versus 163.3; matched pair $t(5) = 4.194$, $P < .01$). For the first 8 hours of the test, response rates were similar; then responses to the paddle that produced stimulation increased (Fig. 1B). Individual animals required varying amounts of time to form the discrimination. Those whose initial rate of responding was high acquired the discrimination more rapidly. Some animals learned in as little as 3 hours; others took as long as 15 hours. In general, pups did not increase their rate of responding to the paddle with the reward until they had received approximately 75 stimulation trains. This was also the case in the experiments with a single paddle.

Electrode placement was verified histologically, as shown in Fig. 2. Only electrodes in the medial forebrain bundle in the area of the lateral hypothalamus support the performance of the operant task. This is a site which, in the adult, has been shown to support very high rates of intracranial self-stimulation (12). Placements that were either medial or dorsal to this location were ineffective (13) during the pretest even at a current range of 30 to 80 μA, and the response rates of these animals did not increase. Thus there is a strong correlation between electrode placement, behavior during the pretest, and learning the operant response.

This work indicates that stimulation of the medial forebrain bundle can reinforce behavior in 3-day-old rat pups. Brain sites that support self-stimulation in adults correspond to projections of catecholamine pathways, and reinforcement is thought to be mediated by the activation of dopamine neurons (14). Development of central norepinephrine and dopamine systems, however, is far from complete in 3-day-old pups. Density of terminals is only 15 to 40 percent of adult levels (1) and, on the basis of axotomy studies, neuronal activity is not present in dopamine pathways as late as 6 days of age, even though these pathways are capable of generating and conducting impulses (15). Either self-stimulation behavior is mediated by another system in the pup, an unlikely possibility considering the similarity of supporting site, or the development of the catecholamine pathways is sufficient to mediate self-stimulation at this age when these pathways are electrically activated.

TIMOTHY H. MORAN
MARK F. LEW
ELLIOTT M. BLASS
Department of Psychology,
Johns Hopkins University,
Baltimore, Maryland 21218

References and Notes

1. L. Loizou, *Brain Res.* **40**, 395 (1972); W. Porcher and A. Heller, *J. Neurochem.* **19**, 1927 (1972); J. T. Coyle and D. Henry, *ibid.* **21**, 61 (1973).
2. J. B. Wirth and A. N. Epstein, *Am. J. Physiol.* **230**, 188 (1976); W. G. Hall, *J. Comp. Physiol. Psychol.* **93**, 977 (1979).
3. J. W. Rudy and M. D. Cheatle, *Science* **198**, 845 (1977).
4. J. T. Kenny and E. M. Blass, *ibid.* **196**, 898 (1977).
5. I. B. Johanson and W. G. Hall, *ibid.* **205**, 419 (1979).
6. J. Olds, *ibid.* **127**, 315 (1958); C. R. Gallistel, in *The Physiological Basis of Memory*, J. A. Deutsch, Ed. (Academic Press, New York, 1973).
7. Only pups weighing between 10.0 and 11.0 g were used in all experiments.
8. The stereotaxic coordinates, taken from the confluence of the midsagittal suture and lambdoidal fissure, were 2.0 mm anterior to the bregma, 1.2 mm lateral to the midline, and 6.5 mm below the skull surface.
9. Results of the threshold test were used as the basis for including pups in the study. Pups responded to brain stimulation by displaying a progressive behavioral sequence. In response to single stimulation trains pups showed mouthing and chewing. Vigorous activation, licking, and stretch responses were emitted to multiple stimulation trains. These responses were a reliable predictor of successful performance in the operant task.
10. Response rates of yoked pups decreased slightly over the test, a result which is not surprising since yoked controls were essentially being reinforced for remaining on the floor of the cup.
11. In some instances pups emitted fewer than 20 responses in the first 10 hours of the test period. Results from these pups were discarded from the study.
12. Y. H. Huang and A. Routtenberg, *Physiol. Behav.* **7**, 419 (1971); E. T. Tolls, in *Brain-Stimulation Reward*, A. Wanquir and E. Rolls, Eds. (North-Holland, Amsterdam, 1976), p. 65.
13. Placements anterior and posterior to this level were effective within a range of 0.2 mm. One placement posterior to this range was ineffective.
14. D. C. German and D. M. Bowden, *Brain Res.* **73**, 381 (1974); C. D. Wise, *ibid.* **152**, 215 (1978).
15. J. C. Cherons, L. Erinoff, A. Heller, P. C. Hoffman, *ibid.* **169**, 545 (1979).
16. We wish to thank M. Williams for her expert histology. This work was supported by grant AM 18560 from the National Institute of Arthritis, Metabolism, and Digestive Diseases (to E.M.B.).

