


A brain for all seasons: An in vivo MRI perspective on songbirds

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Funding information

Fonds Wetenschappelijk Onderzoek,
Grant/Award Numbers: Nr 1115217N, Nr
G030213N; Flemish Impulse funding for
heavy scientific equipment,
Grant/Award Number: 42/FA010100/1230;
Hercules Foundation, Grant/Award Number:
AUHA/012; Interuniversity Attraction Poles
(IAP), Grant/Award Number: "PLASTOSCINE":
P7/17

Abstract

Seasonality in songbirds includes not only reproduction but also seasonal changes in singing behavior and its neural substrate, the song control system (SCS). Prior research mainly focused on the role of sex steroids on this seasonal SCS neuroplasticity in males. In this review, we summarize the advances made in the field of seasonal neuroplasticity by applying in vivo magnetic resonance imaging (MRI) in male and female starlings, analyzing the entire brain, monitoring birds longitudinally and determining the neuronal correlates of seasonal variations in plasma hormone levels and song behavior. The first MRI studies in songbirds used manganese enhanced MRI to visualize the SCS in a living bird and validated previously described brain volume changes related to different seasons and testosterone. MRI studies with testosterone implantation established how the consequential boost in singing was correlated to structural changes in the SCS, indicating activity-induced neuroplasticity as song proficiency increased. Next, diffusion tensor MRI explored seasonal neuroplasticity in the entire brain, focusing on networks beyond the SCS, revealing that other sensory systems and even the cerebellum, which is important for the integration of sensory perception and song behavior, experience neuroplasticity starting in the photosensitive period. Functional MRI showed that olfactory, and auditory processing was modulated by the seasons. The convergence of seasonal variations in so many sensory and sensorimotor systems resembles multisensory neuroplasticity during the critical period early in life. This sheds new light on seasonal songbirds as a model for unlocking the brain by recreating seasonally the permissive circumstances for heightened neuroplasticity.

KEYWORDS

DTI, European starling (*Sturnus vulgaris*), MRI, neuroplasticity, seasonality, sensory systems, song, Songbird, testosterone, thyroid hormone

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1 | WHAT WE KNEW ABOUT THE SEASONAL SONGBIRD BRAIN BEFORE MAGNETIC RESONANCE IMAGING (MRI) WAS INTRODUCED IN THIS MODEL

Seasonality is directly controlled by photoperiod, the fraction of light exposure within a day, which is a reliable predictor of spring and summer for birds in high latitudes in contrast to equatorial songbirds (Gwinner, 2003). The changes in photoperiod can be picked up by endogenous control mechanisms and regulate seasonal processes like seasonal reproduction. Increasing day length (or photostimulation) during spring stimulate secretion of gonadotropin-releasing hormone (GnRH) and consequent gonadal maturation, as preparation for breeding (Yoshimura et al., 2003). However, breeding is limited in time. Prolonged exposure to long photoperiods during summer has a second effect—the induction of photorefractoriness, where birds no longer respond to long day lengths with gonadal maturation (Nicholls et al., 1988). This dual role of long photoperiods is required to impart the asymmetry in breeding seasons. Typically, photorefractoriness is associated with a massive decrease in hypothalamic GnRH and subsequent gonadal regression, essentially a reversal to a prepubertal condition. In the autumn, the exposure to short photoperiods, when day length falls below approximately 11.5 h, leads to photosensitivity. During the photosensitive state birds regain the ability to respond to long day lengths in spring (for more info see Bentley, 2009 and Dawson et al., 2001). However, seasonality in songbirds is not limited to seasonal reproduction, but also includes seasonal changes in molt, migration, reproductive behaviors like singing and even structural changes in the related brain regions for song (Figure 1).

Songbirds are equipped with a specific brain network, the so called song control system (SCS), analogous to the human speech network, to support their vocal communication through “species-specific” songs. This neural circuit for song learning and production consists of two major pathways. First, the anterior forebrain pathway (in blue in Figure 2a) originates in HVC (abbreviation used as a proper name but the meaning is High Vocal Centre) and projects to Area X. In turn, Area X projects to the medial part of the dorsolateral nucleus of the anterior thalamus (DLM), continues to the lateral magnocellular nucleus of the anterior nidopallium (LMAN) and finishes again in Area X. Information from this “recursive” loop is relayed to the motor pathway by a connection of LMAN to robust nucleus of arcopallium (RA). This pathway provides feedback which is essential for song learning and song maintenance in adulthood (for review: Bottjer and Johnson, 1997 and Brainard, 2004). Second, the caudal motor pathway (in red in Figure 2a) sends direct projections from HVC to RA. From RA, the pathway continues further to the tracheosyringeal part of the nucleus of the XIIth cranial nerve (nXIIts). Finally, it arrives at the syrinx, which is the avian analogue of the larynx, and nucleus retroambiguus (Ram) a respiratory nucleus. The caudal motor pathway controls the motor aspect of singing behavior.

Next to the SCS, singing behavior is controlled by another set of interconnected nuclei, i.e., the social behavior network, which has been shown to influence the motivation to sing. Many components of the social behavior network express various hormone receptors and affect singing behavior depending on the social context (Goodson, 2005; Heimovics & Riters, 2007; Newman, 1999).

Finally, there is an important role and strong interconnection with the auditory system (in green in Figure 2a), where auditory

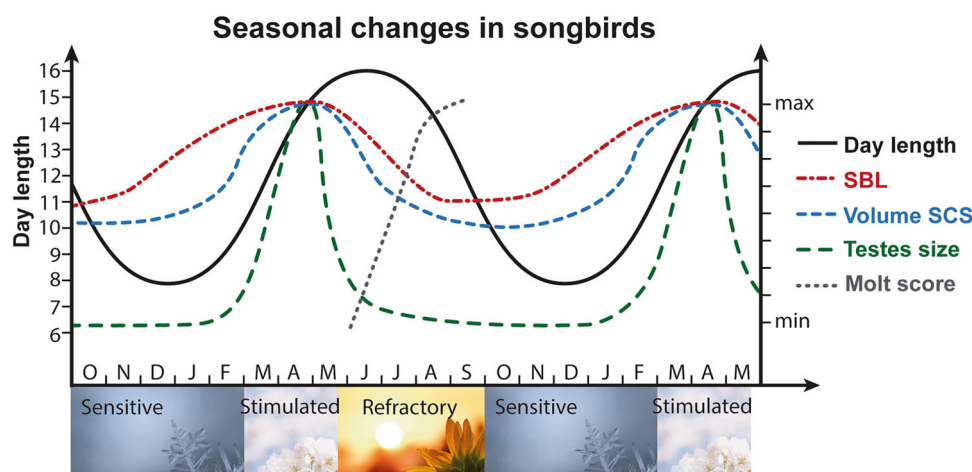


FIGURE 1 Schematic overview of the seasonal changes in the male European starling, a seasonal open-ended learner. The seasonal changes in day length (black solid line, left axis) regulate different seasonal processes. During autumn and winter, when day length drops below 11.5 h, birds become photosensitive. This means they regain the ability to respond to a long day photostimulus. Spring is the breeding season for starlings and the exposure to long days or photostimulation causes gonadal maturation, here reflected by the testes size (green dashed line, right axis). Also reproductive behaviors like singing, reflected by the song bout length (SBL, red dash-dotted line, right axis) reach their maximum. This increased singing behavior is related to increased volume of different song control system (SCS) nuclei (blue dashed line, right axis). Prolonged exposure to long days makes birds photorefractory. They become irresponsive to the long photoperiod and go through molt (gray dotted line, right axis). Testes size and molt scores are based on data from Dawson (2003), song behavior and volume of SCS are based on data from Orije et al. (2021) and Riters et al. (2002)

