Published in final edited form as:

Gen Comp Endocrinol. 2014 May 1; 200: 1–9. doi:10.1016/j.ygcen.2014.02.006.

Increased STAT5 signaling in the ring dove brain in response to prolactin administration and spontaneous elevations in prolactin during the breeding cycle

John D. Buntin^a and Linda Buntin^a

^aDepartment of Biological Sciences, University of Wisconsin-Milwaukee, P.O. Box 413, Milwaukee, WI 53217

Abstract

Prolactin acts on target cells in the central nervous system (CNS) to stimulate behavioral changes associated with parental care in birds, but the signaling mechanisms that mediate these actions have not been characterized. In mammals, the Janus Kinase 2-Signal Transducer and Activator of Transcription 5 (JAK2-STAT5) signaling pathway mediates many of the actions of prolactin. To assess the importance of this pathway in prolactin-sensitive target cells in the avian brain, we measured changes in activated (phosphorylated) STAT5 (pSTAT5) in the forebrain of female ring doves sampled as plasma prolactin levels change during the breeding cycle and in prolactin-treated, non-breeding females. The anatomical distribution of cells exhibiting pSTAT5 immunoreactivity in dove brain closely paralleled the distribution of prolactin receptors in this species. The density of pSTAT5 immunoreactive (pSTAT5-ir) cells were highest in the preoptic area, the suprachiasmatic, paraventricular, ventromedial hypothalamic nuclei, the lateral and tuberal hypothalamic regions, the lateral bed nucleus of the stria terminalis, and the lateral septum. Mean pSTAT5-ir cell densities in these eight brain areas were several fold higher in breeding females during late incubation/early post-hatching when plasma prolactin levels have been observed to peak than in non-breeding females or breeding females sampled at earlier stages when prolactin titers have been reported to be lower. Similar differences were observed between prolactin-treated and vehicle-treated females in all three of the forebrain regions that were compared. We conclude that JAK2-STAT5 signaling is strongly activated in response to prolactin stimulation in the ring dove brain and could potentially mediate some of the centrally-mediated behavioral effects of this hormone.

Keywords

Prolactin; Brain; Avian; JAK-STAT Signaling Pathway

© 2014 Elsevier Inc. All rights reserved.

Corresponding author: Dr. John D. Buntin, Department of Biological Sciences, P.O. Box 413, University of Wisconsin-Milwaukee, Milwaukee, WI 53217, buntin@uwm.edu, Phone: 414-332-0885, FAX: 414-229-3926.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Prolactin plays a critical role in the expression of several behavioral changes necessary for successful parenting in birds [1–3]. Studies in ring doves have demonstrated that parental behaviors can be induced by direct intracranial administration of prolactin [4,5] and that high affinity receptors for prolactin are present in specific forebrain regions [6,7]. While there is convincing evidence that several prolactin-induced behavioral changes in doves are mediated by prolactin receptors in the central nervous system (CNS) [4,5], the possible molecular signaling pathways involved have not been examined in this or any other non-mammalian species. Although the hormone has been reported to influence several different signaling pathways, the Janus Kinase 2 (JAK2) -Signal Transducer and Activator of Transcription 5 (STAT5) pathway is the best documented mediator of prolactin's physiological effects in a wide range of mammalian tissues, including the brain [8–11]. In this pathway, the binding of prolactin to its receptor activates JAK2, which in turn phosphorylates STAT5. Following dimerization and nuclear translocation, phosphorylated STAT5 (pSTAT5) influences the transcriptional activity of prolactin-sensitive genes [8,9]. This study examined the degree to which prolactin influences this signaling pathway in the CNS of female ring doves by measuring changes in pSTAT5 levels in discrete forebrain regions following changes in plasma prolactin concentrations that naturally occur during the breeding cycle and following exogenous prolactin administration.

2. Materials and Methods

2.1 Animals

The adult female ring doves (*Streptopelia risoria*) used in these studies were drawn from a breeding colony maintained at the University of Wisconsin-Milwaukee Animal Care Facility. Prior to use, all birds had received one or more previous breeding experiences, which included successful rearing of young to an independent age. Birds were housed as breeding pairs or as individuals in white acrylic cages (33 cm × 68 cm × 46 cm for pairs; 33 cm × 34 cm × 46 cm for individuals) with wire fronts. All birds were housed in visual but not auditory isolation from birds in neighboring cages under constant temperature (20–22°C) and photoperiod (14 h light/10 h dark; lights on 07:30 h). Water, grit, and seed mixture (consisting of kafir, milo, wheat, hulled oats, buckwheat, and white proso millet), were available to all birds *ad libitum*. Breeding pairs were provided with glass nest bowls and excelsior for nesting material. All of the procedures involving ring doves were approved by the University of Wisconsin-Milwaukee Animal Care and Use Committee.

2.2 Procedure

Changes in pSTAT5 immunostaining during the breeding cycle were analyzed by comparing brain tissue from female doves sampled under the following reproductive conditions (n=5/sampling group): non-breeding (visually isolated from other birds for a minimum of two weeks), pre-laying (2–3 days after pairing with a male breeding partner), early incubation (2 days after the first of the two egg clutch was laid), and late incubation/early post-hatching (14–16 days after egg laying). Because plasma prolactin levels in breeding doves begin to rise near the midpoint of the 14 day incubation period and reach peak levels around the time

of hatching [12,13], prolactin signaling activity would be expected to be higher in doves sampled during the late incubation/early post hatching period than in non-breeding doves or doves sampled at earlier breeding stages.