23 July 1981; revised 28 August 1981

A Brain for All Seasons: Cyclical Anatomical Changes in Song Control Nuclei of the Canary Brain

Abstract. *Male canaries that have reached sexual maturity can, in subsequent years, learn new song repertoires. Two telencephalic song control nuclei, the hyperstriatum ventrale, pars caudale, and nucleus robustus archistriatalis are, respectively, 99 and 76 percent larger in the spring, when male canaries are producing stable adult song, than in the fall, at the end of the molt and after several months of not singing. It is hypothesized that such fluctuations reflect an increase and then reduction in numbers of synapses and are related to the yearly ability to acquire new motor coordinations.*

The song of adult male canaries is a motor skill learned by improvisation (1) and by imitation of other males (2), in either case requiring intact hearing and access to auditory information (3). A male canary has the potential to learn on successive years new and different song repertoires (4). In the following experiment I have tried to identify brain changes in adulthood that relate to this yearly learning of a motor skill.

First-year male canaries (5) hatched in April develop stable adult song by mid-January, when 9 months old. The song patterns developed at that time last for the duration of the breeding season, until approximately mid-June. Canaries sing little if at all during the summer months. A total absence of song characterizes the period of the molt, lasting roughly from mid-August to mid-September. As the molt ends, male canaries start to sing once more, first in the tentative, highly variable manner typical of early plastic song. By early January, birds well into their second year of life have developed a new, stable song repertoire (4).

In the experiment described here, 21 male canaries hatched in mid-April were used. At 10.5 months of age they were caged singly. Nine of these birds were killed the following April, when 12 months old. These birds were then in full reproductive condition and were producing stable adult song. The remaining 12 canaries were paired with females and allowed to breed (6), then killed 5 months later, in mid-September, toward the end of the molt, when 17 months old (7). Blood (1/2 ml) was obtained by intracardiac puncture before birds died (8). The testes and brain were removed after perfusion (9).

Spring and fall volumes were obtained for each of the following brain structures (10–12): two telencephalic nuclei involved in song control, the hyperstriatum ventrale, pars caudale (HVc), and the nucleus robustus archistriatalis (RA) (13); two discreet midbrain nuclei not known to be involved in song control, nucleus rotundus (Rt) and spiriformis medialis (SpM) (14); and the caudal forebrain at the level of HVc, referred to subsequently as caudal forebrain volume (15). This last measurement was taken in order to get an impression of the size of the telencephalon over the rostro-

caudal reaches that include nucleus HVc.

Testis volume and blood androgen levels showed marked seasonal differences, as expected (16).

There was no significant difference between the right and left HVc and between the right and left RA volumes for the birds in the spring and fall samples. Then, the seasonal comparisons correspond to the summed values of the two sides (17).

The ratio of spring to fall (spring:fall) volume for each of the brain anatomical measures and for brain weight is shown in Table 1. Except for the seasonal difference in volume of nucleus SpM, all other differences were significant (18).

