

Fig. 2. Therapeutic effect of SOD and pyran-SOD conjugate on influenza virus-infected mice. Male ddY mice (age: 4 to 6 weeks) were used in the experiments. Mice received the influenza virus (Table 1) by inhalation of virus aerosol at twice the LD₅₀ dose. Ten mice were used in each treated group and 20 mice in the control group. SOD (200 and 1000 U per mouse) and pyran-SOD conjugate (200 U per mouse) were given intravenous injections once daily for four consecutive days from 5 days after virus infection. ●, Control; ○, SOD (200 U per mouse); ●, SOD (1000 U per mouse); ▲, pyran-SOD conjugate (200 U per mouse). The mortality rate of mice did not increase after day 15 to day 30. Mice surviving on day 15 were considered as being cured of the virus infection.

trations were maximal on day 4 and decreased to a very low level on day 8 or later (21). Treatment on days 1 to 4 at the same dose had no therapeutic effect even though viral production was maximal (22). The treatment with pyran-SOD did not affect clearance of the virus from the lung (23). This is a contrast to the treatment of cyclophosphamide, which impairs immune response and prolongs survival at most several days but no cure is seen (1, 3). These results suggest that oxygen radicals produced by the host's delayed response (day 5 to 8 or later) affect the mortality of virus-infected mice.

Pyran copolymer is a well-known interferon inducer (24). Thus, it was possible that the interferon induced by the pyran copolymer exerted the therapeutic effect in these mice. However, the interferon should be effective against viral multiplication (days 1 to 4), but our data showed no effect during these days. Furthermore, no therapeutic effect of pyran copolymer by itself was observed in our experiments (25). Thus, it seems most reasonable to attribute the pronounced therapeutic effect of pyran-SOD conjugate to its enzyme activity as an O₂⁻ scavenger, not as an interferon inducer. These results suggest that activated macrophages and systemic and local xanthine oxidase levels, all generating O₂⁻, are important in the pathogenesis of influenza virus infection.

REFERENCES AND NOTES

1. S. H. Singer, P. Noguchi, R. L. Kirschstein, *Infect. Immun.* **5**, 957 (1972).

2. F. Suzuki, J. Ohya, N. Ishida, *Proc. Soc. Exp. Biol. Med.* **146**, 78 (1974).
3. J. Hurd and R. B. Heath, *Infect. Immun.* **11**, 886 (1975).
4. J. L. Sullivan, R. E. Mayner, D. W. Barry, F. A. Ennis, *J. Infect. Dis.* **133**, 91 (1976).
5. E. Shimomura, F. Suzuki, N. Ishida, *Microbiol. Immunol.* **26**, 129 (1982).
6. R. M. Tate and J. E. Repine, *Am. Rev. Respir. Dis.* **128**, 552 (1983).
7. D. R. Blake *et al.*, *Ann. Rheumat. Dis.* **42**, 89 (1983).
8. C. F. Nathan and R. K. Root, *J. Exp. Med.* **146**, 1648 (1977).
9. J. M. McCord, *N. Engl. J. Med.* **312**, 159 (1985).
10. I. Fridovich, *Science* **201**, 875 (1978).
11. W. R. Waud and K. V. Rajagopalan, *Arch. Biochem. Biophys.* **172**, 365 (1979).
12. C. M. Grum, R. A. Ragsdale, L. H. Ketari, R. H. Simon, *J. Critical Care* **2**, 22 (1987).
13. T. Akaike *et al.*, in preparation.
14. J. M. McCord, S. H. Stokes, K. Wong, *Adv. Inflamm. Res.* **1**, 273 (1979).
15. H. Maeda, M. Ueda, T. Matsumoto, T. Morinaga, *J. Med. Chem.* **28**, 455 (1985).
16. H. Maeda, J. Takeshita, R. Kanamaru, *Int. J. Pept. Protein Res.* **14**, 81 (1987).
17. H. Maeda, T. Matsumoto, T. Konno, K. Iwai, M. Ueda, *J. Protein Chem.* **3**, 181 (1984).
18. H. Maeda, Y. Matsumura, T. Oda, K. Sasamoto, in *Protein Tailoring for Food and Medical Uses*, R. E. Feeney and J. R. Whitaker, Eds. (Dekker, New York, 1986), pp. 353-382.
19. H. Maeda, T. Oda, Y. Matsumura, M. Kimura, *J. Bioactive Compatible Polym.* **3**, 27 (1988).
20. M. Kimura, Y. Matsumura, Y. Miyauchi, H. Maeda, *Proc. Soc. Exp. Biol. Med.* **188**, 364 (1988).
21. T. Akaike, A. Molla, M. Ando, S. Araki, H. Maeda, *J. Virol.*, in press.
22. T. Akaike *et al.*, unpublished observation.
23. We quantitated the infecting virus in the mice lungs treated with pyran-SOD (200 U) by the plaque-forming assay with Madin-Darby canine kidney (MDCK) cells as described (21). The results showed that the infectious virus in the lungs on day 11 or thereafter could not be detected. Thus we concluded that the mice maintained under pyran-SOD cleared out virus.
24. W. Regelson, in *Anionic Polymeric Drugs*, L. G. Donaruma, R. M. Ottenbrite, O. Vogl, Eds. (Wiley, New York, 1980), pp. 303-325.
25. Treatment with 1000 U of pyran-SOD-equivalent pyran copolymer had no effect at the same injection dose schedule in Fig. 2.
26. R. B. Johnston, C. A. Godzik, Z. A. Cohn, *J. Exp. Med.* **148**, 115 (1978).
27. V. Massey, P. E. Brumby, H. Komai, G. Palmer, *J. Biol. Chem.* **244**, 1682 (1969).
28. We thank J. Gandy and M. Fujii for the preparation of manuscript.

