Integrative and Comparative Biology

Integrative and Comparative Biology, volume 57, number 3, pp. 603–618
doi:10.1093/icb/icx036

Society for Integrative and Comparative Biology

SYMPOSIUM

Oxytocin Neurons Exhibit Extensive Functional Plasticity Due To Offspring Age in Mothers and Fathers

Aubrey M. Kelly,[1] Lisa C. Hiura, Alexander G. Saunders and Alexander G. Ophir

Department of Psychology, Cornell University, Ithaca, NY 14853, USA

From the symposium "The Development and Mechanisms Underlying Inter-individual Variation in Pro-social Behavior" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 4–8, 2017 at New Orleans, Louisiana.

[1]E-mail: amk297@cornell.edu

Synopsis The needs of offspring change as they develop. Thus, parents should concomitantly change their investment based on the age-related needs of the offspring as they mature. Due to the high costs of parental care, it is optimal for parents to exhibit a shift from intense caregiving of young offspring to promoting independence in older offspring. Yet, the neural mechanisms that underlie shifts in parental behavior are poorly understood, and little is known about how the parental brain responds to offspring of different ages. To elucidate mechanisms that relate to shifts in parental behavior as offspring develop, we examined behavioral and neural responses of male and female prairie voles (*Microtus ochrogaster*), a biparental rodent, to interactions with offspring at different stages of development (ranging from neonatal to weaning age). Importantly, in biparental species, males and females may adjust their behavior differentially as offspring develop. Because the nonapeptides, vasopressin (VP) and oxytocin (OT), are well known for modulating aspects of parental care, we focused on functional activity of distinct VP and OT cell groups within the maternal and paternal brain in response to separation from, reunion (after a brief period of separation) with, or no separation from offspring of different ages. We found several differences in the neural responses of individual VP and OT cell groups that varied based on the age of pups and sex of the parent. Hypothalamic VP neurons exhibit similar functional responses in both mothers and fathers. However, hypothalamic and amygdalar OT neurons exhibit differential functional responses to being separated from pups based on the sex of the parent. Our results also reveal that the developmental stage of offspring significantly impacts neural function within OT, but not VP, cell groups of both mothers and fathers. These findings provide insight into the functional plastic capabilities of the nonapeptide system, specifically in relation to parental behavior. Identifying neural mechanisms that exhibit functional plasticity can elucidate one way in which animals are able to shift behavior on relatively short timescales in order to exhibit the most context-appropriate and adaptive behaviors.

Introduction

Parenting is an energetically costly and dynamic behavior. The demands on parents change as a function of offspring age such that offspring require different forms of attentiveness and care as they mature. Allocating resources and effort in accordance to offspring's need optimizes offspring survival, and is therefore highly adaptive (Winkler 1987; Clutton-Brock 1991). Such plasticity may take the form of preferentially attending to the needs of more vulnerable offspring or, conversely, favoring offspring that are less vulnerable and more likely to achieve independence and reproductive success (Clutton-Brock 1991; Jeon 2008). In either case, the form of care that offspring at different ages require is fundamentally different. For example, even under ideal conditions, caring for neonatal and weanling-age offspring in the same way would likely be sub-optimal toward meeting the specific needs of the offspring. Differential needs based on offspring age inherently impose a need for behavioral flexibility over time in order for parents to maximize fitness. From this

Advance Access publication September 6, 2017

perspective, selection is likely to favor parents that shift their behavior from intense caregiving for the youngest offspring to promoting offspring independence in older offspring. Thus, selection is likely to differentially shape the ways in which parents react to offspring, behaviorally and mechanistically, at different stages of development.

Research examining the neurobiological underpinnings of parental behavior has largely examined the roles of steroid hormones, prolactin, and the nonapeptides (Bales and Saltzman 2016; Lynn 2016; Numan and Young 2016; Pereira and Ferreira 2016; Kohl et al. 2017). For instance, the nonapeptides, oxytocin (OT) and vasopressin (VP), are involved in modulating parental behaviors, in addition to numerous other social behaviors including affiliation, aggression, and reproductive behaviors (Landgraf and Neumann 2004; Young 2009; Goodson and Thompson 2010). Although the nonapeptide system may be best known for distinguishing differences in behavioral phenotype within and across species (typically reflecting more stable neuroanatomical profiles) (Wang 1995; Goodson 2008; Ophir et al. 2008), it also exhibits properties that allow for rapid neuromodulation and functional plasticity on very short timescales (Stoop 2012). Moreover, because OT and VP are produced in anatomically and functionally distinct cell groups throughout the brain (Kelly and Goodson 2014b), where and the degree to which VP and OT act may vary based on the specific behavioral context facing an animal.

Despite extensive research dedicated to understanding the maternal brain, surprisingly little is known about how the parental brain responds to offspring of different ages. Furthermore, how the brain allows for the plasticity observed in parental behavior as parents shift from intense caregiving to promoting offspring independence is poorly understood (Getz et al. 1994; Rosenfeld et al. 2013). In fact, to our knowledge, there has been no attempt to understand how or if parents exhibit differential concomitant behavioral and neural responses to offspring at different stages of development. We, therefore, conducted an immediate early gene (IEG) experiment to examine the neural and behavioral responses of parents to offspring separation or reunion with offspring after a brief period of separation with offspring of different ages (ranging from neonates to weanlings). To this end, we used prairie voles (*M. ochrogaster*), which are a socially monogamous and biparental rodent. Prairie vole males exhibit all the same parental behaviors as females (e.g., licking/grooming, retrieving, arch-backed huddling) except nursing (Roberts et al. 1998; Lonstein and De Vries 1999). Although the costs of parental care should be similar for male and female prairie voles, the difference in nursing presumably creates different selective pressures and energetic demands on each sex (Trivers 1974). Therefore, the ways in which males and females shift their behavior toward offspring as they develop might vary. We hypothesize that (1) behavioral and neural reactions to pups should vary as a function of age, and (2) mothers should demonstrate more pronounced behavioral and neural reactions to offspring compared to fathers. In addition, because OT has been more extensively linked to aspects of parental behavior compared to VP (e.g., Rilling and Young 2014), we predicted that we would find that OT cell groups were more responsive to our manipulations than VP cell groups. Here we examined plasticity of functional responses of nonapeptide neurons in the brain of both mothers and fathers, and predicted that nonapeptide neural sensitivity to interactions with offspring would differ in parents based on the age of offspring and sex of the parent. Our findings suggest that the OT system may exhibit greater plasticity in relation to parental behavior compared to the VP system. Our results also suggest that preoptic OT and hypothalamic VP cell groups may have similar functions in parental behavior for both mothers and fathers, whereas amygdalar OT and hypothalamic OT cell groups exhibit differential functional responses in mothers and fathers.

Materials and methods

Subjects

All prairie voles used in this study were obtained from our breeding colony, from breeding pairs that were offspring of wild caught animals captured in Champagne County, IL, USA. All animals were housed in standard polycarbonate rodent cages (29 × 18 × 13 cm) lined with Sani-chip bedding and provided nesting material. Animals were kept on a 14L:10D cycle, and were provided with rodent chow (Laboratory Rodent Diet 5001, LabDiet, St. Louis, MO, USA) and water ad libitum. Ambient temperature was maintained at 20 ± 2 °C. All procedures were approved by the Institutional Animal Care and Use Committee of Cornell University (2013-0102).

Design

We used a two-factor design to test the hypothesis that the parental brain responds to interactions with offspring differentially based on the age of the pups.

The first factor describes the manipulation of the family group. Here families (composed of a mother, father, and three pups) were assigned to one of three manipulations: Separate, Reunite, or Together (Fig. 1; see below). The second factor was the age of the offspring at testing. We tested pups on postnatal day (PND) 2, 9, and 21 to account for different stages across pup development. PND 2 pups are not yet capable of independent locomotion, eye opening has not yet occurred, and they are constantly attached to the nipples of the mother unless the mother forcefully detaches the pups (Salo et al. 1994). We chose PND 2 over PND 0 to allow time for a parent–pup attachment to form, and because PND 2 was the youngest age at which pup brains could be successfully and reliably processed via immunocytochemistry. Prairie vole pups exhibit open eyes and are capable of independent locomotion by PND 9 (Solomon 1991; Robison et al. 2016). By PND 21 prairie vole pups are weaned in the lab and can function independently (Carter and Getz 1985).

Behavioral procedure

A total of 200 adult prairie voles ($n = 100$ males; $n = 100$ females) were used to create breeding pairs to serve as parents in our experiment. All adults were between PND 80 and PND 120, sexually naïve, and housed with same-sex siblings until pairing. Males and females were paired to form 100 breeding pairs, whereby size-matched males and females were identified for pairing. We induced sexual receptivity in females by housing them alone and exposing them for 48 h to soiled bedding and nesting material from the males with which they were planned to be paired (Richmond and Stehn 1976; Carter et al. 1980; Dluzen et al. 1981). Males were then introduced into the females' cages and left alone. All subjects were monitored immediately after pairing to ensure the pair did not harm each other. At the end of 21 days after pairing, pairs were monitored closely and checked four times daily for the birth of pups. Of the 100 pairs we created, 94 produced a litter of three pups or greater and were retained for experiments. Litters greater than three were culled to three pups on the day of birth.

