GENE REGULATION BY SONG IN THE AUDITORY TELENCEPHALON OF SONGBIRDS

Claudio V. Mello

Neurological Sciences Institute, Oregon Health and Science University, 505 NW 185th Avenue, Beaverton, OR 97006

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1. ABSTRACT

Inducible gene expression analysis has been successfully used to identify and characterize areas involved in the auditory processing of song in songbirds. When songbirds hear song, *zenk*, a gene encoding a transcriptional regulator linked to synaptic plasticity, is rapidly and transiently induced in several discrete areas of the telencephalon. This phenomenon is most marked in the caudomedial neostriatum (NCM), a major auditory processing area. *zenk* induction by song has proven very useful to investigate the functional organization of NCM as well as to characterize song-responding neurons in this brain area. Combined with data from anatomical and electrophysiological studies, the results of gene expression analysis suggest that NCM is involved in the auditory processing of complex sounds such as song and possibly in the formation and/or storage of song auditory memories. Thus, NCM appears to play a prominent role in perceptual aspects of vocal communication, and potentially in the process of vocal learning in songbirds and other vocal learning avian orders, i.e. parrots and hummingbirds.

2. INTRODUCTION

The analysis of expression of activity-dependent genes yields global maps of brain activation at single-cell resolution in awake unrestrained animals (1, 2, for recent reviews see 3) This approach is therefore useful to reveal brain activation patterns associated with specific stimuli or behavioral contexts. In songbirds and other avian groups, analysis of the immediate early gene *zenk* has been used to generate high-resolution maps of the gene expression response associated with perceptual and motor aspects of vocal communication (4-6). Such studies have generated novel insights into the organization of brain pathways for vocal communication in birds. Some highlights include: 1) The identification of the caudomedial neostriatum (NCM) as an area involved in song auditory processing and possibly in song-related auditory memories that could affect individual recognition, mate selection and vocal learning (7, 8); 2) The discovery of a possible basis for a syllabic auditory representation in NCM (5); 3) The finding that the anterior forebrain pathway within the brain system that controls song is actively engaged in song production during adulthood (9); 4) The finding that the context of singing affects brain activation patterns associated with singing behavior (10); 5) The identification of auditory and vocal control brain areas in parrots and hummingbirds (6), the only known vocal learning birds besides songbirds. This latter comparative approach has revealed striking similarities in auditory and vocal control pathways in all vocal learning avian groups, indicating that these pathways evolved under strong constraints. The present discussion will focus on the analysis of *zenk* expression in response to song auditory stimulation, with emphasis on NCM studies. The currently available literature will be discussed and some important research directions will be indicated.

3. THE *zenk* GENE

zenk is the songbird homologue of *zif-268*, *egr-1*, *ngfi-a* and *krox-24* (11-14) (we use here *zenk* for the gene and ZENK for the protein). It is an immediate early gene whose expression in neuronal cells is generally highly sensitive to membrane depolarization. *zenk* expression levels are typically very low or absent in the absence of activation, but increase rapidly in response to neuronal activation. In contrast, *zenk* expression appears not to occur in glial cells (15, 16). The low expression levels of *zenk* during rest and its high responsiveness to membrane depolarization render *zenk* a sensitive indicator of the previous activation of neuronal cells (1, 7, 17). Importantly, some neuronal populations are known to undergo activation without a concomitant *zenk* expression response. Therefore, a lack of *zenk* expression is not always indicative of a lack of neuronal cell activation. This dissociation between neuronal cell activation and *zenk* expression has been documented for only few brain areas (4, 18, 19) and likely reflects a downregulation of signal transduction components (such as specific membrane receptors, calcium-binding proteins or kinases) that couple membrane depolarization to the nuclear gene expression response (20).

zenk is also a likely candidate gene associated with long-lasting neuronal modification, as its expression has been implicated in neuronal plasticity in different systems. For instance, *zenk* is induced in association with cortical morphological changes in rodents after exposure to an enriched environment (21). *zenk* induction also occurs in association with induction of hippocampal long-term potentiation (LTP) (22-25), a well-studied model of synaptic plasticity in mammals (26). Among several IEGs, the induction of *zenk* correlates best with LTP induction (23-25, 27). In addition, recent evidence from *zenk* knockout mouse lineages shows that *zenk* is actually required for the long-term maintenance of LTP and of some forms of hippocampal-dependent memories (28).

zenk encodes a zinc-finger DNA-binding protein that can potentially regulate the expression of target genes containing a specific ZENK-binding site in their promoters (29-31). Thus *zenk* induction potentially represents an early regulatory event in a genomic cascade triggered by neuronal activation and leading to long-lasting changes in neuronal gene expression (2, 20, 32, 33). Even though a large number (a few hundred) of potential ZENK targets can be identified in GenBank, direct evidence for a transcription regulatory action of ZENK is available for only very few targets. Some of the identified ZENK targets are involved in neuronal function, such as synapsins I and II (34, 35), but it is generally unknown whether these or other potential ZENK targets are regulated in the brain by physiological stimuli.