It is hard to believe that whole brain volume could change seasonally. Yet, if the values obtained for brain weight, caudal forebrain volume, and the volume of the thalamic nucleus Rt are taken at face value, it seems that much of the brain undergoes a significant reduction in volume, from spring to fall, of the order of 15 to 20 percent. Such wholesale seasonal brain changes could be artifactual, however. Birds with larger brains could have been placed, by chance, in the spring rather than in the fall sample. Alternatively, some unknown factor in the treatment of tissues could have caused greater shrinkage or swelling in one of the seasonal groups.

To correct for these possibilities, two subsets of birds were formed. The spring subgroup was composed of the five spring birds with the lightest brains. The fall subgroup was composed of the five fall birds with the heaviest brains. When this was done, the mean brain weights of birds in the two subgroups differed by only 2 percent, yet the spring:fall ratio of HVc and RA volumes remained high, at 1.86 and 1.52, respectively, and significant. Seasonal differences in the volume of Rt, SpM, and caudal forebrain retained their sign, but became smaller and ceased to be significant.

Two other subgroups were formed, this time by choosing the five spring birds with the smallest caudal forebrain volume and comparing them with the five fall birds with the largest caudal forebrain volume. The two subgroups matched in this manner had caudal forebrain volumes that differed by only 2 percent, yet the spring:fall ratio of HVc and RA volumes remained high and significant, at 1.91 and 1.60, respectively. The seasonal differences for Rt, SpM, and brain weight were much smaller and not significant.

The results of comparing subgroups

matched for brain weight and caudal forebrain volume allow us to rule out the possibilities that the seasonal differences in HVc and RA volume resulted from groups unevenly matched for brain weight or that such differences resulted from swelling and shrinkage due to histological artifact.

To establish beyond doubt the reality of the observed seasonal differences in the volume of telencephalic vocal control nuclei, HVc and RA values were normalized by dividing them, for each bird, by the corresponding volume of nucleus Rt. The underlying assumption was that Rt volumes are free from seasonal fluctuations and that they are exposed to the same interactions with brain size and histological artifact as the vocal control nuclei (19). The mean ratio of HVc:Rt for the spring group was 0.764, and for the fall group, 0.463 ($P < .001$). The corresponding mean values for RA:Rt for the spring were 0.608, and for the fall, 0.385 ($P < .001$). The spring:fall ratio for HVc:Rt (0.764:0.463) was 1.65; that for RA:Rt was 1.58. Thus, even when normalized in this manner, HVc and RA were at least 65 and 58 percent, respectively, larger in the spring than in the fall.

In an earlier study (12) it was shown that the volume of HVc and RA does not differ significantly when comparing 2- or 3-year-old male canaries with 1-year-old males. These birds had been killed at the end of the breeding season, when the ratio of HVc volume to Rt volume was 0.735. From this it can be inferred that the spring-to-fall reduction in volume observed in this study would be reversed in the following spring. We cannot tell from the present data whether the spring-to-fall change in HVc and RA volumes occurs on subsequent years.

The extent of HVc and RA volume changes reported here is strikingly similar to that reported for ovariectomized females receiving in adulthood physiological doses of testosterone. This treat-

ment induces adult female canaries to sing in a male-like manner (20). When such testosterone-treated females are compared with cholesterol-treated controls, HVc and RA are, respectively, 90 and 53 percent larger in the testosterone- than in the cholesterol-treated group (21). This increase in volume has been related in nucleus RA to a testosterone-induced growth of extra dendritic length (22). An addition of dendritic length, we may assume, leads to the formation of new synapses. Perhaps the nature of the seasonal changes observed in males, going from spring to fall, is comparable but of reverse sign to that induced by testosterone in adult females.

Since male canaries can learn a new song repertoire every year, one may argue that the seasonal swelling and shrinking of forebrain nuclei involved in song control is related to the seasonal learning and forgetting of a song repertoire (23). If this view is correct, there should be no seasonal changes in HVc or RA volume in species that do not show a yearly change in song repertoire. Evidence in support of this prediction comes from work with zebra finches (24). A temporal relation between song learning and growth of vocal control nuclei is also observed during ontogeny. Juvenile canaries acquire their song at an age when both HVc and RA are showing marked and sustained growth (25), and this relation also applies to young zebra finches (26).

