

Chapter 2

Song Learning in Birds Offers a Model for Neuronal Replacement in Adult Brain

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I dedicate this chapter to a true pioneer, Joseph Altman. He was first to publish evidence that some kinds of neurons of the mammalian brain are produced post-natally and even in adulthood.

The discovery of neurogenesis in adult canaries came as a surprise because it was found in a context where it had not been contemplated, the study of vocal learning. To everybody's disbelief, the new, spontaneously produced neurons replaced numerically others that had died, a process of spontaneous brain self-repair or rejuvenation. I will describe how these discoveries came about and how they have helped us understand the natural history of neurogenesis and neuronal replacement in adult brain. Adult neurogenesis may also shed light on a basic issue of brain function: what limits learning?

2.1 The Study of Vocal Learning

The study of vocal learning in birds was revolutionized in the early 1950s by the use of an instrument, the sound spectrograph, that converted sounds recorded on tape into a two dimensional display, the sound spectrogram (Fig. 2.1), that read like a musical notation with time on the horizontal axis and frequency on the vertical one (Thorpe, 1954). This technique enabled the collection and comparison of sounds gathered during the entire vocal development of individual birds and the side-by-side comparison of the sounds produced by different individuals. The sound-spectrograph enabled the classical work of Thorpe (1958), Marler and Tamura (1964), Marler (1970a) and Konishi (1965), which showed that vocal learning in oscine songbirds: (1) occurred during a sensitive period, usually before sexual maturity; (2) required intact hearing; (3) was preceded by the memorization of an external model, that then guided changes in vocal output until that model was matched. Both Thorpe (1955; Thorpe and Pilcher, 1958) and Marler (1970b)

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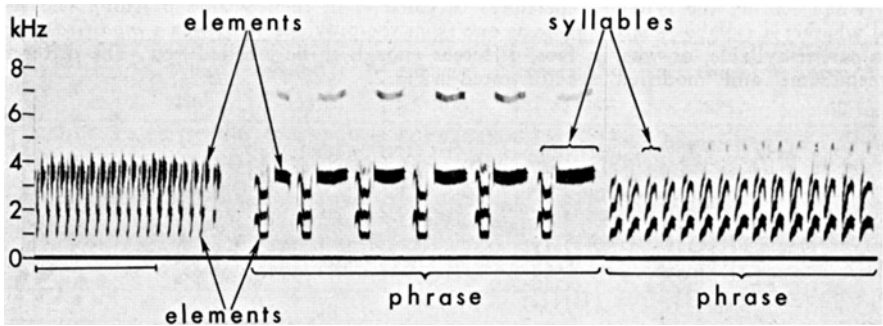


Fig. 2.1 (Figure 1 from Nottebohm and Nottebohm, 1978). A sound spectrogram of canary song. Canaries sing by repeating many times a same “syllable”, then switching to the next syllable, that is also repeated many times, and in this manner eventually producing the full repertoire of syllables that characterizes the song of that individual. Each syllable is composed of 1–3 elements, each followed by a brief silent gap. Each sound is produced at its “fundamental” frequency as well as at higher “harmonics”. In this visual display of sound, frequency in Kilohertz is represented in the vertical axis and time, in seconds, in the horizontal axis. The horizontal bar on the lower left represents a 0.5 s duration

emphasized that song imitation was preceded by a stage of vocal experimentation, called “subsong”, that was reminiscent of infant babbling. These new tools and observations suggested that songbirds would provide a good animal model for studying vocal learning in general, and this drew a lot of interest.

There are two general issues about vocal learning: how it occurs in the individual, including its ontogeny, and how it evolved. Both are related, but we do not know how. Because of this uncertainty, the origins of vocal learning have always been difficult to imagine. It is widely accepted that in the process of evolution new traits emerge from variability in pre-existing ones. Unlearned vocal repertoires, as those found in domestic fowl, *Gallus domesticus* (Konishi, 1963) and ring-doves, *Streptopelia risoria* (Nottebohm and Nottebohm, 1971), develop normally even when individuals are deafened a few days after hatching. How does one go from this situation, thought to be the primitive one, to one in which vocal ontogeny becomes more dependent on hearing – and eventually on imitation – and how does one do this transition so that all intervening evolutionary stages serve animal communication? That was a paradox that, early on, caught my attention (Nottebohm, 1972a).

2.2 Two Sound Sources and Left Hemispheric Dominance

The appeal of vocal learning in birds as a model system became even stronger when work with chaffinches, *Fringilla coelebs*, (the subjects of Thorpe’s 1958 article) showed functional asymmetry in their vocal organ, the syrinx. The chaffinch syrinx consists of right and left anatomically symmetrical and functionally independent halves, each with its own supply of airflow, muscular control and innervation (Fig. 2.2).

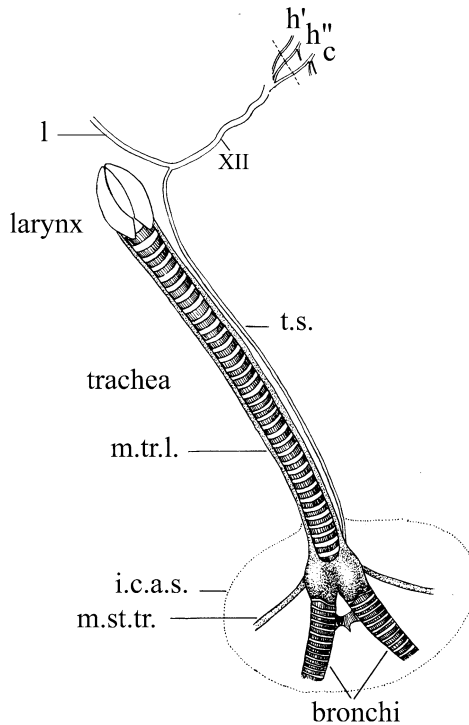


Fig. 2.2 (Figure 3 from Nottebohm, 1971b). Schematic drawing of the vocal tract of songbirds. The syrinx, vocal organ of birds, is at the confluence of the two bronchi and the trachea. It is surrounded by an air space, the interclavicular air sac (icas). The syrinx has several pairs of intrinsic muscles, represented here just as a stippled muscle mass; the sternotrachealis muscle (m.st.tr.) anchors the syrinx to the sternum (not shown). The tracheolateralis muscle (m.tr.l.) runs along the full length of the tracheal and its contraction or relaxation allow the trachea to conform to the neck's posture; the rostral pull of m.tr.l is countered by the caudal pull of the m.st.tr. The larynx is open during phonation, but otherwise does not generate sound. The muscles of trachea and syrinx are innervated by the tracheosyringeal nerve (t.s.), a branch of the hypoglossus nerve (XII). Section of the ts. nerve on one side, or section of its roots (h', h'', c), blocks vocal control on the ipsilateral side of the syrinx

Yet I found that most of the sounds of chaffinch song were produced by the left syringeal half, which therefore was dominant for song. This situation was uncannily reminiscent of handedness in humans. Moreover, this dominance could be reversed if the left syringeal half was denervated early on. This suggested that peripherally both sides were equipotential but that a central bias – like left cerebral dominance for speech and handedness in humans – determined which syringeal half normally did most of the singing (Nottebohm, 1971b, 1972b; DeVoogd et al., 1991). We now had three human traits – vocal learning, left hemispheric dominance and handedness – for which the basic biology could be investigated in songbirds.

The next step was to extend the findings of left syringeal dominance in chaffinches to another songbird, such as the canary, *Serinus canaria*, that could be bred

in captivity. I was eager then to go into the brain to see how the traits that interested me – vocal learning, hemispheric dominance – were represented there and how they developed. A stereotaxic atlas of the canary brain was produced (Stokes et al., 1974), modeled after that of pigeons, *Columba livia*, and using the same terminology (Karten and Hodos, 1967). Once this work was done we set out to uncover the brain pathways that control the performance of the syrinx.

2.3 The Song System

The results were gratifying. Over the next 10 or so years a system of anatomically discreet nuclei and connecting pathways was identified and shown to control vocal output and vocal learning in songbirds (Nottebohm et al., 1976, 1982; Okuhata and Saito, 1987; Bottjer et al., 1984, 1989). I called this array of nuclei and pathways “the song system”. It was the first time that we had, for any vertebrate, an array of nuclei and pathways that was associated, apparently exclusively, with the acquisition and production of a particular learned behavior. This came as a surprise because, under the influence of Karl Lashley (1950), the neural substrate for learned skills in vertebrates was still thought to be widely distributed throughout the brain. Our findings placed the song system conceptually closer to the functional centers conceived by von Holst (1935) and von Holst and St. Paul (1960) and to the simple networks of invertebrates that were proving so helpful in studying simple patterns of behavior (e.g. Huber, 1960; Bullock, 1961; Roeder, 1962; Wiersma, 1962; Wilson, 1961) and that eventually, in the sea slug *Aplysia*, became so important for understanding simple learning paradigms (Kandel, 2006).

The song system can be thought of as consisting of four modules, shown schematically in Fig. 2.3 and in greater detail in Fig. 2.4. The full name of the forebrain nuclei that are part of the song system appears in the legend for Fig. 2.4. **Module #1** is composed of brainstem nuclei that control the muscles for respiration and phonation. **Module #2** is a descending motor pathway in caudal forebrain that tells module #1 what to do (Nottebohm et al., 1976; Yu and Margoliash, 1996; Hahnloser et al., 2002). **Module #3** is a forebrain module that loops through basal ganglia (Area X) and thalamus (DLM) and then returns to anterior forebrain (LMAN), from where it projects to nucleus RA of module #2; module #3 is necessary for song learning but not for the production of learned song (Bottjer et al., 1984; Scharff and Nottebohm, 1991; Brainard and Doupe, 2000; Kao et al., 2005; Oelveczky et al., 2005; Andalman and Fee, 2009; but see Aronov et al., 2008). **Module #4** consists of the ascending auditory pathway and its relays to modules #2 and #3 (Vates et al., 1996). Modules 2 and 3 have their origin in a same nucleus, the High Vocal Center (HVC), which also receives auditory input (Cardin and Schmidt, 2004; Dave et al., 1998; Janata and Margoliash, 1999; Katz and Gurney, 1981; Margoliash, 1986; Vates et al., 1996; Williams and Nottebohm, 1985). The blend of auditory and motor capabilities, the absence of muscle representation and its high position in the descending vocal pathways, make HVC reminiscent of Broca’s area in humans and

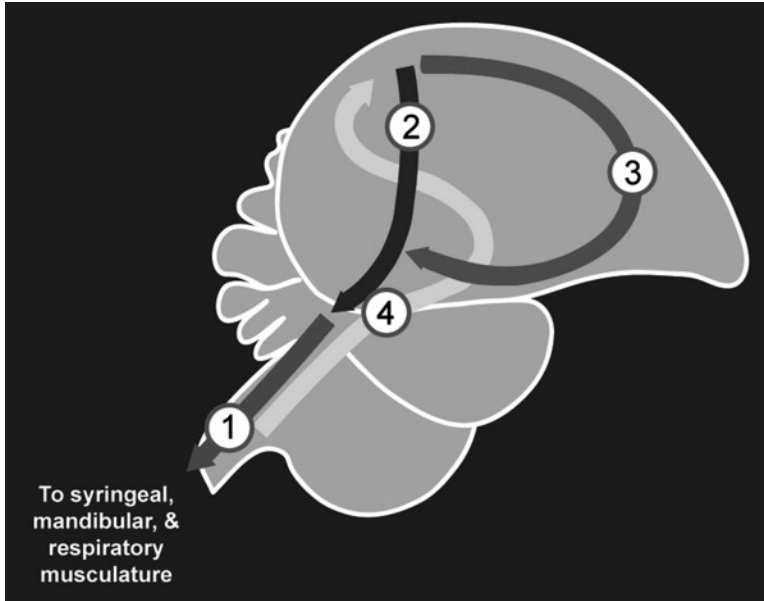


Fig. 2.3 (Figure 4 from Nottebohm, 1993). Schematic diagram of a songbird's brain (rostral to right, caudal to left). The four numbered *arrows* stand for the four song system “modules” described in the text

justify its name as the high vocal center. This name is justified, too, by elegant neurophysiological work that strongly suggests that HVC, more than any other nucleus of the song system, plays a key role in the patterning of learned song (Yu and Margoliash, 1996; Hahnloser et al., 2002; Long and Fee, 2008). Moreover, recent evidence shows synaptic changes occur in this nucleus as new song is learned (Roberts et al., 2010), supporting an earlier correlation between the complexity of learned song and dendritic spine density in HVC (Airey et al., 2000). In addition to the four modules shown, there are feedback loops between different parts of the song system (Vates and Nottebohm, 1995; Vates et al., 1997).