The avian *zenk* homologues share about 70% overall identity at the aminoacid level to the mammalian counterparts (36) (Mello CV, unpublished data). Importantly, the zinc-finger DNA-binding domain is 100% conserved between birds and mammals (29) (Mello, unpublished data). This indicates that the same DNA motif is recognized by the ZENK protein and that the same or similar genes are likely to be its targets. *zenk* expression in avian tissues can be assessed by *in situ* hybridization or immunocytochemistry (ICC), the latter with a commercially available antiserum against the ZENK protein (37).

4. *zenk* REGULATION IN THE BRAIN

zenk expression levels in most avian brain areas is very low or absent in the absence of specific stimulation. This is most clearly seen by *in situ* hybridization analysis of birds killed after overnight individual isolation in soundproof chambers (4, 18). A similar pattern is seen with ICC (37), although basal ZENK expressing cells tend to be more prevalent with ICC than with *in situs*, especially in lateral portions of the telencephalon presumably involved in visual processing. In contrast, basal *zenk* expression is particularly low in auditory processing areas as shown both by *in situ* and ICC.

Most brain regions contain large numbers of neurons that express *zenk* in response to depolarization. This is clearly shown by administration of the GABAergic antagonist metrazole, which leads to widespread depolarization and associated brain *zenk* expression (18, 38). This finding accords with the notion that *zenk* expression in avian brain is highly sensitive to neuronal activation, as also occurs in mammals (1). Interestingly, the areas that clearly lack *zenk* induction after metrazole share the fact that they are primary thalamo-recipient zones for sensory stimuli (auditory, visual and somatosensory), suggesting that *zenk* expression and its regulatory function are dissociated from neuronal activation in these areas. Considering the link between *zenk* and neuronal plasticity, as discussed in the preceding section, such a dissociation could represent a means to maintain brain representations stable at this level of the ascending sensory pathways.

Most importantly, several brain areas respond to song stimulation with a rapid, robust and transient increase in *zenk* expression (Figure 1) (4, 7, 37). Such an effect is observed both in males exposed to the sound of song but without exhibiting a vocal response ("hearing only" birds), and in females, who do not sing in many species, and it can be abolished by deafening through cochlear lesion (9). One can therefore conclude that the *zenk* induction response to song is related to the act of hearing song, although it is potentially modulated by attentional and/or motivation factors associated with hearing. Most *zenk*-labeled cells in song-stimulated birds have clear neuronal morphology (7, 18), whereas cells with clear glial characteristics are not labeled. Thus, the *zenk* expression response to birdsong occurs primarily in neurons, conforming to observations in other systems (17). A small percentage of *zenk*-labeled cells are small and their identity can be very difficult to determine based on Nissl staining alone. These cells possibly represent small-sized neurons (for example, as shown by double-labeling with a neuronal tract-tracer, as in (10)).

zenk levels at peak expression are proportional to the amount of song the bird heard during the exposure period before sacrifice (4, 39). As expected, the ZENK protein response is protracted relative to the mRNA increase, peaking between 1 and 2 hr and decreasing to unstimulated levels only by 6 hr after stimulus onset (37). Although the ICC method provides high spatial (cellular) resolution for ZENK expression mapping, ZENK protein levels at a given time reflect brain activation during a much longer preceding period compared with *zenk* mRNA levels. These factors need to be taken into consideration when choosing a method for *zenk* expression analysis.

