

Complementary Neural Systems for the Experience-Dependent Integration of Mate-Choice Cues in European Starlings

Keith W. Sockman,¹ Timothy Q. Gentner,² Gregory F. Ball¹

¹ Department of Psychological and Brain Sciences, Johns Hopkins University, Baltimore, Maryland 21218

² Department of Organismal Biology and Anatomy, University of Chicago, Chicago, Illinois 60637

Received 4 December 2003; accepted 29 April 2004

ABSTRACT: Choice of a particular mate phenotype may arise out of experience with the very phenotypes under consideration. Female European starlings (*Sturnus vulgaris*) prefer males that sing predominantly long-bout songs over males that sing predominantly short-bout songs, and thus, song-bout length is a phenotypic parameter instrumental in releasing the female's mate choice. The preferred long-bout songs induce higher expression of the immediate early gene (IEG) ZENK in the female auditory telencephalon than short-bout songs do, but this sensitivity to song length depends on the female's recent song experience. Here, we compared the experience-dependent modulation of ZENK with that of another IEG, FOS, and report that ZENK and FOS expression in the caudomedial mesopallium and caudomedial nidopallium show different modulation properties that complement natural variation in song-bout length. As reported previously, ZENK expression was greater in response to novel long-bout than to

novel short-bout songs following a 1-week experience with long-bout but not short-bout songs. In contrast, FOS expression was greater in response to novel long-bout than to novel short-bout songs following a 1-week experience with short-bout but not long-bout songs. Thus, the ZENK and FOS signaling pathways are made sensitive to variation in song length by experiences with songs at opposite ends of the starling song-variation continuum, suggesting the presence of complementary neural systems made sensitive in register with the natural axis of phenotypic variation fundamental to the female's mate choice. © 2004 Wiley Periodicals, Inc. *J Neurobiol* 62: 72–81, 2005

Keywords: auditory telencephalon; bird song; caudomedial mesopallium (CMM, cmHV); caudomedial nidopallium (NCM); FOS; immediate early genes (IEG); mate sampling; plasticity; sexual selection; *Sturnus vulgaris*; ZENK

INTRODUCTION

Choice of a particular mate phenotype may arise out of experience with the very phenotypes under consid-

eration (Rosenqvist and Houde, 1997; van Gossum et al., 2001; Hebets, 2003). This is possible because preliminary sampling of prospective mates may influence the threshold for accepting one (Wiegmann et al., 1996; Jennions and Petrie, 1997). Female songbirds assess the quality of prospective mates and choose between them based on variation in their courtship songs (Searcy and Yasukawa, 1996; Gil and Gahr, 2002). Populations may vary in the locally adaptive prevalence of alternate song phenotypes, and, as a reflection of this "song culture", the song types with which the female has recent auditory experience can affect her song preference (Nagle and Kreutzer, 1997; Freeberg et al., 1999; MacDougall-

Present address: Department of Biology, Coker Hall CB #3280, University of North Carolina, Chapel Hill, NC 27599

Correspondence to: K.W. Sockman (kwssockman@unc.edu).

Contract grant sponsor: NIH/NICHD; contract grant number: Individual NRSA 41854 (to K.W.S.).

Contract grant sponsor: NIH/NINDS; contract grant number: R0135467 (to G.F.B.).

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Published online 8 September 2004 in Wiley InterScience (www.interscience.wiley.com).

DOI 10.1002/neu.20068

Shackleton et al., 2001). The mechanisms giving rise to experience-dependent mate choice are largely unknown (Jennions and Petrie, 1997), but they likely involve neural systems tuned by experience with the phenotypic parameters relevant to the mate-choice decision (Sockman et al., 2002).

Neural control of mate choice based on song experience likely depends on processing in telencephalic auditory regions. In the songbird brain, Field L is the primary telencephalic target of the auditory thalamus (Karten, 1968), and interconnected subregions of Field L project to the caudolateral mesopallium (CLM) (formerly hyperstriatum ventrale, cIHV) and reciprocally to the caudomedial nidopallium (NCM) (formerly neostriatum) (for details on new nomenclature, see Reiner et al., 2004). The NCM and CLM, in turn, share reciprocal connections with the caudomedial mesopallium (CMM), but the CMM itself does not project to or receive from Field L (Vates et al., 1996). Thus, the CMM and NCM are positioned at relatively high levels in the sensory hierarchy.

Responses to songs occur throughout these forebrain regions but with greatly variable selectivity. Neurons in Field L subregions L1 and L3 show greater selectivity to species-specific vocalizations than those in subregions L2a and L2b (Leppelsack and Vogt, 1976; Bonke et al., 1979; Müller and Leppelsack, 1985; Theunissen and Doupe, 1998). Selectivity continues to sharpen in the CMM and NCM (Müller and Leppelsack, 1985), where experience-dependent representational plasticity, at least in the CMM (Gentner and Margoliash, 2003), enables extraction of the complex acoustic features of conspecific song (Leppelsack, 1983; Grace et al., 2003).