2.4 Sexual Dimorphism

Song is a seasonal behavior and, perhaps not surprisingly, the song system of songbirds has androgen and estrogen receptors that make it very sensitive to gonadal hormones (Arnold et al., 1976). This might have prepared us for the next observation, which, however, came as a surprise. Once careful anatomical details were on hand from sufficient individuals, we realized that the song system of canaries was sexually dimorphic: Area X (homologous to the striatum of mammalian basal ganglia, see Fig. 2.3) was 3.8 times larger in male than in female canaries, while

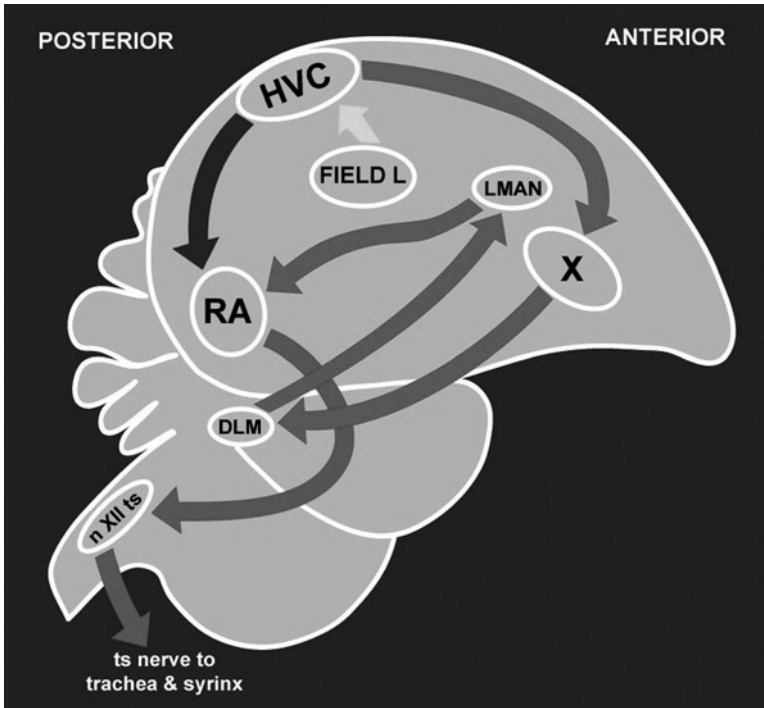


Fig. 2.4 More detailed, but still schematic, representation of the song system's pathways. The shading of the various arrows conforms to that used in Fig. 2.3 and shows which pathways are part of the different modules. The ascending auditory pathway (#4) is here represented as a short arrow that touches HVC. All pathways shown here are ipsilateral

the male / female ratios for HVC and RA were, respectively 3.2 and 2.7. We thought this dimorphism might reflect the fact that whereas male canaries sing relatively large and complex learned song repertoires and the sounds produced are very stereotyped (Waser and Marler, 1977; Marler and Waser, 1977; Nottebohm et al., 1986), female canaries sing much less and when they sing, their song is simpler and the sounds more variable (Nottebohm, 1980b). In zebra finches, *Taeniopygia guttata*, in which females never sing, the male/female differences in size of song nuclei were twice as large as in canaries (Nottebohm and Arnold, 1976).

These observations raised the obvious question. Did the male and female song nuclei have different developmental histories or did the differences observed in adulthood reflect current levels of circulating hormones. There was a precedent for the latter explanation in the rodent preoptic area (Raisman and Field, 1971). Our work and that of others indicates that both mechanisms play a role in the sexual dimorphism of the song system. The size of song nuclei, including HVC, could be made to double by systemic administration of testosterone to adult female canaries over a period of 3–4 weeks. During this time the females started to sing more and more and the sounds produced became more stereotyped and like those of adult

males. However the number of syllable types in their song remained small and their song nuclei were still significantly smaller than those of adult males (Nottebohm, 1980a). Closer inspection revealed that in nucleus RA (Fig. 2.3) the hormone-driven growth in female volume was associated with the growth of dendrites and formation of new synapses (DeVoogd and Nottebohm, 1981; DeVoogd et al., 1985; Canady et al., 1988).

In addition to the role of hormones in adulthood, the sexual dimorphism observed in the song system of temperate area songbirds owes much to differences that emerge during development. The song nuclei of male and female zebra finches – the species now most commonly used in studies of vocal learning in birds – are very similar until day 30 after hatching. After then, many of the cells in the female nuclei atrophy and die and the female song control nuclei shrivel; this effect is prevented by treating the female zebra finches with estrogen before day 30; when these females are given testosterone as adults they sing like males (Gurney and Konishi, 1980; Gurney, 1981; Simpson and Vicario, 1991). In addition to the role of gonadal hormones during ontogeny and in adulthood, other genetic differences between males and females contribute also to the sexual dimorphism of the song system (Wade and Arnold, 1996; Agate et al., 2003, 2004), but that is a story beyond the scope of the present review. What matters, for the present story, is that by 1980 we knew that the anatomy of the adult song system of canaries was very sensitive to testosterone.

2.5 The Late Ontogeny of the Song System

Even as studies of the anatomy and hormonal sensitivity of the song system raced ahead, we also focused on the behavior of song learning – its timing and manner of occurrence. Male canaries in our breeding colonies followed the natural photoperiod of New York State. They started breeding in late March and stopped in mid July. Starting at approximately 40 days after hatching, juveniles produced variable, low amplitude patterns of frequency modulation called “subsung”, reminiscent of the babbling of infants. These sounds occurred in a non-communicatory context, often as the young bird with feathers fluffed and eyes closed seemed to doze. By 3 months of age these sounds were louder and by 4 most of the adult sounds were already present, though still delivered in a variable manner as “plastic song”. Stereotyped adult song was first used in early spring, when the birds were in full breeding condition. In all these respects, canaries were not different from many other songbirds, such as the chaffinch (Thorpe, 1958), that acquire their learned song during their first year of life. Our canaries differed, though, in one important respect, they continued to change their song in successive years and for this reason we called them “open-ended learners” (Nottebohm and Nottebohm, 1978; Nottebohm et al., 1986, 1987).

It was of great interest to me that telencephalic nuclei such as HVC and RA grew dramatically during the juvenile period of song acquisition. By day 15 after hatching

the brain of a young male canary had already achieved full adult weight, yet the volume of HVC 30 days after hatching was 20% that of an adult 1-year-old male. RA at that time had 30% of its adult volume. Both RA and HVC showed a very marked spurt of growth from the thirtieth to the 60th day after hatching. At 60 days the volume of HVC was close to 50% that of an adult 1-year-old male (Nottebohm, 1980b, 1989). If these changes in volume accompanied the learning of a new song, what happened in successive years? Would new episode's of song learning be accompanied by further anatomical changes in HVC and RA?

2.6 Brain Seasonal Changes

The blood testosterone levels of our adult male canaries dropped in mid-summer and soon thereafter the birds started molting their feathers. During the molt, male canaries sang very little if at all and the song of those that sang tended to be variable and reminiscent of that of juveniles (Nottebohm et al., 1987). The size of HVC and RA of males in breeding condition killed in April, when 12 months old, was 1.99 and 1.77 times larger, respectively, than that of other males of the same cohort killed in mid-September, after the end of the molt. Since another study had shown that the size of these two nuclei did not differ significantly between 1-, 2- and 3-year-old males (Nottebohm et al., 1981), I inferred that the reduction in volume observed in late summer was temporary. The results of this seasonal comparison were published in *Science* under the title of "A brain for all seasons" (Nottebohm, 1981). The late summer reduction in volume seen in the song nuclei of the males seemed like the inverse of the doubling in volume seen in the testosterone-treated females. In addition, I indicated that there were also whole brain changes, such that the weight of brains collected in September was 15% lighter than that of brains collected in April. What to make of this?

Towards the end of the 1981 *Science* article I wrote: "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 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 a shedding of synapses, much the way trees grow leaves in the spring and shed them in the fall." This interpretation was in line with the *Zeitgeist* in the neurosciences at that time because it assumed that the plasticity required for learning was vested *solely* in the synapses. It was in line, too, with our observation that in females testosterone induced dendritic growth and the addition of new synapses.

2.7 Space for Learning and Memory

The seasonal ebb and flow of the brain’s space for song fitted well with another observation. Extensive song recordings from 25 adult male canaries of the Belgian Wasserschlager strain were scored for the number of different “song syllables” produced by each bird – “syllables” being the units of repetition produced in canary song (Fig. 2.1). The birds were killed after their song was recorded and the volume of their HVC estimated from serial histological sections. An interesting correlation emerged (Fig. 2.5): a large HVC was a poor predictor of how many different syllables a canary might sing, but birds with a large syllable count tended to have a large HVC. Conversely, if HVC was relatively small, the associated syllable count tended to be relatively small (Nottebohm et al., 1981). This relation between the complexity of learned song and the amount of brain space for learned song was subsequently corroborated in another songbird (Canady et al., 1984) and in studies comparing many species (DeVoogd et al., 1993).

These space/learning relations suggested that brain space for a particular learned skill might be in short supply. However, if birds such as the canary were able to learn new song repertoires every year, then perhaps there was always more space available. The seasonal changes in volume observed in HVC and RA offered a

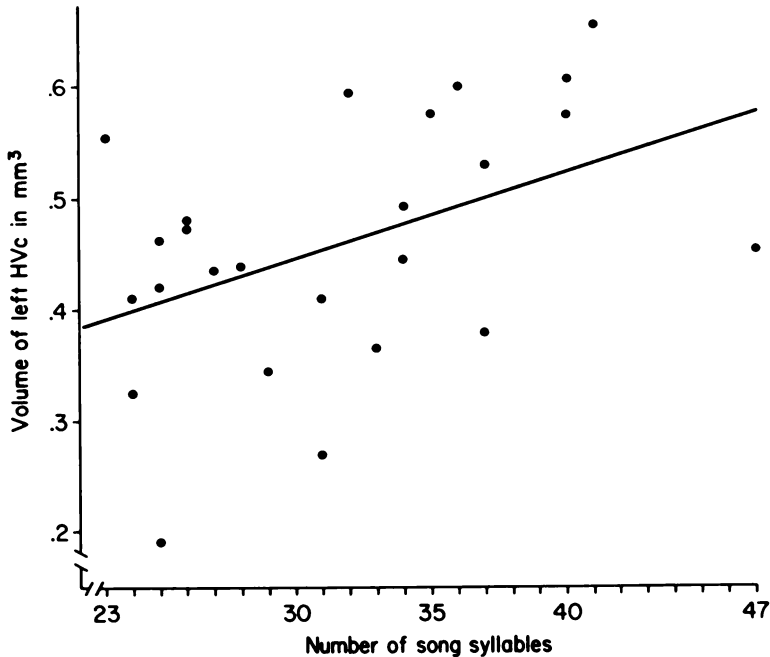


Fig. 2.5 (Figure 1 from Nottebohm et al., 1981). Regression of size of syllable repertoire on the volume of left HVC for 25 adult male canaries

solution to this conundrum: perhaps as songs were discarded, so was the substrate that held them; then, as a new substrate was generated, new songs could be learned once again. This interpretation suggested that a better understanding of the “space” eliminated would provide a clue to how and where the memories of learned song were stored.

2.8 Always the Same Neurons?

Our thinking so far had gone along with the commonly accepted view that learning could be explained by changes in synaptic number and synaptic efficacy. This idea had been advanced initially by two Italians, Lugaro and Tanzi, whose thoughts were quoted and further developed by Santiago Ramon y Cajal in his Croonian Lectures to the Royal Society of London. Cajal said: “Mental training cannot better the organization of the brain by adding to the number of cells; we know that nervous elements have lost the property of multiplication past the embryonic life; but it is possible to imagine that mental exercise facilitates a greater development of the protoplasmic apparatus and of the nervous collaterals in the part of the brain in use. In this way, pre-existing connections between groups of cells could be reinforced by multiplication of the terminal branches of protoplasmic appendix and nervous collaterals. But the preexisting connections could also be reinforced by the formation of new collaterals and protoplasmic expansions.” (Cajal, 1894, p. 466). These “connections” are now called “synapses”. Two researchers Jerzy Konorski (1948) and Donald Hebb (1949) formalized the role of synapses in learning and subsequent workers spelled out the chemistry of how these synaptic changes come about and how long they last (review in Kandel, 2006).

Then, one evening, as I was enjoying a hot, end of day shower I found myself thinking once again about the seasonal changes in HVC and RA volume, and how these changes were more pronounced in HVC than in RA. What were the underlying mechanisms and need they be the same in both cases? We really had no evidence that the dendrites of males grew and withdrew seasonally, this was just an inference based on the observation of dendritic growth in females treated with testosterone. We did not know if in our seasonal comparisons we were always dealing with the same collection of neurons. Could neurons come and go? Might there be conditions under which neurons were born and conditions under which neurons were eliminated and replaced? It was fun to think in this manner, perhaps because it was so different from all I had been taught and because if this budding idea were true, the implications would be big.

There was in my laboratory at the time a very bright and resourceful doctoral student, Steve Goldman, who had not yet chosen a topic for his thesis work. I told him of my fantasy about neuronal comings and goings and to this day remember the amused smile that crossed his face. He knew of a quick way to test it, but, he asked me, “do not talk about this to anyone. People will wonder about the lab if word gets out.” I agreed to keep mum and he told me about tritiated thymidine. 3H-thymidine could be used as a marker of DNA synthesis and therefore as a

presumptive marker of birth date as DNA doubled during the S-phase preceding mitosis. He added that this method was widely used by embryologists staging the order in which different populations of cells were produced (Messier et al., 1958; review in Korr, 1980). 3H-thymidine administered systemically remained in the blood for a short time before it was metabolized and it was only during that brief period that it was available for uptake by cells. This tool would tell us whether new neurons were added when the size of song nuclei doubled.

2.9 3H-Labeled Cells in Adult HVC

We tested for adult neurogenesis in adult female canaries in which, we knew, testosterone treatment would roughly double the volume of HVC and RA. Each bird received six intramuscular injections of 50 uCi of 3H-thymidine at 8 h intervals over a 2 day period, starting at various intervals after the hormone implant. Most birds were killed 37 days after onset of hormone treatment. Brain levels showing HVC and RA were sectioned at 6 um intervals and the sections incubated for autoradiography. After incubation they were stained with cresyl violet. Six kinds of cells were recognized: (1) large (10–18 um soma diameter) cells with clear nuclei and dark-staining, central nucleoli and scant cytoplasm, which were tentatively identified as neurons; (2) smaller cells (6–10 um) containing light nuclei with stippled heterochromatin, small eccentric nucleoli and scant cytoplasm, which we considered astrocytes; (3) small cells (5–8 um) with deeply basophilic cytoplasm, small nuclei and dark karyoplasm, considered to be oligodendrocytes; (4) thin, fusiform perivascular cells, clearly endothelial; (5) very small (4–6 um) cells of variable morphology lining the lateral ventricle over HVC, which were presumed to be ependymal and subependymal cells jointly referred to as ventricular zone cells; (6) cells of uncertain identify. These rather simple criteria, we felt, might under-estimate neuron numbers, but we preferred to err on the conservative side. Using this material, a cell was considered labeled if it had five or more exposed silver grains per nucleus – background was exceedingly low in our material. To validate our light-microscopic criteria for neuronal identification, we cut the brains of some of the 3H-thymidine-treated birds at alternating 1 um and 100 nm intervals. We reasoned that a light microscopic neuronal profile that was one micron thick was very unlikely to include over a labeled cell contaminating material from another cell type, e.g. a glia, that contributed the label, and that an adjacent 100 nm section could be used for electro-microscopy to see if the ultrastructural details of the labeled cell conformed with those of a neuron. Electron microscopy revealed details in labeled cells identified as “neurons” – extensive rough endoplasmic reticulum, homogeneous nucleoplasm, long processes filled with microtubules and in one case an apparent axonal hillock – that were compatible with this identification, but we failed to see in these cells any unambiguous synaptic profiles. The other cell types looked very different under the electron microscope. Our counts indicated that 0.9–2.0% of the cells that we identified under the light microscope as “neurons” were labeled per day of 3H-thymidine treatment when

the birds were killed 18 or more days after the last 3H-thymidine injection, with no labeled “neurons” in the HVC of two birds that received their 3H-thymidine 1–2 days before they were killed. In the latter two cases, however, the ventricular zone above HVC was blanketed with small cells whose nuclei were tritium labeled, while within HVC only endothelial and glia cells were labeled. Under none of the survival times we used were there any 3H-labeled “neurons” in RA.