5. THE CAUDOMEDIAL NEOSTRIATUM

The area that shows the most robust *zenk* induction response to song is the caudomedial neostriatum (NCM; Figure 1). NCM is also the brain area where *zenk* expression has been characterized in most detail, particularly in zebra finches, canaries and starlings. The finding that NCM has a strong response to song was originally somewhat of a surprise, since this area had not been previously directly implicated in the songbird brain's response to song. NCM is located adjacent to field L, the primary auditory area in the avian telencephalon (4). It is not a discrete nucleus, but rather a large expanse of the
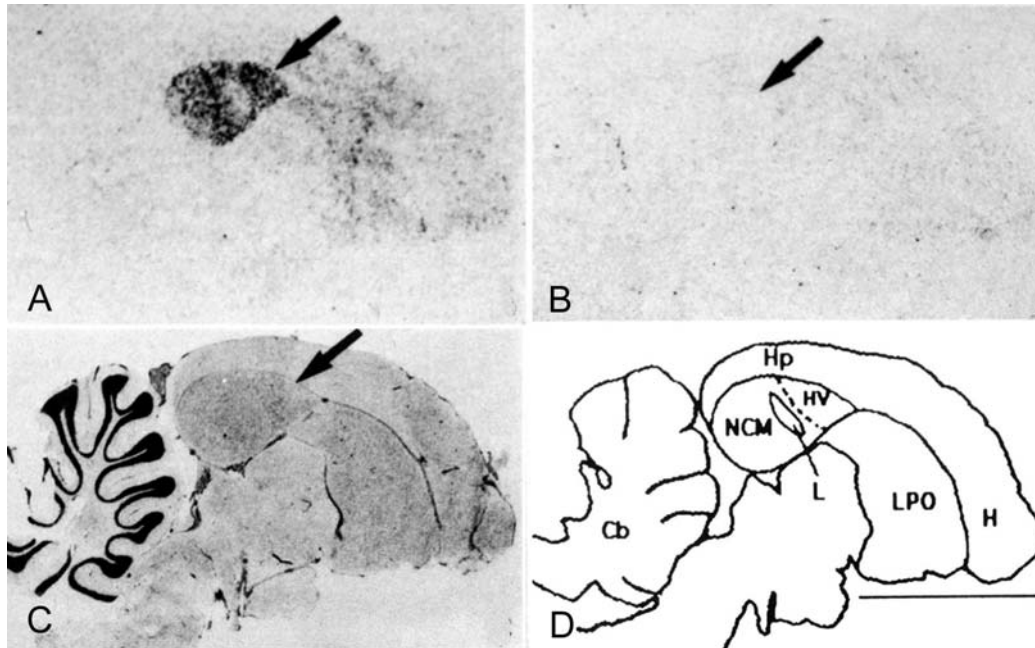
Figure 1. Exposure to song induces *zenk* gene expression in the brain of songbirds (from (7)). Shown are *in situ* hybridization (X-ray film) autoradiograms of medial parasagittal sections hybridized with a radioactively-labeled *zenk* riboprobe. (a) Adult male zebra finch exposed for 45 min. to recorded conspecific song. (b) Unstimulated control. (c) Cresyl violet stained section whose autoradiogram is shown in a. (d) Camera lucida drawing of the section shown in c. The arrows point to the caudomedial telencephalon where areas of high *zenk* expression are located, including the caudomedial neostriatum (NCM) and the caudomedial hyperstriatum ventrale (HV). Bar size, 4mm. Abbreviations: Cb, cerebellum; L, field L, H, hyperstriatum; Hp, hippocampus; LPO, lobus parolfactorius.

caudomedial telencephalon that likely contains subdomains. NCM receives inputs from field L and projects mainly to field L targets (40) (Mello, unpublished data). Thus, NCM is an integral component of central auditory pathways.

Consistent with the anatomical data, electrophysiological responses to song and other auditory stimuli are prevalent throughout NCM (8, 41-44). These responses in NCM are less brisk and have longer latencies than those in auditory field L, and seem to lack the degree of selectivity for specific song elements seen in song control nuclei (for example, see (45)). These observations indicate that NCM occupies an intermediate position between the primary auditory telencephalic area and higher-order auditory processing brain areas. They are also consistent with the conclusions from early studies performed in starlings, where auditory responses were seen over a large portion of the caudal telencephalon, many units showing selectivity for complex stimuli (46).

Also consistent with a role of NCM in song auditory processing is the finding that *zenk* induction in NCM of zebra finches and canaries is highest for same species' (conspecific) song, lower for other species' (heterospecific) song, and even lower or absent for synthetic tones (7). In addition, the *zenk* induction response in NCM, both at the mRNA and protein level, decreases upon repeated presentations of the same song (19), an effect commonly referred to as "habituation", to borrow a

term from the behavioral literature. This habituation is song-specific, as presentation of a novel song re-induces *zenk* expression in habituated birds (19), which provides further evidence that NCM neurons or circuits apparently participate in the discrimination of different conspecific songs. As a large percentage of neurons in NCM show a *zenk* response to any given conspecific song, it is likely that individual NCM neurons show independent *zenk* responses to multiple songs (19). This is consistent with the notion of ensemble coding for song representation, according to which the auditory representation of different songs or song elements involves the recruitment of distinct but partially overlapping groups of neurons from a given neuronal population (5).