I hypothesize that the acquisition of a new motor coordination or of a new auditory-motor integration is made possible or facilitated by the growth of new dendritic segments and the consequent opportunity to form new synapses. The plasticity offered by such a scheme is potentially twofold: to allow for the formation of new interneuronal relations, and to bring into existence synapses that have not yet been altered by previous patterns of use. Seasonal changes in the volume of HVc and RA may reflect the

Table 1. Ratio of spring to fall measures of brain variables.

| Variable | Mean ± standard deviation | | P | Spring: fall ratio |
	Spring	Fall		
HVc* (mm³)	0.884 ± 0.243	0.444 ± 0.105	< .001	1.99
RA* (mm³)	0.519 ± 0.114	0.293 ± 0.058	< .001	1.77
Rt† (mm³)	0.572 ± 0.056	0.481 ± 0.039	< .001	1.19
SpM† (mm³)	0.111 ± 0.015	0.099 ± 0.013	> .05	1.12
Caudal forebrain* (mm³)	7.93 ± 0.120	6.47 ± 0.440	< .001	1.23
Brain weight (g)	0.754 ± 0.065	0.655 ± 0.041	< .001	1.15
HVc:Rt	0.764 ± 0.186	0.463 ± 0.118	< .001	1.65
RA:Rt	0.608 ± 0.213	0.385 ± 0.122	< .001	1.58

*Corresponds to volume reconstruction of left and right structures.   †Corresponds to volume reconstruction of left structures.

amount of plastic substrate that can be exploited for such learning purposes. According to this hypothesis the plastic substrate for vocal learning is renewed once yearly, a growing, then shedding of synapses, much the way trees grow leaves in the spring and shed them in the fall.

The shrinkage of brain nuclei in adulthood, resulting from a loss of dendritic processes, may be likened to a rejuvenating process that reduces the size of a network to an earlier developmental age. Of course, such a process can be labeled "rejuvenation" only if it is followed by a new wave of dendritic proliferation and synapse formation. If rejuvenation of brain circuitry ever becomes possible in humans, being able to induce a retraction of neurites may be found to be the indispensable first step, to be followed by their regrowth. We may now have an animal model for this kind of phenomenon.

FERNANDO NOTTEBOHM
Rockefeller University,
Field Research Center,
Millbrook, New York 12545

