A large-capacity memory system that recognizes the calls and songs of individual birds

(amnesia/cognition/species recognition/electrophysiology/long-term memory)

SEK JIN CHEW, DAVID S. VICARIO, AND FERNANDO NOTTEBOHM

Laboratory of Animal Behavior, The Rockefeller University, New York, NY 10021

ABSTRACT   Auditory responses in the caudomedial neo-striatum (NCM) of the zebra finch (*Taeniopygia guttata*) forebrain habituate to repeated presentations of a novel conspecific song. This habituation is long lasting and specific to individual stimuli. We here test the acoustic and ethological basis of this stimulus-specific habituation by recording extracellular multiunit activity in the NCM of awake male and female zebra finches presented with a variety of conspecific and heterospecific vocalizations, white noise, and tones. Initial responses to conspecific song and calls and to human speech were higher than responses to the other stimuli. Immediate habituation rates were high for all novel stimuli except tones, which habituated at a lower rate. Habituation to conspecific calls and songs outlasted habituation to other stimuli. The extent of immediate habituation induced by a particular novel song was not diminished when other conspecific songs were presented in alternation. In addition, the persistence of habituation was not diminished by exposure to other songs before testing, nor was it influenced by gender or laterality. Our results suggest that the NCM is specialized for remembering the calls and songs of many individual conspecifics.

Vocal communication using songs and calls is an important part of social and reproductive behavior in many songbird species (1). Songbirds learn their songs (and in some cases calls) from conspecific tutors, but include minor variations; as a result, these vocalizations have an acoustic form that is specific to each individual. We have previously shown that playbacks of novel conspecific songs elicit vigorous auditory responses in neurons in the caudomedial neostriatum (NCM) of the zebra finch (*Taeniopygia guttata*) brain. These responses habituate with repeated presentations of the same song, and the habituation persists for up to 48 h (2). We have now tested the specificity of these responses by using a larger variety of stimuli, presented in various temporal combinations to both male and female zebra finches. The results show that the initial activity elicited by noise, tones, and various conspecific or heterospecific vocalizations does not predict whether or not repeated exposure to any of these signals will produce long-term habituation. In addition, long-term habituation remains specific for individual songs and calls even when the bird is exposed to large numbers of similar stimuli that could potentially interfere with this form of memory.

METHODS

Experimental Animals and Surgical Procedures. Seventy-six male and female zebra finches were obtained from our breeding colony or from a local supplier. Under anesthesia, birds were surgically prepared for recording. One to two days later, the awake animals were restrained for recording. Re-cording sessions typically lasted 4–5 h, conforming to an approved animal use protocol. For all procedural details, see ref. 2.

Auditory Stimulation. The learned songs and long calls of male zebra finches, unlearned long calls of female zebra finches, songs of canaries (*Serinus canaria*), Bengalese finches (*Lonchura striata*), silver bills (*Lonchura malabarica*), and speech from human subjects were digitized at 20 kHz (Signal, Engineering Design) to provide a set of conspecific and heterospecific stimuli which the birds had not heard before (Fig. 1). The songs and words from human speech were 1.2–2.0 s long, while calls were 100–400 ms in duration. The canary songs consisted of partial songs with the same duration as the zebra finch songs. Other stimuli included tone sequences (seven pure tones with frequencies from 0.5 to 4 kHz in an ascending–descending series of 1.5 s total duration) and bursts of white noise. The stimuli (peak amplitude 75 decibels) were played from a speaker placed 0.5 m from the bird in a soundproof chamber.

The auditory stimulation protocol (referred to as "training") began by exposing the bird to a fixed number of iterations of a novel stimulus at an interstimulus interval (ISI) of 11 s, measured from the onset of one iteration to the onset of the next. The most commonly used protocol, consisting of 200 repetitions of the same stimulus, lasted 36 min (Table 1, paradigm A). One to 48 h after the bird first heard a novel song, the animal was tested with exposure to 100 iterations of the same stimulus. For delays of >4 h, the birds were trained under free-movement conditions and were restrained only during recordings.

