, but it was not significantly different among MAT-pups females and AGG-pups females. This shows that these areas were similarly activated in maternal females that interacted with the pups and those that had aggressive interactions with them. Furthermore, it should be noted that both the MAT-pups and AGG-pups females presented immunoreactivity to c-Fos in MeA and OB, unlike maternal or aggressive females that interacted with candy. These results suggest that stimuli from the pups cause activation of OB and MeA in maternal and aggressive females, which could indicate that both MeA and OB are part of the positive and negative mechanisms that regulate maternal behaviour in the Mongolian gerbil. In laboratory rats, these two neural regions have multiple connections with both the facilitating (mPOA/BNST) and inhibiting (AHN/VMH) regions of neural circuits of maternal behaviour, as mentioned above [3,7,8,42,48,49].

Plasma estradiol concentrations were significantly higher in maternal females than aggressive females, which could indicate that a high E_2 concentration is required for virgin female Mongolian gerbils to respond maternally. We believe that an immediate maternal response in females can occur because the positive mechanism of the neural circuit of maternal behaviour is ready to respond to the presence of the pups, and that this can happen due to the high concentration of E_2. In addition, E_2 concentrations in plasma were positively and significantly correlated with the number of c-Fos-ir cells in mPOA and BNST. This suggests that high concentrations of E_2 correspond to greater activation of these nuclei involved in facilitating maternal behaviour. Well-designed experiments have shown that administration of high doses of E_2 (100 μg/kg) cause a shortening of the latency of onset of maternal behaviour in virgin female laboratory rats [50–52]. Several studies have suggested that mPOA integrates inputs generated by sensory stimuli from pups and that the hormonal milieu influences this process to facilitate maternal behaviour that inhibits the AHN, VMH, and PAG, which are part of the aggression/fear circuitry [53]. Bales and Saltzman [8] point out that in the absence of hormonal inputs, such as E_2 and oxytocin, MeA stimulation leads to activation of AHN/VMH/PAG, which are neural regions that promote aversion to pups. In a hormonal milieu similar to that of the last phase of pregnancy (high E_2 concentrations), MeA stimulation leads to activation of mPOA/BNST and consequently to the display of maternal behaviour.

Interestingly, the MAT-pups group had significantly higher E_2 concentrations in plasma than the MAT-candy group, which suggests that interaction with the pups stimulates an increase in E_2 levels, possibly to reinforce maternal behaviour. In contrast, aggressive females' interactions with pups were associated with a significant decrease in E_2. We think that the presence of pups could cause stress, which could affect

the synthesis of E_2. Some studies have reported that stress alters the biosynthesis of luteinizing hormone, thus causing a decrease in testosterone levels and consequently the production of one of its metabolites, E_2 [54,55]. Non-sexually aggressive male Mongolian gerbils also show a significant decrease in the concentration of testosterone when they interact with pups [26]. E_2 concentrations between MAT-candy and AGG-candy groups were not significantly different, which supports the idea that unlike candy, multiple stimuli from pups are capable of causing not only neural activation, but also a hormonal response, such as an increase or decrease of E_2 [28,29].

The results of this study suggest the existence of positive and negative mechanisms in the regulation of maternal behaviour in the Mongolian gerbil and that the immediate maternal response could be due to high E_2 concentrations. Future studies should be done using electrolytic or excitotoxic lesions, as well as chemogenetic and optogenetic techniques. Such efforts could confirm that the neural areas activated in maternal and aggressive interactions with pups are part of positive and negative mechanisms of the neural circuits of maternal behaviour in Mongolian gerbils.

Data Availability

No data was used for the research described in the article.

Acknowledgments

This research was supported by Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (IA205721) of Universidad Nacional Autónoma de México. The authors are grateful to Carmen Álvarez and Jesús Delgado Solís for their technical assistance. We express our gratitude to the anonymous reviewers.