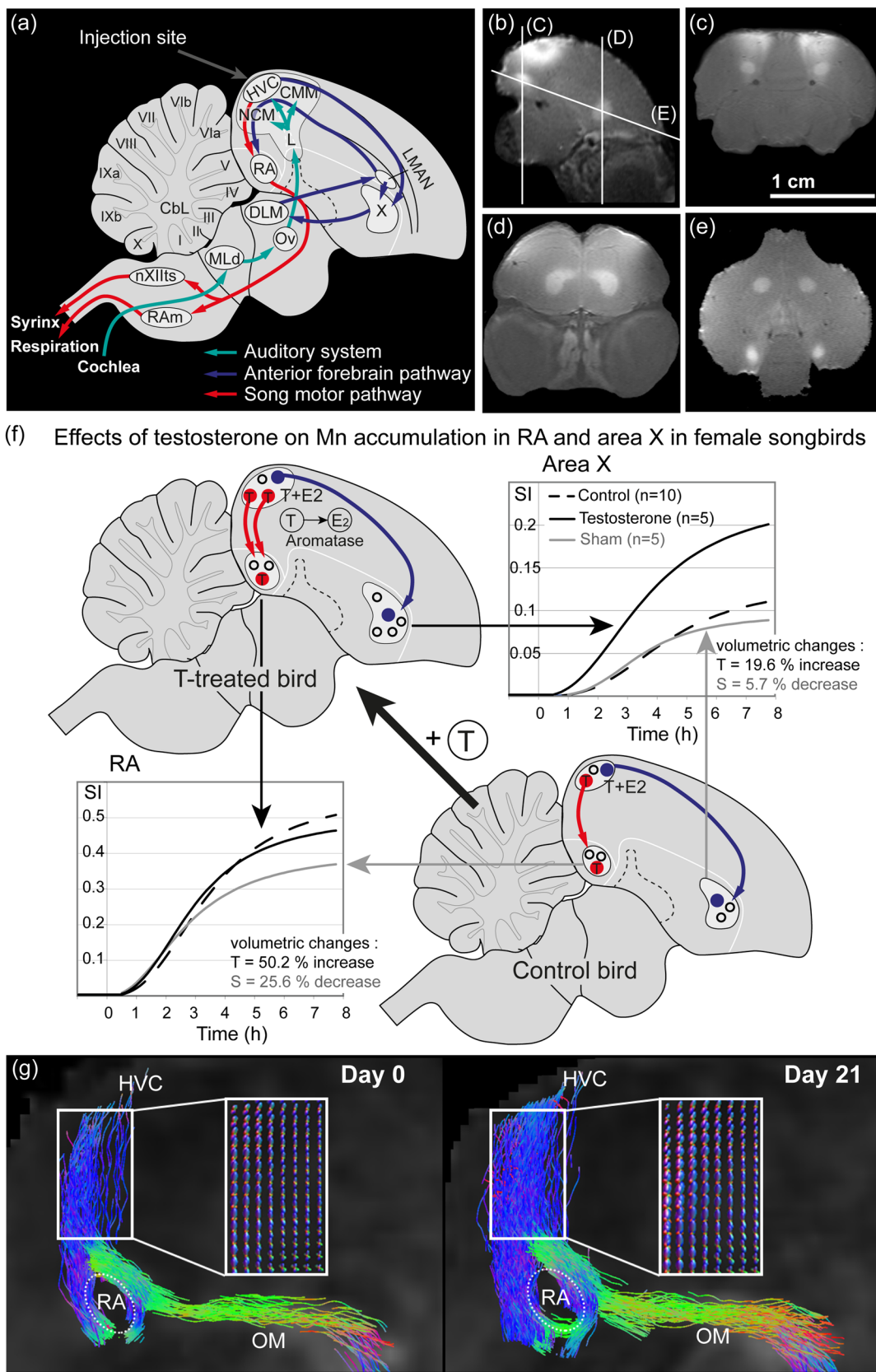


FIGURE 2 (See caption on next page)

information registered in the inner ear, connects to the cochlear nuclei in the brainstem, traversing nucleus mesencephalicus lateralis pars dorsalis, nucleus ovoidalis (Ov, the avian auditory thalamus) and Field L (analogous to the mammalian primary auditory cortex). Field L contacts several secondary and tertiary auditory telencephalic areas situated in the nido- and mesopallium, i.e., the caudomedial nidopallium (NCM) and caudomedial mesopallium (CMM) (Prather, 2013). Because of its many afferent and efferent connections, Field L is often regarded as a relay station (Woolley, 2012).

Based on their song learning ability, songbirds are categorized into closed- and open-ended learners. Closed-ended learners, such as zebra finches, only learn a song during a specific period in early life, the so called critical period (CP). In zebra finches this CP takes place during the first 120 days posthatching (dph) and consists of two partly-overlapping sub-phases, i.e., the sensory and the sensorimotor phase (for review e.g., Doupe and Kuhl, 1999 and Marler and Peters, 2010). During the sensory phase (approximately 20–45 dph), the juvenile birds memorize the song sung by an adult tutor bird. During the sensorimotor phase (approximately 40–90 dph), the juvenile birds will start to vocalize, trying to match their own vocalizations to the previously memorized tutor song. After the sensorimotor phase, zebra finch males will evolve into the crystallization phase (approximately 90–120 dph), where the song will become fixed. During this CP, the heightened neuroplasticity is shaping the SCS to a final mature and stable circuit. Importantly, when the young bird is not exposed to a tutor during the appropriate “critical” time window he will never be able to produce a typical mature zebra finch song as his SCS deprived of proper inputs will not be properly developed.

On the other hand, open-ended learners manage to extend or adapt their song repertoire (or learn new songs) on a yearly basis. Seasonal open-ended learners, like canaries and starlings, experience each year a sensory and sensorimotor phase with “plastic” song

during the photorefractory and photosensitive phase, when circulating testosterone levels are low. Their song crystallizes or stabilizes again during the photostimulated phase when testosterone concentrations are high (Brenowitz et al., 1991; Cornez et al., 2020; Nottebohm et al., 1986; Van Hout et al., 2009) (Figure 1). These seasonal changes in singing behavior are associated with seasonal changes in the volume and structure of song control nuclei (Riters et al., 2002) and this was described for the first time in male canaries (*Serinus canarius*) by Nottebohm (1981). This discovery has led to a research branch of its own: seasonal neuroplasticity of the SCS (some illustrative reviews are: Balthazart et al., 2010, Ball et al., 2002, Rundstrom and Creanza, 2021).

Due to the link with the breeding season, and the fact that in most songbird species only the males sing, SCS neuroplasticity was often studied in relation to the seasonal changes in sex steroid hormones, like testosterone. Moreover, the complex actions of steroid hormones within the brain were already explored in mammals, as estrogen receptors are expressed in brain regions other than the hypothalamus, like hippocampus and cerebral cortex. Furthermore, aromatase, the enzyme that metabolizes testosterone and dehydro-testosterone into estrone and estradiol, is highly expressed in these brain regions critical for memory encoding and consolidation, a feature that is conserved across species, including songbirds (Vahaba & Ramage-Healey, 2015). Interestingly, high aromatase expression found in the NCM (auditory system) of songbirds distinguishes them from other nonvocal learning birds, even though the auditory pathway itself is conserved among bird species (Metzdorf et al., 1999). Social interaction and tutor song playback rapidly increase neuro-estrogen production in NCM, which enhances auditory processing and consolidation of recent auditory experiences. This further implies the role of neuro-estrogens in sensory encoding and vocal communication (Vahaba & Ramage-

FIGURE 2 (a) Schematic overview of the different nuclei and their connections forming the auditory system (green arrows), and anterior forebrain pathway (blue arrows) and song motor pathway (red arrows) of the song control system (SCS). (b–e) In vivo manganese enhanced magnetic resonance imaging (MEMRI) of the male starling brain in photostimulated conditions obtained 6 h after $MnCl_2$ injection into the HVC (a, gray arrow). The sagittal MEMRI image in (b) illustrates the different planes of imaging for subsequent coronal (c, d) and horizontal (e) MEMRI images. Image resolution in the coronal plane is 97 μm (pixel size). Adapted with permission from Van der Linden et al. (2002). (f) Schematic overview of the effects of testosterone (T) implantation in photosensitive female starlings obtained with in vivo dynamic MEMRI data from the same bird before and after T. Comparison of bottom right drawing (brain of a control bird) with the upper left drawing (brain of a T-treated bird) reveals that the song control nuclei, HVC, RA, and Area X increase in volume and the number of RA projecting neurons increases following T-treatment. Repeated MRI acquisitions up to 8 h after Mn^{2+} injection demonstrated changes in the dynamics of Mn^{2+} accumulation in RA (lower left insert) and Area X (upper right insert) as an increase in MRI Signal Intensity. The inserts show the mean sigmoid curves based on the mean parameter values in each group from data obtained in control sham-implanted (gray curves, S) and T-treated birds (black curves, T). The dashed curves represent pooled data for all birds before implantations. The volumetric changes in Area X and RA are summarized in the bottom right corner of the inserts. Adapted with permission from Van der Linden et al. (2004). (g) Sagittal view of in vivo diffusion tensor imaging (DTI) based fiber-tracking of the HVC-RA connection in photosensitive female starling before (Day 0) and 21 days after testosterone implantation. On Day 21 more tracts are going from HVC to RA, surrounding RA and going from RA to the occipitomesencephalic (OM) tract. The inserts show the individual DTI based fiber orientation distributions used to create fiber-tracking of the HVC-RA tract. Color coding of the tractogram: red = left-right orientation, blue = dorso-ventral and green = rostrocaudal. The in-plane resolution is 179 μm . Adapted with permission from Orije et al. (2020). CMM, caudal medial mesopallium; DLM, medial part of the dorsolateral nucleus of the anterior thalamus; E2, estradiol; HVC, used as a proper name (high vocal center); L, field L; LMAN, lateral magnocellular nucleus of the anterior nidopallium; MLD, dorsal lateral nucleus of the mesencephalon; NCM, caudomedial nidopallium; nXII, nucleus XII, tracheosyringeal part; OM, occipitomesencephalic tract; Ov, nucleus ovoidalis; RA, robust nucleus of arcopallium; RAm, nucleus retroambiguus; T, testosterone; X, Area X

Healey, 2018). Since many of the song control areas express several components of androgen signaling (including aromatase, androgen and estrogen receptors), a series of in depth studies in male canaries have looked at the distinct role of androgen signaling within these specific song nuclei. Alward et al. (2017) found that androgens within the RA control syllable and trill bandwidth stereotypy, whereas in the HVC they control song variability in male canaries. Furthermore, local testosterone implantation within the medial preoptic nucleus stimulates the song rate (Alward et al., 2013). Interestingly, also the expression patterns of these androgen signaling components change seasonally within the brain, independent of the volume changes in the song control nuclei. In canaries the estrogen and androgen receptor expression in HVC is higher during photosensitive and early photostimulated state compared to the photorefractory state (Fusani et al., 2000; Gahr & Metzdorf, 1997). In starlings, aromatase activity and androgen receptor density in the telencephalon was the highest during the photosensitive phase (Riters et al., 2001, 2002). Together, these findings suggest a complex interaction between testosterone, song behavior and seasonal neuroplasticity within the SCS.