Physiological Data Analysis. Stereotaxic coordinates were used to place insulated tungsten recording microelectrodes (Microprobe) in caudal NCM. Placement in caudal NCM was confirmed by observing robust responses that rapidly habituated to repetitions of a novel song. As verified histologically, this habituation does not occur in rostral NCM, which, however, gives strong auditory responses (2), or in overlying hippocampus, which gives no auditory responses. Physiological signals were filtered, amplified, and digitized at 20 kHz (Experimenter's Workbench, Datawave, Longmont, CO). Each presentation of an auditory stimulus constituted a trial lasting up to 2500 ms, including a 500-ms silent control interval preceding the stimulus. On each trial, the response magnitude of multi-unit activity was quantified by subtracting the root-mean-square (rms) value during the control period from the value during the response period (from stimulus onset to offset plus 100 ms). These values were then normalized to the response magnitude on the first trial with each stimulus and were plotted as a function of stimulus iteration number. This relationship, which showed systematic decreases in response magnitude (see *Results*), was quantified by computing the linear regression between iteration number and normalized magnitude for the first 100 iterations in either the training or
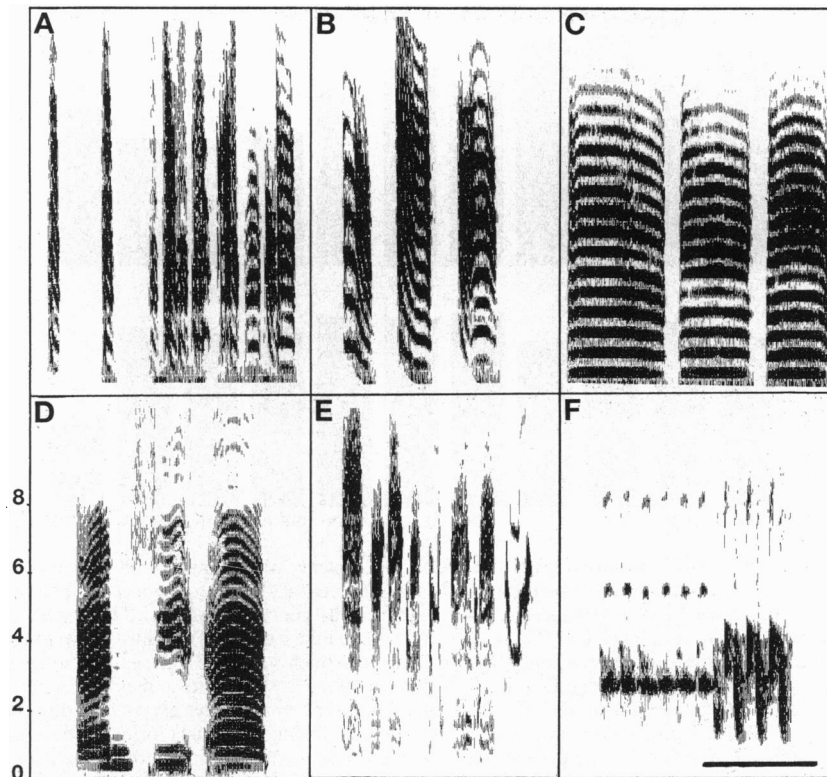
FIG. 1.   Spectrographs of various auditory stimuli used to study habituation in NCM. (*A*) Zebra finch song. (*B*) Long calls of three zebra finch males. (*C*) Long calls of three zebra finch females. (*D*) Speech of human female enunciating the letters "N C M." (*E*) Bengalese finch song. (*F*) Canary song. Horizontal scale bar = 500 ms; vertical scale is in kHz.

testing condition (2). Since this habituation rate was expressed in units of percent change per trial, independent of the absolute levels of activity in any given recording, it could be used to compare responses recorded at different sites and times.

Statistics. Comparisons were made within and across birds and treatment groups. Comparisons of mean habituation rates were made by using the *t* test for independent samples and analysis of variance (ANOVA) procedures for multifactor comparisons. A criterion of $P < 0.05$ (two-tailed) was used for statistical significance.

RESULTS

Habituation Is Stimulus Specific for Each Exemplar of Different Classes of Auditory Stimuli. We sequentially presented several novel exemplars of each class of complex stimulus to male ($n = 2$) and female ($n = 3$) birds, using an 11-s ISI and 50 repetitions of each stimulus in the sequence (Table 1, paradigm B). In both sexes, physiological responses in NCM to each stimulus class, including conspecific female long calls (Fig. 2), conspecific male long calls, and heterospecific vocalizations (not shown), showed a clear pattern of habituation that occurred independently for each exemplar in the sequence, as previously described for responses to conspecific songs (2). At a minimum, this stimulus specificity must depend

on acoustic features which enable the stimuli to be discriminated from each other. The maintenance of the habituation level achieved (Fig. 2, broken line), despite intervening exposure to many similar stimuli, further suggests that an independent neuronal record of each stimulus is being laid down. These properties were examined in a further series of experiments.