The effects of prolactin treatment on pSTAT5 immunostaining were assessed in non-breeding female doves (n=4/treatment group) given seven days of exposure to osmotic minipumps (Alzet model 2001, 1.0 μ l/hr; DURECT Corp., Cupertino, CA) containing ovine prolactin (NIDDK-oPRL21; 3.33 μ g/hr; 80 μ g/day) in 0.87% NaCl/0.01M NaHCO₃ vehicle (3:1 ratio v/v; 1 μ l/hr; 24 μ l/day) or saline/bicarbonate vehicle alone. Pumps were implanted subcutaneously using isoflurane anesthesia. Ovine prolactin was selected because it is widely recognized by prolactin receptors in a variety of different vertebrate tissues [14]. Ovine prolactin is also more potent than turkey prolactin in binding to prolactin receptors in dove brain [15] and in stimulating hyperphagia in doves when given by direct intracranial injection [16].

2.3 Immunohistochemistry

Doves were deeply anesthetized with a chloral hydrate (21.2 mg/ml)/pentobarbital (4.4 mg/ml) solution (7.0mg/kg s.c.) and then perfused transcardially with heparinized 0.01M phosphate-buffered saline followed by 4% paraformaldehyde in 0.1M phosphate buffer. Brains were then removed, post-fixed in 4% paraformaldehyde for 60–90 min, and transferred to 30% sucrose until completely infiltrated. Brains were then cryosectioned at 40 μ m and stored in cryoprotectant at –20°C until use. Following a series of washes in 0.05M Tris-buffered saline (TBS), every third section was processed for immunohistochemistry in 24-well plates using a Vectastain Elite ABC kit (Vector Laboratories, Burlingame CA). Free-floating sections were initially subjected to an antigen-retrieval procedure (5 min incubation at 90°C in 0.01M Tris-HCl, pH 10.0) followed by four washes in TBS and 10 min exposure to 0.03% hydrogen peroxide. Sections were then washed and incubated on an orbital shaker for 72 h at 4°C in TBS with 2% normal goat serum and a 1:500 dilution of anti-phosphoSTAT5 antibody (tyr694; Cell Signaling Technology, Beverly CA). This antibody is specific for the phosphorylated protein, but does not distinguish between phosphoSTAT5a and phosphoSTAT5b. Immunoperoxidase staining was visualized using diaminobenzidine.

Each immunohistochemical assay run consisted of brain sections from one dove in each of the four breeding conditions. For analysis of the effects of prolactin on pSTAT5 immunostaining, each assay contained brain sections from two prolactin-treated and two vehicle-treated birds.

2.4 Quantification of pSTAT5 immunostaining

Following pSTAT5 immunostaining, free-floating sections were mounted on slides and coverslipped. For the breeding cycle study, pSTAT5-immunoreactive (pSTAT5-ir) cells were quantified in eight brain regions that exhibited the highest levels of immunostaining based upon visual inspection of brain sections from breeding birds sampled during the late incubation/early posthatching phase when peak plasma levels of prolactin are reported [12,13]. The areas sampled (see Fig. 1) were the preoptic area (POA), the

suprachiasmatic nucleus of the hypothalamus (SCN), the paraventricular nucleus of the hypothalamus (PVN), the ventromedial nucleus of the hypothalamus (VMN), the tuberal hypothalamic region (TU), the lateral hypothalamic region (LHy), the lateral septum (LS), and the lateral bed nucleus of the stria terminalis (BSTL). To assess whether changes seen in pSTAT5-ir during the breeding cycle paralleled those seen following exogenous prolactin administration, pSTAT5 immunostaining was examined in three of these regions in birds given 7 days of prolactin or vehicle via a subcutaneously implanted osmotic minipump. The areas sampled in these birds were the POA, PVN, and TU.

The number of pSTAT5-ir cells in each area was quantified from digital photomicrographs of each section using a Nikon CoolPix 990 camera mounted on a Nikon Eclipse 400 light microscope. Photomicrographs were taken at 100x in all brain regions. Using public domain Image J software, region of interest (ROI) templates were constructed for each brain region, as described by Buntin et al [20]. To count pSTAT5-ir cells, the optical densities of each digital image were thresholded in each ROI so that only cells showing unambiguous pSTAT5 immunostaining were highlighted and counted by the Image J software. The number of pSTAT5-ir cells in a given ROI was quantified for each section and expressed as cell density (number of cells/ μm^2). The cell density value assigned to each animal for a particular brain region represented the average of four to twenty cell density determinations. The mean $\pm$ standard error of the mean (SEM) number of cell density determinations per animal ranged from 5.9 ± 0.8 for the POA to 12.6 ± 2.1 for the BSTL. All data were collected without the investigator's knowledge of treatment condition or breeding stage.

2.5 Statistical analysis

Missing or damaged tissue precluded an accurate assessment of pSTAT5-ir cell density in the TU from one non-breeding prolactin-infused female and in the POA from one breeding female in the post-hatching group. For the remaining data, SigmaPlot 11 software (Systat Software, San Jose CA) with a two-tailed significance level of $P < 0.05$ was used for statistical analyses. In preliminary analyses, the Levene's median test [21] revealed no significant heterogeneity of variance in pSTAT5-ir cell densities across the four breeding stages in any brain region. Accordingly, one-way analyses of variance tests were used to assess breeding stage differences in each brain region. When significant F values were obtained, the Tukey test was used for pair-wise group comparisons. Differences in pSTAT5-ir cell densities between prolactin-treated and vehicle-treated birds were assessed in each brain region using the Student's *t* test or the non-parametric Mann-Whitney U test [22], depending on whether equal variance assumptions were met. Data are depicted as mean $\pm$ SEM.

