
Acetylcholinesterase in central vocal control nuclei of the zebra finch (*Taeniopygia guttata*)

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The distribution of acetylcholinesterase (AChE) in the central vocal control nuclei of the zebra finch was studied using enzyme histochemistry. AChE fibres and cells are intensely labelled in the forebrain nucleus area X, strongly labelled in high vocal centre (HVC) perikarya, and moderately to lightly labelled in the somata and neuropil of vocal control nuclei robust nucleus of arcopallium (RA), medial magnocellular nucleus of the anterior nidopallium (MMAN) and lateral magnocellular nucleus of the anterior nidopallium (LMAN). The identified sites of cholinergic and/or cholinceptive neurons are similar to the cholinergic presence in vocal control regions of other songbirds such as the song sparrow, starling and another genus of the zebra finch (*Poephila guttata*), and to a certain extent in parallel vocal control regions in vocalizing birds such as the budgerigar. AChE presence in the vocal control system suggests innervation by either afferent projecting cholinergic systems and/or local circuit cholinergic neurons. Co-occurrence with choline acetyltransferase (ChAT) indicates efferent cholinergic projections. The cholinergic presence in parts of the zebra finch vocal control system, such as the area X, that is also intricately wired with parts of the basal ganglia, the descending fibre tracts and brain stem nuclei could underlie this circuitry's involvement in sensory processing and motor control of song.

[Sadananda M 2004 Acetylcholinesterase in central vocal control nuclei of the zebra finch (*Taeniopygia guttata*); *J. Biosci.* **29** 189–200]

1. Introduction

A system of discrete brain nuclei in the telencephalon, thalamus, mesencephalon and rhombencephalon control vocal behaviour in oscine songbirds such as the canary (*Serinus canaria*), zebra finch (*Poephila guttata*), song sparrow (*Melospiza melodia*) and starling (*Sturnus vulgaris*) and in vocalizing birds such as the psittacine budgerigar (*Melop-*

sittacus undulates) and the hill mynah (*Gracula religiosa*). The circuitry that subserves both vocal learning and vocal motor control demonstrates changes in morphology, synaptic connectivity and neurochemistry between seasons in seasonal birds such as the canary (Nottebohm 1981), and between male and female (sexual dimorphism) where only the male sings, or where each sex sings to varied extents (Tramontin and Brenowitz 2000). This structure-

Keywords. Acetylcholinesterase; birdbrain; histochemistry; song; vocal control system; zebra finch

Abbreviations used: A, arcopallium; AChE, acetylcholinesterase; Aid, arcopallium intermediale dorsale; Ail, arcopallium intermediale laterale; AQ, aqueductus cerebrii; Bas, nucleus basalis trigeminalis; CB, cerebellum; ChAT, choline acetyltransferase; DLM, medial nucleus of the dorsolateral thalamus; DM/ICo, dorsomedial part of the intercollicularis; E, entopallium; FLM, fasciculus longitudinalis medialis; GP, globus pallidus; his, histochemistry; HVC, high vocal centre; IP, nucleus interpeduncularis; IR, immunoreactive; LAD, dorsal arcopallial lamina; LaM (LH), mesopallial lamina (lamina hyperstriatica); LMAN, lateral magnocellular nucleus of the anterior nidopallium; LSt, lateral striatum; M, mesopallium; MSt (LPO), medial striatum (lobus parolfactorius); MMAN, medial magnocellular nucleus of the anterior nidopallium; MV, nucleus motorius nervi trigemini; N, nidopallium; N III, nervus oculomotorius; NAs, supralaminar area of the frontal nidopallium; NC, nidopallium caudale; Nif, nucleus interface of the nidopallium; OM, occipitomesencephalic tract; nXIIts, tracheosyringeal portion of the hypoglossal nucleus; PPT, pedunculopontine tegmental nucleus; PSL (LMD), pallial-subpallial lamina (lamina meduallaris dorsalis); RA, robust nucleus of arcopallium; TeO, tectum opticum; TV, nucleus tegmenti ventralis; Uva, nucleus uvaeformis; VP, ventral paleostriatum; VTA, ventral tegmental area; X, area X.

function relation combined with the changes in synaptic efficacy and neurochemical content constitutes a well-suited model to elucidate neuronal correlates of learning and memory processes. The vocal repertoire in each case needs to be acquired. Once acquired it is either retained throughout life in a stereotyped manner, or can be built upon. It may be lost in one season when not in use, and needs to be acquired anew in the next when required.

The vocal control pathway in songbirds is comprised of a well-defined network of interconnected nuclei from the ear to the brainstem, forebrain, back to the brainstem and finally to the syrinx and respiratory muscles. Of these, the song learning pathway (also called the anterior forebrain pathway, shown in red hues – figure 2C) includes the high vocal centre (HVC), area X, lateral magnocellular nucleus of the anterior nidopallium (LMAN) and the thalamic nucleus, medial nucleus of the dorsolateral thalamus (DLM); HVC projects to area X, LMAN projects to robust nucleus of the arcopallium (RA); area X projects to DLM which in turn projects to LMAN and finally to RA (figure 2C). Lesions in LMAN in juveniles which are learning to sing disrupts vocal behaviour, while those of area X during acquisition lead to lack of crystallization of song. The song production pathway (shown in blue hues – figure 2C) includes HVC, RA and brain stem nuclei; HVC projects to RA, dorsal RA projects to mesencephalic, dorsomedial part of the intercollicularis (DM/ICo) that projects (not shown in figure 2C) to nXIIts (tracheosyringeal portion of the hypoglossal nucleus), while ventral RA projects to nXIIts directly (figure 2C). Lesions that destroy any of these nuclei or pathways result in profound and irreversible deficits in song (DeVoogd 1994).

The axonal connectivity between the various vocal control nuclei in the vocalizing species thus far studied seems to be similar (Nottebohm *et al* 1982; Bottjer *et al* 1989; Johnson *et al* 1995), though vocalizing budgerigars have a different set of discrete brain nuclei that elicit vocal behaviour (Striedter 1994). While mimicking birds and songbirds belong to different orders – psittacines and passerines, and probably evolved vocalization independently, their neurochemical profile and content could be similar as the subserved behaviour is the same, indicating that despite independent evolution, the neural mechanisms that underlie vocal learning are similar. Such a functional neuroanatomy has been greatly benefited by comparative studies. While comparisons in neurochemistry among bird species demonstrating the same behaviour would be indicative of direct structure-chemistry-function relations, comparisons of chemistry-structure between birds and mammals have demonstrated corresponding regions and therefore homologies.

Various techniques have been used to find equivalents between mammalian and avian brain structures and among different avian species, the most common being screening of the distribution of acetylcholine either for receptor types

and their densities, the synthesising enzyme choline acetyltransferase (ChAT) or the degrading enzyme acetylcholinesterase (AChE). These studies not only provided detailed descriptions of the distribution of cholinergic systems and receptors but also demonstrated comparable and possibly homologous regions between tetrapod reptiles (Brauth *et al* 1985), birds (Kusunoki 1969; Medina and Reiner 1994) and mammals (Mesulam 1987), indicating that diverse vertebrate forms have similar cholinergic systems from the brainstem to higher order regions in the forebrain and back.

As in mammals parts of the avian basal ganglia demonstrate characteristic cholinergic staining. While the striatum is richly stained with perikarya and fibres, the pallidum is poorly stained (Parent and Olivier 1970). Based on cholinergic staining patterns, basal forebrain groups that innervate the lateral and medial forebrain bundles in birds have specifically been compared to the substantia innominata and nucleus basalis in mammals, while cholinergic neurons in the septal and basal forebrain in birds have been compared to the nucleus of the diagonal band that is cholinergic in mammals (Medina and Reiner 1994).

Among the bird species that have been studied so far, in singing and vocalizing birds the vocal control regions have been studied (Ryan and Arnold 1981; Zuschratter and Scheich 1990; Cookson *et al* 1996), in the pigeon other regions (Waechter 1985; Dietl *et al* 1988; Medina and Reiner 1994), and in the quail regions involved in reproduction (Ball *et al* 1990). The zebra finch (*Taeniopygia guttata castanotis* GOULD) a singing passerine has established itself as an amenable model to study the neuronal correlates of song acquisition and retention (Brainard and Doupe 2002) and sexual imprinting, which determines mate selection on sexual maturity (Immelmann *et al* 1991). Both these early learning paradigms show striking similarities (Bischof 1997).