One hallmark of the CMM and NCM is their stimulus-specific expression of immediate early genes (IEGs) (Clayton, 2000; Mello, 2002; Bolhuis and Eda-Fujiwara, 2003; Mello, 2004). Expression of the IEGs ZENK (the avian homolog of and an acronym for *zif-268*, *egr-1*, *NGFI-A*, and *Krox-24*) and FOS is selective for conspecific over heterospecific song (Mello et al., 1992; Bailey et al., 2002) and increases with strength of song learning (Bolhuis et al., 2000). In terms of mate choice, CMM and NCM ZENK expression in female mountain white-crowned sparrows (*Zonotrichia leucophrys oriantha*) correlates positively with the level of sexual receptivity to a particular song type (Maney et al., 2003).

Several studies on European starlings (*Sturnus vulgaris*) suggest that the length of the male song bout is a signal of his quality and is therefore a phenotypic parameter instrumental in releasing the female's mate choice in this species. Nutritional stress during development reduces average song-bout length (Buchanan

et al., 2003), which itself positively correlates with immunocompetence (Duffy and Ball, 2002) and mating success in the wild (Mountjoy and Lemon, 1996; Eens, 1997). Female starlings show robust preferences in a mate-choice context for songs organized into long bouts over songs organized into short bouts (Gentner and Hulse, 2000); novel long-bout songs, in turn, induce higher ZENK expression in the female CMM and NCM than novel short-bout songs do (Gentner et al., 2000; Sockman et al., 2002). Moreover, prior experience with long-bout songs amplifies the sensitivity of the ZENK response to song bout length, whereas prior experience with short-bout songs attenuates this bout-length sensitivity (Sockman et al., 2002). Thus, when viewed through the lens of ZENK expression in the female CMM and NCM, recent experience with the favored male phenotype makes the female sensitive to the favored category of phenotype. In species where sampling and assessment of the local song culture may affect the acceptance threshold for a prospective mate's phenotype, such a system would seem highly beneficial.

To further characterize the experience-dependent properties of the CMM and NCM in female starlings, we manipulated song experience and then compared subsequent song-induced ZENK with song-induced FOS expression. Whereas ZENK sensitivity to variation in song bout-length arises out of experience with longer songs, here we show that FOS sensitivity to this variation arises out of experience with shorter songs. The results indicate the presence of complementary neural systems made sensitive to variation in mate-choice cues by acoustic experiences at opposing positions in the naturally varying distribution of a phenotypic parameter fundamental to the natural history and fitness of the female.

METHODS

Procedure

We housed 20 wild-caught female European starlings with *ad libitum* access to food and water throughout the study. To synchronize their reproductive cycles, we drove them to the reproductively unresponsive state of photorefractoriness (Nicholls et al., 1988) (confirmed by laparotomy and presence of regressed ovaries) by housing them on a 16-h light–8-h dark (16L:8D) photoperiod. We then restored reproductive competence (i.e., photosensitivity) by transferring them to 8L:16D (Dawson, 1991), and assigned each female to one of four treatment groups: (1) long-bout experience, long-bout stimulus; (2) long-bout experience, short-bout stimulus; (3) short-bout experience, long-bout

stimulus; (4) short-bout experience, short-bout stimulus (Table 1).

We then transferred females to four sound-attenuation chambers (two per chamber) on an 11L:13D photoperiod and, for 7 days, played either long-bout or short-bout songs in each chamber. We presented songs in partially randomized 30-min intervals during the photophase, for a total of 5.5 h of song per day. This constituted the “experience” treatment (Table 1). The first 30-min playback of each day began at photophase onset, and no more than two 30-min playbacks occurred without at least one intervening 30-min silent period. The last 30 min of photophase were always silent. This protocol dispersed song playbacks unpredictably throughout the photophase and, along with our selection of the 11L:13D photoperiod, was our attempt to mimic what free-living females might experience when choosing mates early in the spring.

After completion of the last song playback on day 7 of the experience treatment, we transferred each female to her own sound-attenuation chamber and held her in isolation on 11L:13D through the following day. Beginning 30 min after photophase onset the subsequent day, we exposed one female of each experience-treatment pair to novel long-bout and the other to novel short-bout songs for 30 min. This constituted the “stimulus” treatment phase (Table 1). Ninety minutes after stimulus onset, we anesthetized females (6.5 mg secobarbital, injected i.m.), rapidly decapitated them, and processed their brains as described previously (Sockman et al., 2002). We staggered by 30 min the stimulus treatment for the four pairs of females such that we completed all stimulus treatments by 2.5 h and collected all brains by 3.5 h into the photophase.

We conducted the above experience and stimulus procedures in three runs—twice with eight and once with four females. Successive runs were spaced 1 week apart, such that the first, second, and third cohort spent 44, 51, and 58 days, respectively, on the 8L:16D photoperiod.

Song Recordings

Details of the song recordings have been described previously (Gentner and Hulse, 2000; Gentner et al., 2000). Briefly, for the song sets used in the experience treatment, we recorded a large library of complete song bouts from a single, laboratory-housed male directing song at a female. From these songs we selected 12 exemplars, which, based on bout length, we divided into two sets of six: a 30-min long-bout set and a 30-min short-bout set, with mean bout lengths of 55.2 and 26.0 s, respectively (Table 1). Recordings used for the stimulus treatment were the same, except we recorded the initial library of songs from a different male. The mean bout lengths of the long-bout and short-bout sets used in the stimulus treatment were 55.6 and 25.4 s, respectively. Neither total song nor total silence duration differed among the four 30-min song sets. The two males’ songs shared approximately 1% of their motifs in common, and, in earlier studies in which experience was not manipulated, females preferred the long-bout sets of both

males (Gentner and Hulse, 2000). Because we used songs from one male for both levels of the experience treatment and songs from another for both levels of the stimulus treatment, stimulus songs were always novel, even when the experience and stimulus categories were not (e.g., long-bout experience followed by long-bout stimulus).