The 94 families were randomly assigned to one of nine experimental groups that varied based on the age of the pups (PND 2, PND 9, and PND 21) on the testing day and social condition (Separate, Reunite, or Together) for the IEG experiment. We created these three social conditions to assess the functional reaction of OT- and VP-immunoreactive (-ir) cells in parents following interactions with or separation from pups. The "Separate" condition tested parents' behavioral and neural reactions to being housed apart from pups. The "Reunite" condition was included to determine if nonapeptide cells exhibit a distinct functional profile following the separation and return of offspring. Our control condition allowed parents and pups to remain together ("Together").

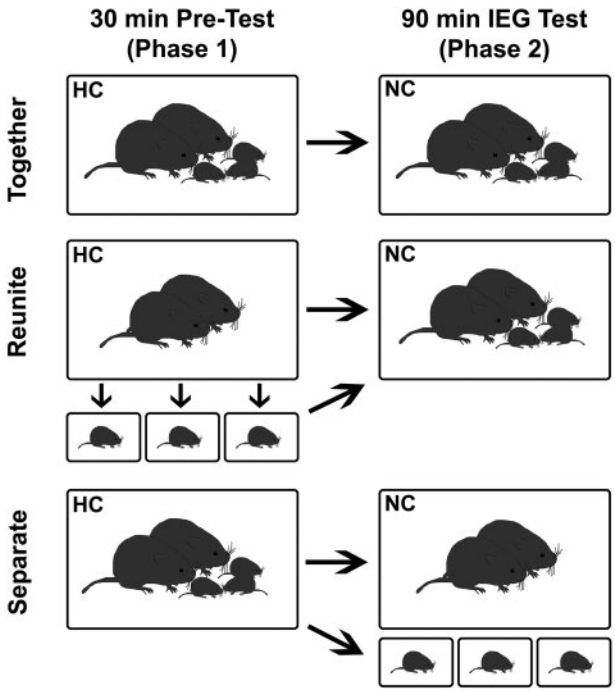

Fig. 1 A schematic representation of treatment groups. Family units were randomly assigned to one of three conditions: Together, Separate, or Reunite. The same procedure was used for family units with pups of each age (PND 2, PND 9, or PND 21). See text for details. HC = home cage. NC = novel cage.

The experiment consisted of two phases: a 30-min home cage phase before the IEG test (Phase 1), and a 90-min novel cage phase (phase 2/IEG). To begin the experiment, in Phase 1, each family unit for all three social conditions (Separate, Reunite and Together) was handled and returned to their home cage, except for the pups in the Reunite condition. Next, the parents were all handled and transferred to a clean novel cage, at which time any functional changes measured by the IEG activation would have been initiated. We, therefore, also refer to the second phase as the IEG test. In the Separate condition, parents and pups were handled and returned to their home cage in the first phase. Next, parents were transferred to a novel cage together, while each pup was transferred to a different novel cage during the IEG test (Phase 2). Thus, parents were separated from their pups for the 90-min test. In the Reunite

condition, pups and parents were handled as in the Separate condition. However, while parents were returned to the home cage in Phase 1, the pups were removed from the home cage and each placed into novel cages during Phase 1. Then the pups and parents were all transferred into the same novel cage in Phase 2 for the 90-min IEG test. In the Together condition, parents and pups were all handled as above, but all were returned to the home cage in the first phase, and then transferred to the same novel cage in Phase 2 for the 90-min IEG test. After the 90-min IEG test, all subjects were sacrificed and brains of parents and pups were collected.

During both phases, at all age groups and across all conditions, the degree to which each animal was handled was mimicked to control for the total quality and quantity of handling. All handling was performed by an experimenter wearing nitrile gloves and all transferring was performed using a plastic beaker. All novel cages contained new wood chip bedding, new shredded nestlet material, contained no food or water, and had a clear Plexiglas lid with air holes for top-down video recording. All animals were video recorded for the full 2 h of testing.

When pups were removed, they were housed individually (and served as subjects for a companion experiment). We controlled for differences in thermoregulatory abilities between PND 2, PND 9, and PND 21 pups that were isolated in the Separate and Reunite conditions by adjusting the temperature in each testing cage using electric heating pads. We used a veterinary grade infrared thermometer (Nasco Product B01377N) to determine how much a novel test cage needed to be heated so that a pup could maintain a temperature consistent with the temperature of pups in a nest with their family. By taking the average temperature from a sample of five families at each pup age, we found that the novel empty cage needed to be heated to 36 °C for PND 2 pups, 34 °C for PND 9 pups, and 30 °C for PND 21 pups.

Group sizes for each age group for the Separate condition were: PND 2, $n = 12$; PND 9, $n = 12$; PND 21, $n = 10$. Group sizes for each age group for the Reunite condition were: PND 2, $n = 8$, PND 9, $n = 11$, PND 21, $n = 9$. Group sizes for each age group for the Together condition were: PND 2, $n = 10$; PND 9, $n = 11$; PND 21, $n = 11$.

Histology and immunocytochemistry

Our study aimed to assess neuronal activation of VP-ir or OT-ir cells using the IEG cFos. cFos functions by rapidly altering gene expression, either positively or negatively, in response to cell surface signals. Most neurons express little or no cFos under baseline conditions, but after stimulation, cFos gene expression is rapidly induced in neurons, with its mRNA peaking within 3-min after acute stimulation and its protein product reaching a maximum 60–90 min after stimulation (Hoffman et al. 1993). We can, therefore, use cFos to obtain important information concerning changes in neuronal activity. It is possible to determine that OT-ergic and VP-ergic cells are activated when cFos is co-expressed in cells positively labeled for VP or OT.

To visualize OT, VP and cFos, subjects were sacrificed by isoflurane overdose and transcardially perfused with 0.1M phosphate buffered saline (PBS) followed by 4% paraformaldehyde. Brains were extracted, post-fixed overnight, and cryoprotected in 30% sucrose in PBS for 48 h prior to sectioning on a cryostat. Tissue was sectioned into three 40 µm series. One series of tissue was immunofluorescently stained for VP and cFos, and a second series of tissue was stained for OT and cFos. Tissue was rinsed 5× for 10 min in 0.1M PBS (pH 7.4), incubated for 1 h in block (PBS + 10% normal donkey serum + 0.03% Triton-X-100), and then incubated for approximately 48 h in primary antibodies diluted in PBS containing 5% normal donkey serum + 0.03% Triton-X-100. Primary antibodies used for the first series were guinea pig anti-VP (1:1000; Peninsula Laboratories, San Carlos, CA) and rabbit anti-Fos (1:200; Santa Cruz Biotechnology, Santa Cruz, CA); primary antibodies for the second series were guinea pig anti-OT (1:1000; Peninsula Laboratories) and rabbit anti-Fos (1:200; Santa Cruz Biotechnology). The primary incubation was followed by two 30-min rinses in PBS. Tissue was incubated for 1 h in a biotinylated donkey anti-guinea pig secondary (1:125; Jackson Immunoresearch, West Grove, PA), rinsed twice for 15 min in PBS, and incubated for 2 h at room temperature in streptavidin conjugated to Alexa Fluor 488 (1:325) and donkey anti-rabbit secondary conjugated to Alexa Fluor 594 (1:200). All secondary antibodies were diluted in PBS containing 5% normal donkey serum + 0.03% Trion-X-100. Alexa Fluor conjugates were obtained from ThermoFisher Scientific (Waltham, MA). Following two 30-min rinses in PBS, sections were mounted on microscope slides and cover-slipped with Prolong Gold antifade containing a DAPI nuclear stain (ThermoFisher Scientific).

Quantification and analysis

To perform cell counts, images were captured using a Zeiss AxioImager II microscope outfitted with an

AxioCam MRm, z-drive, and an Apotome optical dissector (Carl Zeiss Inc., Gottingen, Germany). Flattened z-stack images were used by observers blind to treatment condition to conduct cell counts in Photoshop CS6 (Adobe Systems, San Jose, CA) and Image J (National Institutes of Health, Bethesda, MD) as previously described (Goodson and Wang 2006; Kelly and Goodson 2014a).

In order to account for individual differences in nonapeptide anatomy (e.g., VP-ir and OT-ir cell numbers), we examined the percentage of VP and OT cells that were doubled labeled for cFos (hereafter referred to as VP-cFos or OT-cFos colocalization). VP-cFos colocalization was quantified in the paraventricular nucleus of the hypothalamus (PVN), anterior hypothalamus (AH), and peduncular lateral hypothalamus (PLH). PVN VP-cFos colocalization was quantified at two levels given the extensive size of this cell group. There were no significant differences between rostral and caudal levels, and thus a combined measurement of both levels was used for analysis. OT-cFos colocalization was quantified in the preoptic periventricular nucleus (POPVN; also referred to as the medial part of the medial preoptic nucleus, MPOM; [Okabe et al. 2017], medial bed nucleus of the stria terminalis [BSTm]), and the PVN. For visual representations of these primary hypothalamic and accessory nonapeptide nuclei in rodents see (Wang et al. 1996; Rood and De Vries 2011). We were unable to obtain VP-cFos or OT-cFos colocalization counts in the supraoptic nucleus of the hypothalamus (SON) due to extremely dense labeling of VP-ir and OT-ir neurons that would not allow for accurate quantification of cFos within the nucleus of neuronal cell bodies. Furthermore, we were unable to obtain data from the BST VP cell groups due to extremely low labeling; 70% of our subjects expressed no VP-ir neurons in the BST, which was possibly due to a lack of use of sodium tertraborate in the paraformaldehyde used for perfusions (personal comm. with AH Veenema and GJ De Vries).