Immediate early gene (IEG) expression is elicited in the HVC, a song control nucleus when a bird actively vocalises (Sadananda and Bischof 2002), and in adjacent areas when the bird perceives song (Bolhuis *et al* 2001). Song forms an integral part of courtship behaviour in the zebra finch where only the male sings. Two of the song control nuclei, LMAN and RA are found adjacent to regions in the medial nidopallium and caudal arcopallium respectively. These regions show high metabolic activity using 2-deoxyglucose technique (2-DG) during first courtship (Bischof and Herrmann 1986), which consolidates sexual imprinting.

While neurochemical studies on the distribution of parvalbumin, cytochrome oxidase, calbindin (Braun *et al* 1985) dopamine (Lewis *et al* 1981), tyrosine hydroxylase (Bottjer 1993), vasotocin (Voorhuis and deKloet 1992), and aromatase (Balthazart *et al* 1996) in the zebra finch (*T. guttata*) exist, an elaborate study on cholinergic sites and cholinceptive neurons in the vocal control system of this genus

does not exist. Song learning has also been studied in *Poephila guttata*, another zebra finch species. In this species work on cholinergic projections in the song control system has been done (Ryan and Arnold 1981; Zuschratter and Scheich 1990). However, as there are indications of even interspecific differences existing within the same family (MacDougall-Shackleton and Ball 1999), AChE distribution in the *T. guttata* merits a separate study. Moreover, new information about the vocal control circuitry has not been paralleled with newer information on the neurochemistry of these regions.

The present study gives an account of the cholinergic presence in the vocal control system of this songbird with special reference to the forebrain vocal nuclei, while comparing the data to other singing birds and vocalizing birds. As acquisition and retention of vocalization has established itself as a prime model for study of neural mechanisms underlying learning and memory, and cholinergic systems could underlie acquisition and motor control of song, the study aimed at a delineation of cholinergic and/or cholinceptive regions in vocal control nuclei of the zebra finch forebrain.

2. Materials and methods

Adult male zebra finches (*T. guttata*) were procured from a local dealer. The birds were kept in large cages in a normal photoperiod (14L, 10D), and food was provided *ad libitum*. All birds were fully adult, weighing 10–12 g. The birds were deeply anaesthetized and perfused transcardially with 0.9% NaCl followed by 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The brains were removed, post-fixed and cryo-protected (30% sucrose) overnight at 4°C. Coronal sections (40 µm) were cut on a freezing sliding microtome, collected in PBS, and processed for enzyme histochemistry after a Hedreen *et al* (1985) modification of the Roots-Karnovsky method with silver enhancement, and alternately using the DAB peroxidase method outlined by Vincent (1992).

Following the former method the sections were kept in sodium sulphate (5%), incubated in a mixture containing acetyl thiocholine iodide (0.34 g in 150 ml), 37.5 ml of 0.4% copper sulphate, 37.5 ml of 0.6% glycine in 75 ml of 0.2 M acetate buffer (pH 5.0). Each of these solutions was prepared separately in distilled water, and mixed in the given order just before incubation. This was followed by washes in distilled water, incubation in 1.25% sodium sulphide (pH 7.8) for 1 min, in 1% silver nitrate for 1 min, and finally in 5% sodium thiosulphate solution for 5 min with intermittent washes in distilled water.

The DAB method involved washes in 0.1 M maleic buffer (pH 6.0), incubation in a 1 : 100 dilution of a mixture that contained 6.5 ml maleic buffer, 1.0 ml of 5 mM

potassium ferricyanide, 1.0 ml of 40 mM copper sulphate, and 0.5 ml of 100 mM sodium citrate. Sections were subsequently incubated in 98 ml maleic buffer with 1 ml of the above mixture and 1 ml of 30 mg acetylthiocholine iodide dissolved in 10 ml maleic buffer. Sections were washed in Tris and incubated in DAB (5 mg in 0.5 ml) and hydrogen peroxide (1% H₂O₂) with nickel ammonium sulphate (200 mg in 19.5 ml Tris) to visualize the reaction product (Vincent 1992). All incubations and washes were carried out with the sections in continuous motion. The sections were then mounted onto cleaned, gelatine-subbed slides, dehydrated, and cover-slipped.

A zebra finch brain atlas was used to delineate areas in telencephalon, thalamus and mesencephalon (B E Nixdorf and H-J Bischof, unpublished) with cross-reference from the atlas of the canary (Stokes *et al* 1972). The new nomenclature (Reiner *et al* 2004) has been used throughout while the older terminologies (Karten and Hodos 1967; Stokes *et al* 1972) have been mentioned in brackets. Line drawings were made from the zebra finch atlas and colour photomicrographs with 400 ASA films made using an SLR camera mounted on an Olympus microscope.

The animal protocols used in this study were approved by the Institute's Animal Ethics Committee and were performed under the guidelines given by the CPCSEA, Govt. of India.

3. Results

3.1 General distribution

AChE was found to be distributed throughout the brain that is, in the telencephalon, thalamus, hypothalamus and mesencephalon. The intensity of staining varied, as can be observed in figure 1; regions of intense, dark, moderate, light, very light and no staining were apparent in the telencephalon. All the thalamic and mesencephalic nuclei stained to various degrees, as also brain stem nuclei. In the telencephalon, in some regions stained perikarya could be discerned against a light or very lightly stained background (figure 5B) as also against a dark neuropil stained background on closer observation (figure 3B).

The telencephalon showed very high accumulation of the reaction product in the striatal regions: medial striatum (MSt), lateral striatum (LSt), ventral paleostriatum (VP), while dark to moderate to light staining was observed in parts of the dorsal pallium: entopallium (E), mesopallium (M), hyperpallium (HA, HD), and in parts of the limbic system such as the amygdala, hippocampus, septum and hypothalamus. Very light staining was observed in ventral pallium: nidopallium (N) and the pallidal part – globus pallidus (GP) of the avian basal ganglia (figure 1). Large neurons stained in the medial and lateral forebrain

bundles, as well as in the basal forebrain and septal regions were observed.

The thalamus and rest of the brain stem showed moderate to light staining in various nuclei. The laminated

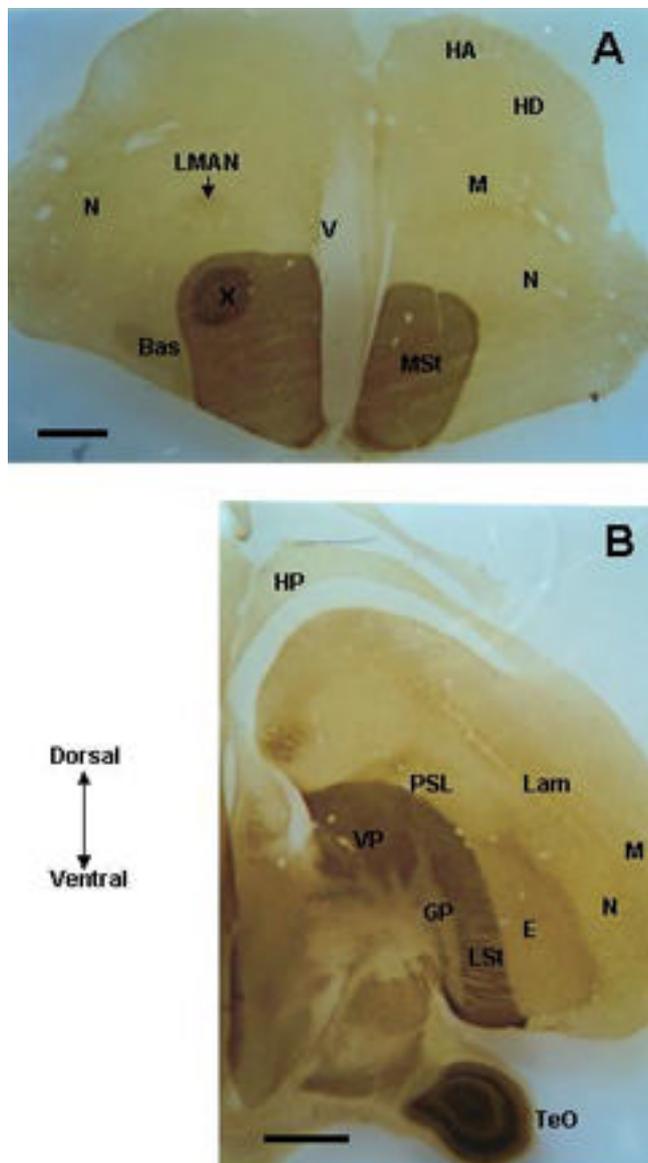


Figure 1. Low power photomicrographs showing different intensities of staining of the AChE reaction product in the brain of a male zebra finch. The two hemispheres in **A** are at different anterior co-ordinates: the one on the right provides a more frontal view than the one on the left. **B** is at around 2.8 mm from the Y point. Intense AChE staining in parts of the striatum (MSt, LSt, VP), vocal control nucleus area X (X), dark to moderate staining in the nucleus of the trigeminal system (Bas), visual entopallium (E), moderate to light staining in the dorsal pallium (HA, HD, M) and very light staining in the ventral pallium (N). No staining is observed in the globus pallidus (GP), while differential staining is observed in the optic tectum lamina (TeO). For more abbreviations see list. Bar scale = 1 mm.