Auditory Responses in NCM Are Selective for Certain Classes of Complex Natural Stimuli. The selectivity of neurons in NCM for acoustic features of the stimulus was determined by comparing the initial response amplitude to novel exem-

Table 1.   Stimulation paradigms

Paradigm	Stimulus iteration sequence
A	200 (S_1)
B	$n(S_1), n(S_2), \ldots, n(S_{16})$; n = 50 or 200
C	200 (S_1, S_2)
D	200 ($S_1, S_2, S_3, \ldots, S_{16}$)

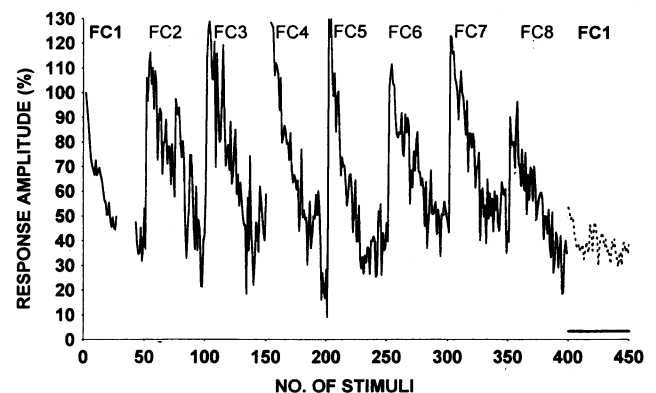$S_1, S_2, \ldots$ = first stimulus (e.g., song), second stimulus, etc.

FIG. 2.   Stimulus-specific habituation to each of eight female long calls presented sequentially (FC1–FC8). Amplitudes were normalized to the initial response to the first stimulus (FC1). Each new stimulus produced strong responses which habituated with repeated exposure. After intervening stimuli, the familiar stimulus was again played (FC1); the responses during this testing period (broken lines, dark bar on x-axis) show that the habituated response level achieved for FC1 was retained even after training with other calls. Similar results were seen for other classes of complex natural stimuli.
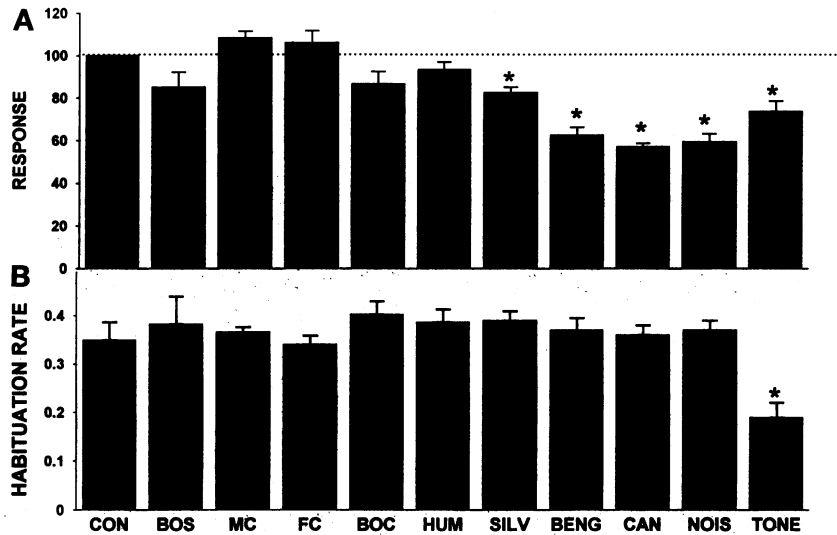
FIG. 3. Selectivity and habituation to novel auditory stimuli in NCM. (*A*) Twenty-nine females and five males were exposed to five novel exemplars of each type of stimulus (10 iterations, ISI = 3.0 s). Bars show the mean ± SEM of the responses, expressed as percentage of the mean for conspecific songs. Data from both sexes were pooled because no significant sex differences were found. The responses to conspecific song (CON), the bird's own song (BOS), conspecific male contact calls (MC), conspecific female long calls (FC), the bird's own long call (BOC), and words from human speech (HUM) were larger than those to silverbill song (SILV), Bengalese finch song (BENG), canary songs (CAN), white noise (NOIS), and pure tones (TONE). *, Responses which were significantly different from the response to conspecific song, as determined by pairwise comparisons for each bird (*t* = 2.51–4.46, *P* = 0.0001–0.01). (*B*) Five females and three males were exposed to three novel exemplars of each type of stimulus (100 iterations, ISI = 11 s). Each bird's mean habituation rate (percent change per trial) for each stimulus over the 100 iterations was calculated (see *Methods*), and the mean of means for the eight birds is shown. For all complex stimuli, the rates were high and not different from those for novel conspecific song (*t* = 0.77–1.44, *P* > 0.05). The pure tone stimuli elicited far weaker habituation (*t* = 3.50, *P* = 0.0015).

