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RESEARCH ARTICLE

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Caudal mesopallial neurons in female songbirds bridge sensory and motor brain regions

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Abstract

Female songbirds use male song as an indicator of fitness and use that information to select their mate. Investigations of the female auditory system have provided evidence that the neurons within the caudal mesopallium (CM) are involved in the processing of songs that a female finds attractive, however, it is not clear how CM may exert its influence on behavioral indicators of mate choice. In the present study, anterograde tracing revealed the efferent connections of the female songbird CM. The results demonstrate connections to other auditory regions previously described in males, as well as novel connections to brain regions implicated in motor control. As in males, CM neurons in females project robustly to the lateral and medial extents of the caudal nidopallium, and to the ventral intermediate arcopallium. In a novel finding that is not present in males, CM neurons also project to the robust nucleus of the arcopallium and to the caudal striatum. Calling behavior and the expression of copulation solicitation displays are key indicators of female mate choice, and the projections found here bridge critical gaps necessary to understand how auditory perception can influence circuits related to the expression of those affiliative behaviors in female songbirds.

KEYWORDS

arcopallium, anterograde tracer, Bengalese finch, caudal striatum, courtship signal, forebrain, nidopallium, RRID: AB_221568, RRID: AB_2633275

1 | INTRODUCTION

Mate choice is a fundamentally important decision that often relies on sensory perception of signals that indicate a sender's fitness and allow the receiver to discriminate among conspecifics. Female songbirds provide an excellent model system in which to study how signal perception influences mate choice, as songs performed by males of their species are a primary means through which females choose their mate (Marler & Zeigler, 2008). Similar to mammals, songbirds possess a network of neural structures responsible for auditory perception. That network comprises an ascending auditory stream, a primary auditory region, and secondary auditory regions, such as the caudal mesopallium (CM) (Butler & Hodos, 2005). It is not yet known, however, how the female songbird brain uses auditory information to influence motor networks or hormonal systems to execute the behavioral indicators of mate choice.

Previous work in female songbirds has demonstrated that CM plays a role in processing and evaluating the attractiveness of male song. Studies using immediate early gene (IEG) markers in female songbirds reveal increased activity in CM in response to hearing songs that the female finds attractive, including conspecific song, female-directed song, and songs containing a high density of attractive song elements (Mello & Clayton, 1994; Monbureau, Barker, Leboucher, & Balthazart, 2015; Woolley & Doupe, 2008). Other studies have shown that CM lesions (at least partial lesions of that structure) are associated with changes in female mate preferences (Brenowitz, 1991; MacDougall-Shackleton, Hulse, & Ball, 1998). Moreover, studies using chronic electrophysiological recordings in parallel with an operant choice paradigm have demonstrated that the response properties within the male and female CM are tuned to aspects of song that are of greater behavioral relevance (Gentner & Margoliash, 2003; Jeanne, Thompson, Sharpee, & Gentner, 2011). Together, these studies point to CM as an important contributor to song perception and female mate choice.

The connectivity of CM in male songbirds suggests that similar connections in females could play important roles in driving mate choice behavior. CM in males provides the primary output of the auditory system to descending structures implicated in motor control, hormonal control, and context-dependent release of dopamine

(Mandelblat-Cerf, Las, Denisenko, & Fee, 2014; Vates, Broome, Mello, & Nottebohm, 1996). In female songbirds, it is unknown where CM projects and how these connections may bias courtship behavior. Although it may be tempting to assume that the female CM is made up of descending connections identical to those in males, sexual dimorphisms in brain structures and behaviors are well documented in songbirds (Nottebohm & Arnold, 1976; Tobari, Nakamura, & Okanoya, 2005). Therefore, interpreting the role of CM in female mate choice requires direct examination of the projections that emanate from CM in female songbirds. Here, we sought to define the efferent projections of CM in female songbirds using an anterograde neural tracer. Our results confirm the existence of connections observed in males and also reveal other novel connections, indicating that the female CM is well positioned to impact neural circuits that are central in the generation of behavioral indicators of mate choice.

2 | METHODS AND MATERIALS

2.1 | Animals

We preformed all experiments using 14 adult (age > 120 days post hatch) female Bengalese finches (BF, *Lonchura striata domestica*) obtained from our breeding colony or from a commercial breeder (Magnolia Bird Farm, CA). Females were identified initially through behavioral observations and verified via histological analysis of sexually dimorphic brain structures (e.g., HVC) (Nottebohm & Arnold, 1976). Subjects were housed in group cages ($41 \times 31 \times 24$ cm^3) that maintained the 15:9 light:dark photoperiod used in our colony. After the experimental procedures, subjects were housed individually to prevent injury and infection.

All procedures were approved by the University of Wyoming Animal Care and Use Committee, and procedures were in compliance with recommendations from that group and state and federal regulations governing the housing of songbirds.