mesencephalic tectum opticum (TeO) was darkly to moderately to lightly stained in the various lamina (figure 1B). The tegmental isthmus nuclei were most conspicuous with very intense staining. Deeper in the tegmentum accumulation of the reaction product occurs in various nuclei; brain stem nuclei such as the substantia nigra, ventral tegmental area (figure 6A) and the reticular formation depict moderate to high staining.

However, since the main aim of this study is to highlight staining in the main telencephalic centres that comprise the vocal acquisition and motor control circuitry, the staining pattern in these is described in detail.

3.2 Central vocal control nuclei

The vocal control nuclei of the zebra finch brain are very conspicuously labelled. Dense to moderate to light AChE staining is observed in all the forebrain nuclei of the vocal control network of the zebra finch. Five forebrain nuclei can be very easily distinguished: the LMAN, MMAN and area X in the frontal telencephalon (figure 2A) and the HVC and RA in the caudal telencephalon (figure 2B). In the frontal basal telencephalon, area X stains very darkly and can be easily distinguished from the surrounding medial striatum (MSt) that is also darkly stained (figures 1A, 3A). In area X the neuropil contains stained fibres and scattered darkly-stained perikarya (figure 3B – shown with arrows). The adjacent lateral striatal region also shows intense labelling (figure 1B).

In the frontal nidopallium*, found just dorsal to MSt and area X lie the LMAN and MMAN (figure 4A) that can easily be distinguished from the surrounding nidopallium that is lowly stained. The nidopallium is separated from the underlying MSt by the pallial-subpallial lamina (PSL) and from the overlying mesopallium by the mesopallial lamina (LaM) (figure 2A). The LMAN is composed of two sub-regions: a core and a surrounding shell that could be clearly differentiated in this study. The shell region does not contain AChE (figure 4B). Within the core two distinct regions could be made out: a centre with diffuse staining of AChE in perikarya and fibers, and a periphery of stained somata with more well-defined cell controls (figure 4B). The shell

*This new term has been adopted by the Avian Brain Nomenclature Forum to appropriately depict an area known earlier as the neostriatum (for more details see <http://avianbrain.org>). Neostriatum is a misnomer having no relation to the mammalian structure of like name; rather this region is the ventral pallium of birds (Puelles *et al* 2000; Striedter *et al* 1998) and constitutes functional units of parts of the mammalian neocortex (Shimizu and Karten 1993). This change and other proposals for change in nomenclature made to depict proper homologies to mammalian structures is published (Reiner *et al* 2004), and have been recently used in an atlas (den Boer-Visser *et al* 2004).

region does not contain AChE (figure 4B). The MMAN, which is located very close to the ventricle (figure 2A) contains perikarya that stain for AChE (figure 4A).

In the caudal telencephalon, the HVC contains AChE-labelled perikarya and the nucleus stands out against the lightly-stained caudal nidopallium (figure 5A). The HVC also contains a core and a shelf region; with AChE staining however the shelf region could not be discerned (figure 5B). The other caudal nucleus RA is lightly stained, but clearly distinguishable from the adjacent arcopallial sub-division (figure 5C), the Aid that is also moderately stained. The sub-division found more laterally (Ail) however is more intensely labelled. The dorsal arcopallial lamina (LAD) shows denser accumulation of the reaction product.

The other telencephalic nucleus, called interfacial nucleus of the nidopallium (Nif), that is involved in the vocal control system is found just ventral to HVC. It could not be distinguished from the surrounding lightly-stained nidopallium. The AChE staining in the thalamic DLM that projects to the LMAN (figure 2C), and is innervated by area X neurons (figure 2C), and in the Uva which projects to the HVC, could not be qualitatively distinguished from other thalamic nuclei, and therefore were not considered in this analysis. The same holds true for the mesencephalic DM/ICo that provides the auditory input. In this study its staining pattern is similar to the adjacent moderately stained auditory nucleus mesencephalic lateralis, pars dorsalis (MLd).

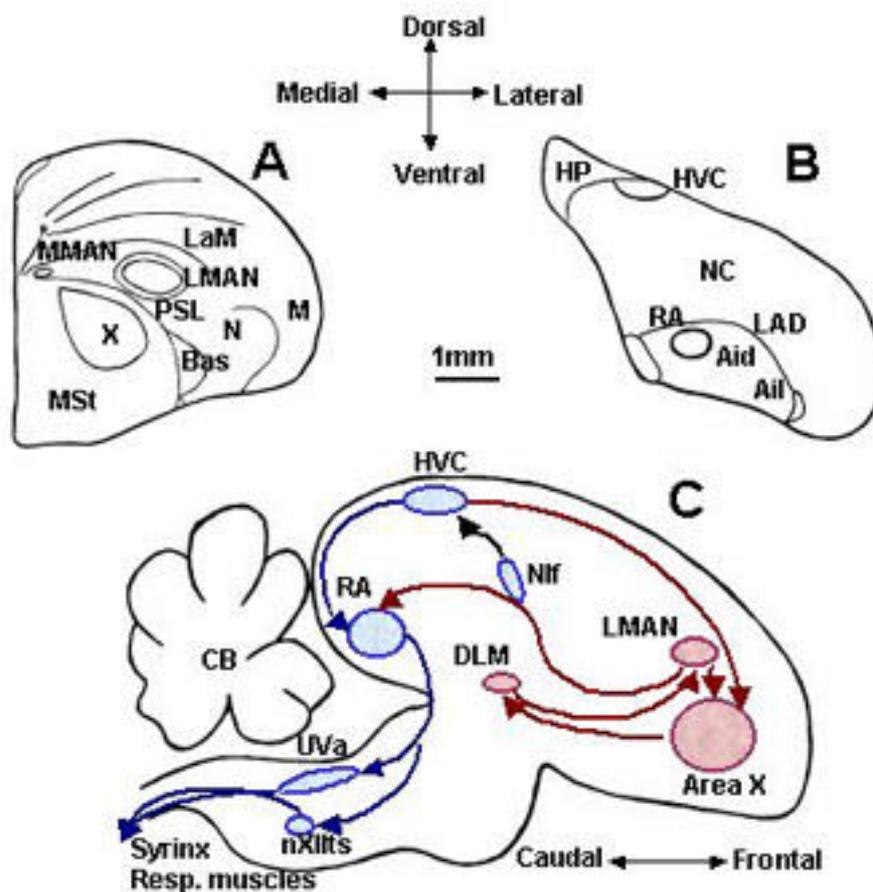


Figure 2. Transverse hemisections through the zebra finch telencephalon showing the occurrence of vocal control nuclei. (A) Area X, LMAN and MMAN in the frontal telencephalon. LMAN and MMAN are embedded in nidopallium (N) that is separated from the dorsal mesopallium (M) by the mesopallial lamina (LaM) and from the striatum by the pallial subpallial lamina (PSL). (B) HVC and RA in the caudal telencephalon. Adjacent to RA is the arcopallium, where the intermediate dorsal (Aid) and intermediate lateral (Ail) subdivisions are seen. (C) Sagittal section of the zebra finch brain showing the vocal control system. The anterior forebrain pathway is shown in red hues (nuclei and projections) while the motor control pathway is shown in blue hues (nuclei and projections). For abbreviations see list.

