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Fos expression in the medial preoptic area and nucleus accumbens of female Japanese quail (*Coturnix japonica*) after maternal induction and interaction with chicks

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ABSTRACT

The neural system underlying maternal caregiving has often been studied using laboratory rodents and a few other mammalian species. This research shows that the medial preoptic area (mPOA) integrates sensory cues from the young that, along with hormonal and other environmental signals, control maternal acceptance of neonates. The mPOA then activates the mesolimbic system that drives maternal motivation and caregiving activities. How components of this neural system respond to maternal experience and exposure to young in non-mammals has rarely been examined. To gain more insight into this question, virgin female Japanese quail (*Coturnix japonica*) were induced to be maternal through four days of continuous exposure to chicks (Maternal), or were not exposed to chicks (Non-Maternal). Chicks were removed overnight from the Maternal group and half the females from each group were then exposed to chicks for 90 minutes (Exposed), or not exposed to chicks (Non-Exposed), before euthanasia. The number of Fos-immunoreactive (Fos-ir) cells was examined as a marker of neuronal activation. As expected, repeated exposure to chicks induced caregiving behavior in the Maternal females, which persisted after the overnight separation, suggesting formation of a maternal memory. In contrast, Non-Maternal females were aggressive and rejected chicks when exposed to them. Exposed females, whether or not they were given prior experience with chicks (i.e., regardless if they accepted or rejected chicks during the exposure before euthanasia), had more Fos-ir cells in the mPOA compared to Non-Exposed females. In the nucleus accumbens (NAC), the number of Fos-ir cells was high in all Maternal females regardless of whether or not they were Exposed to chicks again before euthanasia. In the lateral bed nucleus of the stria terminalis, a site involved in general stress responding, groups did not differ in Fos-ir cells. These data indicate a conserved role for the mPOA and NAC in maternal caregiving across vertebrates, with

the mPOA acutely responding to the salience rather than valence of offspring cues, and the NAC showing longer-term changes in activity after a positive maternal experience even without a recent exposure.

Keywords: immediate early gene, maternal behavior, motivation, mesolimbic, preoptic area, maternal brain, maternal memory

Studies of the neural system underlying maternal caregiving behaviors have long implicated the medial preoptic area (mPOA) as a critical site for the expression of active caregiving behaviors such as offspring carrying and nest building (González-Mariscal & Poindron, 2002; Numan, Fleming and Lévy, 2006; Lonstein, Pereira, et al., 2014). This is because the mPOA receives a variety of sensory inputs from the young, integrates that information with the females' previous and current endocrine status, and then projects to a host of brain sites involved in socially-relevant motivation, affective state, and cognition (Barratt and Fleming, 2011; Kohl et al. 2018; Lonstein, Lévy and Fleming, 2015; Numan, 2012; Swain, 2011).

This role of the mPOA in maternal caregiving is mostly based on work in laboratory rodents showing that lesioning or deactivating the mPOA can severely inhibit the onset and expression of caregiving behaviors (e.g., Fang et al. 2018; Numan et al., 1974; Pereira and Morrell, 2009; Tsuneoka et al. 2013). In addition, exposure to sensory cues from the young or directly interacting with them increases immediate-early gene (IEG) expression in the mPOA of maternally behaving animals (e.g., Da Costa et al. 1997; González-Mariscal et al. 2009; Kalinichev et al. 2000; Numan and Numan, 1994; Fleming and Walsh, 1994; Lonstein et al. 1998; Tsuneoka et al. 2013). However, some research has instead suggested that mPOA activation may be capable of inhibiting maternal care. For example, chemically deactivating the mPOA of late-postpartum rats showing relatively low maternal behavior to their older pups increases their caregiving (Pereira and Morrell, 2009). Furthermore, high Fos expression is not only found in the mPOA of maternally acting female rats, but also in females that are not maternal but forced to be close to the offspring (Sheehan et al., 2000). Such results indicate that

mPOA may be involved in processing the salience or novelty, rather than only the valence, of offspring cues (see also Olazabal and Morrell, 2005).

In addition to the question regarding the function of the mPOA in maternal caregiving, it also is mostly unknown if its roles in caregiving are conserved across vertebrates. This is because the body of research studying the mPOA and its connections in maternal caregiving has been performed almost exclusively in mammals, primarily laboratory rodents (Lonstein et al., 2014, Lonstein, Lévy and Fleming, 2015; Stolzenberg and Numan, 2011) as well as sheep (Perrin et al., 2007) and rabbits (Basurto et al., 2018). Studies on the brain control of maternal caregiving behaviors in non-mammals include a handful of studies of brain IEG expression in birds after displaying reproduction-related nest building (Hall et al., 2014; Kingsbury et al., 2015), incubation of eggs (Sharp et al. 1996), or chick brooding (Buntin et al. 2006; Ruscio and Adkins-Regan, 2004; Thayanaphat et al., 2011), as well as a recent report on tadpole-carrying frogs (Fischer et al., 2019). However, the specific question of how prior maternal experience with chicks, and then re-exposure later, interact to affect activity of the female brain has not been explored in any avian species. While the just-mentioned studies generally support a positive role for the mPOA or its homologous brain region during these activities by showing high IEG expression, more research into the nature of the mPOA's role is needed in non-mammals.