An important question is whether any sex differences exist in the anatomical or functional organization of NCM. Presently no direct evidence is available for such differences in the *zenk* response to song in NCM (47) (Mello, unpublished data). In starlings, where song-based female preferences have been studied in detail (48), the *zenk* response in NCM of females is highest for their preferred conspecific stimuli, i.e. long songs (49). This effect is observed even when taking into account total stimulus duration (for further discussion, see (50)). Overall, the available data on *zenk* expression (as well as electrophysiological studies; see below) in NCM indicate that NCM is tuned to auditory stimuli that are of high behavioral relevance to the recipient bird.
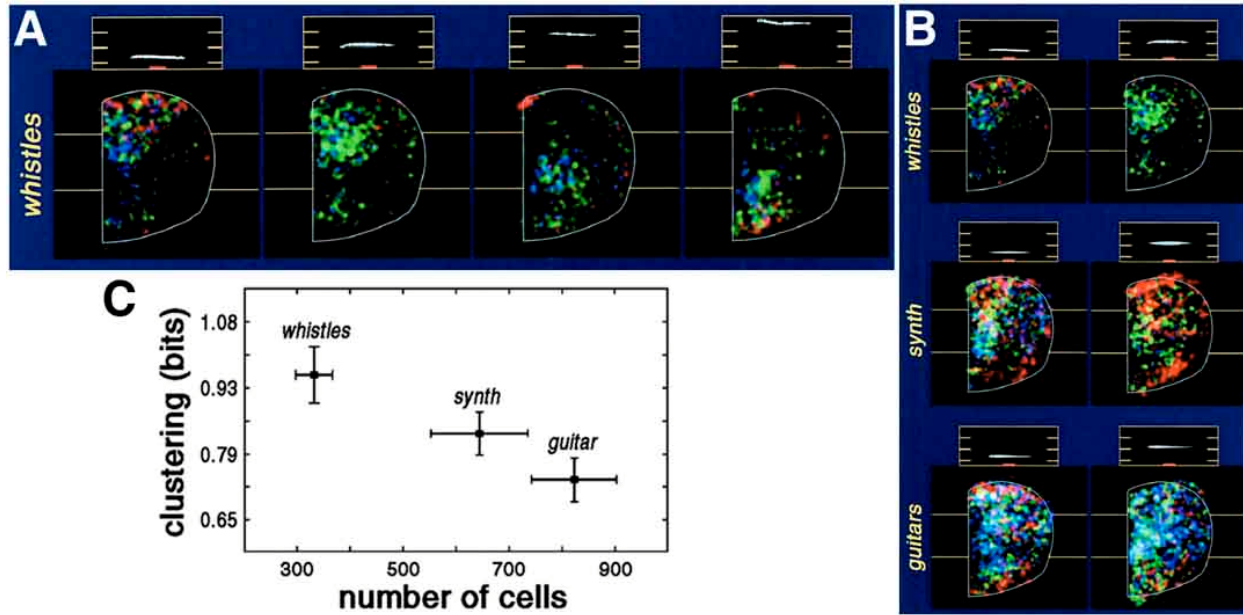
Figure 2. ZENK expression analysis of whistle representation in NCM (modified from (5)). A- ZENK expression maps in NCM of adult female canaries resulting from the presentation of natural canary song whistles. The respective frequencies (left to right) are 1.4, 2.2, 2.8 and 3.5 kHz, as shown in the sonograms (top). B- The maps for natural whistles and artificial stimuli (synthetic tones and digitized guitar notes) of same frequencies differ markedly. C- Patterns elicited by natural whistles and artificial stimuli can be clearly separated by quantifying total cell number and spatial clustering (as detailed in 5, and 52).

Analysis of song-induced *zenk* expression has also been very useful to reveal the internal functional organization of NCM. In this respect, the characteristic structure of canary (Waterslager strain) song proved to be particularly helpful. Canary song is formed of phrases that consist in the repetition of single syllables (5, 51, 52). These syllables are simpler in their acoustic properties than complete songs (for instance, whistles are pure tone-like vocalizations), therefore representing more discrete acoustic stimuli than song. This observation provided the starting point to correlate variations in *zenk* expression patterns in canary NCM with the acoustic properties of the song syllabic stimuli presented to the birds.

The *zenk* expression pattern seen in NCM in response to a whole canary song is complex and difficult to interpret in terms of acoustic analysis. In contrast, presentation of song syllables results in patterns that are topographically organized (5), as can be visualized in maps of ZENK protein expression using an automated mapping system (37, 53). For instance, whistles result in clusters of ZENK-positive cells organized in a frequency-dependent manner within rostral NCM. This organization is revealed with natural whistles but not with synthetic stimuli of equivalent frequencies (Figure 2). It is unclear how NCM can distinguish natural syllables from synthetic ones, but a testable hypothesis is that this discrimination depends on specific acoustic features that are present in natural whistles but absent in synthetic stimuli. Whistle combinations result in patterns that are distinct from the sum of the patterns resulting from the individual component whistles, and syllables with fast frequency modulations result in distributed patterns with little

activation of rostral NCM. Importantly, the patterns resulting from various classes of canary song syllables can be distinguished by mapping the position and labeling intensity of ZENK-positive cells. Analysis of the resulting ZENK expression maps by principal component analysis has revealed that the information present in these maps is sufficient to differentiate the response to various syllabic stimuli. These findings provide evidence for a syllabic auditory representation in canary NCM that is topographically organized, based on stimulus frequency, and tuned to cues present in natural song (5).