The caudalmost sections of the brainstem were mostly damaged during mounting and then processing. The tracheosyringeal nerve nXIIIts (figure 6B) demonstrates accumulation of the reaction product, along with various other stained nuclei.

4. Discussion

The aim of this study was to describe cholinergic and/or cholinceptive structures within the vocal control regions of the zebra finch brain. As only male zebra finches exhibit song behaviour and possess a well-developed vocal control system that subserves it, only males were used in this study. However, other studies indicate that in the absence

of conspicuously labelled male structures, the female brain does demonstrate labelling of either the surround (as in the case of MMAN and LMAN) or reduced diffuse staining (as in the case of area X, RA and HVC) in structures that are less or poorly developed, though in terms of muscarinic receptor content (of area X) females are on par with males (Bernard *et al* 1993). In Nissl-stained sections, a small RA and area X can be distinguished from

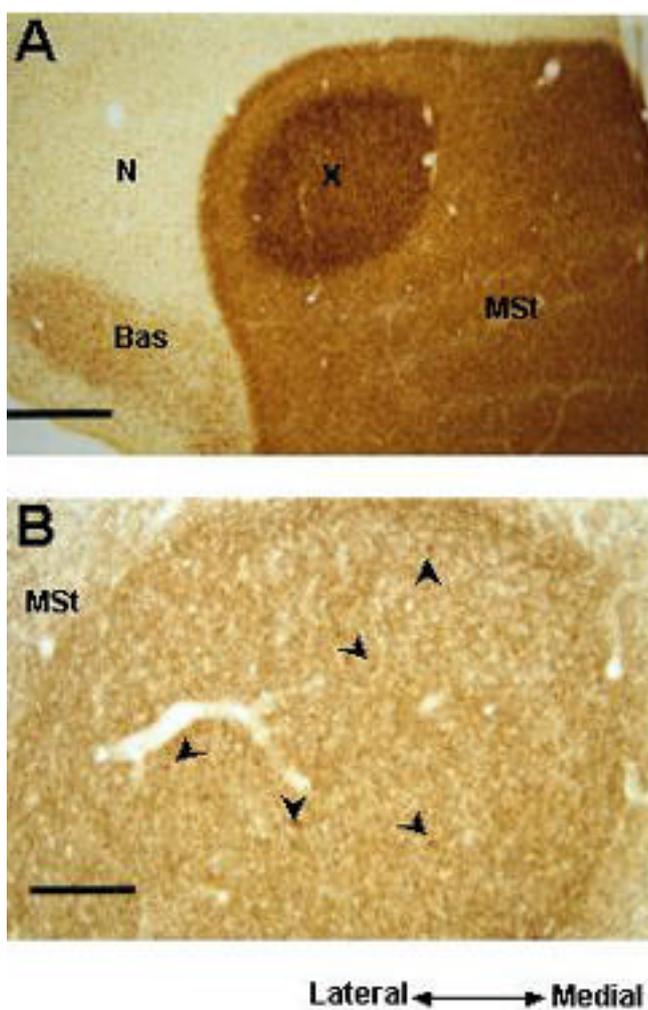


Figure 3. Photomicrographs through the area X. (A) Intensely labelled X distinct from the surrounding MSt. (B) Magnification of area X taken under a lighter filter to highlight the intrasomal reaction product in some individual neurons (marked with arrow-heads). Bar scale **A** = 400 μ m; **B** = 160 μ m. For abbreviations see list.

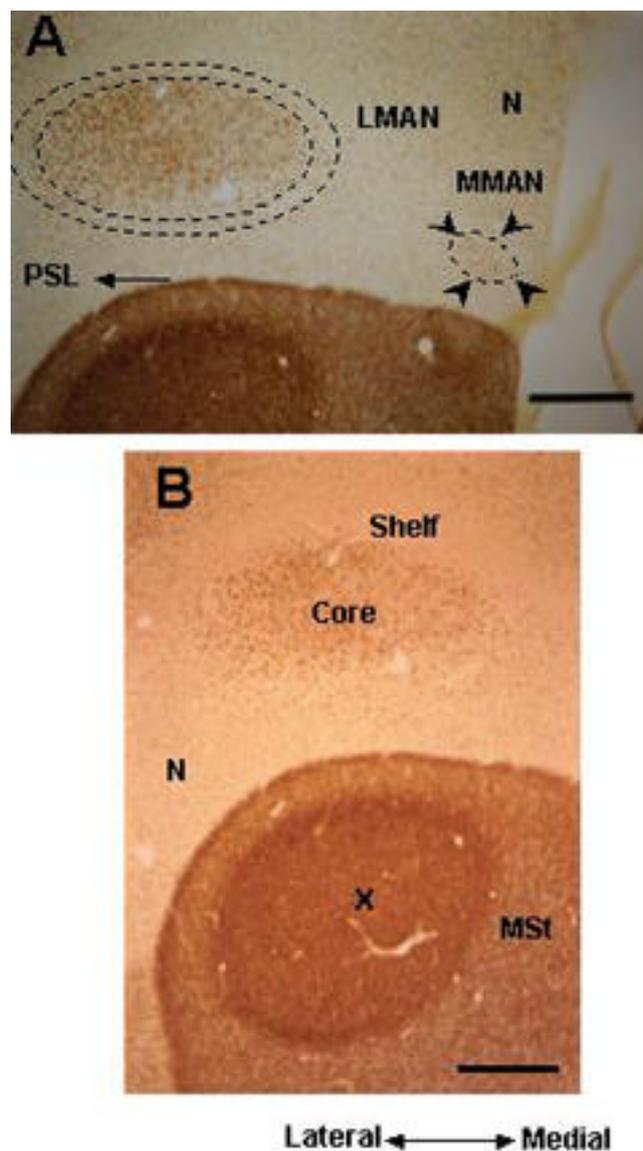


Figure 4. Photomicrographs showing labelling pattern in LMAN and MMAN: In **A** MMAN is encircled, arrows point to demarcation with distinct labelling. LMAN consists of a core and a shell. Only neurons of the core are labelled. In **B** (photographed using a different filter) the shell with no labelling can be clearly made out from the lightly stained N. In the core there appears diffuse staining in the centre with more distinctly labelled somata in the periphery. Bar scale = 400 μ m. For abbreviations see list.

the surround; while for the HVC other staining methods such as estrogen receptor staining define the boundaries of female HVC (Gahr *et al* 1993).

The presence of a dense background staining of AChE is often suggested to be an accurate enough marker for the simultaneous presence of AChE and ChAT and serves as a good indicator, although indirect of both cholinergic and cholinceptive brain regions (Ryan and Arnold 1981). But there are also indications of non-parallel distribution when simultaneous assays of ChAT and AChE have been conducted (Zuschratter and Scheich 1990; Cookson *et al* 1996) which indicate that only a co-occurrence of AChE and ChAT denote cholinergic and cholinceptive nature of neurons, while AChE staining alone denotes cholinceptive neurons. AChE presence in the perikarya and neuropil is probably suggestive of both cholinergic and cholinceptive activity.

