



## Review article

## Prolactin and avian parental care: New insights and unanswered questions

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## A B S T R A C T

Parental care is a critical component of reproductive success for many species, but especially for birds that have high rates of parental care. While ample studies have shown strong, positive correlational relationships between the hormone prolactin and parental care in birds, few studies in a limited number of avian species have performed the causal experiments necessary to elucidate the exact roles of prolactin during these behaviors. Additionally, how prolactin acts in the brain to affect parental behaviors is still virtually unknown with the exception of a small number of studies in very few species. Here, I review what is currently known about prolactin and avian parental care, propose a new hypothesis for prolactin's role in avian parental care, and highlight the gaps in our current understanding of prolactin's role in parental care.

## 1. Introduction

Parental care is critical for offspring survival and reproductive fitness across a range of vertebrate taxa. Birds display an enormous diversity of parental care systems ranging from brood parasitism to cooperative breeding, but the most prevalent form is biparental care (~81% of species, Cockburn, 2006). Even within biparental species, there is great variation in the degree to which males and females participate in the raising of young, making birds an extremely valuable taxon in which to study parental care behaviors. In addition to the study of division of labor between sexes, there are two phases of parental care which can be studied: incubation and the post-hatch care period. Depending on the species, either the breeding male, female, or both will incubate the eggs to keep them warm, which is referred to as brooding. Most incubating birds will form a brood patch, a small defeathered area filled with many blood vessels that efficiently transfer heat to developing eggs (Bailey, 1952). Incubation often requires constant nest attentiveness and the transfer of heat is highly energetically demanding (Buntin et al., 2008; Vleck, 1981). Parents usually decrease feeding or even fast during incubation because they cannot leave the eggs unattended for long periods of time (Buntin et al., 2008; Sherry et al., 1980). Alternatively, a small number of birds bury their eggs underground to use geothermal heat, circumventing the need to incubate (Cockburn, 2006). Others bypass this parental care stage by laying their eggs in other birds' nests, known as brood parasitism (Cockburn, 2006), leaving all parental care duties up to an unsuspecting foster parent.

Post-hatch chick provisioning can be provided by one parent (uniparental care; either the male or female), both parents (biparental care) or by a group of individuals, known as cooperative breeding. In the latter case, non-breeding individuals who are not the biological parents, known as helpers, contribute significant amounts of parental care

towards the young. The amount of post hatch care contributed by an individual is dependent upon the type of offspring that hatch, either altricial or precocial, and the social structure in place for the division of care. For instance, birds can hatch precocial young, which are developmentally mature upon hatching and are usually able to walk and feed on their own, and require relatively little post-hatch care. Alternatively, many birds hatch altricial young, which are developmentally immature, often born with their eyes closed and lacking the ability to thermoregulate or feed on their own. In contrast to precocial young, altricial species require constant brooding and feeding. In both cases, parents will also defend the nest and chicks from predators and other threats. See Fig. 1 for examples of parental care behaviors with precocial and altricial young.

While the neuroendocrine mechanisms of avian parental behavior are still not sufficiently well understood, much focus has been placed on the role of the peptide hormone prolactin (PRL). PRL was initially discovered as a hormone involved in mammalian lactation but has since been discovered to have around 300 different functions including osmoregulation, metabolism, reproduction, feeding behavior, and modulation of the stress response, among many others (Bole-Feysot et al., 1998). Accordingly, the PRL receptor can be found in many tissues throughout the body, but most important for behavior, it is found throughout the central nervous system (Bole-Feysot et al., 1998).

In virtually all birds studied to date, circulating PRL levels are relatively low during non-breeding times, but significantly increase during incubation, the post-hatch care period, or both (Buntin, 1996; Ohkubo, 2017; Smiley and Adkins-Regan, 2016a; Vleck and Vleck, 2011). Numerous correlational studies have shown that high PRL titers have a strong, positive relationship with the expression of parental care behaviors and increased reproductive success (e.g., Chastel et al., 2005; Miller et al., 2009; Ouyang et al., 2011; Riechert et al., 2014; Smiley

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**Fig. 1.** Examples of avian parental care. (A) Wood duck mother with precocial young. Photo by Debra Bangasser. (B) Black-capped Gnatcatcher feeding altricial young. Photo by Andy J. Boyce.

and Adkins-Regan, 2016b). In addition, PRL levels tend to reflect parental investment decisions made during times of increased stress, such as food shortages or extreme weather, with higher levels reported in good breeding years and lower levels reported in poor breeding years (reviewed in Angelier and Chastel, 2009). However, fewer studies in a limited number of avian species have actually manipulated PRL to provide causal evidence for such relationships. In particular, most of the work on PRL's role in incubation has been performed in chickens and turkeys, while PRL's role in post-hatch parental care behavior, including other behaviors related to parental care such as hyperphagia (increased eating), have been limited primarily to ring doves, zebra finches, and penguins. I will briefly summarize the current state of knowledge on PRL and avian parental care, including both peripheral and central effects of PRL, and describe how PRL interacts with previous reproductive experience, stimuli from the nest, eggs, and chicks, and other hormonal systems. Finally, I will propose a new hypothesis about PRL's role in avian parental care and will highlight some critical missing information that is still needed in order to fully understand PRL's many roles in avian parental care.

## 2. Peripheral prolactin and avian parental care

### 2.1. Regulation of prolactin secretion in birds

Prolactin is produced in lactotroph cells in the anterior pituitary gland and is primarily under stimulatory control from vasoactive intestinal peptide (VIP). VIP is released from neurons in the infundibular nuclear complex in the hypothalamus which project to the median eminence, and acts via its receptors on the lactotroph cells to stimulate PRL gene expression and the release of PRL into the circulation (Kahtane et al., 2003; Sharp et al., 1998; Xu et al., 1996). In incubating turkey hens, when PRL is highest, there is a greater number of immunoreactive VIP neurons and increased VIP concentrations in the hypophyseal portal vein correspond with increased circulating levels of PRL (reviewed in Ohkubo, 2017). In addition, lactotroph cells size and number of PRL-producing cells increase in the pituitary, which coincides with the major elevation in circulating PRL levels (reviewed in Ohkubo, 2017). Administration of VIP-antibodies (passive immunization against VIP) prevents the increase in PRL during this time (Sharp et al., 1989).

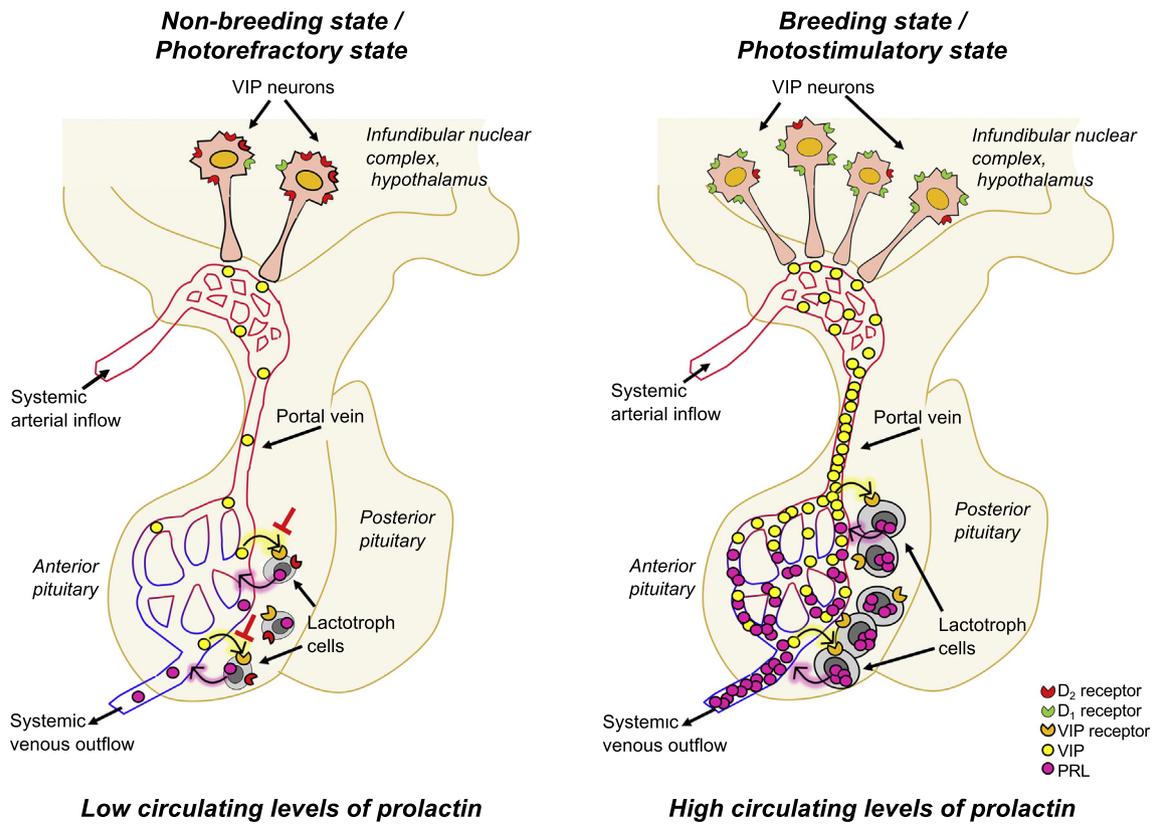
In contrast to mammals, where dopamine (DA) tonically inhibits PRL secretion, DA released from neurons in the infundibular nuclear complex has both stimulatory and inhibitory effects on avian PRL release, depending on which DA receptor subtype it acts upon (Youngren et al., 1995, 1996, 1998). DA acting at the  $D_1$  receptor stimulates VIP neurons to enhance PRL secretion (Youngren et al., 1996, 2002), while DA acting at the  $D_2$  receptor antagonizes the actions of VIP (El

Halawani et al., 1991). Relative expression levels of  $D_1$  and  $D_2$  receptors differ by reproductive state depending on whether there are high or low circulating levels (Ohkubo, 2017). DA can also regulate PRL release at the level of the anterior pituitary by inhibiting the actions of VIP release (Xu et al., 1996). The DA signaling pathways involved in PRL regulation have been primarily studied in mammalian models (for a review see Fitzgerald and Dinan, 2008). Mechanistic studies on DA's role in stimulating and inhibiting PRL release in birds are still required, especially in both seasonally and non-seasonally breeding birds (see next paragraph). For a summary of PRL regulation depending on reproductive state, refer to Fig. 2.