Interestingly, a close analysis of ZENK expression patterns suggests that auditory representations in NCM are affected by auditory experience. For example, the range of whistle frequencies represented in rostral NCM (5) appears to correspond to the range of frequencies that is present in canary song (51, 52, 54) and that juveniles usually hear in a canary aviary. In addition, ZENK expression habituates with repeated syllable presentations (5). In zebra finches, *zenk* gene induction by song has been shown to be developmentally modulated (55), emerging in conjunction with the song learning period. Furthermore, ZENK expression levels in the NCM of adults in response to a given song stimulus correlate with the degree to which the birds copy that particular song during the song learning period (56). Nevertheless, a direct and conclusive demonstration that auditory experience modulates auditory representations in NCM is still lacking. Further studies on how auditory experience affects the auditory representation of syllables in canaries may prove to be very informative in this regard.
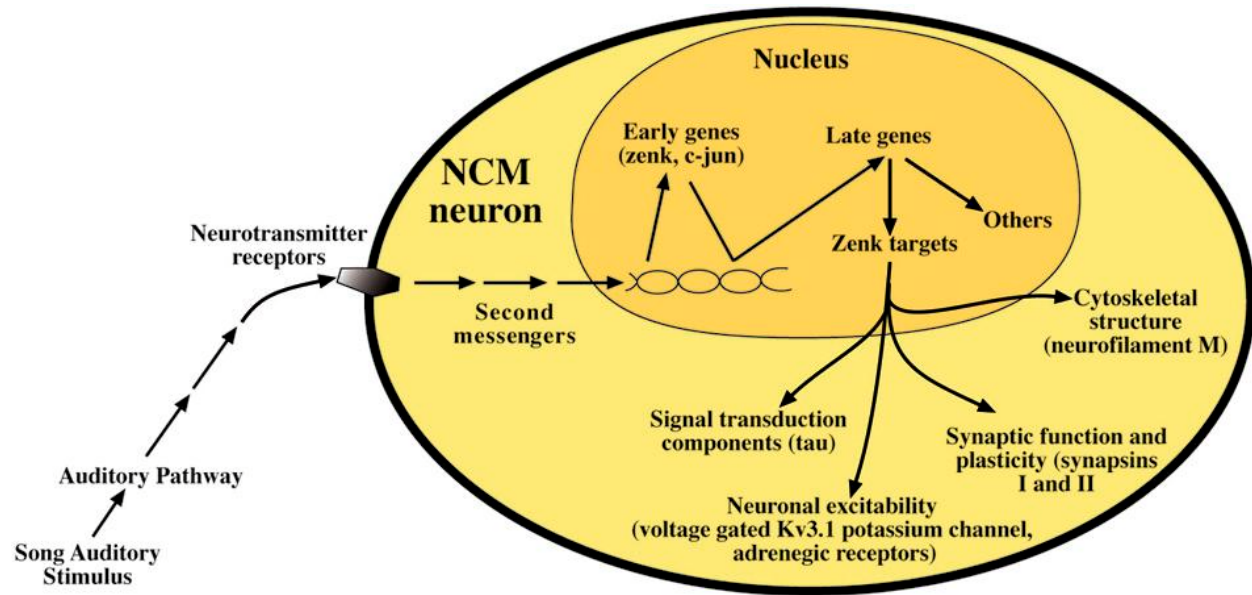
Figure 3. Schematic representation of the gene induction response to song in a given NCM neuron. Indicated is the flow of activity triggered by song exposure and leading to the activation of NCM neurons, followed by the induction of early activity-dependent genes and subsequently of their transcriptional targets. *zenk* and its candidate targets (based on the identification of genes with a ZENK-binding motif in their promoter region through GenBank searches) are depicted as a representative example.