References and Notes

1. M. Metfessel, *Science* 81, 470 (1935).
2. H. Poulsen, *Z. Tierpsychol.* 16, 173 (1959); M. S. Waser and P. Marler, *J. Comp. Physiol. Psychol.* 91, 1 (1977).
3. P. Marler and M. S. Waser, *J. Comp. Physiol. Psychol.* 91, 8 (1977).
4. F. Nottebohm and M. E. Nottebohm, *Z. Tierpsychol.* 46, 298 (1978).
5. All work was done with males from the Rockefeller University Field Research Center closebred strain of Belgian Waserschlager canaries. These birds were kept indoors; light:dark schedules followed the photoperiod of central New York State.
6. The purpose of breeding these birds was to ensure that they would undergo the hormonal changes normally associated with reproductive maturity and the shift from spring to summer and fall condition.
7. Birds were killed by ether overdose.
8. Blood samples obtained from the right auricle were allowed to clot and retract overnight at 2°C. Serum was then separated by centrifugation and stored at −40°C until analysis. Plasma androgen (testosterone + probably dihydrotestosterone) levels were measured by radioimmunoassay (RIA) [V. L. Gay and J. T. Kerlan, *Arch. Androl.* 1, 239 (1978); I. Lieberburg, L. C. Krey, B. S. McEwen, *Brain Res.* 178, 207 (1979)].
9. Birds were perfused through the left ventricle with 0.9 percent saline followed by 10 percent Formalin in 0.9 percent saline. After perfusion, the testes were removed and placed in 10 percent Formalin in 0.9 percent saline. The head was placed in a stereotaxic head-holder, and the brain was blocked rostrally in the transverse plane with a knife so as to reproduce the plane of section of our canary atlas [T. C. Stokes, C. M. Leonard, F. Nottebohm, *J. Comp. Neurol.* 156, 337 (1974)]. The brain was then removed from the skull and stored in 10 percent Formalin in 0.9 percent saline for 1 to 2 weeks, then transferred to a 30 percent sucrose in 10 percent Formalin solution for 2 to 4 days. After 24 hours in sucrose solution the brain sinks to the bottom of the jar, and it was then that brains were weighed. For weighing, brains were removed from the sucrose solution and gently placed on tissue paper to absorb excess wetness, then weighed in the same solution, including the rostral section that had been blocked off. After sucrose, the brains were placed in the gelatin-albumen embedding medium, where they remained for 2 to 4 weeks. Embedding and sectioning proceeded as described in the canary atlas mentioned above. Frozen sections were cut with a repeat interval of 50, 50, and 25μm. One of the 50-μm series was collected sequentially into 50 percent ethyl alcohol, mounted on chrome-alum (chromium potassium sulfate) coated slides and stained with cresyl violet, a Nissl substance stain for cell bodies.
10. Volume of brain structures was reconstructed through the use of a microprojector and polar planimeter (11,12).
11. F. Nottebohm and A. P. Arnold, *Science* 194, 211 (1976).
12. F. Nottebohm, S. Kasparian, C. Pandazis, *Brain Res.* 213, 99 (1981).
13. F. Nottebohm, T. M. Stokes, C. M. Leonard, *J. Comp. Neurol.* 165, 457 (1976). The volume of each right and left nucleus HVc and RA was reconstructed separately.
14. 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)]. In canaries and zebra finches, part of the telencephalic input to SpM may come from RA or from part of the archistriatum close to RA [F. Nottebohm, D. B. Kelley, J. A. Paton, unpublished observations; M. E. Gurney, thesis, California Institute of Technology (1980)]; if this projection comes in fact from RA, it is a small one. Nucleus SpM has not yet been studied in songbirds to ascertain its role, if any, in song control. For purposes of the present study only the volumes of the left Rt and left SpM were reconstructed. From earlier observations (11,12), we know that the right and left volumes of these nuclei are symmetrical. When normalizing the volume of HVc and RA, each bird's total (left + right) HVc and RA volume was divided by twice the volume of its left Rt.
15. To reconstruct the caudal forebrain volume at the level of HVc, we measured the summed area of three telencephalic sections. For each bird, one of these sections was the one that showed the largest cross section through HVc; the other two sections were taken, respectively, 200 μm more rostral and 200 μm more caudal. For each bird the right and left reconstructed volumes were added, obtaining a unitary value for caudal forebrain volume.