2.2 | Stereotaxic microsurgery

Each subject was anesthetized with a 3% isoflurane gaseous solution and fixed in a stereotaxic apparatus with ear and beak bars angled 45° below horizontal. Bilateral craniotomies were made above CM at specific coordinates in relation to the bifurcation of the mid-sagittal sinus (1.95 mm anterior, 1.33 mm lateral). Glass micropipettes with an outer diameter of 25–35 μm were filled with 10% BDA (biotinylated dextran amine, 10,000 MW, Molecular Probes, OR) in phosphate buffered saline (PBS, 0.1 M) and lowered into CM (0.85 mm ventral) using a negative holding current (−7.0 μA) to prevent BDA leakage from the electrode. BDA was then iontophoretically injected into CM (+7.0 μA, 7 s on/off, for 7 min), followed by a 5 min period with the electrode unmoved to facilitate diffusion away from the injection site. After that waiting period, the electrode was withdrawn using the same negative holding current described above. A silicone elastomer (Kwik-Sil, World Precision Instruments, FL) was used to cover all craniotomies, followed by applications of an adhesive (Vetbond, 3M, MN) to close the scalp, as well as focal application of a local analgesic (2.5% Lidocaine and 2.5% Prilocaine, HI-Tech Pharmacal, NY). Subjects were then placed under a heating lamp for 1 hr and monitored to ensure no signs of distress or pain before being placed in an individual housing cage.

2.3 | Brain tissue processing

Following a survival period for BDA to completely fill neuronal processes (average: 6 days, range: 5–7 days), subjects were deeply anesthetized with an overdose of isoflurane and transcardially perfused through the left ventricle with physiological saline followed by 4°C 4% paraformaldehyde (PFA) in PBS. The brain was then carefully removed and placed in 4% PFA for 24 hr before being transferred to a 30% sucrose PFA cryoprotecting solution for 72 hr. Sagittal sections were cut at 40 μm thickness on a freezing microtome and placed individually in wells containing phosphate buffer. Tissue sections were then placed on gelatin coated slides to dry overnight.

The following day, brain sections underwent immunohistochemistry to locate and visualize BDA. Sections were rehydrated with PBS and then washed for 20 min in methanol containing 0.3% hydrogen peroxide to block endogenous peroxidases. The peroxidase blocker was washed out via 3 baths of PBS for 15 min each. The tissue was then incubated for 1 hr in a 1% bovine serum albumin in PBS mixture to saturate endogenous proteins. BDA was localized by applying the avidin–biotin detection ABC Elite Kit (Vector Laboratories, CA) to the tissue for 1 hr in a humidification box, followed by three washes of PBS containing 0.1% Triton X-100 (PBST) for 15 min each. A DAB substrate (3,3–diaminobenzidine, ImmPACT DAB, Vector Laboratories, CA) was used as the chromogen to visualize BDA, providing a brown reaction product, and that reaction was followed by two washes in PBS for 15 min each. The tissue was then bathed for 5 min in cresyl violet, to produce a light stain to facilitate the location of nuclear groups, before being dehydrated in ascending alcohols and cleared in xylenes. The glass slides were then coverslipped with a Krystalon mounting medium (Fisher Scientific, PA) and stored at 4°C.

In an additional two birds, we injected an adeno-associated virus (AAV) into the same location in CM to induce expression of green fluorescent protein (GFP; serotype 1; product number AAV1.CMV.PI.eGFP.WPRE.bGH from Penn Vector Core, University of Pennsylvania School of Medicine). Previous studies have shown that GFP-encoding AAVs are an excellent means of labeling somas at the site of injection with virtually no retrograde transport, as fewer than one neuron per brain was found distant from the injection site (Chamberlin, Du, de Lacalle, & Saper, 1998). Additional work has revealed that serotype 1 is well suited for transduction of foreign genes in cortical neurons and anterograde transport from those infected somas (Zingg et al., 2017). This served as an additional verification that the projections we observed were efferents from CM.

We injected, recovered, and monitored the bird just as we did in surgeries for BDA injection. After 21 days following AAV injection, we perfused the bird and collected and processed the neural tissue. In histological analysis, we washed the sectioned (40 μm) tissue three times for 5 min each in PBS, washed once more for 15 min in PBST,