Immunocytochemistry

We performed immunocytochemistry for ZENK and FOS on alternate, 40 μm , sagittally cut sections, as previously described for ZENK (Sockman et al., 2002). For FOS, we incubated tissue for 17 h at 4°C with a 1:8000 dilution of an antibody directed against a synthetic fragment of the *c-fos* protein product in chickens (*Gallus domesticus*) (D’Hondt et al., 1999). The *egr-1* and FOS antibodies specifically recognize the female starling ZENK and FOS proteins, respectively (D’Hondt et al., 1999; Sockman et al., 2002).

Quantification and Statistical Analyses

We conducted all quantification procedures blind to the experimental condition of each animal and using a method detailed previously (Gentner et al., 2000). Briefly, we sampled from the CMM and NCM, for which the anatomical boundaries have been described (Gentner et al., 2000). The samples were 640 \times 480 pixel eight-bit grayscale digital microscope-images (200 \times magnification; 412 \times 309- μm image area). For each IEG, we quantified every fourth-cut section from the midline to 1120 μm bilateral. For the CMM, we placed the sampling window as dorsocaudally as possible [Fig. 1(A)]. Due to possible heterogeneity of IEG induction within the NCM (Ribeiro et al., 1998; Gentner et al., 2000; Maney et al., 2003), we sampled from two locations in the NCM. For the dorsal sample (NCMd), we placed the sampling window as ventrocaudally as possible in the dorsal half of the NCM, and for the ventral sample (NCMv) we placed the sampling window as ventrally as possible [Fig. 1(A)].

We counted the number of ZENK-immunoreactive (ZENK-ir) and FOS-ir cells in each digitized image using an automated NIH Image (v1.62, National Institutes of Health, Bethesda, MD) routine (see Gentner et al., 2000) and verified the accuracy by comparing automated with manual counts on a subset of images. Using the mean count of an image and its contralateral counterpart, we analyzed the immunoreactive cell count (dependent variable) using a single, hierarchically nested repeated-measures general linear model, with brain section (laterally from the midline) nested within brain area (CMM, NCMd, NCMv) nested within protein (ZENK, FOS) nested within stimulus (long-bout, short-bout) and with experience and run as between-subjects factors. Due to the split-plot design of this experiment (Table 1), subject refers to the female pair during the experience phase of the study, not to the individual female. Because our primary interest concerned differences between ZENK and FOS in their experience-dependent song-type sensitivity, the model term of primary interest is the protein

Table 1 Song Treatments and Experiment Design

Treatment	Photoperiod	Treatment Duration	Male Used for Recording	Song per 30-min Set (min)	Mean Long/Short Bout-Length (s)	Experiment Design
Pretreatment	8L 16D	6–8 weeks		Isolation from male song		<pre> graph TD A["isolation from male song n = 4 females x 5"] --> B["long bout n = 2 x 5"] A --> C["short bout n = 2 x 5"] B --> D["long bout n = 1"] B --> E["short bout n = 1"] C --> F["long bout n = 1"] C --> G["short bout n = 1"] </pre>
Song experience	11L 13D	1 week	A	27.5	55.2/26.0	
Song stimulus	11L 13D	30 min	B	27.5	55.6/25.4	

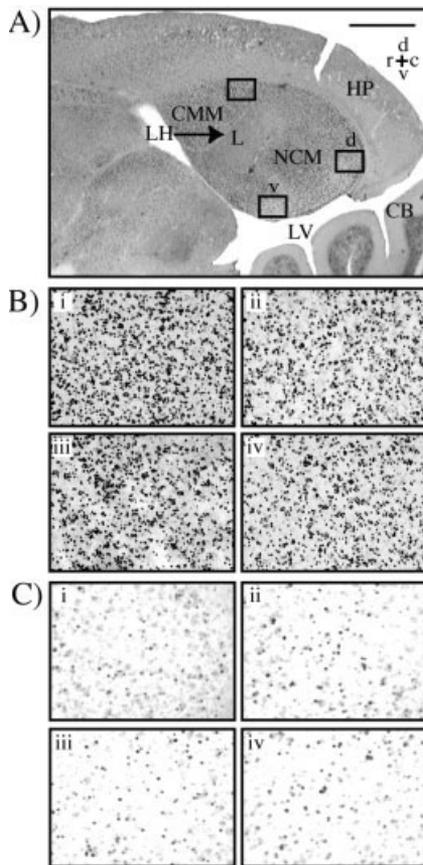


Figure 1 Photomicrographs of the female European starling brain. (A) Sagittal section at the level of the caudomedial mesopallium (CMM) and caudomedial nidopallium (NCM). Rectangles indicate locations of images collected for quantification of ZENK- and FOS-ir cell counts. Scale bar = 1 mm. d, dorsal; v, ventral; r, rostral; c, caudal; CB, cerebellum; HP, hippocampus; L, field L; LH, lamina hyperstriatica; LV, lateral ventricle. Representative photomicrographs ($412 \times 309 \mu\text{m}$) of (B) ZENK-ir and (C) FOS-ir cells in the CMM for each experimental treatment group: (i) long-bout experience, long-bout stimulus; (ii) long-bout experience, short-bout stimulus; (iii) short-bout experience, long-bout stimulus; and (iv) short-bout experience, short-bout stimulus.