We scored behavioral interactions between parents and pups during the 90-min IEG phase of the experiment for the two conditions where parents and offspring were co-housed during this phase (Together and Reunite). Recall that pups were not in the same cage during the Separate condition making it impossible to score parent–pup interactions during this period. Behavior in the first 10 min of the IEG phase of the test was scored using Observer XT (version 8.5, Noldus Information Technology, Leesburg, VA, USA). We quantified parental licking/grooming of pups, pup retrievals, and the amount of time parents spent in contact with pups. Unfortunately, licking/grooming and pup retrievals were inconsistent for different age groups and were too rare in older pups to perform analyses across families with different pup ages. We, therefore, only report analyses for parent contact time with pups.

Behavioral and neural data were analyzed using Linear Mixed Models (LMM) in SPSS 24 (IBM Analytics, USA). Sex of the parent, pup age, and treatment condition were analyzed as fixed factors, while the parent subject was assigned as a random factor to control for the influence each parent may have had on each other during the test. Neural activity data were analyzed using the arcsine transformation of the proportion of the number of double labeled neurons out of the total number of neurons in the cell group. All post hoc pairwise comparisons were adjusted using the Bonferonni correction. Because several of the mothers were pregnant at the time of testing, LMMs were run, in mothers only, for neural activity in each cell group, and for contact behavior, with pregnancy status (yes or no), pup age, and treatment condition as fixed factors. There were no significant effects or interactions involving pregnancy (all $P \geq 0.079$), and thus pregnancy as a factor was excluded from the models presented below for mothers and fathers. For ease of visualization, in figures, data are represented as the percentage of VP or OT cells that were co-expressed with cFos (%VP-cFos or %OT-cFos colocalization) rather than the arcsine transformed data. All data are presented as means $\pm$ standard error.

Results

The time parents spent in contact with their pups was scored for families in the Together and Reunite conditions; parents in the Separate condition did not have access to their pups, preventing us from evaluating parent–pup contact behavior in this condition. All main effects and interactions were significant and are listed in Table 1, and all pairwise comparison are summarized in Table S1.

Analysis of neural activity (as indicated by cFos colocalization) in distinct VP and OT cell groups revealed several differences in the way the maternal and paternal brain respond to separation from and/or reunion with offspring of different ages. Overall, OT, but not VP, cell group neuronal activity was sensitive to the age of the pups. Furthermore, OT, but not VP, cell group activity varied based on the sex of the parent. For each cell group, all main effects and interactions are summarized in Table 1.

Table 1 Main effects and interactions for mean parent–pup contact and VP-cFos and OT-cFos colocalization

	Main effects			Two-way interactions			Three-way interaction
	Sex	Condition	Pup age	Sex × condition	Sex × pup age	Condition × pup age	Sex × Condition × pup age
VP cell groups							
PVN VP	$P = 0.122$	**$P = 0.037$**	$P = 0.823$	$P = 0.601$	$P = 0.209$	$P = 0.847$	$P = 0.919$
AH VP	$P = 0.089$	$P = 0.696$	$P = 0.286$	$P = 0.274$	$P = 0.172$	$P = 0.363$	$P = 0.426$
PLH VP	$P = 0.340$	$P = 0.147$	$P = 0.330$	$P = 0.530$	$P = 0.852$	$P = 0.531$	$P = 0.638$
OT cell groups							
PVN OT	**$P = 0.010$**	$P = 0.381$	**$P < 0.001$**	**$P = 0.044$**	$P = 0.093$	$P = 0.576$	$P = 0.696$
POPVN OT	$P = 0.858$	**$P = 0.053$**	**$P < 0.001$**	$P = 0.516$	$P = 0.135$	$P = 0.431$	$P = 0.828$
BST OT	$P = 0.107$	$P = 0.792$	**$P = 0.022$**	**$P = 0.007$**	$P = 0.221$	$P = 0.570$	$P = 0.204$
Parent–pup contact	**$P < 0.001$**	**$P < 0.001$**	**$P < 0.001$**	**$P = 0.011$**	**$P < 0.001$**	**$P = 0.001$**	**$P = 0.047$**

Bold font highlights statistically significant differences.

Because the primary goal of this experiment was to examine nonapeptide neuronal function, we present below VP- and OT-cFos colocalization data. However, we did observe base anatomical differences based on sex and pup age in some VP-ir and OT-ir cell groups irrespective of cFos, which are detailed in the **Supplementary Materials, Results**.

Parent–pup contact behavior

Not surprisingly, mothers spent more time in contact with pups compared to fathers (main effect of Sex, $P < 0.001$: Supplementary Fig. S1A), and parents spent more time in contact with younger (PND 2 and PND 9) pups compared to older (PND 21) pups (main effect of pup age, $P < 0.001$; Supplementary Fig. S1B). Parents also spent more contact time with pups upon being Reunited after 30 min of separation compared to the condition in which they remained together (main effect of condition, $P < 0.001$; Supplementary Fig. S1C). Pairwise comparisons (shown in Fig. 2 and Supplementary Table S1) revealed that mothers of PND 21 pups exhibited significantly more contact time with pups in the Reunite condition compared to the Together condition ($P < 0.001$). In contrast, fathers of PND 2 and PND 21 pups, but not PND 9 pups, spent more contact time in the Reunite condition than when they remained together (PND 2 fathers, $P < 0.001$; PND 9 fathers, $P = 0.380$; PND 21 fathers, $P = 0.001$).

Mothers appeared to exhibit a ceiling effect with pup contact. We observed no significant differences in parent–pup contact time between mothers with PND 2 pups compared to PND 9 pups. We also found no difference between mothers that were reunited or remained together with either PND 2 or PND 9 pups (all $Ps > 0.257$). Interestingly, mothers with PND 21 pups exhibited less contact time with pups than mothers with PND 2 pups and PND 9 pups in the Together condition (both $Ps < 0.001$), indicating that mothers spend less time overall with pups at weaning age underline baseline family conditions. However, for mothers that were Reunited with pups after a period of separation, mothers with PND 21 pups exhibited less time spent in contact with pups compared only to mothers with PND 2 pups ($P = 0.044$).

Fathers did not show many behavioral differences toward pups across conditions or ages of pups. The one exception to this was that reunited fathers with PND 2 pups spent significantly more time in contact with pups compared to reunited fathers with PND 9 pups ($P = 0.001$).

VP-cFos colocalization in the PVN

Analysis of the percentage of VP-ir neurons expressing cFos in the PVN in parents from the nine treatment groups yielded a main effect of condition ($P = 0.037$; Supplementary Fig. S2A), with parents in the Together group having significantly lower amounts of VP-cFos colocalization compared to the parents in the Separate group ($P = 0.053$).

VP-cFos colocalization in the AH and PLH

We observed no main effects or significant interactions for VP-cFos colocalization in the AH or PLH (all $P \geq 0.089$). The behavioral functions of extra-hypothalamic nonapeptide cell groups are studied comparatively less than the VP-OT cell groups of the PVN and SON. To our knowledge, the AH VP cell group has not been examined in relation to parental care or stress response, and our results here

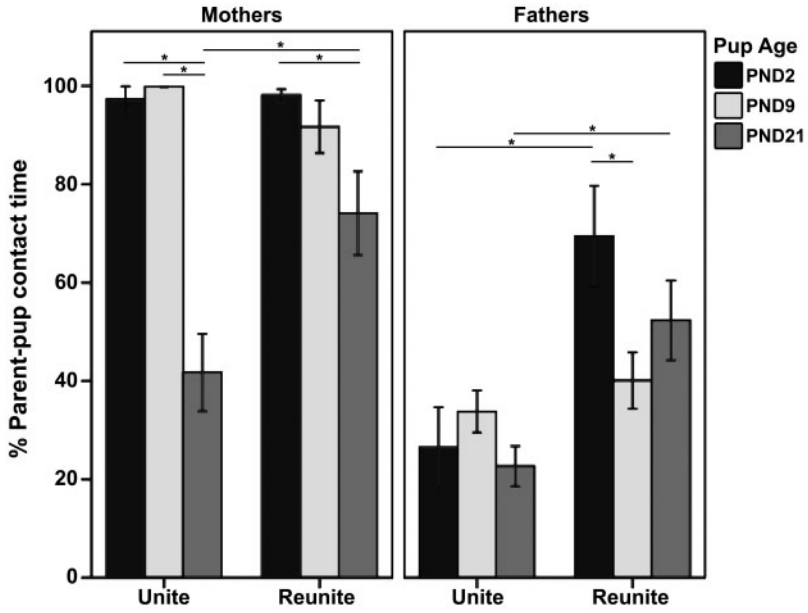

Fig. 2 Mean (±SEM) parent–pup contact time for mothers and fathers that were Together or Reunited with offspring of different ages (PND 2, black; PND 9, light gray; PND 21, dark gray) during the first 10 min of the 90-min IEG test. *indicates $P \leq 0.05$.

suggest that this cell group is not strongly involved in the modulation of parental responses to offspring. Moreover, despite the comparable size of the PLH VP cell group to other extra-hypothalamic VP-OT cell groups in rodents (e.g., AH, BST), the functional significance of the VP neuronal population of the PLH is particularly understudied. Unfortunately, our results here provide little insight into the behavioral functions of this lateral hypothalamic cell group other than that this cell group is not sensitive to separation from or reunion with a familial bond.