16. For each bird, the testicular weights used for obtaining means and standard deviations were gotten by adding the weights of the right and left testis: spring, 253.2 ± 41.9 mg; fall, 1.8 ± 0.9 mg. There was a parallel difference in serum androgen levels, as measured by RIA (8). The spring and fall androgen levels were, respectively, 1.65 ± 1.24 ng/ml and 0.13 ± 0.22 ng/ml [$t(19) = 4.21$, $P < .05$].
17. For right-left comparisons [Wilcoxon matched-pairs signed-ranks test, spring RA and HVc, $T(9) \geq 16$, $P > .05$; fall RA and HVc, $T(12) \geq 20$, $P > .05$].
18. Significance of seasonal differences for all brain measures listed in Table 1 was tested with two-tailed *t*-tests; significance was rejected at $P > .05$.
19. Table 1 shows that Rt may show seasonal changes in size. Thus, use of Rt volume to normalize each bird's HVc and RA values may be an overly stringent way of looking at the magnitude of seasonal changes in HVc and RA; it is reassuring that marked seasonal differences persist under those circumstances.
20. S. L. Leonard, *Proc. Soc. Exp. Biol. Med.* 41, 229 (1939); H. H. Shoemaker, *ibid.*, p. 299; F. M. Baldwin, H. S. Goldin, M. Metfessel, *ibid.* 44, 373 (1940); E. H. Herrick and J. O. Harris, *Science* 125, 1299 (1957).
21. F. Nottebohm, *Brain Res.* 189, 429 (1980).
22. T. DeVoogd and F. Nottebohm, *Science* 214, 202 (1981).
23. Song "learning" and song "forgetting" are used here to refer not so much to the acquisition and loss of an auditory memory, but rather to the conversion of that memory into a motor program, with the consequent matching of an auditory model. The learning of such a motor program seems to require brain space (12).
24. Male zebra finches learn their single song during the first 3 months after hatching [K. Immelmann, in *Bird Vocalizations*, R. A. Hinde, Ed. (Cambridge Univ. Press, London, 1969), p. 61]. No new songs are learned in adulthood. In adult male zebra finches, the volume of HVc and RA shows no specific volume changes even many months after castration [A. P. Arnold, *Brain Res.* 185, 441 (1980)]. Since the amount of singing in male zebra finches is testosterone-dependent [E. Pröve, *J. Ornithol.* 115, 338 (1974); Z. *Tierpsychol.* 48, 47 (1978); A. P. Arnold, *J. Exp. Zool.* 191, 309 (1975)], we may infer that in this species a drop in testosterone levels and a reduction in pathway use do not, by themselves, lead to gross changes in the volume of HVc and RA. The physiology of a spring to fall seasonal change probably involves more than a change in levels of gonadal hormones, so that a better test of the prediction offered is still required.
25. F. Nottebohm, *Prog. Psychobiol. Physiol. Psychol.* 9, 85 (1980).
26. M. E. Gurney, thesis, California Institute of Technology (1980).
27. I thank L. Crane for helping with the logistics of these experiments and for removing the brains. S. Kasparian did the RIA measurements of plasma androgen levels, with the help of C. Harding and L. C. Krey, in laboratory space made available by B. McEwen. T. J. DeVoogd, J. A. Paton, and M. E. Nottebohm offered helpful editorial comments. Supported by PHS grant 5R01 MH 18343 and by Rockefeller Foundation grant RF 70095 for research in reproductive biology.

12 May 1981; revised 3 August 1981

Site-Specific, Sustained Release of Drugs to the Brain

Abstract. A dihydropyridine–pyridinium salt type of redox system is used in a general and flexible method for site-specific or sustained delivery (or both) of drugs to the brain. A biologically active compound linked to a lipoidal dihydropyridine carrier easily penetrates the blood-brain barrier. Oxidation of the carrier part in vivo to the ionic pyridinium salt prevents its elimination from the brain, while elimination from the general circulation is accelerated. Subsequent cleavage of the quaternary carrier–drug species results in sustained delivery of the drug in the brain and facile elimination of the carrier part.

The delivery of drugs to the brain is often seriously limited by transport and metabolism factors and, more specifically, by the functional barrier of the endothelial brain capillary wall called the blood-brain barrier (1). Site-specific delivery and sustained delivery of drugs to the brain are even more difficult, and no useful simple or general methods to achieve them are known. We now report a general method, useful for site-specific and controlled delivery of various drugs, which is achieved by affecting the bidirectional movement of the drugs in and out of the brain with a dihydropyridine ⇌ pyridinium salt redox system.

The dihydropyridine ⇌ pyridinium salt type of redox delivery system was first successfully used for delivery to the brain of N-methylpyridinium-2-carbal-