plars of 11 classes of auditory stimuli in male and female birds (Fig. 3*A*). Response magnitude was measured on the first 10 trials presented at an ISI of 3.0 s because preliminary testing suggested that this minimized the confounding effect of response habituation (3). The response to the bird's own song could be tested only in males, as only male zebra finches sing. The strongest responses were obtained to all classes of conspecific vocalization, and to human voice; heterospecific songs and artificial stimuli produced significantly weaker responses than conspecific song. The bird's own vocalizations produced responses similar in strength to those elicited by conspecific songs or calls. Similarly, reversed songs or calls produced response amplitudes almost identical to their normal counterparts (see Fig. 4). There were no significant sex differences in responses to any stimuli, although responses to conspecific songs in males tended to be slightly larger than in females.

Rates of Immediate Habituation to Different Classes of Auditory Stimuli Are Similar. We have previously shown that the habituation rate (measured as described in *Methods*) can be used to test whether a given song stimulus is novel to the bird (with a high rate near 0.35), or familiar (with a low rate near 0.15) (2). In the present experiment, we compared rates obtained for novel exemplars of the 11 stimulus classes. When these were presented at an 11-s ISI, all classes of stimuli elicited auditory responses which habituated at high rates indistinguishable from those for novel conspecific songs, with the exception of the tone stimuli, which produced significantly lower rates of habituation (Fig. 3*B*). Again, no sex differences were found.

Forward and Reversed Conspecific Stimuli Habituate Independently. The stimulus specificity of habituation may depend on a variety of acoustic features of the stimulus, including spectral components, temporal pattern, and amplitude modulation. By comparing the responses to forward and reversed playbacks of a stimulus, we asked if habituation was sensitive to the temporal profile of frequency modulation and/or to syllable order. Following exposure to a forward song, presentation of the reversed song was treated as a novel stimulus; initial response amplitude was almost identical in both cases

(paired *t* test for means of the first five responses, *t* = 0.33–0.56, *P* > 0.05). We asked the same question, using the shorter, monosyllabic long calls of both sexes (Fig. 4). Reversed calls of both sexes were regarded as novel and distinct from their forward equivalents, just as was seen for conspecific song. This distinction must reflect a very precise acoustic discrimination; in particular, the female long call, which has a nearly symmetrical profile in time, with little or no frequency modulation (Fig. 1*C*), provides minimal cues to the direction of the stimulus.

Lack of Sex Differences in Long-Term Habituation. Having detected no sex differences in the specificity of habituation to
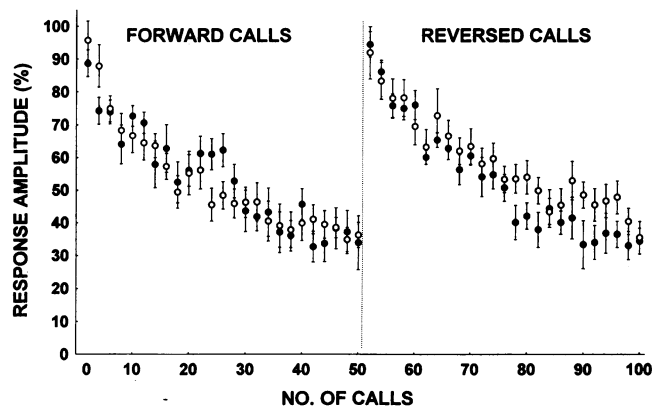

FIG. 4. Independent habituation to forward and reversed long calls. Four birds (two male, two female) were sequentially exposed to a novel long call and the same call played in reverse (50 iterations each; ISI = 11 s). Four male (●) and four female (○) call exemplars were used. The mean responses ± SEM for every two trials with all forward and reversed stimuli are plotted as a function of iteration number. Forward and reversed versions of the same call generated equally vigorous responses with similar rates of habituation. The identical experiment was conducted in another four birds (two male, two female), except that the reversed call was presented before its forward version, with identical results (not shown).