3. Results

3.1. Effects of reproductive state

Breeding female doves sampled when plasma prolactin levels have been observed to peak around the time that their eggs hatch showed a non-uniform distribution of pSTAT5-ir cells with high levels of immunostaining confined exclusively to target cell nuclei in the eight brain regions examined (Fig. 2 and 3). By contrast, reduced levels of pSTAT5

immunostaining were observed in these same brain regions in non-breeding females and females sampled during the pre-laying period of intense courtship and nest-building activity when lower plasma prolactin levels have been reported (Fig. 2 and 3). In seven of the eight brain regions examined, pSTAT5-ir cell densities differed significantly across the four reproductive conditions (Fig. 3; $F(3,16) = 5.21$, $P < 0.02$ for POA; $F(3,16) > 12.0$, $p < .001$ for VMN, SCN, PVN, SL, BSTL, and LH). Females sampled during the late incubation/early post-hatching period showed pSTAT5-ir cell densities in these areas that were 5–49 times higher than those seen in non-breeding females or females sampled during the pre-laying period ($P < 0.025$ for POA; $P < .001$ for all other comparisons). Late incubating/early brooding females also showed significantly higher pSTAT5-ir cell densities in the VMN, SCN, PVN, LH, BSTL, and SL than females sampled during early incubation ($P < 0.001$).

Although average pSTAT5-ir cell density values in the TU were also highest in birds sampled in the late incubation/early post-hatching period, females in all groups showed large within-group variances in this region that precluded the detection of statistically significant differences across the four reproductive conditions. Nevertheless, in a pattern similar to that seen in the other seven brain regions, a significant difference was observed in the TU when pSTAT5-ir cell densities in non-breeding females and breeding females during the pre-laying and early incubation phases were combined and compared with those of females sampled during the late incubation/early post-hatching phase (48.3 ± 16.7 vs 138.4 ± 31.3 ; $t(18) = 2.66$, $P < 0.02$).

3.2. Effects of prolactin treatment

Prolactin-treated doves exhibited the same pattern of nuclear localization of the pSTAT5 antibody as did breeding doves sampled around the time of hatching. As seen in Fig. 4 and 5, pSTAT5-ir cell densities in the POA, PVN, and TU were over nine times higher in prolactin-treated doves than in vehicle-treated doves ($t(6) = 3.61$, $p < .02$; Mann-Whitney $U = 0$, $P < 0.03$; $t(5) = 7.56$, $P < 0.001$, respectively).

4. Discussion

The distribution of pSTAT5 immunostaining that we observed in dove brain closely paralleled the pattern of prolactin receptor distribution as previously determined by *in vitro* autoradiography and densitometry [6,7]. A similar correspondence between prolactin receptor distribution and pSTAT5-immunostaining has been reported in the mouse brain, although disparities in distribution were seen in some brain regions [23]. The fact that pSTAT5-ir was confined to the cell nucleus in brain areas with high concentrations of pSTAT5-ir cells is also consistent with the documented role of pSTAT5 in transcriptional regulation [24]. While it is possible that other ligand-receptor interactions contributed to the pSTAT5-ir that we detected in some brain areas (see below), it is likely that most of the changes that we observed reflected changes in prolactin signaling in the brain. This conclusion is supported by two observations: first, brain regions with high pSTAT5-ir cell density showed fluctuations in immunostaining that closely parallel reported changes in plasma prolactin titers during the dove breeding cycle [12,13], and secondly, the differences in POA, PVN, and TU pSTAT5-ir cell densities that were observed between non-breeding prolactin-treated females and vehicle-treated controls were similar in magnitude to those

observed between parent doves sampled when peak plasma levels of prolactin are reported and untreated non-breeding doves sampled when prolactin titers reportedly reach their nadir. From these results, we conclude that the JAK2-STAT5 signaling pathway could mediate at least some of the effects of prolactin in the dove CNS, and that phosphorylated STAT 5 can be usefully employed as a functional marker of prolactin receptor activation in the dove brain. Similar conclusions have been drawn from studies in rats and mice, which exhibit fluctuations in brain pSTAT5-ir as a function of prolactin treatment, spontaneous changes in circulating prolactin levels during pregnancy and lactation, and previous reproductive experience [25–28].

Despite the fact that the TU was the only brain region that failed to exhibit significant differences in pSTAT5-ir cell densities across the four individual reproductive conditions, it is likely that it resembles the other areas sampled in responding to prolactin by exhibiting increased STAT5 phosphorylation. Two observations support this conclusion. First, pSTAT5-ir cell densities in the TU were significantly higher in the five late incubation/early post-hatching females than in the remaining 15 females in the other three reproductive conditions. Secondly, prolactin-treated non-breeding doves showed significantly higher pSTAT5-ir cell densities in this region than did vehicle-treated controls. The failure to detect significant fluctuations across breeding stages was primarily due to the fact that pSTAT5-ir cell densities in the TU of females in all four breeding conditions showed large within-group variances. In addition, mean pSTAT5-ir cell densities in the five non-breeding females were three to 25 times higher in the TU than in the other seven brain regions sampled. In part, these high pSTAT5-ir cell densities in the TU could reflect heightened sensitivity of these neurons to baseline concentrations of prolactin in plasma, as has been reported in the mammalian counterpart to this region, the arcuate nucleus [29]. Alternatively, or in addition, high baseline and highly variable TU cell densities could reflect dynamic fluctuations in STAT5 phosphorylation by other molecules with receptors in the mediobasal hypothalamus that influence their target cells, at least in part, via the STAT5 pathway. Signaling molecules in mammalian species that fit this description include leptin [30,31], growth hormone [32], tumor necrosis factor- α [33], granulocyte macrophage colony stimulating factor [34,35], erythropoietin [36], and interleukin-7 [37]. Interestingly, the high baseline pSTAT5-ir cell densities and high within-group variability that were observed in untreated, non-breeding doves were not seen in non-breeding doves given osmotic minipumps filled with vehicle. At present, the physiological basis for this disparity remains unclear.

