studying the chronic irritative focus (*1*), the epileptic human temporal lobe (*1*), and the isolated cortical slab (*14*). Our studies differ in that we have used a naturally occurring behavioral model in which it is possible to control many seizure variables. Unlike researchers who detailed structural changes (*1, 14*), we did not examine tissue previously subjected to exogenous agents, chronic scarring, or long-term surgical manipulation. Further work is needed to clarify (i) the time course of the development of seizure pattern and spine density differences between SS and SR gerbils in ontogeny and (ii) whether the hippocampus, and area CA3 in particular, is a primary site in ictogenesis.

The spine decrease in SS strata oriens and pyramidale may represent a general loss of spines over the entire neuron. It could also imply that the receptive surface has been reduced for inputs of a certain character only, since CA3 contains afferents stratified according to both chemical content and site of origin (*15, 16*). The concept of balance between systems of different chemical content or regional origin with synapse on a common target is one with powerful explanatory potential for episodic phenomena such as seizures (*16*). One consequence of regional spine thinning on dendrites distal to the cell soma could be the functional enhancement of proximal inputs. Since the distributions of pre- and postsynaptic structures only partially overlapped, we can come to no firm conclusion regarding the interaction between spine and tuft findings; still, it is possible that the SS is distinguished from the SR preparation by different proportions of mossy tuft input relative to the postsynaptic spiny array.

Our findings provide a possible basis for further investigation through the use of dynamic anatomophysiological techniques to uncover the role of hippocampal neurons in this naturally occurring model of epilepsy (*17*).

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11. Intraventricular perfusion with 2 percent glutaraldehyde, 2 percent paraformaldehyde, and 0.1*M* sodium cacodylate followed pentobarbital overdose.
12. Criteria for tuft selection were (i) tuft diameter greater than 2.5 μm; (ii) tuft must enclose at least one spine; (iii) at least two synaptic plaques (postsynaptic darkenings) must be visible; and (iv) vesicles must be present within tuft boundaries.
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A Test for Responsiveness to Song Structure and Programming in Female Sparrows

Abstract. *Female song sparrows, primed with implants of estradiol, gave the solicitation display for copulation in response to acoustic stimulation with song. This technique demonstrated that female song sparrows respond more strongly to conspecific song than to alien songs, that they discriminate on the basis of both overall temporal pattern and syllabic structure, that they respond more to several song types than to repetitions of one song, and that they are most responsive to several song types if the songs are organized in bouts of a single type, as they are normally delivered by a male song sparrow. These results demonstrate a substantial correspondence between the structure and programming of the singing behavior of male birds and female responsiveness to song.*

Although mate attraction has long been thought to be one of the functions of male birdsong (*1*), little is known of effects of mate attraction on the evolution of singing behavior. This gap in our knowledge is a consequence of the lack of a sensitive, reliable, and widely applicable experimental test for measuring female preference for different songs (*2, 3*). We report on a technique for measuring the effects of several aspects of singing behavior on courtship intensity in female song sparrows (*Melospiza melodia*). The results demonstrate a close match between female responsiveness and the distinctive features of male song sparrow singing behavior.

After treatment with estradiol, female song sparrow response to songs was measured by number and intensity of copulation solicitation displays. King and West and their co-workers (*4*) used copulation solicitation display as a response measure in female brown-headed

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cowbirds (*Molothrus ater*) that had not been treated with estradiol; however, untreated female song sparrows and most other songbirds do not exhibit solicitation display in captivity. Since massive doses of estradiol induce spontaneous solicitation display in white-crowned sparrows (*Zonotrichia leucophrys*) (*5*) we tried lower doses of estradiol to prime female song sparrows to perform solicitation display, reasoning that they would then respond to appropriate songs with the display.

First we investigated whether female song sparrows would solicit more in response to conspecific songs than they would to heterospecific songs. Six female sparrows caught in the wild were given Silastic implants containing 17β-estradiol (*6*). Each female was then isolated in an acoustic chamber. For several days, 3-minute song bouts, assembled by repeating a single song of (i) a male song sparrow, (ii) a male of the sympatric swamp sparrow (*Melospiza georgiana*), or (iii) a male of the allopatric chaffinch (*Fringilla coelebs*) were played to the isolated females (*7*). Song bouts were presented in random order on each day. An observer scored each female display on a scale of 1 to 3 (*8*), and scores were summed over the entire song bout. The response to song sparrow song (mean score 2.8) was significantly greater than to swamp sparrow song (mean score 0, $N = 10$, $T = 0$, $P < .01$, Wilcoxon matched-pairs signed-ranks test), or to chaffinch song (mean score 0, $N = 10$, $T = 0$, $P < .01$) (Fig. 1A) (*9*).