We inferred from these results that if new neurons were recruited into adult HVC, they were not produced by the division of existing HVC neurons, because if that had been the case, labeled neurons would have occurred in HVC even at short survival times. The short-term heavy labeling of cells in the ventricular zone above HVC suggested that this might be the place of birth of the new neurons. Unexpectedly, the numbers of labeled neurons were similar in the females that received testosterone as in those that received cholesterol or empty silastic implants. Since male and female carduelines were known to continue to modify their calls in adulthood (Mundinger, 1970), we speculated that the new neurons of females might be involved with this kind of vocal learning. Finally, since the numbers of cells we called neurons seemed to be recruited at a rather high rate, yet the size of the female HVC did not change significantly between successive years (Nottebohm et al., 1981), we thought that perhaps the new cells replaced numerically older ones that had died, a phenomenon for which there was a precedent in the olfactory epithelium (Graziadei and Monti Graziadei, 1978).

I invited Pasko Rakic, the Yale developmental neurobiologist and a great skeptic of adult neurogenesis, to be a member of Steve Godman’s thesis committee and he graciously accepted. He came to the laboratory and looked over the histological material. He accepted the results, but we all acknowledged that there were still loose ends and that much more work needed to be done. Those results were scrutinized, too, by the great embryologist Viktor Hamburger, who communicated them for publication in the *Proceedings of the National Academy of Sciences* and that is how the first report of adult neurogenesis in songbirds came to be (Goldman and Nottebohm, 1983).

Our results and interpretations were tentative because: (1) There could be in the brain cells that by some criteria looked neuronal, yet played some other role. (2) We were not sure that the labeling we saw resulted from DNA synthesis that accompanied mitotic events. Successive 3H-thymidine injections might create labeling cycles whereby tritium incorporated into DNA during spontaneous, on-going DNA repair induced damage that was followed by more DNA repair and 3H-labeling, followed by more damage and repair and so on. (3) Even if the cells were neurons, we had no evidence that they were part of functioning circuits and that they played a specific role in behavior. (4) The whole phenomenon could be a laboratory artifact that resulted from the highly restricted and monotonous conditions under which our captive birds were kept. Because of these uncertainties, our claims were no less vulnerable to criticism than those of Joseph Altman, of whose work we had learned while preparing our results for publication. Altman and others had claimed to have evidence of post-natal and adult neurogenesis in mammals (Altman and Das, 1965; Altman, 1969; Bayer et al., 1982; Kaplan and Hinds, 1977), but their

work had met strong resistance. To overcome this resistance, our evidence would have to be more compelling.

2.10 Hope for a New Neurology

I organized in 1984 a conference in New York on the topic of “Hope for a New Neurology”, underwritten by Jane and Peter Pattison’s Institute for Child Development Research. The intention was to review what was known about a number of brain neurodegenerative disorders, evidence for recovery following lesion, the possible therapeutic value of transplants and the emerging field of adult neurogenesis. Among those invited was Shirley Bayer, Joseph Altman’s wife, who continued his work. Altman, who had retired, was in the audience. I have often regretted that I did not invite Joseph Altman himself to be a speaker, for he would have had so much to say about a subject he knew so well. Also there was Pasko Rakic, whose work on brain development I admired. I knew Rakic was not convinced that adult neurogenesis was real and I wanted him present as a critic. It was in that context that he said: “Extraordinary claims require extraordinary evidence”. Of course, the claims he found extraordinary were extraordinary only when placed next to the 100-year old dogma that adult neurogenesis couldn’t, shouldn’t and didn’t happen. Rakic felt that evidence for adult neurogenesis in mammals would be believable only when the facts in its favor could not be explained in any other way. Rakic (1985a,b) felt that he knew what he was talking about. He had looked for evidence of post-natal or adult neurogenesis in close to 10,000 sections from the brains of 10 Rhesus macaques of both sexes that had received injections of 3H-thymidine at ages ranging from 6 months to 5 years. He did not find a single “heavily labeled” (15–20 exposed silver grains) cell that met his criteria of neuronal identity. His definition of “heavily labeled” was that of a neuron with at least half the maximum number of exposed silver grains found over the nucleus of other cell types known to continue to divide in adulthood. I admired Rakic’s integrity and high standards of proof, but could not understand how the same man that was willing to accept the possibility that adult neurogenesis occurred in birds still argued so vehemently against its occurrence in mammals. Possibly he thought about these matters as a medical man. If adult neurogenesis did not occur in primates – and he had found zero evidence of it – and only rarely if at all in other mammals, then the data from other species were irrelevant to “hopes for a new neurology”. His was a defensible position.

2.11 The Role of Joseph Altman

The negative results reported by Rakic pertained to macaques, and he did not rule out the possibility that earlier reports of post-natal neurogenesis in rodents and cats might be correct. Among those earlier reports were those of Joseph Altman (1962),

to whom I alluded earlier. It is of interest that although Altman gained attention in the minds of many as first to challenge Cajal's assertion – and that of many neuroanatomists that came after him – that no new neurons were produced after the end of embryonic development, he (Altman) thought it had been done before. To quote from lines 6–10 of Altman and Das (1965), “It was recognized in the nineteenth century (Lahousse, 1888; Schaper, 1894; Cajal, 1911) that a class of small nerve cells, the granule cells, shows a high rate of proliferative activity in the cerebellar cortex in young animals. Likewise, good quantitative evidence was obtained some time ago (Sugita, 1918) of an increase in the total number of nerve cells in the cerebral cortex of rats up to the 20th day after birth.” All the same, Altman's initial and subsequent findings on adult neurogenesis (e.g. Altman, 1963) were resisted and this lingering resistance was in evidence at the time of the Hope for a New Neurology conference on April 16–18, 1984. Reasons for resistance were technical and not trivial. For example, it was possible that in some cases “label” over the nucleus of a neuron came from an overlying fragment of nuclear glia. Thin (1 μ m), contiguous labeled sections of “neurons” and adjacent thinner ones inspected under the electron microscope were necessary to establish the source of label and to bolster the neuronal identification. In 1984 the evidence of this kind for cells born in adult mammalian brain was sparse (Kaplan and Hinds, 1977) or inadequate. The electron-microscopic evidence of post-natal neurogenesis presented by Kaplan (1985) at the Hope for a New Neurology conference came from a 9-day-old rat, hardly an adult. Moreover, it was possible that DNA duplication occurred in differentiated cells leading to polyploidy, without this necessitating the formation of new neurons. It was even possible, as Altman acknowledged, that the 3H-label seen in the nucleus of cells resulted not from the synthesis of new DNA during the S-phase of mitosis, but from a slow turnover of DNA. It is of interest that Altman (1962) countered this concern by pointing out that some neurons showed intense labeling as soon as 1 day after 3H-thymidine injection, which is now considered also too soon for a cell to be born, migrate and differentiate into its adult neuronal phenotype. Finally, skeptics adduced that the neuronal identity of labeled cells had not been sufficiently established. Perhaps, under some conditions, synaptic contacts were made on glia? The point is, a skeptic can always find some reason to doubt what it does not want to believe and it is up to the scientist making the claim to meet all these potential difficulties so that, at the end, the interpretation offered is the only one, or most likely one to account for all known facts.

As suggested above, some of the original skepticism may have been justified. In addition, Altman reported in his 1962 paper in *Science* that bilateral electrolytic lesions in the lateral geniculate body of “young adult” rats, followed by intraperitoneal injections of 3H-thymidine, resulted in the labeling of neurons in cortex, including “a few labeled pyramidal cells”. This claim, without intervening electrolytic lesions, appears again in his 1963, *Anatomical Record* publication, where labeled cortical neurons (including a photograph of a labeled pyramidal cell) appeared after administration of 3H-thymidine to young adult rats and adult cats of unknown age. Some 48 years later, the topic of endogenous addition of new neurons to the adult mammalian cortex remains controversial, with only a small scatter of

positive entries (Gould et al., 1999; Magavi et al., 2000; Dayer et al., 2005) and a persistence of negative reports (Breunig et al., 2007; Koketsu et al., 2003; Kornack and Rakic, 2001; Rakic, 2002).

There was also an interesting semantic issue. Cajal (1894) had stated that the central nervous system ceased to add new neurons “past the embryonic life”, but presumably this need not mean that there was a sudden stop at the moment of hatching or when a mammal was born. Some species are born at earlier developmental stages than others – with extremes, for example in marsupials. Even among rodents, mice and rats are born at an earlier developmental stage than guinea pigs. In short, in some species processes associated with embryogeny continue in parts of the brain that are still developing after birth. Two examples of this were known at the time Altman started his work – one was the cerebellum (Uzman, 1960; Miale and Sidman, 1961); the other was the hippocampus (Angevine, 1965). A third area, the subependymal layer in the anterior reaches of the lateral ventricle was known to continue to show mitotic activity into adolescence and adulthood (Bryans, 1959; Messier et al., 1958). Altman’s focus on these three areas showed, after 1965, that new cells that he identified as granular neurons (“microneurons”) continued to be added post-natally to cerebellum, olfactory bulb and dentate gyrus, and that this addition continued into adulthood in the case of the latter two structures. His inferences from these observations were extensively presented in his reviews (Altman, 1967, 1969 and 1970). Altman’s overarching theory was that macroneurons formed early in the development of the brain and established “hard-wired” circuits. Microneurons – in some cases at least – developed late because the fine details of their connectivity were influenced by events that happened late, when the young animals first encountered their post-natal environment. He was not dogmatic about this view, but rather, echoing the style of his 1962 title (“Are new neurons formed in the brains of adult mammals?”), his thoughts this time also came with a question mark, and I quote from his 1967 review (p.741): “Is the proliferation of the precursors of microneurons delayed in altricial mammals until birth because their function is a post-natal one – that of adjusting the genetically specified programs of behavior in accordance with information about specific environmental conditions as it is acquired by the growing animal?” It was a good question that placed order on the facts known to him. Moreover, Altman used his insights about the late development of some brain circuits to explain why some kinds of learning might be difficult, or absent in young altricial mammals (Altman and Bulut, 1976). We now remember Joseph Altman for the things that, in the 1960s, he got right: (1) Olfactory bulb granule cells continue to be produced after birth and into adulthood and are born in the subventricular zone of the lateral ventricles, from where they migrate rostrally to the bulb; (2) Granule cells of the dentate gyrus continue to be produced post-natally and into adulthood, as was confirmed by the beautiful work of his wife, Shirley Bayer, that presented her results at the 1984 Hope for a New Neurology conference; (3) post-natal neurogenesis in cerebellum; (4) the late neurogenesis that occurs in some brain systems of altricial mammals can explain the late occurrence of some kinds of learning. He was the first, in modern times, to challenge the 100 year old dogma that neurogenesis stops at birth and, despite strong opposition, persevered with this claim and was right.

Moreover, though the information accumulated by Altman during the 1960s was in the public domain, he was one of few that not only believed in it, but also used it to formulate bold new hypotheses. His titles reflected caution, preferring the use of “post-natal” to that of “adult” neurogenesis (Altman, 1967, 1970).

In some ways, the great battle fought in 1984 between Pasko Rakic and those that wanted to discard the “no new neurons in adulthood” dogma was a matter of emphasis. It was a matter of the greatest interest to neurosurgeons and neurologists to know what kinds of repair potential were spontaneously available in the brain of their patients, and what kinds might be “turned on”. That is why observations on primates or other mammals were so important, because they were the closest to potential clinical applications. But there was also a deeper issue. Was neurogenesis in the adult vertebrate brain possible at all? Where, if anywhere in the adult brain, were new neurons produced? From what cell types? Could neurons born in adult brain migrate from birth site to work site? Would they be able to join existing circuits? If these feats were possible in the brains of some vertebrates, then the “no neurons in adulthood” dogma was overly severe and the study of adult neurogenesis became not only more interesting but also a source of hope.

By the time of the 1984 “Hope for a New Neurology” conference postnatal neurogenesis was known to occur in a number of cold-blooded vertebrates whose body, eyes and brain continued to grow after sexual maturity (Anderson and Waxman, 1985; Birse et al., 1980; Easter, 1983; Graziadei and DeHan, 1973; Johns, 1982; Leonard et al., 1978; Raymond and Easter, 1983); in addition, adult neurogenesis had been shown to occur in the olfactory epithelium of mammals (Graziadei and Monti Graziadei, 1978, 1985). These reports had not met the level of skepticism generated by Joseph Altman’s claims about new neuron formation in the brain of adult mammals. Perhaps the evidence from cold-blooded vertebrates and from the olfactory mucosa was not seen as relevant to the “no neurons in adulthood” dogma and so no big battle was fought over those observations.

Ironically, both sides could claim a measure of victory in the disputes over neurogenesis in the brain of warm-blooded vertebrates. No embryologist doubted in 1984 or doubts now that the vast majority of the neurons present in the central nervous system of eutherian mammals are produced during embryogeny and not later. By this measure, the “no new neurons in adulthood dogma” was 99.9% right. Yet, if the exceptions discovered by Altman were confirmed, the consequences for the brain sciences could be great. We found the field polarized in this manner when we started our work on neurogenesis in songbirds and we went after the basic data that would test the reality of this phenomenon and, if real, its manner of occurrence.