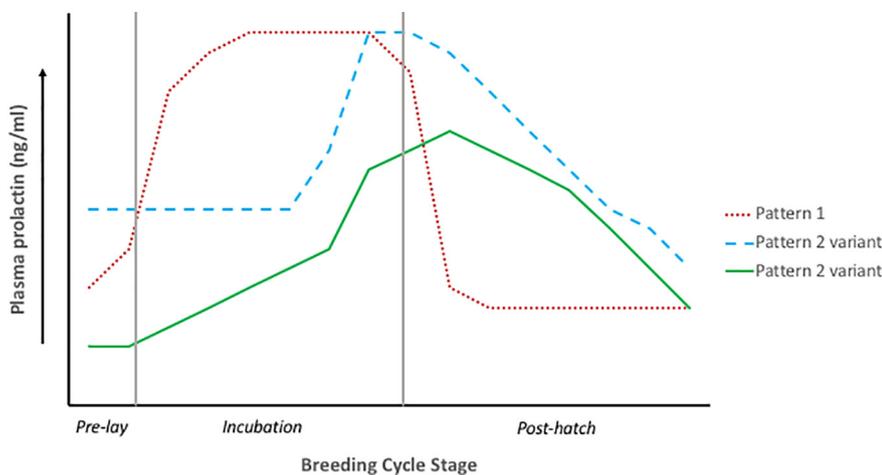
In many seasonally breeding birds living in temperate zones, PRL is under photoregulatory control, with increases in PRL co-occurring with increases in day length and the onset of the breeding season (Dawson et al., 2001). Active immunization against VIP prevents photo-induced PRL secretion in turkeys (El Halawani et al., 1996). Near the end of the breeding season, as day length becomes shorter, birds enter a photorefractory state in which they no longer respond to day light, and PRL slowly declines back to low levels (Dawson et al., 2001).  $D_2$  receptors increase during times of photorefractoriness in turkey hens (Ohkubo, 2017), consistent with its role in suppressing PRL release. Christensen and Vleck (2008) confirmed that zebra finches (*Taeniopygia guttata*), which are nonphotoperiodic breeders that are capable of breeding any time of the year, also respond to VIP with increased PRL. VIP has also been shown to increase PRL in other passerines including three subspecies of white crowned sparrows (*Zonotrichia leucophrys gambelii*), dark-eyed juncos (*Junco hyemalis*), Mexican jays (*Aphelocoma ultramarina*), blue jays (*Cyanocitta cristata*), Florida scrub-jays (*Aphelocoma coerulescens*), and California scrub jays (*Aphelocoma californica*) (Maney et al., 1999; Vleck and Patrick, 1999), establishing that VIP is indeed the primary releasing factor of PRL in birds. While DA has been less studied in nonphotoperiodic breeding birds, bromocriptine, a  $D_2$  receptor agonist, has successfully been used to lower PRL in a number of species including house finches (Badyaev and Duckworth, 2005), domestic chicken hens (Reddy et al., 2007), emperor penguins (*Aptenodytes forsteri*; Angelier et al., 2006), Adélie penguins (*Pygoscelis adeliae*; Cottin et al., 2014; Thierry et al., 2013), and zebra finches (Smiley and Adkins-Regan, 2018a) suggesting DA regulation of PRL is relatively similar across avian species.

### 2.2. Prolactin and incubation

In virtually all birds studied to date, PRL is high during some part of incubation. Although there are a variety of trajectories in which the increase in PRL occurs after egg laying, these patterns can be divided roughly into two categories: birds that show an increase in PRL immediately after laying the first egg and species which the increase in



**Fig. 2.** Differences in the regulation of PRL secretion based on reproductive state. In non-laying hens, dopamine acts primarily at D<sub>2</sub> receptors on vasoactive intestinal peptide (VIP) neurons in the hypothalamus and the anterior pituitary cells to inhibit VIP's actions, which normally stimulate PRL release, resulting in low levels of circulating prolactin. In incubating hens, dopamine acts primarily at D<sub>1</sub> receptors on VIP neurons in the hypothalamus, which are upregulated in this state, releasing VIP to act on lactotroph cells in the anterior pituitary to stimulate PRL release into the circulation. The lactotroph cells increase in size and number during breeding to increase the production and release of PRL into the circulation.



**Fig. 3.** Three examples of the changes in circulating PRL levels that are observed in birds. Birds which hatch precocial young tend to have high levels of PRL immediately after egg laying but decrease rapidly after chick hatching (Pattern 1, red dotted line). Other species which hatch altricial young tend to show gradual increases in PRL over incubation (Pattern 2 variant, green solid line) or increases in PRL mid-way through incubation (Pattern 2 variant, blue dashed line). In these two latter patterns, PRL tends to remain high throughout the post-hatch care period, gradually returning to low, baseline levels as chicks approach fledging. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

PRL occurs after clutch completion (see Fig. 3). This increase occurs in both sexes when both males and females contribute to incubation and/or the provisioning of chicks. However, if only one parent incubates, then this increase tends to be higher or may only occur in the incubating sex. This includes species such as Wilson's phalaropes (*Phalaropus tricolor*) that have a sex-reversed role in which the male performs the majority of incubation (Oring et al., 1988).

PRL generally rises soon after egg laying commences and remains high until the chicks hatch in birds that have large clutch sizes and precocial young (Ohkubo, 2017). Elevated PRL is necessary to maintain in contact with the eggs while brooding. Passive immunization against

VIP in bantam hens, which reduces PRL, induces nest abandonment, but can be rescued by administration of PRL (reviewed in Buntin, 2010). Administration of PRL can induce incubation behavior in non-laying hens when given in conjunction with sex steroid priming (reviewed in Buntin, 1996).

In contrast, many birds that lay smaller clutches and hatch altricial young show the second pattern of a delayed increase in PRL until after the full clutch of eggs are laid. In contrast to the species which display the pattern above, PRL appears to play little or no role in stimulating the onset of incubation. In fact, injections of progesterone were more effective than PRL in stimulating incubation in ring doves (Lehrman,

1958; Lehrman and Brody, 1961), suggesting egg brooding may be an extension of earlier nesting behavior, which is stimulated by gonadal hormones. High PRL is, however, likely required for incubation maintenance. Incubating ring doves injected with twice daily doses of PRL maintained incubation readiness during a ten-day nest deprivation period compared to vehicle-treated controls (Janik and Buntin, 1985). The role of PRL during incubation maintenance has not been as well studied in other altricial hatching species beyond the ring dove, therefore, is difficult to generalize at this time.

While high PRL is necessary for maintaining incubation behavior for species with both PRL patterns, it is important to note that tactile contact with the eggs is also critical for maintaining both incubation behavior and high circulating levels of PRL. Contact with artificial eggs alone can stimulate an increase in PRL, brood patch development, and the initiation of incubation of the egg within 1–2 days (Massaro et al., 2007), exemplifying the bidirectional relationship between high PRL and incubation behavior. Nest disruption, egg removal, or anesthesia or denervation of the brood patch disrupts incubation and causes PRL levels to decline, while replacing the nest or eggs can reinstate high levels of PRL (reviewed in Buntin, 1996). Injecting hens with PRL during nest removal prevents the decreased interest in incubation that is normally observed in nest-deprived hens (reviewed in Buntin, 1996).

There are some notable exceptions to the patterns described above. First, penguins and other seabirds, which take long foraging trips during breeding times, maintain high PRL levels while they are away at sea despite being devoid of all egg contact for many days, suggesting PRL is under an endogenously timed mechanism (Vleck and Vleck, 2011). Experimentally lengthening or shortening the incubation period had no effect the trajectory of the PRL profile during the post-hatch period in Adélie penguins (Vleck et al., 2000) or grey-headed albatrosses (*Diomedea chrysostoma*) and black-browed albatrosses (*Diomedea melanophris*) (Hector and Goldsmith, 1985), further supporting this hypothesis. Second, bantam hens treated with anti-PRL antiserum will maintain interest in incubation for 1–3 days before deserting the nest for good (Lea et al., 1981). Because this effect on behavior is not immediate, it is likely under the control of multiple and redundant neuroendocrine mechanisms. Third, PRL increases are still observed in the brood parasitic brown-headed cowbird, which lay their eggs in other birds' nests and therefore provide no parental care to their offspring (Dufty et al., 1987). This increase in PRL may be under photoperiodic control (as described above) or play a role in anti-gonadal actions which co-occur after egg laying (see Ohkubo, 2017 for a review). Therefore, while PRL plays a critical role in incubation, clearly other factors are at play that also support incubation and there are likely species differences in how PRL levels are maintained throughout the incubation period.

