

Changes in Stereotyped Central Motor Patterns Controlling Vocalization Are Induced by Peripheral Nerve Injury

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Adult male zebra finches (*Taeniopygia guttata*), as closed-ended learners, normally crystallize their songs at 90 days of age, and the song remains fixed throughout life (Price, 1979). We show that injuring the tracheosyringeal nerve(s) (each of which innervates the ipsilateral half of the syrinx, the avian vocal organ) results in a short-term deficit in the syllables forming adult male song; this deficit disappears after its nerve regeneration. However, when adult males were followed for a period of several weeks after unilateral tracheosyringeal nerve injury, long-term changes occurred in the temporal patterning of song. Syllables were deleted, remaining portions of the song were linked, and new syllables were added. Syllables with call-like morphology were less likely to be deleted from and more likely to be added to the song. Deletions were most often contiguous chunks of syllables. Changes in the temporal patterning of song occurred during specific periods following nerve injury, were completed within 100 days after nerve transection, and were not dependent upon regeneration of the ts nerve. The resulting newly formed song patterns were stable, remaining unchanged up to 1 year later. The ability of adult male zebra finches to make specific types of changes to crystallized song indicates that some form of vocal plasticity remains even after song learning is completed, though this plasticity may be restricted to a subset of song characteristics. The limitations on the types of changes that are possible may reflect how song is centrally organized. © 1992 Academic Press, Inc.

Some species of passerine birds, such as the canary (*Serinus canaria*), are "open-ended" song learners, repeating portions of the song learning process every year (Nottebohm, 1981). In contrast, other species, including the zebra finch (*Taeniopygia guttata*), are "critical period" learners: an adult male

zebra finch's stereotyped song remains fixed after song development is completed at 90 days (Immelmann, 1969). It has been thought that neither auditory (Price, 1979) nor proprioceptive (Bottjer & Arnold, 1984) feedback is necessary for the maintenance of the stereotyped central motor pattern that controls crystallized zebra finch song. However, Nordeen and Nordeen (1992) have recently reported that auditory feedback is necessary for the long-term maintenance of syllable structure in crystallized song.

Damage to the neural structures controlling the vocal organ, the syrinx, alters the morphology of song to varying degrees, depending upon the species, side, and site of the lesion or nerve transection (Crane, Price, & Nottebohm, unpublished manuscript; Nottebohm, 1971; Nottebohm, Stokes, & Leonard, 1976; Nottebohm & Nottebohm, 1976). In canaries, which are open-ended learners, regeneration (if the injury was to peripheral nerves) may result in the recovery of song syllable morphology (Nottebohm, 1971). More intriguingly, recovery from unilateral injuries to the peripheral or central song control system can be observed, in the absence of regeneration, during the singing season subsequent to the injury (Nottebohm, Manning, & Nottebohm, 1979), indicating that central reorganization takes place during the process of seasonal relearning of song. The well-described seasonal and yearly changes in the neural substrate for song learning (Nottebohm, 1981) presumably underlie canaries' ability to recover from unilateral injury to the song system as well as open-ended learners' yearly recapitulation of some stages of song learning.

In contrast, any compensation for the effects of injury to the song control system in zebra finches must either take place without the neural changes that underlie yearly relearning or, alternately, in-

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duce those changes. This study examines the long-term effects of peripheral nerve injury upon the stereotyped songs of adult male zebra finches and so attempts to discriminate between the neural plasticity that remains in the adult zebra finch song system and that which is restricted to development.

MATERIALS AND METHODS

Subjects

Adult male zebra finches (aged 100 days to 18 months) were obtained from a breeding colony at The Rockefeller University Field Research Center in Dutchess County, New York. All birds remained in the breeding aviary until they were more than 90 days old; song reaches its final form in normally reared birds at 80–90 days (Price, 1979). Birds were then housed in single-sex flight cages until the initiation of the study, at which time each bird was individually caged. During the study, subjects were maintained on 14L:10D illumination.

Surgery

We injured the right ($n = 12$) or both ($n = 5$) tracheosyringeal (ts) nerve(s). Each ts nerve innervates the ipsilateral half of the syrinx (the avian vocal organ). Birds were anesthetized with 0.05 ml of a 50:50 mixture of ketamine (Ketalar; 10 mg/ml) and xylazine (Rompun; 20 mg/ml) supplemented when necessary by inhaled Metofane. Feathers were removed from the ventral surface of the neck and an incision was made in the skin over the middle portion of the trachea. At this level, the tracheosyringeal nerves course along either side of the trachea, surrounded by a thin layer of connective tissue and muscle fibers. The nerve was dissected free of surrounding tissue and injured. The incision was then sutured and sealed with collodion.

Five types of injury were used: (a) the right nerve was crushed ($n = 3$); (b) the right nerve was transected ($n = 3$); (c) a section approximately 1 mm long was cut out of the right nerve ($n = 3$); (d) the right nerve was transected and the distal portion of the nerve, amounting to 1–2 cm, was pulled out ($n = 3$); and (e) the right nerve was transected and the distal end pulled out, and after an interval of 5–9 weeks, the left ts nerve was similarly transected and the distal end removed ($n = 5$).

After 12–15 weeks, the ts nerves of the birds that had received injuries only to their right ts nerves (groups 1–4) were visually examined for evidence of regeneration. Visible evidence of nerve regrowth was found after all types of injury except transection

plus removal of the distal portion of the nerve. The ts nerve was then resected distal to the original injury to evaluate the contribution of any regenerated fibers to song. Upon completion of the study, syringes were examined to evaluate possible atrophy consequent to denervation. Muscle atrophy was only observed in one case, after the most severe type of nerve injury (removing the distal portion of the nerve). Birds with successive unilateral ts nerve resections as this type of injury often leads to severe respiratory difficulty and death.

