curs in the presence of visual targets. (ii) The appropriate behavior is predominantly visual; about 70 percent consists of scanning. (iii) Although the infants were active, relatively little of their motor activity was directed toward the targets; of this only about half seemed to be coordinated with visual response to the target. (iv) There is virtually no evidence that the behavior toward graspable and nongraspable objects differs in any way. The observed rates of responding in our first experiment were so low that differences for the two target conditions could scarcely have been detected. But in the second experiment higher frequencies of relevant behavior were observed, so that real differences should have been detectable. Only one of the six differences tested was statistically significant, and was small.

Our findings are consonant with an earlier report on the development of visually elicited reaching (*7*). We conclude that, although infants show definite interest when visual targets are displayed, they express their interest primarily through visual exploration; these experiments thus do not support the hypothesis of some form of advanced or higher-order processing of the properties of a distal stimulus. In particular the experiments do not allow the interpretation that infants in the first 2 weeks of life readily differentiate visually presented objects from their representations.

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Sexual Dimorphism in Vocal Control Areas of the Songbird Brain

Abstract. *In canaries and zebra finches, three vocal control areas in the brain are strikingly larger in males than in females. A fourth, area X of the lobus parolfactorius, is well developed in males of both species, less well developed in female canaries, and absent or not recognizable in female zebra finches. These size differences correlate well with differences in singing behavior. Males of both species learn song by reference to auditory information, and females do not normally sing. Exogenous testosterone induces singing in female canaries but not in female zebra finches. This is believed to be the first report of such gross sexual dimorphism in a vertebrate brain.*

In many species of animals, males and females exhibit different patterns of behavior, especially in contexts related to courtship and reproduction (*1*). Recent evidence suggests that structural differences in male and female central nervous systems may contribute to these differences in behavior (*2*). We have discovered a striking sexual dimorphism in song control areas of the brain of the canary (*Serinus canarius*) and the zebra finch (*Poephila guttata*), which can be related to behavioral differences between the two sexes.

Adult male canaries have a complex song repertoire learned by reference to auditory information (*3*, *4*). Female canaries do not normally sing, although they will produce a song similar to that of the males when administered testosterone (*5*, *6*); the song, however, is considerably less varied than that of males (*6*). Female canaries also produce a variety of other calls (*7*), and, as in the case of other carduelines (*8*), some of these calls may be learned.