Song structure can be divided into two components: the note clusters or syllables that make up the song, the syllable structure, and the way in which these components are reiterated in time, the temporal pattern. In a second set of trials, we tested whether female song sparrows prefer song sparrow syllables to swamp sparrow syllables and song sparrow temporal patterns to swamp sparrow patterns. Three female song sparrows were presented with bouts of three computer-edited songs: (i) a song composed of song sparrow syllables in a song sparrow temporal pattern, (ii) a song composed of swamp sparrow syllables in a song sparrow temporal pattern, and (iii) a song composed of song sparrow syllables in a swamp sparrow temporal pattern (*10*). Female response was greatest to the song with conspecific syllables and a conspecific temporal pattern (mean score 7.2). (Fig. 1C) Response was significantly lower to the song with conspecific syllables and a heterospecific temporal pattern (mean score 3.9, $N = 11$, $T = 4$, $P < .01$). Response was also significantly lower to the song with conspecific temporal pattern and hetero-specific syllables (mean score 1.9, $N = 12$, $T = 3$, $P < .01$) (*11*). Thus both syllable structure and temporal pattern are important in evoking solicitation display from female song sparrows.

Male song sparrows from New York populations typically sing eight to ten different versions of the species song with a certain range of individual variability. Studies of other bird species suggest that females may prefer to mate with males that have large repertoires of song types (*3*). We tested for a preference for larger repertoires in female song sparrows by presenting them with song bouts containing either a single song type or four song types (*12*). Eight females were each tested on 4 days (*13*). Order of presentation of the bouts was determined randomly for each female on each day. The response to bouts that contained four song types (mean score 9.9) was significantly higher than the response to bouts with a single song (mean score 5.1, $T = 46$, $N = 24$, $P < .01$). In addition, mean response scores for individual females (across days) were significantly higher for four-song bouts than for single-song bouts ($T = 4.5$, $N = 8$, $P < .05$) (Fig. 1B) (*14*).

There are several ways in which a male could program the delivery of the different types of song. A male could sing with immediate variety, switching song types after every song; alternatively, a male could sing with eventual variety, repeating a single song several times before switching (*15*). Male song sparrows sing with eventual variety, repeating a single song type approximately eight times, on the average, before switching to another type. In a final experiment, we tested whether female song sparrows would respond preferentially to song bouts structured with eventual variety over bouts structured with immediate variety. The immediate variety bout contained 32 songs of four types, ordered ABCDABCD and so on, and the eventual variety contained 32 songs of the same four types, ordered AAAAAAAABBBBBBBB and so on (*16*). The two types of bouts were presented in an order determined randomly for each female on each day. The eight females tested (*17*) solicited more strongly in response to the eventual-variety bout (mean score 12.3) than to the imme-