However, a common drawback of these studies is that whereas song behavior and hormone fluctuations can be monitored longitudinally, a similar longitudinal readout of the structural neuroplasticity within the same animals is not possible with common histological approaches. This causes a lot of inter-subject variability and limits correlation analyses between structural neuroplasticity, song behavior and hormone levels. Furthermore, beside structural changes it is also relevant to investigate functional changes within the brain across seasons. In vivo MRI is a tomographic method based on the presence of water protons (H⁺) in tissues of living creatures. This imaging technique ticks all the boxes where its major benefits to study neuroplasticity clearly lie in its versatility, as the MRI signal can be sensitized to a wide range of biological phenomena, its noninvasive nature, which allows repeated measures and correlation to additional metrics such as changing hormone levels and behavioral tests, and its ability to virtually slice through the brain in any direction and extract data driven information from the entire brain at once (J. Hamaide et al., 2016).

2 | SCOPE OF THE REVIEW

In this review, we will discuss the recent advances in songbird seasonal neuroplasticity research from an in vivo MRI perspective. The reported data were acquired in our team on the European starling, a seasonal songbird in which both males and females sing. As far as we know there are no other MRI data available on seasonal neuroplasticity in songbirds.

Although this review illustrates clearly how cutting-edge technology drives new discoveries, the applied methodology as such will not be the focus as these details can be found in the material and methods section of the publications we refer to. It is important to know that MRI can make virtual slices through a living subject creating voxels (volume elements) with different types of contrast

correlating to a broad set of physiological phenomena resulting in spatial anatomical and functional information. For a broader view on the use of in vivo MRI to study neuroplasticity we refer to our review (Hamaide et al., 2016), for more specific use of MRI to study neuroplasticity in songbirds we refer to (Hamaide et al., 2018a; Van der Linden et al., 2009) and for more details on the functional MRI (fMRI) methods adapted to songbirds we refer to (Poirier et al., 2010; Van Ruijsevelt et al., 2013). In this review we discuss how in vivo MRI was applied (i) to explore seasonal changes in the SCS and brain wide, (ii) to study the contribution of hormones in seasonal neuroplasticity (iii) to identify which photoperiod coincides with heightened neuroplasticity and (iv) to identify the structural and functional neural correlate and substrate of song performance and song perception.

3 | MONITORING CHANGES IN VOLUME OF THE SCS AND IN SONG PRODUCTION IN PHOTOSTIMULATED AND PHOTOREFRACTORY STARLING: THE EARLY MRI WORK

The focus in songbird neurosciences has been the SCS and the first in vivo MRI studies showed that using conventional T1- and T2-weighted sequences, which reveal changes in fat or water content respectively, no anatomical contrast of the SCS could be observed even at a very high spatial resolution (Van der Linden et al., 1998; Verhoye et al., 1998). To visualize the song control and auditory system, other MRI methods had to be employed and since then several neuro MRI techniques have been used successfully in songbirds.

About the same time as Van der Linden and coworkers implemented MRI in songbirds, different research labs used manganese-enhanced MRI (MEMRI) to investigate brain activation to e.g., somatosensory stimulation (Lin & Koretsky, 1997), or to perform in vivo tract tracing experiments (Pautler et al., 1998). Manganese (Mn²⁺) is used as a paramagnetic contrast agent that influences the T1 MRI signal. It is a biological Ca²⁺ analogue, picked up by voltage gated Ca²⁺ channels in neurons, axonally transported to the synapse and even travels transsynaptically. Given the nodular arrangement of the songbird brain, injection of an isosmotic solution of MnCl₂ in one song control nucleus was hypothesized to successfully visualize the entire SCS. To test this hypothesis, MnCl₂ was injected locally in the HVC of anaesthetized adult European starlings (Van der Linden et al., 2002). HVC is situated on the caudo-dorsal surface of the telencephalon making it an ideal target for stereotactic injections, and sends afferent projections to Area X and RA. Six hours after injection, both RA and Area X appeared hyperintense on the T1-weighted images and their shape clearly matched previous descriptions from immunohistochemistry (Figure 2b–e). Furthermore, volumetric analysis of Area X and RA confirmed the previously described disparity of these nuclei between both sexes (Bernard et al., 1993). This was the first in vivo image of

the SCS in a songbird brain opening the way for repeated measures studying the impact of seasons and hormones on the brain.

MEMRI not only informs us about the structural connections, but also reveals alterations in functional properties of distinct cell populations based on their Ca^{2+} signaling reflecting neuronal activity (Brini et al., 2014). Consequently, besides anatomical contrast enhancement, Mn^{2+} can serve as a highly sensitive and direct readout of neuronal activation (Lin & Koretsky, 1997). The functional properties of particular cell groups in HVC (i.e., HVC to RA and HVC to Area X projecting neurons) were evaluated by performing dynamic MEMRI measurements. After injection of MnCl_2 in HVC, the change in signal intensity in RA and Area X was monitored over time (Figure 2f), reflecting different characteristics of the activity of the Mn^{2+} transporting neurons in HVC. In general, a change in the kinetics of Mn^{2+} could be caused by a change in activity or a morphological change in the number or density of projections from HVC.

After this validation, dynamic MEMRI was applied to further disentangle the intricate relationships between behavioral, hormonal and neuroplastic changes observed in starling. Van Meir and coworkers confirmed the seasonal volumetric changes in song control nuclei of female starlings by demonstrating that Area X and RA were larger in photostimulated (spring) compared to the photorefractory starling (summer) (Van Meir et al., 2006). Furthermore, 6-week testosterone treatment in adult photosensitive female starlings resulted in a significant volume increase in RA and Area X as demonstrated with MEMRI (Van Meir et al., 2004). Moreover, both the rate and total amount of Mn^{2+} transported to Area X and to RA increased, following the testosterone treatment (Figure 2f) (Van der Linden et al., 2004). These data point towards a cell- and pathway-specific effect of testosterone as two distinct cell populations within the same nucleus i.e., HVC to Area X and HVC to RA-projecting neurons in HVC, display differentially altered Mn^{2+} kinetics upon hormone treatment. Especially, the dynamics of accumulation altered in HVC to Area X projecting neurons, suggesting a testosterone induced change in activity, since HVC to Area X-projecting neurons are known to remain stable in adult songbirds (Alvarez-Buylla et al., 1988). This finding was later on supported by the neuronal correlate of the testosterone induced song rate in female starlings at the level of the mesopallial lamina which harbors HVC to Area X projections (Orije et al., 2020). In contrast, kinetic changes in the HVC to RA-projecting neurons could also be due to morphological changes in the number or density of projections, since it was shown that testosterone can increase the recruitment and/or survival of newly generated HVC to RA-projecting neurons in canaries (Rasika et al., 1994). This finding is further corroborated by tract tracings performed in later diffusion tensor imaging (DTI) studies (Orije et al., 2020) (Figure 2g). Furthermore, Van Meir et al. (2006) tested for possible relationships between song production and the size and functional properties of the song control nuclei in adult female starlings. They observed that female starlings that sung at high song rate during the photostimulated stage showed a higher Mn^{2+} accumulation in RA in both photostimulated and photorefractory stage compared to female birds that sung less. This hints to an activity-dependent functional intensification of the song control pathways.

4 | EXPLORING SEASONAL CHANGES IN THE BRAIN: EXTENDING THE FOCUS BEYOND THE SCS

4.1 | Microstructural changes between photostimulated and photorefractory stage in male starling brains

Targeted MEMRI permits volume quantification and the deduction of several properties related to functional connectivity between distinct brain areas. However, it does not inform on changes in structural connectivity or microstructural tissue properties. Nor could information beyond the song control nuclei be obtained if not targeted specifically with MnCl_2 .

DTI on the other hand, is known to be sensitive to anatomical and microstructural properties of the brain, and became at that time increasingly used in small animal preclinical brain wide investigations (e.g., Kim et al., 2003; Lin et al., 2001; Xue et al., 1999). This method visualizes mainly myelinated fiber connections based on the directionality of the water hindrance in brain tissue. Generally, five DTI parameters can be evaluated from diffusion weighted data, i.e., fractional anisotropy (FA), mean diffusivity (MD) and the three eigenvalues. FA is a measure for the directionality of water and is calculated as an abstract value ranging from 0 (isotropic) to 1 (fully anisotropic). For example, water protons in fibers and myelinated axons experience highly anisotropic diffusion as these water protons can only diffuse along and not perpendicular to the fibers, which makes that FA is extremely sensitive to detect alterations in white brain matter. The eigenvalues (λ_1 , λ_2 , λ_3) refer to the geometrical properties of the diffusion tensor (along three axes) estimated within a voxel. They are used to calculate MD and FA. Importantly, however, even though less regularly described, the eigenvalues are valuable parameters to understand the origin of particular changes in MD and FA. De Groof and coworkers optimized a protocol to obtain the first DTI data in a songbird, the European starling (Figure 3a), which illustrated nicely how songbird brains differ from mammalian brains (De Groof & Van der Linden, 2010). This is not only in their overall anatomy but also in the absence of large white matter bundles, like the corpus callosum in mammals, whereas songbirds have three smaller commissure's connecting both hemispheres. Most interhemispheric connections from the SCS, auditory and visual system run through different white matter laminae (Jarvis et al., 2005), whereas certain connections between nuclei are clearly visible as a separate white matter tract crossing the gray matter, the best example is the HVC-RA tract (Karten et al., 2013) (Figure 2g).

This first DTI study in starlings consisted of 8.5 h scans visualizing the entire SCS and provided additional anatomical contrast on several other important circuitries such as the auditory and visual system (De Groof et al., 2006). The obtained anatomical contrast on several song control nuclei and auditory regions obtained by DTI was most evident on FA maps as it was mainly caused by the visualization of fiber capsules surrounding the nuclei (Figure 3a).