The present study was conducted to test the hypothesis that the putative function of the mPOA to mediate the salience, rather than the valence, of offspring cues in female rats extends to female Japanese quail (*Coturnix japonica*). To accomplish this, we used a 2 x 2 experimental design to examine Fos expression in the mPOA in virgin females that were induced to show maternal caregiving by exposing them to chicks (i.e., maternal sensitization) and in females not induced to show caregiving. Induction of maternal behavior after exposure to chicks has been

demonstrated in quail (Bertin and Richard-Yris, 2004, Lumineau et al., 2019; Aigueperse et al. 2020), and also females' different maternal styles toward them (Pittet et al., 2014a). Groups of females from the sensitized and non-sensitized conditions were then later exposed to chicks or not before euthanasia for brain analysis of Fos. In addition to measuring Fos expression in their mPOA, we also measured Fos expression in the nucleus accumbens (NAC) and lateral ventral bed nucleus of the stria terminalis (BSTL). The NAC processes both the salience and valence of rewarding stimuli in many animals, including birds (Cooper and Knutson, 2008; Levita et al. 2009; Riters et al., 2013; Zachar et al., 2017), so may somewhat differ than the mPOA in its response to chicks. The BSTL is part of the “extended amygdala” system underlying endocrine and anxiety-like responses to stressors (Cecci et al., 2002; Morilak et al. 2003; Walker et al. 2009), including in birds (Nagarajan et al., 2014). Thus, the BSTL is expected to differentiate between sensitized and non-sensitized females exposed to chicks before euthanasia, given that the latter group typically shows fear and aversion during their very first exposure to young (Ruscio and Adkins-Regan, 2004).

Methods

Subjects

Forty-five 3.5-month old virgin female Japanese quail (*C. japonica*) purchased from a commercial line (Les Cailles de Chanteloup, Corps-Nuds, France) were used as subjects and housed in individual wire cages (51 × 40 × 35 cm) with food and water available *ad lib*. Cages were maintained in the same room under a 12:12 hour light/dark cycle (lights on 08:00 h) and installed with heaters to keep the temperature at 20 ± 1 °C. Under this photoperiod, females were sexually developed and had stopped laying eggs the week before maternal induction. Observing

maternal responsiveness under LD 12:12 is consistent with reproduction in quail, as they can show precocious winter sexual development in southern Europe; for instance, 50% of the closely related *Coturnix c. coturnix* show signs of sexual development even in December (LD 9:13) (Fontoura et al., 2000). To induce maternal brooding, we used fertilized eggs purchased from the same commercial line that were hatched in our laboratory. At hatching, chicks were group-housed in cages ($98 \times 35 \times 42$ cm) containing food, water, and a heater until the fostering procedure began the next evening. All animal procedures were approved by the Ethics Committee of the French Ministry of Research (authorization number APAFIS#26924-2020082411233900) in accordance with the European Communities Council Directive (2010/63/EU). All efforts were made to minimize animal stress and pain.

Induction of brooding, re-exposure to chicks, and behavioral observations

Twenty-five of the adult females (group Maternal = M) were induced to show maternal behavior by placing four hatchlings in their cages for 4 days. The 20 control females were housed in similar individual cages and not exposed to chicks (Group Non-Maternal = NM). For the Maternal females, the induction procedure was as described previously by our group (Aigueperse et al., 2017) and induces an endocrine state similar to naturally maternal females, including high plasma levels of prolactin (Richard-Yris et al., 1987). Chicks were gently introduced within an opaque nest box ($18 \times 18 \times 18$ cm) where the female had been placed one hour before at the evening of hatching day. During the night, tactile and vocal stimulation from chicks induce expression of maternal care by the female (Richard-Yris and Leboucher, 1987). The next morning, the nest box was opened and females were characterized using instantaneous scan sampling (each female was observed every 30 minutes during the first morning) as maternal

if the young were warm, food was being offered, and maternal calls (cooing and food calls) were emitted. Observations of maternal behavior performed on post-hatching day 3 using 5-min focal scans confirmed that females were providing maternal care to the chicks and not displaying aggressive or rejection behaviors toward them. Two females failed to become maternally induced, so were removed from the study, leaving 23 successfully sensitized females.