From a functional standpoint, it is significant that many of the dove brain sites where increased STAT5 phosphorylation occurred following exogenous prolactin administration or during spontaneous elevations in plasma prolactin levels during the breeding cycle are also sites at which prolactin acts to stimulate parental behavior in this species. As in mammals [38,39], the preoptic area is a critical component of the prolactin-sensitive circuitry underlying parental behavior. Immunohistochemical mapping of the immediate early gene *fos* reveals that neurons in the POA, LH, and SL, which our results indicate are rich in pSTAT5 and which previous studies indicate are rich in prolactin receptors [6,7], are strongly activated in parent doves following exposure to their young [20]. In addition, axon-sparing lesions of the POA lead to profound deficits in prolactin-induced parental regurgitation feeding behavior in doves, which is necessary for parental transfer of food to

the young [40]. Finally, as discussed below, the POA, TU, and VMN are all implicated as sites of prolactin action in promoting the dramatic increase in food intake shown by parent doves while provisioning their young.

One of the best documented effects of prolactin in the mammalian brain is its short loop feedback action involving the stimulation of tuberoinfundibular dopamine neurons in the arcuate nucleus of the hypothalamus, which release their contents into the median eminence to inhibit pituitary prolactin secretion [41]. Prolactin receptor mRNA or protein have been detected in these neurons [23,43,44] and they show STAT5 phosphorylation in response to prolactin treatment [25,27,28]. Prolactin receptors are also plentiful in the dove TU, which is the avian counterpart of the ARC [6,7], and the present results further suggest that prolactin could signal in this region via STAT5 phosphorylation. Although the neurochemical identity of these prolactin-sensitive neurons cannot be identified until dual-label in situ hybridization or immunohistochemistry studies are conducted, they are unlikely to be dopaminergic. Although the TU reportedly contains a population of dopaminergic neurons in zebra finches [44,45] and budgerigars [46], few dopaminergic neurons are found in this region in many other birds, including doves [47], pigeons [48,49], canaries [50], chickens [51–53] and Japanese quail [54]. These differences in the localization of dopamine neurons in avian and mammalian hypothalamus also imply differences in the dopaminergic regulation of prolactin secretion. In most of the avian species that have been studied, DA neurons in the caudal hypothalamus are mainly localized to a region dorsal or dorsolateral to the TU [47–54]. In the turkey, there is evidence that these dopaminergic neurons indirectly increase prolactin secretion by stimulating a population of vasoactive intestinal peptide (VIP) neurons in the TU, which in turn release their contents into the median eminence to increase pituitary prolactin secretion [55,56]. That VIP is the principal prolactin releasing factor in birds is well established [55,57] and there appears to be a sizable population of VIP neurons in the TU region of many avian species [see 47 for review]. Given these observations and the importance of short-loop feedback regulation by prolactin in mammalian species, it would be interesting to explore the possibility that the pSTAT5-ir cells that we detected in the dove TU are VIP neurons.

Other neurons in the TU that could conceivably respond to prolactin by exhibiting increased pSTAT5 phosphorylation are those that secrete the orexigenic peptides neuropeptide Y (NPY) and agouti-related peptide (AGRP). Prolactin is elevated in dove plasma during the post-hatching period when parent doves increase their food intake to provision their young [12,13], and because prolactin dramatically elevates food intake in non-breeding doves when administered via intracerebroventricular or subcutaneous injection [5,58], it is likely to be a major hormonal stimulus for this “parental hyperphagia”. Previous microinjection studies have shown that the TU and two other hypothalamic regions of high pSTAT5 and prolactin receptor density, the POA and VMN, are sites at which prolactin stimulates this hyperphagic response [59]. NPY and AGRP have both been shown to increase food intake in doves [60,61], and neurons that synthesize these orexigenic peptides are concentrated in the dove TU [62,63]. It is likely that these neurons are responsive to prolactin because the numbers of immunoreactive NPY and AGRP cells in the dove TU increase in response to prolactin administration and during the post-hatching phase of the breeding cycle when peak prolactin levels and increased parental foraging are observed [62,63]. However, it remains to be

determined if prolactin influences these cells directly or indirectly. Parallel studies in the rat indicate that prolactin increases NPY expression in the dorsomedial nucleus of the hypothalamus, but not in the arcuate nucleus [64].

Neuroanatomical and electrophysiological studies in rats have demonstrated that prolactin acts via STAT5 to influence oxytocin neurons in the PVN [27,28,65]. Because mesotocin, the avian homolog of oxytocin, is produced in this same region of the avian hypothalamus [66–69] it would be fruitful to investigate the possibility that the neurons in the dove PVN that exhibit increased STAT5 phosphorylation are mesotocinergic. Indirect support for prolactin involvement in mesotocin expression is provided by a recent series of studies by Thayananuphat and colleagues [69] in turkeys. These authors found that the number of mesotocin-ir neurons in the PVN increase when plasma prolactin levels are elevated during late incubation. An effect of prolactin on mesotocin in birds would be functionally consistent with its oxytocin-regulating effect in mammals, since both neuropeptides influence social and parental behavior [70–72]. In support of this idea, Thayananuphat et al. [69] also reported that mesotocinergic neurons in the PVN and other brain regions showed increased cfos mRNA expression when poults were introduced to incubating hens. Furthermore, blocking the action of mesotocin with an oxytocin receptor antagonist also interferes with the onset of brooding behavior and the neuronal activation that occurs in incubating turkey hens given poults [69]. Oxytocin receptor antagonists also exert inhibitory effects on social behavior in female zebra finches as measured by several indices, including pair bond formation [70], preference for spending time in large groups, and preference for familiar individuals [71]. Such effects closely parallel the reported effects of oxytocin on mammalian social and maternal behavior [see 72 for review].