\times experience \times stimulus interaction. We do not discuss the main effect of protein, $F(1, 4) = 485.41$, $p < 0.0001$, because it is not possible to distinguish differences between the two immunocytochemistry procedures from differences in protein expression. Although the main effect of run was reliable in a previous study (Sockman et al., 2002), it was less so in the present study, $F(2, 4) = 4.37$, $p = 0.10$, and, because here it was not of primary interest, we do not discuss it further. We also do not discuss most of the numerous interactive effects inherent in large, multifactor analyses such as ours, because they were not of central interest.

The time course for induction and decay of immediate

early genes is rapid (Clayton, 2000), with protein expression in the songbird CMM and NCM peaking approximately 90 min and falling to low levels approximately 4 h after stimulus onset (Mello and Ribeiro, 1998). Consequently, at the start of the stimulus phase of the experiment, IEG induction in the CMM and NCM would likely be basal in all females. IEG induction quantified at the end of the study would be a response to the novel stimulus songs immediately preceding brain collection, and the experience treatment would therefore exert an effect only by mediating this stimulus response. ZENK counts compiled in the present study are from animals used previously (Sockman et al., 2002), although analyses and results are unique to the present study. Figures depict adjusted least-squares means ± 1 SEM and p -values from the models described above and from post hoc least-significant difference procedures.

RESULTS

We observed ZENK- and FOS-ir cells throughout the CMM and NCM (Fig. 1). For both IEG proteins, the immunoreactive cell count was greater in response to the long-bout stimulus than in response to the short-bout stimulus [stimulus: $F(1, 4) = 18.88$, $p = 0.012$] (Fig. 2). However, the magnitude of the response bias toward the long-bout stimulus differed between IEG proteins. The 1.4-fold difference between the long-bout and short-bout stimuli for FOS-ir cell counts (see Fig. 2) was slightly, but reliably, greater than the 1.2-fold difference between stimuli for ZENK-ir cell

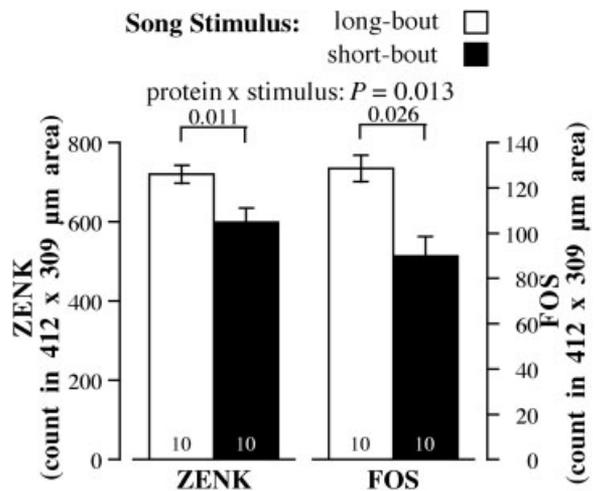


Figure 2 Song-induced ZENK-ir (left panel) and FOS-ir (right panel) cell counts in the auditory telencephalon of the female European starling with respect to novel long- (white bars) and novel short-bout (black bars) song stimuli (mean \pm S.E.M.). Indicated are the p -values for the effect depicted and for post hoc analyses. At the base of each bar is the number of experiment units.

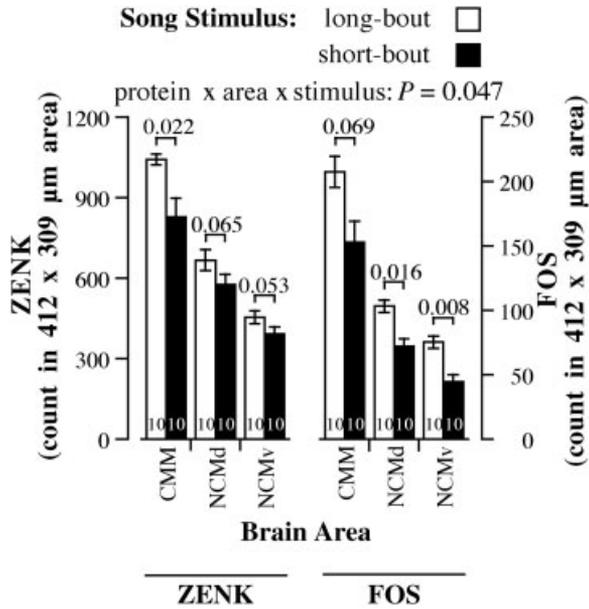


Figure 3 Song-induced ZENK-ir (left panel) and FOS-ir (right panel) cell counts of the female European starling with respect to brain area and with respect to novel long- (white bars) and novel short-bout (black bars) song stimuli (mean \pm S.E.M.). Indicated are the p -values for the effect depicted and for post hoc analyses. At the base of each bar is the number of experiment units.

counts [protein \times stimulus: $F(1, 4) = 18.10$, $p = 0.013$]. In addition, expression of each IEG protein showed anatomical variation in its sensitivity to the stimulus (Fig. 3), with the brain area of greatest sensitivity dependent on the IEG protein expressed (protein \times area \times stimulus: $F(2, 8) = 4.61$, $p = 0.047$). Specifically, the area with the largest relative response-bias toward the long-bout stimulus was the CMM for ZENK-ir cell counts but the NCMv for FOS-ir cell counts.