OT-cFos colocalization in the PVN

Analysis of the percentage of OT-ir neurons expressing cFos in the PVN yielded numerous significant differences. We observed a main effect of sex ($P = 0.010$), with fathers exhibiting significantly higher amounts of OT-cFos colocalization than mothers. We also found a significant sex × condition interaction ($P = 0.044$; Fig. 3A), showing that within the Separate condition, fathers exhibited significantly more OT-cFos colocalization compared to mothers ($P < 0.001$), and for mothers, those separated from pups exhibited significantly less OT-cFos colocalization compared to mothers under baseline family conditions in the Together group ($P = 0.039$). Furthermore, we observed a main effect of pup age ($P < 0.001$; Fig. 4A), with parents with PND 21 pups exhibiting significantly lower amounts of PVN OT neural activity compared to parents with PND 2 and PND 9 pups (both $P < 0.001$).

OT-cFos colocalization in the POPVN

Analysis of the percentage of OT-ir neurons expressing cFos in the POPVN yielded a main effect of condition ($P = 0.053$), with parents in the Separate condition tending to exhibit more OT-cFos colocalization than parents in the Reunite condition ($P = 0.065$). Moreover, similar to PVN OT neural activity, we found a main effect of pup age ($P < 0.001$; Fig. 4B), with parents of PND 21 pups exhibiting significantly less POPVN OT-cFos colocalization compared to parents of PND 2 ($P < 0.001$) and PND 9 pups ($P = 0.002$).

OT-cFos colocalization in the BSTm

Consistent with both hypothalamic OT cell groups just discussed, we observed a main effect of pup age ($P = 0.022$; Fig. 4C), with parents of PND 21 pups exhibiting significantly lower amounts of OT-cFos colocalization compared to parents of PND 2 ($P = 0.021$), but not PND 9 pups ($P = 0.175$). We also found a significant sex × condition interaction ($P = 0.007$; Fig. 5), which revealed that within the Reunite condition, mothers exhibit significantly more OT-cFos colocalization than fathers ($P = 0.004$). In addition, within fathers, those in the Separate condition exhibited greater OT-cFos colocalization compared to fathers in the Reunite condition ($P = 0.020$).

Discussion

In this study, we used the biparental prairie vole to examine parental neural and behavioral responses to

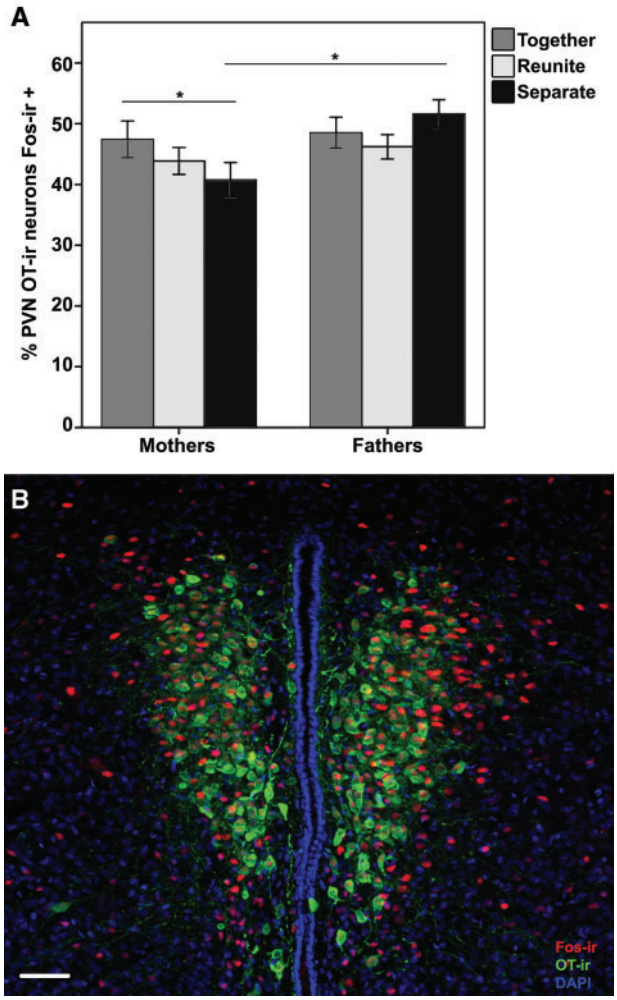

Fig. 3 (A) Mean (±SEM) percentage of OT immunoreactive (ir) neurons expressing cFos-ir in the PVN for mothers and fathers that were Together (dark gray), Reunited (light gray), or Separated (black) with offspring during the 90-min IEG test. *Indicates $P \leq 0.05$. **(B)** Representative immunocytochemical staining of OT (green; Alexa Fluor 488), cFos (red; Alexa Fluor 594), and DAPI nuclear stain (blue; ProLong Gold) in the PVN of a father with PND 9 pups in the Separate condition. Scale bar = 50 μm.

offspring at three different stages of development: neonatal offspring (PND 2), perinatal offspring (PND 9), and weanling-aged offspring (PND 21). Each of these ages corresponds to ages of rapid brain growth in rodents (Gottlieb et al. 1977) and captures distinct points of maturation in social and physical development. We utilized a simple design of examining IEG responses to baseline conditions of parents interacting with their pups, separating parents from their pups, or reuniting parents with their pups after a period of brief separation. Our experiment sheds light on plasticity in parental responsiveness to offspring over the course of offspring development and may reveal some of the mechanisms important for differential responses to offspring under a variety of social contexts. Overall, we observed several differences in both the neural and behavioral responses of mothers and fathers to context-dependent interactions with offspring of different ages. Our findings suggest that the OT system may play a particularly important role in modulating parental behavioral plasticity given that OT neuronal function in each cell group examined here exhibited a main effect of pup age, whereas no VP cell groups were significant for pup age. Below we discuss the observed behavioral and neural differences in more detail. Taken together, these differences might reflect the result of different selective pressures that operate on males and females and the varying trade-offs they experience between ensuring offspring survival and their own survival.

Parental behavioral responses to offspring separation varies based on the age of offspring

Studies in rats and mice have demonstrated that a period of separation from pups results in increased bouts of maternal care upon reunion with offspring (Pryce et al. 2001; Garoflos et al. 2008; Stamatakis et al. 2015). Our behavioral results in prairie voles are consistent with these studies. We found that parents exhibited increased contact time with pups upon being reunited after a 30-min period of separation. Interestingly, post hoc analyses revealed that this effect is pup age dependent, and differed by sex of the parent. For example, our results indicated that mothers of younger offspring (PND 2 and PND 9) appear to have reached an upper limit in the degree to which they provide care for pups, irrespective of treatment condition. Indeed, mothers were almost constantly in contact with young pups. This result is consistent with the observation that prairie vole pups exhibit a strong and consistent nipple attachment through roughly PND 10 (Salo et al. 1994). In contrast, mothers that were not separated from older (PND 21) pups showed relatively lower levels of contact. However, if they were separated and then reunited with pups, mothers demonstrated a significant increase in pup contact time upon reunion. Taken together, mothers provided intense care to younger offspring independent of separation events, but reduced care of older pups as they matured and became independent. Nevertheless, mothers increased their caregiving after being separated from weaning-aged pups. In this way, mothers appear to act more like fathers when interacting with older pups. To this point, fathers that remained cohoused with pups provided modest parental care.

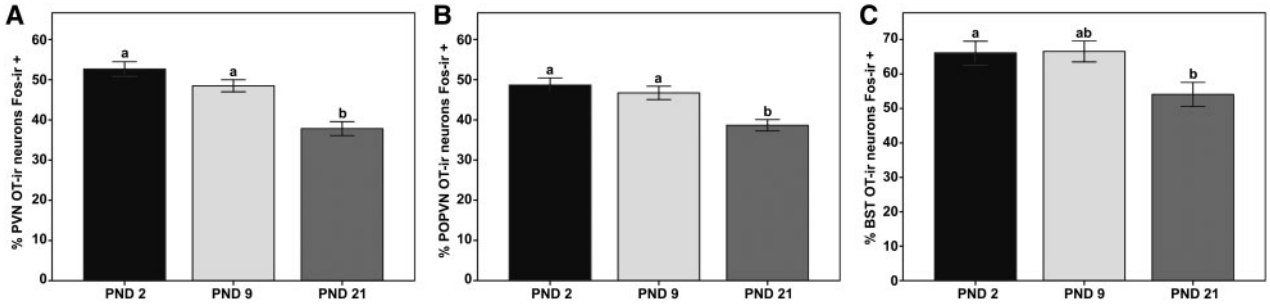

Fig. 4 Mean (±SEM) percentage of OT immunoreactive (ir) neurons expressing cFos-ir in the **(A)** PVN, **(B)** POPVN, and **(C)** BST for mothers and fathers with offspring of different ages (PND 2, black; PND 9, light gray; PND 21, dark gray) during the 90-min IEG test. Letters (a, b) represent groups that are statistically similar; different letters indicate $P \leq 0.05$.

