THE CONDOR

JOURNAL OF THE COOPER ORNITHOLOGICAL SOCIETY

Volume 86 Number 3 August 1984

The Condor 86:227–236

BIRDSONG AS A MODEL IN WHICH TO STUDY BRAIN PROCESSES RELATED TO LEARNING

FERNANDO NOTTEBOHM

ABSTRACT.—The last fifteen years have yielded an ever increasing amount of information about brain pathways for song control in songbirds. I review here aspects of this work which suggest that the size of brain networks for song control may limit how much can be learned. In addition, sustained learning in adulthood may relate to plasma levels of gonadal hormones and to the replacement of dendrites, synapses and neurons. Mechanisms involved in this pathway "rejuvenation" may be similar to mechanisms for brain self-repair.

The activity and functional interconnections of neurons and of the circuits they form changes during learning (Fifkova and van Harreveld 1977, Kandel 1978, Alkon and Crow 1980, Kandel and Schwartz 1982). These changes can be long-lasting, constituting memories. The number and complexity of neuronal circuits present in the central nervous system will determine how much information can be processed; the degree to which these circuits can be modified by experience will determine how much information can be learned. I will use song learning in Common Canaries (*Serinus canaria*) to argue in favor of three interrelated hypotheses: 1) the number of modifiable circuits determines learning potential; 2) as learning takes place modifiable circuits become committed, subtracting from the initial learning potential; 3) replacement of synapses and neurons in adulthood restores learning potential, but possibly at the expense of earlier memories. These hypotheses are offered as stimulation for further work and as a way of focusing attention on a system that is unusually well suited for the study of brain processes for learning a complex skill.

Song learning is the process of acquiring a song repertoire by reference to auditory models (Thorpe 1958, Marler and Tamura 1964, Konishi 1965, Nottebohm 1968, Immelmann 1969). The model and its imitation can be recorded on tape and converted into a two-dimensional visual display, the sound spectrograph. This conversion is quick and objective (Hopkins et al. 1974) and allows one to count the number of sounds learned, describe the stages in learning and time when they occur.

Song in birds is produced by a specialized organ, the syrinx (Greenewalt 1968, Nottebohm 1975) controlled by well-defined brain nuclei. A relatively large forebrain nucleus, the hyperstriatum ventralis, pars caudalis (HVc) projects to a smaller forebrain nucleus, robustus archistriatalis (RA), which in turn projects to an even smaller pool of hypoglossal motor neurons; the latter motor neurons give rise to the tracheosyringeal (ts) branch of the hypoglossus nerve, which innervates the muscles of the trachea and syrinx (Fig. 1). The challenge, then, is to understand how this system operates, and what limits its learning potential.

THE RELATION BETWEEN PERCEPTION AND PRODUCTION

When a bird learns to sing, it modifies a motor program until the vocal output generated matches an auditory model. During this process, the circuits involved in sound production and sound perception are part of a control loop. Some units that control output must also be able to "hear" the consequences. Not surprisingly, a major auditory projection abuts on the nucleus HVc (Kelley and Nottebohm 1979). Some HVc neurons respond to a diversity of sounds, including song (Katz and Gurney 1981, McCasland and Konishi 1981) and can do so in a very selective manner (Margoliash 1983). Some HVc neurons fire during song production (McCasland and Konishi 1981). Thus, HVc neurons show properties that one might expect from a nucleus involved in the perception and production of learned vocalizations. In this sense, HVc might be functionally analogous to Broca's area for speech control in the

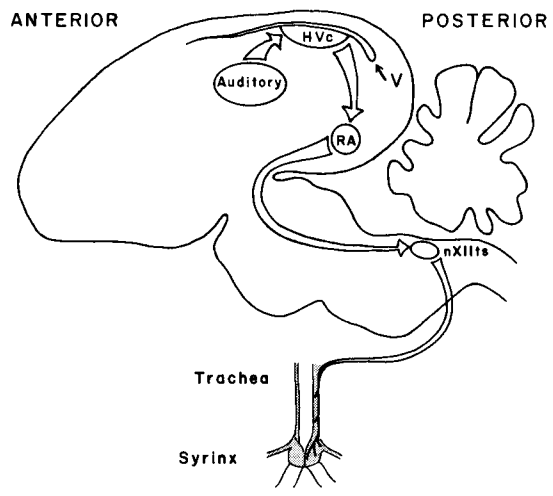

FIGURE 1. Schematic sagittal section of adult canary brain showing components of the song control system. Open arrows indicate direction of information flow. The pathways indicated by the open arrows are ipsilateral. Tracheal and syringeal muscles are indicated by light stippling. Abbreviations: V, forebrain lateral ventricle; nXIIts, tracheosyringeal part of nXII.

human frontal lobe (Ojemann and Mateer 1979). The RA, in turn, has been likened, in terms of connectivity, to layer five of the mammalian motor cortex (Nottebohm et al. 1976).

Though avian circuits for perception and production of sound may overlap and share components, the acquisition of auditory memories and the development of song to match such memories may be controlled by different factors. This is shown by the following examples: 1) song learning as a motor skill can start well after the auditory model is acquired (Marler 1970); 2) auditory memories that are useful in song recognition can be acquired by female songbirds that normally do not sing (Miller 1979, Baker et al. 1981).

CRITICAL-PERIOD AND OPEN-ENDED LEARNERS

Many songbirds, such as the Common Chaffinch (*Fringilla coelebs*), White-crowned Sparrow (*Zonotrichia leucophrys*) and Zebra Finch (*Poephila guttata*) have, as juveniles, a "critical period" for song learning (Thorpe 1958, Marler and Tamura 1964, Immelmann 1969). Members of these species retain their learned song programs intact for several years even after loss of hearing (Konishi and Nottebohm 1969). Other songbirds, such as the canary and other cardueline finches, are open-ended learners (Mundinger 1970, Nottebohm and Nottebohm 1978). They can alter their song from year to year by retaining some sounds, dropping others, modifying still others and adding new ones (Nottebohm and Nottebohm 1978, and unpubl.). After being deafened in adulthood, such a bird gradually forgets its song repertoire (Nottebohm et al. 1976). The distinction between critical-period and open-ended learners is useful because it should help to isolate circuit features that limit or encourage vocal learning and forgetting in adulthood.