Experience affected the stimulus-induced expression patterns of each IEG protein differently, as indicated by the model effect of primary interest—the protein \times experience \times stimulus interaction, $F(1, 4) = 14.45$, $p = 0.019$. We compared the mean values of each protein in each of the four treatment groups to explore the detail of this interaction effect. Following long-bout experience, ZENK-ir cell counts in response to the novel long-bout stimulus were 1.3-fold greater than those in response to the novel short-bout stimulus, a difference post hoc tests revealed as considerably more reliable than the 1.1-fold difference between stimuli following short-bout experience (Fig. 4, left panel). Following long-bout experience, FOS-ir cell counts in response to the long-bout stimulus were 1.2-fold greater than those in response to the short-

bout stimulus, a difference the post hoc tests indicated as much less reliable than the 1.8-fold difference between stimuli following short-bout experience (Fig. 4, right panel). In other words, whether the IEG protein most sensitive to variation in the song stimulus was ZENK or FOS depended on the females' song experience. It is possible that greater statistical power would yield reliable differences for all four comparisons. But regardless of statistical power, our finding, that the IEG most sensitive to stimulus variation depended on experience, is highly repeatable, as indicated by the p -value for the protein \times experience \times stimulus interaction (above). This protein- and experience-dependent sensitivity to the stimulus did not vary with brain area [protein \times experience \times stimulus \times area: $F(2, 8) = 1.13$, $p > 0.2$].

DISCUSSION

The female European starling bases her mate choices, in part, on the average length of the males' song-bouts or correlates thereof. The two stimuli

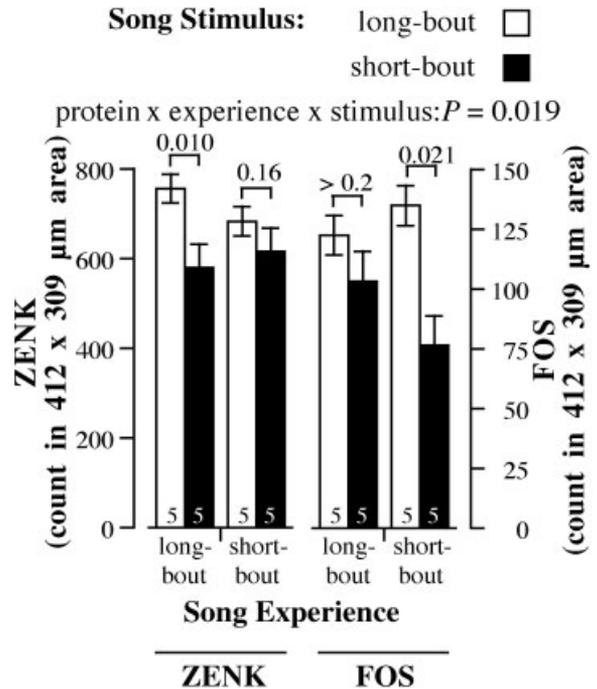


Figure 4 Experienced-based modulation of song-induced ZENK-ir (left panel) and FOS-ir (right panel) cell-count in the auditory telencephalon of the female European starling (mean \pm S.E.M.). Novel long-bout stimulus (white bars); novel short-bout stimulus (black bars). Indicated are the p -values for the effect depicted and for post hoc analyses. At the base of each bar is the number of experiment units.

we contrasted, long-bout song and short-bout song, thus differ in register with an axis of phenotypic variation highly relevant to the species's natural history and fitness. The results of this study reveal complementary perceptual systems that differ from one another by which end of the natural continuum of song experience makes them sensitive to variation in song type. Whereas experience toward one end of the natural continuum of song variation, long-bout songs, makes the ZENK system sensitive to bout length, experience toward the other end of this continuum, short-bout songs, makes the FOS system sensitive to bout length (Fig. 4). This is apparently due to the separate actions of long-bout songs on ZENK expression and short-bout songs on FOS expression. When variation in song experience is not a factor, ZENK and FOS differ only slightly with respect to bout-length sensitivity (Fig. 2), and they also differ with respect to the forebrain region of greatest sensitivity (Fig. 3).

The differential modulation of ZENK and FOS expression by long- and short-bout song experience, respectively, suggests that the separate IEG pathways are sensitive to separate acoustic features. That is, experience with long-bout songs appears to enhance subsequent ZENK expression in response to songs that share common long-bout features, whereas experience with short-bout songs appears to suppress subsequent FOS expression in response to songs that share common short-bout features. Because the songs presented during the experience and the stimulus phases of the experiment were different, the acoustic features that ultimately engender these sensitivities must supercede the explicit components of any one song (Sockman et al., 2002). Therefore, we attribute the bout-length sensitivity of ZENK to features common to our sets of long-bout songs or, potentially, all long-bout songs (Sockman et al., 2002). In contrast, we attribute the bout-length sensitivity of FOS to features common to our sets of short-bout songs or, possibly, all short-bout songs. Although the data do not rule out the possibility that short-bout and long-bout experience have effects on the ZENK and FOS pathways, respectively, such effects appear to be secondary.