References

[1] J.S. Lonstein, M. Pereira, I.J. Morrell, A.C. Marler, Parenting behavior, in: M. T. Plant, J.T. Zeleznik, F.D. Albertini, L.R. Goodman, E.A. Herbison, M. M. McCarthy, J.L. Muglia, S.J. Richards (Eds.), The Physiology of Reproduction, Elsevier, Amsterdam, 2015, pp. 2371–2437.
[2] R.S. Bridges, B.C. Nephew, Neuroendocrine control: maternal behavior, in: L. R. Squire (Ed.), Encyclopedia of Neuroscience, Elsevier Academic Press, St. Louis/San Diego, 2009, pp. 333–341.
[3] M. Numan, T.R. Insel, The Neurobiology of Parental Behavior, Springer-Verlag, New York, USA, 2003.
[4] C. Dulac, L.A. O'Connell, Z. Wu, Neural control of maternal and paternal behaviors, Science 345 (6198) (2014) 765–770, https://doi.org/10.1126/science.125329.
[5] J. Kohl, A.E. Autry, C. Dulac, The neurobiology of parenting: a neural circuit perspective, BioEssays 39 (2017) 1–11, https://doi.org/10.1002/bies.201600159.
[6] R. Lin, Y. Li, M. Luo, A neural circuit driving maternal behavior, Neuron 1 (98) (2018) 192–207, https://doi.org/10.1016/j.neuron.2018.03.025.
[7] M. Numan, Neurobiology of Social Behavior: toward an Understanding of the Prosocial and Antisocial Brain, Academic Press, CA, 2014.
[8] L.K. Bales, W. Saltzman, Fathering in rodents: neurobiological substrates and consequences for offspring, Horm. Behav. 77 (2016) 249–259, https://doi.org/10.1016/j.yhbeh.2015.05.021.
[9] S.R. Bridges, A quantitative analysis of the roles of dosage, sequence, and duration of estradiol and progesterone exposure in the regulation of maternal behavior in the rat, Endocrinology 114 (3) (1984) 930–940, https://doi.org/10.1210/endo-114-3-930.
[10] H.I. Siegel, J.S. Rosenblatt, Hormonal basis of hysterectomy-induced maternal behavior during pregnancy in the rat, Horm. Behav. 6 (1975) 211–222, https://doi.org/10.1016/0018-506X(75)90008-2.
[11] M.J. Baum, B.J. Everitt, Increased expression of c-Fos in the medial preoptic area after mating in male rats: role of afferent inputs from the medial amygdala and midbrain central tegmental field, Neuroscience 50 (1992) 627–646, https://doi.org/10.1016/0306-4522(92)90452-8.
[12] L.M. Coolen, H.J. Peters, J.G. Veening, Fos immunoreactivity in the rat brain following consummatory elements of sexual behavior: a sex comparison, Brain Res. 738 (1996) 67–82, https://doi.org/10.1016/0006-8993(96)00763-9.
[13] G.E. Hoffman, M.S. Smith, J.G. Verbalis, c-Fos and related immediate early gene products as markers of activity in neuroendocrine systems, Front. Neuroendocrinol. 14 (3) (1993) 173–213, https://doi.org/10.1006/frne.1993.1006.
[14] M. Koch, G. Ehret, Parental behavior in the mouse: effects of lesions in the entorhinal/piriform cortex, Behav. Brain Res. 42 (1991) 99–105, https://doi.org/10.1016/S0166-4328(05)80044-0.
[15] A. Lee, S. Clancy, A.S. Fleming, Mother rats bar-press for pups: effects of lesions of the mPOA and limbic sites on maternal behavior and operant responding for pup reinforcement, Behav. Brain Res. 108 (2000) 213–231, https://doi.org/10.1016/S0166-4328(98)00109-0.