After the M group received four days of exposure to chicks, the chicks were removed and subjects remained alone overnight for ~15 hours. This period of separation has been shown to be appropriate to induce Fos expression after re-exposure to chicks in parental ring doves (Buntin, Berghman and Buntin, 2006). Half of each of the M and NM groups then had four chicks placed in their cages for 90 min before sacrifice (Exposed) and the other half of the M and NM groups did not (Not Exposed). Thus, the final four groups in this 2 x 2 design were Maternal/Exposed (M/E, $n = 12$), Maternal/Non-Exposed (M/NE, $n = 11$), Non-Maternal/Exposed (NM/E, $n = 10$) and Non-Maternal/Non-Exposed (NM/NE, $n = 10$).

Females' behavior was observed during the first 20 min of exposure to chicks (or not), using instantaneous scan sampling every 30 sec, as well as focal sampling. The behavior of 2 NM/E and 1 M/NE females was not observed due to technical problems. For each instantaneous scan, females' activity (feeding, drinking, moving, exploring, pacing and freezing), self-comfort behaviors (resting, preening, or dustbathing), and chick warming were recorded. For warming, the latency to begin warming chicks was recorded and a covering posture (i.e., the female is in high posture over chicks) was differentiated from a non-covering posture (i.e., female is lying on her side not allowing chicks to be warmed). Focal sampling was continuously performed to record the number of females' vocalizations, the number of vigilance postures, the number of aggressive and neglectful behaviors displayed towards chicks, the number of warming attempts

made by the chicks that were accepted or rejected by the female, and the number of contact breaks made by the female (see Table 1 for detailed descriptions).

Euthanasia and Tissue Collection

At the end of the 90 min of exposure or no exposure to chicks, subjects were deeply anaesthetized with an overdose of sodium pentobarbital (100 mg/kg) and transcardially perfused with 4% paraformaldehyde, 0.2% glutaraldehyde, 15% saturated picric acid solution, in 0.01 M phosphate buffer. Brains were removed, cryoprotected in 20% sucrose for 24 h at 4 °C, frozen on dry ice and stored at -75 °C until sectioning. Brains were later sectioned at 30 µm on a cryostat (Leica Biosystems, Nanterre, France) into a series of four, stored in a sucrose-based cryoprotectant solution, and maintained at -18 °C until immunohistochemical processing.

Fos immunohistochemistry and quantification

Immunostaining for Fos protein was performed as previously described in quail (Charlier et al., 2005). Briefly, one set in the series for each animal was rinsed and then incubated in 0.6% H₂O₂ for 20 min, rinsed again, and blocked in 2% normal goat serum for 30 min. Tissue was incubated overnight at 4 °C in a primary antiserum (generous gift from Dr. L. Arckens, KUL, Belgium) that was raised against avian Fos protein and has been validated in quail (D'Hondt et al. 1999; 1:1000). After rinsing, tissue was exposed to a secondary biotinylated goat anti-rabbit antiserum (Dako, 1:400) for 90 min, rinsed, then incubated for 90 min in the VECTASTAIN® Elite ABC-HRP Kit, Peroxidase (Vector, 1:1000). All reagents were in 0.01 M PBS containing 0.1% Triton X-100 (PBST). Cells were visualized with a DAB Substrate Kit Peroxidase (HRP)

with Nickel following the manufacturer's instructions. Sections were mounted and the slides coverslipped.

Slides were randomized and analyzed by a single observer who was naive to the subjects' group placement (J.S.L.). Images of the brain sections were captured by automated scanning of the slides using Axioscan Z1 (ZEISS). The microscope's illumination level and magnification were standardized across subjects. The number of Fos-immunoreactive cells in two hemisections of the anterior mPOA, four hemisections of the NAC, and four hemisections of the BSTL were quantified using Image J (NIH). A rectangular region of interest (ROI) covering the entire area of each brain site was created and used for all subjects (Figure 1). Cell nuclei containing any visible Fos-immunoreactivity were included in the quantification. The average number of Fos-ir cells for each subject from the sections analyzed for each site was used for data analyses. An inability to find highly appropriate section matches, or damaged tissue, for a few subjects resulted in final sample sizes of 8-11/group for this Fos analysis.