Mate choice could reflect not just preference for the favored phenotype but also a relative aversion to the less favored phenotype. Because preference and aversion are not mutually exclusive, a single mate-sampling experience may drive both phenomena concurrently and thereby establish a push-pull relationship that sets the threshold for choice relative to the idiosyncratic local distribution of phenotypes. Complementary processing systems made

sensitive by sampling experiences at opposite ends of the ethologically relevant axis of phenotypic variation would seemingly enable such a hypothetical scenario. Given the natural history of the European starling, the complementary ZENK and FOS systems of the auditory forebrain may help to establish the attractiveness of any given song within the context of the prevailing song culture. Importantly, however, the song experiences of this study do not reverse or eliminate either the ZENK or FOS response-bias toward the long-bout stimulus. Rather, ZENK and FOS expression are always greater in response to the long-bout than to the short-bout stimulus, regardless of experience (Fig. 4). However, the magnitude of this bias toward the long-bout stimulus varies with experience and IEG protein. Therefore, in the starling forebrain, perhaps innate or developmentally organized biases exist, and can be modulated but not eliminated or reversed in adults. Such a "hard-wired" response might help to establish a lower bound on what can serve as an aversive mate-choice cue.

We do not know what might enable these auditory response properties. Nonetheless, experience-dependent representational plasticity of cortical neurons has been described in most sensory systems (Gilbert et al., 2001; Calford, 2002), and in the mammalian auditory system, such plasticity is linked to coincident activation of both cholinergic (Bakin and Weinberger, 1996; Kilgard and Merzenich, 1998) and catecholaminergic (Bao et al., 2001) neurotransmitter systems. In rats, where expression of *zif-268* (a mammalian homologue to ZENK) is required for specific forms of long-term potentiation in the dentate gyrus (Jones et al., 2001), noradrenergic activity selectively modulates light-induced FOS but not ZENK expression in the visual cortex (Yamada et al., 1999). Thus, one might hypothesize that in both mammals and birds, the IEGs ZENK and FOS help to mediate the actions of separate neurotransmitter systems that continuously shape the real-time responses of single cells, and thus the system, to future stimuli.

Local and sex-based variation between the expression patterns of different IEGs have been found previously (Yamada et al., 1999; Mower and Kaplan, 2002; Bailey and Wade, 2003; Reti and Baraban, 2003), but, the specific consequences of such differences are difficult to predict based on current knowledge. Given the vagaries of eukaryotic transcription, the cascade of molecular events following expression of any particular transcription factor, such as ZENK or FOS, is likely to vary from system to system, cell to cell, and even with a cell's

recent synaptic experience (Guzowski, 2002). Generally, however, IEG induction has been implicated in a variety of processes including synaptic remodeling necessary for the formation and stability of new memories, in memory retrieval (Clayton, 2000; Jones et al., 2001; Guzowski, 2002; Fleischmann et al., 2003), and in memory reconsolidation (Bozon et al., 2003). Implication of the songbird auditory system in mnemonic processing (Bolhuis and Eda-Fujiwara, 2003) is consistent with recent findings that song selective responses in the CMM are closely tied to the specific experiences of individual birds (Gentner and Margoliash, 2003). Thus, selective storage or selective retrieval of song-type memories based on sampling experience might be reflected in the differential IEG induction we observed.

It is compelling that the stimulus dimension revealing the difference between experience-dependent IEG expression patterns holds such well-defined ethological relevance. ZENK responses in the female NCM that are biased toward songs preferred in a mate-choice context have been reported in other species (Eda-Fujiwara et al., 2003; Maney et al., 2003), and, in mountain white-crowned sparrows, song-induced ZENK induction in the female CMM and NCM correlates positively with the female's frequency of copulation-solicitation behavior (Maney et al., 2003). This raises the possibility that neuronal activity in these structures might influence song-mediated mate choice, which itself may depend on recent song-sampling experience (Nagle and Kreutzer, 1997; Freeberg et al., 1999; MacDougall-Shackleton et al., 2001).

Our results raise the hypothesis that the forebrain ZENK and FOS expression-patterns work in a complementary fashion by contributing to mechanisms that underlie behavioral preferences for long-bout songs. This could arise either from an affiliation toward a favored phenotype that is represented in starlings by an elevated ZENK response to the favored long-bout songs or from a relative aversion to the less favored phenotype that is represented by a reduced FOS response to the less favored short-bout songs. In social systems where choosing a particular mate depends on the prevalence of different mate types in the population (Badyaev and Qvarnström, 2002), such culturally conditioned neural responses would seem highly beneficial.

We thank R.D. Fernald for logistical support.

