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REVIEW

The neuroethology of avian brood parasitism

Kathleen S. Lynch

ABSTRACT

Obligate brood-parasitic birds never build nests, incubate eggs or supply nestlings with food or protection. Instead, they leave their eggs in nests of other species and rely on host parents to raise their offspring, which allows the parasite to continue reproducing throughout the breeding season. Although this may be a clever fitness strategy, it is loaded with a set of dynamic challenges for brood parasites, including recognizing individuals from their own species while growing up constantly surrounded by unrelated individuals, remembering the location of potential host nests for successful reproduction and learning the song of their species while spending time being entirely surrounded by another species during a critical developmental period, a predicament that has been likened to being 'raised by wolves'. Here, I will describe what we currently know about the neurobiology associated with the challenges of being a brood parasite and what is known about the proximate mechanisms of brood parasite evolution. The neuroethology of five behaviors (mostly social) in brood parasites is discussed, including: (1) parental care (or the lack thereof), (2) species recognition, (3) song learning, (4) spatial memory and (5) pair-bonding and mate choice. This Review highlights how studies of brood parasites can lend a unique perspective to enduring neuroethological questions and describes the ways in which studying brood-parasitic species enhances our understanding of ecologically relevant behaviors.

KEY WORDS: Song learning, Parental behavior, Social behavior, Spatial memory, Cowbird, Blackbird

Introduction

Avian obligate brood parasites use a Machiavellian-like reproductive strategy in which they leave their eggs in the nest of another species rather than providing parental care for their own young. These birds leave demanding tasks such as nest building, egg incubation and nestling provisioning entirely to another species, which allows the parasite to continue laying eggs throughout the breeding season. Consequently, brood-parasitic species have traded off parental care for enhanced reproductive output. For example, in at least one parasitic species, it has been documented that 120 eggs were laid by the same female in a single breeding season – an egg-laying rate that is roughly equivalent to 9.2 times the female's body mass (Kattan, 1995). This is in stark contrast with most songbird species that lay 10–12 eggs per season at most if they are fortunate enough to raise two broods in a season, which is a labor-intensive endeavor.

Although it may appear that avian brood parasites take an idle approach to ensuring parental care for their offspring, the parasitic strategy presents significant challenges for both adults and young brood parasites. For instance, the young brood parasite faces the

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predicament of being 'raised by wolves' (Searcy and Nowicki, 2019) because they spend critical developmental stages surrounded by the wrong (i.e. heterospecific) species. This increases the risk of mis-imprinting, species recognition errors and mistakes in social learning, including song learning (ten Cate and Vos, 1999; Slagsvold and Hansen, 2001; Slagsvold et al., 2002). In addition, breeding adults need excellent spatial navigation skills to locate and remember nests that may be potential hosts for their young and they must coincide their egg laying with the host's egg laying to optimize the parasite's chances at avoiding detection. Thus, parasites must also remember the stage of each potential host nest (i.e. egg laying, incubation or nestling provisioning). Each of these predicaments represents new challenges and the neural adaptations associated with these challenges may provide unique insight into long-standing neuroethological questions. Yet, examining proximate mechanisms to understand the evolution of the brood-parasitic strategy and the molecular, neural and hormonal adaptations that allow parasitic species to meet the challenges associated with their lifestyle have been almost entirely overlooked.

By integrating studies of proximate and ultimate mechanism, we may finally examine brood-parasitic behavior from each of Tinbergen's four perspectives (i.e. causation, function, development and phylogeny; Bateson and Laland, 2013) rather than focusing solely on perspectives provided by behavioral ecologists. The purpose of the present Review is to highlight the ways in which parasitic species serve as a natural system to address enduring neuroethological questions concerning parental care, species recognition, song learning, spatial memory and development of social behaviors, including pair bonding and mate choice.

Loss of parental care in brood parasites

Parental care has been lost in birds seven independent times, resulting in roughly 99 brood-parasitic species. There are three proposed origins for brood parasitism that exists in cuckoos, cowbirds, honeyguides, Old World finches and one South American duck (Lanyon, 1992; Sorenson and Payne, 2002; Powell et al., 2014; Fig. S1A). Brood parasitism arises in many forms, including (1) facultative parasitism, in which birds switch between parasitizing nests and building their own, (2) intra-specific parasitism, which involves occasionally leaving eggs in the nest of conspecifics, and (3) obligate brood parasitism, in which birds never perform parental responsibilities. This Review will focus on obligate brood parasites.

Behavioral ecologists have provided many excellent explanations as to how obligate brood parasitism may have evolved. These explanations include the evolution of highly specialized diets that increased time spent foraging at the expense of time spent on parental activities (Davies, 2000). Obligate brood parasitism can also be considered a form of 'bet hedging' that dilutes the risk of losing an entire brood to a predator or environmental unpredictability (Hamilton and Orians, 1965; Payne, 1977; Rothstein, 1993, 1994; Winfree, 1999; Antonson et al., 2020). Although behavioral ecologists have provided many alternative explanations for the

evolution of brood parasitism, very few studies have examined parasitism from the proximate perspective. The few proximate studies of brood parasites that have been done mostly focus on one family of parasites: the *Icteridae* (i.e. blackbirds). Parasitic icterids are within the *Molothrus* genus (i.e. cowbirds) and inhabit North, Central and South America. Consequently, not only are there few mechanistic studies of parental care in brood parasites but also the studies that have been conducted are largely focused on cowbirds of the Americas. This Review will describe two proximate explanations that have been explored to understand the loss of parental care in cowbirds, including the prolactin-insensitivity hypothesis and neotonic gene expression in the brain of parasitic cowbirds.

The prolactin-insensitivity hypothesis

Investigations into how brood parasites lost parental care mostly focus on the role of prolactin (Höhn, 1959; Selander, 1960; Selander and Kuich, 1963; Selander and Yang, 1966; Dufty et al., 1987; Ball, 1991). Prolactin is an anterior pituitary peptide hormone that is often referred to as the 'parental hormone'. However, prolactin actually serves over 300 functions (Bole-Feysot et al., 1998) and there are examples in which prolactin and parental behavior are disconnected. Brood parasites are among the top examples of this disconnect (Angelier et al., 2016), which leads to questions concerning inter-species variation in prolactin's role in parental care and whether prolactin is even necessary or sufficient for parental behaviors. In brown-headed cowbirds (*Molothrus ater*), circulating prolactin levels are not significantly different from those in red-winged blackbirds (*Agelaius phoeniceus*), a closely related non-parasitic blackbird species (Höhn, 1959; Fig. S1B). Seasonal-related fluctuations in prolactin occur in brown-headed cowbirds as is also the case in parental birds (Dufty et al., 1987; Buntin, 1996). However, there are fewer prolactin receptors in brown-headed cowbirds relative to red-winged blackbirds (Ball, 1991), specifically in a brain region critically involved in regulating parental care in nearly all vertebrates that exhibit parental behaviors: the preoptic area (POA; Fig. S2). Exogenous prolactin in female brown-headed cowbirds does not stimulate development of a brood patch, nest building or egg incubation even if administered with estradiol, progesterone or a combination of these hormones (Selander, 1960; Selander and Kuich, 1963; Selander and Yang, 1966; Lynch et al., 2020), although these hormones have been linked to parental behavior in other bird species (Angelier et al., 2016). These collective studies indicate a disconnect between prolactin and parental care in brood-parasitic species.