CIRCUITS GROW AS VOCAL LEARNING PROCEEDS

Birds that learn their song and calls go through a "subsong" stage, which Charles Darwin likened in *The Descent of Man* to the babbling of infants (Thorpe and Pilcher 1958). As in babbling, the sounds produced are of low volume and have no communicatory function (Thorpe and Pilcher 1958). During subsong the young bird may learn the relation between some efferent commands and the resulting auditory feedback; once this relation is established, particular sounds may be more easily imitated. The subsong experience may also bias the later selection of models (Nottebohm 1972).

The subsong of male canaries first appears by day 40 after hatching, and lasts two to three weeks. It develops into "plastic" song. During the period of plastic song, the units of repetition, or syllables, become defined and new ones are added until, by eight months of age the repertoire is stable and stereotyped. This stable repertoire changes little during the next six months, while the bird is in breeding condition (unpubl. observ.).

The brain of a 15-day-old male canary weighs as much as that of an eight-month-old reproductively mature adult, but HVc first becomes recognizable at 30 days of age, when it is one-eighth of its adult volume. The size of HVc triples from day 30 to day 60 after hatching. The rate of growth slows thereafter, and stops during the seventh month (Fig. 2). Nucleus RA develops in a similar way, although the extent of growth is not as marked (unpubl. observ.). This suggests that during ontogeny, circuit space for song control grows at the same time that new syllable types are added and perfected. We do not know if circuit growth results from an increase in the number and size of neurons, their processes, or the synapses they form, but all of these are likely candidates. Neither do we know if circuit growth occurs because learning is taking place, or if learning is taking place because circuit growth makes it possible. In either case, learning would occupy circuit space.

SEASONAL WAXING AND WANING OF CIRCUIT SPACE

As a male canary comes to the end of its first breeding season, its song becomes unstable again for several months; in the middle of this period of instability, song may cease for sev-

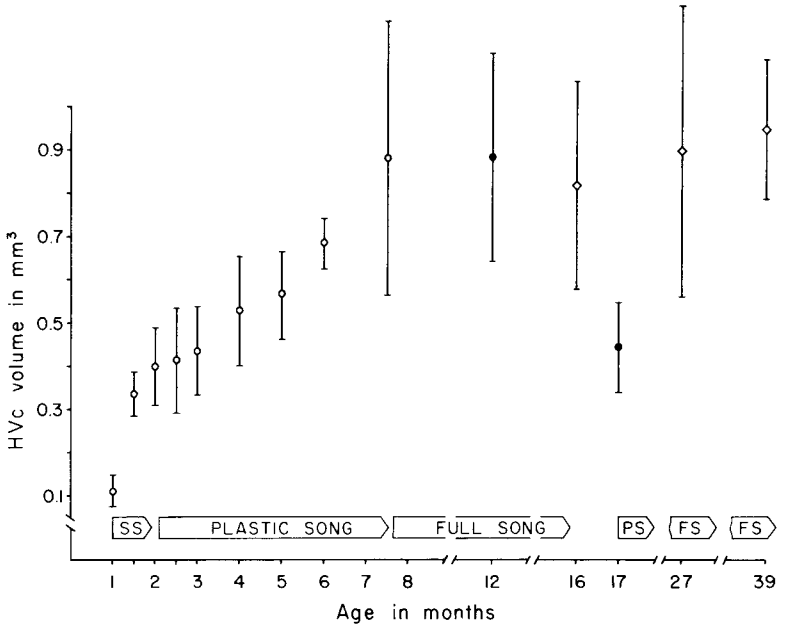

FIGURE 2. Relation between age, HVc volume, and stage in song development in male canaries. Open circles for the first 6 months correspond to means from four birds, the open circle at 7.5 months of age corresponds, respectively, to groups of 9 and 12 birds (Nottebohm 1981); the diamonds at 16, 27 and 39 months correspond, respectively, to groups of 10, 9 and 8 birds (Nottebohm et al. 1981). The vertical bars indicate one standard deviation. Abbreviations: SS, subsong; PS, plastic song; FS, full song.

eral weeks. By late summer, when song is unstable, HVc size is half what it was in the spring (Nottebohm 1981) and comparable to that of a three-to-four-month-old male canary in plastic song (Fig. 2). New syllables are added throughout the second year of life, particularly during the summer and fall months, at the time of song instability. When the following breeding season is fully underway, the new song repertoire is once again stable and HVc has regained the volume lost during the previous summer. RA goes through similar, though lesser seasonal changes (Nottebohm 1981). Seasonal changes in the size of HVc and RA do not occur in adult male White-crowned Sparrows, a critical-period species that learns its song once during juvenile life (Baker et al. 1984). Taken together, this evidence from juvenile and adult canaries as well as that from other species suggests that learning is aided by the availability of new, uncommitted circuit space.

BIGGER CAN MEAN BETTER

The size of nucleus HVc and RA has a three-fold range in the adult close-bred Waserschlager canaries used in my laboratory. There is also a three-fold range in the number of syllable types such birds produce. These two variables are related in a significant manner. Male canaries with large song repertoires tend to have large HVcs and large RAs. Male canaries with small HVcs and small RAs tend to have small song repertoires (Nottebohm et al. 1981).

Large song repertoires are more effective than small song repertoires in inducing nest-building and ovulation in female canaries (Kroodsma 1976). From a male canary's point of view, a large HVc may be "better" than a small one.

A similar relationship between song complexity and size of HVc and RA has been found in two critical-period species, the Zebra Finch (Nottebohm and Crane, unpub. observ.) and the Marsh Wren (*Cistothorus palustris*). The latter species occurs throughout the United States, but its song complexity varies considerably among populations (Kroodsma and Verner 1978). California populations have song repertoires that are three times as large as those recorded in New York's Hudson Valley. Although the body and brain of the western birds are slightly smaller than those of the eastern ones, HVc and RA are 40% and 30% larger, respectively, in the western than in the eastern birds (Canady et al., in press). Circuit space for a learned skill seems to be related to how much of that skill is learned. Causality and direction of this relation have not been established.