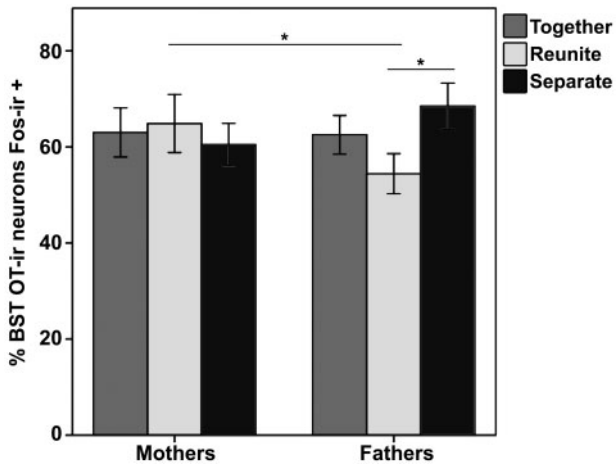

Fig. 5 Mean (±SEM) percentage of OT immunoreactive (ir) neurons expressing cFos-ir in the BST for mothers and fathers that were Together (dark gray), Reunited (light gray), or Separated (black) with offspring during the 90-min IEG test. *indicates $P \leq 0.05$.

However, fathers of PND 2 and PND 21 pups demonstrated a relative increase in pup contact time upon reunion, whereas contact time for PND 9 pups remained modest across conditions. These results indicate that fathers care for pups of all ages, but that a period of (unexpected) separation induced fathers to increase their care for pups. The lack of difference in contact time for fathers of PND 9 pups is interesting. The apparent increase in attentiveness to PND 2 pups may suggest that prairie vole fathers are predisposed to be more responsive to pups when they are most vulnerable (i.e., neonates). Why fathers appeared to be relatively more responsive to PND 21 pups, particularly compared to PND 9 pups, is intriguing. This result may reflect the dyadic interactions of two independently mobile actors. In other words, increased contact time could be attributable to fathers that might engage in active contact-seeking with PND 21 pups, or attributable to pup mobility and their motivation to seek social interaction with fathers. Parent–pup interactions are dynamic and involve multiple forms of reciprocal actions (e.g., physical, vocal, etc.) of two independent organisms (Lucion and Bortolini 2014). It is, therefore, important to consider that pups exhibit changes in behavioral independence and motivation as they age, thus influencing the types of interactions they have with their parents.

Nonapeptide modulation of maternal behavior

Despite knowing a great deal about the maternal brain, surprisingly little is known specifically about the functional role of nonapeptide neurons in maternal behavior. The vast majority of research on the maternal brain involves examination of nonapeptide receptors or nonapeptide release as assessed via microdialysis (Bester-Meredith and Marler 2012; Bosch and Neumann 2012; Bridges 2015; Champagne and Curley 2016). Despite the numerous studies examining the impact of maternal separation on offspring (Lukas et al. 2010; Yu et al. 2015; Banqueri et al. 2017), extremely little is known about the consequences of offspring separation on mothers. Furthermore, very little is known about how the maternal brain responds to pups of different ages. In the present experiment, we observed several differences in nonapeptide neuron sensitivity within the maternal brain. The majority of differential responses we observed involved functioning within OT cell groups. Below we discuss our findings of VP and OT neuronal functioning for each distinct cell group in the light of previous studies examining the functional involvement of these neuromodulators in maternal behavior.

PVN and maternal behavior

In amniote vertebrates, the nonapeptide neuronal populations in the PVN represent one of the largest nonapeptide-positive cell groups in the brain (Kelly and Goodson 2014b). PVN VP, and OT neurons

exhibit numerous axonal projections throughout the basal forebrain and midbrain, and also send projections to the pituitary to exert peripheral effects (Mikami et al. 1978; De Vries et al. 1985). The nonapeptide cell groups of the PVN are very likely to be important for the modulation of maternal behavior. For example, electrolytic lesions of the PVN impair the onset of maternal behavior and reduce maternal aggression (Insel and Harbaugh 1989; Consiglio and Lucion 1996). Studies in rats and sheep have shown that OT is released in the PVN during parturition and suckling (Landgraf et al. 1992; Neumann et al. 1993; Da Costa et al. 1996). Although PVN VP release is not associated with suckling and lactation (Neumann et al. 1993), VP mRNA expression in the PVN increases around parturition and the onset of lactation (Windle et al. 1997; Walker et al. 2001; Bosch et al. 2007). Furthermore, VP receptor (V1aR) density increases in the PVN as female prairie voles approach parturition (Ophir et al. 2013). Consistent with these studies, our findings also suggest a primary role of the PVN in the modulation of maternal behavior given that both the PVN OT and VP cell groups were responsive to the presence and absence of offspring in different contexts.

The close links between OT action in pregnancy, lactation, and maternal care (Bosch and Neumann 2012) are likely important confounds that are difficult to disentangle. Although we were able to statistically rule out pregnancy as a factor influencing PVN OT neural activity, we cannot account for the concomitant influence that PVN OT neural functioning is likely to have on both maternal behavior, and lactation and suckling. Notably, although mothers that were separated from their pups were in a general state of lactation, they were not actively nursing pups during the IEG phase of the experiment. It is also interesting that, within these mothers, PVN OT neural activity differed between mothers with younger pups (PND 2 and 9) and older pups (PND 21). Specifically, OT-cFos colocalization decreased in mothers as pups got older. Furthermore, mothers separated from their pups (regardless of age) exhibited a significant decrease in PVN OT neural activity compared to mothers that were never separated from their pups. This decrease in PVN OT activity in mothers separated from their pups likely reflects a lack of social contact with their offspring. This interpretation would be consistent with other IEG studies showing that positive social contact elicits PVN OT neural activity in birds and mammals (Kelly and Goodson 2014a; Barrett et al. 2015; Hathaway et al. 2016).

Interestingly, we observed only a main effect of condition (parents that remained together, were separated, or were reunited) when examining PVN VP neural activity. Parents that were separated from their pups exhibited greater amounts of PVN VP-cFos colocalization compared to parents that were never separated from their pups (Together condition). This result might reflect a general stress response to disruption of the family unit in the light of the fact that stressors (primarily social isolation and physical stressors) are known to increase PVN VP-cFos colocalization and PVN VP mRNA expression in rodents (Bartanusz et al. 1994; Landgraf et al. 2007; Pirnik et al. 2009). Our findings suggest that being separated from offspring (regardless of age), even while still in the presence of the pair-bond partner, may be a stressful event for a biparental animal like the prairie vole.

Together, these findings demonstrate that the PVN OT cell group exhibits different functional properties based on the age of the pup and may play a more plastic role in the modulation of parental behavior over the course of offspring development than PVN VP neurons. Furthermore, while PVN OT and VP neurons are responsive to different parent–pup social contexts, the sex of the parent matters. Indeed, these differences in neural activity could reflect lactation in mothers, stress response, and/or differences in parent–pup interactions.

POPVN and maternal behavior

We also examined neural activity in a second, smaller, hypothalamic OT cell group: the POPVN. To our knowledge, no other studies have examined OT neuronal function in this cell group in relation to parental behavior. These neurons are located in the preoptic area, which is a brain region that has been extensively implicated in maternal behavior, particularly the medial preoptic area (MPOA) (Tsuneoka et al. 2013; Kuroda and Numan 2014; Numan and Young 2016). There are large densities of OT receptors (OTR) in the MPOA (Meddle et al. 2007), which likely receive extracellular OT released from POPVN OT cells (the nearest OT cells). Blocking OTR in the MPOA impairs maternal behavior in lactating rats (Bosch and Neumann 2012) and blocks the onset of maternal behavior in parturient rats (Pedersen et al. 1994). Similar to what we observed with the PVN OT cell group, we found a main effect of pup age on POPVN OT neural activity, suggesting that the POPVN may exhibit functional plasticity in the modulation of parental behavior across the development of offspring. Consistent with this interpretation, a study utilizing reversible local neural inactivation examined the role of the MPOA in the regulation of maternal behavior

throughout the postpartum period, and found that the MPOA is differentially engaged throughout postpartum in facilitating maternal responses to pups of different developmental stages (Pereira and Morrell 2009).

Similar to our findings with the PVN VP cell group, we observed a main effect of Condition for POPVN neural activity. Interestingly, post hoc analyses revealed the pattern of this main effect trends in the same direction as the PVN VP cell group, such that POPVN OT-cFos colocalization was greater in parents that were separated from their offspring. This suggests that POPVN OT neurons, much like PVN VP neurons, may play a role in modulating stress response.

Potential functional significance of AH, PLH and BST activation

To our knowledge, no other studies have examined a functional role of extrahypothalamic VP neurons in parental behavior. We found no differences in VP neural activity in the AH or PLH. AH VP is best known for its involvement in aggression and flank marking (Albers 2012). For example, VP infused into the AH decreases aggression in female Syrian hamsters, while the opposite effect is found for males (Gutzler et al. 2010). However, studies examining AH VP involvement in flank marking also suggest that VP in this region may play a role in social recognition (Albers 2012). Depending how strongly AH VP is involved in social recognition, we may have seen an increase in AH VP activity in parents that were reunited with their pups after separation. However, we observed no such effect, suggesting that perhaps AH VP involvement in social recognition is context dependent (also see Zheng et al. 2013a). It is worth noting that our paradigm in the present study is substantially different from the design of standard social recognition tests, and thus our findings here cannot rule out the possibility that AH VP is involved in social recognition in prairie voles. Unfortunately, given our lack of results for the PLH VP cell group, the functional role of PLH VP continues to remain unknown aside from the conclusion that this neuronal population may not be involved in modulating aspects of parental behavior and stress response.