One possible mechanism to explain the prolactin–parental care disconnect in brood parasites is prolactin insensitivity. This possibility was examined by comparing POA transcriptomes and candidate genes in surrounding hypothalamic regions of prolactin-treated and untreated female brown-headed and bronzed *(Molothrus aeneus)* cowbirds. Transcriptome comparisons revealed only four transcripts were differentially expressed between prolactin-treated and untreated birds (Lynch et al., 2020; Fig. 1A,B), none of which were relevant to parental or other social behaviors. Candidate gene analysis also revealed no significant differences in transcript abundance between treatments in surrounding hypothalamic brain regions (Fig. S2), which included the paraventricular nucleus (PVN), ventromedial hypothalamic nucleus (VMN), lateral hypothalamic (LH) and tuberal hypothalamic regions (TU; Lynch et al., 2020; Fig. 1C–J). The eight candidate genes examined in hypothalamic regions surrounding the POA were neurotransmitters and structural plasticity-related genes that help regulate parental care, including prolactin receptors (PR), arginine vasotocin (avian

homolog of vasopressin), mesotocin (avian homolog of oxytocin), galanin, prostaglandin synthase, corticotropin releasing hormone, stathmin and mesencephalic astrocyte-derived neurotrophic factor (see Table S1 for function of genes and corresponding citations). Transcriptomic and candidate gene comparisons both indicate that the POA and surrounding hypothalamic brain regions exhibit insensitivity to prolactin, at least at the mRNA level.

Although transcriptome comparisons indicate prolactin insensitivity, this does not appear to be the case at the behavioral level as the hormonal milieu necessary for the onset of parental care in other birds and mammals does not stimulate maternal-like behavior in female brown-headed cowbirds. On the contrary, it appears to stimulate nestling avoidance (Lynch et al., 2020). Female brown-headed cowbirds treated with estrogen, estrogen plus prolactin, or saline were placed in approach/avoidance tests with nests containing sounds of begging nestlings or random tones. Saline-treated females spent more time near nestling begging sounds than random tones; however, this pattern reversed in females treated with either estrogen or estrogen and prolactin, resulting in significant interactions between hormone treatment and type of sound (Fig. 2A; Lynch et al., 2020). In this case, hormone-treated females avoided the begging nestling sound by spending more time near tones. Females treated with estrogen or estrogen plus prolactin made significantly more visits to empty food cups with random tones compared with nests with begging nestling sounds (Fig. 2B; Lynch et al., 2020). These results did not disentangle the effects of estrogen versus prolactin on nestling avoidance in cowbirds because both groups of hormone-treated females avoided the nestling begging sounds. Nonetheless, these behavioral results support earlier studies from Selander and others, which show that hormonal manipulations stimulating parental care in other birds do not stimulate physiological or parental-related behavior in cowbirds, including the appearance of a brood patch, or nest building and egg incubation behaviors (Selander, 1960; Selander and Kuich, 1963; Selander and Yang, 1966).

Prolactin insensitivity in parasitic birds may occur via a lower overall density of prolactin receptors, a lower density of specific receptor isoforms or both. Recent POA transcriptome comparisons between brown-headed and bronzed cowbirds versus red-winged blackbirds did not detect prolactin receptor (PR) as being differently expressed between parasitic and non-parasitic species (Lynch et al., 2019). However, comparing PR as the only gene of interest revealed lower PR abundance in female brown-headed cowbirds compared with red-winged blackbirds in the POA and surrounding hypothalamic regions (Lynch et al., 2020; Fig. 3), confirming results reported by Ball (1991). However, lower PR abundance was not identified in bronzed cowbirds compared with red-winged blackbirds. Thus, it is not entirely clear whether lower PR abundance in the POA is a conserved mechanism across various brood-parasitic species. The existence of multiple PR isoforms in mammals (and turkeys) suggests an alternative possibility (Bole-Feysot et al., 1998; Grattan et al., 2001). These multiple isoforms are tissue specific such that one isoform exists in the brain and all types fluctuate in various tissues with reproductive state (Pitts et al., 2000). Thus, it is possible that different receptor isoforms may activate different intracellular pathways and it may be this difference that matters in parental care, not merely the density of prolactin receptors.

Neotony in gene expression

Although the prolactin-insensitivity hypothesis does appear to have at least some support, there are still alternative possibilities, such as the retention of juvenile-like (neotenic) gene expression (Lynch
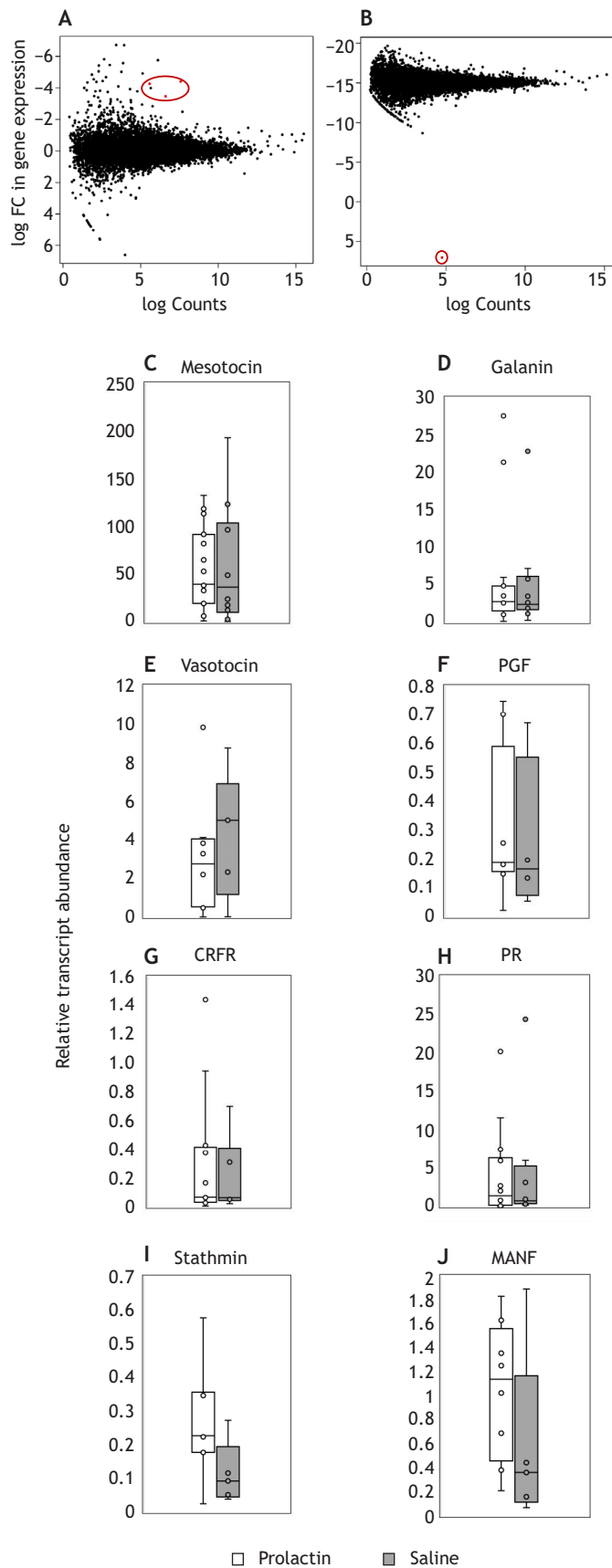
Fig. 1. Sensitivity of mRNA expression to prolactin in parasitic cowbirds. This study focused on brain regions involved in regulating parental care in female parasites; mRNA transcript abundance was measured in the preoptic area (POA; A,B) and surrounding hypothalamic brain regions (C–J) of cowbirds that were either treated with prolactin or untreated (saline). (A,B) Results generated using Next Generation Sequencing (NGS) reveal negligible effects of prolactin treatment on transcript abundance (fold change, FC) in (A) brown-headed and (B) bronzed cowbirds. Red dots indicate four transcripts that did have altered expression patterns between prolactin treatments in bronzed and brown-headed cowbirds. (C–J) The expression of eight individual candidate genes involved in the regulation of parental care, examined using real-time quantitative PCR (qPCR). The graphs illustrate combined data from both bronzed and brown-headed cowbirds that were prolactin treated or untreated (Lynch et al., 2020). PGF, prostaglandin synthase F; CRFR, corticotropin releasing factor receptor; PR, prolactin receptor; MANF, mesencephalic astrocyte-derived neurotrophic factor.