3 January 1989; accepted 28 March 1989

Categorical Perception of a Natural Stimulus Continuum: Birdsong

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A fundamental issue in perception and communication is how continuously varying stimuli are partitioned into discrete categories. In swamp sparrow songs, note duration is a critical feature distinguishing two note categories with different roles in song construction. Pairs of songs with initial notes from different categories contrast more in their effects on territorial males than song pairs with initial notes differing by the same amount but taken from within one note category. The results indicate categorical perception by wild swamp sparrows.

CATEGORIZATION IS A BASIC PERCEPTUAL process by which animals recode variable stimuli into discretely different categories. This recoding is thought to reduce neural information-processing requirements and to increase the speed and accuracy of critical perceptual judgments (1). Categorization of visual and auditory stimuli by humans has been widely documented, especially in speech perception where the partitioning process compensates for the variability in our pronunciation of words (2, 3). Much animal research has been devoted to identifying vocal features involved in species recognition (4), but little is known about whether animals categorize vocal signals of their own species, as humans do (5).

One hallmark of stimulus categorization is the "category boundary effect" (3) in which the discriminability of physically equivalent steps along a stimulus continuum is nonuniform. As a result, stimulus pairs

astride a category boundary are distinguished more readily than stimulus pairs within a category. Thus, one approach to identifying categorical perception is to measure how well subjects discriminate equal differences along a stimulus continuum (3). An alternative approach used here is to assess how stimulus variation influences natural, unconditioned responses. This approach poses a different question by testing which stimulus variants are meaningfully different instead of simply discriminably different. The dependence upon naturally occurring responses rather than arbitrary operant responses serves to reveal the nature of perceptual categories that are meaningful within a communication system.

Research indicated a possible role for categorization in birdsong perception. Songs of the swamp sparrow (*Melospiza*

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georgiana) consist of a 2-s trill of identical "syllables," each composed of two to five notes (Fig. 1a). Analyses of songs recorded throughout the species' geographic range revealed that notes can be sorted into approximately six types or categories with some intergradation between them (6, 7). Two note categories in particular play distinct roles in syllable structure in different populations. In New York, category 1 notes are most common in the initial position of three-note syllables, and category 6 notes occupy the terminal position. In songs from northern Minnesota, this note-order rule is reversed: category 6 notes predominate in the first position and category 1 notes in the terminal position (6, 8).

Population differences in note usage are perceptually important to swamp sparrows (8). New York birds of both sexes respond more strongly to songs with New York patterning than to songs with the same notes rearranged into the Minnesota pattern. These three observations—variation within note categories, population differences in note usage, and behavioral salience—suggested that acoustic distinctions between note categories are sufficiently critical to be perceived by swamp sparrows categorically rather than continuously.