AChE sometimes is also found to co-exist with non-cholinergic neurotransmitters, as this study also observed

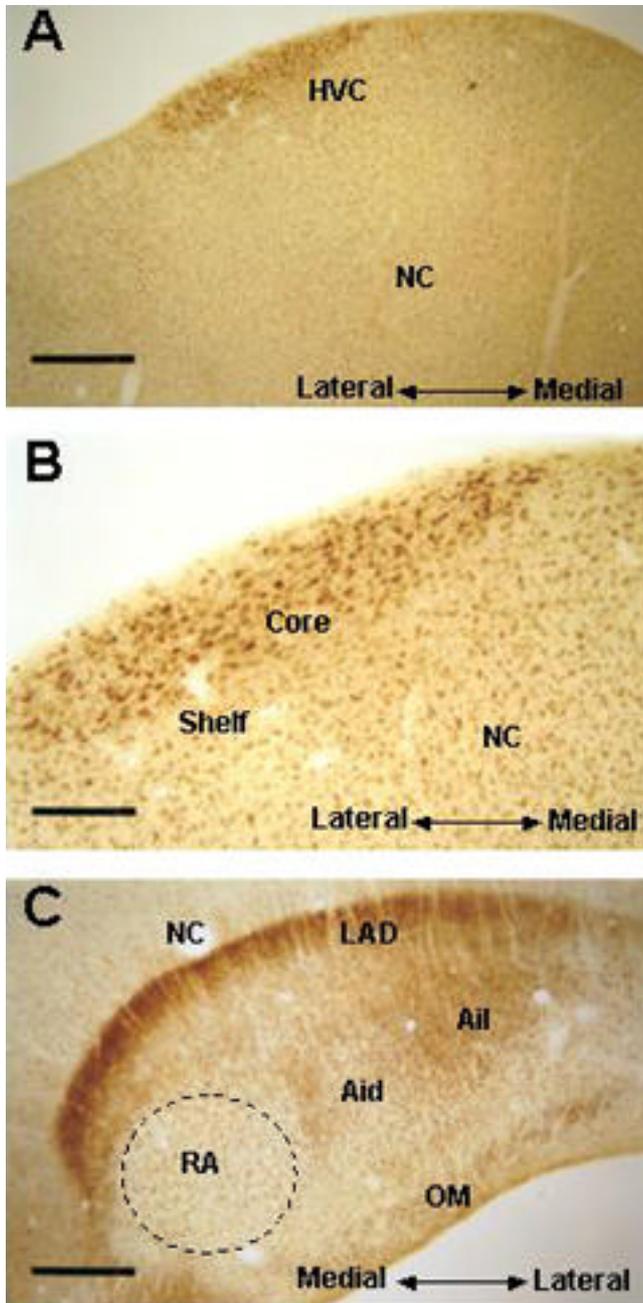


Figure 5. AChE staining in the HVC and RA of the caudal telencephalon. (A) Intensely stained perikarya in the HVC core. (B) Magnification of A. The HVC shelf cannot be distinguished from the surrounding nidopallium caudale (NC). (C) Lowly labelled RA (encircled) embedded in the arcopallium. The Aid subdivision is less intensely labelled than the Ail. Note the intense labelling in the fibre tract LAD. OM is the fibre tract for arcopallial efferents. Bar scale A, C = 400 μ m; B = 160 μ m. For abbreviations see list.

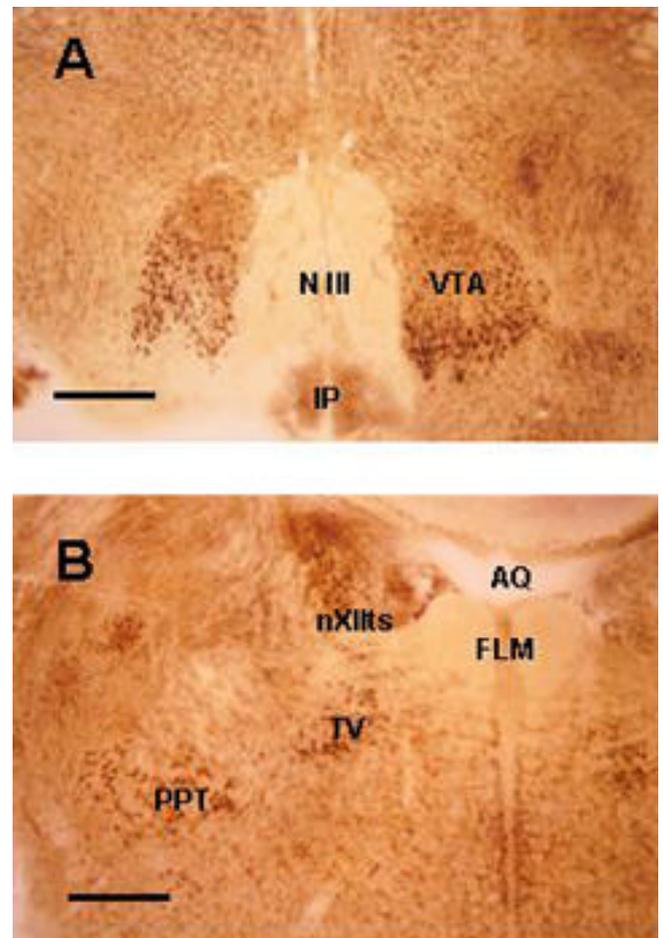


Figure 6. AChE presence in brainstem nuclei. (A) Dopaminergic ventral tegmental area (AVT) considered homologous to A10 depicting accumulation of the reaction product in ventral neurons. (B) Frontal part of nXIIIs, the caudal part of which projects to the syringeal muscles, depicting AChE activity. Bar scale = 400 μ m. For other abbreviations see list.

in monoamine brain stem regions such as the catecholaminergic substantia nigra, and the dopaminergic ventral tegmental area. Other studies have shown that AChE hydrolyses substances like P, met- and leu-enkephalin (Chubb *et al* 1980, 1982), and could degrade other neuropeptides as well. This could explain the very widespread staining observed in the thalamus, mesencephalon and other brain stem regions.

However, distribution of AChE-his, ChAT-IR, distribution of muscarinic, nicotinic and other acetylcholine receptor sites have served as good indicators of cholinergic systems in the bird brain (Sorenson and Chiapinelli 1992), and based on this many direct homologies to mammalian cholinergic regions, fibre tracts and nuclei have been drawn (Medina and Reiner 1994), making it a suitable technique in comparative analysis of functional neuroanatomy.

4.1 Vocal control regions

Song control regions stain for AChE in the perikarya, neuropil or both and are well demarcated from the surround. While the HVC, LMAN, MMAN and RA have dark to moderate to light staining in somata, area X contains a very intensely stained neuropil and some more intensely stained, scattered perikarya. This is indication of cholinergic input to the song control system. Cholinergic afferents could be from basal forebrain groups, from the septum, brain stem nuclei or one or the other vocal control nucleus. Alternately, cholinergic input could also be from local intrinsic neurons.

The existence of high to moderate staining somata and neuropil in the vocal control regions is indicative of cholinergic and/or cholinceptive transmission. The latter could be true for the HVC; it has been shown to contain AChE-stained somata but no or very sparse ChAT staining (Ryan and Arnold 1981; Zuschratter and Scheich 1990), a moderate density of muscarinic receptors (Ball *et al* 1990), and a high number of binding sites for nicotinic ligand bungaratoxin (Watson *et al* 1988). HVC of the songbird is comparable to the NLC in budgerigars where AChE-labelled somata, fibers and varicosities and ChAT-IR fibres and varicosities have been described (Cookson *et al* 1996).

The HVC is innervated by MMAN (Bottjer *et al* 1989), which expresses moderate AChE in this study. The expression of AChE in MMAN was first shown in *P. guttata* (Ryan and Arnold 1985). MMAN has no apparent counterpart in budgerigars (Striedter 1994; Cookson *et al* 1996), though two frontal forebrain vocal control nuclei are present, one in the mesopallium and another in the nidopallium, which show AChE and ChAT labelled somata, fibres and varicosities (Cookson *et al* 1996) indicating both cholinergic transmission and cholinception.

The HVC projects to area X, a part of the basal ganglia which is highly positive for AChE and contains compo-

nents of the sensori-motor basal ganglia (Farries and Perkel 2002). It contains dopaminergic receptors (Dietl and Palacios 1988; Casto and Ball 1993) and is innervated by the dopaminergic VTA (Lewis *et al* 1981) that stains for AChE in this study. When compared to the surrounding striatal LPO, area X is intensely labelled in perikarya and neuropil, which has also been shown in all other songbird and quail brain studies using AChE-his, ChAT-IR, and muscarinic receptor autoradiography (Zuschratter and Scheich 1990; Ryan and Arnold 1981; Ball *et al* 1990; Bernard *et al* 1993). However nicotinic receptor autoradiography does not distinguish area X from the surrounding LPO (Watson *et al* 1988). In the budgerigar, the magnocellular part of the medial striatum (MStm) that is located at a topographically similar position to area X in songbirds also labels more intensely for ChAT than the surrounding MSt (Cookson *et al* 1996).