HORMONES INDUCE SYNAPTOGENESIS IN ADULTHOOD IN "OPEN-ENDED" SPECIES

Evidence suggests that gonadal hormones are important for setting the size of adult HVc and RA because both these nuclei are several times

larger in males than in females (Nottebohm and Arnold 1976). Neurons in the male RA have dendrites that are longer than those in the corresponding female cell type (DeVoogd and Nottebohm 1981a). Part of this difference may result from hormonal influences early in ontogeny, as has been shown in the Zebra Finch (Gurney and Konishi 1980, Gurney 1981), but there is also a role for adult hormonal levels.

Female canaries do not normally sing. However, adult females treated with physiological doses of testosterone develop male-like song and show a marked increase in the size of HVc and RA (Nottebohm 1980). The increase in RA volume results not from the addition of new neurons (Goldman and Nottebohm 1983), but, in part at least, from dendritic growth. The dendrites of an RA cell type are 49% longer after testosterone treatment than in controls (DeVoogd and Nottebohm 1981b). This increased length is accompanied by a net gain of 51% in the number of RA synapses (DeVoogd et al. 1982). Nucleus RA is the point of exit from forebrain for telecephalic pathways controlling song. The testosterone-induced synaptogenesis on RA neurons presumably represents changes in circuitry that are relevant to the newly acquired behavior.

As mentioned earlier, the size of nucleus RA changes seasonally in male canaries. Such changes are accompanied by changes in gonadal function. In late summer, testes are 1/140 of their spring volume, and blood androgen levels are close to zero. Whereas testosterone in females induces growth of RA dendrites and synaptogenesis, a drop in testosterone levels in males may induce a temporary and reversible retraction of synapses and dendrites. If so, this may be important for the seasonal and yearly changes in the learned song repertoire.

Testosterone treatment fails to induce song in adult female Zebra Finches, a critical-period species, and the size of their HVc and RA is not affected (Arnold 1980). In White-crowned Sparrows, also a critical-period species, seasonal fluctuations in gonadal function affect the occurrence of song but not the size of HVc and RA (Baker et al. 1984). It seems likely that critical-period and open-ended learners differ importantly in the way that the song control system of adults responds to changes in hormone levels. The cellular and molecular bases for this difference remain to be discovered.

NEUROGENESIS IN ADULTHOOD

The magnitude of the seasonal and hormone-induced changes in the volumes of nucleus HVc and RA raised the possibility that new network space might result not only from new dendrites and new synapses, but also from the addition of neurons (Nottebohm 1980). If so, this addition might be governed by gonadal hormones, possibly testosterone or its metabolites. To test this hypothesis, one-year-old female canaries were treated with testosterone and subsequently received three daily injections of radioactively labeled thymidine, a marker of DNA synthesis (Korr 1980). As many as 1.5% of all HVc neurons were labeled per day of ^{3}H-thymidine treatment. Surprisingly, the percentage of labeled neurons did not differ between testosterone- and cholesterol-treated control birds, although only the former developed male-like song (Goldman and Nottebohm 1983). From this we concluded that testosterone-induced masculinization of the female song-control system was not necessary to induce neuronal labeling. Instead, phenomena underlying neuronal labeling seemed to occur spontaneously in adult female canaries. The issue of whether or not this neuronal labeling is under hormonal control remains open, as our cholesterol-treated females had intact ovaries.

Had we administered ^{3}H-thymidine to canary embryos, then the subsequent presence of labeled neurons would have been interpreted in the customary way, as evidence of neuronal birth that had occurred by mitosis a few hours after the injection of the label. Our subjects were adults, however, so it was possible, for example, that the label had been incorporated into the nuclei of fully differentiated neurons. To test for this possibility, adult female canaries were given ^{3}H-thymidine for two days and killed one or two days later. These birds had no labeled neurons in HVc. Instead, their HVc was overlain by a band of labeled ventricular-zone cells (Goldman and Nottebohm 1983). This suggested that the new neurons were born in the ventricular zone, from whence they migrated into HVc and differentiated. This ventricular-zone origin of neurons may not differ from that observed during embryogeny (Jacobson 1970, Korr 1980). Had neuronal labeling resulted from either DNA repair or genomic replication without mitosis, leading to polyploidy, then it would have occurred in situ. In this case, the birds sacrificed one or two days after the last ^{3}H-thymidine injection would have had labeled neurons throughout HVc. The process of neuronal migration and differentiation in adult HVc apparently takes longer than one or two days.

In all of these experiments, there were no significant differences in the numbers of silver grains overlying the nuclei of labeled neurons, glia and endothelial cells (Nottebohm and Kasparian 1983). The mitotic origin of new glia and endothelial cells in the nervous system

of other adult animals has been well documented (Jacobson 1970, Alberts et al. 1983). In the presence of ^{3}H-thymidine the nuclei of such new cells are labelled. Since, in our canaries, the extent of label seen over neuronal, glial and endothelial nuclei was comparable between these three cell types, it seems parsimonious to conclude that the steps leading to labeling were in all three cases the same: ^{3}H-thymidine incorporation during the S-phase of DNA synthesis which precedes cell division.

How sure could we be that the new neuron-like cells labeled with ^{3}H-thymidine were in fact neurons? We had used standard anatomical criteria accepted by others as adequate for neuronal identification, but the possibility remained that we might have been tricked into calling neurons a new cell type which, though neuron-like, was not really part of neuronal circuits. Two lines of evidence reassure us that our original identification was correct. Firstly, it has been possible to show in material prepared for electron microscopy that the labeled neurons receive synapses (Burd and Nottebohm 1984). Secondly, we also know that the labeled neurons are working neurons. Adult male and female canaries received two daily injections of 50 μCi of ^{3}H-thymidine for 14 days, which labeled many HVc neurons, as ascertained one month after the last injection. The ^{3}H-thymidine treated birds were allowed to survive for three to four weeks, then anesthetized. Single neurons in HVc were then penetrated with hollow electrodes, and changes in electric potential were recorded in response to auditory stimuli. After obtaining this physiological description, the HVc cells recorded were filled with horseradish peroxidase (HRP) and the birds killed. After adequate histological treatment, the position and fine anatomical details of each cell recorded and filled with HRP were described. In all cases the cells that had yielded neuronal physiological profiles also had typically neuronal anatomy, with dendrites and axons. When subsequently processed for autoradiography, 9% of these HVc cells proved to have radioactively labeled nuclei. Thus, not only are new neurons born in adulthood and recruited into HVc, but also they are integrated into existing circuits (Paton and Nottebohm, in press).