The studies on syllabic representation in canaries (5), as well as studies in starlings (49), indicate the existence of functional NCM subdomains that respond distinctly to different acoustic features of song. It is unclear, however, what mechanisms underlie this differential response. One intriguing hypothesis is that the functional organization of NCM is determined largely by the connectivity patterns of NCM subdomains. This hypothesis is based on limited available neuroanatomical data suggesting that different portions of NCM have different connectivities (40, 57). For example, rostral but not caudal NCM receives robust inputs from subfields L1 and L3 (40). Testing further this hypothesis will require the use of small injections of anterograde and retrograde neuronal tract-tracers to investigate the connectivity of NCM sub-domains that respond to distinct acoustic features of song.

zenk expression in NCM and other auditory areas is higher in juveniles than in adults, as has also been generally observed in the mammalian cortex (58, 59). Song-induced *zenk* expression is not observed at 20 days of age (59), even though auditory responses in NCM are present at that age (55). Basal *zenk* expression later declines, and *zenk* induction by song presentation is detectable in NCM by day 30 (59), within the initial period for song learning, increasing further thereafter. *zenk* expression in the NCM of adult males has also been tested in response to the songs heard during the song learning period (56). *zenk* levels were found to correlate with the number of song elements the birds copied from their tutors' songs. Thus, there occur significant changes both in the organization of the auditory forebrain and in song-induced *zenk* expression patterns in NCM in parallel with song learning. Song-induced gene expression in

NCM could thus be related in the formation and/or storage of song auditory memories, and thereby exert a significant effect on song learning. Vocal learning is often affected by social context, arousal, motivation and hormonal state (for example, see reviews in (60))., but the impact that such variables may have on song-regulated *zenk* expression has not been studied.

6. A POSSIBLE FUNCTION FOR *zenk* INDUCTION IN NCM

Based on the fact that immediate early genes such as *zenk* encode transcriptional regulators, it is reasonable to hypothesize that song exposure triggers a gene regulatory cascade in song-responding neurons (Figure 3). We expect such a cascade to contain early and late genes, according to their induction kinetics, as well as regulatory and effector elements. Regulatory elements would consist of factors such as ZENK and c-jun (also regulated by song in NCM; (61)), which act on the transcription of downstream (target) genes, whereas effector elements would consist of genes encoding protein products that exert direct effects on neuronal morphology and/or function (33, 62). The regulation of the latter could potentially result in long-term changes in song-responsive neurons. A large-scale search for song-regulated genes has been initiated using differential gene screening methods (63). Preliminary evidence suggests that a large number of genes, perhaps several hundred, are regulated by song in NCM. It is of interest to note that a consortium including several songbird investigators has been working towards generating a large annotated EST (expressed sequence tags) collection representing unique genes expressed in the zebra finch brain. This will represent a large advance in the field, facilitating the expression analysis of particular candidate
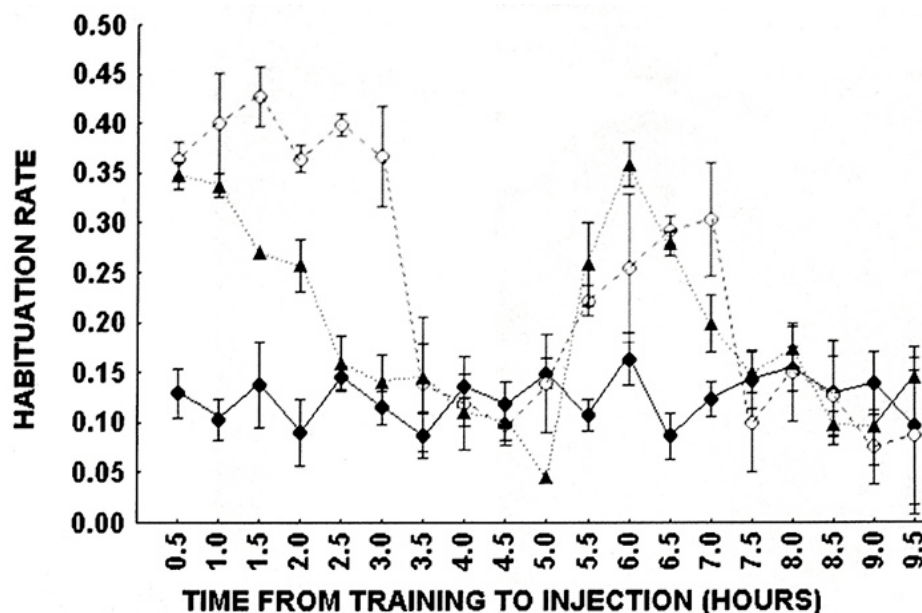
Figure 4. Long-term habituation of evoked auditory responses to song in NCM is dependent on gene expression (from (8)). Plotted are the values of habituation rates (linear derivatives of the habituation curves of the electrophysiological response to repeated song presentation) +/- SEM as a function of the interval from onset of stimulation to the time of local injection of cycloheximide (CYC; solid triangles), actinomycin (ACT, open diamonds) or saline (solid diamonds) in NCM. All habituation rates presented here were recorded 10 h after habituation to repeated song presentation, a time when songs are normally well remembered (low habituation rates). Notice that CYC and ACT were effective in blocking long-term habituation (recorded rates different from rates of control saline injections) at 0.5-3.0 and 5.5-7.0 h and at 0.5-2.0 and 5.5-6.5 h respectively.

genes in songbirds and enabling the construction of a representative brain DNA collection for microarray analysis. This approach should greatly accelerate the rate of discovery of song-regulated genes in the brain.