### 2.3. Prolactin and post-hatch parental care

PRL tends to decrease shortly after chick hatching in species that have precocial young. A premature reduction in circulating PRL can be caused by swapping eggs for chicks in incubating hens (Leboucher et al., 1993; Richard-Yris et al., 1998; Sinpru et al., 2018). However, it may be the loss of egg stimuli, rather than the presence of chick stimuli, that is responsible for this drop in PRL in naturally breeding hens. For species with precocial young, post-hatch parental care behaviors are usually limited to leading young to food sources and brooding the chicks for about a week or so after hatching. Nonetheless, parental behavior in mallard duck hens (*Anas platyrhynchos*) is positively correlated with PRL levels after hatching and PRL injections facilitate parental responses towards chicks in turkeys, pheasants, and chickens (reviewed in Buntin, 2010). However, it should be pointed out that PRL is not required for the onset of maternal care, as simply housing hens with chicks can stimulate the onset of maternal behavior, albeit with a slower onset than what is observed in normally breeding hens (Richard-Yris et al., 1987). In these species, PRL may play more of a role in

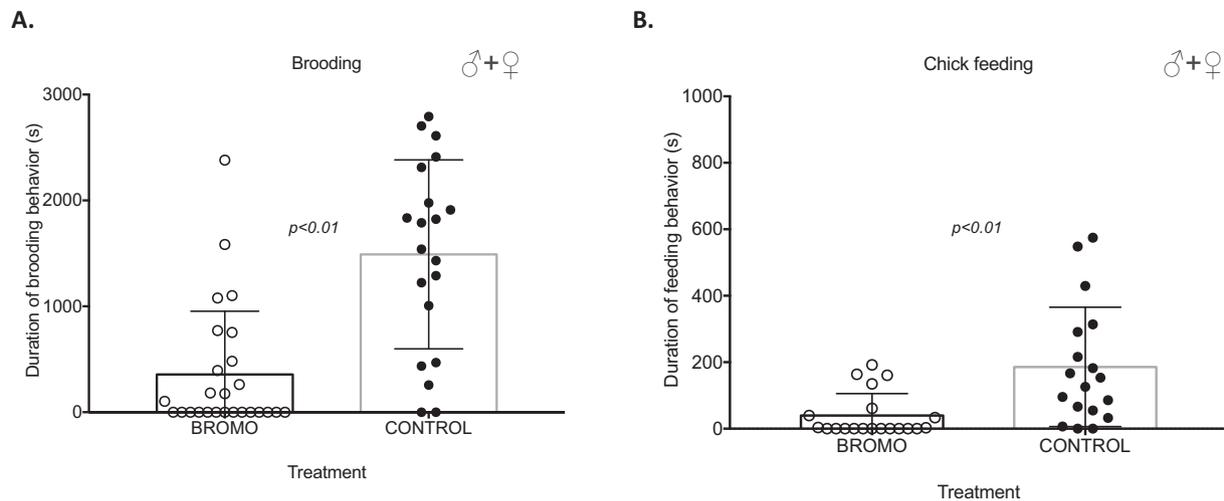
heightening the responsiveness to chicks, rather than stimulating the parental behavior motor acts per se.

In altricial hatching species, prolactin remains elevated for a large portion of the post-hatch care period, and declines at a much slower rate compared to precocial species. Similar to the role of egg stimuli for maintaining high PRL levels in precocial hatching species, high PRL is maintained by the presence of young in altricial hatching species. For example, the peak in PRL remained higher for longer in pied flycatchers (*Ficedula hypoleuca*) when chicks were repeatedly swapped out for 1–3 day chicks whereas the decline in PRL occurred quicker when newly hatched chicks were swapped out for older ones (3 days old) (Silverin and Goldsmith, 1990). Interestingly, this effect only occurred in females, which continue to brood chicks, and not males in which parental care is limited to feeding the chicks, suggesting tactile input from chicks is required for maintaining high PRL levels in this species. This study, again, highlights the bidirectional relationship between high PRL levels and parenting behaviors. It should be noted throughout that while high PRL may be required to show parental care, high levels PRL are dependent on being in contact with the egg/chick stimulus. This may be to ensure that high PRL levels only occur when parental behavior is appropriate (i.e., in response to fertile eggs that require incubating or to young, dependent chicks that require provisioning).

The role of chick stimuli in eliciting parental care behaviors and the release of PRL is exemplified by the fact that many cooperative breeding helpers also show an increase in PRL when provisioning young. Helpers generally contribute significant amounts of parental care although they have not undergone mating or egg laying. Elevated PRL concentrations have been positively associated with levels of provisioning by helpers in red-cockaded woodpeckers (*Picoides borealis*; Khan et al., 2001), Florida scrub-jays (*Apheloma e. coerulesens*; Schoech et al., 1996), and Harris' hawks (*Parabuteo unicinctus*; Vleck et al., 1991) helpers. In fact, during the post-hatch period Harris' hawk male helpers have higher circulating PRL concentrations than breeding males and females (Vleck et al., 1991). These male helpers also provide more food to nestlings than breeders do, which likely explains the high levels of PRL. Whether this increase in PRL is solely driven by egg/chick stimuli or a rise in PRL precedes helping behavior is not yet known.

Published causal evidence for a role of PRL in post-hatch parental care is limited to three types of birds: ring doves, zebra finches, and penguins. Daniel Lehrman (Lehrman, 1955) was the first to show that PRL administration into reproductively experienced ring doves facilitated the onset of regurgitation behavior. Since then, several lines of work indicate that PRL may have multiple functions in ring doves. First, PRL may influence the attractiveness of chicks. Moore (1976a) showed that 24 h of squab (ring dove chick) exposure alone was enough for non-breeding ring doves to form a preference for squabs over eggs in incubating males. However, administering PRL injections accelerated this preference (Moore, 1976b), suggesting PRL may make the squabs more appealing or salient to the parents. Second, PRL stimulates crop milk, which is produced by the epithelial mucosal cells along the wall of the crop sac organ and is regurgitated by both males and females to feed the squabs (Buntin, 1996). This is a unique trait that has evolved in ring doves and other species in the pigeon and dove family, and is critical for squab provisioning and survival. Third, both peripheral and intracerebroventricular (ICV) injections of PRL facilitate regurgitation feeding bouts, even when administered at levels that are too low to stimulate crop-milk production (Buntin et al., 1991). Injecting PRL has a much greater effect on regurgitation feeding in experienced ring dove females compared to inexperienced females (Wang and Buntin, 1999), suggesting there are complex interactions between PRL and reproductive experience to regulate this behavior. Together, these studies suggest PRL plays a role in both promoting parental attentiveness towards young as well as promoting appropriate nutrient provisioning to the young. However, because crop milk production is unique to ring doves, it has been difficult to generalize these findings to other birds.

To test whether PRL has a causal role in post-hatch care in avian



**Fig. 4.** Effects of lowering PRL on zebra finch parental care. Bromocriptine (BROMO) treatment, which lowers circulating PRL, significantly decreased the amount of time spent brooding and chick feeding behavior on day 2 post-hatch. Data above includes both males and females. Data reproduced from Smiley and Adkins-Regan, 2018a.