Song Recording

All songs were recorded as “directed song” sung to a female (Sossinka & Böhner, 1980). Recordings were made using Scotch XS II tape, a Marantz microphone, and a Marantz PDM 221 cassette recorder. Each recording session included at least 10 song bouts (each consisting of several songs), or, if the birds were not ready singers, they were rerecorded the next day. All birds were recorded immediately before surgery, 1 and 3 days postoperatively, and weekly thereafter. After 6 months, the 12 birds that received unilateral ts nerve injuries were sacrificed and the extent of regeneration and syringeal atrophy was determined through visual examination and photography. Song recordings were made immediately prior to resection of the nerve and on the first day the bird sang following surgery. The five males with injuries to both nerves were recorded for a total of 18 months after the second injury. The songs of four intact adult males housed under identical conditions were also recorded over the course of the study to ascertain whether changes in song appear normally.

Song Analysis

A Kay Sonagraph and a sound analysis package (SoundEdit, Farallon Computing) run on a Macintosh II computer were used to generate sonograms. Zebra finch song (see Fig. 1) begins with a series of repeated introductory notes followed by the song itself, a 0.5- to 2-s stereotyped sequence of distinct syllables. Songs can be repeated without intervening introductory notes to form a song bout (Sossinka & Böhner, 1980).

All songs from each recording session were examined. For both pre- and postoperative recordings, the longest song (including all syllables produced during that session) was used as the standard song for analysis. All changes to songs were evaluated against the baseline provided by the preoperative