Neurobiology: Chew *et al.*

Proc. Natl. Acad. Sci. USA 93 (1996)   1953

individual stimuli, in response amplitude, or in habituation rates to novel stimuli, we asked if the duration of long-term habituation to various classes of stimuli was similarly independent of the listener's sex. Five exemplars of each of four different stimulus classes were played (200 iterations, ISI = 11 s) to three males and three females, and the habituation rates to these familiar stimuli were measured 24 h later. Zebra finch songs and female calls remained well habituated, with low rates typical of familiar songs (range 0.08–0.17), but habituation to male calls and canary songs was lost, and showed high rates typical of novel songs (range 0.34–0.40). The mean habituation rates were the same in males and females for all stimuli. Thus, in both sexes, the duration of habituation for conspecific songs and female contact calls exceeds that for canary song and male contact calls. This suggests that the mechanisms of this neuronal memory in NCM are similar in males and females, despite considerable sex differences in the motor pathways for the production of learned vocalizations (4, 5).

Lack of Interhemispheric Differences in Habituation. To rule out any contribution of laterality differences to our results, which were collected in both left and right NCM, we tested explicitly for hemispheric differences by recording simultaneously in NCM at symmetrical stereotaxic locations on both sides. There was no significant difference in the mean habituation rates obtained on the two sides for both novel and familiar songs (n = 648 songs in 63 birds of both sexes; time from training to testing = 1–100 h; paired t test for means, P > 0.05), and a regression analysis showed that the rates on the two sides for each stimulus were related with a slope not significantly different from unity (t test for the significance of the regression coefficient, t = −0.851, P > 0.05).

Lack of Influence of Time of Day and Ambient Illumination on Habituation. The study of long-term habituation used training to testing intervals that varied systematically from hours to days. Because birds are active diurnally and testing was often done at night, we needed to ascertain the effect of circadian influences on the comparability of our results. To do this, we presented 16 training songs (200 iterations each, ISI = 11 s) (Table 1, paradigm B) beginning either at 6 a.m. with lights on, or at 6 p.m. with lights off (n = 6 birds per group) and measured the time course of long-term habituation. The training sessions lasted about 10 h. Testing commenced at 8 p.m. for songs used to train during the day, and at 8 a.m. for songs used to train at night. The songs were tested in the reverse order to that with which they were used for training, resulting in a training-to-testing interval of 4 h for the 16th song and 19 h for the first song, within the period of long-term habituation for conspecific songs. Testing occurred with lights either on or off (n = 3 birds per group). There was no effect of these manipulations or of the relative order in which the training stimuli were presented. In all cases the habituation rates for the familiar songs were significantly different from those to novel songs (t = 4.63–8.51, P < 0.05). The habituation rates were all low, typical of familiar songs, and there were no significant differences between the time of day when training occurred, between the 1st and 16th songs, or between rates obtained during testing with the lights on or off (lights on: 1st song, day-trained = 0.172, night-trained = 0.105; 16th song, day-trained = 0.092, night-trained = 0.126; lights off: 1st song, day-trained = 0.089, night-trained = 0.108; 16th song, day-trained = 0.143, night-trained = 0.115).

Effects of Intervening Auditory Signals on Short-Term and Long-Term Habituation. The specificity and long-term habituation phenomena described above were studied in experiments that included presentation of a number of other stimuli during the delay between training and testing for any given stimulus. To validate our results, we systematically investigated the possibility of interference by other conspecific songs, using various playback paradigms shown in Table 1. We then tested for changes in response strength and habituation rate in three

different time periods: during training, within the first hour after training, and long-term (≥1 h). Findings are listed below.

(i) *No interference from other songs during training.* To determine if acquisition of habituation to a novel song was sensitive to interference by other conspecific songs, we recorded NCM responses during a modified training protocol in which another song (either novel or familiar) alternated with 200 successive iterations of a novel stimulus (Table 1, paradigm C). We used a 5-s ISI, which resulted in a 10-s interval between repetitions of the test stimulus (Fig. 5). The presence of an intervening song had no effect; the habituation rate for each test song was indistinguishable from rates obtained for songs with no intervening stimulus. Responses recorded to the intervening song (for which the original test song could be considered an intervening stimulus) were consistent with the bird's prior exposure to each song; high habituation rates were seen for each novel song, and low rates for each familiar song.