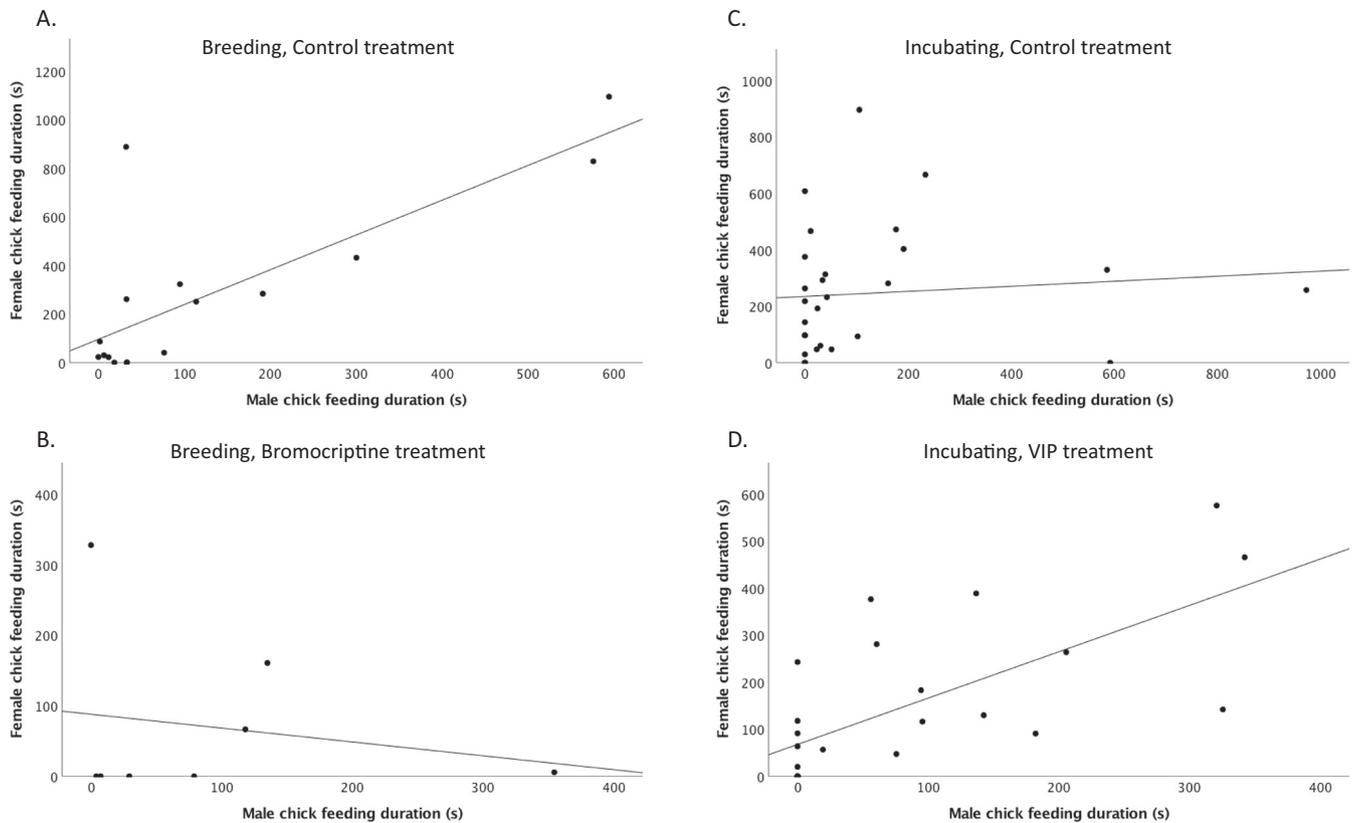
species that do not produce crop milk, I examined the role of PRL in post-hatch parental care of zebra finches, a biparental songbird species, in two different studies (Smiley and Adkins-Regan, 2018a, 2018b). In the first study, we treated male and female pairs with bromocriptine (a DA D<sub>2</sub> receptor agonist, which suppresses PRL) during the last three days of incubation and the first two days of post-hatch care, when PRL levels were found to be the highest (Smiley and Adkins-Regan, 2016a). This treatment either eliminated or greatly reduced the amount of time pairs spent brooding and feeding the chicks, compared to pairs which received a vehicle control treatment, see Fig. 4 (Smiley and Adkins-Regan, 2018a). In most cases, these parents hardly visited the nest during the time when the drug was most active. For the second manipulation VIP was administered to pairs which had recently laid eggs with the hypothesis that increasing PRL (via VIP) would initiate a quicker onset of parental care behavior towards foster chicks, relative to controls. Surprisingly, increasing PRL did not alter the mean levels of parental behavior displayed towards foster chicks. Instead, we found that early incubating birds, regardless of treatment, could show a substantial amount of baseline parental care behavior towards 1–4-day old chicks that were placed in the nest (Smiley and Adkins-Regan, 2018b). Interestingly, however, VIP manipulations made male and female pairs' behavior more similar. In saline treated pairs, it was typically the case that the females performed the majority of behaviors towards chicks and males would perform very little (although there were a few cases where the sex difference was reversed). When both members of the pair were administered VIP, the time spent attending to the chicks was more similar, suggesting PRL could be playing a role in synchronizing behaviors between pairs (see Fig. 5). Notably, this synchronized feeding behavior has been observed in both laboratory (Smiley and Adkins-Regan, 2016b) and free-living (Mariette and Griffith, 2012) breeding zebra finches, and this pair coordination was disrupted by bromocriptine (which lowered PRL); see Fig. 5 (Smiley and Adkins-Regan, 2018a). Wild zebra finches that are more coordinated in their parental behaviors have greater reproductive success (Mariette and Griffith, 2015), possibly due to more efficient and consistent parental provisioning. Other studies in great tits (*Parus major*) have found that pairs with more similar hormone levels also have greater reproductive success (Ouyang et al., 2014). This synchrony in behaviors observed in zebra finch pairs may have been due to the fact that VIP elicits a maximal PRL response (Christensen and Vleck, 2008), therefore males and females would have more similar hormone levels than control pairs. In contrast to ring doves, in both studies these PRL manipulations affected both experienced and inexperienced birds

similarly. That is, experience did not buffer against the effect of bromocriptine, nor did it provide any additive effects to the VIP treatment.

A final line of evidence for a more indirect role of PRL during post-hatch parental care comes from penguins. Decreasing PRL in Adélie penguin males before egg laying caused a reduction in incubation behavior, reduced egg temperatures, and decreased the number of chicks produced (Thierry et al., 2013). Lowering PRL in Adélie males during the beginning of the guard phase (right after chick hatching), however, did not affect chick growth or survival or food foraging durations, but did reduce their diving effort as measured by time-depth recorders (Cottin et al., 2014). Therefore, it could be hypothesized that PRL plays more of a role in motivating the parents to collect food and return to the chicks during the post-hatch period, while the actual feeding behavior may be stimulated by the chicks, themselves. Another intriguing set of studies in Emperor penguins supports the idea that PRL is involved in parental motivation to be in close association with chicks. Unlike species such as red footed boobies in which PRL declines right after nest failure occurs (Chastel and Lormée, 2002), high PRL levels do not decline in failed/unsuccessful penguin breeders (Garcia et al., 1996; Vleck et al., 2000). In fact, failed penguin breeders are often still motivated to care for young and will attempt to kidnap or adopt other chicks to care for when theirs do not succeed. Lowering PRL reduced this kidnapping behavior in Emperor penguins, although it was not completely eliminated (Angelier et al., 2006). Accumulating evidence, at least in penguin species, supports a role for PRL in prompting birds to return and maintain close association with chicks/eggs, highlighting again, the importance of chick stimuli in stimulating the actual behavior (e.g., regurgitation) itself.

#### 2.4. Prolactin and stress

Another area of research that has received a lot of attention is the PRL stress response during various life history stages. In addition to the traditionally measured cortisol/corticosterone (CORT) stress response to a standard capture-restraint stress protocol (Wingfield and Sapolsky, 2003), an adaptive PRL stress response may also have an important role in mediating the trade-off between allocating resources to self-maintenance and parental care. PRL usually decreases in response to stress and the rate at which PRL decreases in response to a stressor is thought to be directly proportional to the amount parental effort exerted (Angelier and Chastel, 2009). This idea has been supported by studies showing that parents can attenuate the PRL stress response during times of breeding, but not during other times when parental effort is not



**Fig. 5.** Correlations between male and female chick feeding behaviors under various conditions. Each point represents a male-female pair. For all figures, the durations of females' chick feeding are plotted on the y-axis and durations of males' chick feeding behavior are plotted on the x-axis. (A) Breeding birds on day 2 post-hatch given vehicle control treatment (oral administration of peanut oil) showed highly positively correlated behaviors, meaning males and females fed chicks for similar amounts of time. (B) Administrations of bromocriptine on day 2 post-hatch, which lowers PRL, disrupted the correlation observed in (A). (C) Early incubating pairs administered a vehicle control treatment (saline injection) and given foster chicks did not show correlated chick feeding behaviors. Instead, most males did not show much chick feeding behavior while the females show a varied amount of chick feeding behavior. (D) Increasing PRL to breeding levels, via VIP injections, resulted in incubating male-female pairs showing more similar amounts of chick feeding behaviors, similar to the pairs in (A) which have naturally high PRL. Data reproduced from Smiley and Adkins-Regan, 2018a, 2018b.

required. For example, Chastel et al. (2005) found that successful parents showed an attenuated PRL decline in response to stress whereas failed breeders showed a greater decline in PRL to stress. Manx shearwaters (*Puffinus puffinus*), Eurasian hoopoes (*Upupa epops*), and little auks (*Alle alle*) showed a similar pattern during times of high parental investment, relative to times of lower parental investment (Riou et al., 2010; Schmid et al., 2011; Wojczulanis-Jakubas et al., 2018). However, these patterns are not always the response across bird taxa. For instance, Krause et al. (2015) found no effect of stress at any stage of the breeding cycle on PRL in white crowned sparrows. In several other species, increases in PRL or no change in PRL have also been reported (reviewed in Angelier et al., 2016). A variety of other factors may influence this stress response, including age of the individual or whether it is a long or short-lived species. (For a complete review on this topic, see Angelier and Chastel, 2009). While the PRL stress response may be able to provide additional information on parental care effort, it is still unclear how PRL and CORT work together during these responses, or whether PRL and CORT affect parental care via separate mechanisms in parallel of one another.