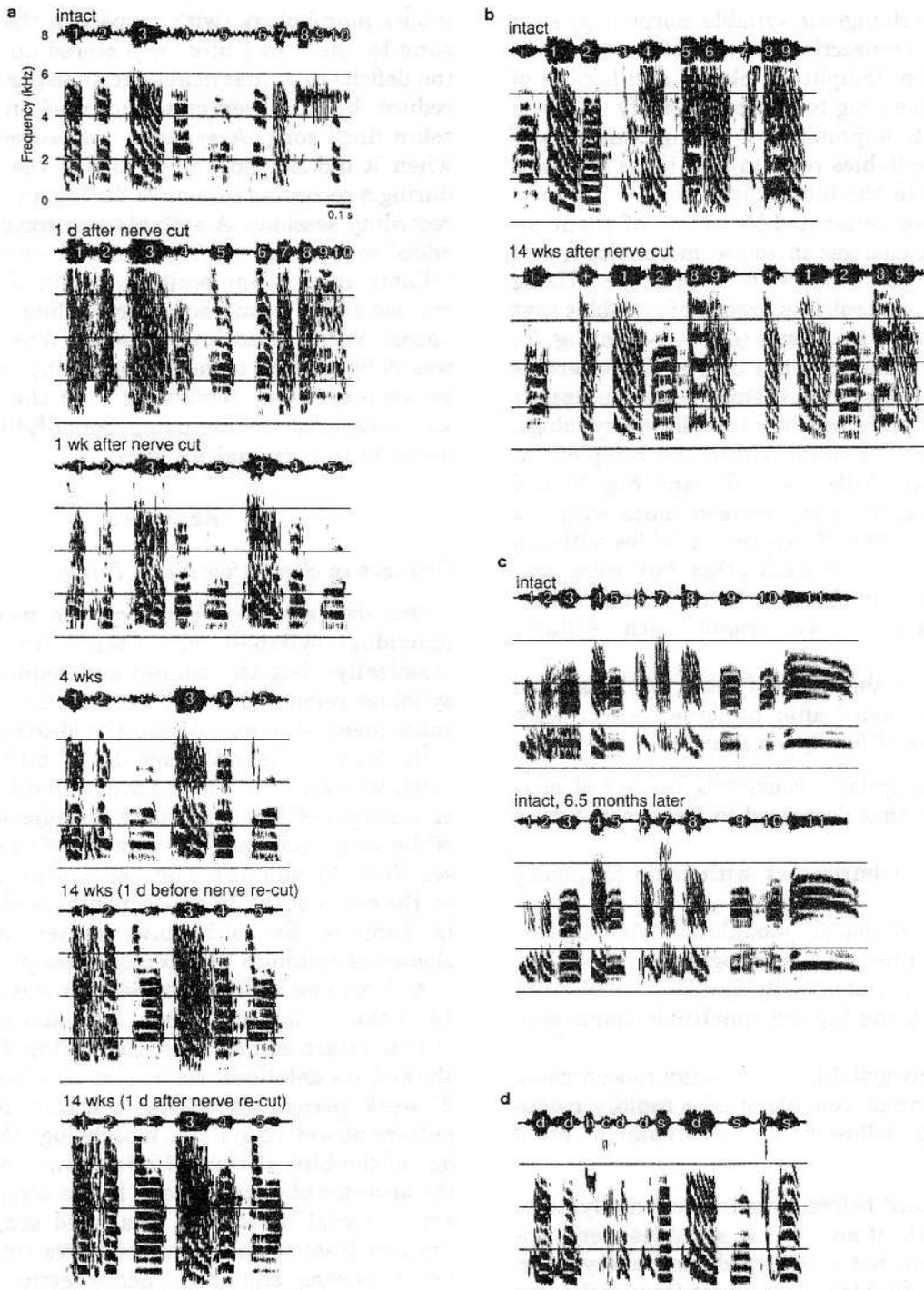


FIG. 1. Changes in song after tracheosyringeal nerve transection. In all portions of this figure, the time/amplitude trace corresponding to the song is shown along the 8-kHz line. When the morphology has been altered, the amplitude profile of a syllable and its temporal placement are often the best cues to identifying a syllable. (a) The song of an adult male zebra finch aged 170 days at the time of right ts nerve injury. At 1 day postoperatively, the morphology of all syllables deteriorated, but the temporal sequence and time/amplitude profile of the song were conserved. One week after nerve injury, syllables 6–10, an optional chunk terminating the song, were dropped. At 4 weeks after injury, a new syllable, closely resembling the original syllable 5, was inserted between syllables 2 and 3; the morphology of all song syllables improved. This song pattern persisted unchanged during the following 70 days, at which time the right ts nerve was recut. With respect to the song recorded immediately prior to recutting the nerve, the song recorded 1 day after the second injury showed little alteration in syllable morphology and no temporal changes, indicating that the right ts nerve made no contribution to the reconstituted song sequence. (b) A second example of song alterations after ts nerve injury. The right ts nerve was injured when the bird was 150 days old. The song stabilized by 100 days after the initial injury, after a simple downsweep had been added to the beginning of the song, syllables 3–7 had been deleted, and an elision linked the remaining syllables. (c) The song of an intact male recorded at 260 days of age and again 6.5 months later. Syllable morphology and sequence are unchanged.

song. Given the changes in syllable morphology seen after ts nerve transection, it was often useful to examine the time/amplitude plot or oscillogram of the postoperative song to reliably identify syllables by their amplitude profile and timing. Analyses of postoperative syllables refer to how those syllables were classified in the intact bird's song.

Syllables were delineated by means of silent intervals, abrupt changes in sound morphology, and high-low-high transitions in amplitude. Using these methods, we broke up groups of syllables that were not separated by silence (see c-d-e in Fig. 2). Although such complexes are treated elsewhere as single syllables, the birds themselves often appear to treat the subunits within a complex as syllables, copying the smaller units within the complex independently (see Williams, 1990, and Fig. 2) and breaking song production between units within a complex (Cynx, 1990). When two syllables within a song closely resembled each other but were consistently produced in characteristic locations within the song sequence, we scored each syllable independently.

All syllables in the songs of intact birds, as well as the syllables added after ts nerve section, were classified as one of five types (Fig. 1d):

downsweep: a syllable consisting mainly of multiple harmonics that decreased in frequency during delivery;

stack: multiple harmonics with little frequency modulation;

click: a train of sharply modulated notes appearing as vertical lines on the sonogram;

high note: a syllable with few (1-3) frequency components and the highest amplitude component at >2 kHz;

V note: a short syllable with energy concentrated at high frequencies, consisting of a rapidly modulated upsweep followed by a similarly rapid downsweep.

All songs recorded before surgery were analyzed to determine which, if any, of the syllables were "optional" and were not produced during every song. Such optional syllables are distinct from call notes in that they are usually given as part of the song, are not repeated many times but are given only once, and are often sequences of two to four syllables (see Sossinka & Böhner, 1980). In this study, the term "optional" designates only those syllables that were optional in the preoperative song.

Changes in syllable morphology and syllable sequence were evaluated for songs from all postoperative recording sessions. The deficit in each syl-

lable's morphology (with respect to the syllable as sung by the intact bird) was scored on a scale of 0 (no deficit) to 3 (maximal deficit), using a blind procedure, by an observer experienced in evaluating zebra finch song. A syllable was scored as deleted when it did not appear in any of the songs sung during a recording session or during any subsequent recording sessions. A syllable was considered to be added to the song when it appeared consistently and reliably in a given position within the song, remained stable in subsequent recordings, and did not appear in the preoperative song. When a syllable was deleted from or added to a song, the interval between syllables remaining from the intact song was measured (in ms using SoundEdit) and compared to the original interval.

RESULTS

Changes in Song after Nerve Injury

One day after nerve injury, the morphology of individual syllables was altered (in some cases drastically), but the timing and sequence of song syllables remained intact (see Fig. 1a). During the subsequent 14 weeks, 51 (32.1%) of the original 159 syllables were deleted from the 17 birds' songs, 10 syllables were added to the songs, and 11 "elisions," or linkages of two previously nonadjacent portions of the song, occurred (for examples of these changes, see Figs. 1a and 1b). The net and average effects on the songs of the 17 experimental birds are shown in Table 1. Songs became shorter, and certain classes of syllables also became less prevalent.

At least one addition or deletion was observed in 14 of the 17 subjects' songs. In contrast, the songs of four intact adult males, containing 45 syllables, showed no deletions, additions, or elisions over a 27-week period, remaining constant in temporal pattern as well as syllable morphology (Fig. 1c). The age of the bird at time of injury was not related to the severity of changes seen in the song. The three experimental birds with unaltered songs received the two least severe forms of nerve injury (two ts nerve crushes, and one ts nerve section). However, as each type of injury induced all three types of song change, all injury types were pooled for analysis.

Syllables Added and Deleted

No relation between the severity of the initial deficit in syllable morphology (as evaluated during the first recording session after injury) and the probability that the syllable would subsequently be deleted was found. However, it is possible that the

visual evaluation of syllables as represented in a sonogram does not correspond to the auditory changes perceived by the bird singing the syllable and that the perceived deficit helped to determine which syllables were deleted.

Whether a syllable was retained, deleted, or added to a song during the period of change depended in part on its morphology when intact. The distribution of syllable types (see Materials and Methods and Fig. 1 for descriptions) in the songs of operated and control birds were not significantly different ($\chi^2(4) = 4.63, p > .3$). The distribution of deleted syllable types was nonrandom ($\chi^2(4) = 10.2, p < .05$): downsweeps were deleted at chance levels (34.4%); stacks were deleted less often than expected (21.7%); and clicks (40%), v notes (50%), and high notes (75%) were deleted more often than expected (Table 1). Added syllables reflected a similar trend: 80% of the added syllables were stacks, 20% were downsweeps, and no v notes, high notes, or clicks were added to songs (Table 1), a distribution that differs significantly from that of syllable types in the original songs ($\chi^2(4) = 13.23, p < .02$). The effect of these changes was to maintain the proportion of downsweeps in songs; decrease the proportion of high notes, clicks, and v notes; and increase the representation of stacks within songs.