We also examined functional responses of OT neurons in the BSTm, part of the extended amygdala. Few studies have yielded positive results suggesting a role for BST OT in maternal behavior. However, one study showed that OTR expression is upregulated during lactation in rats (Bosch et al. 2010). Importantly, some studies indicate that OTR expression in the prairie vole BST is very weak given that it is below detection thresholds using conventional autoradiography (Ophir et al. 2013; Zheng et al. 2013b) but see (Insel and Shapiro 1992; Bales et al. 2007). Thus, these findings in rats may not directly translate to prairie voles. Moreover, OT release in the BST does not differ between lactating maternal rats and females under non-maternal/non-lactating conditions (Bosch and Neumann 2012). As a result, BST OT has not been considered important for maternal behavior. Our data are largely consistent with this conclusion given that a sex × condition interaction revealed that BST OT neural activity differed between groups only in fathers. However, we did find that BST OT neural activity differed in mothers based on the age of their offspring, with higher levels of BST OT-cFos colocalization in mothers with neonates (PND 2) compared to mothers with weanling age pups (PND 21). If this activity difference does not reflect lactation or pregnancy, as suggested by other studies, then our results may reflect changes in olfactory social information-processing as offspring develop. A different subdivision of the BST, the posterior BST (pBST) has been heavily implicated in playing an essential role in transmitting chemosensory social information and modulating olfactory-guided social behavior (Petrulis 2013). In addition, OTRs in the pBST of female hamsters receive OT from the nearby BSTm and facilitate scent marking, and OTRs in the pBST of male and female rats promote social recognition (Martinez et al. 2010; Dumais et al. 2016). Therefore, it is possible that the BST OT functional changes observed across the development of offspring may reflect differences in olfactory processing of the changes in pheromones that pups release as they mature (Bind et al. 2013).

Taken together, these findings suggest that BSTm OT neurons in females may play a role in processing olfactory information from pups, however, where this peptide binds in prairie voles, and how sensitive that area may be, remain open questions. Generally, OT neurons within the BSTm appear to exhibit functional plasticity across the development of offspring, a result that is consistent with the other OT cell groups we examined.

Nonapeptide modulation of paternal behavior

While there is substantial research that investigates the neurobiology of maternal behavior, far fewer studies have examined the paternal brain. This likely stems from the fact that most studies examining parental behavior are conducted in uniparental species, like rats or mice. However, studies in biparental rodents (Bales and Saltzman 2016) and birds

(Lynn 2016) have elucidated important mechanisms underlying paternal care.

Perhaps the most striking neural finding for fathers in the present study is that paternal OT neural activity changes as offspring develop (as indicated by the main effects of pup age for the PVN, POPVN, and BST OT cell groups). This is particularly interesting because activity in the paternal brain is not confounded by lactation, nursing, or pregnancy, as is the case with mothers. Nevertheless, fathers do exhibit neural changes associated with the birth of pups (Bamshad et al. 1993, 1994; Bester-Meredith et al. 1999). As discussed in the section above for mothers, these changes in OT neural activity as pups develop may reflect changes in father–pup interactions, stress response, and/or olfactory-processing of pup pheromones.

Unfortunately, the data investigating nonapeptide changes in the paternal brain are somewhat contradictory. For example, one study in prairie voles reported no differences in PVN OT mRNA expression following parturition (Wang et al. 2000), whereas other studies have found that PVN OT-ir neuron numbers are higher in fathers compared to virgin male prairie voles (Kenkel et al. 2014) and that there are more PVN OT-ir fibers in fathers compared to virgin male mandarin voles (Song et al. 2010). The latter findings suggest a role for PVN OT in paternal behavior. Interestingly, in the present study, a significant sex × condition interaction revealed that PVN OT differential functional responses for parents that remained together, were separated, or were reunited with their pups were found only in mothers. Taken together, while the PVN OT cell group may be involved in paternal behavior, the precise behavioral functions of this neuronal population in relation to paternal behavior remain to be determined.

Aside from the main effects of condition observed in the PVN VP and POPVN OT cell groups, which may reflect a stress response to being separated from pups (see above), we only observed significant neural differences in fathers in the BSTm OT cell group. Little is known about the potential involvement of BST OT in paternal care, however, OT in the pBST has been implicated in social recognition and olfactory processing (see above) (Petrulis 2013; Dumais et al. 2016). Interestingly, here we found that fathers separated from their pups exhibited significantly higher amounts of BST OT-cFos colocalization, but only compared to fathers that were reunited with their pups after a brief period of separation. The lack of a difference between the Separate and Together fathers poses a conundrum. These results in fathers likely do not reflect a stress response to being separated from pups; if so, we should have also observed a significant difference between the Separate and Together fathers. To our knowledge, only one other study has examined BST OT neural activity in relation to parental care in males and found no difference in BST OT activity (when virgin males were exposed to pups versus a novel object; (Kenkel et al. 2012). However, the disagreement between our study and Kenkel et al. (2012) may reflect differences between neural functioning associated with alloparental and paternal care, or with differences attributable to sexual experience (virgin versus sexually experienced males). Together, the precise role of BST OT in paternal behavior remains unknown. Nevertheless, our findings suggest that this cell group might play a role in some aspect of father–pup interactions.

Despite its importance in offspring survival and condition in both human and non-human species (Kleiman 1977; Thomas and Birney 1979; Wolff and Macdonald 2004), there is an alarming lack of data examining nonapeptide neuronal function in relation to paternal care. Even fewer studies have manipulated VP or OT signaling to determine the role of peptide release or receptor binding in paternal behavior (Bales and Saltzman 2016). One study found that a cocktail of OT and VP antagonists reduced alloparental care in virgin male prairie voles, however this was an intracerebroventricular infusion, so the findings do not provide brain region-specific insight (Bales et al. 2004). Another study in prairie voles manipulated VP receptors and found that blockade of V1aRs in the LS decreased paternal behavior (Wang et al. 1994). The LS is heavily innervated by the PVN and BST VP and OT cell groups (De Vries and Buijs 1983). Importantly, the nonapeptide receptors exhibit promiscuity, such that V1aRs and OTRs can bind both VP and OT (Manning et al. 2008; Song et al. 2014). Thus, the effects of V1aR blockade on paternal behavior (Wang et al. 1994) could reflect the blockade of OT and/or VP from binding to septal V1aRs. Given that we observed functional differences based on the context of interactions with pups in the PVN VP, POPVN OT, and BST OT cell groups of fathers, future studies examining nonapeptide modulation of paternal care should consider preferentially investigating a role for these cell groups over other nonapeptide cell groups.

Conclusion

Mechanisms that exhibit functional plasticity on relatively short timescales and enable animals to adapt

behavior in context appropriate ways have likely been favored by selection. For instance, such plasticity can allow animals to shift parental care from intense caregiving to promoting offspring independence over the course of just a few weeks. The ability to shift behavioral responses based on the needs of offspring not only enhances the potential of reproductive success for offspring, but also enhances parental fitness and survivability (Winkler 1987; Clutton-Brock 1991), and would therefore be extremely adaptive. In the present experiment, we examined plasticity within the nonapeptide system in order to elucidate neural mechanisms that relate to shifts in parental behavior as offspring develop. Although the nonapeptides are well-known for being involved in the modulation of parental behavior, little is known about how the nonapeptide system may differentially respond to pups at different stages of development. Here, we show that OT neuronal functioning is sensitive to the age of pups whereas VP neuronal function is not. Thus, although both OT and VP are involved in the modulation of parental behavior, functional plasticity of the OT system may be more intimately associated with the behavioral plasticity exhibited by parents as their offspring develop. To our knowledge, these results are among the first to show that nonapeptide neurons exhibit differential responses in parents based on the age of offspring. This should be taken into consideration when conducting studies that examine parental responses to offspring. For example, studies examining alloparental care and maternal separation often use offspring of varying ages as stimuli, and it is important to note that parental neural and behavioral responses may differ based on the age of the offspring stimuli, particularly for mothers. Lastly, these findings provide insight into the plastic capabilities of the nonapeptide system. While previous studies have demonstrated that the parental brain undergoes nonapeptide anatomical changes pre- and postpartum, we now show that the nonapeptide system in a biparental rodent also exhibits functional plasticity across the development of pups.

Acknowledgments

We are grateful to Dustin Rubenstein and Ben Dantzer for their invitation to participate in this issue associated with the SICB symposium. This symposium was supported by the US National Science Foundation (IOS-1634027), the Division of Animal Behavior, Division of Comparative Endocrinology, Division of Ecology and Evolution, and the Division of Neurobiology of the Society for Integrative and Comparative Biology. We are also grateful for statistical help from the Statistics Consulting Center at Cornell University, and to Eileen Chun and Ruth Witmer for assistance with cell counts.

Funding

The authors acknowledge the support from the National Institutes of Health (*Eunice Kennedy Shriver* National Institute of Child Health and Human Development HD081959 to A.M.K. and HD079573 to A.G.O.), and the National Science Foundation IOS-1634027 to A.G.O.