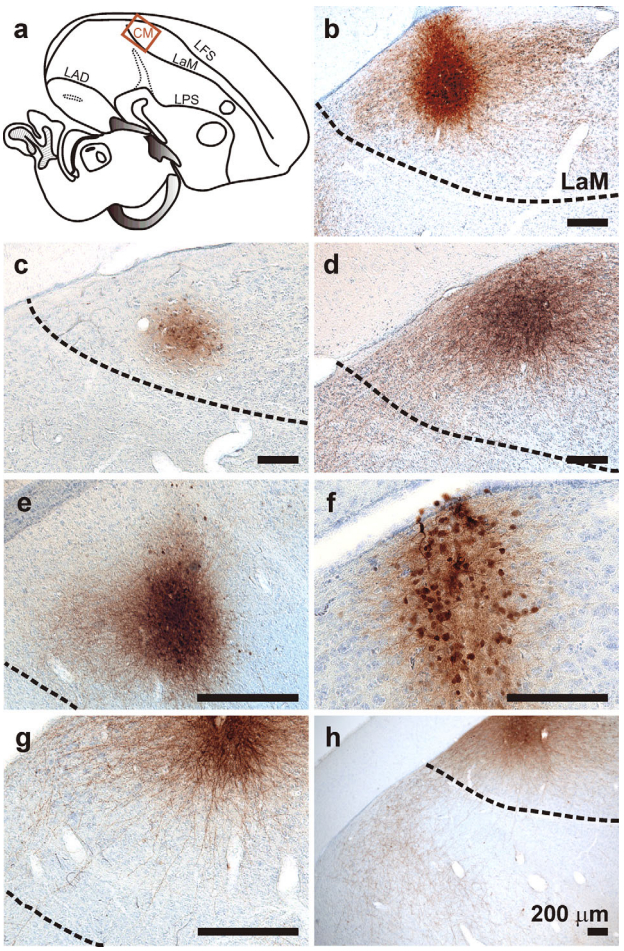

FIGURE 1 Tracer injections lay within CM. (a) Schematic of a parasagittal section of the female Bengalese finch brain adapted from the OHSU male zebra finch atlas (Oregon Health & Science University, Portland, OR 97239; http://www.zebrafinchatlas.org). The red box indicates the approximate location of the photographs displayed in the panels below. (b–e) Injections of the 10K BDA tracer into the caudal mesopallium (CM) resulted in densely labeled sites spanning approximately 200–300 μm and lying entirely within CM. (f) Individually labeled somas were evident in CM. (g) Projections and varicosities were evident within CM, indicating local projections, and (h) projections extended across lamina mesopallialis to target sites described in subsequent figures. LAD = lamina arcopallius dorsalis; LaM = lamina mesopallialis (LaM); LFS = lamina frontalis superior; LPS = lamina pallio-subpalliallis; Scale bars = 200 μm [Color figure can be viewed at wileyonlinelibrary.com]

then washed the tissue a final time in PBS for 5 min. We then washed the tissue in PBST containing 5% goat serum for 30 min, followed by application of a primary antibody (1:1000 dilution of mouse IgG2a anti-GFP in PBS, Invitrogen, RRID: AB_221568) in a humidification chamber overnight at 4 C. The following morning, we washed the tissue three times in PBS for 10 min each then applied a secondary antibody (1:500 dilution of goat antimouse IgG Alexa Fluor 488 in PBS, Invitrogen, RRID: AB_2633275) in a humidification chamber for 1 hr at room temperature. After two final washes in PBS for 15 min each, we coverslipped the tissue (Krystalon) and stored the tissue at 4°C until visualization under the microscope.

Images of BDA-injected brains were obtained using an Olympus BX51 Fluorescence Microscope (Olympus, PA) equipped with an RT-SE camera (SPOT 9.4 Slider-6, MA) and analyzed with SPOT software (version 5.1, MA). All images of BDA-injected brains were taken under bright-field conditions and whole-image contrast and brightness modifications were made to increase the resolution of the BDA-filled processes. Variations in background color across the images emerged from slight differences in the magnitude of cresyl violet stain. Images of AAV-injected brains were taken using a Zeiss 710 laser scanning confocal microscope (Zeiss, NY).

3 | RESULTS

3.1 | Tracer injections reveal projections of caudal mesopallium neurons

Injections of BDA were restricted to the CM ($N = 22$ hemispheres). Those injection sites were on average approximately 250 μm in diameter, located ventral to the lamina frontalis superior (LFS) and dorsal to the lamina mesopallialis (LaM) (Figure 1). When viewed under the microscope, all CM injections contained clusters of BDA-labeled cell somas accompanied by meandering processes that were a mixture of axons and dendritic arborizations. As shown in Figure 1b–h, our placement of CM lesions was consistent across birds. We typically observed a CM injection site with dense labeling spanning 200–300 μm in the medial-lateral direction and 200–300 μm in the anterior-posterior direction. As is also evident in Figure 1b–h, that site always resided in the posterior portion of CM. Because there are no clear boundaries that distinguish the medial versus the lateral portion of CM, we are not able to state to what degree we may have labeled cells in each of those subdivisions within CM. Given the primarily anterograde nature of 10K BDA (Reiner, Veenman, & Honig, 1993), CM projections were identified by the presence of varicosities that were outside the injection site. We interpreted those BDA-labeled axons and varicosities as terminals at target sites.

The densest projections from CM were to other auditory regions within the nidopallium that have been described elsewhere in both male and female songbirds (Vates et al., 1996). Specifically, dense fields of axons and varicosities were found in the caudal nidopallium (NC) ($n = 22$ hemispheres) (Figure 2) and along the ventricular border in the most caudal portion of the NC (cNC) ($n = 22$ hemispheres) (Figure 3). Several BDA-labeled somas were also found within the NC. This somatic labeling outside of CM indicates retrograde labeling, which is known to occur with the use of 10K BDA (Lei, Jiao, Del Mar, & Reiner, 2004) and which reveals bidirectional interconnectivity between CM and NC. Retrograde labeling of somas was also seen in Field L, which is the primary input of auditory information to CM (Bonke, Bonke, & Scheich, 1979; Vates et al., 1996). Therefore, CM receives input from Field L and projects to NC. Labeling of somas in NC suggest that CM and NC may be reciprocally connected, but characterizing the projections from NC to CM awaits further investigation.