Effect of Position within the Song on Changes to Syllables

Contiguous chunks of syllables were more likely to be deleted than individual syllables (Table 2). Given the known number of deleted syllables in each song, the probability that a chunk of each pos-

TABLE 2
Size and Position of Deleted Syllable Chunks

Chunk size	Chunk position		
	Initial	Internal	Terminal
1	0	4	0
2	1	1	2
3	0	0	0
4	0	1	0
5	0	0	2
6	1	1	3
Total chunks	2	7	7
Total syllables	8	16	32

Note. Chunk size was measured in syllables. Initial chunks included the first syllable in the song, terminal chunks included the final syllable in the song, and internal chunks included neither the first nor the last song syllable.

sible size would be deleted was calculated (Appendix). Chunking of deletions was significantly greater than would have been expected by chance ($\chi^2(7) = 320.1, p < .001$). No bird added more than two syllables to its song, making analysis of how added syllables were grouped inconclusive.

Optional syllables, defined as any syllable that was omitted from at least one song during preoperative song recording (see Materials and Methods), were deleted more often than other song syllables (68.4% vs 27.1%; $\chi^2(1) = 7.13, p < .01$). When optional syllables were deleted, they were invariably deleted as an integral chunk; no syllables that were part of an optional string remained after a deletion of any other syllable within that string.

Deletions within the song were not evenly dis-

TABLE 1
Changes in Syllable Distribution after ts Nerve Injury

	Song syllables	Optional syllables	Stacks	Downsweeps	High notes	Clicks	V notes	Added syllables	Deleted syllables
Preop song									
Totals	159	19	44	93	12	6	5	—	—
Means \pm SE	9.4 \pm .5	1.1 \pm .4	2.6 \pm .2	5.4 \pm .3	0.7 \pm .2	0.4 \pm .1	0.3 \pm .1	—	—
Postop song ^a									
Totals	118	6 ^b	44	65	3	3	3	10 ^c	51
Means \pm SE	6.9 \pm .6*	0.4 \pm .2*	2.6 \pm .2	3.8 \pm .4*	0.2 \pm .1*	0.2 \pm .1	0.2 \pm .1	0.6 \pm .2	3.0 \pm .7

Note. Totals for the entire sample ($n = 17$) and means (\pm standard error) are shown. Syllable types are described under Materials and Methods and in Fig. 1. Significant decreases in postoperative song (t test, $p < .05$) are shown with asterisks.

^a The syllables in postoperative songs include both those that remained from preoperative song and those that were added to the songs.

^b For the postoperative song, "optional syllables" refers to syllables remaining from the preoperative song that were scored as optional in the preoperative song.

^c Eight of the 10 added syllables were stacks and two were downsweeps.

tributed among initial, internal, and terminal chunks ($\chi^2(2) = 7.01, p < .05$). Terminal chunks were deleted more often than internal chunks, and initial chunks were deleted least often (Table 2). The possibility that there may be more internal chunks than terminal or initial chunks only accentuates the trend toward preferential deletion of terminal chunks. The high probability of deletion for optional syllables, 89% of which fell into terminal chunks, may in part account for this distribution. Preferential effects by position were also seen in the addition of syllables: four syllables were added to the ends of songs, five syllables to the beginnings of songs, while only one syllable was inserted into a song. Despite these tendencies to favor certain positions within the sequence of song syllables for both additions and deletions, no position within the song was excluded from any type of observed change.

The addition of syllables appeared to be largely independent of syllable deletions: five birds both deleted and added syllables to their songs, two birds only added syllables, and six birds only deleted syllables.

As syllables near the ends of songs were subject to deletion at higher rates, any syllable type that occurred primarily at the ends of songs would also be preferentially deleted, a trend which could account for selective deletion of certain syllable types. Exact comparisons of deletion rates cannot be made because defining chunk position is difficult unless the chunk is deleted, but it is clear that high notes were deleted from within internal chunks at a high rate (7/10, or 70%) comparable to that with which optional syllables were eliminated from the ends of songs (13/19, or 68%). Positional effects cannot then account for selective deletion of syllable types.

Elisions

When a syllable (or chunk of syllables) is deleted, the resulting song can be delivered in two ways: (1) the deleted syllables can be replaced by silent intervals corresponding to the missing sounds, or (2) all trace of the deleted syllables can be eliminated by splicing the remaining portions of the song together, forming an elision. Silent intervals replaced deleted syllables only in three instances; in each of these cases, only one syllable was deleted, that deleted syllable was part of a syllable complex (several syllables delivered in close succession) and was separated from an adjacent syllable by less than 6 ms. None of the remaining deletions fit this profile, and all resulted in elisions within the song or be-

tween subsequent songs. In every case where an elision was recorded, it appeared simultaneously with the first instance of the deletion; birds did not first sing a song with a silent interval and later remove that interval.

Changes by Song Class

No two subjects' songs were identical, but at least half of the syllables within each subject's song fell into one of three song classes or sets of songs including groups of similar syllables. These birds had presumably copied portions of their songs from a common model. However, a given sequence of syllables, shared by several birds, did not change in a systematic manner after nerve injury. The boundaries of deleted chunks varied between songs, different syllables were deleted and preserved, and additions were of different types and in different locations (Fig. 2).