Male zebra finches have a single song type, which, as in the canary, is devel-

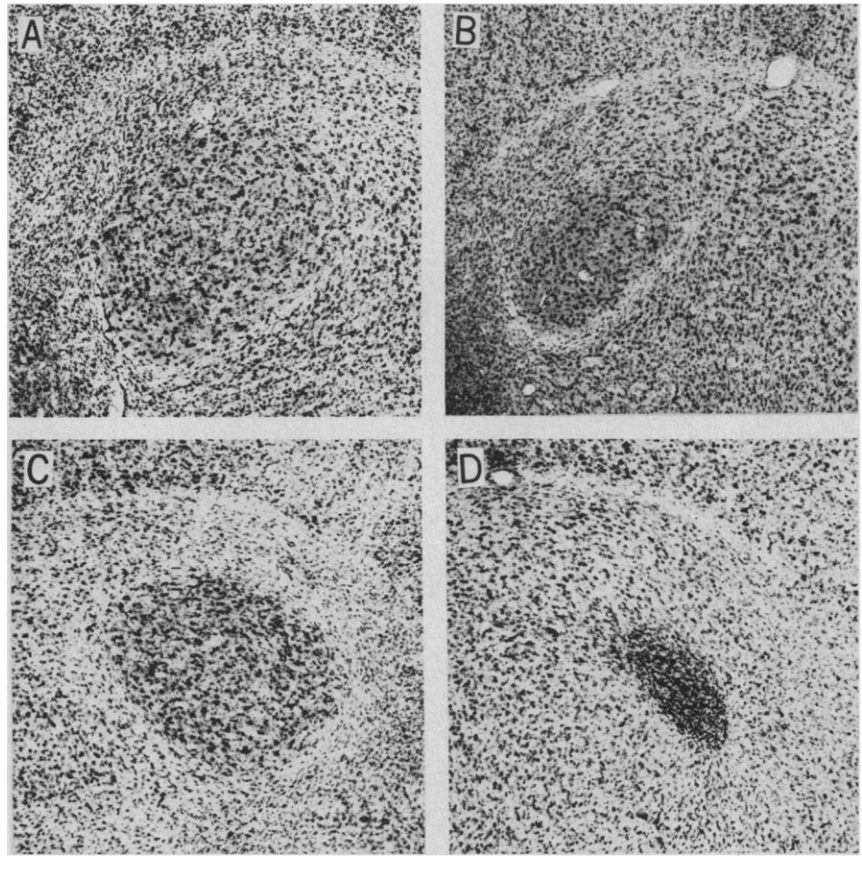

Fig. 1. Frontal sections through the robust nucleus of the archistriatum (RA) in a male (A) and female canary (B) and a male (C) and female zebra finch (D). The canary photographs are from the left hemisphere, and those of zebra finch are from the right. For each of the four birds shown, the rostro-caudal level corresponds to the largest area of RA seen in this plane of section. The relatively unstained eyebrow-shaped structure is the lamina archistriatalis dorsalis, which separates the neostriatum (dorsal) from the archistriatum (ventral). The prominent ellipsoidal nucleus is RA. Cresyl violet–stained sections, 50 μm thick (×42).

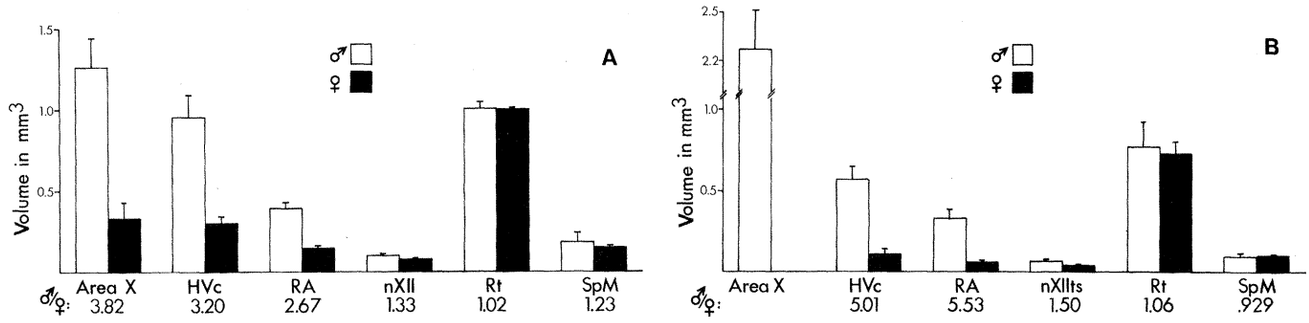

Fig. 2. The volumes occupied by four neural regions associated with vocal behavior (area X, HVc, RA, and nXII) and by two regions not associated with vocalization (Rt and SpM) in male and female canaries (A) and zebra finches (B). Each bar represents the mean of the total (right plus left) volumes of each area sampled (*18, 26*), and the vertical line above the bar is the standard deviation of the individual values. The ratio of the male to the female mean is given for each region.

oped by reference to auditory information (*9*). Female zebra finches do not sing even when testosterone propionate is implanted when they are adults (*10*). The vocal repertoire of female zebra finches is otherwise small, consisting of contact and enticement notes produced in a variety of circumstances (*11*). Because of this simplicity, the calls may develop normally in the absence of auditory models.