REFERENCES

- Badyaev AV, Qvarnström A. 2002. Putting sexual traits into the context of an organism: a life-history perspective in studies of sexual selection. *Auk* 119:301–310.
- Bailey DJ, Rosebush JC, Wade J. 2002. The hippocampus and caudomedial neostriatum show selective responsiveness to conspecific song in the female zebra finch. *J Neurobiol* 52:43–51.
- Bailey DJ, Wade J. 2003. Differential expression of the immediate early genes FOS and ZENK following auditory stimulation in the juvenile male and female zebra finch. *Mol Brain Res* 116:147–154.
- Bakin JS, Weinberger NM. 1996. Induction of a physiological memory in the cerebral cortex by stimulation of the nucleus basalis. *Proc Natl Acad Sci USA* 93:11219–11224.
- Bao S, Chan VT, Merzenich MM. 2001. Cortical remodeling induced by activity of ventral tegmental dopamine neurons. *Nature* 412:79–83.
- Bolhuis JJ, Eda-Fujiwara H. 2003. Bird brains and songs: neural mechanisms of birdsong perception and memory. *Anim Biol* 53:129–145.
- Bolhuis JJ, Zijlstra GGO, den Boer-Visser AM, Van der Zee EA. 2000. Localized neuronal activation in the zebra finch brain is related to the strength of song learning. *Proc Natl Acad Sci USA* 97:2282–2285.
- Bonke D, Scheich H, Langer G. 1979. Responsiveness of units in the auditory neostriatum of the guinea fowl (*Numida meleagris*) to species-specific calls and synthetic stimuli. I. Tonotopy and functional zones of field L. *J Comp Physiol A* 132:243–255.
- Bozon B, Davis S, Laroche S. 2003. A requirement for the immediate early gene *zif 268* in reconsolidation of recognition memory after retrieval. *Neuron* 40:695–701.
- Buchanan KL, Spencer KA, Goldsmith AR, Catchpole CK. 2003. Song as an honest signal of past developmental stress in the European starling (*Sturnus vulgaris*). *Proc R Soc Lond B* 270:1149–1156.
- Calford MB. 2002. Dynamic representational plasticity in sensory cortex. *Neuroscience* 111:709–738.
- Clayton DF. 2000. The genomic action potential. *Neurobiol Learn Mem* 74:185–216.
- D'Hondt E, Vermeiren J, Peeters K, Balthazart J, Tlemçani O, Ball GF, Duffy DL, Vandesande F, Berghman LR. 1999. Validation of a new antiserum directed towards the synthetic c-terminus of the FOS protein in avian species: immunological, physiological and behavioral evidence. *J Neurosci Methods* 91:31–45.
- Dawson A. 1991. Effect of daylength on the rate of recovery of photosensitivity in male starlings (*Sturnus vulgaris*). *J Reprod Fert* 93:521–524.
- Duffy DL, Ball GF. 2002. Song predicts immunocompetence in male European starlings (*Sturnus vulgaris*). *Proc R Soc Lond B* 269:847–852.
- Eda-Fujiwara H, Satoh R, Bolhuis JJ, Kimura T. 2003. Neuronal activation in female budgerigars is localized