Recovery of Song Syllable Morphology

The morphology of syllables remaining in the song often recovered substantially from the initial deficits seen after ts nerve injury. The recovery of normal syllable morphology followed a consistent pattern (Fig. 3). After the initial deficit in morphology appeared, syllable production remained stable or improved slightly during the following 2-3 weeks. The period from 3 to 6 weeks after the initial nerve injury was often marked by instability in syllable morphology (see Weeks 3 and 4 in Fig. 3). Following this period of instability, syllable morphology stabilized in a form similar to that of the original syllable. Such instability of syllable morphology followed by recovery was seen only in birds that later showed evidence of nerve regeneration. Resectioning the nerve after recovery of syllable morphology caused deterioration to the level of the deficit seen after the original injury (Fig. 3). Syllables added to songs after the initial nerve injury were not affected by subsequent resection of the nerve.

Timing of Song Changes

Deletions occurred as early as the first week and as late as 14 weeks after nerve section (Fig. 4). All deletions of optional syllables took place during an initial peak (1-3 weeks postoperatively) in the number of syllable deletions. The number of syllables deleted rose to a second peak at 6 weeks postinjury. An interval of 25 days followed during which no syllables were deleted. A third episode of syllable deletions occurred at 10-14 weeks after the initial

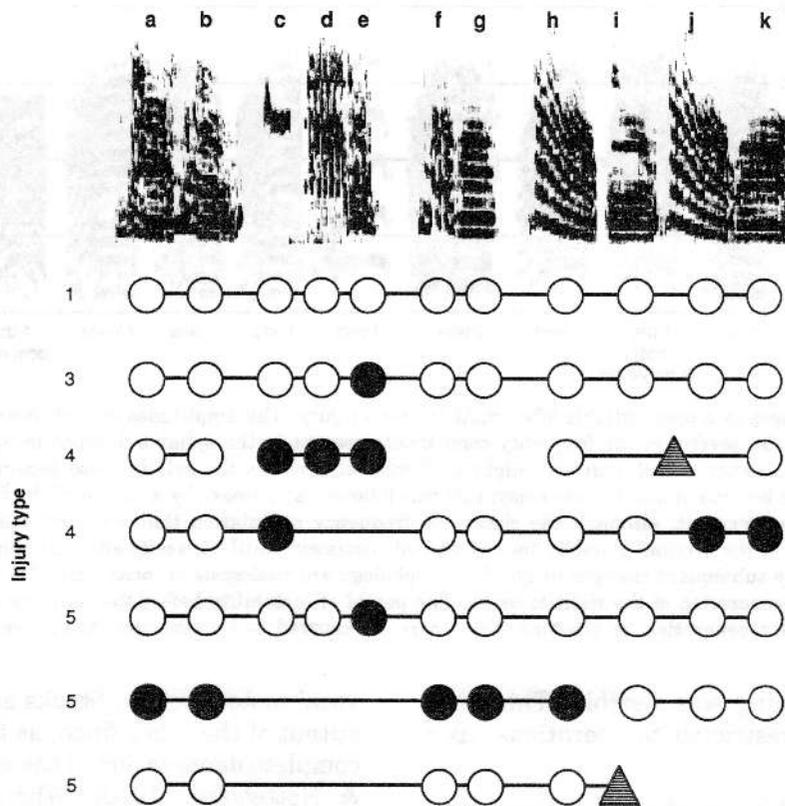


FIG. 2. Similar songs were not altered in the same manner after *ts* nerve injury. The songs of the seven birds shown included similar syllables, presumably learned from a common source. Syllables present in the songs of intact males are shown with circles; those sung in a continuous sequence are connected by a line (breaks in the line indicate changes in order or omission of some syllables). Syllables that were deleted after *ts* nerve injury are represented by filled circles, those that remained are represented by white circles, and the position of added syllables is shown by striped triangles. For example, the second song shown included the syllable sequence a-b-c-d-e-f-g-h-i-j-k before *ts* nerve injury; syllable e was deleted after *ts* nerve injury. The third song shown included syllable sequences a-b, c-d-e, and h-k before injury; after nerve injury, syllables c-d-e were deleted and a new syllable was added between syllables h and k. The temporal spacing of syllables is only shown accurately for the three songs that included all 11 syllables. Injury type is designated by numbers corresponding to descriptions in the Materials and Methods.

injury. Injury type did not appear to be a determinant of the timing of the deletions of syllables: birds with all but one injury type (nerve crush) deleted syllables in all three phases regardless of whether *ts* nerve regeneration occurred.

Additions were restricted to the period between 2 and 7 weeks postinjury (Fig. 4). No relation between syllable type and the timing of additions was observed.

The songs of all subjects stabilized within 4-5 months after the initial injury. The five birds that were followed for 18 months after nerve injury confirmed this observation; all song changes occurred within 4 months after injury, and the song remained stereotyped for 1 year thereafter.

DISCUSSION

After *ts* nerve injury, syllables were dropped from crystallized song, previously separated portions of

song were stitched together, and new syllables were added over a period of 100 days. These changes in the song pattern appear to be centrally controlled and permanent: regeneration of the *ts* nerve did not result in recovery of the original song pattern, re-injuring the *ts* nerve did not affect the new song pattern, and the altered song pattern became stable within 4 months after injury. Changes in the song after *ts* nerve injury were restricted to specific types of modifications to the existing song pattern; a completely novel song never appeared. The types of changes we observed in crystallized song can be summarized as follows:

1. Syllables with novel morphology were not produced. Any modifications of previously crystallized syllables can be attributed to the peripheral effects of nerve injury, and syllables added to song were call-like and similar to vocalizations produced before injury.