Swamp sparrow song notes are simple frequency sweeps, varying in duration, minimal and maximal frequencies, and in the rate and direction of frequency modulation. By

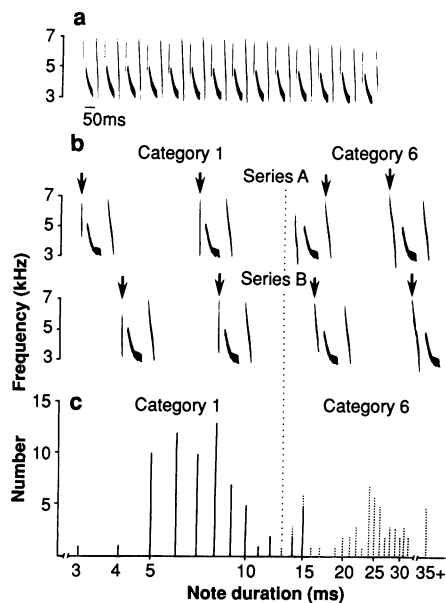


Fig. 1. (a) A complete swamp sparrow song composed of repetitions of a three-note syllable. (b) The two stimulus series of syllables differing in initial note duration. Syllables are aligned according to the duration of the initial note, indicated by arrows. (c) Distribution of category 1 (solid bars) and 6 (broken bars) notes according to note duration. The dotted line at 13 ms denotes the optimal decision boundary between note categories based on duration.

linear discriminant analysis we identified the acoustic characteristic most effective in classifying category 1 and category 6 notes (9). By note duration alone we were able to classify 93% of these two note categories. Geometric mean durations were 6.6 ms for category 1 notes and 25.1 ms for category 6 notes. There was variation within note categories, with some intergradation. Notes shorter than 13 ms, the optimal classification boundary, were classified as category 1, and longer notes as category 6 (Fig. 1c).

If swamp sparrows perceive song notes categorically, the auditory contrast between two notes on opposite sides of the 13-ms boundary should be more salient than equally different pairs of within-category notes taken from either side of the boundary. We predicted that territorial reactions of males to playback of an array of songs incorporating notes forming a continuum between categories 1 and 6 would vary nonmonotonically. Alternatively, if perception of note duration is continuous, the response function should be monotonic.

To create experimental songs, we selected two series of four notes each, taken from our sample of natural songs. In series A the notes were 3, 7, 14, and 27 ms long; in series B durations were 4, 8, 16, and 31 ms (Fig. 1b) (10). The two shortest notes in each series fell within the category 1 range and the two longest were within the category 6 range. The second and third notes in each series straddled the boundary between categories 1 and 6. Each was edited into first position in a song syllable, with two other notes, the same in all cases, in second and third positions. The resulting eight three-note syllables differed only in the first note (Fig. 1b) (11). Each was repeated to create 16-syllable songs of normal duration.

A habituation procedure was used to compare territorial responses of wild male swamp sparrows to pairs of test songs (12). One song was played, at the rate of four songs per minute, in 3-min-long blocks, with each block followed by 3 min of silence. Playback and silence alternated until responses habituated by at least 75% of the value in the first playback block, for two consecutive blocks. Two blocks of a second song followed, alternating with silence. Initial notes in the first (habituation) song and the second (test) song differed by a single step within a series. The extent of revival of the habituated response by the test song reflects the degree of perceptual contrast of the two songs. All 12 possible combinations of song pairs (counterbalanced by order) were used twice, along with eight controls in which habituation and test songs were identical, making 32 comparisons possible. These comparisons fell into four groups:

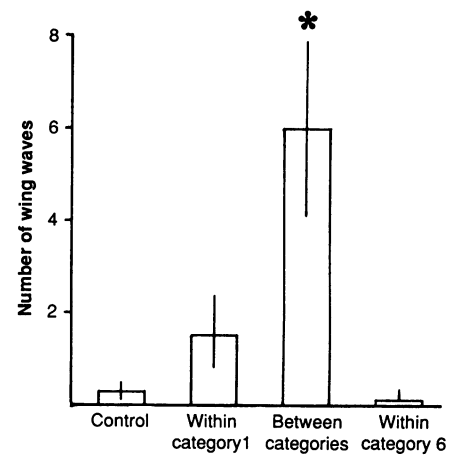


Fig. 2. Means (\pm standard errors) of territorial responses to the first block of playback of a test song after switching from a habituation song. Responses were significantly higher ($P < 0.05$, Kruskal-Wallis ANOVA) when habituation and test songs contained initial notes of different categories (between-categories 1 and 6, $n = 8$), than when both songs were identical (control, $n = 7$) or contained different initial notes taken from the same category (within-category 1, $n = 8$; within-category 6, $n = 7$).

controls; within-category-1 pairs; between-category 1 and 6 pairs; and within-category-6 pairs (13). The response measured was the frequency of the aggressive "wing-wave" display, in which male sparrows raise and flutter one or both wings, often with crest erection and vocalization (14). Thirty individuals responded and were used in the analysis.