The majority of cell types found in area X have been shown to resemble typical mammalian striatal cells in firing properties (Farries and Perkel 2002). Neurochemical studies showing distribution of NADPH-diaphorase, substance P, dopamine and adenosine 3'-5' monophosphate regulated phospho protein-32 (DARPP-32), parvalbumin and ChAT also demonstrate similar features (Zuschratter and Scheich 1990; Wallhäusser *et al* 1995; Reiner *et al* 2004). However, area X also contains a cell type that resembles typical mammalian pallidal cells in its projections to the thalamus, physiological (Bottjer *et al* 1989) and neurotransmitter (GABAergic – Luo and Perkel 1999) and neuropeptide LANT6 (Lys8-Asn9-NeuroTensin8-13 – Reiner *et al* 2004) features. It projects to the thalamic DLM, which in turn projects to LMAN that projects to the RA (see figure 2C) of the motor control pathway (Johnson *et al* 1995) somewhat similar to the basal ganglia pathways in mammals. The cells that stained intensely for AChE in this study would probably be comparable to the striatal interneurons called long lasting after hyperpolarization (LA) cells which stain positively for ChAT in zebra finch area X (Farries and Perkel 2002) and in mammalian striatum (Kawaguchi 1993).

LMAN that is distinctly labelled in this study demonstrates a shell that has no AChE presence. While the core is made of magnocellular neurons, the shell is made of parvicellular neurons. In this study at higher magnification, two different sets of moderately labelled somata may be observed in the core: one found in the centre showing diffuse neuropil labelling in addition to labelled somata, the other with more distinctly labelled perikarya with no neuropil labelling found in the periphery. The LMAN core has no subdivisions as such, though tracing studies demonstrate a topographic innervation of the thalamic DLM and a topographic projection onto different regions of the RA (Johnson *et al* 1995). ChAT-IR fibres have been reported to be more in the periphery than in the centre of

the nucleus (Zuschratter and Scheich 1990). LMAN could be compared topologically to NAs of budgerigars, which demonstrates very little ChAT-IR. It also demonstrates a low density of muscarinic cholinergic receptors (Ball *et al* 1990). Taken together, all the evidence along with that of this study is indicative of sparse cholinergic input to LMAN.

RA that controls the downstream motor pathway, shows a moderate to low AChE labelling in this study which supports data provided for Poephila (Ryan and Arnold 1981; Zuschratter and Scheich 1990), is very low in ChAT fibre content (Zuschratter and Scheich 1990), and low in muscarinic (Ball *et al* 1990), and nicotinic (Watson *et al* 1988) receptor content. The RA in budgerigars shows AChE stained somata, but very few ChAT labelled fibres indicating that RA does not receive a substantial cholinergic input in either songbirds or vocalizing birds.

While LMAN core projects to RA, the shell projects to the adjacent arcopallium dorsale (Ad in Johnson *et al* 1995; Bottjer *et al* 2000) that belongs to the sensori-motor and limbic system of zebra finches. The Ad demonstrates more ChAT labelling than the ventral part (Medina and Reiner 1994) and has a high content of muscarinic receptors (Wächtler and Ebinger 1989). It includes parts of the intermediate dorsal (Aid) and intermediate lateral (Ail) subdivisions that have been identified in other avian species, such as chick, duck and pigeon. In this study, the Ail stains more for AChE. Aid and Ail form part of the sensori-motor cortex in pigeons (Zeier and Karten 1981). While research on the songbird arcopallium is sparse (Korte and Bischof 1997; Sadananda and Bischof 2003), evidence from chick (Davies *et al* 1997) and duck (Dubbeldam *et al* 1997) also indicate the sensori-motor nature of the Aid and Ail.

4.2 Cholinergic transmission

If AChE expression in HVC neurons is indication of cholinergic transmission, these neurons receive projections from different sub-layers of the higher auditory centre called field L and vocal control nucleus Nif, both of which are not conspicuously labelled in this study. HVC is innervated by a moderately stained MMAN. HVC projects to a cholinergic and/or cholinergic rich area X and a moderately to lightly staining RA, which in turn projects to well stained nXIIIts. RA receives projections from LMAN and projects to a moderately stained (this study) DM/ICo (Bottjer *et al* 1989). DM/ICo however stains distinctly for AChE in somata and neuropil of Poephila (Ryan and Arnold 1981), and demonstrates heavier muscarinic density than the adjacent areas (Ball *et al* 1990) in song sparrow.

In the forebrain, the cholinergic neurons would need to be located in HVC and LMAN, while area X and RA would need to be cholinergic. However, RA is very low in

both muscarinic and nicotinic receptor content (Ball *et al* 1990; Watson *et al* 1988), while LMAN and HVC neurons seem to be sparsely cholinergic (Zuschratter and Scheich 1990; Ryan and Arnold 1981; Watson *et al* 1988; Ball *et al* 1990) and rather cholinergic. On the other hand, the RA projections downstream to DM/ICo and to nXIIIts seem to be cholinergic. Rather, cholinergic transmission in most of the vocal control nuclei therefore, while not being restricted to projecting cholinergic neurons could constitute intrinsic small local connections like those in the mammalian cortex and striatum. HVC, RA, LMAN and MMAN are found in regions considered homologous to some regions of the mammalian cortex, while area X consists of striatal components.

4.3 Vocal acquisition and motor control

How cholinergic transmission, either from local circuit neurons or some putative projection neurons is involved in vocal acquisition, retention and its motor control is not clear. However, the effects of steroids are better understood, and with steroids affecting cholinergic functions in mammals being well established, it could suggest similar mechanisms also in birds. The differentiation of vocal control nuclei post-natally and the maintenance of song behaviour in adults depends on estrogens and androgens (Gurney and Konishi 1980; deVoogd 1994). Juvenile female zebra finches when treated with estrogen develop a male-like song control system, and treatment when adult with androgens leads to singing behaviour (Pohl-Apel and Sossinka 1984).

In mammals there are indications of estrogens increasing the activity of both ChAT and AChE, and more specifically, of inducing a cholinergic-mediated sprouting of synapses in the dentate gyrus (Stone *et al* 1998). Studies in the quail indicate that androgens modulate the density of adrenergic and muscarinic receptors in the mesencephalic ICo (Ball and Balthazart 1990). Further downstream in the syrinx, castration elicits amelioration of AChE and ChAT activity (Luine *et al* 1980) and testosterone replacement influences the number of ACh receptors (Bleisch *et al* 1984).

In mammals, cholinergic projections from brain stem nuclei to higher order regions and back modulate sleep, locomotory behaviour, aggression, learning and memory and motivation. More specifically, there is evidence for cholinergic modulation via reticular activity on visual cortex plasticity (Bear and Singer 1986). Similar activity-dependent plasticity phenomena are observed in the post-natal development of vocal behaviour and its subserving structures in birds (deVoogd 1994). The ACh content in LMAN and HVC increases 2-fold from day 30 to 50 after which it declines, while the increase in RA is 7-fold after

around day 50 after which it declines (Sakaguchi and Saito 1989). These time frames correlate with profound morphological (reduction in spine densities – Wallhäusser-Franke *et al* 1995, axons of RA projecting neurons – Herrmann and Arnold 1991, and NMDA receptor sites – Aamodt *et al* 1995) in LMAN, and hodological changes in each of the nuclei (HVC begins to project to area X, and extends projecting axons to RA – Konishi and Akutagawa 1985, and DLM projects to LMAN – Bottjer 1987).

Thus, given the influence of steroids on development of the vocal control nuclei and the modulatory effects of steroids on cholinergic systems, cholinergic transmission may play a role in the acquisition of song, which involves both sensory and motor learning as has been shown in other stable-learning paradigms (Davies and Horn 1983) such as filial imprinting. An increase in receptor sites for ACh occurs following passive avoidance learning (Rose *et al* 1980). The same is observed following imprinting (Bradley and Horn 1981) with a concomitant increase in AChE expression (Haywood *et al* 1975).

5. Conclusions

The distribution of AChE expressing neurons in vocal control regions as outlined by the present study demonstrates similarities to that of *Poephila*, other songbirds, and to a certain extent to budgerigars. The budgerigar's vocal control counterparts demonstrate similarities to song control nuclei in terms of cholinergic-cholinoceptive neurochemistry despite differences in circuitry (Striedter 1994). This indicates that despite divergent evolution, there does exist homoplasy in cholinergic systems. Moreover, songbird studies such as this that demonstrate conspicuous cholinergic and/or cholinoceptive activity in vocal control regions implicate it in the plastic events at the synapse level during sensory acquisition and motor learning of song. In addition, the cholinergic presence in parts of the zebra finch vocal control system such as area X that is intricately wired with parts of the basal ganglia, descending fibre tracts and brain stem nuclei could underlie this circuitry's involvement not only in sensory processing, but also motor control and cognition.