associated with a failure to transition into an adult-like behavioral state. POA transcriptome comparisons between two parasitic species and juvenile or adult red-winged blackbird were used to evaluate whether gene patterns looked more like a juvenile or adult pattern (i.e. neotony; Lynch et al., 2019). Neotenic gene patterns in the cowbird POA were apparent in both concordantly differentially expressed (CDE) genes (genes differentially expressed in both cowbirds compared with redwings) and differentially expressed (DE) genes (genes that differ in either brown-headed or bronzed cowbirds relative to redwings). Roughly 78% of CDE transcripts exhibited juvenile-like expression in parasitic cowbirds (Fig. 4; Lynch et al., 2019). Among DE genes, 76% and 74% exhibited neotenic patterns in brown-headed or bronzed cowbirds, respectively. This transcriptome comparison demonstrates evidence for shifts toward neotenic expression in the POA of adult parasites. These results suggest that brood-parasitic behavior may be mediated by a failure to transition to an adult-like, parental state. This neotenic gene pattern may occur by altering existing mechanisms through shifts in developmental timing rather than the evolution of novel genetic variants.

Species recognition and social learning
Password-based species recognition

The conventional view that early sensory experience is critical for appropriate species recognition in songbirds (Brenowitz and Beecher, 2005) presents a significant challenge for young brood parasites, which are raised with little contact with related individuals. One means by which young brood parasites may recognize their own species is using passwords that identify other parasites of the same species. A password can be any unique identifier that is unlearned and salient from a very early age, including vocalizations or other phenotypic attributes that identify conspecifics and initiate social learning (Hauber et al., 2001). The chatter vocalization produced by brown-headed cowbirds is a good candidate for a password because it is unlearned, not sex specific, and produced throughout the breeding season (Burnell and Rothstein, 1994; Hauber et al., 2001). Hauber and colleagues (2001) tested cowbird nestling responses to cowbird chatter relative to other call types, including the chatter of heterospecific species. Their results confirmed that cowbird nestlings preferentially respond to cowbird chatter and preferentially approach chatter once they fledge the nest. However, the neural mechanisms that guide these presumably innate preferential responses to this chatter (password) are not at all clear.

Activity-dependent gene expression has been used in brown-headed cowbirds and red-winged blackbirds to investigate neural mechanisms of password-based species recognition in auditory

et al., 2019). Neoteny is characterized by the retention of juvenile traits in adults. In the case of brood parasites, it is possible that neotenic gene expression, particularly in key brain regions, is
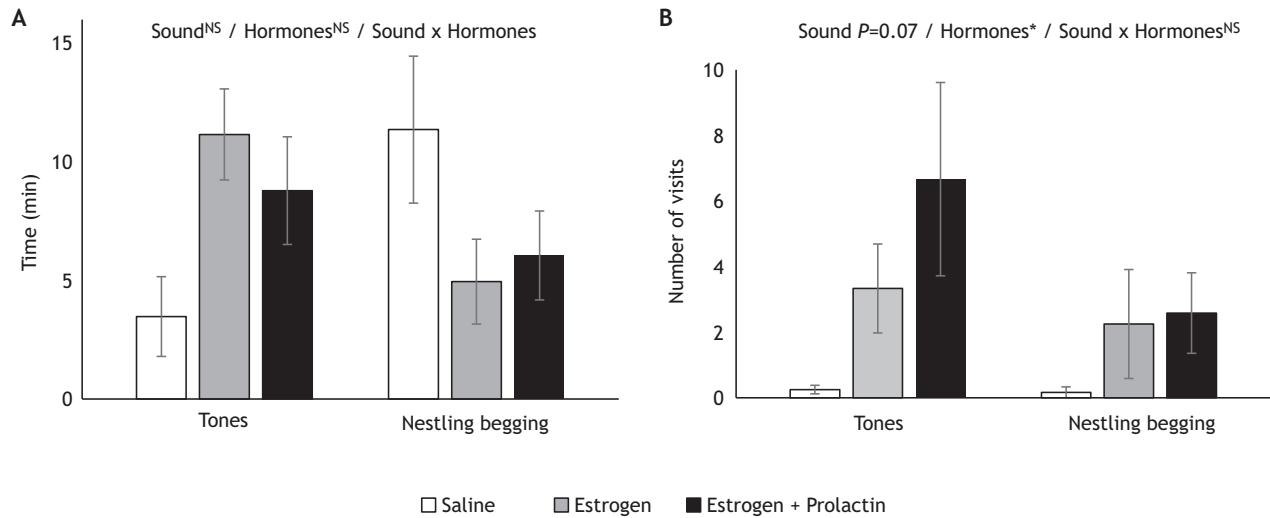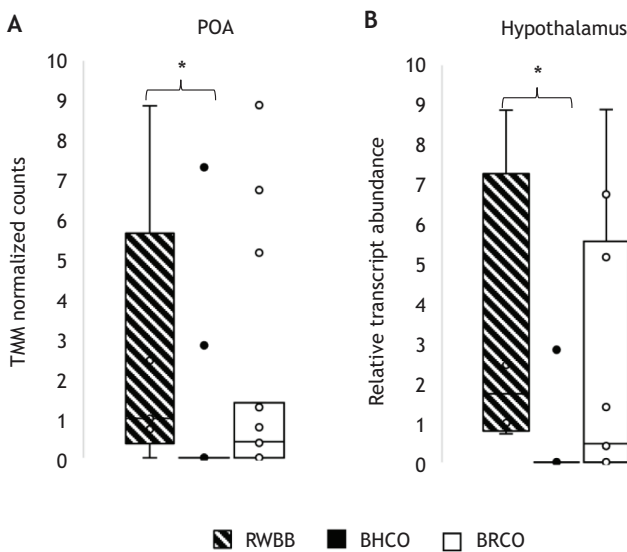
Fig. 2. Behavior of prolactin-treated and untreated parasitic cowbirds. Hormone-treated (estrogen, estrogen+prolactin) or untreated (saline) birds were free to approach or avoid the sound of begging nestlings coming from a nest or the sound of random single-frequency tones broadcast from a cup. The behaviors measured include (A) time spent near nests with begging nestling sounds versus food cups with tones, and (B) number of visits to nests versus food cups. Visits were defined as perching on or in the structure. All subjects were adult female brown-headed cowbirds. NS, not significant, *significant at α≤0.05. Adapted from Lynch et al. (2020).