Dense projections from CM into the arcopallium were also found in the majority of brain hemispheres that received BDA injections.

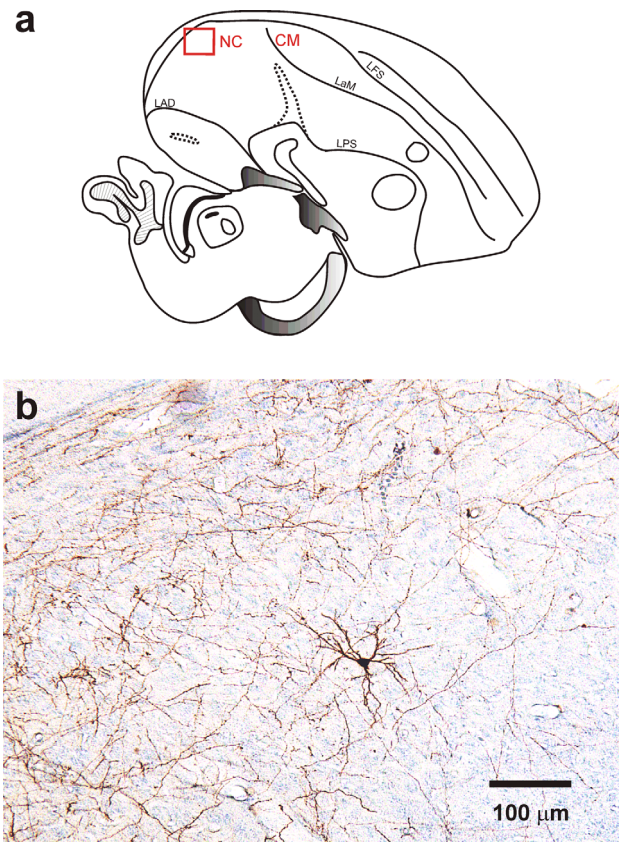

FIGURE 2 CM is reciprocally interconnected with NC. Injections of tracer into CM resulted in dense projections to the caudal potion of the nidopallium (NC), evident as axons and varicosities. Somas in NC were also labeled by CM projections (e.g., center of panel b), indicating that CM and NC are reciprocally interconnected. Layout and lamina abbreviations as in Figure 1. Scale bar = 100 μm [Color figure can be viewed at wileyonlinelibrary.com]

Specifically, labeled axons descended ventrally through the nidopallium and across the lamina arcopallius dorsalis (LAD) before terminating in the robust nucleus of the arcopallium (RA) ($n = 15$ hemispheres) (Figure 4). In female songbirds, RA is much smaller compared to males and can be difficult to detect (Tobari et al., 2005). To facilitate identification of RA and surrounding regions such as AIV, we used a contrast-enhancing cresyl violet stain. Projections from CM to RA were quantified using the criterion of two or more discrete axonal processes with terminations within the "cigar shaped" female RA (Figure 4). Labeled axons also descended from the nidopallium into the arcopallium and terminated in an area that stereotaxic coordinates indicate is the ventral portion of the intermediate arcopallium (AIV) ($n = 20$ hemispheres) (Figure 5).

Projections from CM were also found within the caudal striatum (CSt) ($n = 12$) (Figure 6). Labeled terminal fields within the CSt resided primarily along the rostral side of the lamina pallio-subpalliallis (LPS), with a minority of labeled fibers extending rostrally toward the globus pallidus (GP). Notably, no CM efferents were observed within the GP or the lateral striatum.

In a subset of our subjects ($n = 2$ subjects, 2 hemispheres), we placed unilateral BDA injections to investigate whether the projections described above were ipsilateral, contralateral, or both. Data from these unilateral injections revealed patterns of projections within the ipsilateral hemisphere that were identical to the pattern of projections that was evident in subjects that received bilateral CM injections ($n = 2$ hemispheres, 2 birds, Figure 7). In no case did those unilateral injections reveal projections into the contralateral hemisphere.

In each of the two birds that received AAV injections to induce expression of GFP in CM somas and their projections, we observed a region of dense somas in CM and projections to the NC, the arcopallium and the CSt ($n = 2$ hemispheres, 2 birds, Figure 7). Furthermore, we searched across all regions of the brain and brainstem, and we did not find any labeled somas outside of our injection site, suggesting that we had no cases of retrograde labeling. Therefore, we conclude that all of the pathways described above are efferent from CM and are exclusively ipsilateral (Figure 8).