If our studies in doves are broadly representative of other avian species, it would appear that the remarkable commonalities in prolactin function and target cell sensitivity in the CNS that have been documented in birds and mammals [1] could be mediated through similar signal transduction mechanisms. While JAK2-STAT5 is the best characterized prolactin signaling pathway in mammalian brain [10,11,25], other pathways are also implicated. For example, recent studies in the rat indicate that intracerebroventricular injections of prolactin increase the phosphorylation of two other STAT family proteins (STAT 1 and 3) and activate the mitogen-activated protein kinase signaling pathway in corticotrophin releasing hormone (CRH), oxytocin, and vasopressin neurons in the PVN and supraoptic nucleus of the hypothalamus [73]. In light of such evidence, further exploration of the importance of these and other pathways in mediating prolactin action in the avian brain will be required to adequately determine the degree to which the underlying signaling mechanisms are evolutionarily conserved.

Acknowledgments

This work was supported by NIMH grant 1 R56 MH41447. We also wish to acknowledge Dr. A.F. Parlow and the National Hormone and Pituitary Program (NHPP) for supplying the purified ovine prolactin used in these studies.

Abbreviations

JAK2	Janus Kinase 2
STAT5	Signal Transducer and Activator of Transcription 5
pSTAT5	phosphorylated STAT5
ir	immunoreactivity
POA	preoptic area
SCN	suprachiasmatic nucleus of the hypothalamus
PVN	paraventricular nucleus of the hypothalamus
VMN	ventromedial nucleus of the hypothalamus
LH	lateral hypothalamic area
TU	tuberal hypothalamic area
SL	lateral septum
BSTL	lateral bed nucleus of the stria terminalis

References

1. Ziegler TE. Hormones associated with non-maternal infant care: a review of mammalian and avian studies. *Folia Primatol (Basel)*. 2000; 71:6–21. [PubMed: 10686483]
2. Angelier F, Chastel O. Stress, prolactin and parental investment in birds: a review. *Gen Comp Endocrinol*. 2009; 163:142–148. [PubMed: 19351537]
3. Buntin JD. Neural and hormonal control of parental behavior in birds. *Adv Study Behav*. 1996; 25:161–213.
4. Buntin JD, Becker GM, Ruzycki E. Facilitation of parental behavior in ring doves by systemic or intracranial injections of prolactin. *Horm Behav*. 1991; 25:424–444. [PubMed: 1937430]
5. Buntin JD, Tesch D. Effects of intracranial prolactin administration on maintenance of incubation readiness, ingestive behavior, and gonadal condition in ring doves. *Horm Behav*. 1985; 19:188–203. [PubMed: 4007798]
6. Fechner JH Jr, Buntin JD. Localization of prolactin binding sites in ring dove brain by quantitative autoradiography. *Brain Res*. 1989; 487:245–254. [PubMed: 2731045]
7. Buntin JD, Ruzycki E, Witebsky J. Prolactin receptors in dove brain: autoradiographic analysis of binding characteristics in discrete brain regions and accessibility to blood-borne prolactin. *Neuroendocrinology*. 1993; 57:738–750. [PubMed: 8367036]
8. Chilton BS, Hewetson A. Prolactin and growth hormone signaling. *Current Top Devel Biol*. 2005; 68:1–23.
9. Boyle-Feysot C, Goffin V, Edery M, Binart N, Kelly PA. Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. *Endocr Rev*. 1998; 19:225–268. [PubMed: 9626554]
10. Grattan DR, Xu J, McLachlan MJ, Kokay IC, Bunn SJ, Hovey RC, Davy HW. Feedback regulation of PRL secretion is mediated by the transcription factor, signal transducer, and activator of transcription 5b. *Endocrinology*. 2001; 143:3935–3940. [PubMed: 11517172]
11. Lerant A, Kanyicska B, Freeman ME. Nuclear translocation of STAT5 and increased expression of Fos related antigens (FRAs) in hypothalamic dopaminergic neurons after prolactin administration. *Brain Res*. 2001; 904:259–269. [PubMed: 11406124]