forebrain regions, including the caudal medial mesopallium (CMM) and the caudal medial nidopallium (NCM). These regions are homologous to the mammalian auditory cortex (Jarvis et al., 2005) and are involved in the recognition of learned vocalizations such as song, which is used in courtship or territorial defense in songbirds (Louder et al., 2019a,b). These higher order auditory cortex regions (NCM and CMM) are prominent neural substrates for auditory recognition in many oscine birds, including brood-parasitic species

Fig. 3. Prolactin receptor transcript abundance in the preoptic area (POA) and surrounding hypothalamic regions of parasitic cowbirds and non-parasitic blackbirds. (A) Comparison of prolactin receptor (PR) expression in the POA using Next Generation Sequencing. TMM, normalization using trimmed mean of M values. (B) Comparison of PR expression in the hypothalamic regions surrounding the POA using qPCR. Pooled hypothalamic regions include: paraventricular nucleus, ventromedial hypothalamic nucleus, and lateral and tuberal hypothalamic regions. RWBB, red-winged blackbird; BHCO, brown-headed cowbird; BRCO, bronzed cowbird. Adapted from Lynch et al. (2020). *P≤0.05.
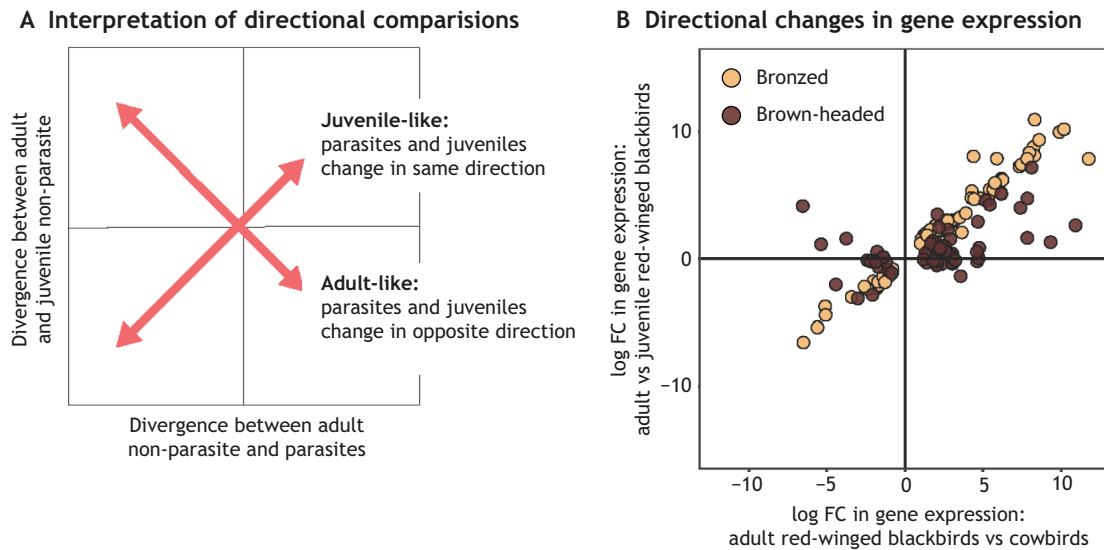
(Louder et al., 2016). To examine the possibility that the NCM and CMM are involved in the processing of unlearned vocal passwords, juvenile and adult male brown-headed cowbirds were exposed to conspecific chatter (password) or dove coos (heterospecific unlearned vocalizations). Activity-dependent gene expression was measured in the NCM and CMM using ZENK (zif268, egr-1, NGFIA and krox-24) immunoreactivity (ZENK-ir) across ages and species. ZENK-ir was elevated in response to conspecific chatter relative to dove coos in both juvenile and adult male cowbirds in the NCM (Fig. 5). In the CMM, however, this pattern only appeared in adult male cowbirds whereas juveniles exhibited no ZENK-ir differences when exposed to chatter or coos (Fig. 5; Lynch et al., 2017), indicating that these regions, particularly the NCM, exhibit selective activity-dependent responses to unlearned vocalizations. Moreover, the NCM exhibits chatter-selective ZENK-ir responses in juveniles that have heard few (if any) conspecific vocalizations, suggesting it is involved in species recognition in young parasites. In contrast, the CMM exhibits chatter-specific ZENK-ir induction only in adults that are presumably familiar with the sound, indicating this region guides species recognition and discrimination after the parasite has encountered conspecifics and experienced conspecific sounds.

Differences in activity-dependent gene expression in the auditory forebrain of juvenile cowbirds also highlight the functional differences between the NCM and CMM, which has been an enduring question in songbird sensory neurobiology. For instance, in the cowbird, ZENK-ir in the CMM appears to be dependent on song familiarity, whereas this is not the case in the NCM (Fig. 5). A follow-up comparative study explored whether functional differences in the NCM and CMM are an adaptation in parasitic species (Lynch et al., 2018). Again, activity-dependent gene expression was measured in the NCM and CMM, but this time in juvenile male red-winged blackbirds exposed to conspecific chatter, dove coos or cowbird chatter. The results revealed that activity-dependent gene expression in the CMM, but not the NCM, was selectively evoked in response to conspecific chatter relative to cowbird chatter or dove coos (Fig. 6A). Because red-winged blackbird nestlings are surrounded by conspecifics from birth, they

A Interpretation of directional comparisions

B Directional changes in gene expression

Fig. 4. Neotenic gene expression in the POA of adult female brown-headed cowbirds. The log fold-change (FC) in gene expression was calculated between adult cowbirds and adult or juvenile red-winged blackbirds for 81 genes found to be concordantly differentially expressed (CDE) in the POA of two brood parasites. (A) The model demonstrates the pattern of log fold-change in gene expression if cowbird gene expression better resembles that of an (1) adult or (2) juvenile red-winged blackbird. (B) The actual pattern of CDE genes of both brown-headed and bronzed cowbirds (Lynch et al., 2019).

are presumably familiar with their species' chatter sounds from day one. This further supports a role for the CMM in responding to sound familiarity as this result does not occur in the CMM of juvenile cowbirds that are not yet familiarized with their own species' chatter (Fig. 6B; Lynch et al., 2018). This comparative study identified functional differences in the CMM and NCM, but also revealed that the NCM is possibly co-opted in parasitic auditory systems to recognize unlearned and unfamiliar vocalizations. The conclusion that the CMM responds to song or sound familiarity is consistent with functional studies in other songbirds (Gentner et al., 2004) and highlights the power of comparisons between parasitic and non-parasitic species to help understand the functional
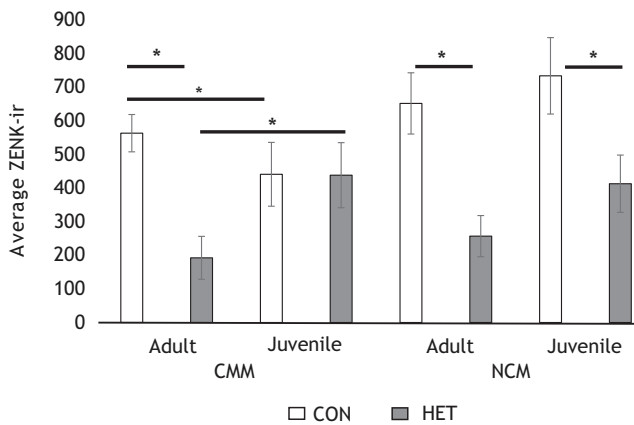