2.12 Are They Really Adult-Born Neurons?

The procedure we used to test for adult neurogenesis in female canaries also yielded positive results in males. Moreover, in both cases new neurons occurred through much of the telencephalon, so the phenomenon was not restricted to the

song system. However, there were no neurons that met stringent criteria for ^3H -labeling outside of telencephalon: none in the diencephalon, mesencephalon, cerebellum or brain stem. Since the telencephalon had been traditionally associated with learning and higher brain functions, maybe we had to think of the new cells in that context (Nottebohm, 1984). But we first had to resolve two questions: (1) was the labeling observed evidence of birth? (2) Were the new cells really neurons? The answer to the first of these questions came from a low-tech observation. We got the answer to the first of these questions by comparing the number of exposed silver grains over the nuclei of endothelial, glia and neuronal nuclei in HVC. The numbers were very similar for all three cell types. True, peaks of label (45 and 40 exposed silver grains, respectively) occurred over the nuclei of glial and endothelial cells, compared to 25 exposed silver grains in neurons, but the rest of the histogram distributions were very similar in the three cell types. Perhaps glia and endothelia collected more label because of their greater proximity to capillary blood? What mattered was that there was not an order of magnitude difference in amount of labeling when comparing glia and endothelia vs. neurons. The distributions of label argued, instead, for a common process, the formation of new DNA during the S-phase preceding mitosis (Nottebohm, 1985).

But were the labeled “neurons”, neurons? Our initial evidence from electron microscopy had been a bit disappointing in that we found no synaptic profiles on the soma of HVC labeled “neurons” from adult female canaries (Goldman and Nottebohm, 1983). This issue was revisited by Gail Burd, an electron-microscopist in my laboratory. The protocol she followed was similar to that used by Steve Goldman, but she used HVC samples from adult male canaries, rather than females as in the previous study. Not only did she show in this material that contiguous 1- μm thick sections of a same “neuron” showed nuclear labeling – further reducing the likelihood of glial “contamination” – but she found that these cells received in their somata three different kinds of synapses, characterized by synaptic vesicles of different size and density. Electron-microscopic examination of these cells revealed also other standard features of neuronal ultra-structure (Burd and Nottebohm, 1985). We felt this was encouraging, but still not conclusive.

The next test was more stringent and, as so often in my career, I was lucky that the right person, John Paton, a neurophysiologist of impeccable standards, was at that time in my laboratory. The approach, again, was simple: we knew that if we injected adult canaries twice daily with ^3H -labeled thymidine for a period of 2 weeks and killed the birds 1 month after the last injection, approximately 10% of the neurons in HVC reached our labeling criterion. This meant that if we used in such birds an electrode to penetrate cells in HVC, one in ten of neurons randomly entered would be labeled. We used beveled micropipettes containing a solution of horseradish peroxidase (HRP) in KCl. The pipette was slowly advanced into HVC. Penetrations of cells were accompanied by a negative d-c shift in electrical potential and cells were identified as neurons by the presence of spontaneous or depolarization-induced action potentials. Many of the neurons penetrated responded to noise bursts and the latencies of any time-locked synaptic potentials were noted. Then, over a period of 20 min, the HRP was iontophoretically injected into the cell and

the stereotaxic coordinates of the cell injected were recorded. Then the bird was killed and the brain prepared for histology. The HRP reached into all corners of the cells injected, but did not spill out, giving a beautiful, Golgi-like, rendering of soma and processes, down to the level of synaptic spines. The full anatomy of these cells was photographed and drawn, as seen in 100 μ m-thick sections. When these cells were later cut into thinner sections and the sections were covered with a photographic emulsion, the nuclei of seven of the cells had over them a rich collection of exposed silver grains. We inferred that, as expected, approximately one in ten of the HVC neurons that had been randomly penetrated had been born during (or after) the 2 weeks of 3H-thymidine treatment that had ended 30 days prior to electrode penetration; four of these seven cells had responded to sound. We now had direct evidence that at least some of the labeled HVC cells that we had identified as neurons were, indeed, neurons and that some of these cells had been recruited into existing, functioning circuits. I believe this experiment turned the tide on how other scientists felt about adult neurogenesis (Paton and Nottebohm, 1984).

Four years later this result was confirmed using a totally different and much simpler approach. This time we used a combination of 3H-thymidine as a birth-date marker and fluorogold as a retrograde tracer. By making injections of fluorogold in Area X or RA in canaries pretreated with 3H-thymidine, we were able to show that whereas the great majority of HVC neurons that project to Area X (Fig. 2.4) are born before hatching, those that project to RA (HVC→RA projection neurons) are born after hatching and during the very period when song is learned as an auditory-motor skill (Alvarez-Buylla et al., 1988a) as well as later in adulthood.

Yet, it seemed wise to remain skeptical. For adult neurogenesis to be an indisputable fact, we had to show where the new cells came from and how they reached their destination. It is only when all parts of a story fit together, that one is confident that this is the story that explains all observed facts.

2.13 Birth and Migration of New Neurons

Again, good fortune interceded, this time in the person of Arturo Alvarez-Buylla (pre-viewed in the above ref.), an extraordinarily able experimentalist whose thesis work focused on the origin and migration of new neurons in adult avian brain. We already suspected that, as during embryogeny, the new neurons were born in ventricular walls (Goldman and Nottebohm, 1983), but we needed stronger evidence that this was so.

Soon after the initial, 1983, publication, Daniel Buskirk joined my laboratory and started a search for monoclonal antibodies that might identify cell types or phenomena involved in the production / recruitment of new neurons into adult brain. It was the kind of approach that some belittle as a “fishing expedition” and that, with a bit of luck can generate key clues. Mice were injected intraperitoneally with a suspension of homogenized adult canary brain. Spleen cells from these

mice were fused with mouse myeloma cells and plated into tissue culture dishes, followed by screening for positive hybridomas. We then screened for antibodies that recognized markers restricted to the telencephalon and associated with the ventricular zone. Our “expedition” came up with the nicest “fish” we could have hoped for: a monoclonal antibody, 40E-C, that stained cells with small bodies in the walls of the lateral ventricle and long, undividing processes that dove into the forebrain’s parenchyma. Follow up work by Alvarez-Buylla showed that this antibody recognized vimentin. We had stumbled upon radial glia, a cell type familiar to embryologists but not known to occur in adult birds (Alvarez-Buylla et al., 1987) or mammals.

But this observation got still better. When the antibody that stained for vimentin was combined with cresyl violet, we were able to see an uncanny association between the vimentin-positive radial fibers and small, darkly staining elongated cells that were closely apposed to these fibers and showed their same orientation (Fig. 2.6). This kind of cell had not been described before in the parenchyma of adult telencephalon. We thought these might be young, migrating neurons (Alvarez-Buylla et al., 1988b). To test this interpretation, adult male canaries received two intramuscular injections of 3H-thymidine 12 h apart and were killed at various intervals thereafter. One day after the last injection there were no labeled neurons, but lots of labeled cells in the ventricular wall of the lateral ventricle. By 3 days, still no labeled neurons, but small, elongated, darkly staining labeled cells were seen a short distance from the ventricular zone. This collection of labeled cells continued to grow and move further away from the lateral

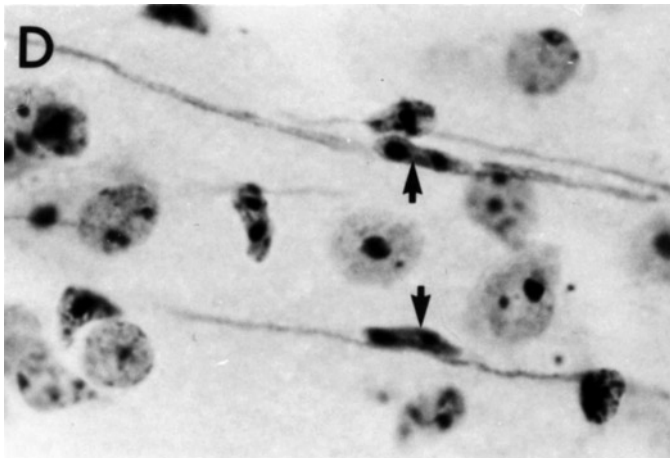


Fig. 2.6 (Figure 2D from Alvarez-Buylla et al. 1988b). A cresyl violet stain that reveals cell nuclei and an antibody stain that recognizes vimentin allow us to see the relation between young migrating neurons (small, elongated nucleus) and the vimentin positive fibers of radial glia in adult canary forebrain

ventricle in successive days. By day 20 it had reached the furthest corners of the telencephalon and on that day, too, the first labeled, differentiated neurons were seen. Thereafter, the cohort of small, elongated, darkly staining labeled cells dwindled and, at the same time the number of labeled neurons steadily increased. We felt that we now had further evidence that the neurons were born in the ventricular zone, and from there migrated, as spindle-shaped, young neurons, guided by radial glia fibers (Fig. 2.6), until they reached, presumably, a signal that triggered the change from the migratory to the sedentary phenotype. When we compared the maximum count (at 20 days) of migratory young neurons to the number of adult, labeled neurons, we inferred that only one third or less of the cells that migrated away from the ventricular zone were still present, as neurons, when our counts stopped, 40 days after 3H-thymidine injection. As during embryogeny, the system overproduced neurons and only a fraction was incorporated for longer durations of time. We felt that the story of adult neurogenesis had, with these details, been firmed up a bit more (Alvarez-Buylla and Nottebohm, 1988).

2.14 Neuronal Stem Cells

We had identified the presumptive birth-site. But who were the parents? To answer this question, adult canaries received a single injection of 3H-thymidine and were killed 1 h later. Virtually all of the labeled ventricular zone cells were in the walls of the forebrain's lateral ventricle. Apparently, cells on the walls of other ventricles seldom divided in adulthood, as already reported for the adult mammalian brain (Korr, 1980; Smart, 1961). In other, similarly treated adult canaries the strip of tissue (ventricular zone, VZ) adjacent to the lateral ventricle was excised and its cells dissociated and spread on a glass slide, where they formed a monolayer of easily identified cells. The cells were then reacted with the anti-vimentin antibody and incubated for autoradiography. At 1 h survivals, 80% of the VZ cells labeled with 3H-thymidine (20 times above background) were vimentin positive and a good many of these had the long, undividing process typical of radial glia. Since the highest concentrations of labeled VZ cells of adult canaries coincided with those regions where the greatest number of migrating neurons first appeared, we suggested that neurons were probably born there by division of radial cells (Alvarez-Buylla et al., 1990b). If so, then the very cells responsible for neurogenesis were the same as the ones that seemed to guide their migration (Fig 2.6). This arrangement was confirmed some years later in work that Alvarez-Buylla did after he left my laboratory (Alvarez-Buylla et al., 1998). Subsequently, Alvarez-Buylla and co-workers showed that the neuronal stem cells of adult mammalian brain are also glia – radial glia and astrocytes (Doetsch et al., 1999; Seri et al., 2001; Alvarez-Buylla et al., 2002; Merkle et al., 2004;

Alvarez-Buylla and Lim, 2004). Furthermore, work in developing mammalian brain has also shown that radial glia are progenitors of neurons (Kriegstein and Alvarez-Buylla, 2009). Time and again I have been gratified by how well our findings in birds generalized to mammals.

2.15 The Relation Between Behavior, Adult Neurogenesis and Neuronal Replacement

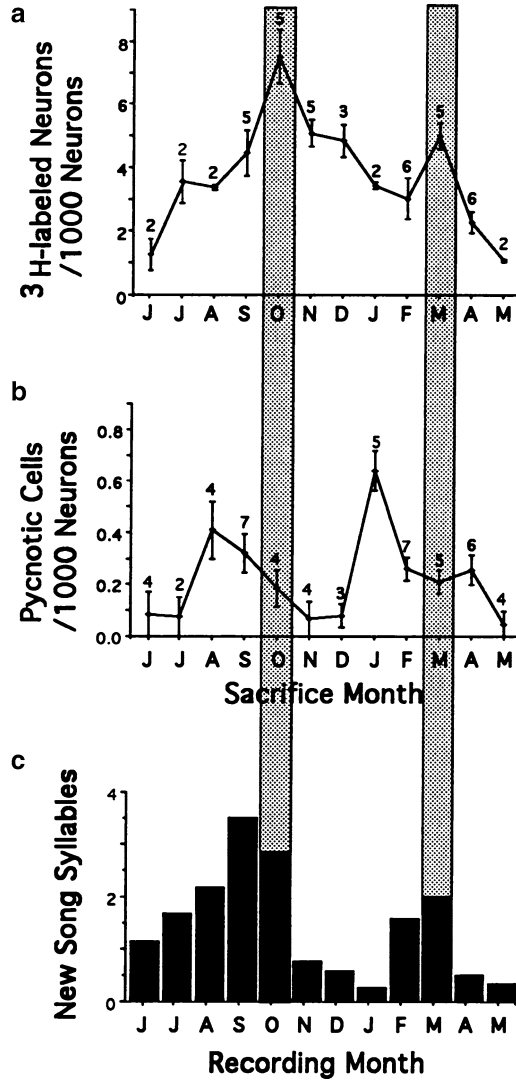
My initial interest had been the biology of vocal learning. This had led to studies of adult neurogenesis. But what was the relation between neurogenesis and behavior? The fact that in canaries HVC neuron numbers did not increase with age suggested that when new HVC neurons were recruited they replaced – numerically at least – older ones that died (Nottebohm, 1985). A similar conclusion had been reached by Bayer (1985) in her study of the dynamics of neuronal recruitment and total neuronal numbers in the olfactory bulb of adult rodents. But what were the behavioral correlates of replacement, if any? For example, did the incidence of neurogenesis in HVC change seasonally, accompanying changes in song?

To test this idea, adult canaries received intramuscular injections of 3H-thymidine every month of the year and were killed 1 month later and the proportion of labeled neurons in HVC was counted. This proportion showed two yearly peaks, in October and March. These peaks were preceded 2 months earlier by peaks in the ratio of pycnotic cells to total number of neurons in HVC, suggesting that we were dealing with a process of replacement (Kirn et al., 1994) (Fig. 2.7). Pycnotic staining is thought to identify dying cells, but of course, by the time a cell is that far gone it is not possible to know if it had been a neuron.