The production of new neurons does not lead to a long-term change in the total number of neurons in HVc. No differences in HVc neuron numbers have been seen between one- and two-year-old adult female canaries (Nottebohm and O'Loughlin, unpubl.). Therefore, the recruitment of new neurons must be accompanied by neuronal death. Otherwise, at a recruitment rate of 1.5% per day the number of HVc neurons would double over a 50-day period. Thus, "new" neurons must replace "old" ones. We do not know whether neurogenesis occurs at the same rate throughout the year.

In contrast to what was observed in HVc, we found no labeled neurons in RA (Goldman and Nottebohm 1983). This suggests that new neurons are added to parts of a network in a selective manner, there to replace other neurons. We do not know whether the new neurons are themselves eventually replaced. If such replacement of new neurons occurs, then we will have discovered a new type of neuron that lasts for a period of weeks or months of adult life and then is replaced.

WHAT IS THE FUNCTION OF NEW NEURONS?

The addition of new neurons to the vocal control nucleus of adult female canaries that were not treated with testosterone was intriguing because such females do not normally sing. However, they may develop a preference for some songs they hear, as in White-crowned Sparrows (Baker et al. 1981) and Zebra Finches (Miller 1979). Since HVc has access to auditory information, could song recognition be its main role in females? In this case, the replacement of HVc neurons in adulthood could be related to perceptual, rather than motor, learning.

The possible perceptual and motor roles of neurogenesis in female canaries could not be separated because, as shown for other carduelines, adult females may continue to alter their call repertoire (Mundinger 1970), a phenomenon that may be similar to song learning.

To separate these possibilities, we treated male Zebra Finches with ^{3}H-thymidine well after the end of their critical period for song learning. If neuronal replacement was related just to the acquisition of song as a learned motor skill, then it would cease after the skill had been mastered. If, however, it was related to some other ongoing phenomenon, such as song recognition, then it would continue to occur after the end of the critical period for song learning. A small fraction (0.26%) of HVc neurons was labeled per day of ^{3}H-thymidine treatment in adult male Zebra Finches (Nottebohm and Kasparian, unpubl. observ.). If this proportion of labeled neurons represented the daily recruitment rate, then the number of HVc neurons would double in about 300 days. Thus, although neurogenesis occurs in a song control nucleus its significance need not be restricted to motor learning. Despite the interest of these speculations and their value because of the experiments they suggest, it is important to remember that no direct evidence at present

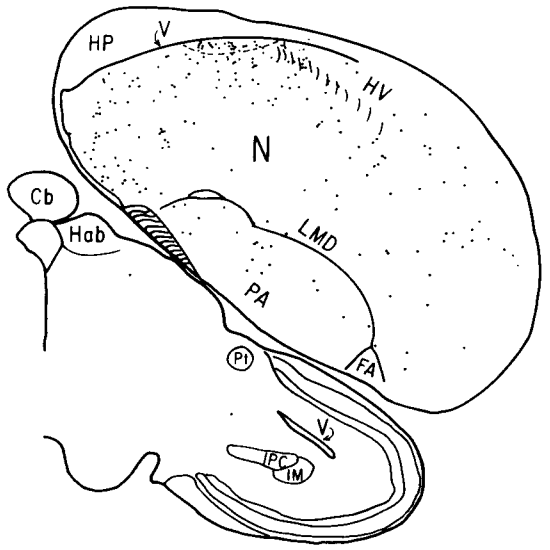

FIGURE 3. Distribution of labelled neurons in cross section of adult female canary brain. This bird received 50 μCi of ^{3}H-thymidine at 12-h intervals for 14 days and was killed 26 days after the last injection. Whereas a total of 228 labelled neurons occur in the forebrain part of this section, exclusive of hippocampus, only two labelled neurons occur in the midbrain. Abbreviations: Cb, cerebellum; FA, tractus fronto-archistriatalis; Hab, habenula; Hp, hippocampus; HV, hyperstriatum ventralis; IM, nucleus isthmi, pars magnocellularis; IPC, neucleus isthmi, pars parvocellularis; LMD, lamina medullaris dorsalis; N, neostriatum; PA, paleostriatum augmentatum; Pt, nucleus pretectalis; V, ventricle (Stokes et al. 1974). Broken line indicates ventral border of nucleus HVc.

links neuronal replacement in HVc with learning of any kind.

A FOREBRAIN CONSTANTLY REBUILDING ITSELF?

Recent evidence suggests that adult neurogenesis in the canary brain is not limited to the song control system. Labeled neurons are found not just in HVc, but also in various parts of the forebrain (Fig. 3). In the analysis thus far, virtually no labeled neurons have been found in the hypothalamus, septum, thalamus, optic lobe, cerebellum or medulla. Neurogenesis is best represented in parts of the hippocampus and in the forebrain, that part of the brain usually credited with complex perception and the control of goal-oriented behaviors and learning (Nottebohm and Kasparian 1983).

Evidence of forebrain neurogenesis in adulthood has now been obtained from male and female canaries, male and female Zebra Finches, male parakeets (Manogue and Nottebohm, unpubl. observ.) and male and female doves (Nottebohm and Cohen, unpubl. observ.). These birds represent three avian orders, Passeriformes, Psittaciformes and Columbiformes, so it seems fair to assume that the phenomenon occurs widely among birds.

New neurons continue to be formed in the adult forebrain, yet brain weight does not increase after the first year (Nottebohm et al. 1981). It seems reasonable to assume that, as in HVc, new neurons replace old neurons. That is, the adult forebrain is constantly rebuilding itself, or at least rebuilding parts of some circuits.