[16] J.S. Lonstein, J.M. Stern, Site and behavioral specificity of periaqueductal gray lesions on postpartum sexual, maternal, and aggressive behaviors in rats, Brain Res. 804 (1998) 21–35, https://doi.org/10.1016/S0006-8993(98)00642-8.
[17] S.W. Newman, The medial extended amygdala in male reproductive behavior. A node in the mammalian social behavior network, Ann. N. Y. Acad. Sci. 877 (1999) 242–257, https://doi.org/10.1111/j.1749-6632.1999.tb09271.x. ⟨https://ui.adsabs.harvard.edu/link_gateway/1999NYASA.877.242N⟩.
[18] Y. Tsuneoka, T. Maruyama, S. Yoshida, K. Nishimori, T. Kato, M. Numan, K. O. Kuroda, Functional, anatomical, and neurochemical differentiation of medial preoptic area subregions in relation to maternal behavior in the mouse, J. Comp. Neurol. 521 (7) (2013) 1633–1663, https://doi.org/10.1002/cne.23251.
[19] J. Kohl, B.M. Babayan, N.D. Rubinstein, A.E. Autry, B. Marin-Rodriguez, V. Kapoor, K. Miyamishi, L.S. Zweifel, L. Luo, N. Uchida, C. Dulac, Functional circuit architecture underlying parental behaviour, Nature 556 (2018) 326–331, https://doi.org/10.1038/s41586-018-0027-0.
[20] R. Lin, Y. Li, M. Luo, A neural circuit driving maternal behavior, Neuron 1 (98) (2018) 192–207, https://doi.org/10.1016/j.neuron.2018.03.025.
[21] A.M. Tartaglione, S. Farioli Vecchioli, S. Calamandrei, Altered responsiveness to pups in virgin female mice of the BTBR strain: insights from pattern of c-Fos expression in brain regions involved in maternal behavior, Behav. Brain Res. 410 (1938–53) (2021) 113365–113368.
[22] C.A. Ribeiro, S. Musatov, A. Shteyler, S. Simanduyev, I. Arrieta-Cruz, S. Ogawa, W. D. Pfaff, siRNA silencing of estrogen receptor-α expression specifically in medial preoptic area neurons abolishes maternal care in female mice, PNAS 109 (40) (2012) 16324–16329, https://doi.org/10.1073/pnas.1214094109.
[23] A.S. Fleming, C. Luebke, Timidity prevents the virgin female rat from being a good mother: emotionality differences between nulliparous and parturient females, Physiol. Behav. 27 (5) (1981) 863–868, https://doi.org/10.1016/0031-9384(81)90054.
[24] R.W. Elwood, Paternal and maternal behaviour in the Mongolian gerbil, Anim. Behav. 23 (1975) 766–772, https://doi.org/10.1016/0003-3472(75)90104-9.
[25] R.S. Sikes, The Animal Care and Use Committee of the American Society of Mammalogists, 2016 guidelines of the American Society of Mammalogists for the use of wild mammals in research and education, J. Mammal. 97 (3) (2016) 663–688, https://doi.org/10.1093/jmammal/gyw078.
[26] L. Romero-Morales, M. Cárdenas, M. Martínez-Torres, B. García-Saucedo, A. Carmona, J. Luis, Neuronal activation associated with paternal and aversive interactions toward pups in the Mongolian gerbils (*Meriones unguiculatus*), Horm. Behav. 105 (2018) 47–57, https://doi.org/10.1016/j.yhbeh.2018.07.010.