Two earlier studies had shown that the song of adult male canaries became particularly unstable during September and February, when many new song syllables were added (Nottebohm et al., 1986, 1987). These changes in behavior occurred between the peaks in HVC cell death and the peaks in new neuron recruitment. The relative magnitude of the changes in behavior (higher in summer than in winter) bore a close relation to the relative magnitude of the neuronal recruitment peaks, also higher in late summer than in late winter (Kirn et al., 1994) (Fig. 2.6).

These results of our 1994 study suggested that song stereotypy was weakened by the death of neurons in HVC and that the resulting vocal variability allowed the birds to master new sounds. The subsequent increase in new neuron and total neuronal numbers occurred as stereotypy was, once more, reinstated. Other observations, described in the next section, suggest how the cellular and behavioral changes come about.

Fig. 2.7 (Figure 3 from Kirn et al., 1994). Monthly relation between neuronal recruitment and cell death in nucleus HVC and appearance of new song syllables in 1–2 year old male canaries. (a) Mean (plus or minus standard error) of 3H-labeled neurons per 1,000 HVC neurons in birds killed at different times of year 1 month after 3H-thymidine injection. (b) Number of pycnotic cells per 1,000 HVC neurons. For (a) and (b) the number of birds in each monthly sample is indicated above the *error bar* and the first letter of each month indicates when the birds were killed. (c) Mean number of new syllable types that appeared in the song of six adult male canaries each month during their second year of life. The *shaded bars* linking (a–c) emphasize timing of the two peaks in neuronal recruitment and their relation to peaks in cell death and syllable acquisition



2.16 Use and Disuse as Regulators of New Neuron Survival

Work on neurogenesis emphasizes counts of neurons labeled with a birth-date marker. For practical reasons, those counts must often wait until the new cells have reached their destination and acquired an adult neuronal phenotype. By that time it is not possible to say how many of the new neurons were born and what fraction survived. The number present at the time of the count is the number “recruited” into that part of the brain and this number, as we shall see, continues to change.

The blood testosterone levels of adult canaries fall after midsummer and at that time the song of these birds becomes more hesitant and variable (Nottebohm et al., 1987). How do these two changes relate to the new neurons? Testosterone does not regulate the production of new HVC neurons, but does promote the survival of those born in adulthood (Rasika et al., 1994). In this manner daylength, gonadal function, reproduction, song and neuronal replacement are part of a seasonal choreography that recurs every year. Though there is no direct proof for this, it seems plausible that the song syllables no longer in use and the cells that encode them are discarded to make room for new cells that can then engage in the mastery of new song syllables (Kirn et al., 1994). This view is supported by the observation that a significant fraction of the HVC→RA neurons present in the HVC of adult male canaries in early spring disappears by early fall, while those present in early fall are still present in the following spring (Kirn et al., 1991; Kirn and Nottebohm, 1993; Nottebohm et al., 1994).

We wondered to what extent the effect of testosterone on new neuron survival was mediated directly by its action on androgen sensitive neurons (Arnold et al., 1976), and how much was mediated indirectly by promoting higher levels of singing. The two variables were teased apart by looking at the performance of adult male canaries in early fall, a time when, unlike in the spring, amount of singing is not driven by testosterone. Two groups were set up: birds in one group had intact testis, those in the other had been castrated in early September. The rate of singing between these two groups, tested for 1 month starting 15 days after the date of castration, did not differ. All birds received 3H-thymidine systemically during post-castration days 10–14. When castrates and intact birds that sang comparable amounts were compared, the number of 3H-labeled HVC neurons was 2.6 times higher in intact controls than in castrates. However, in castrates with plasma testosterone levels that were undetectable, the mean amount of singing was positively related to the number of new neurons. This outcome suggests that singing and gonadal factors promote, separately, the recruitment of new neurons into HVC circuits and that when they exert this effect together they do so in an additive manner (Alvarez-Borda and Nottebohm, 2002).

The next experiment tested directly how singing contributes to new neuron survival. New HVC neurons of adult canaries are already in place 8 days after these cells are born (Kirn et al., 1999); this relatively brief period probably results from the proximity of their birth site – the floor (ventricular zone) of the lateral ventricle overlying HVC (Scott and Lois, 2007) – to their final destination. Yet, even after the cells have made the transition from their migratory to their sedentary stage they are not yet “safe”. Their survival must be confirmed by “use”. We compared the number of new HVC neurons in two groups of adult male canaries matched for age. In one group the birds were able to sing unhindered for the full 38 days that intervened between injection of the birth date marker bromodeoxyuridine (BrdU) and the day they were killed. In the other group, singing was discouraged by a person that sat in the room where the birds were housed; this person waved a hand at any bird that started to sing. This protocol was followed during daylight hours from day 31 to day 38 after BrdU treatment. When the birds were killed, the number of new

HVC neurons was 63% higher in the birds that sang freely during all 38 days before they were killed. This effect presumably reflects how pathway use affects new neuron survival. In that same study we showed that the expression of brain-derived neurotrophic factor (BDNF) in the HVC of adult male canaries allowed to sing was directly proportional to the number of songs produced per hour. This increased expression is accompanied by higher concentrations of BDNF in HVC (Li et al., 2000). An earlier study had shown that BDNF infused into the HVC of adult canaries promotes the survival of new HVC neurons (Rasika et al., 1999). Under normal conditions, both singing and rising testosterone levels up-regulate BDNF expression and thereby promote the survival of new HVC neurons (Rasika et al., 1999; Alvarez-Borda and Nottebohm, 2002; Alvarez-Borda et al., 2004).

2.17 New Neurons as a Vehicle for Learning

I pointed out earlier that song nuclei HVC and RA (Fig. 2.4) develop late during ontogeny. In the case of HVC, but not RA, many new neurons continue to be added during the very weeks and months when juvenile canaries first acquire their song. As discussed earlier, many of these new neurons are HVC→RA *projection* neurons (Alvarez-Buylla et al., 1988a, 1990a, 1992). In zebra finches, too, the neurons that project from HVC to RA are added at the time song is first learned (Nordeen and Nordeen, 1988). This is important. It will be remembered that Joseph Altman had promoted the hypothesis that only inter-neurons were added after birth (the granule cells of cerebellum, hippocampus and olfactory bulb), but clearly that is not the case in the song system. Moreover, the very elegant work of Hahnloser et al. (2002) with zebra finches strongly suggests that the sparse firing of HVC→RA projection neurons encodes the program for learned song (Long and Fee, 2008). If so, the memory of learned song may be in the connections formed by the new cells at the time song is acquired. Taken together, these observations suggest that in zebra finches and canaries availability of uncommitted cells is important for learning song as a sensory-motor skill, whether this be in juveniles or, in the case of adult canaries, seasonally.

Given these correlations, does the recruitment of HVC→RA neurons in adulthood differ between species that, like the zebra finch learn their song only once, before sexual maturity, and canaries that modify their song every year? The answer is yes. Many more new neurons are added to the HVC of adult canaries than to that of zebra finches (Alvarez-Buylla et al., 1990a), which brings us to the next issue. Zebra finches normally master their learned song before sexual maturity (Immelmann, 1969), which occurs around post-hatching day 80; most of the pupil's modification of vocal output takes place between post-hatching days 45 and 65. After then and until day 80 or 90 the changes that occur have to do with increased song stereotypy. New songs presented after day 65 are not imitated (Boehner, 1990). However, new songs presented after day 65 are imitated if the bird had, until then, been denied access to a live model it could imitate (Eales, 1985).

2.18 Manipulations of Learning Load Alter New Neuron Recruitment

The recruitment of HVC→RA neurons in socially reared juvenile zebra finches allowed to imitate a model drops markedly after post-hatching day 65 and by day 150 is only one-fifth of that on day 65. However, the recruitment rate remains significantly higher in isolates unable to imitate an external model because none is available (Fig. 2.8). A possible interpretation is that when an expected peak in memory load, which normally occurs during the sensitive period for song learning, is delayed, juvenile rates of new neuron recruitment remain high, as if waiting for this event (Wilbrecht et al., 2006).

We now know that the vocal variability required for song learning depends, probably, not just on new neurons but also on the “noisy” output from LMAN (Fig. 2.4) to RA (Kao et al., 2005; Oelveczky et al., 2005) and on rising BDNF levels in nucleus RA (Kittelberger and Mooney, 2004). When that output from LMAN to RA is blocked, immediate song stereotypy follows (Scharff and Nottebohm, 1991; Brainard and Doupe, 2001) and no further vocal learning is possible (Bottjer et al., 1984). However, we do not know if and how the events in HVC affect the role of LMAN in driving output variability. The neurons in HVC that project to Area X (Fig. 2.4) and that are born before hatching (Alvarez-Buylla et al., 1988a,b) may hold the clue for integrating events in HVC and LMAN, but I will not enter into those details here (Mooney, 2009).

The HVC→RA→nXIIIts descending pathway (Fig. 2.4) of the male zebra finch song system is much the same and predominantly uncrossed on each side of the brain and both sides play an important role in the production of learned song (Williams et al., 1992). The number of 30-day-old HVC→RA neurons counted in

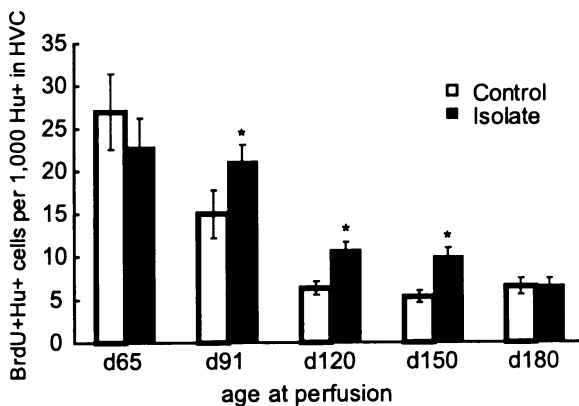


Fig. 2.8 (Figure 1b from Wilbrecht et al., 2006). An excess of new neurons is added to nucleus HVC after post-hatching day 65 and until post-hatching day 150, a time when isolates, but not socially reared controls, can still imitate new songs

90-day-old zebra finches was the same in birds that had learned their song by imitation as in birds that had been prevented from learning by early deafening or bilateral lesion of nucleus LMAN (Wilbrecht et al., 2002a,b). One might be tempted to conclude, from this, that the recruitment of new HVC→RA neurons at that age is not affected by learning, but two other sets of data suggest this would be the wrong conclusion. One set we have already discussed: recruitment of these cells remains higher in isolates that were not able to imitate an external model. The other set has to do with a nerve-cutting experiment. As discussed earlier, the syrinx of songbirds has right and left independent sound sources. If the right or left syringeal half of male zebra finches is denervated before the onset of song learning, then that side of the syrinx and the higher pathways that innervate it are not able to play a major role in song imitation. Nonetheless, these birds are able to produce close copies of a model song. In them, the HVC on the intact side must assume a disproportionately important role in the acquisition and production of learned song. The number of 30-day-old neurons counted in the HVC of the intact side at 90 days of age was in these birds 60% higher than on the denervated side, whose numbers were the same as in intact controls. Presumably during song learning the intact side acquired most of the skill normally shared by the two sides (Wilbrecht et al., 2002a). This difference was eliminated if unilateral nerve section was accompanied by deafening or by bilateral lesions of LMAN. Apparently the act of song learning by reference to an external model only affected the recruitment of neurons involved with song learning. Thus, while there is a developmental program that arrives at a “normal count” of neurons in adult HVC regardless of experience (see also Burek et al., 1991), an extra learning load (Wilbrecht et al., 2002a) or the delay of an expected learning load (Wilbrecht et al., 2006) can alter this count during the time song would be normally mastered.

If the above results, taken together, seem a bit confusing, this is probably because we are not yet very advanced in how we think about what HVC does. This point is emphasized by the next experiment. Male zebra finches reared and housed socially until 4–5 months of age were given systemic injections of 3H-thymidine. Two hours after the last injection individual birds were moved into a larger aviary that they shared either with just a female or with 20 other pairs of adult zebra finches. The injected birds were killed 40 days after this change in social setting and the number of 3H-labeled neurons in HVC was counted. To our surprise, the birds housed with the larger group had 2.5 more labeled neurons than those housed just with a mate (Lipkind et al., 2002). Since the change in social setting occurred well after the end of the sensitive period for new song learning we are left to wonder why the difference in social setting affected new neuron survival. Not only is this a reminder of how poorly we understand the behavior we study, but also a reminder of the importance of including in our studies natural variables, such as the complexity of the social group in which an animal lives. Moreover, it is important to remember that much as HVC plays a key role in the production of learned song (encoding), it may also be involved in song perception (decoding), as suggested by the “motor theory for song perception in birds” (Williams and Nottebohm, 1985).

2.19 Evidence from Other Songbird Systems

Most of our work on adult neurogenesis and neuronal replacement focused on song learning because an understanding of how vocal learning came about continued to be a central concern for my laboratory. However, I was worried that laboratory work on just one system might be misleading and therefore I wanted to extend it to other systems, other species, and particularly to animals living in the wild. The latter goal was met by a study of neuronal recruitment and turnover in the hippocampus of free-ranging chickadees, *Parus atricapillus*. This study showed that large neurons continued to be added in adulthood to the hippocampus of these forest songbirds, particularly at times of year (later summer, early fall) when chickadees cached many food items that they later had to retrieve. Recruitment of these cells was twice as high in free-ranging individuals as in others caught in the wild and then housed in a large outdoors aviary (Fig. 2.9). Unlike reports in the hippocampus of laboratory bred and laboratory housed rodents, the new cells recruited into the chickadee hippocampus lived only for a few weeks or months (Fig. 2.10). Perhaps after then, the spatial information held by those cells was no longer relevant to the animal's needs (Barnea and Nottebohm, 1994, 1996).