Fig. 5. Auditory neural responses to chatter in juvenile and adult brown-headed cowbirds. Neural activity in response to chatter (the presumptive password in brood parasites) was measured using protein labels of activity-dependent gene expression (ZENK immunoreactivity, ir) in two auditory forebrain regions: the caudomedial nidopallium (NCM) and caudal medial mesopallium (CMM). Juvenile (hatch year) birds were within 30 days of nest fledging, whereas after hatch year birds are adults. Juveniles have little prior experience with conspecific chatter, whereas adults have presumably encountered conspecific vocalizations in their lifetime. CON, conspecific chatter exposure; HET, dove coo exposure. Both vocalizations were unlearned. Adapted from Lynch et al. (2017). *$P \leq 0.05$.

differences across brain regions and to provide unique insight into whether species recognition is influenced by innate templates, sensory experience or both.

Password-based social learning
The password hypothesis posits that effective passwords initiate social learning and therefore social learning should not occur prior to encountering the password. To investigate whether this is the case, song-naive juvenile male and female cowbirds were exposed to canary song paired with chatter (the password) or dove coos to identify whether chatter enhances the acquisition of specific song structures and promotes the expression of neuroplasticity-related genes (Louder et al., 2019a,b). The results show that songs of juvenile cowbird males exposed to canary songs with chatter had less disorder and better resembled the tutor songs compared with songs of cowbirds exposed to song paired with dove coos (Louder et al., 2019a,b), indicating better song acquisition. Also, males exposed to chatter paired with canary song exhibited genomic signatures of neuroplasticity after exposure to familiar canary song (Louder et al., 2019a,b). Neuroplasticity-related genes were differentially expressed between chatter-exposed and coo-exposed males, including probable glutamate receptor, aromatase, teneurin-1 and caspase-6 genes (Louder et al., 2019a,b). Differential expression of genes in password- and non-password-exposed males may be related to habituation, a non-associative form of learning that appears to be password dependent. Gene ontology (GO) analysis of differentially expressed genes identified significant categories indicative of neural plasticity, including 'regulation of nervous system development', 'regulation of axonogenesis' and 'regulation of neuron differentiation' (Louder et al., 2019a,b). There were more genes in these categories in the chatter treatment relative to controls and several are involved in auditory learning and neuroplasticity in songbirds (Louder et al., 2019a,b). Genes involved in long-term memory were differentially expressed between chatter- and non-chatter-exposed female cowbirds (Louder et al., 2019a,b). These genes presumably indicate learning via habituation and occur in chatter-exposed females; they include NR4A2, NR4A3 (nuclear receptor subfamily
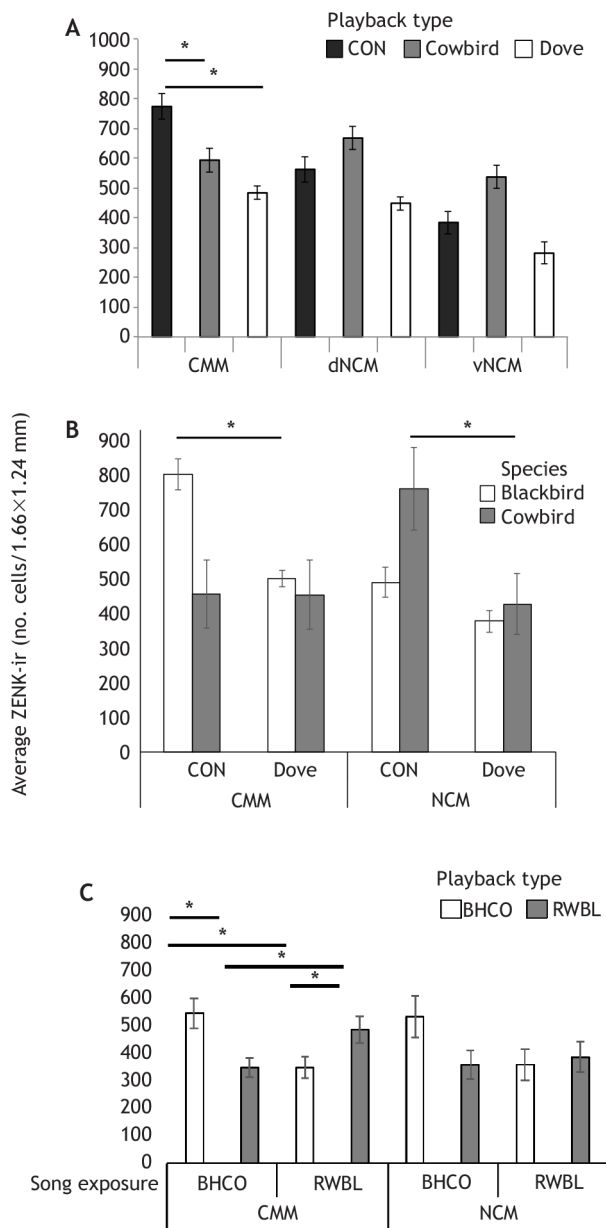
Fig. 6. Specificity of responses to chatter and song in cowbirds. The studies in A and B examined whether auditory responses to chatter calls (the presumptive password in brood parasites) are specific to cowbirds or whether these responses also occur in closely related non-parasitic blackbird species; the study in C examined whether brood-parasitic species exhibit neural signatures of mis-imprinting after prolonged exposure to heterospecific songs during development. Neural activity in juvenile parasitic and non-parasitic blackbirds was assessed using protein labelling of activity-dependent gene expression (ZENK-ir) within higher-order auditory processing regions: the CMM and NCM (dNCM, dorsal NCM; vNCM, ventral NCM). (A) Juvenile male red-winged blackbirds were exposed to conspecific (CON) chatter, cowbird chatter or dove coos. All stimuli were unlearned vocalizations. (B) Juvenile brown-headed cowbirds and red-winged blackbirds were exposed to either conspecific chatter or dove coos. (C) Juvenile (≤38 days) brown-headed cowbirds were subjected to prolonged exposure to either brown-headed cowbird (BHCO) or red-winged blackbird (RWBL) songs. On the test day, birds were exposed to either the familiar song (pre-exposed song) or an unfamiliar song (not pre-exposed). All stimuli were learned vocalizations. Adapted from Lynch et al. (2017, 2018). *$P \leq 0.05$.

group A 2 and 3), EGR-1 (early growth response 1) and FOSL-2 (FOS related antigen 2), all of which play a role in song familiarity in female zebra finches (Dong et al., 2009). These results indicate

that passwords can initiate social learning, including song learning in both males and females.