We attempted to further challenge the memory capacity of the habituation process in NCM in two birds by presenting 16 consecutive novel conspecific songs at an ISI of 3.5 s, resulting in a 56-s interval between iterations of any one stimulus, and repeating this song sequence 200 times (Table 1, paradigm D). The mean habituation rate for each of these songs (0.364 ± 0.024) was not significantly different from that to single novel songs (Table 1, paradigm A). Thus, it appears that the specificity of habituation to a repeated stimulus is not affected by the auditory context in the interval between iterations. This result further implies that an independent trace of each stimulus presented (analogous to working memory) persists for at least 56 s, because even with that interval between iterations of any given stimulus, habituation was effectively induced.

(ii) *Minimal interference from other songs presented soon after training.* We tested the independence of habituation to individual stimuli soon after training by initially exposing the bird to 60 iterations of a novel song, then testing the habituation rate for that stimulus 30 min later in two conditions: either after presenting 60 iterations of each of two additional novel
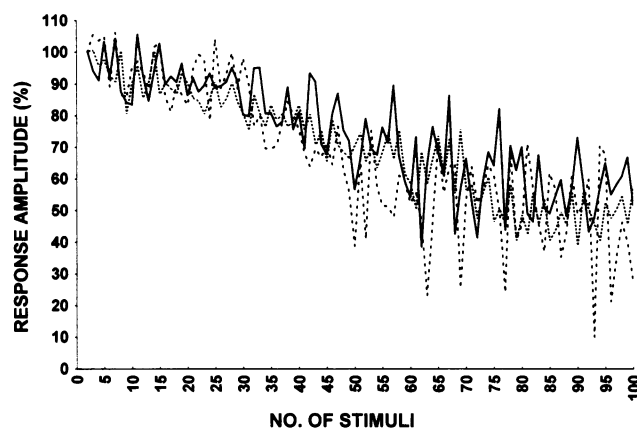

FIG. 5. Effect of intervening conspecific songs on habituation to novel songs during training. Three female birds were trained with a novel conspecific song (100 iterations, ISI = 10 s) under each of three conditions: (*i*) no other song was played (·····); (*ii*) another novel song (———) was alternated with the first at 5-s intervals between stimuli; and (*iii*) a familiar song (- - - -), played 2 h earlier, was alternated with the first at 5-s intervals. In all conditions, the 5-s interval resulted in an effective ISI of 10 s between repetitions of any given stimulus. Results from a representative recording show that the habituation to the novel song in conditions *ii* and *iii* followed a course identical to that seen in condition *i*. Habituation rates were not significantly different in the three conditions. Although they were collected in alternation with responses to a novel song, responses to the intervening stimuli had habituation rates that reflected the bird's prior experience with each stimulus—e.g., the rate for each familiar stimulus was not different from that for other familiar stimuli tested without intervening stimuli.
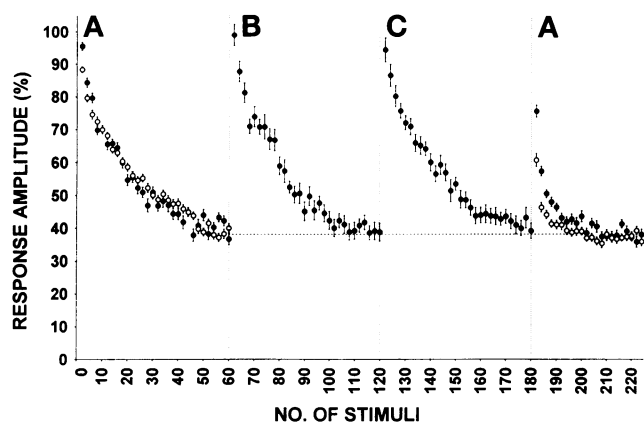
FIG. 6.   Effect of interference on habituation soon after training. Six birds were exposed to a novel song, A (60 iterations, ISI = 10 s, duration of training 10 min), followed either by 20 min of silence (○) or by 20 min of exposure to two novel songs, B and C (●) (60 iterations each, ISI = 10 s). Song A was then played again 30 min after the onset of training. The graphs plot the mean ± SEM of responses for nine different sets of songs, normalized to the first response to A. The habituation rates to A tested at 30 min were not significantly different in the silent and interference conditions. However, in both cases, the first few responses to A were higher than expected if there had been no intervening silence, based on the habituated response to the 60th iteration of A (broken horizontal line). In addition, the 61st–65th responses to A (stimuli 181–185) in the interference condition were significantly higher than those for the silent condition ($t = 2.82$–3.69, $P = 0.005$–0.020). The responses in the interference and silent conditions returned to the habituated level by the 68th and 63rd iteration, respectively.