12. Goldsmith AR, Edwards C, Koprucu M, Silver R. Concentrations of prolactin and luteinizing hormone in plasma of doves in relation to incubation and development of the crop gland. *J Endocrinol.* 1981; 90:437–443. [PubMed: 7276801]
13. Buntin JD, Hnasko RM, Zuzick PH, Valentine DL, Scammell JG. Changes in bioactive prolactin-like activity in plasma and its relationship to incubation behavior in breeding ring doves. *Gen Comp Endocrinol.* 1996; 102:221–232. [PubMed: 8998966]
14. Nicoll, CS.; White, BA.; Leung, FC. Evolution of prolactin, its functions, and its receptors. In: MacLeod, RM.; Scapagnini, U., editors. *Central and Peripheral Regulation of Prolactin Function.* Raven Press; New York: 1980. p. 11-25.
15. Buntin JD, Ruzycki E. Characteristics of prolactin binding sites in the brain of the ring dove (*Streptopelia risoria*). *Gen Comp Endocrinol.* 1987; 65:243–253. [PubMed: 3817448]
16. Buntin JD, Figge GR. Prolactin and growth hormone stimulated food intake in ring doves. *Pharmacol Biochem Behav.* 1989; 31:533–540. [PubMed: 3251237]
17. Karten, HJ.; Hodos, W. *A Stereotaxic Atlas of the Brain of the Pigeon.* The Johns Hopkins University Press; Baltimore: 1967.
18. Kuenzel, WJ.; Masson, M. *A Stereotaxic Atlas of the Brain of the Chick (Gallus domesticus).* The Johns Hopkins University Press; Baltimore: 1988.
19. Reiner A, Perkel DJ, Bruce LL, Butler AB, Csillag A, Kuenzel W, Medina L, Paxinos G, Shimizu T, Striedter G, Wild M, Ball GF, Durand S, Gütürkün O, Lee DW, Mello CV, Powers A, White SA, Hough G, Kubikova L, Smulders TV, Wada K, Dugas-Ford J, Husband S, Yamamoto K, Yu J, Siang C, Jarvis ED. Revised nomenclature for avian telencephalon and some related brainstem nuclei. *J Comp Neurol.* 2004; 473:377–414. [PubMed: 15116397]
20. Buntin L, Berghman LR, Buntin JD. Patterns of fos-like immunoreactivity in the brains of parent ring doves (*Streptopelia risoria*) given tactile and non-tactile exposure to their young. *Behav Neurosci.* 2006; 120:651–664. [PubMed: 16768617]
21. Brown MB, Forsythe AB. Robust tests for the equality of variances. *J Am Stat Assoc.* 1974; 69:364–367.
22. Siegel, S. *Nonparametric Statistics for the Behavioral Sciences.* McGraw-Hill; New York: 1956.
23. Brown RSE, Kokay IC, Herbison AE, Grattan DR. Distribution of prolactin-responsive neurons in the mouse forebrain. *J Comp Neurol.* 2010; 518:92–102. [PubMed: 19882722]
24. Darnell JE Jr. STATs and gene regulation. *Science.* 1997; 277:1630–1635. [PubMed: 9287210]
25. Ma FY, Anderson GM, Gunn TD, Goffin V, Grattan DR, Bunn SJ. Prolactin specifically activates signal transducer and activator of transcription 5b in neuroendocrine dopaminergic neurons. *Endocrinology.* 2005; 146:5112–5119. [PubMed: 16123156]
26. Brown RSE, Herbison AE, Grattan DR. Differential changes in responses of hypothalamic and brainstem neuronal populations to prolactin during lactation in the mouse. *Biol Reprod.* 2011; 84:826–836. [PubMed: 21178171]
27. Sjoeholm A, Bridges RS, Grattan DR, Anderson GM. Region-, neuron-, and signaling pathway-specific increases in prolactin responsiveness in reproductively experienced female rats. *Endocrinology.* 2011; 152:1979–1988. [PubMed: 21363933]
28. Sapsford TJ, Kokay IC, Ostberg L, Bridges RS, Grattan DR. Differential sensitivity of specific neuronal populations of the rat hypothalamus to prolactin action. *J Comp Neurol.* 2012; 520:1062–1077. [PubMed: 21953590]
29. Brown RSE, Piet R, Herbison AE, Grattan DR. Differential actions of prolactin on electrical activity and intracellular signal transduction in hypothalamic neurons. *Endocrinology.* 2012; 153:2375–2384. [PubMed: 22416085]
30. Mutze J, Roth J, Gerstberger R, Hübschle T. Nuclear translocation of the transcription factor STAT5 in the rat brain after systemic leptin administration. *Neurosci Lett.* 2007; 417:286–291. [PubMed: 17353091]
31. Gong Y, Ishida-Takahashi R, Villanueva EC, Fingar DE, Münzberg H, Meyers MG. The long form of the leptin receptor regulates STAT5 and ribosomal protein S6 via alternate mechanisms. *J Biol Chem.* 2007; 282:31019–31027. [PubMed: 17726024]