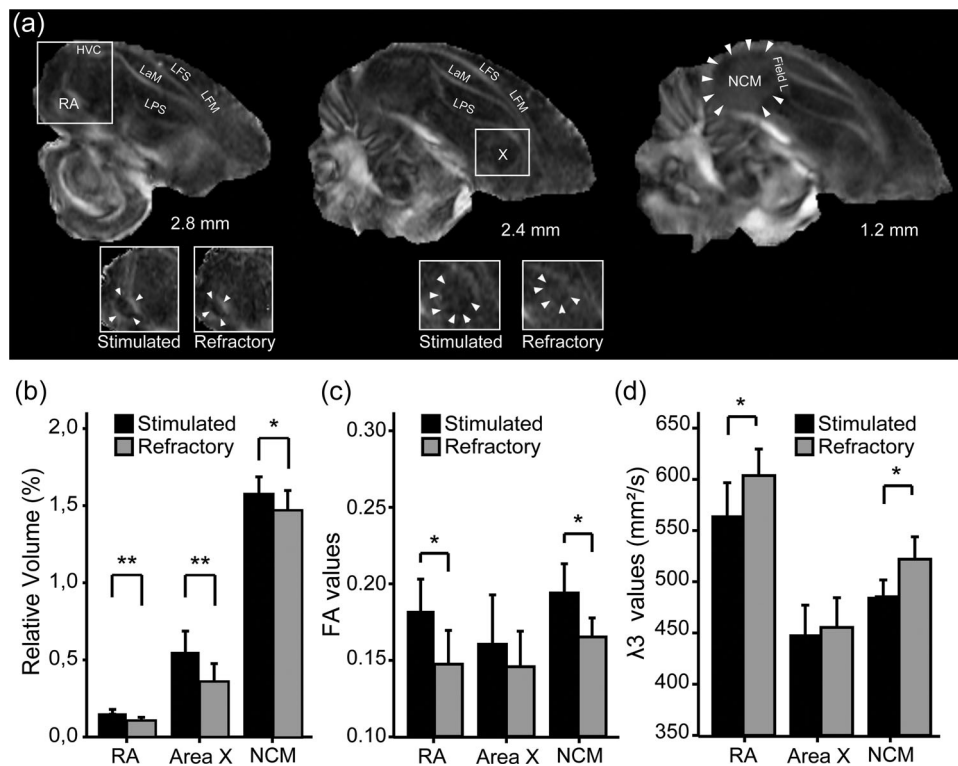
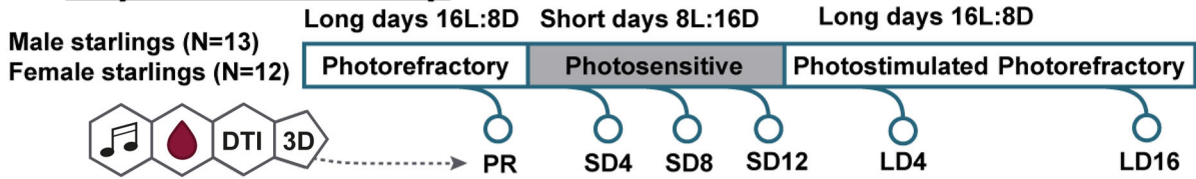


FIGURE 3 (a) Sagittal in vivo diffusion tensor imaging (DTI) based fractional anisotropy (FA) maps of a photostimulated male starling with an indication of position from the midline at the level of RA, Area X, and NCM. The inserts show volume differences of Area X and RA respectively of the same subject at two different photoperiods, (photostimulated in spring and photorefractory in summer). The in-plane resolution is 98 μ m. (b–d) Overview of the mean seasonal changes of RA, Area X, and NCM in their volume (b), DTI based FA values (c) and DTI based eigenvalue 3 (λ_3) (d) of 9 birds. Error bars correspond to SDs. (* p = 0.05, ** p = 0.01). Because of the significant photoperiodic change in entire telencephalon volume (1.6%), the volumes of RA, Area X, and NCM are expressed relative to the volume of the corresponding telencephalon hemisphere (in %). Adapted with permission from De Groof et al. (2009). HVC, used as a proper name (high vocal center); LaM, mesopallial lamina; LFM, supreme frontal lamina; LFS, superior frontal lamina; LPS, pallial-subpallial lamina; NCM, caudomedial nidopallium; RA, robust nucleus of arcopallium; X, Area X

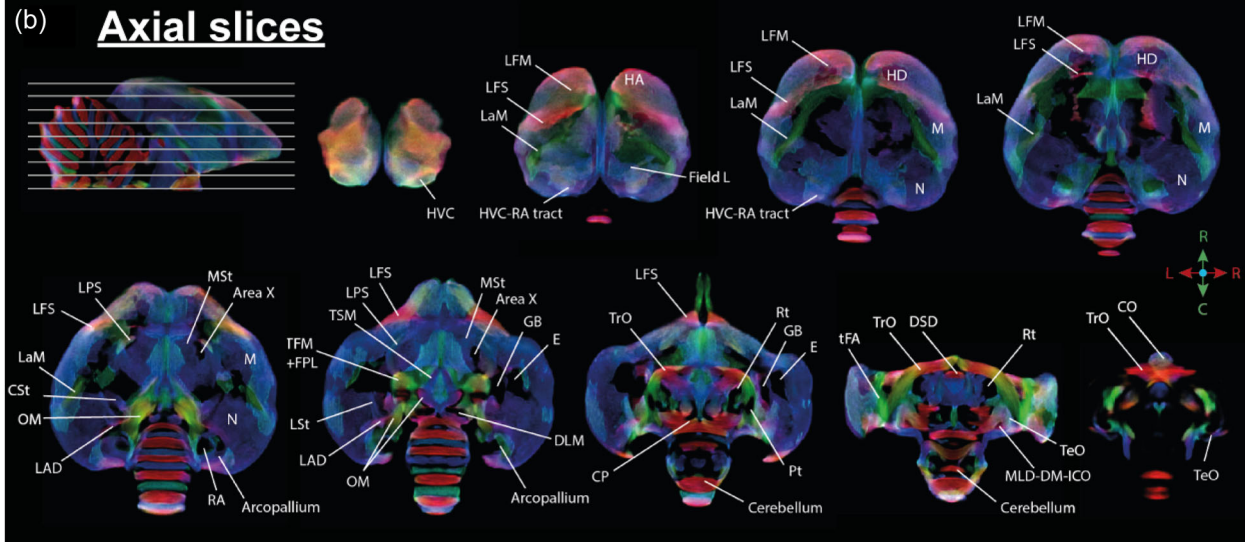
Subsequent DTI studies monitored neuronal changes between different photoperiodic stages (photostimulated in spring vs. photorefractory in summer) focusing on the brain connections rather than the brain nuclei (De Groof et al., 2008, 2009). The earlier acquired MEMRI data already hinted towards intensified connections translated in differential transport of Mn^{2+} from HVC to the projection areas and those connections could now be quantified structurally using DTI. Male starlings were repeatedly imaged during the different photoperiodic stages. Manual delineation of the ROIs resulted in two different outcomes, firstly, the volumes of the gray matter areas could be extracted (Figure 3b) and, secondly, the DTI parameters (FA, MD, AD, λ_1 , λ_2 , λ_3) (Figure 3c,d) which inform on the intrinsic tissue properties, were analyzed. This entire brain approach using DTI uncovered that the entire telencephalon showed significant volume changes between the two photoperiodic stages. RA, Area X and the auditory region NCM showed a volume decrease towards the photorefractory stage (De Groof et al., 2009) (Figure 3b). The first two are in line with previous reports and the MEMRI studies, but the change in NCM was a novel finding. No direct correlations could be found between plasma testosterone levels and volume changes in these regions, which suggests that additional factors, besides gonadal

testosterone, are required to trigger this structural neuroplastic response. The volume decrease in Area X could be linked to previous studies that showed a change in neuron size and spacing in adult male song sparrows between photoperiodic stages (Thompson & Brenowitz, 2005). The volume decrease in NCM was mirrored by a significant reduction in FA and an increase in λ_3 towards the photorefractory stage (Figures 3c,d and 6c). NCM contains aromatase expressing cells, which are known to show seasonal plasticity in canaries (Fusani et al., 2000) displaying highly complex branching patterns and large cell nuclei in the photostimulated stage (Saldanha et al., 2000). This makes them a likely candidate to contribute to the alterations in microstructural tissue characteristics picked up by DTI. Several fiber tracts showed a reduction in FA towards the photorefractory stage, i.e., the HVC-RA tract, tractus occipito mesencephalicus, the LaM, LPS and the commissura posterior (De Groof et al., 2008). Furthermore, also the fiber capsules surrounding Area X and RA displayed a lower FA in the photorefractory compared to the photostimulated stage. These wide-spread changes in connectivity provide additional proof that the entire songbird brain undergoes dramatic structural remodeling when going through the different photoperiodic stages. De Groof and coworkers managed to