Acknowledgements

This study was supported by a Young Scientist Project Grant from the DST-SERC, New Delhi (SR/FTP/LS-192/2000). Access to the microtome facility of the Department of Applied Botany, Mangalore University is acknowledged. I thank Dr Petra von Gemuenden, University of Augsburg, Germany for supply of some literature.

References

Aamodt S M, Koslowski M R, Nordeen E J and Nordeen K W 1992 Distribution and developmental change in [³H]MK-801

- binding within zebra finch song nuclei; *J. Neurobiol.* **23** 997–1005
- Ball G F, Nock B, Wingfield J C, McEwen B S and Balthazart J 1990 Muscarinic cholinergic receptors in the songbird and quail brain: a quantitative autoradiographic study; *J. Comp. Neurol.* **298** 431–442
- Ball G F and Balthazart J 1990 Steroid modulation of muscarinic and α_2 -adrenergic receptor density in the nucleus Intercollicularis of the japanese quail; *Eur. J. Neurosci.* **2** 828–835
- Balthazart J, Absil P, Foidart A, Houbart M, Harada N and Ball G F 1996 Distribution of aromatase-immunoreactive cells in the forebrain of zebra finches (*Taeniopygia guttata*): implications for the neural action of steroids and nuclear definition in the avian hypothalamus; *J. Neurobiol.* **31** 129–148
- Bear M F and Singer W 1986 Modulation of visual cortical plasticity by acetylcholine and noradrenaline; *Nature (London)* **320** 172–176
- Bernard D J, Casto J M and Ball G F 1993 Sexual dimorphism in the volume of song control nuclei in european starlings: assessment by a Nissl stain and autoradiography for muscarinic cholinergic receptors; *J. Comp. Neurol.* **334** 559–570
- Bischof H-J and Herrmann K 1986 Arousal enhances [¹⁴C]-2-deoxyglucose uptake in four forebrain areas of the zebra finch; *Behav. Brain Res.* **21** 215–221
- Bischof H-J 1997 Song learning, filial imprinting and sexual imprinting: three variations of a common theme?; *Biomed. Res. (Suppl. 1)* **18** 133–146
- Bleisch W V, Luine V N and Nottebohm F 1984 Modification of synapses in androgen-sensitive muscle: hormonal regulation of acetylcholine receptor number in the songbird syrinx; *J. Neurosci.* **4** 786–792
- Bolhuis J J, Hetebrij E, den Boer-Visser A M, de Groot J H and Zijlstra G O 2001 Localized immediate early gene expression related to the strength of song learning in socially reared zebra finches; *Eur. J. Neurosci.* **13** 2165–2170
- Bottjer S W 1987 Ontogenetic changes in the pattern of androgen accumulation in song-control nuclei of male zebra finches; *J. Neurobiol.* **18** 125–139
- Bottjer S W 1993 The distribution of tyrosine hydroxylase immunoreactivity in the brain of male and female zebra finches; *J. Neurobiol.* **24** 51–69
- Bottjer S W, Halsema K A, Brown S A and Miesner E A 1989 Axonal connections of a forebrain nucleus involved with vocal learning in zebra finches; *J. Comp. Neurol.* **279** 312–326
- Bottjer S W, Brady J D and Cribbs B 2000 Connections of a motor cortical region in zebra finches: relation to pathways for vocal learning; *J. Comp. Neurol.* **420** 244–260
- Bradley P and Horn G 1981 Imprinting: a study of cholinergic receptor sites in parts of the chick brain; *Exp. Brain Res.* **41** 121–123
- Brainard M S and Doupe A J 2002 What songbirds teach us about learning; *Nature (London)* **417** 341–358
- Braun K, Scheich H, Schachner M and Heizmann C W 1985 Distribution of parvalbumin, cytochrome oxidase activity and [¹⁴C]-2-deoxyglucose uptake in the brain of the zebra finch. I. Auditory and vocal motor systems; *Cell Tiss. Res.* **240** 101–115
- Brauth S E, Kitt C A, Price D L and Wainer B H 1985 Cholinergic neurons in the telencephalon of the reptile *Caiman crocodiles*; *Neurosci. Lett.* **58** 235–240
- Casto J M and Ball G F 1993 Characterization and localization of D1 dopamine receptors in the sexually dimorphic vocal control nucleus, Area X and the basal ganglia of european starlings; *J. Neurobiol.* **25** 767–780