Responses differed significantly among groups (Fig. 2) [$\chi^2(3) = 10.9$, $P = 0.01$], and the most were given by the between-category group. The two within-category groups and the controls did not differ significantly from one another (15).

These results show that territorial male swamp sparrows partition this natural continuum of notes, at the boundary identified by acoustic analysis, into two categories known to play different roles in song organization. Thus, sounds that are natural units in song production are also natural perceptual units. We suggest that categorization allows birds to assign stimuli quickly and accurately to the correct note category, thereby resolving ambiguities resulting from intergradations between categories (Fig. 1c). The reliability of judgments made by birds about the categorization and sequencing of song notes is thus augmented by categorical perception, much as humans enhance sensitivity to phonemic contrasts in speech.

REFERENCES AND NOTES

1. E. Rosch, in *Cognition and Categorization*, E. Rosch and B. B. Lloyd, Eds. (Erlbaum, Hillsdale, NJ, 1978), pp. 27–48; R. J. Herrnstein, in *Animal*

Cognition, H. L. Roitblat, T. G. Bever, H. S. Terrace, Eds. (Erlbaum, Hillsdale, NJ, 1984), pp. 233–261.

2. A. M. Liberman, F. S. Cooper, D. P. Shankweiler, M. Studdert-Kennedy, *Psychol. Rev.* **74**, 431 (1967); P. K. Kuhl, *Brain Behav. Evol.* **16**, 374 (1979); S. Hamad, Ed., *Categorical Perception* (Cambridge Univ. Press, Cambridge, 1987).
3. C. C. Wood, *J. Acoust. Soc. Am.* **60**, 1381 (1976); B. H. Repp, in *Speech and Language: Advances in Basic Research and Practice*, N. J. Lass, Ed. (Academic Press, New York, 1983), vol. 10, pp. 243–335.
4. P. H. Becker, in *Acoustic Communication in Birds*, D. E. Kroodsma and E. H. Miller, Eds. (Academic Press, New York, 1982), vol. 1, pp. 213–252; H. C. Gerhardt, in *The Evolution of the Amphibian Auditory System*, B. Fritsch, M. J. Ryan, W. Wilczynski, T. E. Hetherington, W. Walkowiak, Eds. (Wiley, New York, 1988), pp. 455–483.
5. C. T. Snowdon and Y. V. Pola, *Anim. Behav.* **26**, 192 (1987); G. Ehret and B. Haack, *Naturwissenschaften* **68**, 208 (1981); N. Masataka, *Primates* **24**, 40 (1983); B. May, D. B. Moody, W. C. Stebbins, *J. Acoust. Soc. Am.* **85**, 837 (1989).
6. P. Marler and R. Pickert, *Anim. Behav.* **32**, 673 (1984).
7. C. W. Clark, P. Marler, K. Beeman, *Ethology* **76**, 101 (1987).
8. E. Balaban, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 3657 (1988); *Behaviour* **105**, 292 (1988).
9. We measured 220 notes sampled from 59 different syllables recorded from 45 individuals in Dutchess County, New York. Notes were digitized at a sampling rate of 25 kHz at 12-bit precision with a PDP-11/23 minicomputer [Engineering Design, *SIGNAL Software Manual* (Engineering Design, Belmont, MA, 1987)]. Measurement methods are described in D. A. Nelson, *Condor* **91**, 120 (1989). A *k*-means cluster analysis [L. Engelman and J. A. Hartigan, in *BMDP Statistical Software Manual*, W. J. Dixon, Ed. (Univ. of California Press, Berkeley, 1985), pp. 464–473] with four variables (maximal and minimal frequencies, note duration, FM rate) was used to identify 70 category 1 notes and 52 category 6 notes.
10. Durations were chosen to represent approximately equal intervals on a log scale ($\bar{x} = 0.307$, $SD = 0.030$). All notes were frequency down-sweeps taken from natural songs.
11. Notes were stored as files in a digital computer and manipulated using a synthesis program [see Engineering Design in (9)]. A constant 13-ms interval was used after notes in first and second positions, with a 34-ms intersyllable interval, which is similar to the species means for three-note syllables. Song durations ranged from 2212 to 2670 ms.
12. D. A. Nelson, *J. Comp. Psychol.* **101**, 25 (1987).
13. The observer was ignorant of stimulus identity, and the order of presentation was randomized. Field trials were completed between 3 June and 10 July 1988 at three sites in Dutchess County under procedures described in (12).
14. We counted the number of 5-s intervals within each playback block in which at least one wing-wave occurred (36 maximum possible). To correct for individual variation, the number of wing-waves in the block prior to song shift was subtracted from the number of wing-waves in the first block after song shift. A Kruskal-Wallis analysis of variance was used to test for heterogeneity in difference scores among groups, followed by pair-wise comparisons at $P < 0.05$ [W. J. Conover, *Practical Nonparametric Statistics* (Wiley, New York, 1980)].
15. The number of blocks to reach the habituation criterion did not differ among groups (grand mean = 4.8 ± 2.29 blocks, Kruskal-Wallis $\chi^2 = 1.48$, $P > 0.60$).
16. We thank D. Kroodsma for loaning tape recordings. The Institute of Ecosystem Studies at the Mary Flagler Cary Arboretum, Millbrook School, and Dutchess County Infirmary gave permission to work on their properties. Supported by grant MH 14561 to P.M., Biomedical Research Support grant BRSG S07 RR07065 to The Rockefeller University, and a fellowship from the Charles H. Revson Foundation to D.A.N.