### 3. Central prolactin, prolactin receptors, and avian parental care

#### 3.1. The PRL receptor and signaling cascades

The PRL receptor (PRLR) is a membrane-bound receptor that is part of the class 1 cytokine receptor superfamily and is widely expressed in various tissues of vertebrates, including the brain (reviewed in Bole-

Feysot et al., 1998; reviewed in Ben-Jonathan et al., 2007). Although the PRLR can influence multiple signaling pathways, activation of the PRLR primarily recruits the Janus Kinase 2 (JAK2)-Signal Transducer and Activator of Transcription 5 (STAT5) pathway to alter gene expression in the nucleus. In birds, similar to mammals, when PRL couples with the PRLR, JAK2 is activated and becomes phosphorylated, leading to the phosphorylation of STAT5 (pSTAT5), which then dimerizes. The pSTAT5 dimer translocates to the nucleus resulting in signal transduction and gene expression alteration (for a more detailed review, see Chilton and Hewetson, 2005). pSTAT5 is a commonly used marker for recent PRLR activity (Brown et al., 2010; Buntin and Buntin, 2014). Indeed, Buntin and Buntin (2014) showed that PRL-treated ring doves exhibited increased central pSTAT5 immunoreactivity, relative to vehicle-treated controls. Our current understanding of the PRLR signal transduction pathway regulation comes primarily from mammalian studies, however. More work on the molecular regulation of avian PRLR signaling, including the role of inhibitory proteins such as suppressors of cytokine signaling proteins (SOCS) or phosphotyrosine phosphatases (PTP) is needed to fully understand how the actions of PRL can affect avian behavior and other PRL-mediated effects.

#### 3.2. The role of central PRL action during avian parental care

Data from peripheral PRL manipulation studies support a role for PRL in several aspects of avian parental care behavior. However, the remaining question is whether these effects of PRL are mediated by central PRLRs. However, it is not yet well established whether PRL's

**Table 1**  
Summary table of the brain areas in which prolactin receptors (PRLR) have been detected in birds. Where available, significant differences in the PRLR distribution between males and females or reproductive stage are reported.

Areas	Species	Relative density	Methods used	Sex measured in	Breeding stage measured in	Sex differences	Breeding stage differences	Citation
Anterior hypothalamus, rostral	Ring dove ( <i>Streptopelia risoria</i> )	Moderate	Autoradiography	Males and females	Non-breeding			Fechner and Buntin, 1989; Hnasko and Buntin, 1993
Bed nucleus of the stria terminalis, lateral	Zebra finches ( <i>Taeniopygia guttata</i> )	Moderate	Immunohistochemistry	Males and females	Non-breeding and breeding			Smiley et al., unpublished obs
Bed nucleus of the stria terminalis, medial	Zebra finches ( <i>Taeniopygia guttata</i> )	Moderate	Immunohistochemistry	Males and females	Non-breeding and breeding			Smiley et al., unpublished obs
Caudal Nidopallium	Dark-eyed Juncos ( <i>Junco hyemalis</i> )	Low	Autoradiography	Males	Seasonal breeding differences: Spring, Summer, and Fall			Deviche and Buntin, 1992; unpublished obs
	Ring dove ( <i>Streptopelia risoria</i> )	Low	Autoradiography	Males and females	Non-breeding			Fechner and Buntin, 1989; Hnasko and Buntin, 1993
Cerebellum	Pigeons	Low	Radio ligand receptor assay	Males and females	Not stated			Muccioli et al., 1988
	Zebra finches ( <i>Taeniopygia guttata</i> )	Low	Immunohistochemistry	Males and females	Non-breeding and breeding			Smiley et al., unpublished obs
Choroid plexus	Wilson's Phalarope ( <i>Phalaropus tricolor</i> )	Low	Autoradiography	Males and females	non-Breeding		incubating males had a lower mean specific binding scores compared to nonincubating males for all areas measured below	Fechner and Buntin, 1989; Hnasko and Buntin, 1993
	Zebra finches ( <i>Taeniopygia guttata</i> )	High	Immunohistochemistry	Males and females	Non-breeding and breeding			Smiley et al., unpublished obs
Cortex	Dark-eyed Juncos ( <i>Junco hyemalis</i> )	Low	Autoradiography	Males	Seasonal breeding differences: Spring, Summer, and Fall		Lowest during summer (relative to spring or fall)	Deviche and Buntin, 1992; unpublished obs
	Ring dove ( <i>Streptopelia risoria</i> )	High	Autoradiography	Males and females	Non-breeding			Fechner and Buntin, 1989; Hnasko and Buntin, 1993
	Pigeons	Low	Radio ligand receptor assay	Males and females	Not stated			Muccioli et al., 1988
Dorsolateral thalamic area	Wilson's Phalarope ( <i>Phalaropus tricolor</i> )	High	Autoradiography	Males and females	Incubating and non-incubating males; non-breeding females *Note: Females do not provide care in this species	Nonincubating males exhibiting higher binding activity than females		Buntin et al., 1998
Entopallium	Zebra finches ( <i>Taeniopygia guttata</i> )	Moderate	Immunohistochemistry	Males and females	Non-breeding and breeding			Smiley et al., unpublished obs
Fasciculus diagonalis Brocae	Ring dove ( <i>Streptopelia risoria</i> )	Moderate	Autoradiography	Males and females	Non-breeding			Fechner and Buntin, 1989; Hnasko and Buntin, 1993

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Table 1 (continued)

Areas	Species	Relative density	Methods used	Sex measured in	Breeding stage measured in	Sex differences	Breeding stage differences	Citation
Globus pallidus	Zebra finches ( <i>Taeniopygia guttata</i> )	Moderate	Immunohistochemistry	Males and females	Non-breeding and breeding			Smiley et al., unpublished obs
Hippocampus	Zebra finches ( <i>Taeniopygia guttata</i> )	High	Immunohistochemistry	Males and females	Non-breeding and breeding			Smiley et al., unpublished obs
Hyperpallium apicale	Dark-eyed Junco ( <i>Junco hyemalis</i> )	Moderate	Autoradiography	Males	Seasonal breeding differences: Spring, Summer, and Fall		Highest in fall (relative to spring or summer)	Deviche and Buntin, 1992; unpublished obs
	Ring dove ( <i>Streptopelia risoria</i> )	High	Autoradiography	Males and females	Non-breeding			Fechner and Buntin, 1989; Hnasko and Buntin, 1993
Hyperpallium densocellulare	Ring dove ( <i>Streptopelia risoria</i> )	Low	Autoradiography	Males and females	Non-breeding			Fechner and Buntin, 1989; Hnasko and Buntin, 1993
	Zebra finches ( <i>Taeniopygia guttata</i> )	High	Immunohistochemistry	Males and females	Non-breeding and breeding			Smiley et al., unpublished obs
Hypothalamus	Ring dove ( <i>Streptopelia risoria</i> )	Low	Autoradiography	Males and females	Non-breeding			Fechner and Buntin, 1989; Hnasko and Buntin, 1993
	Pigeons	Moderate	Radio ligand receptor assay	Males and females	Not stated			Muccioli et al., 1988
Infundibular tract	Zebra finches ( <i>Taeniopygia guttata</i> )	High/moderate	Immunohistochemistry	Males and females	Non-breeding and breeding		Higher expression in breeders, compared to non-breeders	Smiley et al., unpublished obs
Lateral dorsal nucleus of thalamus	Zebra finches ( <i>Taeniopygia guttata</i> )	High/low	Immunohistochemistry	Males and females	Non-breeding and breeding			Smiley et al., unpublished obs
	Zebra finches ( <i>Taeniopygia guttata</i> )	Moderate	Immunohistochemistry	Males and females	Non-breeding and breeding			Smiley et al., unpublished obs
Lateral geniculate nucleus	Wilson's Phalarope ( <i>Phalaropus tricolor</i> )	Moderate	Autoradiography	Males and females	incubating and non-incubating males; non-breeding females *Note: Females do not provide care in this species			Buntin et al., 1998
	Dark-eyed Junco ( <i>Junco hyemalis</i> )	High	Autoradiography	Males	Seasonal breeding differences: Spring, Summer, and Fall			Deviche and Buntin, 1992; unpublished obs
Lateral septal nucleus	Ring dove ( <i>Streptopelia risoria</i> )	Moderate	Autoradiography	Males and females	Non-breeding			Fechner and Buntin, 1989; Hnasko and Buntin, 1993
	Zebra finches ( <i>Taeniopygia guttata</i> )	Moderate	Immunohistochemistry	Males and females	Non-breeding and breeding			Smiley et al., unpublished obs
Lateral septal nucleus	Wilson's Phalarope ( <i>Phalaropus tricolor</i> )	Moderate	Autoradiography	Males and females	Incubating and non-incubating males; non-breeding females *Note: Females do not provide care in this species			Buntin et al., 1998
	Ring dove ( <i>Streptopelia risoria</i> )	Moderate	Autoradiography	Males and females	Non-breeding			Fechner and Buntin, 1989; Hnasko and Buntin, 1993

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Table 1 (continued)