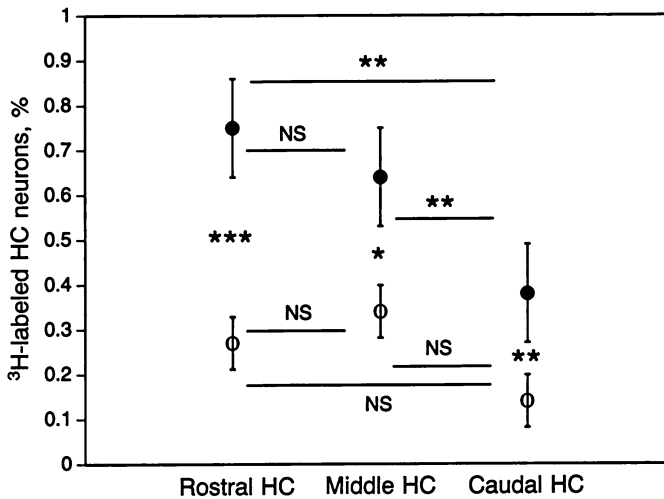


Fig. 2.9 (Figure 6 from Barnea and Nottebohm, 1994). Mean (plus or minus standard error of the mean) percentage of ^3H -labeled hippocampal complex (HC) neurons in adult wild-caught chickadees. Six of these birds had been living in the wild until killed in November (*filled circles*); four had been housed in a large outdoor aviary during the 3 months before they were killed, also in November (*open circles*). Each of the 10 birds received a single dose of 50 μCi of ^3H -thymidine and was killed 6 weeks later. Two trends are shown in this figure: (1) a higher percentage of ^3H -labeled HC neurons was present rostrally than caudally in the free-ranging birds, a twofold difference; (2) the percentage of new HC neurons was twofold higher in the free-ranging than in the captive birds. Differences between groups were not significant (NS) or reached significance levels of $P < 0.01$ (*), $P < 0.001$ (**), or $P < 0.0001$ (***)

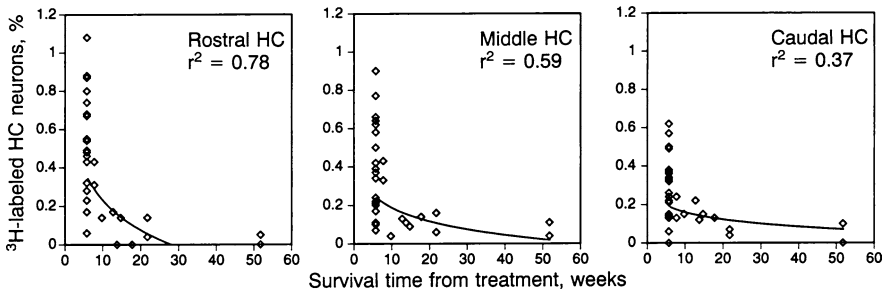


Fig. 2.10 (Figure 5 from Barnea and Nottebohm, 1994). Survival curves of 3H-labeled neurons in the rostral, middle and caudal HV of free-ranging chickadees treated with a single dose of 3H-thymidine at different times of year and then killed 6–52 weeks later. Many fewer new neurons are present after the 6-week survival

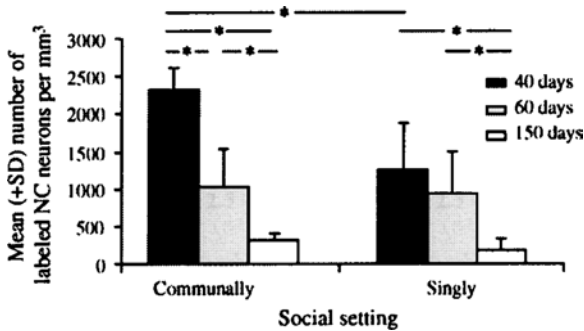


Fig. 2.11 (Figure 3 from Barnea et al., 2006). Mean (plus or minus SD) number of 3H-labeled neurons per mm³ in the caudal nidopallium (NC) of adult zebra finches that received systemic injection of 3H-thymidine and were then housed singly or communally in large outdoors aviaries (* indicates $P =$ or $<$ than 0.05). Birds were killed 40, 60 or 150 days after the change in social setting. Differences present at 40 days disappear at longer survivals

Another study focused on how changes in the social conditions under which adult zebra finches were housed affected the survival of neurons born immediately before the social change. This approach had already yielded evidence that social setting affected the survival of new neurons in HVC, which I mentioned at the end of the previous section. In a follow up, we looked at a part of the caudal forebrain, the nidopallium caudale, NC, thought to integrate auditory and non-auditory sensory information. A change to a more complex social environment – sharing an outdoor aviary with many zebra finches vs. sharing it with no other birds had a marked effect on up-regulating NC neuronal recruitment. As in the case of the chickadees, the increased recruitment was followed by the eventual demise of most cells so that by 150 days only a small fraction of the originally recruited cohort still remained (Fig. 2.11). It was of interest, too, that survival of the new neurons depended on part of the brain sampled, e.g. more rostral or more caudal, as if in some parts of the brain memories were up-dated more frequently than in others (assuming memory storage in these cells!) (Barnea et al., 2006).

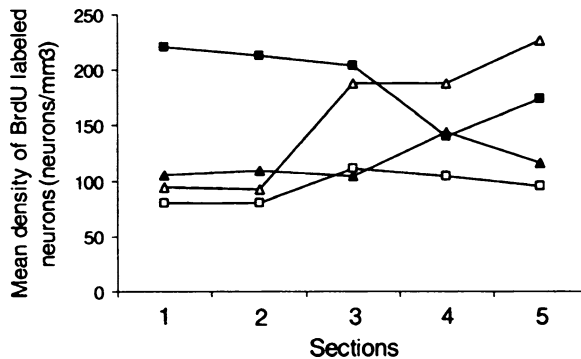


Fig. 2.12 (Figure 5 from Adar et al., 2008). Mean density of BrdU-labeled neurons per mm^3 in five sections of nidopallium of adult male zebra finches. BrdU was administered systemically at 4 months of age and birds were moved to a novel social setting either 30 or 90 days after the last injection of BrdU. The new social setting consisted of being housed with a female the male had not encountered before or 40 other zebra finches of both sexes the bird had not encountered before. Birds in either of these two settings occupied outdoors aviaries of the same size. All birds were killed 40 days after entering the new social setting. In the four groups shown here *open symbols* correspond to birds that encountered social change 1 month after the last BrdU injection; *filled symbols*, birds that encountered social change 90 days after BrdU injection. *Triangles*, complex social setting; *squares*, housed as pairs. Group sizes were six or seven birds. The most rostral section is 1, the most caudal, 5

A further study of the NC of adult zebra finches (Adar et al., 2008) showed that position and age of “new” neurons at the time of a social change as well as the nature of the social change influenced the survival of the new cells. A complex social setting favored the survival of caudal 1-month old neurons; a simple social setting favored the survival of rostral, 3-months old neurons. Perhaps the rostrally placed, older cells were too set in their ways to cope with a surge in information load, a situation that, however, encouraged the survival of younger, caudal neurons (Fig. 2.12). Apparently a same stimulus can prolong the survival of some cells and doom others. Studies that focus on a single cohort of same age cells, obtained from a single anatomical location may miss the rather complex strategies a brain might use to manage its replaceable neurons. A replaceable neuron vested with information may be a humbug or a precious asset, depending on past and present memory loads.

2.20 Overview

The song system is likely to yield the first description of how the vertebrate brain acquires a complex learned behavior and the role of late born neurons in this process.

I reviewed how my laboratory discovered evidence of widespread neuronal recruitment in adult avian brain. That evidence was scrutinized and challenged and held suspect until reliability of the birth dating process, reliability of neuronal

identification, evidence of function, and origin of the new cells and manner of migration were established. When these matters were resolved, one big question remained: Why new neurons?

Joseph Altman suggested a hypothesis: experience gleaned post-natally sculpted the fine wiring of circuits handling that information. He saw this role vested in the late born granule cells of mammalian cerebellum, hippocampus and olfactory bulb. We have found that in songbirds the new neurons added to the forebrain of juveniles and adults have a much wider distribution than in mammals and are not confined to granule cells or inter-neurons. Our best-studied example, the HVC→RA neurons, are projection neurons. As in Altman's hypothesis, however, they are involved with a late-developing function, song learning.

If late-born neurons are required for the mastery of late-developing skills, then we might predict that some of the neurons required for vocal learning in humans would also develop late, as language is acquired, and that new waves of these neurons would be recruited when new languages are learned and perhaps this would also apply to manual skills, to the mastery of music and to some sports. Clearly, there is need for much more comparative data. But even if there is a correlation between late developing skills and post-natally born neurons, there was no prediction that neuronal replacement would be encountered in the juvenile and adult brain or that there would be specific types of cells that belonged to the replaceable category. As we became aware of replaceable neurons, many new questions came to mind: what was special about these cells, about their functions, about the information they held and the way they held it? Did these cells age in a particularly fast way, were they susceptible to death by over-use? Moreover, why was adult neurogenesis so much more common in birds than in mammals?

During my formative years I had been taught that neural plasticity had to do with the nature of connections between existing neurons. Information was stored in the synapses and could be altered by changing the number and efficacy of synapses between cells that were already in place. Since these changes could, in principle, happen again and again, the adult brain had virtually unlimited space for all its learning needs, which could be satisfied by neurons already in place. Neuroscientists had not, by the mid 1980s, predicted the existence of ephemeral neurons, of cells born in adulthood, in captive or free ranging animals, that would be recruited for a few weeks or months or a year and would then disappear and be replaced by others.

Why, then does the healthy brain replace some of its neurons? Might it be that in some systems the whole neuron, rather than each synapse, is the unit of learning? Might it be that the very susceptibility of synapses to change makes them unreliable repositories for memory? Might long-lasting changes in gene expression be required to lock up irreversibly a cell's functional modifications associated with learning thus securing for long periods a particular memory? If so, might this come at the expense of that cell participating in new learning events?

The need to replace neurons could result, too, from age restrictions on what a neuron can do. Young neurons may be able to sculpt their connections in ways that older neurons cannot, regardless of participation in earlier learning events.

An overproduction of new neurons with modifiable connections may provide many alternatives from which to choose by use and disuse, with only a fraction of the new cells persisting for longer periods of time. Restriction by age and restriction by learning could be complementary processes and the consequence would still be that new neurons have a learning flexibility that older neurons lack. Learned song, the circuits that support it and the cells within these circuits that continue to be added offer good material to test the role of new neurons and the manner in which this role is achieved.

If neurons, rather than synapses are the units of long-term memory, new principles of brain economics emerge. Synapses are too numerous to count, but neuron numbers are finite. Assigning memory space to skills mastered may deny “space” to memories still to be acquired. May be neuronal replacement clears the brain of idle cells or of unwanted memories and of the cells that hold them, and makes room for new cells and the new memories they may store. The 10-year life span of a songbird is five times longer than that of a mouse yet the brain of a canary and that of a mouse weigh approximately the same, one gram. Neuronal replacement may be much more common in birds than in mammals because the brain of the former must be able to learn many more things over a longer span of time.

But even as I offer the above speculations, I should warn that the link between song learning and the recruitment of new HVC→RA neurons remains to be tested and until then should remain suspect. Recent, unpublished observations by Clare Walton, a doctoral student in my laboratory, show that the number of HVC→RA neurons in adult male zebra finches doubles during the first 2 years after song is mastered, though the song itself changes relatively little after sexual maturity. Do the new neurons partake in song production? Do they learn this role? We do not know. It is not inconceivable that neurogenesis in juvenile and adult brain has little or nothing to do with learning, but is related to other, still unimagined needs.

The account I have offered is almost painfully rustic in the extent to which it leaves out the myriads of questions that come to mind about the molecular biology of adult neurogenesis and neuronal replacement. Some of these questions occurred to me too and I would have liked to address them, if funding had been available. However, it is probably just as well that I did not, for I now realize that this will be the work of another life time. Lucky those that will carry on with these studies.

The discovery of adult neurogenesis, of its mechanisms and of its use in neuronal replacement offer, potentially, a whole new approach to brain rejuvenation and brain repair. Knowing that young neurons can be added to old brains and that in some systems can replace, numerically, other neurons that have died should allow a new approach to the study of brain aging. As molecular ways to control the production, migration, differentiation, connections and survival of new neurons become available, it is unthinkable that the clinical potential of replaceable neurons will go unexplored. A quarter century after the 1984 Hope for a New Neurology conference in New York, I am confident that the adult brain's potential to produce and replace neurons will eventually be harnessed for clinical purposes. But that is not why I did this work. I did it because I fell in love with the song of birds and wanted to understand how vocal learning came to be.