32. Bennett E, McGuinness L, Gevers EF, Thomas GB, Robinson IC, Davey HW, Luckman SM. Hypothalamic STAT proteins: regulation of somatostatin neurons by growth hormone via STAT5b. *J Neuroendocrinol.* 2005; 17:186–194. [PubMed: 15796771]
33. Rizk NM, Stammers D, Preibisch G, Eckel J. Leptin and tumor necrosis factor- α induce the tyrosine phosphorylation of signal transducer and activator of transcription proteins in the hypothalamus of normal rats *in vivo*. *Endocrinology.* 2001; 142:3027–3032. [PubMed: 11416024]
34. Reed JA, Clegg DJ, Smith KB, Tolod-Richer EG, Matter EK, Picard LS, Seeley RJ. GM-CSF action in the CNS decreases food intake and body weight. *J Clin Invest.* 2005; 115:3035–44. [PubMed: 16276414]
35. Lee J-Y, Muenzberg H, Gavrilova O, Reed JA, Berryman D, Villanueva EC, Louis GW, Leininger GM, Bertuzzi S, Seeley RJ, Robinson GW, Myers MG Jr, Hennighausen L. Loss of cytokine-STAT5 signaling in the CNS and pituitary gland alters energy balance and leads to obesity. *PLoS One.* 2008; 3:e1639. [PubMed: 18286195]
36. Teng R, Gavrilova O, Suzuki N, Chanturiya T, Schimel D, Hugendubler L, Mammen S, Yver DR, Cushman SW, Yamamoto M, Hsu LL, Noguchi CT. Disrupted erythropoietin signaling promotes obesity and alters hypothalamus proopiomelanocortin production. *Nat Commun.* 2011; 2:520. [PubMed: 22044999]
37. Macia L, Viltart O, Delacre M, Sachot C, Héliot L, DiSanto JP, Wolowczuk I. Interleukin-7, a new cytokine targeting the mouse hypothalamic arcuate nucleus: role in body weight and food intake regulation. *PLoS One.* 2010; 5:e9953. [PubMed: 20376352]
38. Numan M, Stolzenberg DS. Medial preoptic area interactions with dopamine neural systems in the control of the onset and maintenance of maternal behavior in rats. *Front Neuroendocrinol.* 2009; 30:46–64. [PubMed: 19022278]
39. Bridges RS, Numan M, Ronsheim PM, Mann PE, Lupini CE. Central prolactin infusions stimulate maternal behavior in steroid-treated, nulliparous female rats. *Science.* 1990; 87:8003–8007.
40. Slawski BA, Buntin JD. Preoptic area lesions disrupt prolactin-induced parental feeding behavior in ring doves. *Horm Behav.* 1995; 29:248–266. [PubMed: 7557926]
41. Moore KE. Interactions between prolactin and dopaminergic neurons. *Biol Reprod.* 1987; 36:47–58. [PubMed: 3552068]
42. Kokay IC, Grattan DR. Expression of mRNA for prolactin receptor (long form) in dopamine and pro-opiomelanocortin neurons in the arcuate nucleus of pregnant and lactating rats. *J Neuroendocrinol.* 2005; 17:827–835. [PubMed: 16280030]
43. Augustine RA, Kokay IC, Andrews ZB, Ladyman SR, Grattan DR. Quantitation of prolactin receptor mRNA in the maternal rat brain during pregnancy and lactation. *J Mol Endocrinol.* 2003; 31:221–232. [PubMed: 12914538]
44. Bottjer SW. The distribution of tyrosine hydroxylase immunoreactivity in the brains of male and female zebra finches. *J Neurobiol.* 1993; 24:51–69. [PubMed: 8093477]
45. Bharati IS, Goodson JL. Fos responses of dopamine neurons to sociosexual stimuli in male zebra finches. *Neuroscience.* 2006; 143:661–670. [PubMed: 17027163]
46. Roberts TF, Cookson KK, Heaton KJ, Hall WS, Brauth SE. Distribution of tyrosine hydroxylase-containing neurons and fibers in the brain of the budgerigar (*Melopsittacus undulatus*): general patterns and labeling in vocal control nuclei. *J Comp Neurol.* 2001; 429:436–454. [PubMed: 11116230]
47. den Boer-Visser AM, Dubbeldam JL. The distribution of dopamine, substance P, vasoactive intestinal polypeptide and neuropeptide Y immunoreactivity in the brain of the collared dove (*Streptopelia decaocto*). *J Chem Neuroanat.* 2002; 23:1–27. [PubMed: 11756007]
48. Reiner, A.; Karle, EJ.; Anderson, KD.; Medina, L. Catecholamine perikarya and fibers in the avian nervous system. In: Smeets, WJ.; Reiner, A., editors. *Phylogeny and Development of Catecholamine Systems in the CNS of Vertebrates*. Cambridge University Press; Cambridge: 1994. p. 135-181.
49. Kiss JZ, Péczei P. Distribution of tyrosine hydroxylase (TH)-immunoreactive neurons in the diencephalon of the pigeon (*Columba livia domestica*). *J Comp Neurol.* 1987; 257:333–346. [PubMed: 2881951]

50. Appelants D, Ball GF, Balthazart J. The distribution of tyrosine hydroxylase in the canary brain: distribution of a specific and sexually dimorphic catecholaminergic innervation of the telencephalic song nuclei. *Cell Tissue Res.* 2001; 304:237–259. [PubMed: 11396718]
51. Moons L, van Gils J, Ghijsels E, Vandesande F. Immunocytochemical localization of L-dopa and dopamine in the brain of the chicken (*Gallus domesticus*). *J Comp Neurol.* 1994; 346:97–118. [PubMed: 7962714]
52. Sartsoongnoen N, Kosonsiriluk S, Prakobsaeng N, Songserm T, Rozenboim I, El Halawani M, Chaiseha Y. The dopaminergic system in the brain of the native Thai chicken, *Gallus domesticus*: localization and differential expression across the reproductive cycle. *Gen Comp Endocrinol.* 2008; 159:107–115. [PubMed: 18765240]
53. Prakobsaeng N, Sartsoongnoen N, Kosonsiriluk S, Chaiyachet O, Chokchaloemwong D, Rozenboim I, El Halawani M, Porter T, Chaiseha Y. Changes in vasoactive intestinal peptide and tyrosine hydroxylase immunoreactivity in the brain of nest-deprived native Thai hen. *Gen Comp Endocrinol.* 2011; 171:1889–196.
54. Bailhache T, Balthazart J. The catecholaminergic system of the quail brain: immunocytochemical studies of dopamine beta-hydroxylase and tyrosine hydroxylase. *J Comp Neurol.* 1993; 329:230–256. [PubMed: 8095939]
55. El Halawani, ME.; Youngren, O.; Chaiseh, Y. Neuroendocrinology of prolactin regulation in the domestic turkey. In: Dawson, A.; Chaturvedi, CM., editors. *Avian Endocrinology*. Narosa Publishing House; New Delhi: 2001. p. 233-244.
56. Bhatt R, Youngren O, Kang S, El Halawani M. Dopamine infusion into the third ventricle increases gene expression of hypothalamic vasoactive intestinal peptide and pituitary prolactin and luteinizing hormone beta subunit in the turkey. *Gen Comp Endocrinol.* 2003; 130:41–47. [PubMed: 12535623]
57. Sharp PJ, Dawson A, Lea RW. Control of luteinizing hormone and prolactin secretion in birds. *Comp Biochem Physiol C Pharmacol Toxicol Endocrinol.* 1998; 119:275–282. [PubMed: 9827000]
58. Buntin JD, Figge GR. Prolactin and growth hormone stimulate food intake in ring doves. *Pharmacol Biochem Behav.* 1988; 31:533–540. [PubMed: 3251237]
59. Hnasko RM, Buntin JD. Functional mapping of neural sites mediating prolactin-induced hyperphagia in doves. *Brain Res.* 1993; 623:257–266. [PubMed: 8221107]
60. Strader AD, Buntin JD. Neuropeptide Y: A possible mediator of prolactin-induced feeding and regulator of energy balance in the ring dove (*Streptopelia risoria*). *J Neuroendocrinol.* 2001; 13:286–392.
61. Strader AD, Schiöth H, Buntin JD. The role of the melanocortin system and the melanocortin-4 receptor in ring dove (*Streptopelia risoria*) feeding behavior. *Brain Res.* 2003; 960:112–121. [PubMed: 12505663]
62. Strader AD, Buntin JD. Changes in agouti-related peptide during the ring dove breeding cycle in relation to prolactin and parental hyperphagia. *J Neuroendocrinol.* 2003; 15:1046–1053. [PubMed: 14622434]
63. Ramakrishnan S, Strader AD, Wimpee B, Chen P, Smith MS, Buntin JD. Evidence for increased neuropeptide Y synthesis in mediobasal hypothalamus in relation to parental hyperphagia and gonadal activation in breeding ring doves. *J Neuroendocrinol.* 2007; 19:163–171. [PubMed: 17280589]
64. Chen P, Smith MS. Regulation of hypothalamic neuropeptide Y messenger ribonucleic acid expression during lactation: role of prolactin. *Endocrinology.* 2004; 145:823–829. [PubMed: 14617575]
65. Kokay IC, Bull PM, Davis RL, Ludwig M, Grattan DR. Expression of the long form of the prolactin receptor in magnocellular oxytocin neurons is associated with specific prolactin regulation of oxytocin neurons. *Am J Physiol Regul Integr Comp Physiol.* 2006; 290:R1216–1225. [PubMed: 16410399]
66. Goossens N, Blähser S, Oksche A, Vandesande F, Dierickx K. Immunocytochemical investigation of the hypothalamo-neurohypophysial system in birds. *Cell Tissue Res.* 1977; 184:1–13. [PubMed: 922855]