Areas	Species	Relative density	Methods used	Sex measured in	Breeding stage measured in	Sex differences	Breeding stage differences	Citation
Lateral striatum	Zebra finches ( <i>Taeniopygia guttata</i> )	Moderate	Immunohistochemistry	Males and females	Non-breeding and breeding			Smiley et al., unpublished obs
	Zebra finches ( <i>Taeniopygia guttata</i> )	Moderate	Immunohistochemistry	Males and females	Non-breeding and breeding			Smiley et al., unpublished obs
Medial dorsal nucleus of thalamus	Zebra finches ( <i>Taeniopygia guttata</i> )	Moderate	Immunohistochemistry	Males and females	Non-breeding and breeding			Smiley et al., unpublished obs
	Wilson's Phalarope ( <i>Phalaropus tricolor</i> )	High	Autoradiography	Males and females	Incubating and non-incubating males; non-breeding females *Note: Females do not provide care in this species			Buntin et al., 1998
Medial habenula nucleus	Dark-eyed Juncos ( <i>Junco hyemalis</i> )	Low	Autoradiography	Males	Seasonal breeding differences: Spring, Summer, and Fall		Highest in fall (relative to spring or summer)	Deviche and Buntin, 1992; unpublished obs
	Ring dove ( <i>Streptopelia risoria</i> )	High	Autoradiography	Males and females	Non-breeding			Fechner and Buntin, 1989; Hnasko and Buntin, 1993
Medial hypothalamus	Wilson's Phalarope ( <i>Phalaropus tricolor</i> )	Moderate	Autoradiography	Males and females	Incubating and non-incubating males; non-breeding females *Note: Females do not provide care in this species			Buntin et al., 1998
	Dark-eyed Juncos ( <i>Junco hyemalis</i> )	High	Autoradiography	Males	Seasonal breeding differences: Spring, Summer, and Fall		Lowest during summer (relative to spring or fall)	Deviche and Buntin, 1992; unpublished obs
Medial septal nucleus	Zebra finches ( <i>Taeniopygia guttata</i> )	Moderate	Immunohistochemistry	Males and females	Non-breeding and breeding			Smiley et al., unpublished obs
	Zebra finches ( <i>Taeniopygia guttata</i> )	Moderate	Immunohistochemistry	Males and females	Non-breeding and breeding			Smiley et al., unpublished obs
Mesopallium	Dark-eyed Juncos ( <i>Junco hyemalis</i> )	Low	Autoradiography	Males	Seasonal breeding differences: Spring, Summer, and Fall			Deviche and Buntin, 1992; unpublished obs
	Ring dove ( <i>Streptopelia risoria</i> )	Low	Autoradiography	Males and females	Non-breeding			Fechner and Buntin, 1989; Hnasko and Buntin, 1993
Nidopallium	Zebra finches ( <i>Taeniopygia guttata</i> )	High	Immunohistochemistry	Males and females	Non-breeding and breeding			Smiley et al., unpublished obs
	Wilson's Phalarope ( <i>Phalaropus tricolor</i> )	Low	Autoradiography	Males and females	Incubating and non-incubating males; non-breeding females *Note: Females do not provide care in this species			Buntin et al., 1998
	Ring dove ( <i>Streptopelia risoria</i> )	Low	Autoradiography	Males and females	Non-breeding			Fechner and Buntin, 1989; Hnasko and Buntin, 1993
	Zebra finches ( <i>Taeniopygia guttata</i> )	Moderate	Immunohistochemistry	Males and females	Non-breeding and breeding			Smiley et al., unpublished obs

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Table 1 (continued)

Areas	Species	Relative density	Methods used	Sex measured in	Breeding stage measured in	Sex differences	Breeding stage differences	Citation
Nucleus accumbens	Dark-eyed Juncos ( <i>Junco hyemalis</i> )	High	Autoradiography	Males	Seasonal breeding differences: Spring, Summer, and Fall			Deviche and Buntin, 1992; unpublished obs
	Zebra finches ( <i>Taeniopygia guttata</i> )	High	Immunohistochemistry	Males and females	Non-breeding and breeding			Smiley et al., unpublished obs
	Ring dove ( <i>Streptopelia risoria</i> )	Moderate	Autoradiography	Males and females	Non-breeding			Fechner and Buntin, 1989; Hnasko and Buntin, 1993
Nucleus intercollicularis	Dark-eyed Juncos ( <i>Junco hyemalis</i> )	Low	Autoradiography	Males	Seasonal breeding differences: Spring, Summer, and Fall		highest in fall (relative to spring or summer)	Deviche and Buntin, 1992; unpublished obs
	Ring dove ( <i>Streptopelia risoria</i> )	Low	Autoradiography	Males and females	Non-breeding			Fechner and Buntin, 1989; Hnasko and Buntin, 1993
Nucleus isthmi	Zebra finches ( <i>Taeniopygia guttata</i> )	High/low	Immunohistochemistry	Males and females	Non-breeding and breeding			Smiley et al., unpublished obs
	Dark-eyed Juncos ( <i>Junco hyemalis</i> )	Low	Autoradiography	Males	Seasonal breeding differences: Spring, Summer, and Fall			Deviche and Buntin, 1992; unpublished obs
Nucleus mesencephalicus lateralis, pars dorsalis	Ring dove ( <i>Streptopelia risoria</i> )	High	Autoradiography	Males and females	Non-breeding			Fechner and Buntin, 1989; Hnasko and Buntin, 1993
	Dark-eyed Juncos ( <i>Junco hyemalis</i> )	Low	Autoradiography	Males	Seasonal breeding differences: Spring, Summer, and Fall			Deviche and Buntin, 1992; unpublished obs
Nucleus ovoidalis	Ring dove ( <i>Streptopelia risoria</i> )	Low	Autoradiography	Males and females	Non-breeding			Fechner and Buntin, 1989; Hnasko and Buntin, 1993
	Ring dove ( <i>Streptopelia risoria</i> )	Moderate	Autoradiography	Males and females	Non-breeding			Fechner and Buntin, 1989; Hnasko and Buntin, 1993
Nucleus paramedianus internus thalami	Ring dove ( <i>Streptopelia risoria</i> )	Low	Autoradiography	Males	Seasonal breeding differences: Spring, Summer, and Fall			Deviche and Buntin, 1992; unpublished obs
	Dark-eyed Juncos ( <i>Junco hyemalis</i> )	Low	Autoradiography	Males and females	Non-breeding		(predominately in males)	Fechner and Buntin, 1989; Hnasko and Buntin, 1993
Nucleus rotundus	Ring dove ( <i>Streptopelia risoria</i> )	Moderate	Autoradiography	Males and females	Non-breeding			Fechner and Buntin, 1989; Hnasko and Buntin, 1993
	Dark-eyed Juncos ( <i>Junco hyemalis</i> )	Low	Autoradiography	Males	Seasonal breeding differences: Spring, Summer, and Fall			Deviche and Buntin, 1992; unpublished obs
Nucleus subrotundus	Ring dove ( <i>Streptopelia risoria</i> )	Low	Autoradiography	Males and females	Non-breeding			Fechner and Buntin, 1989; Hnasko and Buntin, 1993
	Zebra finches ( <i>Taeniopygia guttata</i> )	Moderate	Immunohistochemistry	Males and females	Non-breeding and breeding			Smiley et al., unpublished obs
Nucleus taeniae of the amygdala	Wilson's Phalarope ( <i>Phalaropus tricolor</i> )	Moderate	Autoradiography	Males and females	Incubating and non-incubating males; non-breeding females			Buntin et al., 1998
	Zebra finches ( <i>Taeniopygia guttata</i> )	Moderate	Immunohistochemistry	Males and females	Non-breeding and breeding			Smiley et al., unpublished obs
Nucleus taeniae of the amygdala thalami	Wilson's Phalarope ( <i>Phalaropus tricolor</i> )	Moderate	Autoradiography	Males and females	Incubating and non-incubating males; non-breeding females			Buntin et al., 1998
	Zebra finches ( <i>Taeniopygia guttata</i> )	Moderate	Autoradiography	Males and females	Non-breeding and breeding			Smiley et al., unpublished obs

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Table 1 (continued)