Five adult male canaries, five adult female canaries, six adult male zebra finches, and seven adult female zebra finches (*12*) were anesthetized and then perfused with saline followed by 10 percent formalin in physiological saline. The brains were removed, blocked, fixed, embedded, and sectioned; the sections were mounted on glass slides (*13, 14*). All brains were weighed before being embedded (*15*). Serial sections cut at 10 to 50 μm were mounted, stained with cresyl violet for cell bodies or silver stain (Fink-Schneider) (*14*) for unmyelinated nerve fibers, and viewed under the light microscope. The volume of certain brain structures was measured as follows. A microprojector (Bausch and Lomb) projected magnified (×53) images of cresyl violet–stained sections on drawing paper. A perimeter was then drawn around the regions of interest, and the area enclosed was measured with a polar planimeter. These areas were then multiplied by the thickness of the sections, and the resulting volume was corrected for the frequency of sampling (for example, multiplied by 2 if every other section was measured). The sum of all such products for a given brain region was an estimate of its volume.

We made drawings of four cytoarchitectonically distinct brain structures: (i) area X of the lobus parolfactorius (LPO), (ii) the hyperstriatum ventrale, pars caudale (HVc), (iii) the robust nucleus of the archistriatum (RA) (Fig. 1), and (iv) the hypoglossal nucleus of the medulla (nXII) (*16*). Nottebohm *et al.* (*4*) have described these structures in the canary and presented anatomical and behavioral evidence that they are part of the vocal control system of the canary brain. The brain of the male zebra finch includes areas that are similar to these four regions in position and cytoarchitecture; we thus presume that they have a similar role in vocalization (*17, 18*). We also drew and estimated the volume of two thalamic brain structures not related to vocal control, the nucleus rotundus (Rt) and the nucleus spiriformis medialis (SpM), which were chosen because of their discrete boundaries (*19*).

In both the canary and the zebra finch, the four vocal control areas are markedly larger in males than in females ($P < .02$, two-tailed t-test) (Figs. 1 and 2). These differences are highly significant. That there were no such differences in the volume estimates of the two structures not related to vocal control or in total brain weight ($P > .2$) (*15*) suggests that the differences are specific to song areas and related to a sexual difference in vocal behavior. This conclusion is also supported by the observation that the sexual differences in volume are more marked in zebra finches than in canaries, as would be expected from the total absence of song in female zebra finches.

In zebra finches, we detected no differences between the right and left sides of any of the four vocal and two nonvocal areas. Similarly, there was no systematic difference between the volumes of the right and left area X, HVc, RA, Rt, and SpM in canaries. This result is intriguing in light of the marked left hemispheric dominance for vocal control described for this species (*4, 20*). There was significant difference between the volumes of the right and the left hypoglossal nuclei of canaries (*21*). The larger size of the left side in all ten male and female canaries may be related to the left hypoglossal dominance for song control in this species (*22*).

The size ratio of male to female canary brain areas increases as one goes from the hypoglossal nucleus to RA, HVc, and area X, that is, as one goes to structures further removed from the motoneurons innervating the syringeal musculature (*23*). This graded series of ratios suggests that the "higher" (that is, further removed) neural regions in this system are involved in some aspect of vocal performance in males that is specifically absent or underrepresented in females. It may be that the higher centers are responsible for a disproportionately large share of the neural operations controlling vocal learning and size of vocal repertoire; therefore, in females, who normally do not sing, these areas should be less well represented. The zebra finch brain departs from this pattern in that the volume ratio of male to female brains is somewhat smaller in HVc than in RA (Fig. 2), and area X is not recognizable in the female.

In both male and female canaries, area X contains larger cell clusters than the surrounding LPO; perhaps as a result, cresyl violet stains area X darker than the surrounding tissue (*4*). In Fink-Schneider stains of unmyelinated fibers, area X is discriminable from the rest of LPO because it contains a rich mesh of fibers, some of which are projections from HVc (*24*). Area X in male zebra finches is similar in these respects [see also (*19*)]. However, in the corresponding area of the female zebra finch brain area X is not recognizable, which suggests that it is grossly modified or absent. We have assumed it to be absent (Fig. 2). It is not clear how the disproportionately large size of area X in the male zebra finch compared with that of the canary might relate to differences in behavior.