[27] J.I. Morgan, D.R. Cohen, J.L. Hempstead, T. Curran, Mapping patterns of c-fos expression in the central nervous system after seizure, Science 2377 (1987) 7–192, https://doi.org/10.1126/science.3037702.
[28] B. Kirkpatrick, W.J. Kim, R.T. Insel, Limbic system fos expression associated with paternal behavior, Brain Res. 658 (1994) 112–118, https://doi.org/10.1016/S0006-8993(09)90016-6.
[29] B. Kirkpatrick, I.R. Williams, B.M. Slotnick, C.S. Carter, Olfactory bulbectomy decreases social behavior in male prairie voles (*Microtus ochrogaster*), Physiol. Behav. 55 (1994) 885–889, https://doi.org/10.1016/0031-9384(94)90075-2.
[30] W.J. Loskota, P. Lomax, M.A. Verity, A Stereotaxic Atlas of the Mongolian Gerbil Brain (*Meriones unguiculatus*), Ann Arbor Science, Publisher Inc., Michigan, USA, 1974.
[31] A. Martínez, G. Ramos, M. Martínez-Torres, L. Nicolás, A. Carmona, M. Cárdenas, J. Luis, Paternal behavior in the Mongolian gerbil (*Meriones unguiculatus*): estrogenic and androgenic regulation, Horm. Behav. 71 (2015) 91–95, https://doi.org/10.1016/j.yhbeh.2015.04.009.
[32] R.W. Elwood, Changes in the responses of male and female gerbils (*Meriones unguiculatus)* towards test pups during the pregnancy of female, Anim. Behav. 25 (1977) 46–51, https://doi.org/10.1016/0003-3472(77)90066-5.
[33] E.C. Stack, C. Numan, The temporal course of expression of c-Fos and Fos B within the medial preoptic area and other brain regions of postpartum female rats during prolonged mother-young interactions, Behav. Neurosci. 114 (3) (2000) 609–622, https://doi.org/10.1037/0735-7044.114.3.609.
[34] E.C. Stack, R. Balakrishnan, M.J. Numan, M. Numan, A functional neuroanatomical investigation of role of the medial preoptic area in neural circuits regulating maternal behavior, Behav. Brain Res. 131 (1–2) (2002) 17–36, https://doi.org/10.1016/S0166-4328(01)00370-9.
[35] M. Numan, M.J. Numan, Importance of pup-related sensory inputs and maternal performance for the expression of Fos-like immunoreactivity in the preoptic area and ventral bed nucleus of the stria terminalis of postpartum rats, Behav. Neurosci. 108 (2) (1995) 379–394, https://doi.org/10.1037/0735-7044.109.1.135.
[36] M. Numan, Medial preoptic area and maternal behavior in the female rat, J. Comp. Physiol. Psychol. 87 (4) (1974) 746–759, https://doi.org/10.1037/h0036974.
[37] C.D. Jacobson, J. Terkel, R.A. Gorski, C.H. Sawyer, Effects of small medial preoptic area lesions on maternal behavior: retreiving and nest building in the rat, Brain Res. 194 (2) (1980) 471–478, https://doi.org/10.1016/0006-8993(80)91226-3.
[38] M. Numan, K.P. Corodimas, M.J. Numan, E.M. Factor, W.D. Piers, Axon-sparing lesions of the preoptic region and substantia innominata disrupt maternal behavior in rats, Behav. Neurosci. 102 (3) (1988) 381, https://doi.org/10.1037/0735-7044.102.3.381.
[39] M. Kalinichev, J.S. Rosenblatt, J.I. Morrell, The medial preoptic area, necessary for adult maternal behavior in rats, is only partially established as a component of the