Areas	Species	Relative density	Methods used	Sex measured in	Breeding stage measured in	Sex differences	Breeding stage differences	Citation
Nucleus tractus septomesecephalicus	Wilson's Phalarope ( <i>Phalaropus tricolor</i> )	Moderate	Autoradiography	Males and females	Incubating and non-incubating males; non-breeding females *Note: Females do not provide care in this species			Buntin et al., 1998
	Dark-eyed Juncos ( <i>Junco hyemalis</i> )	Low	Autoradiography	Males	Seasonal breeding differences: Spring, Summer, and Fall			Deviche and Buntin, 1992; unpublished obs
Oculomotor nerve	Ring dove ( <i>Streptopelia risoria</i> )	Low	Autoradiography	Males and females	Non-breeding			Fechner and Buntin, 1989; Hnasko and Buntin, 1993
	Zebra finches ( <i>Taeniopygia guttata</i> )	High/moderate	Immunohistochemistry	Males and females	Non-breeding and breeding		higher expression in breeders, compared to non-breeders	Smiley et al., unpublished obs
Olfactory bulb	Dark-eyed Juncos ( <i>Junco hyemalis</i> )	High	Autoradiography	Males	Seasonal breeding differences: Spring, Summer, and Fall			Deviche and Buntin, 1992; unpublished obs
	Ring dove ( <i>Streptopelia risoria</i> )	Low	Autoradiography	Males and females	Non-breeding			Fechner and Buntin, 1989; Hnasko and Buntin, 1993
Optic Chiasm	Dark-eyed Juncos ( <i>Junco hyemalis</i> )	Low	Autoradiography	Males	Seasonal breeding differences: Spring, Summer, and Fall			Deviche and Buntin, 1992; unpublished obs
	Ring dove ( <i>Streptopelia risoria</i> )	Low	Autoradiography	Males and females	Non-breeding			Fechner and Buntin, 1989; Hnasko and Buntin, 1993
Optic tectum	Zebra finches ( <i>Taeniopygia guttata</i> )	Moderate	Immunohistochemistry	Males and females	Non-breeding and breeding			Smiley et al., unpublished obs
	Dark-eyed Juncos ( <i>Junco hyemalis</i> )	Moderate	Autoradiography	Males	Seasonal breeding differences: Spring, Summer, and Fall		Lowest during summer (relative to spring or fall)	Deviche and Buntin, 1992; unpublished obs
Parahippocampus	Ring dove ( <i>Streptopelia risoria</i> )	High	Autoradiography	Males and females	Non-breeding	(Predominantly in females)		Fechner and Buntin, 1989; Hnasko and Buntin, 1993
	Wilson's Phalarope ( <i>Phalaropus tricolor</i> )	Moderate	Autoradiography	Males and females	Incubating and non-incubating males; non-breeding females *Note: Females do not provide care in this species			Buntin et al., 1998
Paraventricular nucleus	Dark-eyed Juncos ( <i>Junco hyemalis</i> )	High	Autoradiography	Males	Seasonal breeding differences: Spring, Summer, and Fall		Lowest during summer (relative to spring or fall)	Deviche and Buntin, 1992; unpublished obs
	Zebra finches ( <i>Taeniopygia guttata</i> )	Moderate	Immunohistochemistry	Males and females	non-breeding and breeding			Smiley et al., unpublished obs
Posterior medial hypothalamus	Ring dove ( <i>Streptopelia risoria</i> )	Low	Autoradiography	Males and females	Non-breeding			Fechner and Buntin, 1989; Hnasko and Buntin, 1993
	Zebra finches ( <i>Taeniopygia guttata</i> )	High	Immunohistochemistry	Males and females	Non-breeding and breeding			Smiley et al., unpublished obs

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Table 1 (continued)

Areas	Species	Relative density	Methods used	Sex measured in	Breeding stage measured in	Sex differences	Breeding stage differences	Citation
Preoptic area	Wilson's Phalarope (Phalaropus tricolor)	High	Autoradiography	Males and females	Incubating and non-incubating males; non-breeding females *Note: Females do not provide care in this species			Buntin et al., 1998
Preoptic area, anterior	Ring dove (Streptopelia risoria)	High	Autoradiography	Males and females	Non-breeding			Fechner and Buntin, 1989; Hnasko and Buntin, 1993
	Dark-eyed Junco (Junco hyemalis)	High	Autoradiography	Males	Seasonal breeding differences: Spring, Summer, and Fall	Lowest during summer (relative to spring or fall)		Deviche and Buntin, 1992; unpublished obs
Preoptic area, medial	Zebra finches (Taeniopygia guttata)	High/low	Immunohistochemistry	Males and females	Non-breeding and breeding		Higher expression in breeders, compared to non-breeders	Smiley et al., unpublished obs
	Zebra finches (Taeniopygia guttata)	High/low	Immunohistochemistry	Males and females	Non-breeding and breeding		Higher expression in breeders, compared to non-breeders	Smiley et al., unpublished obs
Pretectal area	Ring dove (Streptopelia risoria)	Low	Autoradiography	Males and females	Non-breeding			Fechner and Buntin, 1989; Hnasko and Buntin, 1993
Robust nucleus of arcopallium	Zebra finches (Taeniopygia guttata)	Moderate	Immunohistochemistry	Males and females	Non-breeding and breeding			Smiley et al., unpublished obs
	Dark-eyed Junco (Junco hyemalis)	Low	Autoradiography	Males	Seasonal breeding differences: Spring, Summer, and Fall	Lowest during summer (relative to spring or fall)		Deviche and Buntin, 1992; unpublished obs
Subrotundus nucleus	Ring dove (Streptopelia risoria)	Low	Autoradiography	Males and females	Non-breeding			Fechner and Buntin, 1989; Hnasko and Buntin, 1993
	Ring dove (Streptopelia risoria)	Moderate	Autoradiography	Males and females	Non-breeding			Fechner and Buntin, 1989; Hnasko and Buntin, 1993
Suprachiasmatic nucleus	Ring dove (Streptopelia risoria)	High	Autoradiography	Males and females	Non-breeding			Fechner and Buntin, 1989; Hnasko and Buntin, 1993
	Ring dove (Streptopelia risoria)	Low	Autoradiography	Males and females	Non-breeding			Fechner and Buntin, 1989; Hnasko and Buntin, 1993
Tractus septomesencephalicus	Ring dove (Streptopelia risoria)	Moderate	Autoradiography	Males and females	Incubating and non-incubating males; non-breeding females *Note: Females do not provide care in this species			Fechner and Buntin, 1989; Hnasko and Buntin, 1993
	Ring dove (Streptopelia risoria)	High/moderate	Immunohistochemistry	Males and females	Non-breeding and breeding		Higher expression in breeders, compared to non-breeders	Smiley et al., unpublished obs
Tuberal nucleus	Dark-eyed Junco (Junco hyemalis)	High	Autoradiography	Males	Seasonal breeding differences: Spring, Summer, and Fall	Lowest during summer (relative to spring or fall)		Deviche and Buntin, 1992; unpublished obs

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Table 1 (continued)

Areas	Species	Relative density	Methods used	Sex measured in	Breeding stage measured in	Sex differences	Breeding stage differences	Citation
Ventral tegmental area	Zebra finches ( <i>Taeniopygia guttata</i> )	High	Immunohistochemistry	Males and females	Non-breeding and breeding			Smiley et al., unpublished obs
Ventromedial hypothalamus	Ring dove ( <i>Streptopelia risoria</i> )	High	Autoradiography	Males and females	Non-breeding			Fechner and Buntin, 1989; Hnasko and Buntin, 1993
	Zebra finches ( <i>Taeniopygia guttata</i> )	High	Immunohistochemistry	Males and females	Non-breeding and breeding			Smiley et al., unpublished obs

effects on avian care are directly mediated by the central PRLRs. This is in part because so few studies have mapped out the PRLR distribution in the avian brain. The data that are available on PRLRs are limited to five avian species: pigeons (Muccioli et al., 1988), ring doves (Fechner and Buntin, 1989; Hnasko and Buntin, 1993), Wilson's Phalaropes (Buntin et al., 1998), Dark-eyed Juncos (Deviche and Buntin, 1992; unpublished obs), and zebra finches (Smiley et al., unpublished obs), which are summarized in Table 1. PRLRs have also been investigated in cowbirds, redwing blackbirds, and European starlings, but data are not currently available beyond conference abstracts (Ball et al., 1998, 1990). When comparing across avian species, PRLRs are most prevalent in the hypothalamus. Most notably, PRLRs are repeatedly found to be high in the preoptic area (POA), ventromedial hypothalamus (VMH), and the tuberal regions of the hypothalamus (TU; homologue of the mammalian arcuate nucleus). High levels of PRLR mRNA have also been found in the pituitary, basal hypothalamus, and POA in domestic and bantam hens (Ohkubo et al., 1998a, 1998b). While these results have been found for both males and females, to date, most studies describing the PRLR distribution have not had a large enough sample size to detect significant sex differences. This does not, however, rule out the possibility that sex differences in the PRLR distribution do exist in the brain.

Although there is a similar distribution of PRLRs across the species tested so far, the role of PRL at these sites has rarely been tested in birds. Intracerebroventricular (ICV) infusions of PRL into the lateral ventricle increased nest visits and nesting activity in turkeys (Youngren et al., 1991) and the number of feeding invitations and regurgitation bouts towards foster squabs in non-breeding, reproductively experienced male and female ring doves (Buntin et al., 1991). Note that these effects only occur in reproductively experienced ring doves, suggesting the brain is not as sensitive to the actions of PRL until reproductive experience is gained. Lesions to the POA, VMH, or lateral hypothalamus (LHy) disrupt the onset of incubation and the rise in plasma PRL that accompanies incubation (Youngren et al., 1989).

The POA is a well-known parental “hot-spot” across vertebrates (O’Connell and Hofmann, 2011). Axon-sparing lesions of the POA disrupt the PRL-induced regurgitation feeding behavior in non-breeding, reproductively experienced ring doves given foster squabs (Slawski and Buntin, 1995). Although the studies of direct actions of PRL in the POA are limited, intriguing evidence from brown-headed cowbirds suggests that central sensitivity to PRL is different between species, depending on parental system, which may affect behavior. As mentioned earlier, brown-headed cowbirds, despite showing no parental care, still experience a seasonal rise in circulating PRL. However, PRLR were less prevalent in the POA, compared to two other songbird species, while PRLR distributions were relatively similar in other brain regions examined (Ball et al., 1998; 1990). This suggests that brown-headed cowbirds have a lowered central sensitivity to PRL in the POA and therefore do not experience the parental effects of PRL. The idea that the PRLR distribution changes across the breeding cycle, which corresponds with an increase in PRL, was tested in zebra finches in which the PRLR distributions were compared between non-breeders and breeders (day 2 post-hatch). PRLRs were significantly upregulated in areas that may be important to parental care including the POA, VMH, and TU (Smiley et al., unpublished obs). These results are similar to the finding that PRLR mRNA concentrations are higher in the anterior pituitary and basal hypothalamus in incubating hens relative to laying or out-of-lay hens, which corresponds to the time in which PRL is most active (Ohkubo et al., 1998a). However, it is at odds with the results that dark-eyed junco males show a lower density of PRLR in the POA and TU during the summer (breeding times) relative to the spring or fall (Deviche and Buntin, 1992; unpublished obs), despite the fact that males and females contribute roughly equally to chick feeding and defense and play a crucial role to the survivorship of young (Wolf et al., 1988). Whether an up or downregulation of PRLRs actually has an effect on behavior or is a species-specific phenomenon has yet to be tested.