Supplementary data

Supplementary data available at *ICB* online.

References

Albers HE. 2012. The regulation of social recognition, social communication and aggression: vasopressin in the social behavior neural network. Horm Behav 61:283–92.

Bales KL, Kim AJ, Lewis-Reese AD, Sue Carter C. 2004. Both oxytocin and vasopressin may influence alloparental behavior in male prairie voles. Horm Behav 45:354–61.

Bales KL, Plotsky PM, Young LJ, Lim MM, Grotte N, Ferrer E, Carter CS. 2007. Neonatal oxytocin manipulations have long-lasting, sexually dimorphic effects on vasopressin receptors. Neuroscience 144:38–45.

Bales KL, Saltzman W. 2016. Fathering in rodents: neurobiological substrates and consequences for offspring. Horm Behav 77:249–59.

Bamshad M, Novak MA, De Vries GJ. 1993. Sex and species differences in the vasopressin innervation of sexually naive and parental prairie voles, *Microtus ochrogaster* and meadow voles, *Microtus pennsylvanicus*. J Neuroendocrinol 5:247–55.

Bamshad M, Novak MA, de Vries GJ. 1994. Cohabitation alters vasopressin innervation and paternal behavior in prairie voles (*Microtus ochrogaster*). Physiol Behav 56:751–8.

Banqueri M, Mendez M, Arias JL. 2017. Behavioral effects in adolescence and early adulthood in two length models of maternal separation in male rats. Behav Brain Res 324:77–86.

Barrett CE, Arambula SE, Young LJ. 2015. The oxytocin system promotes resilience to the effects of neonatal isolation on adult social attachment in female prairie voles. Transl Psychiatry 5:e606.

Bartanusz V, Aubry JM, Steimer T, Baffi J, Kiss JZ. 1994. Stressor-specific increase of vasopressin mRNA in paraventricular hypophysiotrophic neurons. Neurosci Lett 170:35–8.

Bester-Meredith JK, Marler CA. 2012. Naturally occurring variation in vasopressin immunoreactivity is associated with maternal behavior in female peromyscus mice. Brain Behav Evol 80:244–53.

Bester-Meredith JK, Young LJ, Marler CA. 1999. Species differences in paternal behavior and aggression in *Peromyscus* and their associations with vasopressin immunoreactivity and receptors. Horm Behav 36:25–38.

Bind RH, Minney SM, Rosenfeld S, Hallock RM. 2013. The role of pheromonal responses in rodent behavior: future directions for the development of laboratory protocols. J Am Assoc Lab Anim Sci 52:124–9.

Bosch OJ, Musch W, Bredewold R, Slattery DA, Neumann ID. 2007. Prenatal stress increases HPA axis activity and impairs maternal care in lactating female offspring: implications for postpartum mood disorder. Psychoneuroendocrinology 32:267–78.

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

Bosch OJ, Pfortsch J, Beiderbeck DI, Landgraf R, Neumann ID. 2010. Maternal behaviour is associated with vasopressin release in the medial preoptic area and bed nucleus of the stria terminalis in the rat. J Endocrinol 22:420–9.

Bridges RS. 2015. Neuroendocrine regulation of maternal behavior. Front Neuroendocrinol 36:178–96.

Carter CS, Getz LL. 1985. Social and hormonal determinants of reproductive patterns in the prairie vole. Neurobiology:18–36.

Carter CS, Getz LL, Gavish L, McDermott JL, Arnold P. 1980. Male related pheromones and the activation of female reproduction in the prairie vole (*Microtus ochrogaster*). Biol Reprod 23:1038–45.

Champagne FA, Curley JP. 2016. Plasticity of the maternal brain across the lifespan. New Dir Child Adolesc Dev 2016:9–21.

Clutton-Brock TH. 1991. The evolution of parental care. Princeton (NJ): Princeton University Press.

Consiglio AR, Lucion AB. 1996. Lesion of hypothalamic paraventricular nucleus and maternal aggressive behavior in female rats. Physiol Behav 59:591–6.

Da Costa AP, Guevara-Guzman RG, Ohkura S, Goode JA, Kendrick KM. 1996. The role of oxytocin release in the paraventricular nucleus in the control of maternal behaviour in the sheep. J Neuroendocrinol 8:163–77.

De Vries GJ, Buijs RM. 1983. The origin of the vasopressinergic and oxytocinergic innervation of the rat brain with special reference to the lateral septum. Brain Res 273:307–17.

De Vries GJ, Buijs RM, Van Leeuwen FW, Caffe AR, Swaab DF. 1985. The vasopressinergic innervation of the brain in normal and castrated rats. J Comp Neurol 233:236–54.

Dluzen DE, Ramirez VD, Carter CS, Getz LL. 1981. Male vole urine changes luteinizing-hormone-releasing hormone and norepinephrine in female olfactory-bulb. Science 212:573–5.

Dumais KM, Alonso AG, Immormino MA, Bredewold R, Veenema AH. 2016. Involvement of the oxytocin system in the bed nucleus of the stria terminalis in the sex-specific regulation of social recognition. Psychoneuroendocrinology 64:79–88.

Garoflos E, Stamatakis A, Rafrogianni A, Pondiki S, Stylianopoulou F. 2008. Neonatal handling on the first postnatal day leads to increased maternal behavior and fos levels in the brain of the newborn rat. Dev Psychobiol 50:704–13.

Getz LL, McGuire B, Hofmann J, Pizzuto T, Frase B. 1994. Natal dispersal and philopatry in the prairie vole (Microtus ochrogaster): settlement, survival, and potential reproductive success. Ethol Ecol Evol 6:267–84.

Goodson JL. 2008. Nonapeptides and the evolutionary patterning of sociality. Prog Brain Res 170:3–15.

Goodson JL, Thompson RR. 2010. Nonapeptide mechanisms of social cognition, behavior and species-specific social systems. Curr Opin Neurobiol 20:784–94.

Goodson JL, Wang Y. 2006. Valence-sensitive neurons exhibit divergent functional profiles in gregarious and asocial species. Proc Natl Acad Sci USA 103:17013–7.

Gottlieb A, Keydar I, Epstein HT. 1977. Rodent brain growth stages: an analytical review. Biol Neonate 32:166–76.

Gutzler SJ, Karom M, Erwin WD, Albers HE. 2010. Arginine-vasopressin and the regulation of aggression in female Syrian hamsters (Mesocricetus auratus). Eur J Neurosci 31:1655–63.

Hathaway GA, Faykoo-Martinez M, Peragine DE, Mooney SJ, Holmes MM. 2016. Subcaste differences in neural activation suggest a prosocial role for oxytocin in eusocial naked mole-rats. Horm Behav 79:1–7.

Hoffman GE, Smith MS, Verbalis JG. 1993. c-Fos and related immediate early gene products as markers of activity in neuroendocrine systems. Front Neuroendocrinol 14:173–213.

Insel TR, Harbaugh CR. 1989. Lesions of the hypothalamic paraventricular nucleus disrupt the initiation of maternal behavior. Physiol Behav 45:1033–41.

Insel TR, Shapiro LE. 1992. Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. Proc Natl Acad Sci U S A 89:5981–5.

Jeon J. 2008. Evolution of parental favoritism among different-aged offspring. Behav Ecol 19:344–52.

Kelly AM, Goodson JL. 2014a. Personality is tightly coupled to vasopressin-oxytocin neuron activity in a gregarious finch. Front Behav Neurosci 8:55.

Kelly AM, Goodson JL. 2014b. Social functions of individual vasopressin-oxytocin cell groups in vertebrates: what do we really know? Front Neuroendocrinol 35:512–29.

Kenkel WM, Paredes J, Yee JR, Pournajafi-Nazarloo H, Bales KL, Carter CS. 2012. Neuroendocrine and behavioural responses to exposure to an infant in male prairie voles. J Neuroendocrinol 24:874–86.

Kenkel WM, Suboc G, Carter CS. 2014. Autonomic, behavioral and neuroendocrine correlates of paternal behavior in male prairie voles. Physiol Behav 128:252–9.

Kleiman DG. 1977. Monogamy in mammals. Q Rev Biol 52:39–69.

Kohl J, Autry AE, Dulac C. 2017. The neurobiology of parenting: a neural circuit perspective. Bioessays 39:1–11.

Kuroda KO, Numan M. 2014. The medial preoptic area and the regulation of parental behavior. Neurosci Bull 30:863–5.

Landgraf R, Kessler MS, Bunck M, Murgatroyd C, Spengler D, Zimbelmann M, Nussbaumer M, Czibere L, Turck CW, Singewald N et al. 2007. Candidate genes of anxiety-related behavior in HAB/LAB rats and mice: focus on vasopressin and glyoxalase-I. Neurosci Biobehav Rev 31:89–102.

Landgraf R, Neumann I, Russell JA, Pittman QJ. 1992. Push-pull perfusion and microdialysis studies of central oxytocin and vasopressin release in freely moving rats during pregnancy, parturition, and lactation. Ann N Y Acad Sci 652:326–39.

Landgraf R, Neumann ID. 2004. Vasopressin and oxytocin release within the brain: a dynamic concept of multiple and variable modes of neuropeptide communication. Front Neuroendocrinol 25:150–76.