Mis-imprinting avoidance

Imprinting is arguably the most important form of learning needed for accurate species recognition. But this presents yet another challenge for young brood parasites as imprinting on characteristics of host parents or siblings can lead to significant deficits in fitness via misdirected courtship and aggression (ten Cate and Vos, 1999; Slagsvold and Hansen, 2001; Slagsvold et al., 2002). For instance, non-parasitic songbirds imprint on foster parents in cross-fostering experiments, which results in an adult that responds to heterospecifics during courtship and aggressive interactions (ten Cate and Vos, 1999; Slagsvold et al., 2002). Young brood parasites are only exposed to species-specific signals, including plumage, behavioral displays and acoustic traits, after locating flocks of other juvenile and adult conspecifics (Freeberg et al., 1995) and, therefore, they may spend extended periods of time surrounded by heterospecifics. The NCM and CMM were examined in juvenile male brown-headed cowbirds after prolonged exposure to red-winged blackbird songs to explore intrinsic mis-imprinting avoidance mechanisms (Lynch et al., 2017). However, rather than identifying a neural basis for mis-imprinting avoidance, this study revealed that young cowbirds do exhibit neural signatures of mis-imprinting. Young male juvenile cowbirds did not exhibit song-selective responses in the CMM unless provided with recent and prolonged experience of song. The type of song, however, had no significant impact on the song-selective response. Instead, song familiarity had the greatest influence on activity-dependent gene expression within the CMM. In contrast, song familiarity had no effect on activity-dependent gene expression in the NCM (Fig. 6C). Thus, in juvenile male cowbirds, ZENK-ir in the CMM is dependent on previous prolonged exposure to song, rather than whether song was conspecific or heterospecific (Fig. 6C). This indicates that neural signatures of mis-imprinting do occur within the auditory forebrain in juvenile cowbirds, but whether this is entirely maladaptive is not clear as cowbirds should learn heterospecific song for eavesdropping during nest searching, which may potentially improve their odds of finding and parasitizing an active nest.

Song learning

Brood-parasitic cowbirds are within the order Passeriformes (suborder Oscine; hereafter referred to as songbirds). Songbirds typically learn songs from adult male conspecific tutors early in development (Marler and Peters, 1987; Brainard and Doupe, 2002; Williams, 2004; Brenowitz and Beecher, 2005), which is a time frame when brood-parasitic young have negligible exposure to conspecific songs. Songbirds are frequently considered as either 'closed-ended' (age-restricted) or 'open-ended' learners, with the latter having a protracted time frame for song learning (Marler and Peters, 1982; Marler and Peters, 1987; Brainard and Doupe, 2002; Brenowitz and Beecher, 2005). Brood-parasitic songbirds do not fit either of these categories as they do not require adult male tutors during development (King and West, 1977; Brenowitz and Beecher, 2005) and they do not add new songs throughout their lifetime as is the case for open-ended learners (Brenowitz and Beecher, 2005). They also cannot be restricted to learning songs during early development because they may require over a year to locate adult conspecifics (Payne, 1977). Thus, the neuroplasticity that defines the critical time frame for song learning (if there is one) remains an open question.

Parvalbumin (PV) and perineuronal nets (PNN) are neuroplasticity markers that respectively indicate the onset and closure of sensitive periods for vocal learning in songbirds (Balmer et al., 2009). These markers were examined in male cowbirds to determine whether neuroplasticity patterns in juveniles and adults approximated that of an open-ended learner or an entirely novel pattern. PNN and PV were compared across juvenile and adult brown-headed cowbirds and red-winged blackbirds (i.e. open-ended learners; Marler et al., 1972). Both PNN and PV were quantified within song-control regions, including the nucleus HVC, RA (robust nucleus of the arcopallium), Area X and LMAN (lateral magnocellular nucleus of the anterior nidopallium). After controlling for differences in nucleus volume, the RA was found to have species differences in PNN and PV+PNN/nuclei and a significant interaction between species and age (Fig. 7A; Cornez et al., 2020), indicating these markers are dependent on both age and species. Also, total PNN counts were higher in adult cowbirds relative to those in red-winged blackbirds but not in juveniles (Cornez et al., 2020; Fig. 7B). Because the RA is involved in stereotypy of song structure and syringeal motor control (Nottebohm, 1999; Wild, 2004; Brenowitz and Beecher, 2005; Alward et al., 2017; Fig. 7A), this suggests that onset and closure of neuroplasticity associated with a sensitive period are specific to a region involved in motor control of song.

Singing-dependent PNN was investigated by comparison of male and female cowbirds because results in the RA point toward song production and stereotypy as driving the differences between species (Cornez et al., 2020). Because female cowbirds do not sing, a sex-difference comparison further explored the idea that differences in song production were driving the observed differences in PNN/PV. After controlling for sex differences in nuclei volume, robust sex differences in the density of PNN, PV and PV+PNN occurred in the RA, but not in other song control nuclei (Cornez et al., 2020; Fig. 7C). With respect to differences across ages, PNN and PV+PNN/nucleus were significantly greater in adult males relative to juvenile males in both species in the HVC, RA and Area X. This indicates that both cowbirds and red-winged blackbirds display elevated PNN and PV+PNN as they enter adulthood, regardless of whether they experienced conspecific songs during early development. These correlative results suggest that parasitic songbirds have co-opted the song-learning system of an open-ended learner rather than exhibiting an entirely novel system. By using a similar neural plasticity pattern to open-ended learners, cowbirds may possess a protracted song-learning time frame that allows them additional time to locate conspecifics and begin song learning.

Nest searching
Spatial memory and the hippocampus
Brood parasites have long offered a unique perspective for studies of spatial memory and the hippocampus because female parasites must be proficient at remembering host nest locations (Norman and Robertson, 1975) and the respective stages of each nest (i.e. egg laying, incubation or nestling provisioning). Parasitizing nests at the appropriate time substantially improves the odds of egg acceptance by the host, whereas the placement of eggs in nests with no host eggs or nestlings can result in egg ejection or nest desertion (White et al., 2009). Cowbirds also repeat nest visits to evaluate potential egg rejection by the host (Hoover and Robinson, 2007) and remove host eggs (Sealy, 1992). All of this implies that cowbirds maintain a spatial map of their parasitized nests and constantly update their memory of available host nests so they can recall which nests have completed nesting or have been depredated or abandoned, allowing
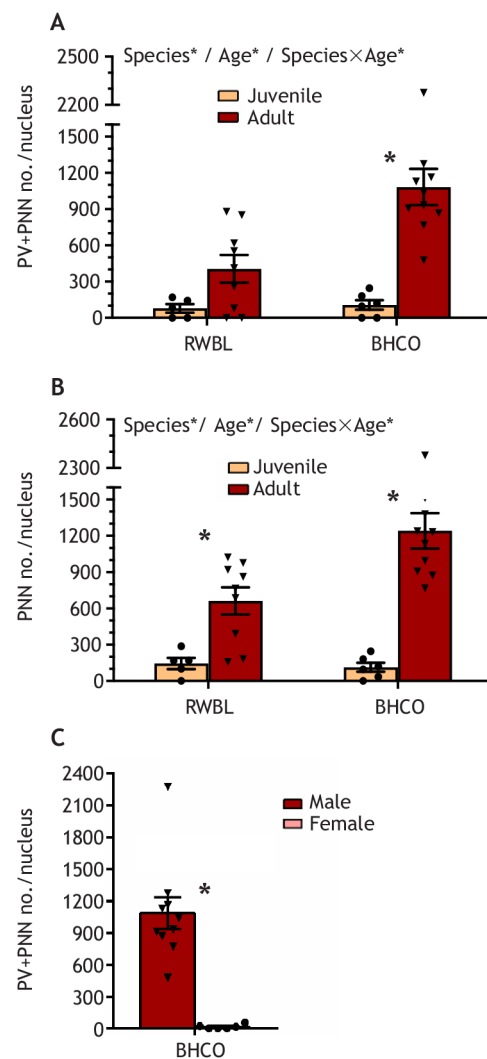
Fig. 7. Neural plasticity of song learning in cowbirds. Neural plasticity in the song system was compared in juvenile and adult brown-headed cowbirds (BHCO) and non-parasitic red-winged blackbirds (RWBL) to determine whether neuroplasticity patterns in brood parasites approximated those of a songbird with open-ended learning or an entirely novel pattern. Neural plasticity was measured using perineuronal nets (PNN) and parvalbumin (PV); both PNN and PV were quantified within all song-control regions but only the data for the robust nucleus of the arcopallium (RA) are shown; the nucleus volume was included in the analysis for all subjects. (A) Comparison of the number of cells in the RA of male cowbirds and blackbirds with co-localized PNN and PV. (B) Comparison of number of cells in the RA of male cowbirds and blackbirds with PNN only. (C) Comparison of the number of cells with co-localized PV+PNN in the RA of male and female brown-headed cowbirds. *$P \leq 0.05$. Adapted from Cornez et al. (2020).

