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)].
11. K. Immelmann, *Zool. Jahrb. Abt. Allg. Zool. Physiol.* **90**, 1 (1962).
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*).
14. G. E. Schneider, *Science* **163**, 895 (1969).
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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28 May 1976

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."

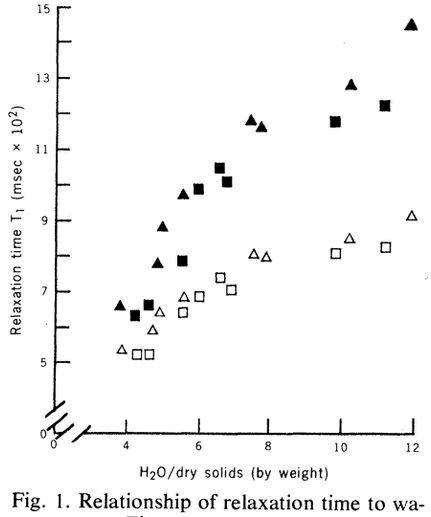

Fig. 1. Relationship of relaxation time to water content. The open symbols refer to original data as given by Beall *et al.* [figure 2B in (*1*)]. The closed symbols refer to the corrected relaxation time. Triangles (open and closed) denote Chinese hamster ovary cells; squares (open and closed) denote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

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

Thus it is the *corrected* value of T, T_{corr}, as given by Eq. 3, that is linear in $(a + b)/a \propto x$ (note that a remains fixed as water is added, and $a + b$ is the total weight of the water).

To test this, values of T_{obs} were taken from figure 2B of (*1*); the corresponding corrected values were then calculated from Eq. 3 with $T_b = 2500$ msec. In Fig. 1 we plot T_{corr} versus x; also shown are the original data. It is our contention that the corrected graph supports the linear theory.

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7 June 1976

In our report (*1*), we did not consider it necessary to present a detailed analysis of T_1 as a function of cellular hydration as suggested by Brownstein and Tarr for three reasons. First, a plot of $1/t_{1(obs)}$ versus $1/x$ (where x is grams of H_2O per gram of dry solids) did not prove to be linear over the entire range of hydration. Second, Raaphorst *et al.* have shown that, as the concentration of water in CHO cells is decreased, the T_1 values for the water protons first decrease and then increase [see figure 3 of (*2*)]. Third, the relaxation times of water protons were shown to change independently of cellular hydration during the HeLa cell cycle [see figure 2A of (*1*)].

The analysis of Brownstein and Tarr demonstrates a linear relationship between their T_{corr} and hydration over the range of 4 to 8 g of H_2O per gram of dry solids. Above 8 g of H_2O per gram of dry solids, there is a change in slope (see their figure). This is the exact observation we made. Therefore, their comment does not add anything new.

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23 August 1976

Serotonin Depression

Åsberg *et al.* (*1*) have observed that the distribution of spinal fluid 5-hydroxyindoleacetic acid (5-HIAA) in some depressed patients was bimodal and inferred that there may be a subgroup of patients with "serotonin depression." There are alternative explanations for this result.

Brain serotinin is known to have a very large 24-hour rhythm (*2*), comparable to the range of variation noted by Åsberg *et al.* Furthermore, there has been speculation that the phase of 24-hour rhythms may be altered in depressed patients. The bimodal distribution reported by the authors could thus be caused by a bimodal distribution in the phases of 24-hour serotonin rhythms without any alteration in total 24-hour serotonin turnover. Furthermore, since one obtains a bimodal distribution by sampling any sinusoidal or square-wave rhythmic function at random phases, a similar result might be obtained if the phases of 24-hour serotonin rhythms were random in depressed patients and uncoupled from social synchronizers.

There is evidence that serotonergic neurons mediate the appearance of slow-wave sleep (*3*). Many depressed patients may sleep poorly at night, but some also nap during the day. Therefore, if a rhythm disturbance is critical, one might expect 5-HIAA levels to correlate better with measures of sleep than with the overall depression ratings, especially correlating cerebrospinal fluid (CSF) 5-HIAA with the amount of sleep in the hours immediately before the CSF sample is obtained. While it would be most interesting to quantify slow-wave sleep electroencephalographically, it might be sufficient to estimate the patients' sleep subjectively to test whether sleep timing accounts for the heterogeneity of CSF 5-HIAA in depressed subjects.

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12 March 1976; revised 21 July 1976