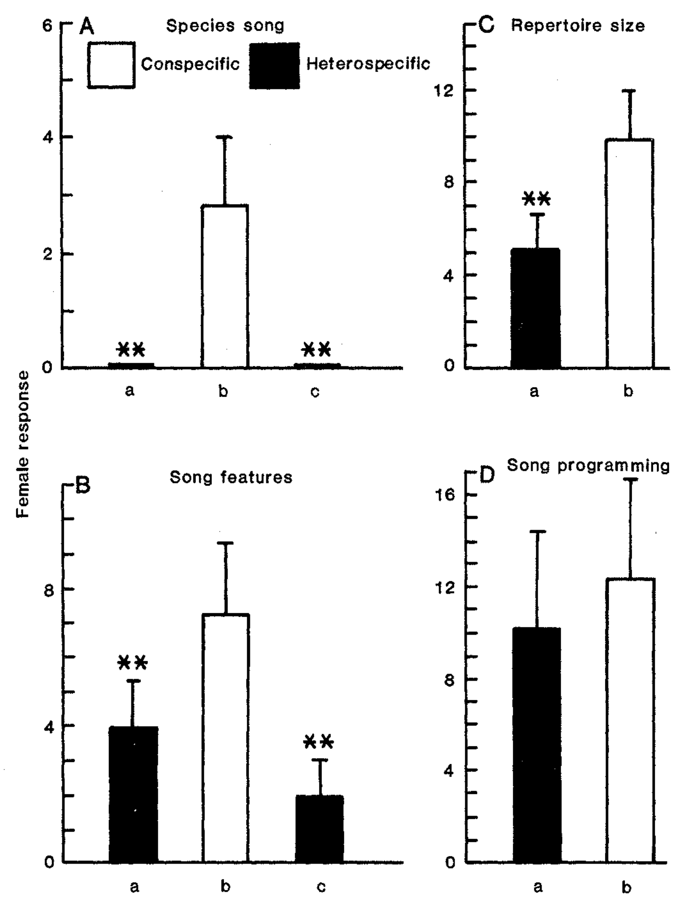

Fig. 1. Copulation solicitation scores of female song sparrows in response to singing behavior arranged in a conspecific or a heterospecific pattern. (A) Response to songs of different species: (a) swamp sparrow song, (b) song sparrow song, and (c) chaffinch song. (B) Response to synthetic songs consisting of mixtures of conspecific and heterospecific features: (a) song sparrow syllables in a swamp sparrow temporal pattern, (b) song sparrow syllables in a song sparrow temporal pattern, and (c) swamp sparrow syllables in a song sparrow temporal pattern. (C) Response to bouts of different numbers of song types: (a) four-song types and (b) single-song types. (D) Response to bouts with different programming of song types: (a) immediate variety (ABCDABCD) and (b) eventual variety (AAAAAAAAABBBB). Histograms give mean response scores plus 1 standard error; **indicates that the response shows a statistically significant difference ($P < .01$) from the conspecific pattern by the Wilcoxon matched-pairs signed-ranks tests.

diate-variety bout (mean score 10.1), but the difference was not significant ($T = 29$, $N = 14$, $P > .05$). However, the mean responses for individual females (over days) were significantly higher for eventual variety than for immediate variety ($T = 0$, $N = 6$, $P < .05$) (*18*) (Fig. 1D). Thus it appears that female song sparrows prefer eventual variety.

These results demonstrate a substantial match between the singing behavior exhibited by adult male song sparrows and the type of song structure and programming that provoke the strongest response from female song sparrows. Female song sparrows solicit more strongly to conspecific than they do to heterospecific songs. For both main elements of song structure, syllable structure and temporal pattern, female song sparrows prefer conspecific patterns to heterospecific ones. Male song sparrows sing bouts that contain multiple song types, and female song sparrows respond more strongly to a sequence of several song types than they do to a bout of a single song type. Finally, male song sparrows order their song types with eventual variety, suggesting that females prefer such an ordering to immediate variety. The match between male behavior and female responsiveness should be advantageous to both sexes; males should benefit from stimulating females to copulate with them, and females should benefit from responding to and copulating with only conspecific males. It remains to be determined whether female song sparrows also use individual difference between conspecific males in song structure and song bout structure as a basis for choosing particular males as mates.