them to avoid putting eggs in inactive nests. Thus, female parasites should have excellent spatial memory skills and the corresponding neural modifications that accompany that ability.

Studies of ecologically relevant spatial memory and the role of the hippocampus in nest searching are rooted in the adaptive specialization hypothesis, which suggests that fitness increases when the neural bases of behavior are modified via selection (Krebs et al., 1989; Krebs, 1990). This can produce sex differences in spatial memory. For example, increased demand for spatial ability in female brown-headed cowbirds is reflected in hippocampal volume. Female cowbirds have a larger hippocampal volume than males and red-winged blackbirds,

both of which do not locate and remember nests (Sherry et al., 1993; Guigueno et al., 2016). Female-biased sex differences in hippocampal size also occur in shiny cowbirds (*Molothrus bonariensis*), another brood-parasitic species in which only females perform nest searching. In contrast, there is no sex difference in hippocampal volume in screaming cowbirds (*Molothrus rufoaxillaris*), a parasitic species in which both sexes nest search (Reboreda et al., 1996; Scardamaglia and Reboreda, 2014; Scardamaglia et al., 2018). Although female cowbirds have the largest hippocampus (relative to the size of the telencephalon) among male and female cowbirds and red-winged blackbirds, there is also a female-biased sex difference in hippocampal volume in red-winged blackbirds (Guigueno et al., 2016). Brown-headed cowbirds of both sexes, however, have a relatively larger hippocampus compared with red-winged blackbirds, and breeding conditions do not effect relative hippocampal size in either species, suggesting that nest searching during the breeding season does not influence the relative size of the hippocampus in female brown-headed cowbirds (Guigueno et al., 2016).

Sex differences in hippocampal volume also translate into superior spatial memory in females. Trained female brown-headed cowbirds made significantly fewer spatial memory errors and took more direct paths to locate hidden food sources compared with males (Guigueno et al., 2014). There was no sex difference in motivation to perform this search task, as measured by the time each sex spent actively searching for the hidden food location. There was also no effect of photoperiod-dependent breeding condition on memory for hidden food sources in this open-field spatial search task (Guigueno et al., 2014). However, the female's superior spatial memory cannot be generalized to all spatial tasks and is likely associated only with tasks that resemble nest searching. For example, trained female and male brown-headed cowbirds were tested in a delayed-matching-to-sample touchscreen task, which requires memory for a location in the immediate visual field without movement though a spatial environment (Guigueno et al., 2015). Breeding condition did affect performance on touchscreen tasks and males outperformed females on this spatial memory task at the shortest retention interval. Although females and males were similar in their performance on the color touchscreen task, females exhibited enhanced performance on this task when in breeding condition (Guigueno et al., 2015), although the behavioral relevance of this enhanced performance is unclear.

Hippocampal neurogenesis

There are multiple alternative mechanisms that may underlie sex- and species-specific hippocampal volume differences. These include structural modification of pre-existing hippocampal neurons, such as changes in soma volume or dendritic arborization, addition of glial cells, rate of neuronal death, dispersion of existing neurons or new neuron recruitment and cell proliferation (Leuner and Gould, 2010; Tronel et al., 2010; McDonald and Kirn, 2012). All of these mechanisms may enhance spatial memory of generalist parasites that leave their eggs in roughly 200 different host species nests (Davies, 2000). The idea that hippocampus-specific cell proliferation may be associated with this task was investigated using doublecortin (DCX), an endogenous protein that marks neurogenesis (Gleeson et al., 1999; Balthazart et al., 2008; LaDage et al., 2010; Melleu et al., 2013). DCX is expressed in neurons for roughly 20–30 days after mitosis is complete and plays a vital role in microtubule scaffolds that guide newborn neurons during migration (Francis et al., 1999; Gleeson et al., 1999; Balthazart et al., 2008; LaDage et al., 2010; Melleu et al., 2013). The results showed that female brown-headed

cowbirds generate more new neurons and fibers (as measured by DCX+ cover) than males in the subventricular zone, a region that contains precursor cells that derive new neurons (Alvarez-Buylla et al., 1990). This sex difference, however, was absent in red-winged blackbirds (Guigueno et al., 2016). DCX+ cover was also higher in the hippocampus post-breeding compared with the breeding condition in brown-headed cowbirds. This contrasts with red-winged blackbirds where higher DCX+ cover occurs in the breeding compared with post-breeding condition. Moreover, DCX+ cover is also higher during post-breeding conditions compared with breeding conditions in brown-headed cowbirds within the subventricular zone, whereas there were no seasonal differences in DCX+ cover in red-winged blackbirds in this region (Guigueno et al., 2016). The post-breeding increase in DCX+ immunoreactivity in cowbirds may relate to replacing older neurons with new neurons encoding new information in preparation for the upcoming breeding season. This would allow the hippocampus to prepare for the immediate acquisition of critical spatial information required for nest searching (Barnea and Pravosudov, 2011). Although it is unknown whether neurogenesis in the avian hippocampus is responsible for increasing brain region volume, as is the case in mammals (Bayer et al., 1982; Crespo et al., 1986), it does counteract losses from cell death in the avian hippocampus (Kirn and Nottebohm, 1993; Scharff, 2000). However, it is still unclear whether new cells serve distinct roles in spatial memory or memory persistence as is the case in other animals, including mammals (Akers et al., 2014; Epp et al., 2016).