songs or after the bird sat in silence during the training-to-testing interval (Fig. 6). Sixty iterations was sufficient to induce significant long-lasting habituation and enabled testing after short delays because training lasts only 10 min. Under both conditions, the habituation rates at 30 min were low and significantly different from those elicited by a novel song (interference mean = 0.127, control mean = 0.119, $t = 5.05$–8.21, $P < 0.05$), indicating that the stimulus remained familiar despite exposure to the intervening songs. The rates in the two conditions were not significantly different from each other. However, there was a small influence of the intervening song exposure. In this paradigm, the first few responses to the familiar stimulus during testing were typically larger than the subsequent responses, although they did not significantly alter the habituation rate. In the condition where intervening songs were presented, these first few enhanced responses were slightly, but reliably, higher than when the bird had sat in silence (Fig. 6).

(iiia) *No difference in long-term habituation for multiple songs vs. a single song.* We previously found that, in birds trained with 200 iterations each of 16 successive songs, habituation to these songs was still present at 38 h but was spontaneously and completely lost by 48 h (2). This duration for habituation might have reflected the 16-song memory load, so we trained eight birds (four of each sex) with 200 iterations (ISI = 11 s) of a single novel song. Four birds were kept in silence (Table 1, paradigm A) and four birds were exposed to 200 iterations each of 15 other songs for a total of 16 songs (Table 1, paradigm B); at 48 h, the habituation rate to the original song was determined. In all the birds, the original song elicited a high habituation rate comparable to that elicited by novel songs (single song = 0.367, following multiple songs = 0.378). The rates for single song and multiple song exposure were not significantly different. We infer that the duration of long-term habituation arising from exposure to a given song is comparable when the birds are trained with 1 or 16 songs.

(iiib) *No long-term effect of training order for multiple songs.* The cumulative effect of interference on long-term habituation was also tested in another four birds sequentially exposed to 200 iterations each of 16 songs with an 11-s ISI (Table 1, paradigm B). Testing was then performed with the songs in reversed order from how they had been first presented: the last song played was tested first, and the first song played was tested last. As a result, training-to-testing intervals ranged from one to 16 h. There was no independent effect of training order; the habituation rates for the 1st and 16th songs were low and not significantly different from each other (1st song = 0.114, 16th = 0.092).

DISCUSSION

We used several different classes of sounds to study the basis of stimulus specificity in NCM, a part of the brain known to be selectively activated by complex auditory stimuli (2, 6). Neurons in NCM were more responsive to conspecific vocalizations than to other sound classes, including the songs of other bird species and artificial sounds, although they also had strong responses to human speech. Zebra finch vocalizations and speech have similar fundamental frequencies and complex harmonic structure compared to the other stimuli used (Fig. 1), and these acoustic features may contribute to the strong initial responses to speech. Unlike the neurons of the high vocal center (HVC) (7) and other nuclei of the song system (8, 9), NCM neurons in male zebra finches did not give stronger responses to the bird's own song than to other conspecific songs. However, since NCM neurons do prefer conspecific stimuli, these neurons could contribute to the processing that results in the special selectivity for the bird's own song in HVC; recent anatomical results suggest the existence of indirect connections between NCM and structures afferent to HVC (10).

All complex novel stimuli induced similar high rates of immediate habituation during training. Thus, response amplitudes and immediate habituation rates do not clearly predict the eventual duration of long-term habituation. For example, conspecific songs produced habituation that was maintained for 48 h, whereas human speech sounds, which elicited response amplitudes similar to those for conspecific sounds, produced habituation which was lost by 3 h after training (2). Apparently, the duration of habituation is determined by factors that are not reflected in the extracellular multi-unit activity we recorded in NCM and that may involve other brain structures. Interestingly, although the initial response amplitude in NCM was the same for both forward and reversed long calls (which are acoustically very similar), these calls were discriminated; after habituation reduced the response to a forward long call, the same call played backwards was treated as a novel sound by NCM neurons. Thus, the sound analysis circuit accessed by recording from NCM is very sensitive to subtle stimulus differences, which it can then memorize.