neural circuit that supports maternal behavior in juvenile rats, Behav. Neurosci. 114 (1) (2000) 196, https://doi.org/10.1037/0735-7044.114.1.196.
[40] A. Lee, S. Clancy, A.S. Fleming, Mother rats bar-press for pups: effects of lesions of the MPOA and limbic sites on maternal behavior and operant responding for pup-reinforcement, Behav. Brain Res. 100 (1–2) (1999) 15–31, https://doi.org/10.1016/S0166-4328(98)00109-0.
[41] M. Numan, M. Numan, A lesion and neuroanatomical tract-tracing analysis of the role of the bed nucleus of the stria terminalis in retrieval behavior and other aspects of maternal responsiveness in rats, Dev. Psychobiol. 29 (1) (1996) 23–51, https://doi.org/10.1002/(SICI)1098-2302(199601)29:1%3C23::AID-DEV2%3E3.0.CO;2-O.
[42] M. Numan, The Parental Brain: Mechanisms, Development, and Evolution, Oxford University Press, 2020.
[43] T.P. Sheehan, J. Cirrito, M.J. Numan, M. Numan, Using c-Fos immunocytochemistry to identify forebrain regions that may inhibit maternal behavior in rats, Behav. Neurosci. 114 (2) (2000) 337–352, https://doi.org/10.1037/0735-7044.114.2.337.
[44] N.S. Canteras, The medial hypothalamic defensive system: hodological organization and functional implications, Pharm. Biochem. Behav. 71 (2002) 481–491, https://doi.org/10.1016/S0091-3057(01)00685-2.
[45] R.S. Bridges, P.E. Mann, J.S. Coppeta, Hypothalamic involvement in the regulation of maternal behaviour in the rat: inhibitory roles for the ventromedial hypothalamus and the dorsal/anterior hypothalamic areas, J. Neuroendocrinol. 11 (1999) 259–266, https://doi.org/10.1046/j.1365-2826.1999.00322.x.
[46] T.A. Rizvi, M. Ennis, M.T. Shipley, Reciprocal connections between the medial preoptic area and the midbrain periaqueductal gray in rat: a WGA-HRP and PHA-L study, J. Comp. Neurol. 315 (1) (1992) 1–15, https://doi.org/10.1002/cne.903150102.
[47] M. Numan, Neurobiology of Social Behavior: Toward an Understanding of the Prosocial and Antisocial Brain, Academic Press, 2014.
[48] P. Brunton, J. Russell, Maternal brain adaptations in pregnancy, in: Knobil and Neill's Physiology of Reproduction, Elsevier, 2015.
[49] G.G. Carter, S.G. Wilkinson, Common vampire bat contact calls attract past food-sharing partners, Anim. Behav. 116 (11) (2016) 45–51, https://doi.org/10.1016/j.anbehav.2016.03.005.
[50] H.I. Siegel, J.S. Rosenblatt, Progesterone inhibition of estrogen-induced maternal behavior in hysterectomized-ovariectomized virgin rats, Horm. Behav. 6 (1975) 223–230, https://doi.org/10.1016/0018-506X(75)90009-4.
[51] R.S. Bridges, A quantitative analysis of the roles of dosage, sequence, and duration of estradiol and progesterone exposure in the regulation of maternal behavior in the rat, Endocrinology 114 (3) (1984) 930–940, https://doi.org/10.1210/endo-114-3-930.
[52] J.S. Rosenblatt, A.D. Mayer, An analysis of approach/withdrawal processes in the initiation of maternal behavior in the laboratory rat, in: Behavioral Development, Routledge, 2013, pp. 199–252.
[53] M. Liang, J. Zhong, H.X. Liu, O. Lopatina, R. Nakada, A.M. Yamauchi, H. Higashida, Pairmate dependent pup retrieval as parental behavior in male mice, Front. Neurosci. 8 (2014) 186, https://doi.org/10.3389/fnins.2014.00186.
[54] E. Xiao, L. Xia-zhang, M. Ferin, Inadequate luteal function is the initial clinical cyclic defect in a 12-day stress model that includes a psychogenic component in the Rhesus monkey, J. Clin. Metab. 87 (5) (2002) 2232–2237, https://doi.org/10.1210/jcem.87.5.8500.
[55] Q. Dong, A. Salva, C.M. Sottas, E. Niu, M. Holmes, M.P. Hardy, Rapid glucocorticoid mediation of suppressed testosterone biosynthesis in male mice subjected to immobilization stress, J. Androl. 25 (6) (2004) 973–981, https://doi.org/10.1002/j.1939-4640.2004.tb03170.x.