- and related to male song complexity. *Eur J Neurosci* 17:149–154.
- Eens M. 1997. Understanding the complex song of the European starling: an integrated ethological approach. *Adv Stud Behav* 26:355–434.
- Fleischmann A, Hvalby O, Jensen V, Strekalova T, Zacher C, Layer LE, Kvello A, Reschke M, Spanagel R, Sprengel R, et al. 2003. Impaired long-term memory and NR2A-type NMDA receptor-dependent synaptic plasticity in mice lacking c-Fos in the CNS. *J Neurosci* 23:9116–9122.
- Freeberg TM, Duncan SD, Kast TL, Enstrom DA. 1999. Cultural influences on female mate choice: an experimental test in cowbirds, *Molothrus ater*. *Anim Behav* 57:421–426.
- Gentner TQ, Hulse SH. 2000. Female European starling preference and choice for variation in conspecific male song. *Anim Behav* 59:443–458.
- Gentner TQ, Hulse SH, Duffy D, Ball GF. 2000. Response biases in auditory forebrain regions of female songbirds following exposure to sexually relevant variation in male song. *J Neurobiol* 46:48–58.
- Gentner TQ, Margoliash D. 2003. Neuronal populations and single cells representing learned auditory objects. *Nature* 424:669–674.
- Gil D, Gahr M. 2002. The honesty of bird song: multiple constraints for multiple traits. *Trends Ecol Evol* 17:133–141.
- Gilbert CD, Sigman M, Crist RE. 2001. The neural basis of perceptual learning. *Neuron* 31:681–697.
- Grace JA, Amin N, Singh NC, Theunissen FE. 2003. Selectivity for conspecific song in the zebra finch auditory forebrain. *J Neurophysiol* 89:472–487.
- Guzowski JF. 2002. Insights into immediate-early gene function in hippocampal memory consolidation using antisense oligonucleotide and fluorescent imaging approaches. *Hippocampus* 12:86–104.
- Hebets EA. 2003. Subadult experience influences adult mate choice in an arthropod: exposed female wolf spiders prefer males of a familiar phenotype. *Proc Natl Acad Sci USA* 100:13390–13395.
- Jennions MD, Petrie M. 1997. Variation in mate choice and mating preferences: a review of causes and consequences. *Biol Rev* 72:283–327.
- Jones MW, Errington ML, French PJ, Fine A, Bliss TVP, Garel S, Charnay P, Bozon B, Laroche S, Davis S. 2001. A requirement for the immediate early gene *Zif268* in the expression of late LTP and long-term memories. *Nat Neurosci* 4:289–296.
- Karten HJ. 1968. The ascending auditory pathway in the pigeon (*Columba livia*). II. Telencephalic projections of the nucleus ovoidalis thalami. *Brain Res* 11:134–153.
- Kilgard MP, Merzenich MM. 1998. Cortical map reorganization enabled by nucleus basalis activity. *Science* 279:1714–1718.
- Leppelsack HJ. 1983. Analysis of song in the auditory pathway of songbirds. In: Evert JP, Capranica BR, Ingle DJ, editors. *Advances in vertebrate neuroethology*. New York: Plenum Press, p 783–799.
- Leppelsack HJ, Vogt M. 1976. Responses of auditory neurons in forebrain of a songbird to stimulation with species-specific sounds. *J Comp Physiol* 107:263–274.
- MacDougall-Shackleton SA, MacDougall-Shackleton EA, Hahn TP. 2001. Physiological and behavioural responses of female mountain white-crowned sparrows to natal- and foreign-dialect songs. *Can J Zool* 79:325–333.
- Maney DL, MacDougall-Shackleton EA, MacDougall-Shackleton SA, Ball GF, Hahn TP. 2003. Immediate early gene response to hearing song correlates with receptive behavior and depends on dialect in a female songbird. *J Comp Physiol A* 189:667–674.
- Mello CV. 2002. Mapping vocal communication pathways in birds with inducible gene expression. *J Comp Physiol A* 188:943–959.
- Mello CV. 2004. Gene regulation by song in the auditory telencephalon of songbirds. *Front Biosci* 9:63–73.
- Mello CV, Ribeiro S. 1998. ZENK protein regulation by song in the brain of songbirds. *J Comp Neurol* 393:426–438.
- Mello CV, Vicario DS, Clayton DF. 1992. Song presentation induces gene expression in the songbird forebrain. *Proc Natl Acad Sci USA* 89:6818–6822.
- Mountjoy DJ, Lemon RE. 1996. Female choice for complex song in the European starling: a field experiment. *Behav Ecol Sociobiol* 38:65–71.
- Mower GD, Kaplan IV. 2002. Immediate early gene expression in the visual cortex of normal and dark reared cats: differences between fos and *egr-1*. *Mol Brain Res* 105:157–160.
- Müller CM, Leppelsack HJ. 1985. Feature extraction and tonotopic organization in the avian forebrain. *Exp Brain Res* 59:587–599.
- Nagle L, Kreutzer ML. 1997. Adult female domesticated canaries can modify their song preferences. *Can J Zool* 75:1346–1350.
- Nicholls TJ, Goldsmith AR, Dawson A. 1988. Photorefractoriness in birds and comparison with mammals. *Physiol Rev* 68:133–176.
- Reiner A, Perkel DJ, Bruce LL, Butler AB, Csillag A, Kuenzel W, Medina L, Paxinos G, Shimizu T, Striedter G, et al. 2004. Revised nomenclature for avian telencephalon and some related brainstem nuclei. *J Comp Neurol* 473:377–414.
- Reti IM, Baraban JM. 2003. Opiate withdrawal induces *Narp* in the extended amygdala. *Neuropsychopharmacology* 28:1606–1613.
- Ribeiro S, Cecchi GA, Magnasco MO, Mello CV. 1998. Toward a song code: evidence for a syllabic representation in the canary brain. *Neuron* 21:359–371.
- Rosenqvist G, Houde A. 1997. Prior exposure to male phenotypes influences mate choice in the guppy, *Poecilia reticulata*. *Behav Ecol* 8:194–198.
- Searcy WA, Yasukawa K. 1996. Song and female choice. In: Kroodsma DE, Miller EH, editors. *Ecology and evo-*

- lution of acoustic communication in birds. Ithaca, NY: Cornell University Press, p 454–473.
- Sockman KW, Gentner TQ, Ball GF. 2002. Recent experience modulates forebrain gene-expression in response to mate-choice cues in European starlings. *Proc R Soc Lond B* 269:2479–2485.
- Theunissen FE, Doupe AJ. 1998. Temporal and spectral sensitivity of complex auditory neurons in the nucleus HVc of male zebra finches. *J Neurosci* 18:3786–3802.
- van Gossum H, Stoks R, De Bruyn L. 2001. Reversible frequency-dependent switches in male mate choice. *Proc R Soc Lond B* 268:83–85.
- Vates GE, Broome BM, Mello CV, Nottebohm F. 1996. Auditory pathways of caudal telencephalon and their relation to the song system of adult male zebra finches. *J Comp Neurol* 366:613–642.
- Wiegmann DD, Real LA, Capone TA, Ellner S. 1996. Some distinguishing features of models of search behavior and mate choice. *Am Nat* 147:188–204.
- Yamada Y, Hada Y, Imamura K, Mataga N, Watanabe Y, Yamamoto M. 1999. Differential expression of immediate-early genes, c-fos and zif268, in the visual cortex of young rats: effects of a noradrenergic neurotoxin on their expression. *Neuroscience* 92:473–484.