Data analyses

Mann-Whitney tests using XLSTAT 3.0 (©Addinsoft 2011) compared M/E and NM/E females on their behavior during the first 20 min of the 90-min interaction with chicks before euthanasia. Because we expected that the maternally induced M/E females would not reject chicks and instead show caregiving behaviors, compared to rejection and avoidance expected from the inexperienced N/E females, we performed one-sided Mann-Whitney U tests. Fos data were normally distributed and analyzed using 2 x 2 ANOVAs to determine main effects and interactions between the two factors (Maternal Induction - M or NM; Exposure - E or NE). Pearson's r was used to determine correlations between behavior and the number of Fos-ir cells

in the two groups of quail Exposed to chicks 90 min before euthanasia. Statistical significance in all cases was indicated by $p < 0.05$.

Results

Females' behavior during exposure to chicks before euthanasia

As expected, the maternally induced and experienced M/E females significantly differed from the non-induced NM/E on several behaviors when they were exposed to chicks before euthanasia (Table 2). The M/E females had a greater frequency of successful solicitation requests from chicks ($U = 106.5, p = 0.001$), and tended to vocalize more in the presence of the chicks ($U = 75, p = 0.054$), when compared to the NM/E females. Moreover, the M/E females were faster to begin warming the chicks ($U = 4, p = 0.0001$), and then spent more time both warming ($U = 115, p = 0.0001$) and displaying the covering posture over them ($U = 48, p = 0.028$).

In contrast, only 5 out of the 10 NM/E females warmed chicks for any duration of time, and spent more time with chicks in the uncovering posture ($U = 12, p = 0.0275$). The NM/E females also showed more rejection of chicks (higher frequency of warming requests refused: $U = 10.5, p = 0.0006$, more escapes from chicks: $U = 3, p < 0.0001$), and a higher frequency of aggression toward chicks ($U = 23.5, p = 0.0048$). Crushing the chicks ($U = 37, p = 0.061$) and vigilance ($U = 75, p = 0.055$) were expressed at low levels and did not significantly differ between groups. The NM/E females showed fewer contact breaks from chicks compared to the M/E females ($U = 87.5, p = 0.034$), but this was due to the fact that they had a low level of direct contact with chicks in the first place. Instead of positively interacting with chicks, the NM/E

female spent more time in general activity ($U = 26.5$, $p = 0.0145$) and exhibiting fearful behaviors ($U = 25$, $p = 0.010$), but spent less time showing self-comforting behaviors ($U = 107$, $p = 0.001$).

Brain Fos immunoreactivity

In the mPOA, the main effect of maternal induction was not significant ($F(1,28) = 0.21$, $p = 0.654$), but there was a significant main effect of exposure to chicks before euthanasia ($F(1,28) = 8.07$, $p = 0.008$) with the females exposed to chicks beginning 90 min before euthanasia (groups M/E and NM/E) having more Fos-ir cells compared to the Non-Exposed females (groups M/NE and NM/NE; Figure 2A; Figure 3). The interaction between maternal induction and exposure to chicks before euthanasia was not significant ($F(1,28) = 0.38$, $p = 0.543$).

In contrast to the mPOA, there was a significant main effect of maternal induction in the NAC ($F(1,34) = 8.42$, $p = 0.006$), with maternally induced females (groups M/E and M/NE) together having more Fos-ir cells compared to the females that were never maternally induced (groups NM/E and NM/NE; Figure 2B). There was no significant main effect of exposure to chicks before euthanasia ($F(1,34) = 1.82$, $p = 0.186$), and no significant interaction between the two factors ($F(1,34) = 1.93$, $p = 0.174$).

In the BSTL, there were no significant main effects of maternal induction ($F(1,35) = 0.91$, $p = 0.347$) or exposure to chicks before euthanasia ($F(1,35) = 0.081$, $p = 0.778$), and no significant interaction between the two factors ($F(1,35) = 3.311$, $p = 0.077$; Figure 2C), on the

number of Fos-ir cells.

There were no significant correlations between the number of Fos-ir cells in any brain site and behavior within each of the M/E or the NM/E groups. While there were a few significant correlations between the number of Fos-ir cells and behavior found when the two groups were collapsed into one, visualizing these correlations revealed that they were due to the main effects of group (i.e., two clustered groups were seen at the ends of the regression line; see Aggarwal and Ranganathan, 2016).

Discussion

The mPOA is a well-studied node in the brain's social behavior network (Newman, 1999; Goodson, 2008; O'Connell and Hofmann, 2015) because it receives sensory inputs from conspecifics, is exquisitely sensitive to steroid and peptide hormones, and projects to sites outside the network that are involved in motivation, affect, and cognition (Barratt and Fleming, 2011; Kohl et al. 2018; Lonstein, Lévy and Fleming, 2015; Numan, 2012; Swain, 2011). The importance of the mPOA for social behavior includes maternal caregiving, particularly active maternal behaviors such as carrying young and nest building (González-Mariscal & Poindron, 2002; Numan, Fleming and Levy, 2006; Lonstein, Pereira, et al., 2014). This is supported by many lines of research spanning decades, including studies demonstrating that exposure to offspring sensory cues and/or interacting with the young increases IEG expression in the mPOA (e.g., Da Costa et al. 1997; González-Mariscal et al. 2009; Numan and Numan, 1994; Fleming and Walsh, 1994; Lonstein et al. 1998; Tsuneoka et al. 2013).