(a) **Experimental setup**



(b) **Axial slices**



(c) **Time effect**

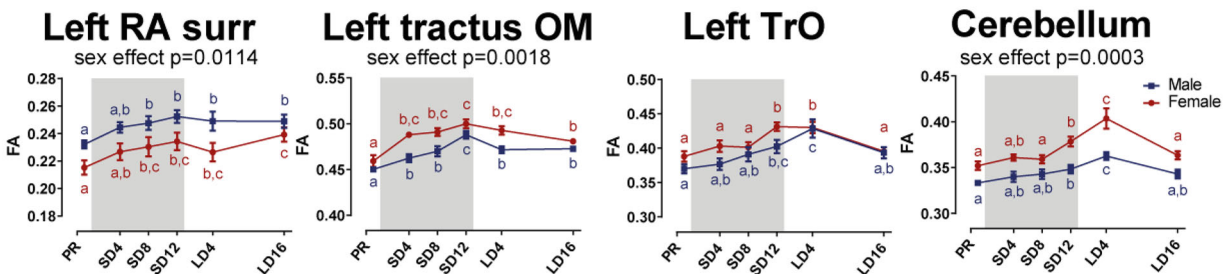
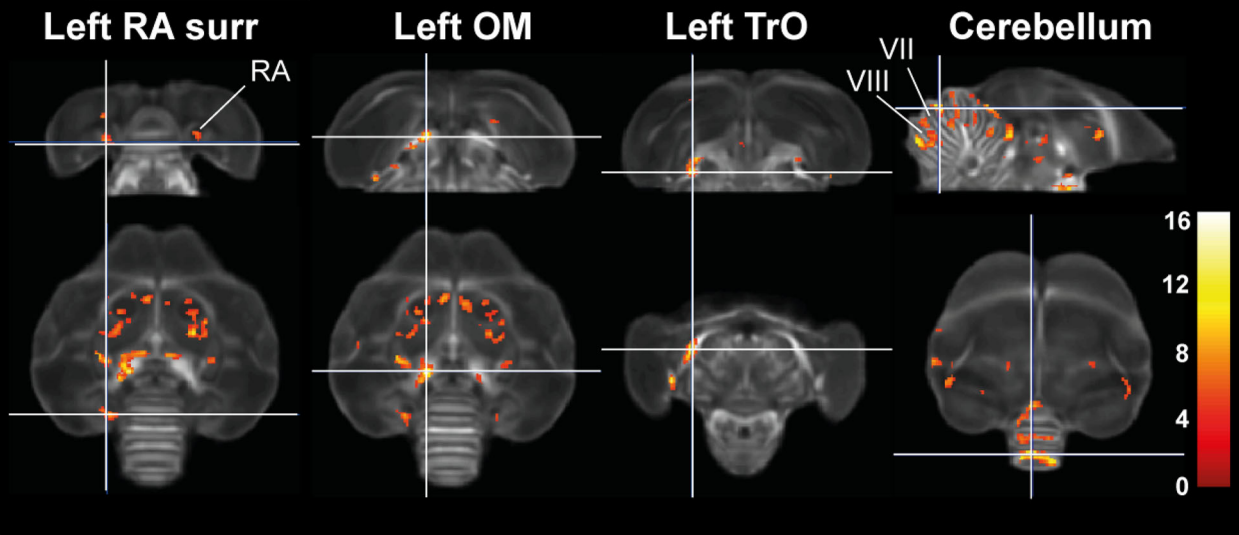


FIGURE 4 (See caption on next page)

relate the changes observed by DTI to a decreased number of axonal projections possibly combined with demyelination by comparing the in vivo DTI to ex vivo myelin stained tissues. Additionally, comparison to previously published histological findings, exposed parallels between changes in DTI parameters and alterations in neuronal density and soma size, showing that DTI is a sensitive technique to pick up known microstructural changes in vivo.

4.2 | The photosensitive period is a “sensitive window” where both sexes experience heightened “multisensory” neuroplasticity

The spatio-temporal dynamics of seasonal neuroplasticity were further explored in an extensive longitudinal DTI study in male and female starlings by Orije et al. (2021) (Figure 4a). The DTI protocol was further optimized by reducing the acquisition time from 8.5 h to 40 min, while the in-plane resolution decreased from 97 to 179 μm (pixel size). A shift was made from the SCS hypothesis driven approach (as described in previous sections) to a data-driven voxel-based approach allowing hypothesis free discovery of plasticity changes within the entire brain. This type of data analysis requires a template or atlas to which the individual data can be spatially normalized (De Groof et al., 2016). Therefore, a high-resolution population based template was created from male and female starling DTI data, which visualizes the different fiber tracts within the starling brain (see Figure 4b). Furthermore, this study extended the view beyond the traditional photostimulated versus photorefractory stage comparisons and included more time points, specifically during the photosensitive period, to establish the timing of neuroplasticity events. The study also confirmed that both females and males present a gradual increase in circulating testosterone during the late photosensitive phase. Besides seasonal changes in FA in the

surroundings of several song control nuclei, also many other regions experienced seasonal microstructural changes in both sexes, including tracts involved in the auditory and the visual system (Figure 4c). Even more interesting was the finding that specific cerebellar lobules, related to the processing of song and sensory information, displayed seasonal neuroplasticity. The cerebellum has never been a target in seasonal studies before. Prior studies looked at the anatomy of the avian cerebellum, which has a strict topographical organization similar to mammals (Arends & Zeigler, 1991), and recently identified a cerebello-thalamic-basal ganglia pathway, which could influence song processing (Hamaide et al., 2018b; Person et al., 2008; Pidoux et al., 2018). Since several sensory systems, including the cerebellum which integrates this sensory information, experience heightened neuroplasticity in preparation of the breeding season, this can be defined as multisensory neuroplasticity. Furthermore, most of the neuroplastic changes start during the photosensitive period when gonadal hormone levels are still low. These periods of heightened neuroplasticity could be seen as reoccurring sensitive windows for sensory and sensory motor system plasticity resembling the initial multisensory plasticity during CPs early in life.

4.3 | Male and female starlings both experience neuroplasticity but display differential underlying mechanisms and singing behavior

The before mentioned longitudinal study by (Orije et al., 2021) determined the temporal profile of multisensory neuroplasticity, but also established sex differences in seasonal neuroplasticity and related them to the song behavior. The data show that the majority of seasonal neuroplasticity occurred similarly in both male and female starlings but some specific parts of the SCS (the surroundings of bilateral HVC and left RA) and auditory system (left NCM) only showed increased FA

FIGURE 4 (a) Overview of the experimental setup where we repeatedly monitored song behavior, hormone plasma levels and structural neuroplastic changes using Diffusion Tensor Imaging (DTI) and structural 3D scans in male and female starlings. (b) Population-based tractogram created from the DTI images of male and female starlings with indications of the different lamina, interconnecting tracts, nuclei, and brain regions displayed on axial slices throughout the brain. The in-plane resolution is 179 μm . (c) Overview of representative regions that displayed seasonal changes in DTI based fractional anisotropy (FA) values over time, including the surroundings of song control nuclei like RA, tracts related to the song control and visual system, like the OM tract and optic tract (TrO) and even within the cerebellum. The statistical parametric maps display the significant regions with a change in FA over time on axial and coronal sections throughout the brain (upper and lower row, respectively). The results are displayed and overlaid on the population FA map. The T-values are color-coded according to the scale on the right. The longitudinal FA changes over time extracted from the ROI-based cluster are displayed in the graphs underneath where the gray area indicates the photosensitive period of short days (SD). Significant sex differences are reported with their p value. Different letters denote significant differences by comparison with each other in post hoc t tests with $p < 0.05$ (Tukey's HSD correction for multiple comparisons) comparing the different time points to each other. If two time points share the same letter, the FA values are not significantly different from each other. Adapted with permission from Orije et al. (2021). CA, anterior commissure; CO, optic chiasm; CP, posterior commissure; CSt, Caudal part of the lateral striatum; DLM, medial part of the dorsolateral nucleus of the anterior thalamus; DSD, Decussatio supraoptica dorsalis; E, Entopallium; FPL, lateral forebrain bundle; GP, globus pallidus; HA, apical part of the hyperpallium; HD, densocellular part of the hyperpallium; HVC, used as a proper name (high vocal center); LAD, dorsal arcopallial lamina; LaM, mesopallial lamina; LFM, supreme frontal lamina; LFS, superior frontal lamina; LMAN, lateral magnocellular nucleus of the anterior nidopallium; LPS, pallial-subpallial lamina; LSt, lateral striatum; M, mesopallium; MLD-DM-ICO, intercollicular nucleus complex; MSt, medial striatum; N, nidopallium; NCM, caudomedial nidopallium; OM, occipito-mesencephalic tract; Pt, pretectal nucleus; RA, robust nucleus of arcopallium; Rt, Nucleus rotundus; surr, surroundings; TeO, optic tectum; tFA, fronto-arcopallial tract; TFM, Tractus thalamo-frontalis; TrO, optic tract; TSM, septopallio-mesencephalic tract