Preliminary observations suggest that the neurons born in adulthood fall into a narrowly defined category. So, for example, long projection neurons, such as those of the archistriatum (e.g., RA), which form connections outside of the forebrain, are not labeled with ^{3}H-thymidine. Conversely, the axons of labeled HVc neurons seem to branch and terminate within HVc (Paton and Nottebohm, in press). Thus, the new neurons may function as local circuit interneurons. If the new neurons replace others of the same kind, then we would have a category of replaceable interneurons.

NEUROLOGICAL COMPARISONS BETWEEN BIRDS AND OTHER ANIMALS

Findings in birds allow us to relate circuit space, synaptogenesis and neurogenesis in adulthood to specific behavioral skills. I will now briefly review some earlier descriptions of synaptogenesis and neurogenesis in other kinds of adult animals.

SYNAPTOGENESIS

It has been proposed that the adult complement of synapses arises during ontogeny by a process of functional selection, whereby some connections become stable and others degenerate (Eccles 1973, Changeux et al. 1973, Changeux 1974). In addition, Changeux (1974) suggested that there are learning periods when motility of some nerve terminals gives them the ability to establish transiently a multiplicity of contacts. Subsequent electrical activity would then stabilize some of these synapses.

Experiential factors may do more than modulate the effectiveness of existing synapses. The dendrites of some cortical neurons of adult rats will grow when the animal is exposed to increased environmental complexity, possibly leading to the formation of new synapses (Greenough 1975, Uylings et al. 1978, Juraska et al. 1980).

Cotman and Nieto-Sampedro (1982) have suggested that synaptic growth, and thereby effectiveness, can be induced by changes in neuronal activity which may result from the occurrence of natural stimuli. Secondly, they

have suggested that in some parts of the brain, such as the hippocampus, synapses are constantly formed and unformed. Part of this constant change may reflect changing patterns of use, but these workers have proposed that in some parts of the brain synaptic turnover occurs as part of an inexorable cycle of synaptic birth, growth and break-up (Nieto-Sampedro et al. 1982). Carlin and Siekevitz (1983) have more recently reviewed the evidence on synapse plasticity and suggested that in many parts of the brain, during learning, a subset of existing synapses undergoes division, so that, for example, where previously contacts between two neurons were represented by 1,000 synapses, now they are represented by 2,000. Thereby the influence of one neuron on another would be strengthened considerably, and the information conveyed would gain greater salience. In sea slugs (*Aplysia*) formation and elimination of synapses have been related to processes of sensitization and habituation (Bailey and Chen 1983).

NEUROGENESIS

In the past, there has been little speculation about the role of adult neurogenesis, probably because good examples of this phenomenon have been rare (Korr 1980). This is so even though the first tentative evidence of neurogenesis in adulthood appeared over 20 years ago (Altman 1962). Three kinds of examples of adult neurogenesis have since been described. First, olfactory neurons in rodents are constantly replaced by new neurons that arise from underlying stem cells (Graziadei and Monti-Graziadei 1979). These neurons are found in the olfactory epithelium and are not really part of the central nervous system. The process of renewal, in this case, has been attributed to peripheral wear and tear of a cell type that is particularly exposed to environmental agents. Second, birth of new neurons in adulthood has been described in the hippocampus, olfactory bulb and occipital cortex (Kaplan and Hinds 1977, Kaplan 1981, Bayer et al. 1982). With one exception, the addition of new neurons in these systems has been interpreted as a process of sustained growth leading to a net gain in neuron numbers. The exception is the case of new olfactory bulb neurons which, it has been suggested, replace older olfactory bulb neurons (Altman 1969, Bayer 1983). In a third category fall reports of neurogenesis in the adult retina and elsewhere in the central nervous system of fish (Leonard et al. 1978, Johns and Fernald 1981, Easter 1983, Raymond and Easter 1983). These examples concern cases of sustained growth in species where body and brain growth continues in adulthood well after the age of sexual maturity.

Several methodological factors could have contributed to the paucity of reports of mammalian neurogenesis in adulthood. Negative results could stem from limited access of the injected thymidine to brain cells, incomplete anatomical and temporal sampling, or wrong assumptions regarding the survival of new, labeled neurons. The avian material proves that adult neurogenesis is possible and that there is no obstacle, in principle, to the incorporation of new neurons into existing networks. Even in the avian brain, regional variations in neuronal recruitment occur. If neurogenesis proves to occur at a lower rate in mammalian than in avian tissue, this does not preclude the existence of latent mechanisms of neurogenesis, as could be used in brain self-repair, or the possibility that adult neurogenesis could be induced. Even if the adult mammalian brain were to be declared incapable of adult neurogenesis, the principles governing the migration and differentiation of neurons in the adult avian brain could be used to guide the acceptance of introduced neuroblasts, and their migration and differentiation and integration into functional circuits. In these various ways, work on avian brains could contribute importantly to matters of human clinical interest.

Altman (1970), who pioneered in the field of post-natal neurogenesis, noted that in all cases known to him involving late-developing structures such as cerebellum, hippocampus and olfactory bulb, the newly recruited neurons were microneurons that acted as local interneurons. He saw this as a developmental means for adding "fine wiring," sensitive to experiential factors, to an otherwise rigid, genetically determined connectivity. This view could be extended into adulthood and integrated with the view on neuronal replacement presented here: when new learning must take place in a system with limited circuit space, new "fine wiring" is necessary.

"USED" DNA VS. "FRESH" DNA

The neuronal replacement in adult forebrain inferred from the avian material poses some interesting questions. For example, why should such a process occur? After all, if dendrites can grow and retract, and synapses can be formed and shed, what extra advantage is to be gained by the replacement of whole neurons? Kandel and Schwartz (1982) have suggested that the formation of long-term memories may require the synthesis of new macromolecules, and thus the expression of new genes. The new mac-

romolecules would give permanence to synaptic changes coding for shorter-term memory. I would like to go one step further and suggest that the genome of some neurons that partake in the formation of long-term memories may, in some instances, be affected irreversibly. Some genes may be turned on, or off, or otherwise modified in an irreversible manner, by cytoplasmic conditions that are determined by the position, connectivity and past history of that cell. For such a cell, modification by experience leading to long-term memory formation would be the achievement of final differentiation. The only way to restore to that circuit the flexibility required for learning would be to replace the old cell by one with a freshly minted genome, new cytoplasm, and new connections.