The few prior studies on IEG expression in the female bird mPOA suggested that its role in maternal activities was evolutionary conserved. For instance, female turkeys incubating eggs

for four weeks followed by a brief exposure to live chicks showed more *c-fos* mRNA in the anterior mPOA compared to females remaining with the eggs and not provided chicks (Thayananuphat et al., 2011). Similarly, ring doves studied seven days after hatching and acutely exposed to their chicks after an overnight separation showed more Fos-ir cells in the anterior mPOA compared to unexposed ring doves (Buntin et al., 2006). A study of virgin quail that involved a sensitization procedure of five 20-min exposures to chicks across three days, with euthanasia immediately after the final exposure, found low number of Fos-ir cells in the mPOA, but higher ZENK-ir cells in the sensitized compared to non-sensitized mPOA (Ruscio and Adkins-Regan, 2004). Motherhood-related cues stimulating Fos expression in the bird mPOA do not even need to be associated with the chicks themselves, as just incubating eggs (Sharp et al., 1996) or building a nest (Hall et al., 2014; Kingsbury et al., 2015) can be sufficient.

However, some studies in laboratory rats suggest that the mPOA can instead inhibit caregiving behaviors (Pereira and Morrell, 2009; Sheehan et al., 2000; Olazabal and Morrell, 2005) and that the mPOA is more involved in processing the high salience or novelty of offspring, rather than only their valence. The previous Fos studies in birds were not designed to address this question, so in the present study of Japanese quail we had groups of virgin females that were maternally sensitized through continuous exposure to chicks for three days or not, and then exposed to chicks or not before euthanasia (with the sensitized group separated from chicks overnight). Thus, about half of our females exposed to chicks 90 min before sacrifice were maternally experienced and familiar with chick cues, while the other half exposed to chicks just before sacrifice had never been seen them before. We found that all adult females exposed to chicks 90 min before euthanasia, whether or not they had previous experience with young, had more Fos-ir cells in the anterior mPOA compared to females that were not exposed to chicks

soon before euthanasia. This result supports the hypothesis that activity in the female quail mPOA is associated with processing the salience of chicks, rather than their affective valence. This is supported by studies in laboratory rodents demonstrating that a group of virgin female mice that were mostly non-maternal and partially infanticidal during their first exposure to pups had almost the same high levels of Fos-ir in the mPOA as a group of lactating females caregiving for the young (Matsushida et al., 2015), that virgin male mice that attack pups (presumably finding them aversive) show similarly high levels of Fos in the mPOA as males that positively interact with pups (Tachikawa, Yoshihara, Kuroda, 2013), and that calcium transients in the mouse mPOA are similar between male mice not paternal when given pups and conspecific females that act maternally (Wei et al., 2018). It seems likely that the phenotype and function of Fos-ir cells differ in these cases where the mPOA expresses similar levels cellular activity despite opposite behavioral responses to young. In the case of spontaneously parental or previously experienced animals, perhaps Fos is expressed mostly in excitatory cells or disinhibitory cells that permit caregiving, but in experienced animals other populations of cells are activated that prevent caregiving and elicit attacks on pups. Lonstein et al. (2002) and Tsuneoka et al. (2013) have found that a variety of cell phenotypes in the mPOA express Fos after females rats and mice interact with pups, and that even in maternally experienced postpartum females >50% of the Fos-ir in their mPOA occurs within inhibitory GABAergic cells. Lastly, instead of only reflecting salience of young Fos expression in the female quail mPOA could be regulated by both salience and valence, depending on previous caregiving experience and regardless of recent behavior toward young. That is, high levels Fos in experienced females could reflect the chicks' positive valence, while in inexperienced females

the same levels of Fos could reflect the salience of chicks and even be involved in initiating the positively-valenced process of sensitization.

The mPOA is central to processing information not only related to young, but also other social and environmental cues, such those involved in aggressive and sexual behaviors (e.g., Charlier et al., 2005, Robertson et al., 1991; Smith and Pfaus, 1997; Gobrogge et al., 2007). It remains to be determined what specific subregion(s) of the mPOA and or specific cell type(s) are specifically stimulated and required to process any defined sensory information. A few studies suggest activation of specific neurons associated with a defined behavior such as caregiving (Lonstein and Devries, 2000; Wu et al., 2014, Nutsch et al., 2014), but the requirement of individual mPOA neurons to process the salience of different types of sensory information is currently unknown in any animal.