References

- Adar, E., Nottebohm, F., Barnea, A. 2008. The relationship between nature of social change, age and position of new neurons and their survival in adult zebra finch brain. *J. Neurosci.* 28:5394–5400.
- Agate, R.J., Grisham, W., Wade, J., Mann, S., Wingfield, J., Schanen, C., Palotie, A., Arnold, A.P. 2003. A neural, not gonadal, origin of brain sex differences in a gynandromorphic finch. *Proc. Natl. Acad. Sci. USA* 100:4873–4878.
- Agate, R.J., Choe, M., Arnold, A.P. 2004. Sex differences in structure and expression of the sex chromosome genes CHD1Z and CHD1W in zebra finches. *Mol. Biol. Evol.* 21:384–396.
- Airey, D.C., Kroodsmas, D.E., DeVoogd, T.J. 2000. Differences in the complexity of song tutoring cause differences in the amount learned and in dendritic spine density in a songbird telencephalic song control nucleus. *Neurobiol. Learn. Mem.* 73:274–281.
- Altman, J. 1962. Are new neurons formed in the brains of adult mammals? *Science* 135:1127–1128.
- Altman, J. 1963. Autoradiographic investigation of cell proliferation in the brains of rats and cats. *Anat. Rec.* 145:573–591.
- Altman, J. 1967. Postnatal growth and differentiation of the mammalian brain, with implications for a morphological theory of memory. In “The Neurosciences, a Study Program”, G.C. Quarten, T. Melnechuck, F.O. Schmidt, eds., pp. 723–743. The Rockefeller University Press, New York.
- Altman, J. 1969. DNA metabolism and cell proliferation. In “Structural Neurochemistry”, A. Lajtha, ed. Plenum Press, New York/London, Handbook of Neurochemistry, vol II, pp. 137–182.
- Altman, J. 1970. Postnatal neurogenesis and the problem of neural plasticity. In “Developmental Neurobiology”, pp.197–237.
- Altman, J., Bulut, F.G. 1976. Organic maturation and the development of learning capacity. In “Neural Mechanisms of Learning and Memory”, M.R. Rosenzweig, E.L. Bennett, eds., pp. 236–240. MIT Press.
- Altman, J., Das, G.D. 1965. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *J Comp Neurol* 124:319–335.
- Alvarez-Borda, B., Nottebohm, F. 2002. Gonads and singing play separate, additive roles in new neuron recruitment in adult canary brain. *J. Neurosci.* 22:8684–8690.
- Alvarez-Borda, B., Haripal, B., Nottebohm, F. 2004. Timing of brain-derived neurotrophic factor exposure affects life expectancy of new neurons. *Proc. Natl. Acad. Sci. USA* 101:3957–3961.
- Alvarez-Buylla, A., Lim, D.A. 2004. For the long run: maintaining germinal niches in the adult brain. *Neuron* 41:683–686.
- Alvarez-Buylla, A., Nottebohm, F. 1988. Migration of young neurons in adult avian brain. *Nature* 335:353–354.
- Alvarez-Buylla, A., Buskirk, D.R., Nottebohm, F. 1987. Monoclonal antibody reveals radial glia in adult avian brain. *J. Comp. Neurol.* 264:159–170.
- Alvarez-Buylla, A., Theelen, M., Nottebohm, F. 1988a. Birth of projection neurons in the higher vocal center of the canary forebrain before, during and after song learning. *Proc. Natl. Acad. Sci. USA* 85:8722–8726.
- Alvarez-Buylla, A., Theelen, M., Nottebohm, F. 1988b. Mapping of radial glia and of a new cell type in adult canary brain. *J. Neurosci.* 8:2707–2712.
- Alvarez-Buylla, A., Kirn, J.R., Nottebohm, F. 1990a. Birth of projection neurons in adult avian brain may be related to perceptual or motor learning. *Science* 249:1444–1446.
- Alvarez-Buylla, A., Theelen, M., Nottebohm, F. 1990b. Proliferation “hot spots” in adult avian ventricular zone reveal radial cell division. *Neuron* 5:101–109.
- Alvarez-Buylla, A., Ling, C-Y., Nottebohm, F. 1992. High vocal center growth and its relation to neurogenesis, neuronal replacement and song acquisition in juvenile canaries. *J. Neurobiol.* 23:396–406.
- Alvarez-Buylla, A., Garcia-Verdugo, J.M., Mateo, A.S., Merchant-Larios, H. 1998. Primary neural precursors and intermitotic nuclear migration in the ventricular zone of adult canaries. *J. Neurosci.* 18:1020–1037.

- Alvarez-Buylla, A., Seri, B., Doetsch, F. 2002. Identification of neural stem cells in the adult vertebrate brain. *Brain Res. Bull.* 57:751–758.
- Andalman, A.S., Fee, M.S. 2009. A basal ganglia-forebrain circuit in the songbird biases motor output to avoid vocal errors. *Proc. Natl. Acad. Sci. USA* 106:12518–12523.
- Anderson, M.J., Waxman, S.G. 1985. Neurogenesis in adult vertebrate spinal cord in situ and in vitro: a new model system. In “Hope for a New Neurology”, F. Nottebohm, ed., *Ann. N.Y. Acad. Sci.*, vol 457, pp. 213–233.
- Angevine, J.B. 1965. Time of neuron origin in the hippocampal region. An autoradiographic study in the mouse. *Exp. Neurol. Suppl.* 2:1–70.
- Arnold, A.P., Nottebohm, F., Pfaff, D.W. 1976. Hormone concentrating cells in vocal control and other areas of the brain of the zebra finch. *J. Comp. Neurol.* 165:487–512.
- Aronov, D., Andalman, A.S., Fee, M.S. 2008. A specialized forebrain circuit for vocal babbling in the juvenile songbird. *Science* 320:630–634.
- Barnea, A., Nottebohm, F. 1994. Seasonal recruitment of new neurons in the hippocampus of adult, free-ranging black-capped chickadees. *Proc. Natl. Acad. Sci. USA* 91:11217–11221.
- Barnea, A., Nottebohm, F. 1996. Recruitment and replacement of hippocampal neurons in young and adult chickadees: an addition to the theory of hippocampal learning. *Proc. Natl. Acad. Sci. USA* 93:714–718.
- Barnea, A., Mishal, A., Nottebohm, F. 2006. Social and spatial changes induce multiple survival regimes for new neurons in two regions of the adult brain: an anatomical representation of time? *Behav. Brain Res.* 167:63–74.
- Bayer, S.A. 1985. Neuron production in the hippocampus and olfactory bulb of the adult rat brain: addition or replacement? In “Hope for a New Neurology”, F. Nottebohm, ed., *Ann. N.Y. Acad. Sci.*, vol 457, pp. 163–172.
- Bayer, S.A., Yackel, J.W., Puri, P.S. 1982. Neurons in the rat dentate gyrus granular layer substantially increase during juvenile and adult life. *Science* 216:890–892.
- Birse, S.C., Leonard, R.B., Coggeshall, R.E. 1980. Neuronal increase in various areas of the nervous system of the guppy, *Lebistes*. *J. Comp. Neurol.* 194:291–301.
- Boehner, J. 1990. Early acquisition of song in the zebra finch, *Taeniopygia guttata*. *Anim. Behav.* 39:369–374.
- Bottjer, S.W., Miesner, E.A., Arnold, A.P. 1984. Forebrain lesions disrupt development but not maintenance of song in passerine birds. *Science* 224:901–903.
- Bottjer, S.W., Halsema, K.A., Brown, S.A., Miesner, E.A. 1989. Axonal connections of a forebrain nucleus involved with vocal learning in zebra finches. *J. Comp. Neurol.* 279:312–326.
- Brainard, M.S., Doupe, A.J. 2000. Interruption of a basal ganglia-forebrain circuit prevents plasticity of learned vocalizations. *Nature* 404:762–766.
- Brainard, M.S., Doupe, A.J. 2001. Postlearning consolidation of birdsong: stabilizing effects of age and anterior forebrain lesions. *J. Neurosci.* 21:2501–2517.
- Breunig, J.J. et al. 2007. Everything that glitters isn’t gold: a critical review of postnatal neural precursor analyses. *Stem Cell Res.* 1:612–627.
- Bryans, W.A. 1959. Mitotic activity in the brain of the adult rat. *Anat. Rec.* 133:65–71.
- Bullock, T.H. 1961. The origins of patterned nervous discharge. *Behaviour* 17:48–59.
- Burd, G.D., Nottebohm, F. 1985. Ultrastructural characterization of synaptic terminals formed on newly generated neurons in a song control nucleus of the adult canary forebrain. *J. Comp. Neurol.* 240:143–152.
- Burek, M.J., Nordeen, K.W., Nordeen, E.J. 1991. Neuron loss and addition in developing zebra finch song nuclei are independent of auditory experience during song learning. *J. Neurobiol.* 22:215–223.
- Cajal, S.R. 1894. The Croonian Lecture. La fine structure des centres nerveux. *Proc. R. soc. Lond. B* 55:444–467.
- Cajal, S.R. 1911. *Histologie du Systeme Nerveux*, vol. 2, pp. 80–119. Maloire, Paris.
- Canady, R.A., Kroodsma, D.E., Nottebohm, F. 1984. Population differences in complexity of a learned skill are correlated with brain space involved. *Proc. Natl. Acad. Sci. USA* 81:6232–6234.

- Canady R.A., Burd, G.B., DeVoogd, T.J., Nottebohm, F. 1988. Effect of testosterone on input received by an identified neuron type of the canary song system: a Golgi/EM/Degeneration study. *J. Neurosci.* 8:3770–3784.
- Cardin, J.A., Schmidt, M.F. 2004. Auditory responses in multiple sensorimotor song system nuclei are co-modulated by behavioral state. *J. Neurophysiol.* 91:2148–2163.
- Dave, A.S., Yu, A.C., Margoliash, D. 1998. Behavioral state modulation of auditory activity in a vocal motor system. *Science* 282:2250–2254.
- Dayer, A.G., Cleaver K.M., Abouantoun, T., Cameron, H.A. 2005. New GABAergic interneurons in the adult neocortex and striatum are generated from different precursors. *J. Cell Biol.* 168:415–427.
- DeVoogd, T.J., Nottebohm, F. 1981. Gonadal hormones induce dendritic growth in the adult brain. *Science* 214:202–204.
- DeVoogd, T.J., Nixdorf, B., Nottebohm, F. 1985. Synaptogenesis and changes in synaptic morphology related to acquisition of a new behavior. *Brain Res.* 329:304–308.
- DeVoogd, T.J., Pyskaty, D.J., Nottebohm, F. 1991. Lateral asymmetries and testosterone-induced changes in the gross morphology of the hypoglossal nucleus in adult canaries. *J. Comp. Neurol.* 307:65–76.
- DeVoogd, T.J., Krebs, J.R., Healy, S.D., Purvis, A. 1993. Relations between song repertoire size and the volume of brain nuclei related to song: comparative evolutionary analyses amongst oscine birds. *Proc. R. Soc. Lond. B* 254:75–82.
- Doetsch, F., Caille, I., Lim, D.A., Garcia-Verdugo, J.M., Alvarez-Buylla, A. 1999. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell* 97:703–716.
- Eales, L.A. 1985. Song learning in zebra finches: some effects of song model availability on what is learnt and when. *Anim. Behav.* 33:1293–1300.
- Easter, S.S., Jr. 1983. Postnatal neurogenesis and changing connections. *Trends Neurosci.* 6:53–56.
- Goldman, S.A., Nottebohm, F. 1983. Neuronal production, migration and differentiation in a vocal control nucleus of the adult female canary brain. *Proc. Natl. Acad. Sci. USA* 80: 2390–2394.
- Gould, E., Reeves, A.J., Graziano M.S.A., Gross, C.G. 1999. Neurogenesis in the neocortex of adult primates. *Science* 286:548–552.
- Graziadei, P.P.C., DeHan, R.S. 1973. Neuronal regeneration in frog olfactory system. *J. Cell Biol.* 59:525–530.
- Graziadei, P.P.C., Monti Graziadei, G.A. 1978. Continuous cell renewal in the olfactory system. In “Handbook of Sensory Physiology”, M. Jacobson, ed., vol IX, pp. 55–83. Springer Verlag, Berlin.
- Graziadei, P.P.C., Monti Graziadei, G.A. 1985. Neurogenesis and plasticity of the olfactory sensory neurons. In “Hope for a New Neurology”, F. Nottebohm, ed., *Ann. N.Y. Acad. Sci.*, vol 457, pp. 143–161.
- 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., Konishi, M. 1980. Hormone induced sexual differentiation of brain and behavior in zebra finches. *Science* 208:1380–1383.
- Hahnloser, R.H., Kozhevnikov, A.A., Fee, M.S. 2002. An ultra-sparse code underlies the generation of neural sequences in a songbird. *Nature* 419:65–70.
- Hebb, D.O. 1949. The organization of behavior. John Wiley & Sons, New York.
- Huber, F. 1960. Untersuchungen ueber die Funktion des Zentralnervensystems und insbesondere des Gehirns bei der Fortbewegung und der Lauterzeugung der Grillen. *Z. vergl. Physiol.* 44:60–132.
- Immelmann, K. 1969. Song development in the zebra finch and other estrildid finches. In “Bird Vocalizations”, R.A. Hinde, ed., pp. 61–74. Cambridge Univ. Press, London & New York.
- Janata, P., Margoliash, D. 1999. Gradual emergence of song selectivity in sensorimotor structures of the male zebra finch song system. *J. Neurosci.* 19:5108–5118.

- Johns, P.R. 1982. Formation of photoreceptors in larval and adult goldfish. *J. Neurosci.* 2:178–198.
- Kandel, E.R. 2006. In search of memory. W.W. Norton & Co., New York and London.
- Kao, M.H., Doupe, A.J., Brainard, M.S. 2005. Contributions of an avian basal ganglia-forebrain circuit to real-time modulation of song. *Nature* 433:638–643.
- Kaplan, M.S. 1985. Formation and turnover of neurons in young and senescent animals: an electron microscopic and morphometric analysis. In “Hope for a New Neurology”, F. Nottebohm, ed., *Ann. N.Y. Acad. Sci.*, vol 457, pp. 173–192.
- Kaplan, M.S., Hinds, J.W. 1977. Neurogenesis in the adult rat: electron microscopic analysis of light autoradiographs. *Science* 197:1092–1094.
- Karten, H.J., Hodos, W. 1967. A stereotaxic atlas of the brain of the pigeon (*Columba livia*). The Johns Hopkins Press, Baltimore.
- Katz, L.C., Gurney, M.E. 1981. Auditory responses in the zebra finch’s motor system for song. *Brain Res.* 221:192–197.
- Kirn, J.R., Nottebohm, F. 1993. Direct evidence for loss and replacement of projection neurons in adult canary brain. *J. Neurosci.* 13:1654–1663.
- Kirn, J.R., Alvarez-Buylla, A., Nottebohm, F. 1991. Production and survival of projection neurons in the forebrain vocal center of adult male canaries. *J. Neurosci.* 11:1756–1762.
- Kirn, J., O’Loughlin, B., Kasparian, S., Nottebohm, F. 1994. Cell death and neuronal recruitment in the high vocal center of adult male canaries are temporally related to changes in song. *Proc. Natl. Acad. Sci. USA* 91:7844–7848.
- Kirn, J.R., Fishman, K., Sasportas, A., Alvarez-Buylla, F., Nottebohm, F. 1999. Fate of new neurons in adult canary high vocal center during the first 30 days after their formation. *J. Comp. Neurol.* 411:487–494.
- Kittelberger, J.M., Mooney, R. 2004. Acute injections of brain-derived neurotrophic factor in a vocal premotor nucleus reversibly disrupt adult birdsong stability and trigger syllable deletion. *J. Neurobiol.* 62:406–424.
- Koketsu, D., Mikami, A., Miyamoto, Y., Hisatsune, T. 2003. Nonrenewal of neurons in the cerebral neocortex of adult macaque monkeys. *J. Neurosci.* 23:937–942.
- Konishi, M. 1963. The role of auditory feedback in the vocal behavior of the domestic fowl. *Z. Tierpsychol.* 20:349–367.
- Konishi, M. 1965. The role of auditory feedback in the control of vocalization in the white-crowned sparrow. *Z. Tierpsychol.* 22:770–783.
- Konorski, J. 1948. Conditioned reflexes and neuron organization. Cambridge Univ. Press, London.
- Kornack, D.R., Rakic, P. 2001. Cell proliferation without neurogenesis in adult primate neocortex. *Science* 294:2127–2130.
- Korr, H. 1980. Proliferation of different cell types in the brain. *Adv. Anat. Embryol. Cell Biol.* 61:1–69.
- Kriegstein, A., Alvarez-Buylla, A. 2009. The glial nature of embryonic and adult neural stem cells. *Ann. Rev. Neurosci.* 32:149–184.
- Lahousse, E. 1888. Recherches sur l’ontogenese du cervelet. *Arch. de Biol.* 8:43–110.
- Lashley, K.S. 1950. In search of the engram. In “Physiological Mechanisms in Animal Behavior”. Symposia Soc. Exp. Biol., vol IV, Cambridge Univ. Press, pp. 454–482.
- Leonard, R.B., Goggeshall, R.E., Willis, W.D. 1978. A documentation of an age related increase in neuronal and axonal numbers in the stingray. *J. Comp. Neurol.* 179:13–22.
- Li, X.-C., Jarvis, E.D., Alvarez-Borda, B., Lim, D.A., Nottebohm, F. 2000. A relationship between behavior, neurotrophin expression and new neuron survival. *Proc. Natl. Acad. Sci. USA* 97:8584–8589.
- Lipkind, D., Nottebohm, F., Rado, R., Barnea, A. 2002. Social change affects the survival of new neurons in the forebrain of adult songbirds. *Behav. Brain Res.* 133:31–43.
- Long, M.A., Fee, M.S. 2008. Using temperature to analyse temporal dynamics in the songbird motor pathway. *Nature* 456:189–194.