WHAT DO WE REMEMBER?

I know of no evidence at present that neuronal replacement and synaptic replacement occur in the human brain. If they do occur, one might expect them in parts of the forebrain involved in the processing and storing of sensory information, and, perhaps, motor skills. I would like to borrow a metaphor from photography, using the terms "fine grain" and "coarse grain" to refer to better and poorer resolution of detail. Most of our memories lose their fine grain with time. Might a change of fine to coarse grain in memories occur as the number of neurons or synapses related to that memory diminishes, as these neurons and synapses are replaced by fresh neurons and fresh synapses?

THE ENGRAM REMAINS ELUSIVE

Summarizing his work of 30 years, Karl Lashley (1950) concluded that "all of the cells of the brain are constantly active and are participating, by a sort of algebraic summation, in every activity. There are no special cells reserved for special memories." We have learned a lot more since then about mechanisms that might mediate learning, particularly at the synaptic level (Kandel and Schwartz 1982). Yet we remain ignorant as to what might be the principles governing "learning space." Imagine that each unique memory—a word, a face—occupies a unique point in memory space, each point being defined by the intersection of several information-bearing axes. If this is so, then occupancy of one point by a memory does not "use" space for other memories. In this case, the sum total of memory space equals the sum total of different perceptions of which the organism is capable. Replacing the "used" set of synapses that define a memory point would achieve nothing except allowing for a re-learning of that same memory; it would not make space available for other memories.

Alternatively, imagine memory space as space on the shelves of a library. The total space remains the same, but the books can be moved around and replaced. In this case, space occupied by particular sets of books is unavailable to other sets. Which of these two metaphors applies better to the brain? It would seem that the "unique memory points" concept would be more in line with what is known about connectivity, except for one observation. Complex memories—a word, a face—are aggregates of simpler percepts, of lines and intensity gradients arranged in space, of sound relations arranged in time. These simpler components are not unique to one face or one word, yet memories may have to be encoded in terms of such simpler components, else one would have to postulate a unique cell for each complex memory. The simpler components of a particular face and a particular word may recur repeatedly in other words and other faces, so that the lexicon of components is shared by many memories. The sum total of these components, each of which may have many replicas, may constitute the sum total of memory space.

A comparison of these two metaphors suggests that the brain's memory space may be best defined by properties of both: a series of unique points, with many replicas of each, constituting an abecedary of memory components, the number of replicas of each component determining the size of the shelf space. If this is true, the same memory can be acquired again and again, and held as many separate memories. Two identical inputs, entered at different times, would compete maximally for memory space, while inputs that had less in common would compete less for memory space. Replaceable neurons may be those that are part of the abecedary of memory components. I offer these ideas not as rigorous hypotheses, but as suggestions for thinking about memory space, its renewal, and the physical representation of memories.

HOPE FOR A NEW NEUROLOGY

My emphasis thus far has been on learning and the insights offered into the machinery of learning by the song control system of birds. Brain plasticity used in learning can also be seen as a spontaneous form of brain rejuvenation or repair. At present, neurology relies heavily on methods that remove damaged or abnormal tissue, prevent infection, maintain electrolyte balance and regulate ventricular pressure. We may know enough now to attempt more than this, and in particular to encourage the repair of damaged circuits (Aguayo et al. 1982, Shatz 1982). How can this be best done? Could genes be turned on and off to

induce dendritic retraction and growth, to induce synapse formation and shedding, to induce birth of new neurons, their migration and differentiation? If the system's own stem cells are not available for neurogenesis, then, as has been shown (Björklund and Stenevi 1979, Dunnett et al. 1982, Labbe et al. 1983), fetal brain grafts may be introduced to repair network damage. Until recently these approaches to brain repair would have seemed unthinkable, but now we know differently. The avian data show that new neurons form, migrate and incorporate themselves into existing networks. These processes are possible in the adult brain, contrary to long-held beliefs. The possibility of a confluence of mechanisms for memory updating and network repair, involving replacement of synapses and neurons, seems worth exploring.

ACKNOWLEDGMENTS

I extend my warm thanks to A. P. Arnold, G. Burd, R. Canady, L. A. Crane, T. J. DeVoogd, S. A. Goldman, S. Kasparian, D. B. Kelley, C. M. Leonard, B. O'Loughlin, C. Pandazis, J. A. Paton, M. E. Nottebohm, and T. M. Stokes for all the skill, enthusiasm and knowledge they brought to the various studies that lead to the present synthesis. This work was supported by Public Health Service grants 5R01 MH18343, F32 NS17991 and S07 RR07065. Marta Nottebohm, Gail Burd, John Paton and David Vicario provided helpful editorial comments.

I dedicate this article to all those who struggle for the protection of wildlife and wilderness. I hope that my efforts in the laboratory will help underscore the magic, the beauty and the value of birds.

LITERATURE CITED

AGUAYO, A. J., P. M. RICHARDSON, S. DAVID, AND M. BENFEY. 1982. Early responses to neural injury, p. 91–104. *In* J. G. Nicholls [ed.], Repair and regeneration of the nervous system. Dahlem Konferenzen Springer-Verlag, Berlin.

ALBERTS, S., D. BRAY, J. LEWIS, M. RAFF, K. ROBERTS, AND J. D. WATSON. 1983. Molecular biology of the cell. Garland Publishing, New York.

ALKON, D. L., AND T. J. CROW. 1980. Associative behavioral modification in *Hermissenda*: Cellular correlates. Science 209:412–414.

ALTMAN, J. 1962. Are new neurons formed in the brains of adult mammals? Science 135:1127–1128.

ALTMAN, J. 1969. DNA metabolism and cell proliferation, p. 137–182. *In* A. Lajtha [ed.], Structural neurochemistry. Vol. 3. Plenum, New York.

ALTMAN, J. 1970. Postnatal neurogenesis and the problem of neural plasticity, p. 192–237. *In* W. A. Himwich [ed.], Developmental neurobiology. Thomas, Springfield.

ARNOLD, A. P. 1980. Sexual differences in the brain. Am. Sci. 68:165–173.