The fact that we were able to observe very high Fos expression in the mPOA of our chick-exposed quail, while the only other study of this in chick-exposed quail by Ruscio and Adkins-Regan (2004) did not, is likely due to a number of methodological differences. First, the degree of maternal experience differed. Our sensitized group of quail were continuously exposed to chicks for four days to establish caregiving behaviors, while the sensitized quail tested by Ruscio and Adkins-Regan received a total of 100 minutes with chicks across three days. Second, we separated our sensitized females from chicks overnight and then re-exposed them before sacrifice whereas Ruscio and Adkins-Regan's experiment did not involve a separation. In postpartum laboratory rats, the high number of Fos-ir cells in the mPOA of pup-exposed females can last at least ~48 hours (Stack and Numan, 2000), but a one- or two-day separation from the pups prior to a final re-exposure may be necessary to stimulate maximal levels of activity-coupled Fos in the maternal mPOA. The fact that chronic exposure to a familiar stimulus until

the moment sacrifice elicits submaximal Fos expression has been previously shown for sexual behavior in quail (Can, Domjan and Delville, 2007). In addition to probably maximizing Fos expression, our overnight separation also allowed us to demonstrate for the first time in quail a “maternal memory”, one that persisted for at least ~15 hours. Previous studies have found that maternally sensitized hens with three days of experience with chicks stop emitting maternal calls after being separated from chicks for four days (Leboucher et al., 1990), but that hens with eight days of experience with chicks continue to display some maternal responsiveness a week later if they have distal chick cues in the interim (Richard-Yris and Leboucher, 1986). How long a maternal memory can last in sensitized female quail remains to be determined.

As mentioned above, components of the NAC process both the salience and valence of rewarding stimuli in many animals including birds (Cooper and Knutson, 2008; Levita et al. 2009; Riters et al., 2013; Zachar et al., 2017). We found a significant main effect of maternal sensitization, but not of exposure to chicks again before euthanasia, on the number of Fos-ir cells. This indicates a slower downregulation of experience-driven Fos in the NAC when compared to what was seen in the mPOA. Thus, unlike the mPOA, Fos in the female quail NAC appears to be primarily responding to the maternal experience and not events just prior to sacrifice. This could implicate the NAC in establishing the “maternal memory” displayed by our sensitized and then re-exposed quail. Maternal memory in laboratory rats has been well studied (Bridges, 2016), and can last to some degree for up to 40 days after removal of pups (Bridges and Scanlan, 2005). Establishing a maternal memory in rats requires D1 and D2 receptor activity and protein synthesis in the NAC shell (Li and Fleming, 2003; Parada et al., 2008). Furthermore, maternal memory is prevented by infusing an oxytocin receptor antagonist into the NAC shell subregion (D’Cunha et al. 2011), and oxytocin-facilitated accumbens dopamine release (Kohli et

al., 2019; Succu et al., 2007) while females have their first interactions with young could be critical for establishing maternal memory across a range of vertebrate species.

Although the high Fos expression in both our groups of sensitized quail could have reflected long-term changes in the NAC related to maternal memory, the group with the most Fos in the NAC was the maternally sensitized and re-exposed females (Figure 2B); an independent t-test comparing that group to the maternally sensitized but not re-exposed females approached statistical significance ($t_{20} = 1.80$, $p = 0.08$). We here suggest that maximal Fos in the maternal female quail NAC only results from two processes – maternal experience that elevates Fos to a moderate degree that reflects priming of the NAC that establishes a maternal memory and promotes future responsiveness to rewarding chick cues, and then a larger activation of that primed NAC during later exposure to young. Longer-term or delayed Fos induction has been observed in the hippocampus of laboratory rats undergoing memory consolidation (Igaz et al., 2004; Katche et al., 2010) as well as in other brain sites under other conditions (Pennypacker et al., 1995). It is interesting that microdialysis studies in female laboratory rats have shown that steroid-treated virgins with no maternal experience have lower basal dopamine release in the NAC compared to controls, but a 3-4 fold rise in dopamine release during exposure to pup cues (Afonso et al., 2009; 2013). This suggests that animals that are prepared to be maternal, either from experience with young or hormone priming, have unique NAC function even without the current presence of offspring when compared to animals that are reluctant to exhibit caregiving. Because the control, unprimed females in these microdialysis studies showed almost no rise in dopamine when they were faced with pups, the NAC of the hormone-primed rats may have been altered to be more prepared to respond to rewarding stimuli

thus pup cue positive valence rather than salience, which is generally consistent with our NAC Fos results here.