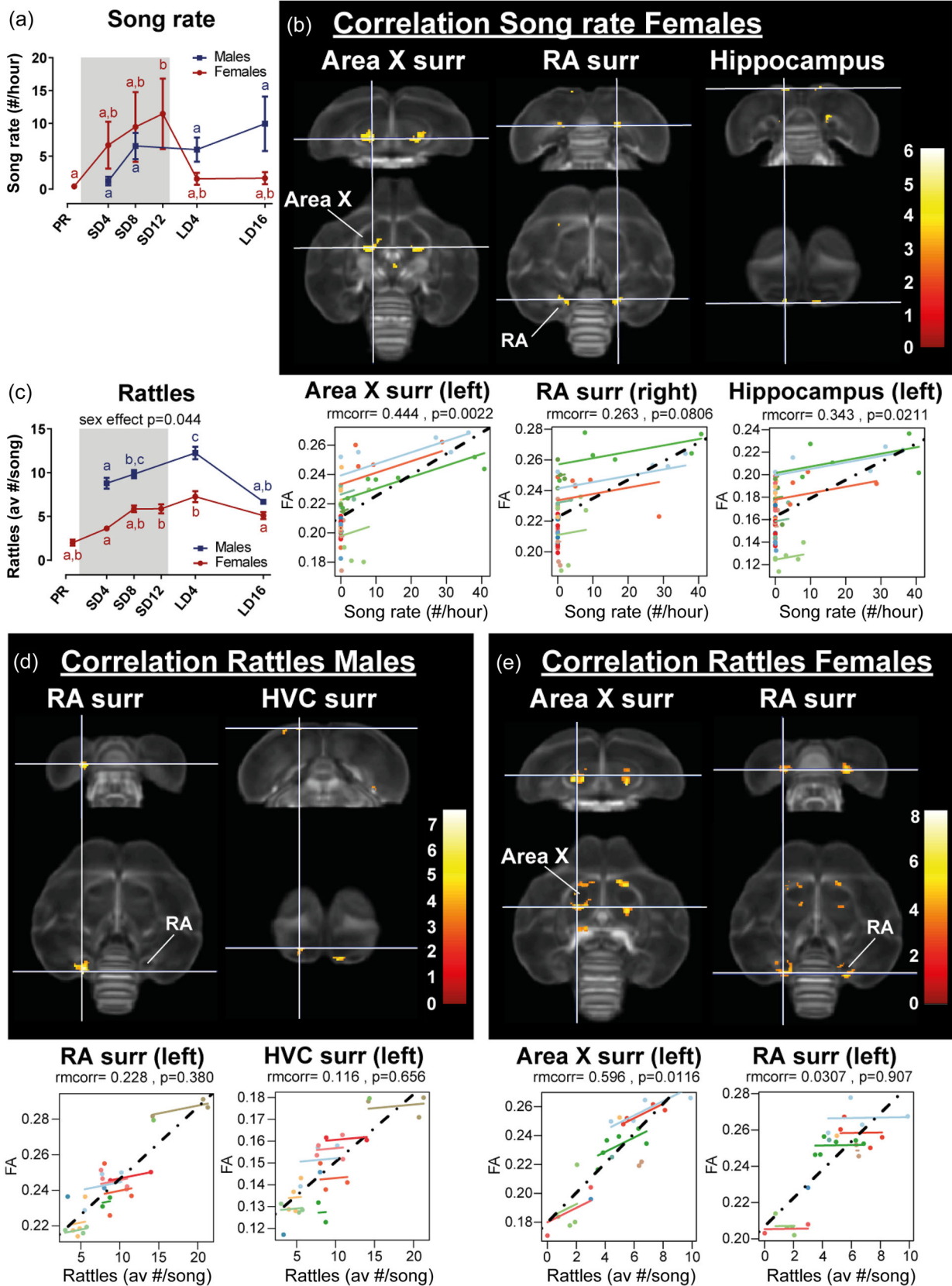


FIGURE 5 (See caption on next page)

values in males and were absent in female starlings. It would be interesting to find out whether this is related to a differential song behavior and since this study monitored several factors simultaneously within the same animals at multiple time points over the seasons, hypotheses like these could be investigated by determining the neuronal correlates of these factors. Such voxel-based correlation methods nail down in an unbiased way the specific regions of which the structural characteristics, like DTI parameters, correlate to a behavioral or hormonal readout. These methods have previously been used to pinpoint neuroplastic regions subserving motor skill learning in humans (Dayan & Cohen, 2011) and song learning in male zebra finches during ontogeny (Hamaide et al., 2020). Additionally, the extracted DTI parameters were further plotted against the correlating factor to distinguish “between-subject” and “within-subject” correlations using repeated measures correlations (Bakdash & Marusich, 2017).

The male and female song behavior differed in several ways. Whereas male starlings sing at every season throughout the year and especially during the photostimulated period, small female starlings rarely sing during any of the photoperiods. The larger females sing most during the photosensitive phase but hardly during the photostimulated phase (Figure 5a), in line with previous findings (Pavlova et al., 2007). The song rate of female starlings correlated with the DTI outcome at the level of the SCS and the hippocampus (Figure 5b). This correlation contributed to the between-subject correlation where proficiently singing female starlings had higher FA values compared to nonsinging females. Additionally, repeated measures correlations uncovered within-subject correlations for example at the surroundings of Area X, indicating a microstructural reorganization of the Area X surroundings as the female songbird increases its song rate. This part of the SCS is known to be involved in song learning during ontogeny (Bottjer & Johnson, 1997). The correlation of song rate at the level of the hippocampus however is quite interesting, since the role of the hippocampus in song behavior is not widely understood. Few studies have indicated that the hippocampus might play a role in specific characteristics of song perception, like identity and temporal context, especially in female zebra finches (Bailey & Wade, 2003; Bailey et al., 2009; Gobes et al., 2009). Hippocampal lesions affected the female

preference for their father's song whereas in male zebra finches, hippocampal lesions during song learning and adulthood did not affect song learning, singing nor song structure (Bailey et al., 2009).

Furthermore, males and females also differ in measures of song complexity. The male song for example is characterized by a higher number of rattle phrases within their song compared to the female song (Figure 5c) (Hausberger et al., 1995; Pavlova et al., 2005). Rattles are complex phrase types and require precise coordination of the respiratory and syringeal muscles to produce clicking sounds (Pavlova et al., 2005). The song data from Orije et al. (2021) were used for more in depth neuronal correlations of DTI with measures of song complexity, like rattles (unpublished additional data analysis falls under ethical dossier of (Orije et al., 2021)). In males, a higher number of rattle phrases correlated with the DTI outcome only in specific parts of the song motor pathway (RA and HVC) (Figure 5d), two regions that experienced neuroplasticity (indicated by FA changes over time). In singing female starlings, a higher number of rattle phrases is also correlated to FA in parts of the anterior forebrain pathway (Area X surroundings) (Figure 5e), which functions as a ‘recursive’ loop providing feedback that is essential for adequate song learning (Brainard, 2004). So whereas adult male starlings involve regions of the auditory and song motor pathway, evident by the DTI changes at the level of NCM, RA and HVC surroundings, adult female rattle phrases involve feedback through the anterior forebrain pathway and the hippocampus. This illustrates how unbiased exploratory MRI studies lead to novel findings, which would not be found with the traditional SCS focus, or the common *ex vivo* neuroscience methods.

5 | HOW DO HORMONAL MANIPULATIONS AFFECT THE SEASONAL NEUROPLASTICITY IN MALE AND FEMALE STARLING: EXTENDING THE FOCUS FROM TESTOSTERONE TO THYROID HORMONES

To unravel the interaction between hormones, song behavior and neuroplasticity, several approaches are possible with *in vivo* MRI. First, correlation analyses can shed light on the interactions between multiple

FIGURE 5 (a) Overview of the seasonal variation in song rate in male and female starlings. The song rate was calculated as the number of complete songs per hour. The gray area indicates the photosensitive period of short days (SD). Different letters denote significant differences by comparison with each other in post hoc *t* tests with $p < 0.05$ (Tukey's HSD correction for multiple comparisons) comparing the different time points to each other. If two time points share the same letter, the song rate is not significantly different from each other. (b) Overview of representative regions with a structural neuronal correlate of song rate to diffusion tensor imaging (DTI) based fractional anisotropy (FA) in female starlings assessed by a voxelbased multiple regression. No significant correlations between song rate and FA were found in male starlings. The statistical parametric maps display the significant regions with a correlation between FA and song rate on axial and coronal sections throughout the brain (upper and lower row, respectively). The results are displayed with $p_{\text{uncorr}} < 0.001$ and $k_E \geq 10$ voxels, and overlaid on the population FA map. The Tvalues are color-coded according to the scale on the right. Below these voxel-based multiple regression map, the identified correlations were further explored with repeated measures correlation. Solid colored lines show the best linear fit for the within-subject correlation using parallel regression lines for individual animals. The dashed line represents the linear fit of the overall Pearson correlation representing the between-subject correlations. Adapted with permission from Orije et al. (2021). (c) Overview of the seasonal variation in average number of rattle phrases within a song bout in male and female starlings. The significant sex difference is reported with the *p*-value. The graphic layout is the same as (a). (d, e) Overview of representative regions with a structural neuronal correlate of rattles to FA in female (d) and male starlings (e) assessed by a voxel-based multiple regression. The image layout is the same as (b). (c–e) present unpublished extended analysis on song data from Orije et al. (2021).

factors measured simultaneously (as discussed in the previous section). Secondly, different hormone manipulations can be applied to study their effects on song behavior and neuroplasticity, similar to what prior histological studies used to unravel these effects. The added advantage of in vivo MRI in these kind of manipulation studies, is that you can follow the same subjects longitudinally and assess the effect of a hormone manipulation on song behavior and neuroplasticity at several time points. Whereas traditional studies could monitor song behavior across time points and only combine this with histology at the endpoint.

As a follow up of the study of Van Meir et al. (2004), Orije et al. (2020) implanted photosensitive female starlings with testosterone but used the same data driven approach introduced above to further uncover the spatio-temporal dynamics of testosterone induced song behavior and neuroplasticity measured by in vivo DTI. They monitored short term effects of testosterone implantation (first days) up to 3 weeks. The volume increase of RA and Area X due to testosterone treatment and the increased projections between HVC and RA confirmed the earlier data obtained with MEMRI (Figure 2g) (Van Meir et al., 2004). Interestingly, testosterone rapidly stimulates the song rate, but measures of song quality, such as song bout length, developed more gradually. While they did not find any significant voxel-based neuronal correlates of DTI parameters to the quick rise in testosterone or song rate, a neuronal correlate to the gradually increasing measures of song quality was found at the level the HVC and RA surroundings and the mesopallial lamina (which harbors connections to and from the anterior forebrain pathway). This study showcases how testosterone treatment causes singing activity-induced neuroplasticity within the SCS.