From the extent of the sexual differences in the volumes of the vocal areas,

we infer that sexual differences are not confined to cell size alone (Fig. 1). The density of cell packing and the amount of neuropil in RA and HVc also differ between males and females (*25*). Males, with a greater commitment to vocal learning, also have more neuropil. The sexual dimorphism in vocal areas of these two songbird species may be related to the fact that, whereas males of both species learn their song by reference to auditory information, females do not normally sing.

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10. On occasion an intact adult female that has had a pellet of testosterone propionate implanted will produce a repetitive vocalization not otherwise heard from members of this species. The manner of delivery of such a sound is reminiscent of song but its physical characteristics differ greatly from normal song [A. P. Arnold, thesis, Rockefeller University (1974)].
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12. Wasserschlager canaries of our own inbred stock ranged in age from 19 to 34 months old. All but two of the zebra finches were descendants of a single pair of domesticated birds and were between 29 and 56 months (usually 29 to 31 months) old.
13. T. M. Stokes, C. M. Leonard, F. Nottebohm, *J. Comp. Neurol.* **156**, 337 (1974). The method was modified in some cases. All canary brains were embedded in gelatin albumin and stained with cresyl violet. They were sectioned serially at 25 or 50 μm, and drawings were made of neuronal regions in single sections at intervals of 100 and 125 μm. All drawings of canary brains were made without prior knowledge of the sex of the animal. Of the zebra finch brains, five male and six female brains were embedded in gelatin albumin, sectioned serially at 25 or 50 μm, and stained with cresyl violet. Drawings were made from sections taken at intervals of 50 to 100 μm. One male and one female zebra finch brain were embedded in paraffin, sectioned serially at 10 μm, and stained with cresyl violet. Of the gelatin albumin–embedded brains of zebra finches, one male and one female brain were sectioned serially at 25, 50, 25, 50 μm, etc., and the 25-μm sections were stained for unmyelinated fibers by the Fink-Schneider method (*14*).
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15. Canary brains were weighed after being fixed in sucrose-formalin, and zebra finch brains were weighed after being fixed in formalin. There was no statistical difference between the two sexes of either species in brain weights (two-tailed *t*-test, $P > .2$). Means and standard deviations of brain weights were 0.74 ± 0.09 g (male canary), 0.68 ± 0.05 g (female canary), 0.53 ± 0.04 g (male zebra finch), and 0.51 ± 0.04 g (female zebra finch).
16. In the case of the motor nucleus of the hypoglossus (nXII), the volume of the entire nucleus was measured in the canaries. It includes an anterior portion innervating the tongue, and a caudal portion (tracheosyringeal portion, nXIIts), which is composed of the motoneurons innervating the vocal organ (syrinx). In the zebra finches, only the volume of nXIIts was measured. This was possible because in each of five males and females, the tracheosyringeal branch of the hypoglossus had been cut unilaterally (three male-female pairs on the left, two on the right) 7 to 9 days before the birds were killed. Under this procedure, the ipsilateral syringeal motoneurons of nXIIts become chromatolytic and swell and serve as a guideline for the limits of nXIIts on the nonchromatolytic side. Since the swelling also enlarges the volume of nXIIts, the values presented in Fig. 2 are derived from twice the volume of the nonchromatolytic side of nXIIts for each zebra finch. In both species the perimeter drawn around the motor nucleus circumscribed all of the motoneuron cell bodies but not the neuropil that surrounds the motor nucleus.
17. The motor nucleus nXIIts innervates the syrinx in both canaries and zebra finches (*4*, *19*).
18. A. Arnold, F. Nottebohm, D. W. Pfaff, *J. Comp. Neurol.* **165**, 487 (1976).
19. In the pigeon, Rt is part of the tectofugal visual pathway [H. J. Karten and A. M. Revzin, *Brain Res.* **2**, 368 (1966); A. M. Revzin and H. J. Karten, *ibid.* **3**, 264 (1966–1967)], and SpM receives input from the telencephalon and projects to the cerebellum [H. J. Karten and T. E. Finger, *ibid.* **102**, 335 (1976)].
20. F. Nottebohm, in *Lateralization in the Nervous System*, S. R. Harnad *et al.*, Eds. (Academic Press, New York, in press).
21. In females, the mean volume of nXII on the right was 0.0332 ± 0.0021 mm^3 (mean ± standard deviation), compared with 0.0436 ± 0.0053 mm^3 on the left (two-tailed *t*-test, $P < .001$). In males, the volume was 0.0495 ± 0.0074 mm^3 on the right and 0.0526 ± 0.0075 mm^3 on the left ($P < .02$). Since neurons on one side of nXIIts were swollen and chromatolytic in the zebra finches (*16*), we could not assess any possible differences in volume between the sides of the brain in that species.
22. F. Nottebohm and M. Nottebohm, *J. Comp. Physiol.* **108**, 171 (1976).
23. The syringeal musculature is larger in male canaries and zebra finches than in females; we have not calculated the male/female ratio of syringeal muscle volume.
24. Observations (*4*) are of male canaries. As in males, area X of female canaries also receives a discrete fiber projection from the ipsilateral HVc (F. Nottebohm, unpublished observations).
25. F. Nottebohm, in preparation; A. P. Arnold, in preparation.
26. In Fig. 2, $N = 5$ for measurements on canaries, and $N = 4$ to 7 for zebra finches.
27. We thank C. M. Leonard and P. Marler for discussion of our results and Y. Holland for technical assistance. Supported by NIMH grant 18343 to F.N. A.P.A. was a NIMH postdoctoral fellow (No. 00559).
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Relaxation Time versus Water Content: Linear or Nonlinear?