The BSTL is part of the extended amygdala system involved in the endocrine and anxiety-like responses to stressors (Cecci et al., 2002; Morilak et al. 2003; Walker et al. 2009). We predicted that the BSTL would particularly differentiate between the groups of maternally sensitized and not maternally sensitized females that were exposed to chicks before euthanasia (i.e., M/E vs. NM/E), given that the latter avoid chicks and show fearful behaviors (Ruscio and Adkins-Regan, 2004; present results). Moreover, the induction of maternal care in quail is regulated by females' emotional reactivity (Pittet et al. 2014b). However, we found no significant main effects of maternal experience or maternal exposure before sacrifice, and no significant interaction between them, on Fos expression in the BSTL. This is consistent with the negative results found by Ruscio and Adkins-Regan (2004), who found almost no difference in the number of Fos or ZENK-ir cells in the BSTL of their maternally sensitized and non-maternal quail.

In sum, the present results provide additional support for a common vertebrate social behavior network by showing that the adult female quail mPOA responds to interactions with chicks with high Fos activity. Our results further suggest that the mPOA does not respond primarily to the valence of the young, but to their salience. The NAC showed a different profile of Fos activity, though, more consistent with its role in valence detection than salience detection. While our study reveals this novel information, it would be valuable in future studies to determine whether these patterns of Fos activity in the female Japanese quail brain extend to other salient social or non-social stimuli. Further work is also needed to understand what key neurohormones, neurotransmitter or peptides may be responsible for altering *c-fos* activity in the

maternal bird brain, which would help us understand the consequences of such region-specific changes in Fos expression following stimulation. While IEG expression is clearly linked to modification of synaptic networks associated with learning and memory (Grimm et al., 1997; He et al., 2002; Countryman et al., 2005; Yosechima et al., 2006; Katche et al., 2010), almost nothing is known about the particular function of neural IEG expression for the expression of social interactions, other than the fact that their normal expression is required for motherhood and other forms of sociality (Brown et al., 1996; Kuroda et al. 2007, 2008; Been et al., 2013; Pitchers et al., 2013; Aleyasin et al., 2018).

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Behavior	Observation Type	Description
Successful request	FOCAL	Quail accepts the chick when it solicits warming
Vocalization	FOCAL	Quail makes cooing, food, or tension calls
Refused request	FOCAL	Quail repels chick when it solicits warming
Escape chicks	FOCAL	Quail runs away or avoids chicks
Vigilance	FOCAL	Quail assumes a high posture and focuses on the environment and the chicks
Contact breaks	FOCAL	Number of contact breaks made by the quail with chicks
Crushing	FOCAL	Quail tramples chicks
Aggression	FOCAL	Quail attacks and/or pecks the chicks
Latency to warm	FOCAL	Latency to warm one or more chicks
% Warming	SCAN	Percentage of scans that quail is warming one or more chicks
% Covering Posture	SCAN	Percentage of scans that quail's body totally covers the chicks
% Uncovering Posture	SCAN	Percentage of scans that quail is lying on its side, not covering chicks
% Activity	SCAN	Percentage of scans that quail is showing an active behavior
% Comfort	SCAN	Percentage of scans that quail preens, dust bathes, or rests

% Fear	SCAN	Percentage of scans that quail freezes, paces, or escapes from chicks
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Table 1: Description of female behaviors recorded by focal and scan observation methods during exposure to chicks.

	MATERNAL CARE		REJECTION				NEGLI- GENCE	ABUSE
	Successful request	Vocalization	Refused request	Escape chicks	Vigilance	Contact breaks	Crushing	Aggression
M/E	15.8 ± 2.4	1.5 ± 0.9	3.1 ± 1.0	0.75 ± 0.4	0.25 ± 0.1	5.25 ± 1.3	1.00 ± 0.4	0.58 ± 0.4
NM/E	3.8 ± 2.1	0 ± 0	12.1 ± 2.0	12.7 ± 3.8	0 ± 0	1.7 ± 0.8	2.20 ± 0.7	4.10 ± 2.0

	WARMING BEHAVIOR				TIME		
	Latency to warm (sec)	% Warming	% Covering Posture	% Uncovering Posture	% Activity	% Comfort	% Fear
M/E	113.9 ± 36.0	68.4 ± 7.3	83.6 ± 8.4	16.4 ± 8.4	25.2 ± 4.1	67.6 ± 7.0	7.1 ± 3.9
NM/E	807.3 ± 126.6	4.9 ± 2.5	40.0 ± 13.2	60.0 ± 13.2	38.9 ± 4.0	33.7 ± 5.8	27.43 ± 6.7

Table 2: Behaviors (Mean \pm SEM) shown by maternally sensitized (M/E) or non-maternally sensitized (NM/E) females during an exposure to chicks before euthanasia. Significant differences are indicated by bold numbers in the higher group ($p < 0.05$).