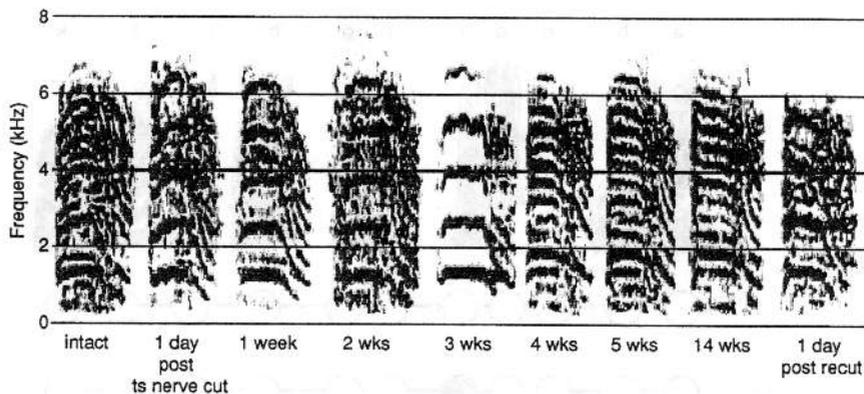


FIG. 3. Successive changes in a song syllable after right ts nerve injury. The amplitudes of each recording were normalized. One day after the ts nerve was cut, several of the frequency components present in the syllable dropped in amplitude. At 1 and 2 weeks postinjury, the syllable's frequency modulation was slightly altered; at 2 weeks the syllable also became more noisy. Three weeks after the injury, the syllable became markedly less noisy; this was followed at 4 weeks by a return of the frequencies that had dropped in amplitude after the nerve was cut, although the change in frequency modulation that was first seen at 1 week persisted. The morphology, similar to that of the original syllable, then remained consistent until 14 weeks after the initial injury; at that time, the right ts nerve was recut. The subsequent changes in syllable morphology are analogous to those seen after the initial injury, indicating that recovery was due to regeneration of the right ts nerve. The period of instability before the recovery of syllable morphology most probably corresponds to this regeneration; in the bird shown here, it occurred 3–4 weeks after the nerve was cut.

2. Temporal patterning was flexible. This plasticity was primarily restricted to operations upon chunks of syllables.

Two classes of syllables, stacks and downsweeps, were preferentially preserved in and added to crystallized song after peripheral nerve injury. This observation, coupled with the disproportionate deletion of high notes, clicks, and v notes, suggests that classes of syllables may differ in (a) the central representation of the sounds that form the syllable, (b) the ability of the partially denervated syrinx to form the sounds, or (c) both of these properties. Stacks and downsweeps are given as calls by both sexes, without being learned in the case of females (Price, 1979, Zann, 1985, Simpson & Vicario, 1990). Males have components of the forebrain circuitry controlling syringeal output that females lack (Nottebohm & Arnold, 1976, Konishi & Akutagawa, 1985, Williams, 1985), and females' calls are not affected by lesions of forebrain song nuclei (Simpson & Vicario, 1990). It may be possible to add female-like calls to song after ts nerve injury because their production is controlled by portions of the song circuitry that do not mediate imitative learning. Under this hypothesis, it would be impossible for adult male zebra finches, without circuits that operate during song learning, to generate the more complex syllables *de novo*. As a consequence, any added syllables would be similar to the calls produced by females. Alternatively, stacks and downsweeps may be preferentially added and retained because they are physically easier to produce with an injured peripheral

vocal motor system. Stacks are the most basic vocal output of the zebra finch, as they are produced after complete denervation of the syrinx (Williams, Cynx, & Nottebohm, 1990). When the syrinx is denervated, it may be difficult or even painful to produce complex syllables and the bird may drop such syllables in favor of the simpler call-like syllables.

The deletion of syllables in chunks suggests that such chunks form an operational unit of internal organization within zebra finch song. In learning their songs, zebra finches extract chunks from the songs of adult males (Williams & Staples, *in press*), and chunking in both learning and in subsequent song production has been shown in the nightingale (Hultsch & Todt, 1989). When a chunk of syllables was deleted, an elision always appeared simultaneously with the deletion and did not gradually eliminate a silent period within the song. Such deletions accompanied by elisions may be the equivalent of a "splice" in the "motor tape" of song. In this view, a chunk of song syllables would occupy temporal space, and removing that chunk would also eliminate the corresponding temporal space within the song. In the three deletions that were not accompanied by elisions, short syllables that were part of a closely related syllable complex disappeared and were replaced by a silent period equal to the length of the syllable. The distinction between deletions accompanied by elisions and the silencing of a single syllable again suggests that syllables may be centrally represented as belonging to chunks within the song (chunk size is variable, and one isolated

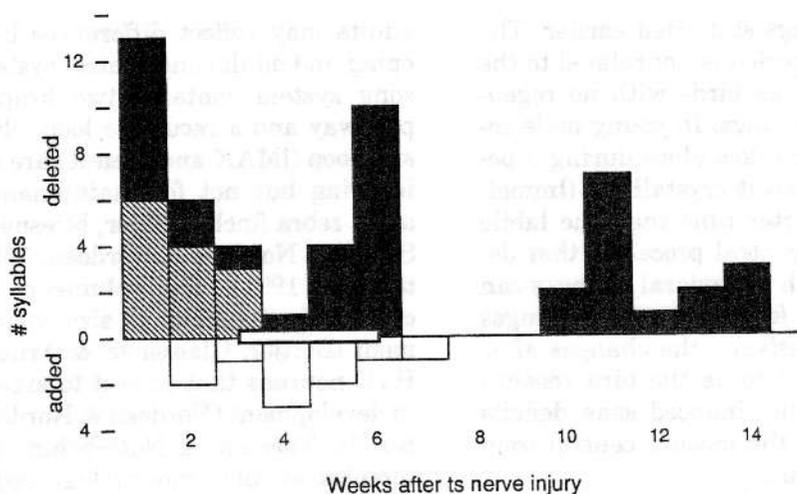


FIG. 4. Timing of syllable deletions (solid bars) and additions (open bars) after tracheosyringeal nerve injury. Deletions of optional syllables are denoted by diagonal hatching. Four of the deleted syllables are not shown here, as the time at which they were dropped from the song could not be ascertained due to insufficiently frequent recording. The horizontal bar separating additions and deletions denotes the period during which instability in syllable morphology was observed.