4 | DISCUSSION

Our findings regarding the efferent connections of CM in females are in general agreement with previous findings in males (Mandelblat-Cerf et al., 2014; Vates et al., 1996). Importantly, we also discovered two novel projections. Similar to males, the female CM projects broadly to all portions of the caudal nidopallium and also to the AIV. In addition

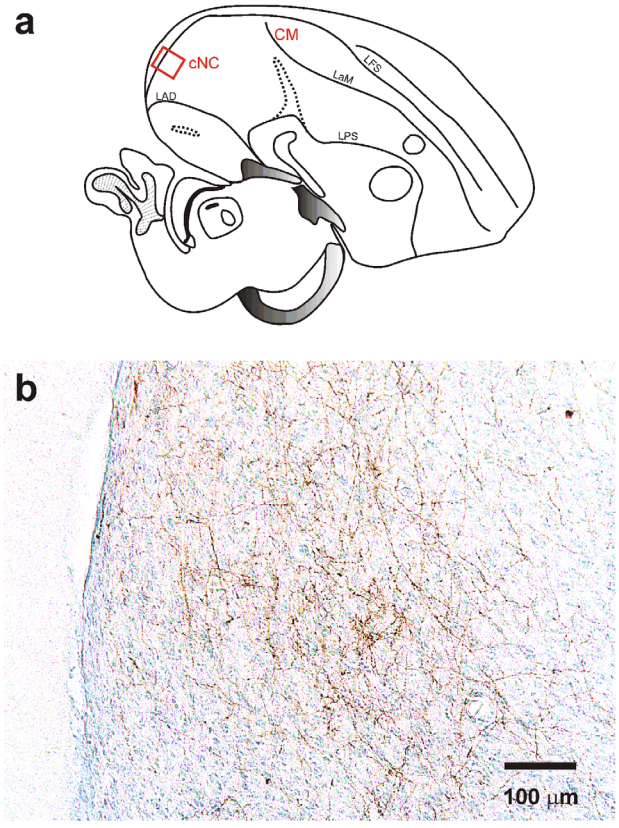

FIGURE 3 CM projects to caudal NC. Injections of tracer into CM revealed BDA-filled varicosities along the ventricular border and the most caudal portion of NC (cNC). Layout and lamina abbreviations as in Figure 1. Scale bar = 100 μm [Color figure can be viewed at wileyonlinelibrary.com]

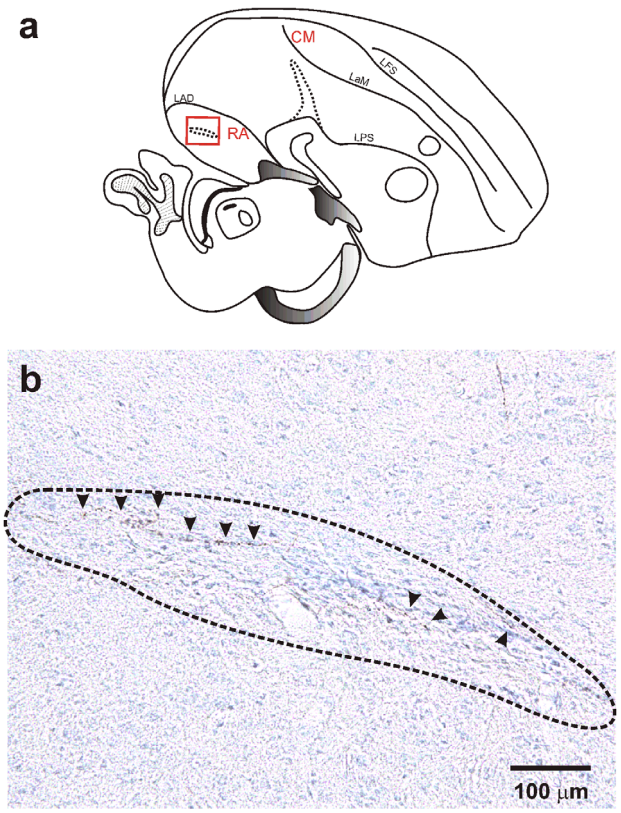

FIGURE 4 CM projects to RA. Injections of tracer into CM resulted in projections of labeled axons and varicosities (black arrows in panel b) into the "cigar shaped" robust nucleus of the arcopallium (RA), indicated by a dashed black outline in panel b. Layout and lamina abbreviations as in Figure 1. Scale bar = 100 μm [Color figure can be viewed at wileyonlinelibrary.com]

to those connections, our study revealed novel projections from CM to RA and from CM to the CSt. These anatomical routes constitute a mechanism through which sensory information can affect brain structures implicated in behavior. More specifically, these connections may underlie the generation of female courtship behaviors in response to perceiving male song (Figure 8).

Bidirectional connectivity between CM and NC has been described previously (Vates et al., 1996), and those interconnected sites are often collectively referred to as the "auditory lobule" of the songbird brain (Cheng & Clayton, 2004; London & Clayton, 2008). The auditory lobule is implicated in song learning in juvenile males and is thought to facilitate the development of song preferences in juvenile female songbirds (Amin, Doupe, & Theunissen, 2007; Gobes & Bolhuis, 2007; London & Clayton, 2008; Murphy, James, Sakata, & Prather, 2017). Similar to CM, the activity of the neurons within NC is selective for natural vocalizations, and that selectivity may help females to assign identity to the vocalizations of specific individuals (Menardy et al., 2012). Neurons in NC undergo rapid habituation in response to repeated song stimuli, with greater habituation occurring in response to conspecific song than in response to heterospecific songs (Chew, Mello, Nottebohm, Jarvis, & Vicario, 1995). Interestingly, approximately half of the neurons within NC are GABAergic with efferent connections to CM (Pinaud et al., 2008). Collectively, these data give rise to a model where NC may provide an increased inhibitory tone onto CM in response to songs that are unfamiliar, heterospecific, or both. In contrast, NC may reduce its inhibition of CM in response to songs that are familiar or conspecific, in turn permitting increased activity within CM and perhaps facilitating the generation of female courtship behaviors.