Lonstein JS, De Vries GJ. 1999. Comparison of the parental behavior of pair-bonded female and male prairie voles (Microtus ochrogaster). Physiol Behav 66:33–40.

Lucion AB, Bortolini MC. 2014. Mother-pup interactions: rodents and humans. Front Endocrinol (Lausanne) 5:17.

Lukas M, Bredewold R, Neumann ID, Veenema AH. 2010. Maternal separation interferes with developmental changes in brain vasopressin and oxytocin receptor binding in male rats. Neuropharmacology 58:78–87.

Lynn SE. 2016. Endocrine and neuroendocrine regulation of fathering behavior in birds. Horm Behav 77:237–48.

Manning M, Stoev S, Chini B, Durroux T, Mouillac B, Guillon G. 2008. Peptide and non-peptide agonists and antagonists for the vasopressin and oxytocin V1a, V1b, V2 and OT receptors: research tools and potential therapeutic agents. Prog Brain Res 170:473–512.

Martinez LA, Albers HE, Petrulis A. 2010. Blocking oxytocin receptors inhibits vaginal marking to male odors in female Syrian hamsters. Physiol Behav 101:685–92.

Meddle SL, Bishop VR, Gkoumassi E, van Leeuwen FW, Douglas AJ. 2007. Dynamic changes in oxytocin receptor expression and activation at parturition in the rat brain. Endocrinology 148:5095–104.

Mikami S, Tokado H, Farner DS. 1978. The hypothalamic neurosecretory systems of the Japanese quail as revealed by retrograde transport of horseradish peroxidase. Cell Tissue Res 194:1–15.

Neumann I, Russell JA, Landgraf R. 1993. Oxytocin and vasopressin release within the supraoptic and paraventricular nuclei of pregnant, parturient and lactating rats: a microdialysis study. Neuroscience 53:65–75.

Numan M, Young LJ. 2016. Neural mechanisms of mother-infant bonding and pair bonding: similarities, differences, and broader implications. Horm Behav 77:98–112.

Okabe S, Tsuneoka Y, Takahashi A, Ooyama R, Watarai A, Maeda S, Honda Y, Nagasawa M, Mogi K, Nishimori K et al. 2017. Pup exposure facilitates retrieving behavior via the oxytocin neural system in female mice. Psychoneuroendocrinology 79:20–30.

Ophir AG, Sorochman G, Evans BL, Prounis GS. 2013. Stability and dynamics of forebrain vasopressin receptor and oxytocin receptor during pregnancy in prairie voles. J Neuroendocrinol 25:719–28.

Ophir AG, Wolff JO, Phelps SM. 2008. Variation in neural V1aR predicts sexual fidelity and space use among male prairie voles in semi-natural settings. Proc Natl Acad Sci U S A 105:1249–54.

Pedersen CA, Caldwell JD, Walker C, Ayers G, Mason GA. 1994. Oxytocin activates the postpartum onset of rat maternal behavior in the ventral tegmental and medial preoptic areas. Behav Neurosci 108:1163–71.

Pereira M, Ferreira A. 2016. Neuroanatomical and neurochemical basis of parenting: Dynamic coordination of motivational, affective and cognitive processes. Horm Behav 77:72–85.

Pereira M, Morrell JI. 2009. The changing role of the medial preoptic area in the regulation of maternal behavior across the postpartum period: facilitation followed by inhibition. Behav Brain Res 205:238–48.

Petrulis A. 2013. Chemosignals and hormones in the neural control of mammalian sexual behavior. Front Neuroendocrinol 34:255–67.

Pirnik Z, Petrak J, Bundzikova J, Mravec B, Kvetnansky R, Kiss A. 2009. Response of hypothalamic oxytocinergic neurons to immobilization stress is not dependent on the presence of corticotrophin releasing hormone (CRH): a CRH knock-out mouse study. J Physiol Pharmacol 60:77–82.

Pryce CR, Bettschen D, Feldon J. 2001. Comparison of the effects of early handling and early deprivation on maternal care in the rat. Dev Psychobiol 38:239–51.

Richmond ME, Stehn RA. 1976. Olfaction and reproductive behavior in microtine rodents. In: Doty RL, editor. Mamm Olfaction Reprod Processess Behav. New York: Academic Press. p. 197–217.

Rilling JK, Young LJ. 2014. The biology of mammalian parenting and its effect on offspring social development. Science 345:771–6.

Roberts RL, Williams JR, Wang AK, Carter CS. 1998. Cooperative breeding and monogamy in prairie voles: influence of the sire and geographical variation. Anim Behav 55:1131–40.

Robison WT, Myers MM, Hofer MA, Shair HN, Welch MG. 2016. Prairie vole pups show potentiated isolation-induced vocalizations following isolation from their mother, but not their father. Dev Psychobiol 58:687–99.

Rood BD, De Vries GJ. 2011. Vasopressin innervation of the mouse (*Mus musculus*) brain and spinal cord. J Comp Neurol 519:2434–74.

Rosenfeld CS, Johnson SA, Ellersieck MR, Roberts RM. 2013. Interactions between parents and parents and pups in the monogamous California mouse (Peromyscus californicus). PLoS One 8:e75725.

Salo AL, Shapiro LE, Dewsbury DA. 1994. Comparisons of nipple attachment and incisor growth among four species of voles (Microtus). Dev Psychobiol 27:317–30.

Solomon NG. 1991. Current indirect fitness benefits associated with philopatry in juvenile prairie voles. Behav Ecol Sociobiol 29:277–82.

Song Z, McCann KE, McNeill JKt Larkin TE, Huhman, KL2ndAlbers HE. 2014. Oxytocin induces social communication by activating arginine-vasopressin V1a receptors and not oxytocin receptors. Psychoneuroendocrinology 50:14–9.

Song Z, Tai F, Yu C, Wu R, Zhang X, Broders H, He F, Guo R. 2010. Sexual or paternal experiences alter alloparental behavior and the central expression of ERalpha and OT in male mandarin voles (Microtus mandarinus). Behav Brain Res 214:290–300.

Stamatakis A, Kalpachidou T, Raftogianni A, Zografou E, Tzanou A, Pondiki S, Stylianopoulou F. 2015. Rat dams exposed repeatedly to a daily brief separation from the pups exhibit increased maternal behavior, decreased anxiety and altered levels of receptors for estrogens (ERalpha,

ERbeta), oxytocin and serotonin (5-HT1A) in their brain. Psychoneuroendocrinology 52:212–28.

Stoop R. 2012. Neuromodulation by oxytocin and vasopressin. Neuron 76:142–59.

Thomas JA, Birney EC. 1979. Parental care and mating system of the prairie vole, *Microtus ochrograster*. Behav Ecol Sociobiol 5:171–86.

Trivers RL. 1974. Parent-offspring conflict. Am Zool 14:249–64.

Tsuneoka Y, Maruyama T, Yoshida S, Nishimori K, Kato T, Numan M, Kuroda KO. 2013. Functional, anatomical, and neurochemical differentiation of medial preoptic area subregions in relation to maternal behavior in the mouse. J Comp Neurol 521:1633–63.

Walker CD, Toufexis DJ, Burlet A. 2001. Hypothalamic and limbic expression of CRF and vasopressin during lactation: implications for the control of ACTH secretion and stress hyporesponsiveness. Prog Brain Res 133:99–110.

Wang Z. 1995. Species differences in the vasopressin-immunoreactive pathways in the bed nucleus of the stria terminalis and medial amygdaloid nucleus in prairie voles (*Microtus ochrogaster*) and meadow voles (*Microtus pennsylvanicus*). Behav Neurosci 109:305–11.

Wang Z, Ferris CF, De Vries GJ. 1994. Role of septal vasopressin innervation in paternal behavior in prairie voles (*Microtus ochrogaster*). Proc Natl Acad Sci U S A 91:400–4.

Wang Z, Zhou L, Hulihan TJ, Insel TR. 1996. Immunoreactivity of central vasopressin and oxytocin pathways in microtine rodents: a quantitative comparative study. J Comp Neurol 366:726–37.

Wang ZX, Liu Y, Young LJ, Insel TR. 2000. Hypothalamic vasopressin gene expression increases in both males and females postpartum in a biparental rodent. J Neuroendocrinol 12:111–20.

Windle RJ, Shanks N, Lightman SL, Ingram CD. 1997. Central oxytocin administration reduces stress-induced corticosterone release and anxiety behavior in rats. Endocrinology 138:2829–34.

Winkler DW. 1987. A general model for parental care. Am Nat 130:526–43.

Wolff JO, Macdonald DW. 2004. Promiscuous females protect their offspring. Trends Ecol Evol 19:127–34.

Young LJ. 2009. The neuroendocrinology of the social brain. Front Neuroendocrinol 30:425–8.

Yu P, Zhang H, Li X, He F, Tai F. 2015. Early bi-parental separation or neonatal paternal deprivation in mandarin voles reduces adult offspring paternal behavior and alters serum corticosterone levels and neurochemistry. Horm Behav 73:8–14.

Zheng DJ, Foley L, Rehman A, Ophir AG. 2013a. Social recognition is context dependent in single male prairie voles. Anim Behav 86

Zheng DJ, Larsson B, Phelps SM, Ophir AG. 2013b. Female alternative mating tactics, reproductive success and nonapeptide receptor expression in the social decision-making network. Behav Brain Res 246:139–47.