28 December 1988; accepted 7 April 1989

Identification of the Molecular Defect in a Family with Spondyloepiphyseal Dysplasia

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Spondyloepiphyseal dysplasias (SED) are a heterogeneous group of inherited disorders characterized by disproportionate short stature and pleiotropic involvement of the skeletal and ocular systems. Evidence has suggested that SED may result from structural defects in type II collagen. To confirm the validity of this hypothesis, the structure of the "candidate" type II collagen gene (COL2A1) has been directly examined in a relatively large SED family. Coarse scanning of the gene by Southern blot hybridization identified an abnormal restriction pattern in one of the affected members of the kindred. Analysis of selected genomic fragments, amplified by the polymerase chain reaction, precisely localized the molecular defect and demonstrated that all affected family members carried the same heterozygous single-exon deletion. As a consequence of the mutation, nearly 90 percent of the assembled type II collagen homotrimers are expected to contain one or more procollagen subunits harboring an interstitial deletion of 36 amino acids in the triple helical domain.

CHONDRODYSPLASIAS, A HIGHLY HETEROGENEOUS group of disorders involving endochondral ossification and displaying abnormal skeletal growth, are believed to result from mutations affecting either the structural integrity of cartilage matrix components or the regulatory pathway of chondrogenesis (1). For example, COL2A1 has been linked to the Stickler syndrome by genetic analysis (2). However, an understanding of the pathogenesis of chondrodysplasias has been greatly hampered by the difficulty in obtaining sufficient amounts of biological material (cartilage) as well as by the inability to maintain differentiated human chondrocytes in culture. Despite these obstacles, biochemical analysis of small cartilage samples from chondrodysplastic individuals has recently suggested that some of these conditions, such as the spondyloepiphyseal dysplasias, Kniest dysplasia, and type II achondrogenesis-hypochondrogenesis, may be associated with type II collagen defects (3). Type II colla-

gen, extracted from cartilage of patients affected by sporadic forms of SED, exhibits abnormalities in SDS-polyacrylamide gel electrophoretic (SDS-PAGE) mobility when compared to normal controls. When cyanogen bromide (CNBr)-cleaved type II collagen molecules from affected cartilage were further examined on SDS-PAGE, decreased electrophoretic mobility of some but not all CNBr peptides suggested an increased degree of hydroxylation and glycosylation progressively NH₂-terminal to the putative site of the mutation. Such a pattern is reminiscent of the metabolic consequences of type I collagen mutations in fibroblasts from osteogenesis imperfecta pa-

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Fig. 1. Eco RI (E) restriction map of COL2A1. Relative positions of exons 45–52, the region spanned by the cDNA clone HC-2, and the oligonucleotides (arrows) used for PCR amplification and sequencing are shown. Below are the respective fragments produced in PCR analysis of the 3.7-kb Eco RI fragment shown in Fig. 2B. Primer sequences, direction of priming (in relation to the coding strand), and positions [referred to the first nucleotide's number in (10)] are as follows: intron 46, nucleotide 1401, forward direction, GAATA-TAGATAGATATGCTGTGCTGACCG; intron 48, nucleotide 2127, reverse direction, AAGAGCT-CAAGCCTCC; intron 48, nucleotide 2386, forward direction, ACAATCCTGGCTGATCTCT; intron 51, nucleotide 3984, reverse direction, AGGCAGTTTGGGCACAGGCAGC; and exon 52, nucleotide 4654, reverse direction, TTGCAACGGATTGTGTTGTTCTG.