It is also important to note that we detect CM efferents within the vicinity of where HVC would be present in the male brain. This is evident in the route along which CM fibers course in their projection caudally to the nidopallium (evident as labeled fibers on the far left in Figure 1b). It is difficult to differentiate, however, whether the terminals found in this area are connections to the regressed (and not morphologically distinct) HVC or whether these are connections to the most dorsal portion of the NC. Additional work using anterograde tracers within the dorsal NC is necessary to determine if the neurons in this region display connectivity characteristic of HVC, especially projections from that site to RA.

Our finding of a projection from CM to RA is an intriguing new link in our understanding of female courtship behaviors, providing a possible bridge from perception-related activity in CM to downstream structures associated with the expression of calls and copulation solicitation displays (CSDs). One of the major targets of the RA in both males and females is the dorsomedial nucleus of the intercollicular complex (DM)

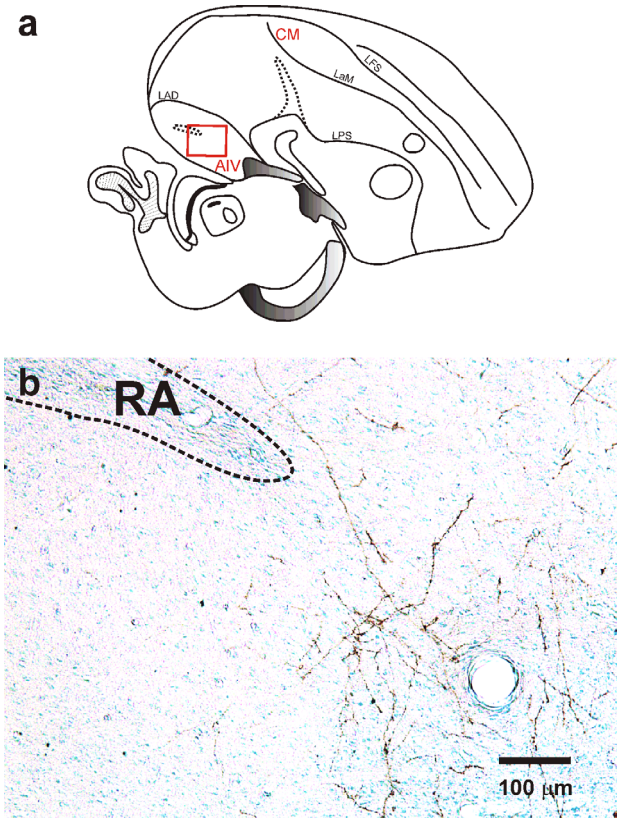

FIGURE 5 CM projects to AIV. Injections of tracer into CM revealed clusters of axons and varicosities in the ventral portion of the intermediate arcopallium (AIV). That area is anterior and inferior of RA, which is indicated by a dashed black line in panel b. Layout and lamina abbreviations as in Figure 1. Scale bar = 100 μm [Color figure can be viewed at wileyonlinelibrary.com]

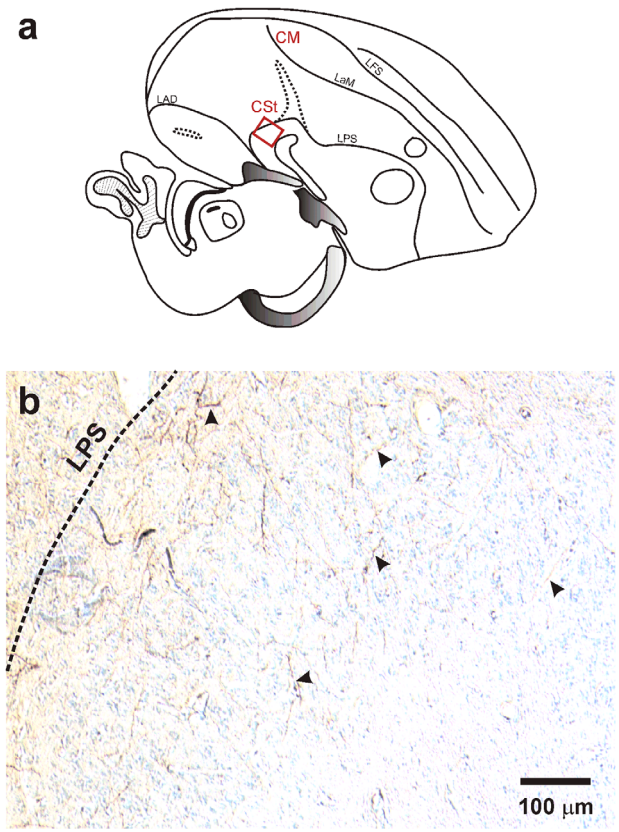

FIGURE 6 CM projects to CSt. Injections of BDA into CM yielded small axonal varicosities (black arrows in panel b) in the caudal striatum (CSt) that lies immediately inferior of the lamina pallio-subpalliallis (LPS, black dashed line in panel b). Layout and lamina abbreviations as in Figure 1. Scale bar = 100 μm [Color figure can be viewed at wileyonlinelibrary.com]