Our recordings from NCM neurons uniformly show that the specificity of long-term habituation to each conspecific vocalization resists interference from the auditory context, even when that includes large numbers of similar sounds presented before, during, or after training with any given vocalization. Apparently, training with each conspecific vocalization establishes a unique auditory memory that is specific to the acoustic features of that vocalization. We infer from experiments with up to 16 stimuli that the NCM system has a fairly large memory capacity and that we did not push against the limits of this capacity. This is concordant with a report that songbirds such as the song sparrow (*Melospiza melodia*) can learn a behavioral discrimination involving 64 different conspecific songs without, apparently, pushing the limits of their memory (11). Moreover, our results show that the auditory context provided by multiple intercalated stimuli (e.g., Table 1, paradigm D),

which may resemble the auditory context of a zebra finch in a large flock of vocalizing birds, had no effect on the recognition of a particular familiar song, arguing for memories that are specific to the sound remembered. We also found that long-term habituation to conspecific songs and calls did not differ between males and females, between hemispheres, or between day-time and night-time training, nor was it influenced by illumination at the time of testing. Overall, our observations demonstrate that the memory system studied in NCM is preferentially activated by conspecific vocal signals and is robustly centered on the acoustic properties of each signal. Presumably, this memory system plays an important role in intraspecific vocal communication. Interestingly, stimulus-specific responses which persist through intervening stimulation are also exhibited in the visual system by neurons of the primate anterior inferior temporal cortex (12).

Other work from this laboratory has shown that expression of an immediate early gene, *ZENK* (also known as *zif-268, egr-1, NGFI-A*, or *Krox-24*), in NCM is preferentially induced by conspecific song (13) and that this genomic response decreases upon repeated presentation of a same song (6). We also know that blockade of RNA or protein synthesis locally in NCM soon after training with a particular stimulus prevents long-term, though not short-term, habituation (2, 14). Although other structures may be involved, these observations strongly suggest that important changes related to memorization of each stimulus take place in NCM and that these changes occur at the level of gene expression, protein synthesis, and electrophysiological responses. Now that we have demonstrated the specificity and independence of long-term memories defined by the responsiveness of NCM neurons to different conspecific vocalizations, this material is ready for analyzing how molecular events in NCM determine the time course of these memories.

1. Kroodsma, D. E. (1982) in *Acoustic Communication in Birds*, eds. Kroodsma, D. E. & Miller, E. H. (Academic, New York), Vol. 2, pp. 1–23.
2. Chew, S. J., Mello, C. V., Nottebohm, F., Jarvis, E. D. & Vicario, D. S. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 3406–3410.
3. Vicario, D. S., Nottebohm, F. & Chew, S. J. (1995) *Soc. Neurosci. Abstr.* **21,** 959.
4. Nottebohm, F. & Arnold, A. P. (1976) *Science* **194,** 211–213.
5. Simpson, H. B. & Vicario, D. S. (1990) *J. Neurosci.* **10,** 1541–1556.
6. Mello, C. V., Nottebohm, F. & Clayton, D. F. (1995) *J. Neurosci.* **15,** 6919–6925.
7. Margoliash, D. (1983) *J. Neurosci.* **3,** 1039–1057.
8. Doupe, A. J. & Konishi, M. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 11339–11343.
9. Vicario, D. S. & Yohay, K. H. (1993) *J. Neurobiol.* **24,** 488–505.
10. Vates, G. E., Broome, B. M., Mello, C. V. & Nottebohm, F. (1996) *J. Comp. Neurol.*, in press.
11. Stoddard, P. K., Beecher, M. D., Loesche, P. & Campbell, S. E. (1992) *Behaviour* **122,** 274–287.
12. Li, L., Miller, E. K. & Desimone, R. (1993) *J. Neurophysiol.* **69,** 1918–1929.
13. Mello, C. V., Vicario, D. S. & Clayton, D. F. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 6818–6822.
14. Chew, S. J., Jarvis, E. D., Mello, C. V., Vicario, D. S. & Nottebohm, F. (1995) *Soc. Neurosci. Abstr.* **21,** 959.