- Chubb I W, Hodgson A J and White G H 1980 Acetylcholinesterase hydrolyses substance P; *Neurosciences* **5** 2065–2072
- Chubb I W, Ranieri E, Hodgson A J and White G H 1982 The hydrolysis of Leu- and Met-enkephalin by acetylcholinesterase; *Neurosci. Lett. Suppl.* **8** S39
- Cookson K K, Hall W S, Heaton J T and Brauth S E 1996 Distribution of choline acetyltransferase and acetylcholinesterase in vocal control nuclei of the budgerigar (*Melopsittacus undulatus*); *J. Comp. Neurol.* **369** 220–235
- Davies D C and Horn G 1983 Putative cholinergic afferents of the chick hyperstriatum ventrale: a combined acetylcholinesterase and retrograde fluorescence labelling study; *Neurosci. Lett.* **38** 103–107
- Davies D C, Csillag A, Székely A D and Kabai P 1997 Efferent connections of the domestic chick archistriatum: a phaseolus lectin anterograde tracing study; *J. Comp. Neurol.* **389** 679–693
- den Boer-Visser A M, Brittijn M L and Dubbeldam J L 2004 *A stereotaxic atlas of the brain of the collard dove, Streptopelia decaocta: using the old and new nomenclature* (Maartenslaan: Shaker)
- DeVoogd T J 1994 The neural basis for the acquisition and production of bird song; in *Causal mechanisms of behavioural development* (eds) J A Hogan and J J Bolhuis (Cambridge: University Press) pp 49–81
- Dietl M M and Palacios J M 1988 Neurotransmitter receptors in the avian brain. I Dopamine receptors; *Brain Res.* **439** 354–359
- Dietl M M, Cortes R and Palacios J M 1988 Neurotransmitter receptors in the avian brain. II Muscarinic cholinergic receptors; *Brain Res.* **439** 360–365
- Dubbeldam J L, Den Boer-Visser A M and Bout R G 1997 Organization and efferent connections of the archistriatum of the mallard, *Anas platyrhynchos* L.; an anterograde and retrograde tracing study; *J. Comp. Neurol.* **338** 632–657
- Farries M A and Perkel D J 2002 A telencephalic nucleus essential for song learning contains neurons with physiological characteristics of both striatum and globus pallidus; *J. Neurosci.* **22** 3776–3787
- Gahr M, Guettinger H-R and Kroodsmas D E 1993 Estrogen receptors in the avian brain: survey reveals general distribution and forebrain areas unique to songbirds; *J. Comp. Neurol.* **327** 112–122
- Gurney M and Konishi M 1980 Hormone induced sexual differentiation of brain and behaviour in zebra finches; *Science* **208** 1380–1383
- Haywood J, Hambley J and Rose S 1975 Effects of exposure to an imprinting stimulus on the activity of enzymes involved in acetylcholine metabolism in chick brain; *Brain Res.* **92** 219–225
- Hedreen J C, Bacon S J and Price D L 1985 A modified histochemical technique to visualize acetylcholinesterase containing axons; *J. Histochem. Cytochem.* **33** 134–140
- Herrmann K and Arnold A P 1991 Lesions of HVc block the developmental masculinizing effects of estradiol in the female zebra finch song system; *J. Neurobiol.* **22** 29–39
- Immelmann K, Pröve R, Lassek R and Bischof H-J 1991 Influence of adult courtship experience on the development of sexual preferences in zebra finch males; *Anim. Behav.* **42** 83–89
- Johnson F, Salan M M and Bottjer S W 1995 Topographic organization of a forebrain pathway involved with vocal learning in zebra finches; *J. Comp. Neurol.* **358** 260–278
- Karten H J and Hodos W 1967 *A stereotaxic atlas of the brain of the pigeon (Columba livia)* (Baltimore, Maryland: John Hopkins)
- Kawaguchi Y 1993 Physiological, morphological and histochemical characterisation of three classes of interneurons in rat neostriatum; *J. Neurosci.* **13** 4908–4923
- Konishi M and Akutagawa E 1985 Neuronal growth, atrophy and death in a sexually dimorphic song nucleus in the zebra finch brain; *Nature (London)* **315** 145–147
- Korte S and Bischof H-J 1997 Afferent connections of an arousal-area in the forebrain of the zebra finch (*Taeniopygia guttata castanotis* GOULD): the archi-neostriatum caudale (ANC); in *Brain and evolution: Proceedings of the 24th Göttingen neurobiology conference* (eds) N Elsner and H U Schnitzler (Stuttgart: Thieme) vol. 2, p 657
- Kusunoki T 1969 The chemoarchitectonics of the avian brain; *J. Hirnforsch.* **11** 477–497
- Lewis J W, Ryan S M, Arnold A P and Butcher L L 1981 Evidence for a catecholaminergic projection to area X in the zebra finch; *J. Comp. Neurol.* **196** 347–354
- Luine V N, Nottebohm F, Harding C and McEwen B S 1980 Androgen affects cholinergic enzymes in syringeal motor neurons and muscle; *Brain Res.* **192** 89–107
- Luo M and Perkel D J 1999 Long-range GABAergic projections in a circuit essential for vocal learning; *J. Comp. Neurol.* **403** 68–84
- MacDougall-Shackleton S A and Ball G F 1999 Comparative studies of sex differences in the song-control system of songbirds; *TINS* **22** 432–436
- Medina L and Reiner A 1994 Distribution of choline acetyltransferase immunoreactivity in the pigeon brain; *J. Comp. Neurol.* **342** 497–537
- Mesulam M-M 1987 Cholinergic neurons, pathways, diseases; in *Encyclopedia of neuroscience* (ed.) G Adelman (Boston: Birkhaeuser) vol. 1, pp 233–235
- Nottebohm F 1981 A brain for all seasons: cyclical anatomical changes in song control nuclei of the canary brain; *Science* **214** 1368–1370
- Nottebohm F, Kelley D B and Paton J A 1982 Connections of vocal control nuclei in the canary telencephalon; *J. Comp. Neurol.* **207** 344–357
- Parent O and Olivier A 1970 Comparative histochemical study of the corpus striatum; *J. Hirnforsch.* **12** 73–81
- Pohl-Apel G and Sossinka R 1984 Hormonal determination of song capacity in females of the zebra finch: critical phase of treatment; *Z. Tierpsychol.* **64** 330–336
- Puelles L, Kuwana E, Puelles E, Bulfone A, Shimamura K, Keleher J, Smiga S and Rubenstein J E R 2000 Pallial and subpallial derivatives in the embryonic chick and mouse telencephalon, traced by the expression of the genes *Dlx-2*, *Emx-1*, *Nkx-2-1*, *Pax-6* and *Tbr-1*; *J. Comp. Neurol.* **424** 409–438
- Reiner A, Laverghetta A V, Meade C A, Cuthbertson S L and Bottjer S W 2004 An immunohistochemical and pathway tracing study of the striatopallidal organisation of area X in the male zebra finch; *J. Comp. Neurol.* **429** 239–261
- Reiner A, Perkel D J, Bruce L, Butler A, Csillag A, Kuenzel M, Medina L, Paxinos G, Shimizu T, Striedter G F, Wild M, Ball G F, Durand S, Güntürkün O, Lee D W, Mello C V, Powers A, White S A, Hough G, Kubikova L, Smulders T V, Wada K, Dugas-Ford J, Husband S, Yamamoto K, Yu J, Siang C and Jarvis E D 2004 Revised nomenclature for avian telencephalic and some related brainstem nuclei; *J. Comp. Neurol.* **473** 377–414
- Rose S P R, Gibbs M E and Hambley J 1980 Transient increase in forebrain muscarinic acetylcholine receptors following passive avoidance learning; *Neurosciences* **5** 169–172
- Ryan S M and Arnold A P 1981 Evidence for cholinergic participation in the control of bird song: acetylcholinesterase distribution and muscarinic receptor autoradiography in the zebra finch brain; *J. Comp. Neurol.* **202** 211–219

- Sadananda M and Bischof H-J 2002 Enhanced fos expression in the zebra finch (*Taeniopygia guttata*) brain following first courtship; *J. Comp. Neurol.* **448** 150–164
- Sadananda M and Bischof H-J 2003 Archistriatal afferents to the lateral neostriatum: the amygdalar influence on mate choice in birds; in *Networks and behaviour: proceedings of the NCBS Neurobiology Symposium*, 4–6 January 2003, Bangalore, p. **b17**
- Sakaguchi H and Saito N 1989 The acetylcholine and catecholamine contents in song control nuclei of zebra finch during song ontogeny; *Dev. Brain Res.* **47** 313–317
- Shimizu T and Karten H J 1993 The avian visual system and the evolution of the neocortex; in *Vision, brain and behaviour in birds* (eds) H P Zeigler, H-J Bischof (Cambridge: MIT) pp 103–114
- Sorenson E M and Chiappinelli V A 1992 Localisation of ³H-Nicotine, ¹²⁵I-kappa-bungaratoxin and ¹²⁶I-alpha bungaratoxin binding to nicotinic sites in the chicken forebrain and mid-brain; *J. Comp. Neurol.* **323** 1–12
- Stokes T M, Leonard C M and Nottebohm F 1972 The telencephalon, diencephalon and mesencephalon of the canary, *Serinus canaria*, in stereotaxic coordinates; *J. Comp. Neurol.* **136** 337–374
- Stone D J, Rozovsky I, Morgan T E, Anderson C P and Finch C E 1998 Increased synaptic sprouting in response to estrogen via an apolipoprotein E-dependent mechanism: implications for Alzheimer's disease; *J. Neurosci.* **18** 3180–3185
- Striedter G F 1994 The vocal control pathways in budgerigars differ from those in songbirds; *J. Comp. Neurol.* **343** 35–56
- Striedter G F, Marchant T A and Beydler S 1998 The “neostriatum” develops as part of the lateral pallium in birds; *J. Neurosci.* **18** 5839–5849
- Tramontin A D and Brenowitz E A 2000 Seasonal plasticity in the adult brain; *TINS* **23** 251–258
- Vincent S R 1992 Histochemistry of endogenous enzymes; in *Experimental neuroanatomy: a practical approach* (ed.) J P Bolam (Oxford: IRL Press) pp 153–171
- Voorhuis T A M and deKloet E R 1992 Immunoreactive vasotocin in the zebra finch brain (*Taeniopygia guttata*); *Dev. Brain Res.* **69** 1–10
- Wächtler K 1985 Regional distribution of muscarinic acetylcholine receptors in the telencephalon of the pigeon (*Columba livia f. domestica*); *J. Hirnforsch.* **26** 85–90
- Wächtler K and Ebinger P 1989 The pattern of muscarinic acetylcholine receptor binding in the avian brain; *J. Hirnforsch.* **30** 409–414
- Wallhäuser-Franke E, Collins C E and DeVoogd T J 1995 Developmental changes in the distribution of NADPH-diaphorase-containing neurons in telencephalic nuclei of the zebra finch song system; *J. Comp. Neurol.* **356** 345–354
- Watson J T, Adkins-Regan E, Whiting P, Lindstrom J M and Podleski T R 1988 Autoradiographic localization of nicotinic acetylcholine receptors in the brain of the zebra finch (*Poephila guttata*); *J. Comp. Neurol.* **274** 255–264
- Zeier H and Karten H J 1971 The archistriatum of the pigeon: organisation of afferent and efferent connections; *Brain Res.* **31** 313–326
- Zuschratter W and Scheich H 1990 Distribution of choline acetyltransferase and acetylcholinesterase in the vocal motor system of zebra finches; *Brain Res.* **513** 193–201

MS received 28 October 2003; accepted 4 May 2004

Corresponding editor: NEERAJ JAIN