What could be, however, the functional consequence(s) of gene activation in response to song presentation in NCM? One intriguing hypothesis derives from the fact that the evoked electrophysiological responses of NCM neurons to song auditory stimuli decrease quickly upon repeated song presentations, as also occurs with *zenk* gene expression (8, 41-43, 55). This "habituation" phenomenon is song-specific, since high-amplitude evoked responses can be restored in NCM by a novel song stimulus, and it can be long-lasting, depending on the presentation protocol used. The habituation rate, a linear regression derivative of the habituation curve, provides a useful index of whether a particular song is "remembered" by NCM neurons (8). While the response to familiar and remembered songs shows no habituation or low habituation rates, the responses to novel or "forgotten" songs have high habituation rates. Auditory responses in NCM are not abolished by habituation, but once below a certain threshold they are no longer capable of eliciting a gene expression response.

Importantly, injections of RNA and protein synthesis inhibitors in NCM close to the time of song exposure do not affect NCM's auditory responses and habituation to song, but interfere with the long-term maintenance of the habituation (Figure 4) (8). This finding establishes that gene expression during the period that follows song presentation is required for a long-term modification in the response properties of song-responsive neurons in NCM. This effect is comparable to the properties described for hippocampal LTP (64) and is consistent with the idea that the gene expression triggered by neuronal activation is necessary for long-term neuronal modification (20, 32, 65) and the establishment of long-lasting memories (66). Although the songbird study did not identify the specific genes required for long-term habituation to song in NCM, the time course of song-induced *zenk* expression suggests that *zenk* is an early regulator in this process (33, 62). Testing this possibility will require methods to selectively block *zenk* expression in NCM during specific time windows.

7. AN AUDITORY PROCESSING CIRCUIT FOR SONG AND FOR LIFE

Several telencephalic areas respond to song presentation with a rapid and robust increase in *zenk* expression, indicating that the neuronal activity in these areas is very sensitive to song stimulation (4, 7, 37). In addition to NCM, *zenk*-expressing areas include nucleus MLd in the midbrain, as well as field L subfields (L1 and L3), the caudal paleostriatum, the caudal hyperstriatum ventrale (cHV), and the shelf and cup regions that are respectively adjacent to, but exclude, song nuclei HVC and RA of the song control system (see below). NCM and other *zenk*-expressing areas are closely associated with central auditory pathways (40, 67), forming a network of
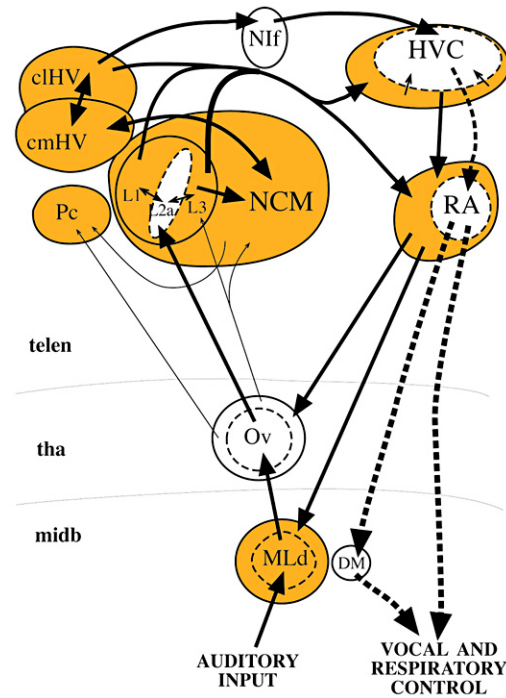
Figure 5. Schematic diagram representing the main projections of auditory structures and of some song control nuclei. Red areas represent regions where the *zenk* gene is induced in response to conspecific song presentation (4); thick solid arrows represent auditory pathways (40, 67) and broken arrows represent connections of the motor pathway for song production (72, 92). Thin solid arrows represent minor or to-be-confirmed projections. Abbreviations: cmHV and clHV, medial and lateral portions of the caudomedial hyperstriatum ventrale; DM, dorsomedial nucleus of the intercollicular complex; L1, L2a, L2b and L3, subdivisions of field L; midb, midbrain; MLd, nucleus mesencephalicus lateralis, pars dorsalis; NCM, caudomedial neostriatum; NIf, nucleus interfacialis; Ov, nucleus ovoidalis; Pc, caudal paleostriatum; RA, nucleus robustus archistriatalis; telen, telencephalon, tha, thalamus.

interconnected structures (Figure 5). This circuit seemingly represents a conserved avian auditory processing system, since similar circuits have been observed in other avian species, including representatives of groups that do not have vocal learning, such as chicken and pigeon (68, 69), as well as vocal learners such as the budgerigar (70). Importantly, the phenomenon of *zenk* expression within the auditory system in response to stimulation with species-specific vocalizations seems to occur in all avian species examined to date (6) and may represent a general phenomenon among birds. In several respects, this auditory processing system bears considerable resemblance to central auditory processing pathways in mammals, including patterns of thalamo-telencephalic projections (67, 68).