BAILEY, C. H., AND M. CHEN. 1983. Morphological basis of long-term habituation and sensitization in *Aplysia*. Science 220:91–93.

BAKER, M. C., S. W. BOTTJER, AND A. P. ARNOLD. 1984. Sexual dimorphism and lack of seasonal changes in vocal control regions of the White-crowned Sparrow brain. Brain Res. 295:85–89.

BAKER, M. C., K. SPITTLER-NABORS, AND D. C. BRADLEY. 1981. Early experience determines song dialect responsiveness of female sparrows. Science 214:819–821.

BAYER, S. A. 1983. ^{3}H-Thymidine-radiographic studies of neurogenesis in the rat olfactory bulb. Exp. Brain Res. 50:329–340.

BAYER, S. A., J. W. YACKEL, AND P. S. PURI. 1982. Neurons in the rat dentate gyrus granular layer substantially increase during juvenile and adult life. Science 216:890–892.

BJÖRKLUND, A., AND U. STENEVI. 1979. Reconstruction of brain circuitries by neural transplants. Trends Neurosci. 2:301–306.

BURD, G. D. AND F. NOTTEBOHM. 1984. Neurogenesis in adulthood: ultrastructural characterization of new neurons in the forebrain of adult canaries. Abs. Soc. Neurosci., vol. 10.

CANADY, R. A., D. E. KROODSMA, AND F. NOTTEBOHM. In press. Population differences in complexity of a learned skill are correlated with brain space involved. Proc. Natl. Acad. Sci.

CARLIN, R. K., AND P. SIEKEVITZ. 1983. Plasticity in the central nervous system: Do synapses divide? Proc. Natl. Acad. Sci. 80:3517–3521.

CHANGEUX, J. 1974. Some biological observations relevant to a theory of learning, p. 281–288. Colloques Internationaux du Centre National de la Recherche Scientific No. 206 "Current Problems in Psycholinguistics."

CHANGEUX J., P. COURRÈGE, AND A. DANCHIN. 1973. A theory of the epigenesis of neuronal networks by selective stabilization of synapses. Proc. Natl. Acad. Sci. 70:2974–2978.

COTMAN, C.W., AND M. NIETO-SAMPEDRO. 1982. Brain function, synapse renewal, and plasticity. Annu. Rev. Psychol. 33:371–401.

DEVOOGD, T., B. NIXDORF, AND F. NOTTEBOHM. 1982. Recruitment of synapses into a brain network takes extra space. Soc. Neurosci. Abstr. 8:140.

DEVOOGD, T. J., AND F. NOTTEBOHM. 1981a. Gonadal hormones induce dendritic growth in the adult brain. Science 214:202–204.

DEVOOGD, T. J. AND F. NOTTEBOHM. 1981b. Sex differences in dendritic morphology of a song control nucleus in the canary: A quantitative Golgi study. J. Comp. Neurol. 196:309–316.

DUNNETT, S. B., W. C. LOW, S. D. IVERSEN, U. STENEVI, AND A. BJÖRKLUND. 1982. Septal transplants restore maze learning in rats with fornix-fimbria lesions. Brain Res. 251:335–348.

EASTER, S. S. 1983. Postnatal neurogenesis and changing connections. Trends Neurosci. 6:53–56.

ECCLES, J. C. 1973. The understanding of the brain. McGraw-Hill, New York.

FIFKOVA, E., AND A. VAN HARREVELD. 1977. Long-lasting morphological changes in dendritic spines of dentate granular cells following stimulation of the entorhinal area. J. Neurocytol. 6:211–230.

GOLDMAN, S. A., AND F. NOTTEBOHM. 1983. Neuronal production, migration and differentiation in a vocal control nucleus of the adult female canary brain. Proc. Natl. Acad. Sci. 80:2390–2394.

GRAZIADEI, P. P. C., AND G. A. MONTI-GRAZIADEI. 1979. Neurogenesis and neuron regeneration in the olfactory system of mammals. I. Morphological aspects of differentiation and structural organization of the olfactory sensory neurons. J. Neurocytol. 8:1–18.

GREENEWALT, C. H. 1968. Birdsong: acoustics and physiology. Smithsonian Inst. Press, Washington, DC.

GREENOUGH, W. T. 1975. Experiential modification of the developing brain. Am. Sci. 63:37–46.

GURNEY, M. E. 1981. Hormonal control of cell form and number in the Zebra Finch song system. J. Neurosci. 1:658–673.

GURNEY, M. E., AND M. KONISHI. 1980. Hormone in-

duced sexual differentiation of brain and behavior in Zebra Finches. Science 208:1380–1383.

HOPKINS, C. D., M. ROSETTO, AND A. LUTJEN. 1974. A continuous sound spectrum analyzer for animal sounds. Z. Tierpsychol. 34:313–320.

IMMELMANN, K. 1969. Song development in the Zebra Finch and other estrildid finches, p. 61–74. *In* R. A. Hinde [ed.], Bird vocalizations. Cambridge Univ. Press, Cambridge.

JACOBSON, M. 1970. Developmental neurobiology. Holt, Rhinehart and Winston, New York.

JOHNS, P. R., AND R. D. FERNALD. 1981. Genesis of rods in teleost fish retina. Nature 293:141–142.

JURASKA, J. M., W. T. GREENOUGH, C. ELLIOT, K. J. MACK, AND R. BERKOWITZ. 1980. Plasticity in adult rat visual cortex, an examination of several cell populations after differential rearing. Behav. Neural Biol. 29:157–167.

KANDEL, E. R. 1978. A cell-biological approach to learning. Soc. Neurosci. Monogr., Bethesda, MD.

KANDEL, E. R., AND J. H. SCHWARTZ. 1982. Molecular biology of learning: modulation of transmitter release. Science 218:433–443.

KAPLAN, M. S. 1981. Neurogenesis in the 3-month old rat visual cortex. J. Comp. Neurol. 195:323–338.

KAPLAN, M. S., AND J. W. HINDS. 1977. Neurogenesis in the adult rat: electron microscopic analysis of light radioautographs. Science 197:1092–1094.

KATZ, L. C., AND M. E. GURNEY. 1981. Auditory responses in the Zebra Finch's motor system for song. Brain Res. 221:192–197.