in the midbrain (Tobari, Okumura, Tani, & Okanoya, 2006; Wild, 1993). DM and its projections provide three major routes through which CM activity may influence female behavior (Figure 8). The first route is the projection from DM to the shell of the auditory thalamic nucleus, ovoidalis (Ov_{shell}), which then projects to the ventromedial nucleus of the hypothalamus and to the overlapping mediobasal hypothalamus (MBH) (Cheng & Peng, 1997; Durand, Tepper, & Cheng, 1992). The MBH is thought to drive the expression of courtship solicitation displays in female songbirds, and IEG studies in female canaries have revealed a correlation between activity in the MBH and in CM in response to songs that the female finds attractive (Cheng & Zuo, 1994; Monbureau et al., 2015). Our results provide a more complete layout of how song perception and the associated activity in CM may affect activity within the MBH via the indirect pathway of CM→RA→DM→Ov_{shell}→MBH (Figure 8).

In addition to hormonal control of CSD expression, the second route through which CM may influence courtship behavior involves innervation of the muscles that control the cloaca, a reproductive organ where contact with the male occurs. Tract-tracing in female canaries has revealed that DM projects to a medullary respiratory center (nucleus retroambigualis, RAm) which in turn projects to spinal motor neurons that control the muscles in the cloaca (Wild & Botelho, 2015). Our results build on those data, providing an additional upstream link through which song perception may shape this circuit and the associated copulatory behaviors: CM→RA→DM→RAm→cloacal motor neurons (Figure 8).

The third route through which CM may influence female behaviors also involves DM but influences call production rather than the production of CSDs. Female calling behavior is strongly correlated with CSD expression and is an indicator of mate preference in some species including the BFs studied here (Bartsch, Hultsch, Scharff, & Kipper, 2016; Dunning, Pant, Bass, Coburn, & Prather, 2014). Moreover, increases in IEG expression in both the CM and MBH are positively correlated with call production in female songbirds (Monbureau et al., 2015). Calling behavior in female songbirds is closely related to activity in DM. Specifically, electrical stimulation in DM drives calling, and DM lesions eliminate calling behavior, indicating that activity in DM is necessary and sufficient for female call production (Fukushima & Aoki, 2000, 2002; Simpson & Vicario, 1990). The results of this study reveal a CM→RA→DM pathway that links perception-related activity in CM with neurons that underlie call production. RA also receives excitatory input from the lateral portion of the magnocellular nucleus of the anterior nidopallium (LMAN), a structure that is as large in females as it is in males (Tobari et al., 2006). LMAN has also been implicated in female song perception and mate preference (Hamilton, King, Sengelaub, & West, 1997). Together, CM and LMAN may both influence the activity of RA→DM projections, providing a mechanism through which both

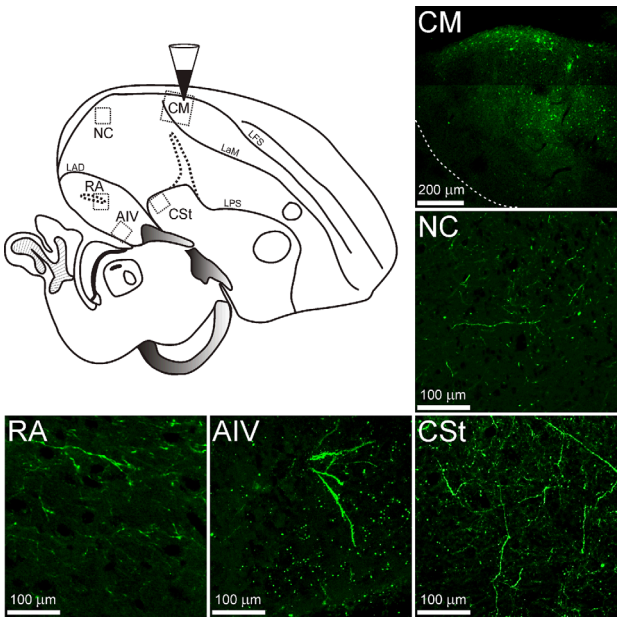

FIGURE 7 Projections detected using BDA were confirmed using adeno-associated viral infection of CM neurons. Injections of AAV1 into CM (top left schematic) yielded infection of somas in CM (top right panel labeled CM) and identification of projections in the caudal nidopallium (NC), the CSt, the ventral portion of the intermediate arcopallium (AIV), and the RA ($n = 2$ hemispheres, 2 birds). Pixels in NC, CSt, AIV and RA panels are background staining from the secondary antibody [Color figure can be viewed at wileyonlinelibrary.com]