It is important to note that the brain nuclei that constitute the song control system do not show increased *zenk* expression in response to song stimulation (4, 7, 9). This system consists of a group of interconnected brain

nuclei that control the syrinx (the avian vocal organ) in coordination with respiratory function. These song nuclei are organized in a direct motor pathway essential for song motor production (71-73), and an anterior forebrain pathway required for vocal learning (74-78). In its basic organization, this system shares important features with motor control pathways of mammals, including a cortical-basal ganglia-thalamo-cortical loop (79, 80) that is likely involved in sensorimotor integration required for normal vocal learning and production. The fact that evoked auditory responses to song can be evoked within song control nuclei under certain circumstances (45, 81-86) has raised the intriguing possibility that the song system may also be involved in perceptual aspects of vocal communication and/or learning. This view has been partly substantiated by data from lesion studies (87, 88, but see 89). Some problems related to this interpretation, however, include the scarcity or absence of evoked auditory responses in awake birds (90, 91), the anatomical specialization of the song system's projections onto vocal and respiratory control centers (92), and the fact that the song system is absent or present only in a rudimentary form in females (93), who typically do not sing but are capable of fine song-related auditory discrimination (for example, see (94)). In addition, the song control nuclei do not present *zenk* expression upon song hearing, but these same areas can express *zenk* in the context of active singing behavior (9, 10). This observation demonstrates that *zenk* expression is not simply repressed within the song system but can be elicited under specific circumstances. Importantly, *zenk* expression during singing relates to the motor control of song production rather than to auditory feedback, as it occurs in singing birds even after deafening (9). Thus, *zenk* expression is a sensitive indicator of activation within the song control circuit. The lack of *zenk* expression in response to song hearing can therefore be interpreted as strong evidence against the activation of the song system by song auditory stimulation in awake birds. Although further analysis is required, these studies collectively suggest that the song control system is not primarily involved in processes such as song auditory processing and perception.

Taken together, the data derived from the existing molecular, anatomical electrophysiological and behavioral studies suggest that song auditory processing occurs primarily in the auditory processing circuits revealed by *zenk* expression analysis. Although the specific role of each *zenk*-expressing area is not known, collectively they compose a system that is likely involved in various aspects of song auditory processing, perception and discrimination. In addition, as evidenced by studies in NCM, for example, this auditory processing system presents properties (e.g., song-specific habituation) that could provide a basic neuronal mechanism related to song auditory memories. Such memories could then play a significant role in social behaviors that are triggered or regulated by conspecific song stimulation, such as territoriality and mate selection and recognition (95). In addition, song auditory analysis and possibly memorization at the level of auditory processing circuits could potentially also play a role in vocal learning. The latter process requires the

memorization of a song auditory model and presumably the comparison between the internalized auditory model and the patterns of vocal production provided through auditory feedback (for instance, see discussion in (96). The potential role of auditory processing circuits in vocal learning, however, remains to be established.

8. PERSPECTIVE

The studies reviewed here demonstrate that song triggers rapid and robust changes in gene expression within auditory processing pathways of the songbird brain. These gene regulatory events, in particular induction of the activity-dependent *zenk* gene in NCM, are potentially linked to neuronal events that underlie song discrimination and auditory memories. Some of the pressing questions to further elucidate the functional significance of song-triggered gene regulation include: 1) testing for a direct link between *zenk* expression and physiological events such as habituation of auditory responses in NCM; 2) identification of molecular targets of *zenk*; 3) characterization of neuronal elements and circuits critically involved in song auditory processing within NCM and other auditory areas; 4) testing for an involvement of NCM and of song-induced *zenk* expression in the establishment and/or maintenance of auditory memories required for vocal communication and vocal learning.

9. ACKNOWLEDGMENT

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Send correspondence to: Claudio V. Mello, M.D. Ph.D., Neurological Sciences Institute, Oregon Health and Science University; 505 NW 185th Avenue; Beaverton, OR 97006; Tel: 503-418-2650, Fax: 503-418-2501, E-mail: melloc@ohsu.edu