PRL also plays an important role in increased food consumption

(hyperphagia) in breeding ring doves. Food intake increases in a dose-dependent manner in response to either systemic or ICV injections of PRL into the POA, VMH, or TU, even when PRL doses are below the level required to stimulate crop sac growth and crop milk production (Buntin, 1989; Buntin and Figge, 1988). However, ring doves with a lesioned VMH are still susceptible to the effects of increased food intake via PRL injections (Buntin et al., 1999), confirming there are multiple and redundant mechanisms which control food intake that PRL can affect. The fact that zebra finches and dark eyed juncos also show a rich concentration of PRLRs in the POA, VMH, and TU suggests that this effect of PRL on food intake may not be unique to ring doves or crop sac feedings.

Consistent with this idea is evidence from both ring doves and zebra finches which shows increased PRLR activity in the POA and VMH at the time of chick hatching, compared to non-breeding times (Buntin and Buntin, 2014; Smiley et al., unpublished obs). pSTAT5 immunoreactivity in the brain mirrors that of circulating PRL; pSTAT5 activity is low during nesting/courtship but increases over incubation, peaking at the time of post-hatch care in ring doves and zebra finches (Buntin and Buntin, 2014; Smiley et al., unpublished obs). Although it is not a direct test of PRL's role in parental behavior, these studies indicate that the PRLRs at these sites are the most active during post-hatch care, supporting the hypothesis that post-hatch parental care behaviors could be mediated by central PRLRs. Whether the PRLR was activated by PRL or some other ligand that can bind to the PRLR is unknown from these studies.

pSTAT5 activity has also been shown to be higher in the LH, supra-chiasmatic nucleus of the hypothalamus (SCN), the paraventricular nucleus of the hypothalamus (PVN), the lateral septum (LS), and the lateral bed nucleus of the stria terminalis (BSTL) in ring doves (Buntin and Buntin, 2014). PRL's role in these regions during breeding has not been examined to know if PRLRs in these regions contribute to parental care behavior. However, it is promising that these data are in agreement with a study that showed increased c-fos activity in the POA, BSTM, and LS when ring doves were exposed to squabs (relative to no exposure controls) (Buntin et al., 2006). Whether the cell types that are activated by c-fos during squab interactions also express PRLRs has yet to be determined. A more controlled study using pSTAT5 in response to specific interactions with eggs or chicks is needed in order to show that the PRLR is activated as a direct consequence of those interactions. A double labeling study with c-fos and pSTAT5 could provide insight into which populations of PRL-responsive neurons are active during parental care to inform future PRLR manipulation studies. Direct manipulation of PRL and the PRLR via the use of receptor antagonists or cannulated ICV delivery of PRL directly into these areas is greatly needed in order to clarify the role of central PRL action during parental care activities.

#### 4. Conclusions: new insights and unanswered questions

PRL plays an important role in stimulating the onset and maintenance in parental care behaviors in birds. However, the way PRL does this is complex as PRL works in interaction with previous reproductive experience, incoming chick stimuli, and other environmental cues to influence behavior. Here, I will highlight a few key points about the PRL-parental care relationship and what we still need to know in order to better understand these relationships.

First, PRL has a role in multiple functions within the scope of parental care. The most well-established roles for PRL can be roughly broken down into two categories: 1) increasing the attentiveness to either egg/chick stimuli and 2) nutrient provisioning. Sensory stimuli, such as the tactile input from eggs or begging calls from chicks, elicits parental behavior alone (e.g., Richard-Yris et al., 1987; Wang and Buntin, 1999). Therefore, I hypothesize that PRL's primary role in parental care is more likely to do with increasing the attention paid to those cues, rather than stimulating the motor act of parental behavior

such as feeding, per se. The more attracted parents are to the egg/chick stimulus, the closer in contact the parents remain with the stimuli and the more parental care can be elicited from these cues. In cases such as penguins and other seabirds, increased PRL may serve to maintain the motivation to return to their eggs/chicks after long foraging trips in which they are away from these stimuli for extended periods of time. In order to more formally test this hypothesis, it would need to be established that PRL increases the saliency of chick/egg cues. This could be done using conditioned place paradigms and testing whether there is a greater preference for chicks when given in conjunction with PRL treatments. Second, it would be worthwhile to determine if PRL has rewarding properties on its own. In this case, birds could be tested to see if they can condition to PRL treatments alone, in absence of egg/chick stimuli.

The second more general role of PRL is in nutrient provisioning to young. While PRL plays a large role in crop-milk production and regurgitation in ring doves, PRL also affects chick feeding behavior in other non-columbiform species, suggesting that PRL's role in chick feeding is not limited to crop sac milk production and function. In zebra finches, bromocriptine had a greater effect on chick feeding behavior than it did on brooding behavior, suggesting feeding behavior is particularly sensitive to the effects of PRL. PRL's role in nutrient provisioning is likely conserved beyond birds as it is also involved in lactation in mammals (Bridges, 2015) and mucous secretion in fish, which provides a food source for young (Whittington and Wilson, 2013). In addition, PRL increases feeding behavior in the parents in order to support the increased energy demands and regurgitation feeding to chicks. While PRL has not been tested for its role in hyperphagia in other species, the fact that zebra finches and other birds have PRLRs in the VMH, POA, and TU – areas where PRL has been shown to play a role in the onset of hyperphagia and regurgitation – suggests that PRL could have similar roles in these processes in other birds. However, there is a striking lack of studies manipulating PRL or the PRLR in the brains of birds to be able to generalize this conclusion. More studies looking at the feeding pathways in birds in relation to parental care and whether they are modulated by hormones such as PRL would be useful in order to understand PRL's role in chick provisioning more generally.

Another important concept is that PRL and parental behavior exhibit a bidirectional relationship. While high PRL levels are required for egg incubation/chick care, the presence of eggs/chicks can also stimulate PRL, making the direction of causality difficult to discern. More studies on the effects of chick and egg stimuli on the secretion of PRL are needed in order to help resolve the chicken-or-egg conundrum of how non-breeding/helper birds can take care of chicks. In those cases, parental care is likely more stimulus driven, rather than hormonally driven, but helpers nonetheless show increases in PRL during time of chick care. How does PRL increase in helpers when they have not undergone the previous nesting, egg laying, or mating processes that parents experience? Relatedly, how does information such as tactile input of eggs get relayed to the hypothalamus to stimulate PRL release in both breeders and non-breeding helpers? The exact mechanism of this feedback loop is not well understood and would likely involve looking at how peripheral PRL targets interact and communicate with central PRL targets.

How do these many functions in parental care become reconciled to describe PRL's role in parental care? One possibility is that PRL serves as an “integrator signal” which processes the current internal and external state of the animal to serve as signal for appropriately timed parental care behavior. Therefore, instead of stimulating the actual expression of the behavior per se, perhaps PRL sends a signal which indicates that is it the right time to display these behaviors. One particular brain area which is likely important for this integration signal is the POA. The POA has been thought to be a relay station between incoming sensory information and reward processing in mammals, which is important for the expression of rodent maternal care (Numan, 2007). It is not yet known whether such circuitries exist in birds in regards to

parental care or whether parental care would be regulated in similar ways in the brain as in mammals. However, given the repeatedly high number of PRLs in the POA and PRL's importance in many different aspects of both avian and mammalian parental behavior, this hypothesis is plausible. Until then, this remains a large, open area in great need of research.

While it is clear that PRL plays a critical role in parental care, *how* exactly it affects parental care is still not well understood. Because sensory stimuli from eggs/chicks is required to elicit parental care, my current hypothesis is that PRL serves as an “integrator signal” to increase attentiveness to these cues in order to elicit the appropriate amount of parental care. The strength of this signal is likely determined by integrating both internal and external breeding information to dictate whether it's an appropriate time to invest in parental care and how much to do so. However, much more work is needed, particularly on the central actions of PRL, before making any such conclusions which support for this hypothesis. Although PRL has been shown to influence egg incubation, egg/nest attentiveness, attractiveness of chicks, and increased feeding and regurgitation, the majority of this work has been performed in ring doves, chickens, and turkeys. These same roles need to be tested in a wider array of avian species with different social life histories, ecological backgrounds, and reproductive systems before they can be generalized to common avian functions.

Finally, two other areas which would greatly benefit from additional research are the role of PRL in parental motivation and the regulation of the HPA axis during parental care. Understanding how PRL affects each of these processes and integrates internal and external information to influence parental care is important for understanding how neuroendocrine mechanisms evolve to support parental care behaviors. More generally, it can elucidate how these types of mechanisms can become co-opted for new social behaviors to give us predictive power to understand how PRL-mediated behaviors may function and evolve in other organisms.

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