- Magavi, S.S., Leavitt, B.R., Macklis, J.D. 2000. Induction of neurogenesis in the neocortex of adult mice. *Nature* 405:951–955.
- Margoliash, D. 1986. Preference for autogenous song by auditory neurons in a song system nucleus of the white-crowned sparrow. *J. Neurosci.* 6:1643–1661.
- Marler, P. 1970a. A comparative approach to vocal learning: song learning in white-crowned sparrows. *J. Comp. Physiol. Psychol.* 71 (monogr.):1–25.
- Marler, P. 1970b. Birdsong and speech development: could there be parallels? *Am. Sci.* 58:669–673.
- Marler, P., Tamura, M. 1964. Culturally transmitted patterns of vocal behavior in sparrows. *Science* 146:1483–1486.
- Marler, P., Waser, M.S. 1977. The role of auditory feedback in canary song development. *J. Comp. Physiol. Psychol.* 91:8–16.
- Merkle, F.T., Tramontin, A.D., Garcia-Verdugo, J.M., Alvarez-Buylla, A. 2004. *Proc. Natl. Acad. Sci. USA* 101:17528–17532.
- Messier, B., Leblond, C.P., Smart, I. 1958. Presence of DNA synthesis and mitosis in the brain of young adult mice. *Exptl. Cell. Res.* 14:224–226.
- Miale, I.L., Sidman, R.L. 1961. An autoradiographic analysis of histogenesis in the mouse cerebellum. *Exp. Neurol.* 4:277–296.
- Mooney, R. 2009. Neural mechanisms for learned birdsong. *Learn. Mem.* 16:655–669.
- Mundinger, P. 1970. Vocal imitation and individual recognition of finch calls. *Science* 168:480–482.
- Nordeen, E.J., Nordeen, K.W. 1988. Sex and regional differences in the incorporation of neurons born during song learning in zebra finches. *J. Neurosci.* 8:2869–2874.
- Nottebohm, F. 1971. Neural lateralization of vocal control in a passerine bird. I. Song. *J. Exp. Zool.* 177:229–261.
- Nottebohm, F. 1972a. The origins of vocal learning. *Am. Nat.* 106:116–140.
- Nottebohm, F. 1972b. 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. 1980a. Testosterone triggers growth of brain vocal control nuclei in adult female canaries. *Brain Res.* 189:429–436.
- Nottebohm, F. 1980b. Brain pathways for vocal learning in birds: a review of the first 10 years. In “Progress in Psychobiology and Physiological Psychology”, J.M.S. Sprague, A.N.E. Epstein, eds., vol. 9, pp. 85–124. Academic Press, New York.
- 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. 1984. Birdsong as a model in which to study brain processes related to learning. *Condor* 86:227–236.
- Nottebohm, F. 1985. Neuronal replacement in adulthood. In “Hope for a New Neurology”, F. Nottebohm, ed., *Ann. N.Y. Acad. Sci.*, vol 457, pp. 143–161.
- Nottebohm, F. 1989. From birdsong to neurogenesis. *Sci. Am.* 260:74–79.
- Nottebohm, F. 1993. The search for neural mechanisms that define the sensitive period for song learning in birds. *Netherlands J. Zoology.*
- Nottebohm, F., Arnold, A.P. 1976. Sexual dimorphism in vocal control areas of the songbird brain. *Science* 194:211–213.
- Nottebohm, F., Nottebohm, M.E. 1971. Vocalizations of breeding behavior of surgically deafened ring doves, *Streptopelia risoria*. *Anim. Behav.* 19:313–327.
- Nottebohm, F., Nottebohm, M.E. 1978. Relationship between song repertoire and age in the canary, *Serinus canarius*. *Z. Tierpsychol.* 46:298–305.
- Nottebohm, F., Stokes, T.M., Leonard, C.M. 1976. Central control of song in the canary, *Serinus canarius*. *J. Comp. Neurol.* 165:457–486.
- Nottebohm, F., Kasparian, S., Pandazis, C. 1981. Brain space for a learned task. *Brain Res.* 213:99–109.
- Nottebohm, F., Kelley, D.B., Paton, J.A. 1982. Connections of vocal control nuclei in the canary telencephalon. *J. Comp. Neurol.* 207:344–357.

- Nottebohm, F., Nottebohm, M.E., Crane, L.A. 1986. Developmental and seasonal changes in canary song and their relation to changes in the anatomy of song-control nuclei. *Behav. Neural. Biol.* 46:445–471.
- Nottebohm, F., Nottebohm, M.E., Crane, L.A., Wingfield, J.C. 1987. Seasonal changes in gonadal hormone levels of adult male canaries and their relation to song. *Behav. Neural. Biol.* 47:197–211.
- Nottebohm, F. 1993. The search for neural mechanisms that define the sensitive period for song learning in birds. *Netherlands J. Zoology*.
- Nottebohm, F., O'Loughlin, B., Gould, K., Yohay, C., Alvarez-Buylla, A. 1994. The life span of new neurons in a song control nucleus of the canary brain depends on time of year when these cells are born. *Proc. Natl. Acad. Sci. USA* 91:7849–7853.
- Oelveczky, B.P., Andalman, A.S., Fee, M.S. 2005. Vocal experimentation in the juvenile songbird requires a basal ganglia circuit. *PLoS Biol.* 3:902–908, e153.
- Okuhata, S., Saito, N. 1987. Synaptic connections of thalamo-cerebral vocal nuclei of the canary. *Brain Res. Bull.* 18:35–44.
- Paton, J.A., Nottebohm, F. 1984. Neurons generated in the adult brain are recruited into functional circuits. *Science* 225:1046–1048.
- Raisman, G., Field, P.M. 1971. Sexual dimorphism in the preoptic area of the brain. *Science* 173:731–733.
- Rakic, P. 1985a. Limits of neurogenesis in primates. *Science* 227:154–156.
- Rakic, P. 1985b. DNA synthesis and cell division in the adult primate brain. In “Hope for a New Neurology”, F. Nottebohm, ed., *Ann. N.Y. Acad. Sci.*, vol 457, pp. 193–211.
- Rakic, P. 2002. Neurogenesis in adult primate neocortex: an evaluation of the evidence. *Nat. Rev. Neurosci.* 3:65–71.
- Rasika, S., Nottebohm, F., Alvarez-Buylla, A. 1994. Testosterone increases the recruitment and/or survival of new high vocal center neurons in adult female canaries. *Proc. Natl. Acad. Sci. USA* 91:7854–7858.
- Rasika, S., Alvarez-Buylla, A., Nottebohm, F. 1999. BDNF mediates the effects of testosterone on the survival of new neurons in an adult brain. *Neuron* 22:53–62.
- Raymond, P.A., Easter, S.S. 1983. Postembryonic growth of the optic tectum in goldfish. I. Location of germinal cells and numbers of neurons produced. *J. Neurosci.* 3:1077–1091.
- Roberts, T.F., Tschida, K.A., Klein, M.E., Mooney, R. 2010. Rapid spine stabilization and synaptic enhancement at the onset of behavioural learning. *Nature* 463:948–952.
- Roeder, K.D. 1962. Neural mechanisms of animal behavior. *Am. Zool.* 2:105–115.
- Schaper, A. 1894. Die morphologische und histologische Entwicklung des Kleinhirns. *Morphol. Jahrb.* 21:625–670.
- Scharff, C., Nottebohm, F. 1991. A comparative study of the behavioral deficits following lesions of various parts of the zebra finch song system: implications for vocal learning. *J. Neurosci.* 11:2896–2913.
- Scott, B.B., Lois, C. 2007. Developmental origin and identity of song system neurons born during vocal learning in songbirds. *J. Comp. Neurol.* 502:202–214.
- Seri, B., Garcia-Verdugo, J.M., McEwen, B.S., Alvarez-Buylla, A. 2001. Astrocytes give rise to new neurons in the adult mammalian hippocampus. *J. Neurosci.* 21:7154–7160.
- Simpson, H.B., Vicario, D.S. 1991. Early estrogen treatment of female zebra finches masculinizes the brain pathway for learned vocalizations. *J. Neurobiol.* 22:777–793.
- Smart, I. 1961. The subependymal layer of mouse brain and its cell production as shown by radioautography after thymidine-H3 injection. *J. Comp. Neurol.* 116:325–348.
- Stokes, T.C., Leonard, C.M., Nottebohm, F. 1974. The telencephalon, diencephalon and mesencephalon of the canary, *Serinus canaria*, in stereotaxic coordinates. *J. Comp. Neurol.* 156:337–374.
- Sugita, N. 1918. Comparative studies on the growth of the cerebral cortex. V., pts. I and II. *J. Comp. Neur.* 30:61–117.

- Thorpe, W.H. 1954. The process of song learning in the chaffinch as studied by means of the sound spectrograph. *Nature* 173:465–469.
- Thorpe, W.H. 1955. Comments on the “bird fancier’s delight” together with notes on imitation in the subsong of the chaffinch. *Ibis* 97:247–251.
- Thorpe, W.H. 1958. The learning of song patterns by birds, with especial reference to the song of the chaffinch, *Fringilla coelebs*. *Ibis* 100:535–570.
- Thorpe, W.H., Pilcher, P.M. 1958. The nature and characteristics of sub-song. *Br. Birds* 51:509–514.
- Uzman, L.L. 1960. The histogenesis of the mouse cerebellum as studied by its tritiated thymidine uptake. *J. Comp. Neurol.* 114:137–159.
- Vates, G.E., Nottebohm, F. 1995. Feedback circuitry within a song learning pathway. *Proc. Natl. Acad. Sci. USA* 92:5139–5143.
- Vates, G.E., Broome, B.M., Mello, C.V., Nottebohm, F. 1996. Auditory pathways of caudal telencephalon and their relation to the song system of adult male zebra finches (*Taeniopygia guttata*). *J. Comp. Neurol.* 366:613–642.
- Vates, G.E., Vicario, D.S., Nottebohm, F. 1997. Reafferent thalamo-“cortical” loops in the song system of oscine songbirds. *J. Comp. Neurol.* 380:275–290.
- von Holst, E. 1935. Ueber den Prozess der zentralnervoesen Koordination. *Pflueg. Arch. ges. Physiol.* 236:149–158.
- von Holst, E., von Saint Paul, U. 1960. Vom Wirkungsgefuege der Triebe. *Naturwissenschaften* 47:409–422.
- Wade, J., Arnold, A.P. 1996. Functional testicular tissue does not masculinize development of the zebra finch song system. *Proc. Natl. Acad. Sci. USA* 93:5264–5268.
- Waser, M.S., Marler, P. 1977. Song learning in canaries. *J. Comp. Physiol. Psychol.* 91:1–7.
- Wiersma, C.A.G. 1962. The organization of the arthropod central nervous system. *Am. Zool.* 2:67–78.
- Wilbrecht, L., Crionas, A., Nottebohm, F. 2002a. Experience affects recruitment of new neurons but not adult neuron number. *J. Neurosci.* 22:825–831.
- Wilbrecht, L., Petersen, T., Nottebohm, F. 2002b. Bilateral LMAN lesions cancel differences in HVC neuronal recruitment induced by unilateral syringeal denervation. *J. Comp. Physiol. A* 188:909–915.
- Wilbrecht, L., Williams, H., Gangadhar, N., Nottebohm, F. 2006. High levels of new neuron addition persist when the sensitive period for song learning is experimentally prolonged. *J. Neurosci.* 26:9135–9141.
- Williams, H., Nottebohm, F. 1985. Auditory responses in avian vocal motor neurons: a motor theory for song perception in birds. *Science* 229:279–282.
- Williams, H., Crane, L.A., Hale, T.K., Esposito, M.A., Nottebohm, F. 1992. Right side dominance for song control in the zebra finch. *J. Neurobiol.* 23:1006–1020.
- Wilson, D.M. 1961. The central nervous control of flight in the locust. *J. Exp. Biol.* 38:471–490.
- Yu, A.C., Margoliash, D. 1996. Temporal hierarchical control of singing in birds. *Science* 273:1871–1875.

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