syllable can form a chunk). In this scheme, chunks are temporal subunits of song and so can be removed or inserted as a unit within the song, while a single syllable within a chunk can be silenced but the time it occupies cannot be removed independently of the other syllables within the chunk. This model of song could explain the weakness of the relationship between syllable type and deletions; syllables affected most severely by ts nerve injury would be deleted, taking with them other syllables in the chunk that suffered only minor deficits.

None of the subjects in this study received an injury restricted to the left ts nerve, which is the less dominant side for song control in the zebra finch (Crane et al., unpublished manuscript). However, injuring the left ts nerve has effects on song similar to those described here (Esposito & Williams, unpublished data). If, as in canaries, hemispheric dominance can be transferred after ts nerve section (Nottebohm et al., 1979), both hemispheres may have the potential to coordinate changes in zebra finch song.

The extended period and multiple peaks of song changes after ts nerve injury suggest that several different processes may be involved in the alteration of crystallized song. As the period of instability in syllable morphology at 3–4 weeks postinjury was always followed by a recovery that in turn was eliminated by resectioning the regenerated ts nerve, we interpret the instability in syllable morphology as a functional correlate of ingrowth of regenerating fibers to the syrinx. Syllable instability also coincided with the addition of new syllables to the song.

However, two observations indicate that syllable addition could not have been directly related to the ingrowth of regenerating ts nerve fibers: (1) 30% of the added syllables were found in the songs of birds that showed no evidence of ts nerve regeneration, and (2) none of the added syllables were affected by subsequent resectioning of the regenerated ts nerve. The timing of syllable addition seems more likely to be associated with a process other than the regrowth of the ts nerve.

Three phases of syllable deletion were observed. All deletions of optional syllables took place during the first phase (1–3 weeks postinjury), and more than 50% of the syllables deleted during this period were optional syllables. If we underestimated the number of optional syllables present in the original song, the proportion of optional syllables among those deleted in the first 3 weeks postinjury may have been even higher. Optional syllables may form a labile class of syllables more prone to deletion and to early deletion than any other class of syllables because of differences in central representation. The second phase of syllable deletions (4–6 weeks after injury) was associated with the period during which we postulate that the regenerating ts nerve fibers reached the syrinx, but deletions during this phase were also seen in the songs of birds that showed no evidence of ts nerve regeneration. The third phase of deletions occurred very late (10–14 weeks after nerve injury).

The final phase of song change after ts nerve injury was the restabilization of the new song. No changes were seen after the fourth month postin-

jury, and many birds' songs stabilized earlier. The termination of the labile period is not related to the regrowth of the ts nerve, as birds with no regeneration did stabilize their songs. In young male zebra finches, song learning takes place during a period of 70 days, after which it crystallizes (Immelmann, 1969), a much shorter time than the labile period we observed. The central processes that define the term during which behavioral changes can take place may be similar for learning and changes during adulthood. Alternatively, the changes after ts nerve injury may stabilize as the bird reaches the limits of how the injury-induced song deficits can be ameliorated using the mature central song system and vocal apparatus.

The overall timing of the delayed song changes we observed after ts nerve injury does not coincide with peripheral changes and may instead correspond to a delayed central response to the initial injury. The time lag might be related to the period required for the central reorganization of connectivity corresponding to changes in the motor program, transfer of song control between hemispheres (Nottebohm & Nottebohm, 1976), or incorporation of a cohort of new neurons within the forebrain song system nuclei (Alvarez-Buylla & Nottebohm, 1988). Similar delays are seen in the degeneration of song after deafening (Nordeen & Nordeen, 1992), suggesting that the processes involved are closely related.

Several factors might initiate or influence males' reorganization of their songs subsequent to ts nerve injury: proprioceptive feedback from the vocal organ (Bottjer & Arnold, 1984), transynaptic changes in the song system consequent to axotomizing the motor neurons (Sumner, 1975), behavioral feedback from the females to whom directed song is sung (King & West, 1983), or inappropriate auditory feedback. Until recently, it had been thought that auditory feedback was not necessary for song maintenance in the adult zebra finch, but changes over a time period similar to that described here are seen after deafening males with crystallized songs (Nordeen & Nordeen, 1992). In many deafened males, song structure shows changes reminiscent of those we see after nerve injury, but in contrast to our study, syllable morphology deteriorates after deafening. The Nordeens' results suggest that the most parsimonious interpretation of our data is that the perceived deficit in song produced after nerve injury is the trigger for changes in the motor program for song.

The restrictions in the plasticity (relative to song learning) that are evident after ts nerve section in

adults may reflect differences between the developing and adult song control systems. The forebrain song system contains two branches, an efferent pathway and a recursive loop. Nuclei in the recursive loop (IMAN and area X) are necessary for song learning but not for maintenance of song in the adult zebra finch (Bottjer, Miesner, & Arnold, 1984; Sohrabji, Nordeen & Nordeen, 1990; Scharff & Nottebohm, 1991). The volume of lateral MAN decreases dramatically in size early in song development (Bottjer, Glaessner, & Arnold, 1985), and the HVC neurons that project to area X are born early in development (Nordeen & Nordeen, 1988; Alvarez-Buylla, Theelen, & Nottebohm, 1988). If imitating complex syllable morphology requires the participation of lateral MAN and other components of the recursive loop of the song system, adult zebra finches might not be able to add syllables other than simpler call-like notes (i.e., stacks and downsweeps) to their songs without recapitulating development and the corresponding changes in IMAN and other components of the recursive loop of the central song system.