In another intervention study, Orije et al. (2022) looked at the potential effect of thyroid hormones on seasonal neuroplasticity in male starlings. Thyroid hormones are known to play a role in early brain development and partake in the regulation of seasonal reproduction in starlings, however little is known about their role in seasonal neuroplasticity. Using in situ hybridization, the authors first established the expression of several thyroid hormone regulating genes within the SCS, suggesting their potential to directly affect the SCS neuroplasticity. Next, hypothyroidism was induced in male starlings using methimazole treatment during the photosensitive and photostimulated period, which not only reduced the circulating thyroid hormones (thyroxine [T4] and triiodothyronine [T3]) to a minimum, but also prevented the testosterone increase upon photostimulation. However, hypothyroidism did not cause a halt to the majority of the neuroplastic changes, only myelination of certain tracts during photostimulation seemed to be affected as deduced from relevant DTI changes. In both control and hypothyroid starlings FA values correlated with song bout length at the level of several song control nuclei, but also at the level of the cerebellum. Remarkably, similar to the song bout length correlation, the FA values of several regions within the SCS and cerebellum had a negative correlation to the circulating thyroid hormone levels. This could be explained since both the control group and hypothyroid group decreased their circulating thyroid hormones during the photosensitive phase, which coincides with the reopening of the

sensitive period of heightened multisensory neuroplasticity (Orije et al., 2021). So whereas we could not find a direct voxel-based neuronal correlation with circulating testosterone in any of the prior correlation studies (Orije et al., 2020, 2021), circulating thyroid hormones were inversely related to the seasonal neuroplasticity at the level of the SCS and the cerebellum. Reducing thyroid hormones during the photosensitive period might be necessary to lift the brakes imposed by the photorefractory period.

6 | SEASONAL FUNCTIONAL CHANGES IN THE NEURONAL SUBSTRATE OF SONG PERCEPTION AND DISCRIMINATION

In vivo DTI in male starlings revealed structural seasonal neuroplasticity in NCM, part of the auditory system, where changes in $\lambda 3$ were suggested to mark altered aromatase expression (De Groof et al., 2008). The question remained what would be the function of these changes in NCM and how would it serve the breeding season? Evidence collected from IEG and electrophysiology studies points towards an important role for NCM in the processing of conspecific song (Chew et al., 1996; Mello et al., 1992). Depending on the time of year, starlings reside in highly different social contexts during which the function of (conspecific) song changes markedly: during the breeding season, starlings live in small groups and song is most important for mate recognition, while during the nonbreeding season, starlings gather in large groups and use song for group recognition (Hausberger, 1997). Furthermore, recent research in canaries (*Serinus canaria*) showed altered neural activity in HVC to conspecific song depending on the seasonal status (Alliende et al., 2013). In canaries, however, the spectro-temporal characteristics of song also change over the seasons. Consequently, no conclusions on seasonally differential song selectivity could be drawn as the different auditory responses might be caused by seasonal changes in the acoustic features of their song. European starling song, on the other hand, use the same acoustic song features over different seasons and could therefore provide a decisive answer on whether the observed structural changes in their auditory system (NCM) serve the seasonally changing social context of their songs.

To that end, we used in vivo auditory fMRI to explore the processing of different kinds of songs in the starling brain within one imaging session and study the modulation of song perception by photoperiod and hormone status. This method with an 'entire brain' approach provides an indirect readout for neuronal activity and this within small voxels which contain hundreds to thousands of neurons. Neuronal activity results in locally increased blood supply (hemodynamic response) creating local changes in the ratio oxygenated/deoxygenated hemoglobin responsible for the so-called blood oxygen-level-dependent MRI contrast (Nair, 2005). As fMRI uses a physiological response as readout for brain activity, the physiological parameters of the anaesthetized bird should be closely monitored and kept stable throughout the MRI acquisition. The implementation of the technique faced several challenges and the first fMRI

experiments in starlings included characterization and validation studies upon three auditory stimuli with differential social value (Van Meir et al., 2005). De Groof et al. (2013) scanned a group of male European starlings twice, once during the photostimulated and once during the photorefractory stage while presenting them with pure tones (control stimulus to compare between different imaging sessions) and four types of unfamiliar starling song, i.e., species specific whistles, individual whistles, species-specific warbling and individual warbling (for an in-depth description of starling song, we refer to (Hausberger, 1997). Voxel-based analyses showed that during the photostimulated stage both dorso-rostral and caudal subareas of NCM showed a differential response when exposed to species-specific songs and songs conveying individual information (Figure 6a,b). During the photorefractory stage this selectivity was only observed in the right dorso-rostral NCM. A ROI-based test for lateralization confirmed that only the right dorso-rostral and/or caudal NCM activate differently to distinct types of conspecific communication signals depending on the photoperiodic or seasonal status (De Groof et al., 2013).

This part of NCM also displayed $\lambda 3$ changes between the same photoperiodic stages investigated here (Figure 6c), which we linked to literature findings of seasonal changes in aromatase expressing cells

(Saldanha et al., 2000). Moreover multiple evidence supports the neuromodulatory role of estrogens on brain and behavior (Remage-Healey et al., 2013). Furthermore, the (rapid) behavioral response to estradiol treatment is dependent on the photoperiod, as observed in song sparrows (Heimovics et al., 2018). To inform whether it is possible to extrapolate the findings obtained in zebra finches to starlings and, if possible, to inform on the spatial extent of the area modulated by local estrogens, auditory fMRI was performed during photostimulated and photorefractory stages, with and without (intraperitoneal) application of vorozole, an aromatase inhibitor (De Groof et al., 2017). Similar to zebra finches, a clear effect of treatment was observed in left NCM and left Field L, and showed a higher neural activity during control compared to vorozole condition. In addition, an interaction between photoperiod and treatment was observed in the caudal subdivision of left NCM. These findings explain the observed seasonal structural and functional changes in NCM through hormonal neuromodulation of the auditory system bringing more emphasis on individual song recognition in the photostimulated than in the photorefractory male starlings. Whether the increase in the volume of NCM during the photostimulated stage (Figure 3b) involves the integration of new cells or is the result of changes in the morphology of aromatase expressing cells was not yet investigated.

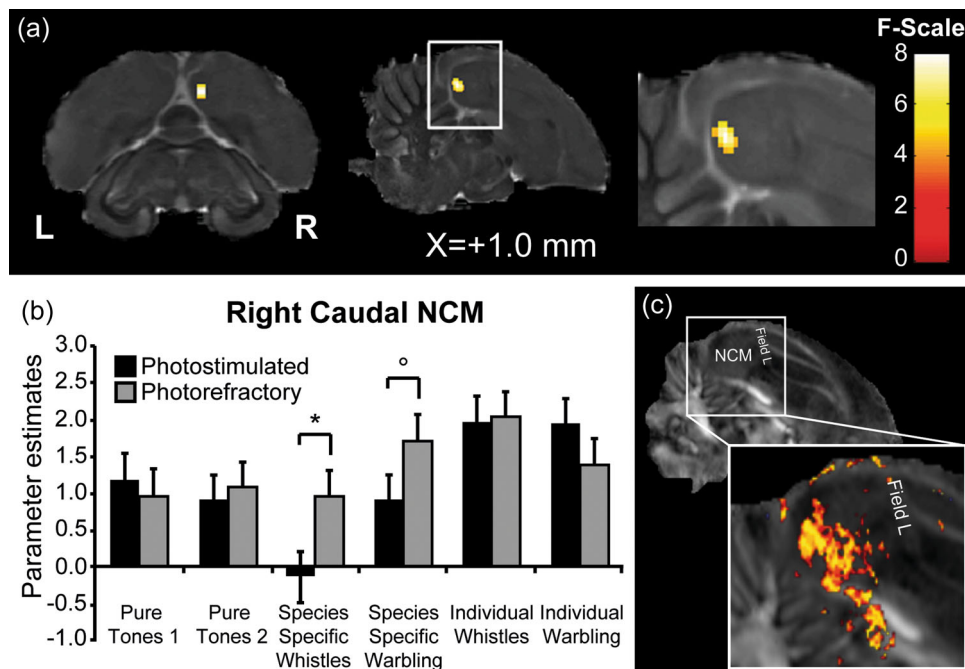


FIGURE 6 (a) Brain activation as observed with auditory functional magnetic resonance imaging (fMRI) and overlaid on coronal and sagittal (1 mm from the midsagittal plane) MRI images of male starlings. The dorsal part of the right caudal NCM (insert) presents a photoperiod dependent differential auditory response to species and individual specific whistles and warbling (F test from a repeated measures ANOVA; $N = 5$). F values are color-coded according to the scale displayed on the right. The in-plane resolution of the fMRI and 3D images are, respectively, 340 and 85 μm . (b) Estimates of the relative (vs. rest) response amplitude (+SEM) of neural activations elicited by the different song stimuli in the fMRI cluster illustrated in (a). The zero level corresponds to the estimated mean activation during rest periods. *Indicates a statistically significant difference between photoperiodic stages ($*p < 0.05$), while $^{\circ}$ indicates a trend ($^{\circ}p < 0.1$). Adapted with permission from De Groof et al. (2013). (c) Result from unpublished voxel-based statistics displaying the photoperiod dependent difference in $\lambda 3$ values derived from diffusion tensor imaging (DTI) data from De Groof et al. (2009) (The ROI-based DTI data are shown in Figure 2). This cluster displaying differences in $\lambda 3$ overlaps with the cluster with differential auditory fMRI responses in the caudal part of NCM as described in (a)