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6. Implants of 17β-estradiol, packed into lengths of Silastic medical-grade tubing (1.96 mm outside diameter) with both ends plugged by adhesive, were placed under the skin of the back where they could be seen easily and removed later. Implants with 15 mm of hormone were given on 16 May and tests with song on 26, 28, and 30 May 1980. Three females that did not give solicitation displays on the first three trials were given a second estradiol implant of the same size on 30 May and tested again on 2, 4, 6, and 8 June (W. A. Searcy, P. Marler, S. S. Peters, *Anim. Behav.*, in press).
7. Each song bout consisted of a single song, 2 to 2.5 seconds long, repeated with 8 seconds between songs. The song sparrow and swamp sparrow songs were recorded from free-living males in Dutchess County, New York. The chaffinch song was from a commercial recording [M. North and E. Simms, *Witherby's Sound-Guide to British Birds* (Witherby, London, 1958)].
8. In copulation solicitation display, a female song sparrow arches her back and brings her tail forward and her head back. The wings are moved away from the body and vibrated. The observer awarded a score of 1 for an incomplete display, 2 for a complete display of short duration (approximately 1 second or less), and 3 for a complete, prolonged display.
9. The N is the number of bird observation days for which nontied response scores were obtained. Since all birds were tested on more than 1 day and the statistics applied to the total observation days, the results of these tests, strictly speaking, apply to the population of all possible scores of the subjects used rather than to the population of all possible individual female song sparrows. For the four subjects that gave solicitation displays in these trials, mean scores (across days) were greater for song sparrow song than for either heterospecific song.
10. The three females used in this experiment were the three given a single implant on 16 May (*6*) and not treated again. Test songs were made by entering natural syllables through the analog-to-digital converter to a PDP 11/10 minicomputer and re-editing the syllables into the desired temporal pattern [S. S. Peters, W. A. Searcy, P. Marler, *Anim. Behav.* **38**, 393 (1980); S. Zoloth *et al.*, *Z. Tierpsychol.* **54**, 151 (1980)].
11. All three subjects gave greater mean responses (across days) to the song with conspecific syllables and conspecific temporal pattern than to either of the two test songs with heterospecific elements.
12. Songs were four different song types taken from the repertoire of a single male recorded in Dutchess County. All bouts consisted of 32 songs repeated with 8 seconds between songs. A different single-song bout was used on each test day, each single-song bout was composed of one of the four song types used in the four-song bout. The four-song bout was assembled with eventual variety (that is, AAAAAAAABBB BBBBB and so on).
13. Six females were given implants (8 to 9 mm) of estradiol on 18 July 1980 and tested on 2, 3, 4, and 5 August. Two females were given implants of the same size on 7 August and tested on 20, 21, 22, and 23 August.
14. In these trials, all eight subjects responded with solicitation displays, and seven gave higher mean responses (across days) to the four-song bouts than to the single-song bouts.
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16. All songs were taken from the repertoire of a single male (not the same one used in the previous experiment) recorded in Dutchess County.
17. These were the same eight females used in the experiment of single-song versus four-song bouts. The group of six was tested on 29, 30, and 31 July and 1 August; the two were tested on 20, 21, 22, and 23 August.
18. Of the eight subjects, one failed to respond, one gave nonzero but tied mean responses, and six gave higher mean responses to eventual variety than to immediate variety tests.
19. We thank M. H. Searcy for drawing the figure. Research was supported by NIMH grant PHS MH 14651.

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Opioid Inhibition of Dopamine Release from Nervous Tissue of *Mytilus edulis* and *Octopus bimaculatus*

Abstract. *Morphine and D-Ala²-Met-enkephalin as well as other opioids suppress potassium-stimulated release of ³H-labeled dopamine from nervous tissue of two marine invertebrates,* Mytilus edulis *and* Octopus bimaculatus. *Naloxone reverses the inhibitory effects in both species. Potassium-stimulated release of ³H-labeled serotonin is not altered by opioids. It is postulated that opiate receptors and their endogenous effectors play a prominent role in regulation of transmitter release in invertebrates.*

Although the importance of aminergic systems in invertebrates has been realized for some time, the role of biologically active small peptides in invertebrate nervous systems has only recently become apparent (*1*). Opioid peptides and various narcotic agents increase dopamine concentrations in certain ganglia of the marine mollusk *Mytilus edulis* (*2*), the freshwater mollusk *Anodonta cygnea* (*3*), and the land snail *Helix pomatia* (*4*). This effect, which requires relatively low concentrations of the agents, is reversed by naloxone, strongly suggesting the involvement of an opiate receptor mechanism. A naloxone-reversible influence of methionine enkephalin and morphine on activity of identified single neurons in *H. pomatia* has been reported (*5*), and opiate binding sites have been characterized in *M. edulis* (*6*), providing further indication that opiate systems play a role in the molluscan nervous system.

Opioids specifically and selectively inhibit the release of dopamine (*7*) and norepinephrine (*8*) as well as other transmitters (*7, 9*) in regions of the mammalian central and peripheral nervous systems; indeed, this may represent a major mode of action of opioid compounds. This action may also be directly mediated by presynaptically localized opiate receptors (*10*). On the basis of our previous studies of the influence of opioids on dopamine concentrations in ganglia of *M. edulis* (*2*), we have examined directly the influence of these substances on dopamine release both in ganglia of *M.*

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