Vocalization requires control of both a vocal organ, to give form to syllables, and the pressure head of air that passes through it, which defines the timing with which syllables are sung. Although the ts nerve is the best-studied output of the central song system, it innervates only the vocal organ. When the ts nerve is sectioned bilaterally, the morphology of all syllables is reduced to stacks but the timing and amplitude of syllables remain intact (Williams et al., 1990). This indicates that the central song system generates a pattern for controlling respiratory musculature that is independent of the ts output. The reorganized song patterns generated as a consequence of ts nerve injury in adult males must be represented centrally both as respiratory bursts and their corresponding vocal gestures. If, as our data indicate, the vocal gestures that control syllable morphology cannot be generated anew after crystallization, most of the changes that adult males can make in their songs are related to the respiratory component, or temporal patterning, of song.

The second branch of the central song system, the efferent pathway, has been related to the temporal patterning of zebra finch song as well as to the production of syllables. Each syllable is preceded by sequentially synchronized firing in the neurons of HVC and RA (McCasland, 1987). Lesioning HVC disrupts the normal temporal patterning of song in adult canaries and zebra finches (Nottebohm et al., 1976; McCasland, 1987). Neurons added to HVC in adulthood project to RA and thence to syringeal

motor neurons. In addition, RA projects to DM of ICo, a midbrain nucleus that is thought to play a role in coordinating respiratory activity and syringeal movements (Wild & Arends, 1987). Although a role for the recursive loop cannot be ruled out, the reorganization of the temporal program of song after its nerve injury seems most likely to be mediated by the efferent pathway.

Our results challenge the idea that closed-ended learners' songs are fixed after song is crystallized. Adult male zebra finches made extensive changes to the motor program for song when the vocal and hence the auditory consequences of running the motor tape were altered. The central song control system retains a degree of plasticity that allows it to respond to a degradation in song production by altering the song in specific ways, even after crystallization in a species that does not relearn its song. This adult song plasticity is distinct from and limited in comparison to song learning, but the limitations themselves may well provide insight into how different aspects of song are organized and controlled by the central nervous system.

APPENDIX

Calculating the Expected Sizes of Deleted Chunks

The problem: If d syllables are deleted from a song n syllables long, what is the probability that a contiguous chunk of c syllables is deleted from the song?

n = total number of syllables in the song

d = number of syllables deleted

c = length of the designated chunk.

First, consider the two cases when the chunk includes the first or the last syllable in the song.

The probability that the first syllable in the song was deleted

$$= \frac{d}{n}.$$

If the first syllable was deleted, the probability that the following syllable was also deleted

$$= \frac{d-1}{n-1}.$$

The probability that the last syllable in the chunk of the designated size was deleted

$$= \frac{d-(c+1)}{n-(c+1)}.$$

The probability that the syllable bounding the last (or first) syllable in a chunk of designated size was *not* deleted

$$= 1 - \frac{d-c}{n-c}.$$

So, the probability that a chunk of the designated size was deleted from the beginning or the end of the song (two cases)

$$\begin{aligned} &= 2 \left(\frac{d}{n} \times \frac{d-1}{n-1} \times \dots \right. \\ &\quad \left. \times \frac{d-(c+1)}{n-(c+1)} \right) \left(1 - \frac{d-c}{n-c} \right) \\ &= 2 \left(\frac{d! (d-c)!}{n! (n-c)!} \right) \left(1 - \frac{d-c}{n-c} \right) \\ &= 2 \left(\frac{d! (n-c)!}{n! (d-c)!} \right) \left(1 - \frac{d-c}{n-c} \right). \end{aligned}$$

Now consider the cases where the deleted chunk does not include the first or last syllable in the song (the chunk is bounded by two nondeleted syllables).

Number of cases

$$= n - (c + 1).$$

The term for the probability that a sequence of syllables of given length will be deleted is the same as before

$$= \frac{d! (n-c)!}{n! (d-c)!}.$$

The term for the probability that the syllable on one side of the chunk will *not* be deleted is the same as before

$$= 1 - \frac{d-c}{n-c}.$$

The term for the probability that the syllable bounding the second side of the chunk will *not* be deleted

$$= 1 - \frac{d-c}{n-(c+1)}.$$

So the full term

$$\begin{aligned} &= (n - (c + 1)) \left(\frac{d! (n-c)!}{n! (d-c)!} \right) \\ &\quad \left(1 - \frac{d-c}{n-c} \right) \left(1 - \frac{d-c}{n-(c+1)} \right) \\ &= (n-d-1) \left(\frac{d! (n-c)!}{n! (d-c)!} \right) \left(1 - \frac{d-c}{n-c} \right). \end{aligned}$$

So the entire expression defining the number of chunks of a given size (c) that would be expected to be deleted from a song of a given length (n) if a

given number of syllables were deleted from the song (d) is

$$\begin{aligned}
 &= 2 \left(\frac{d! (n-c)!}{n! (d-c)!} \right) \left(1 - \frac{d-c}{n-c} \right) \\
 &+ (n-d-1) \left(\frac{d! (n-c)!}{n! (d-c)!} \right) \left(1 - \frac{d-c}{n-c} \right) \\
 &= (1+n-d)(n-d) \left(\frac{d! (n-c-1)!}{n! (d-c)!} \right).
 \end{aligned}$$

The number of syllables deleted within chunks of the given size is determined by multiplying this final term by c .

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