67. Barth SW, Bathgate RAD, Mess A, Parry LJ, Ivell R, Grossman R. Mesotocin gene expression in the diencephalon of domestic fowl: cloning and sequencing of the MT cDNA and distribution of MT gene expressing neurons of the chicken hypothalamus. *J Neuroendocrinol.* 1999; 9:777–787. [PubMed: 9355047]
68. Bons N. The topography of mesotocin and vasotocin systems in the brain of the domestic mallard and Japanese quail: immunocytochemical identification. *Cell Tissue Res.* 1980; 213:37–51. [PubMed: 7459995]
69. Thayananuphat A, Youngren OM, Kang SW, Bakken T, Kosonsiriluk S, Chaiseha Y, El Halawani ME. Dopamine and mesotocin neurotransmission during the transition from incubation to brooding in the turkey. *Horm Behav.* 2011; 60:327–335. [PubMed: 21741977]
70. Goodson JL, Kelly AM, Kingsbury MA. Evolving nonapeptide mechanisms of gregariousness and social diversity in birds. *Horm Behav.* 2012; 61:239–250. [PubMed: 22269661]
71. Goodson JL, Schrock SE, Klatt JD, Kabelik D, Kingsbury MA. Mesotocin and nonapeptide receptors promote estrildid flocking behavior. *Science.* 2009; 325:862–866. [PubMed: 19679811]
72. Neumann ID. Brain oxytocin: a key regulator of emotional and social behaviours in both females and males. *J Neuroendocrinol.* 2008; 20:858–865. [PubMed: 18601710]
73. Blume A, Torner L, Liu Y, Subburaju S, Aguilera G, Neumann ID. Prolactin activates mitogen-activated protein kinase signaling and corticotrophin releasing hormone transcription in rat hypothalamic neurons. *Endocrinology.* 2009; 150:1841–1849. [PubMed: 19022892]

HIGHLIGHTS

- Prolactin-mediated JAK2-STAT5 signaling was examined in dove brain
- Activated-STAT5 cell densities peaked in breeding females with high prolactin levels
- Prolactin-treated doves showed more activated STAT5 than vehicle-treated doves
- JAK2-STAT5 could mediate some of the effects of prolactin in the avian brain

Fig. 1.

Dove brain regions selected for analysis of pSTAT5 immunoreactive (pSTAT5-ir) cell densities. Drawings of coronal sections are taken from the Karten and Hodos [17] pigeon brain atlas. Atlas plate numbers reflect mm anterior (A) to the earbar zero reference. Sources for anatomical nomenclature, localization, and abbreviations: SL, LHy, TU, and POA from Karten and Hodos [17]; PVN, VMN and SCN from Kuenzel and Masson [18]; BSTL from Reiner *et al.* [19].

Fig. 2.

Representative pSTAT5-ir staining patterns in the POA (A–C), SCN (D–F), PVN (G–I) and VMN (J–L) of non-breeding females (C,F,I,L) and breeding females sampled during the late-incubation/early post-hatching period (A,B, D, E, G, H, J, K). Boxes in A,D,G, and J depict areas represented in B,E,H, and K, respectively. Scale bar = 100 μ m.

Fig. 3.

Mean $\pm$ SEM pSTAT5-ir cell densities in the eight forebrain regions sampled in non-breeding untreated females and in breeding females sampled prior to egg laying (pre-lay), during early incubation, and during the late incubation/early post-hatching period (post-hatch). Asterisks denote groups that differed significantly from the post-hatching group.

Fig. 4.

Representative pSTAT5-ir staining patterns in the POA (A–C), PVN (D–F), and TU (G–I) of non-breeding females given exogenous prolactin (A,B,D,E,G,H) and non-breeding females given bicarbonate-saline vehicle (C,F,I). Boxes in A,D, and G, depict areas represented in B,E, and H, respectively. Scale bar = 100 μ m.

Fig. 5.

Mean $\pm$ SEM pSTAT5-ir cell densities in the PVN, POA, and TU of non-breeding females given prolactin and non-breeding females given vehicle. Asterisks denote differences between prolactin-treated and vehicle-treated animals.