7 | SEASONAL FUNCTIONAL CHANGES IN THE NEURONAL SUBSTRATE OF ODOR PERCEPTION AND DISCRIMINATION

The most developed and most sensitive sensory systems in birds are the auditory and the visual system; while rodents use preferentially tactile and olfactory systems. However, the olfactory system of birds has gained more interest (Bonadonna & Mardon, 2013; Caro et al., 2015; Graham et al., 2021) and seasonal changes in olfactory system and odor perception have also been demonstrated in starlings (Clark & Smeraski, 1990). As the olfactory bulb in birds is very tiny as compared to rodents (Figure 7a), fMRI would be far too challenging and we opted for activity induced MEMRI after nostril injections of $MnCl_2$ in starlings as successfully demonstrated by Pautler and Koretsky (2002) when studying the mouse olfactory system. De Groof and co-workers exposed male starling, while in the MRI system, to milfoil, an aromatic herb that birds prefer to include in the nest material and that is considered to have an antiseptic function towards the offspring (Gwinner et al., 2000). The data showed that the olfactory bulb is only able to readily detect or discriminate milfoil odor under photostimulated condition (Figure 7b), while there is less

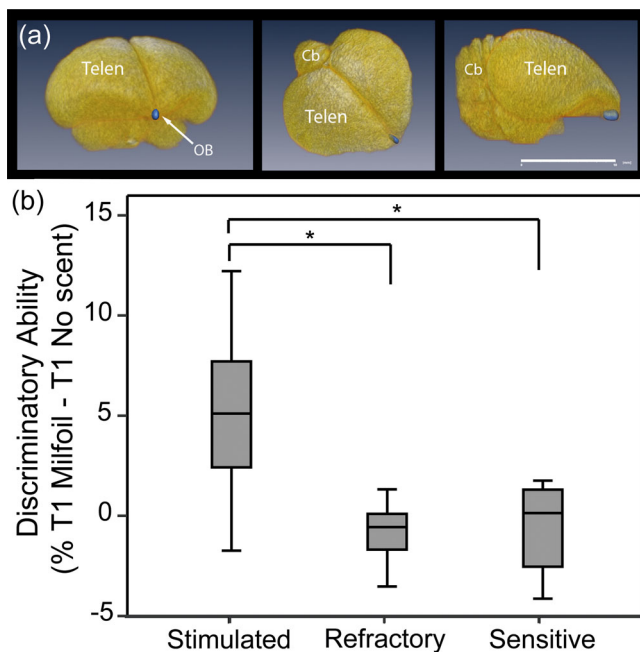


FIGURE 7 (a) 3D rendering of a starling brain from magnetic resonance imaging (MRI) data showing the small size of the olfactory bulb (OB) compared to the rest of the brain (scale bar 1 cm). The in-plane resolution is 195 μm . Cb = cerebellum; Telen = telencephalon. (b) Discriminatory ability $[(T1 \text{ milfoil} - T1 \text{ no scent})/T1 \text{ no scent}]$ of the olfactory bulb (OB) based on mean T1 values from the olfactory bulb obtained from the same individuals ($N = 14$) at different times of the year using dynamic manganese enhanced MRI (MEMRI). In each photoperiod each animal was measured twice, once with a milfoil scent as stimulus, once without any scent. Error bars represent standard deviation ($*p < 0.05$). Adapted with permission from De Groof et al. (2010)

manganese accumulated in the olfactory bulb when milfoil odor is offered under photorefractory conditions due to possible inhibitory mechanisms designed to enhance the sensitivity of the olfactory bulb. The size of the olfactory bulb changes over the year and exogenous testosterone implants have an effect on the (relative) olfactory bulb size during the photorefractory stage but have no effects on the olfactory discrimination capacity (De Groof et al., 2010).

8 | GENERAL CONCLUSION

The development and implementation of in vivo MRI techniques in songbirds has not only allowed us to confirm many of the known seasonal and testosterone-induced changes in neuroplasticity within the SCS in the same bird, but has led to many new and interesting findings. We showed that in fact starlings experience a multisensory neuroplasticity starting during the photosensitive period, which included structural and functional changes in sensory systems like the visual, the auditory and even the olfactory system. Moreover, our MRI data uncovered that the cerebellum, which received thus far very little attention in songbird research, stands out in seasonally and sexually differential neuroplasticity presentation.

The convergence of neuroplastic changes in several sensory and sensorimotor systems, is indicative of the early life multisensory plasticity during CPs and brings further exciting prospects to the study of seasonal songbirds as an animal-model that experiences natural reoccurring sensitive windows of heightened sensory systems neuroplasticity. This is in contrast to mammals that—as far as we know—have “critical” periods for neuroplasticity early in life ending with the stabilization of the main sensory circuits, which form the basis for furthermore complex skill learning and cognition (Reh et al., 2020; Takesian & Hensch, 2013). Nevertheless, studies have found seasonal variations in some shrews and mustelids with the volume or weight of skull, brain and total body decreasing during the winter, the so called Dehnel's phenomenon, which is explained as a reduction in energetic cost when food availability is scarce. In winter, Etruscan shrews (*Suncus etruscus*) present a brain volume reduction located in layer 4 of the somatosensory cortex, a cortex region responsible for (invertebrate) prey detection (Ray et al., 2020). This has been associated with an underlying reduction in the number of inhibitory parvalbumin (PV) expressing interneurons limiting the inhibitory capacity towards somatosensory neurons and lowering their threshold of sensory detection in winter conditions. Our MRI data point to a similar mechanism in starlings with seasonal volume changes in the same direction in the entire telencephalon, in the song control nuclei, the auditory system's NCM and the olfactory bulb (De Groof et al., 2006, 2013; Orije et al., 2021). Moreover, our fMRI readouts point towards a similar mechanism of reduced inhibition in non-breeding or photorefractory conditions. fMRI showed a decreased auditory activation in the caudal NCM to species-specific songs during the photostimulated stage (breeding season), which could indicate an increase in inhibitory activity (De Groof et al., 2013). Based on different earlier studies on activity and aromatase expression in NCM, Remage-Healey et al.

(2008) suggested that auditory-evoked changes in the activity of GABAergic neurons are directly linked to auditory-evoked elevations in forebrain estradiol levels. Similarly, the MEMRI data for olfactory activity showed lower Mn^{2+} transport upon milfoil exposure in the breeding season, suggesting that the starling's olfactory system distinguishes seasonally relevant odors on the basis of presynaptic inhibition with increased inhibition displayed in the breeding season (De Groof et al., 2010). These insights shed new light on seasonal variations in brain volume and function of seasonal songbirds and small mammals. Uncovering the capacity of the brain for seasonally controlled induction of heightened neuroplasticity might help solving the mystery of how to unglue the brain circuits fixed during childhood, raising hopes for treating many brain disorders (Bardin, 2012).

Additionally, the noninvasive aspect of MRI allows the study of longitudinal neuronal changes in the same animals, as well as the data-driven discovery of neuronal correlates with specific behavioral and endocrine changes. This approach led to the discovery that male and female starlings show subtle differences in their song behavior and associated activity-induced neuroplasticity, where males experience seasonal neuroplasticity at the level of NCM, RA, and HVC fiber surroundings, and females rely more on the anterior forebrain pathway and hippocampus (Orije et al., 2021). In view of decades of research pointing to testosterone in the driver's seat of seasonal neuroplasticity under photostimulated conditions, our MRI data could not demonstrate a correlation between circulating testosterone concentrations and any of the fiber network changes as such. However, testosterone clearly boosts the song rate, improving the singing proficiency and song activity-induced neuroplasticity and this already under photosensitive conditions. Our auditory fMRI data demonstrated a clear role of testosterone -via aromatase- as neuro-modulator of seasonally contextual song perception (De Groof et al., 2017). Whereas a direct neuronal correlate to circulating testosterone levels could not be demonstrated in any of our studies, circulating thyroid hormones were shown inversely related to the seasonal neuroplasticity at the level of several song control nuclei and the cerebellum (Orije et al., 2022). As such thyroid hormones might play a more direct role in seasonal reopening and closing of sensitive windows of multisensory neuroplasticity, but this requires further investigation.

Our structural DTI studies focused by default on brain networks or circuits, refocusing the attention in songbird neurosciences away from the song control nuclei itself and towards the fiber network of the SCS and all sensory systems and demonstrated a massive seasonal rewiring of entire SCS, sensory and cerebellar networks. Although many molecular studies on critical and sensitive periods during early life neuroplasticity assigned a key underlying role to PV expressing GABAergic interneuron maturation and perineuronal nets (PNN) surrounding them (for reviews see e.g., Takesian and Hensch, 2013 and Reh et al., 2020), also the contribution of myelin may not be underestimated for its role in white matter or network neuroplasticity (for review see Xin and Chan, 2020). Interestingly where previous studies had not picked up substantial seasonal changes in PV/PNN in starlings (Cornez et al., 2017), our DTI data focusing on fiber connections and fiber surroundings of song control

nuclei discovered sexually differential and substantial seasonal white matter changes often pointing to a contribution of myelin (Orije et al., 2022). This could open a new direction for molecular investigations of seasonal neuroplasticity in songbirds.

ACKNOWLEDGMENTS

The research was funded by a grant from the Research Foundation—Flanders (FWO, project Nr G030213N) and Interuniversity Attraction Poles (IAP) ("PLASTOSCINE": P7/17) awarded to Annemie Van der Linden. Jasmien Orije received a PhD fellowship from the FWO (Nr 1115217N). The MRI equipment was funded by the Flemish Impulse funding for heavy scientific equipment (42/FA010100/1230) and the Hercules foundation funding (Belgium, grant agreement AUHA/012).

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

No new datasets were generated or analyzed for the current review paper.

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How to cite this article: Orije, J. E. M. J., Van der Linden, A. (2022). A brain for all seasons: An in vivo MRI perspective on songbirds. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, 337, 967–984.

<https://doi.org/10.1002/jez.2650>