KELLEY, D. B., AND F. NOTTEBOHM. 1979. Projections of a telencephalic auditory nucleus—field L—in the Canary. J. Comp. Neurol. 183:455–470.

KONISHI, M. 1965. The role of auditory feedback in the control of vocalization in the White-crowned Sparrow. Z. Tierpsychol. 22:770–783.

KONISHI, M., AND F. NOTTEBOHM. 1969. Experimental studies in the ontogeny of avian vocalizations, p. 29–48. *In* R. A. Hinde [ed.], Bird vocalizations. Cambridge Univ. Press, Cambridge.

KORR, H. 1980. Proliferation of different cell types in the brain. Adv. Anat. Embryol. Cell. Biol. 61.

KROODSMA, D. E. 1976. Reproductive development in a female songbird: differential stimulation by quality of male song. Science 192:574–576.

KROODSMA, D. E., AND J. VERNER. 1978. Complex singing behaviors among *Cistothorus* wrens. Auk 95:703–716.

LABBE, R., A. FIRL JR., E. M. MUFSON, AND D. G. STEIN. 1983. Fetal brain transplants: reduction of cognitive deficits in rats with frontal cortex lesions. Science 221: 470–472.

LASHLEY, K. S. 1950. In search of the engram. *In* Physiological mechanisms in animal behaviour. Symp. Soc. Exp. Biol. 4:454–482.

LEONARD, R. B., R. E. COGGESHALL, AND W. D. WILLIS. 1978. A documentation of an age related increase in neuronal and axonal numbers in the stingray, *Dasyatis sabine,* Leseur. J. Comp. Neurol. 179:13–21.

MARGOLIASH, D. 1983. Acoustic parameters underlying the responses of song-specific neurons in the White-crowned Sparrow. J. Neurosci. 3:1039–1057.

MARLER, P. 1970. A comparative approach to vocal learning: song development in White-crowned Sparrows. J. Comp. Physiol. Psychol. 71:1–24.

MARLER, P., AND M. TAMURA. 1964. Culturally transmitted patterns of vocal behavior in sparrows. Science 146:1483–1486.

MCCASLAND, J. S., AND M. KONISHI. 1981. Interaction between auditory and motor activities in an avian song control nucleus. Proc. Natl. Acad. Sci. 78:7815–7819.

MILLER, D. B. 1979. Long-term recognition of father's song by female Zebra Finches. Nature 280:389–391.

MUNDINGER, P. 1970. Vocal imitation and individual recognition of finch calls. Science 168:480–482.

NIETO-SAMPEDRO, M., S. F. HOFF, AND C. W. COTMAN. 1982. Perforated postsynaptic densities: probable intermediates in synapse turnover. Proc. Natl. Acad. Sci. 79:5718–5722.

NOTTEBOHM, F. 1968. Auditory experience and song development in the Chaffinch, *Fringilla coelebs.* Ibis 110:549–568.

NOTTEBOHM, F. 1972. Neural lateralization of vocal control in a passerine bird. II. Subsong, calls, and a theory of vocal learning. J. Exp. Zool. 179:35–49.

NOTTEBOHM, F. 1975. Vocal behavior in birds, p. 287–332. *In* J. R. King, and D. S. Farner [eds.], Avian biology. Vol. 5. Academic Press, New York.

NOTTEBOHM, F. 1980. Testosterone triggers growth of brain vocal control nuclei in adult female canaries. Brain Res. 189:429–436.

NOTTEBOHM, F. 1981. A brain for all seasons: cyclical anatomical changes in song control nuclei of the canary brain. Science 214:1368–1370.

NOTTEBOHM, F., AND A. P. ARNOLD. 1976. Sexual dimorphism in vocal control areas of the songbird brain. Science 194:211–213.

NOTTEBOHM, F., AND S. KASPARIAN. 1983. Widespread labeling of avian forebrain neurons after systemic injections of ^{3}H-thymidine in adulthood. Soc. Neurosci. Abstr. 9:380.

NOTTEBOHM, F., S. KASPARIAN, AND C. PANDAZIS. 1981. Brain space for a learned task. Brain Res. 213:99–109.

NOTTEBOHM, F., AND M. NOTTEBOHM. 1978. Relationship between song repertoire and age in the canary, *Serinus canarius.* Z. Tierpsychol. 46:298–305.

NOTTEBOHM, F., T. M. STOKES, AND C. M. LEONARD. 1976. Central control of song in the canary, *Serinus canarius.* J. Comp. Neurol. 165:457–486.

OJEMANN, G., AND C. MATEER. 1979. Human language cortex: localization of memory, syntax and sequential motor-phoneme identification systems. Science 205: 1401–1403.

PATON, J. A., AND F. NOTTEBOHM. In press. Neurons generated in adult brain are recruited into functional circuits. Science.

RAYMOND, P. A., AND S. S. EASTER. 1983. Postembryonic growth of the optic tectum in goldfish. I. Location of germinal cells and numbers of neurons produced. J. Neurosci. 3:1077–1091.

SHATZ, C. J. 1982. Neural development: Implications for recovery from injury, p. 289–311. *In* J. G. Nicholls [ed.], Repair and regeneration of the nervous system. Dahlem Konferenzen. Springer-Verlag, Berlin.

STOKES, T. C., C. M. LEONARD, AND F. NOTTEBOHM. 1974. The telencephalon, diencephalon, and mesencephalon of the canary, *Serinus canaria,* in stereotaxic coordinates. J. Comp. Neurol. 156:337–374.

THORPE, W. 1958. The learning of song patterns by birds, with special reference to the song of the Chaffinch, *Fringilla coelebs.* Ibis 100:535–570.

THORPE, W. H., AND P. M. PILCHER. 1958. The nature and characteristics of subsong. Br. Birds 51:509–514.

UYLINGS, H. B. M., K. KUYPERS, M. C. DIAMOND, AND W. A. M. VELTMAN. 1978. Dendritic outgrowth in the visual cortex of adult rats under different environmental conditions. Exp. Neurol. 62:658–677.

Rockefeller University, Field Research Center, Tyrrel Road, Millbrook, New York 12545. Received 22 December 1983. Final acceptance 13 April 1984.