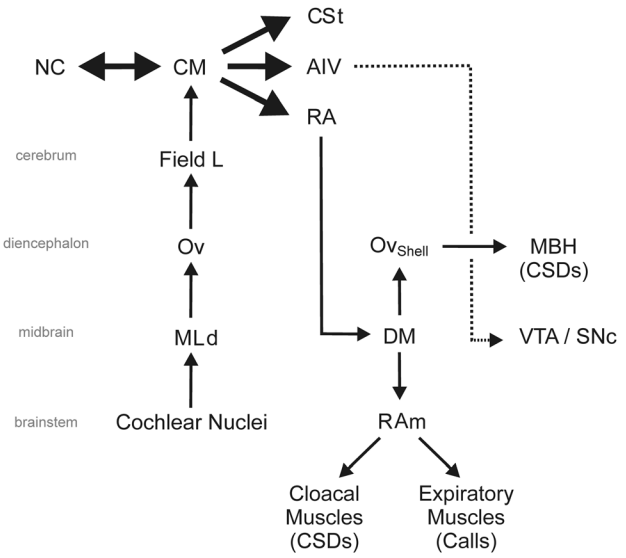

FIGURE 8 A simplified schematic of the connectivity of the female CM, including indirect descending projections that may influence courtship behaviors. Auditory stimuli are processed by the ascending auditory pathway, beginning with cochlear nuclei in the brainstem, then projecting to the dorsal lateral nucleus of the mesencephalon (MLd), which in turns projects to the thalamic site, Ovoidalis (Ov). Field L, the primary thalamorecipient of projections from Ov, then innervates CM. Findings from this study (bold arrows) demonstrate that in females CM projects to the CSt ($n = 12$), AIV ($n = 20$), RA ($n = 15$) and is reciprocally interconnected with NC ($n = 22$). Results from previous work show that neurons in the female RA projects to the midbrain structure DM, which then projects to the brain stem nucleus RAm that then innervates motor neurons that control the expiratory muscles and muscles that control the cloaca (Wild & Botelho, 2015). A connection between AIV and dopaminergic regions in the midbrain (VTA/SNc, dotted arrow) is known to be present in males and is also shown here (Mandelblat-Cerf et al., 2014). As elaborated in the text, these pathways provide a mechanism through which perception-related activity in CM can influence motor performance of behavioral indicators of mate choice

CM and LMAN may influence female expression of behavioral indicators of mate choice.

Our results also reveal projections from CM to a site in the ventral intermediate arcopallium. These projections are categorized as terminals located outside of the RA and clustered dorsoventrally in respect to RA. The AIV in male songbirds has been implicated in vocal motor learning, serving as a driver of motivational state or reinforcement learning through its connections to the ventral tegmental area (VTA) and the substantia nigra pars compacta (SNc) (Mandelblat-Cerf et al., 2014). Both VTA and SNc are midbrain dopaminergic regions that innervate the avian homolog of the mammalian striatum, Area X, in male songbirds (Lewis, Ryan, Arnold, & Butcher, 1981; Person, Gale, Farries, & Perkel, 2008). Interestingly, Area X is a sexually dimorphic structure that shows significant regression in female songbirds, and its involvement in song evaluation is not clear (Tobari et al., 2005). Considering that motivational state and reinforcement learning are likely major players in female learning of mate preferences (Riebel, 2003), future studies aimed at understanding the role of the CM→AIV→VTA/SNc pathway should be a priority (Anderson, 2009; Hernandez & MacDougall-Shackleton, 2004; Nagle & Kreutzer, 1997). Alternatively, these axons terminating in the arcopallium outside of RA may indicate connections to the dorsal arcopallium (Ad) or to an auditory region surrounding RA known as cup (RA_{cup}) (Bottjer & Altenau, 2010; Mello, Vates, Okuhata, & Nottebohm, 1998). In light of the anatomical location of the terminals and previous reports that CM neurons project to AIV neurons in males, it seems most likely that these projections are AIV-projecting CM neurons (Mandelblat-Cerf et al., 2014). Future studies involving coinjection of retrograde and anterograde markers throughout the arcopallium will afford an opportunity to further characterize these cells and others.

An additional novel projection evident in our data was from CM to the CSt. It has been suggested that the avian CSt is analogous to an auditory region in the caudal portion of the mammalian striatum, which receives cortical input from Layers 2 and 3 of the auditory cortex (Jarvis, 2004). Neurons in CM and NCM are also thought to be analogous to neurons in Layers 2 and 3 of auditory association cortex (Karten, 1991). Our data describing a projection from CM to CSt thus provide further evidence for similarities between birds and mammals in the circuitry of their auditory processing sites.

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AUTHORS' CONTRIBUTIONS

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. JLD and JFP contributed to study concept and design; critical revision of the manuscript for important intellectual content; administrative, technical, and material support; and study supervision. JLD and SM involved in acquisition of data. JLD, EJA, and JFP contributed to analysis and interpretation of data. JLD drafted the manuscript and performed statistical analysis. JFP obtained funding.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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