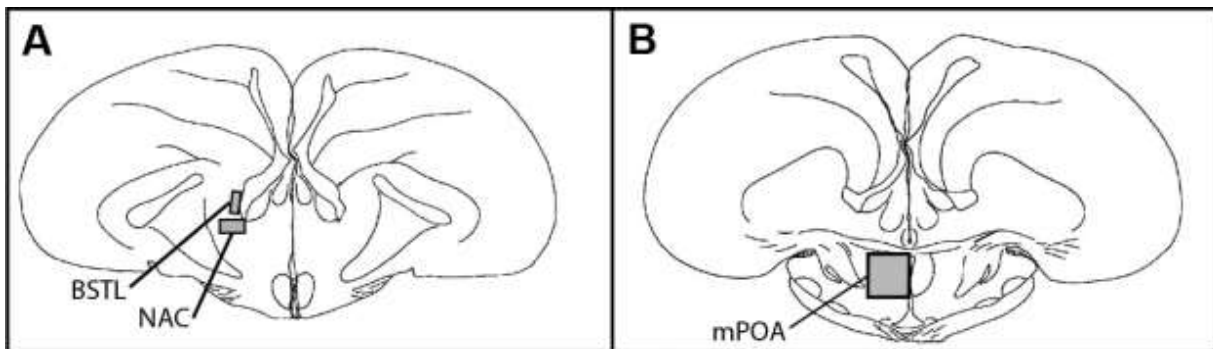
Figure Legends

Figure 1 – Schematic representation of the areas (shown in gray boxes on left side) within the female quail A) NAC and BSTL and B) mPOA that were analyzed for the number of Fos-immunoreactive cells. Figures modified from Kuenzel and Masson's (1988) atlas of the chicken brain.



Figure 2 – Number (Mean \pm SEM) of Fos-immunoreactive cells in the A) mPOA, B) NAC, and C) BSTL of female quail that were induced to be maternal (groups M) or not (groups NM), and later exposed to chicks (groups E) or not (groups NE) soon before euthanasia. Different letters above groups of bars indicate a significant main effect of either sensitized maternal state or of exposure to chicks soon before euthanasia, $p < 0.05$.

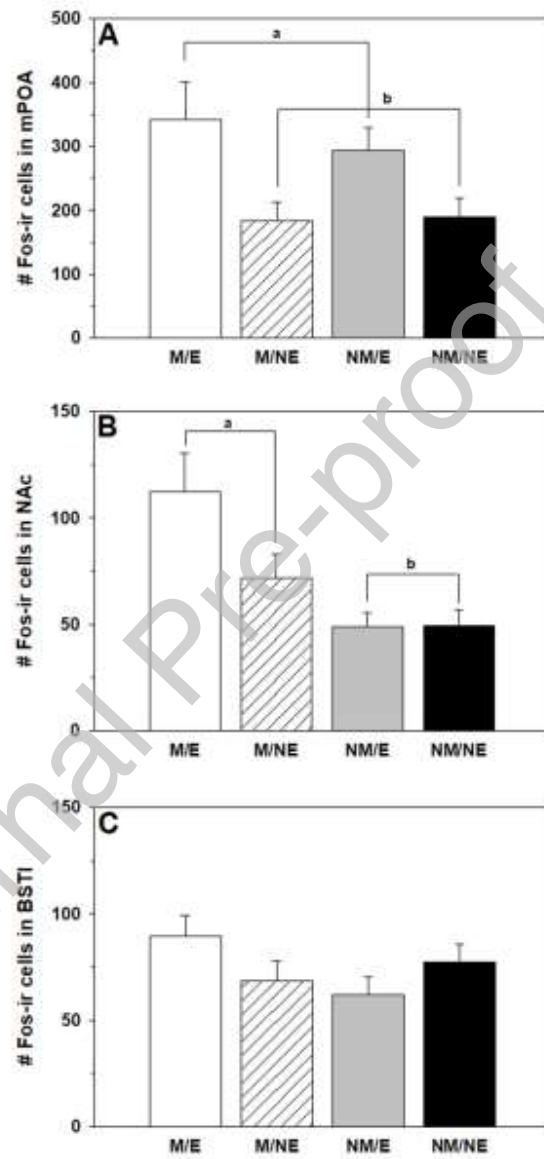


Figure 3 – Photomicrographs of Fos-ir cells in the mPOA of female quail that were induced to be maternal (groups M) or not (groups NM), and later exposed to chicks (groups E) or not (groups NE) soon before euthanasia.