Cultural variation in social behaviors: mate choice and pair bonding

Brown-headed cowbirds exhibit cultural differences in many aspects of their social behavior (Freeberg, 2000; White et al., 2002a,b), which makes this species a potentially powerful system for within-species comparative studies of social behavior. Moreover, cowbird social behavior is almost entirely dependent on early developmental experiences, providing yet another valuable resource to understand how developmental experiences influence social behavior later in life (White et al., 2002a,b). Stark inter-population differences in the social environment stem from the large expansion of the cowbird range across North America (Smith et al., 2000), which led to differences in population density, local climates, sex/age ratios, timing of breeding, vocal dialects, migration movements and mating systems (Rothstein et al., 1986, 1988; King and West, 1977; Johnsgard, 1997; Barnard, 1998; Cristol et al., 1999). Inter-specific differences in cowbird social behaviors arise because some juvenile cowbirds never interact with any adults throughout their first year, whereas other juveniles join a flock with actively breeding adults within their first year (Friedmann, 1929). This stark contrast in social environment between populations generates pronounced variability in aggression, pair-bonding, mate guarding, mate choice and song learning (Freeberg, 2000; White et al., 2002a,b; White et al., 2007). These behavioral patterns persist well into adulthood, which maintains divergent cultures across cowbird populations (Freeberg, 2000; White et al., 2002a).

The divergence of cultures between cowbird populations allows for within-species comparisons of social behaviors including, but not limited to, mate choice and pair-bonding. For instance, although it is bewildering for a non-parental species to form long-term pair-bonds, these bonds exist in six of seven parasitic lineages, including some cowbird species (Feeney and Riehl, 2019). Moreover, pair bonding varies between populations such that it may only appear when density for host nests is low, whereas
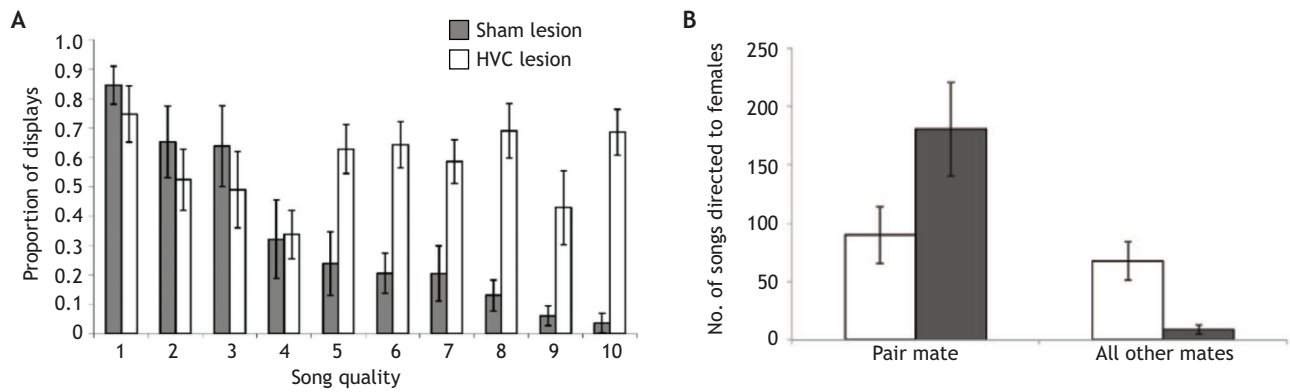
Fig. 8. Connection between the neural basis of mate choice behavior in cowbirds and its impact on the social network. Adult female brown-headed cowbirds living within an established 163 social network were subjected to sham or HVC lesions. (A) The proportion of copulation solicitation displays (CSDs) in HVC- and sham-lesioned females. Songs were ordered by quality based on the number of CSDs females showed to each song in prior years (Maguire et al., 2013). (B) The number of songs males directed to HVC- and sham-lesioned females was used as a proxy of pair bond strength. Songs from the female's pair-mate and from other males are plotted (Maguire et al., 2013).

polygamous behavior dominates when host nests are abundant (Hauber and Dearborn, 2003; Feeney and Riehl, 2019). This provides a powerful resource for neuroethological studies of pair bonding using within-species comparisons and the contribution of early developmental social interactions that may shape pair bonding well into adulthood.

Divergent cultures between populations present a unique system to understand the connections between the neural basis of mate choice decisions and the evolution of social networks. For instance, female brown-headed cowbirds display flexible song preferences depending on early developmental social experiences (King et al., 2003; West et al., 2006). Juvenile females raised with adult females exhibit broad song preference with little consensus across females. In contrast, females raised without adult females exhibit narrow song preferences with concordance across females. These findings indicate that juvenile females exhibit a form of mate copying in which they learn song preference by observing other females (King et al., 2003; West et al., 2006). Thus, opportunities also exist to understand the neuroethology of independent versus non-independent decision making during mate choice trials. In one study of mate choice in cowbirds, female cowbirds were given HVC lesions (Maguire et al., 2013). These females exhibited permissive receptive responses to courting males, as is the case in other female songbirds (Brenowitz, 1991; MacDougall-Shackleton et al., 1998), although they did not become so permissive that they responded to heterospecific songs (Fig. 8A; Maguire et al., 2013). Permissive HVC-lesioned females reduced pair bonding and lacked preferences for dominant males, whereas sham-lesioned females selectively attended to their pair-bonded male (Fig. 8B). The behavioral difference in HVC- and sham-lesioned females created social instability for both males and females within the social network. Because permissive females attracted more male attention, they disrupted male dominance hierarchies, aggression and courtship patterns within the entire social network, indicating that females control some components of the males' social network. Additionally, pair-bonded (non-permissive, sham-lesioned) females produced more undirected vocalizations, possibly as a means of competing with the permissive females that were receiving the bulk of male attention (Maguire et al., 2013). This study highlights a connection between the neural basis of behavior and how the behavior of individuals within a social group can impact the entire network, thus providing a unique perspective on the evolution of social systems and the role individuals can play in structuring social environments.

Conclusions
Obligate brood parasites trade off parental care for increased reproductive output by leaving their eggs and developing offspring in the care of a different species. This puts the young, nestling brood parasite in the predicament of being 'raised by wolves' (Searcy and Nowicki, 2019). This strategy leads to an assortment of challenges for adult, juvenile and nestling parasites and these various challenges likely shape the evolution of the brood parasites' brain, behavior and physiology. Nestling and sometimes even juvenile brood parasites are isolated from their conspecifics, which presents natural and unique opportunities to investigate the neural and molecular basis of social behaviors, especially the ones presented here. However, brood parasitism is not an all-or-none state. There are various other forms of brood parasitism in birds, including facultative brood parasitism. These other forms of parasitism represent even more opportunities for comparative studies that seek to understand the social behaviors discussed here, especially parental care. Moreover, the bulk of the attention has been directed toward cowbirds, particularly brown-headed cowbirds, likely because of the ease with which these birds can be found and caught. However, additional comparative studies both within the Icterid (i.e. blackbird) family and between other parasitic families can offer insight into whether the mechanisms identified and discussed here are conserved mechanisms that drive the evolution of brood parasites or whether there are multiple various independent mechanistic pathways that evolved in the various families of parasitic birds. Because of the novelty of the brood parasites' lifestyle and reproductive strategy, parasitic species offer an opportunity to examine enduring neuroethological questions, particularly concerning social behavior, by asking what the brain looks like in a species that has lost parental care and therefore must face the predicament of being 'raised by wolves'.

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