A recent report by Beall *et al.* (*1*) concerns the relation between spin-lattice relaxation time T_1 and the water content in biological cells. Referring to their figure 2B they state that "It appears that within narrow physiological limits" (presumably for $4 \leq H_2O$/dry solids ≤ 7) "the changing cellular water content and T_1 are linear, but when gross morphological changes become visible, this relationship deviates from linearity."

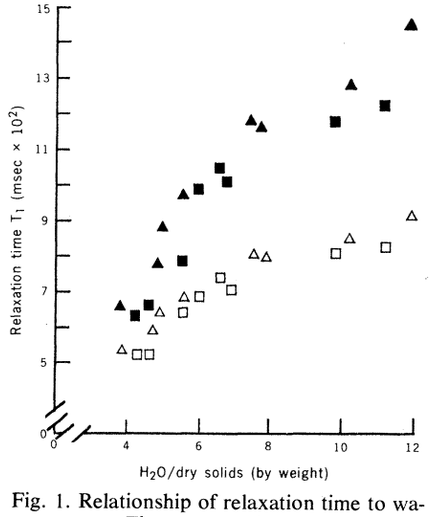

Fig. 1. Relationship of relaxation time to water content. The open symbols refer to original data as given by Beall *et al.* [figure 2B in (*1*)]. The closed symbols refer to the corrected relaxation time. Triangles (open and closed) denote Chinese hamster ovary cells; squares (open and closed) denote HeLa cells.

The purpose of this comment is to point out that under a correct interpretation of "linearity," these data of Beall *et al.* support rather than negate the implied linear relationship. Obviously, T_1 cannot be a linear function of $x = H_2O$/dry solids. This is so since for $x \to \infty$ (bulk water) one must have $T_1 \to T_{free} \cong 2500$ msec. The correct "linearity" relation, alluded to in (*1*), is that of the so-called "two-fraction fast-exchange" model (*2*). One can interpret one fraction as bound water and the other fraction as free water. Then

$$\frac{a+b}{T_{obs}} = \frac{a}{T_a} + \frac{b}{T_b} \qquad (1)$$

where T_{obs} is observed value of T_1, T_a is bound water value of T_1, T_b is free water value of $T_1 \cong 2500$ msec, a is weight of bound water, and b is weight of free water. Hence

$$T_{corr} = \frac{a+b}{a} \quad (1/T_a - 1/T_b)^{-1} \qquad (2)$$

where

$$T_{corr} = (1/T_{obs} - 1/T_b)^{-1} \qquad (3)$$