The Journal of Neuroscience, February 1, 2002, 22(3):624–628

Why Are Some Neurons Replaced in Adult Brain?

Fernando Nottebohm

The Rockefeller University, Field Research Center, Millbrook, New York 12545

I will review recent observations on the production and replacement of neurons in the adult avian brain. I will highlight the fact that the new neurons are temporary, that they replace older ones that have died, and that the spontaneous replacement of neurons calls for a new theory of long-term memory. This theory is new only in relative terms, for it was first proposed 17 years ago (Nottebohm, 1984, 1985).

Neurogenesis affects a minority of cell types in adult brain

New neurons are added to many parts of the adult avian forebrain, but few, if any, are added elsewhere in the CNS of birds (Nottebohm, 1985). At least that has been the case under the conditions and in the species studied so far (mostly the canary *Serinus canaria* and zebra finch *Taeniopygia guttata*). Within the forebrain new neurons are added only to some, narrowly defined circuit positions. For example, the song system of oscine songbirds (Fig. 1) consists of a descending motor pathway necessary for the production of learned song (Nottebohm et al., 1976) and an anterior forebrain pathway necessary for acquisition of the learned pattern (Bottjer et al., 1984, 1989; Scharff and Nottebohm, 1991). These two pathways and their feedback loops consist of ~12 interconnected nuclei distributed from forebrain to medulla. If we assume that each of these nuclei has just two types of neurons, that gives a count of 24 different neuronal types for the whole system. Of these, only three [two in the high vocal center (HVC), part of avian "cortex," and one in area X, part of basal ganglia], or 12% of the total, continue to be produced in adulthood. Thus, adult neurogenesis in songbirds, where it is relatively common, affects only a minority of the cells present in the brain.

Selective, numerical replacement

Neurons produced in adulthood replace other neurons of the same class that have died. The evidence for numerical replacement and for specificity of replacement is as follows. (1) The HVC of canaries reaches adult neuronal numbers at the end of the fourth month after hatching, well before sexual maturity (at 8 months), yet new HVC neurons continue to be added during the remainder of the juvenile period, with no net gain in total numbers (Alvarez-Buylla et al., 1992). (2) New neurons are added to the adult HVC during every month of the year (Kirn et al., 1994), yet, over periods of many months, the total number of HVC neurons remains constant (Kirn et al., 1991). (3) Half of the HVC neurons that project to robust nucleus of the archistriatum (RA) are replaced during a 6 month period by new neurons of the same kind (Kirn and Nottebohm, 1993). (4) When the RA-projecting cells of the HVC are destroyed in adult canaries, there is a marked surge in the recruitment of new cells of the same kind. In contrast, when other HVC projection neurons (HVC to area X) are destroyed, they are not replaced by new cells of the same type (Scharff et al., 2000). Thus, spontaneous or induced replacement occurs only for some cell types.

The life expectancy of new neurons

Only a third of the neurons born in the adult telencephalon on any one day are still present 30 or 40 d later (Alvarez-Buylla and Nottebohm, 1988). In the case of nucleus HVC, half of the new neurons are culled between the second and third week after they were born (Kirn et al., 1999). The culling continues thereafter, depending, for example, on whether or not the birds are allowed to sing. If singing is discouraged, many of the neurons recently added to this song nucleus disappear (Li et al., 2000). Thus, neurons produced in adulthood can have relatively short life spans, measured in days or weeks, or longer ones, measured in months. In the avian material I have examined (song system and hippocampus), very few new neurons live for as long as a year (Nottebohm, 1985; Barnea and Nottebohm, 1994), although the songbirds we study can live up to 10 years. We do not know how long it takes a new neuron to become part of an existing circuit, but a minimum of 10–14 d seems a reasonable guess (Alvarez-Buylla and Nottebohm, 1988; Kirn et al., 1999); during that time the cell has to migrate from its birth site to its work site, grow axonal and dendritic processes, and make connections. There is no information that tells us if surviving a few extra days or weeks will suffice for that cell to play a significant role in brain function. It is clear, though, that an understanding of the role of neuronal replacement in adult brain must take into account the fact that the great majority of new neurons are transient and will, in due course, be replaced.

Factors promoting new neuron survival

The mechanisms thought to promote the death or survival of new, replaceable neurons have been studied at some length in the HVC of canaries and zebra finches. HVC controls a seasonal behavior, song, and neuronal replacement in HVC is very seasonal (Kirn et al., 1994; Nottebohm et al., 1994). The mechanisms that regulate neuronal replacement in HVC seem to work as follows. Changes in photoperiod promote changes in blood testosterone levels. Higher blood testosterone levels promote a higher incidence of singing, particularly during the breeding season. Both testosterone and singing promote in an additive manner (B. Alvarez-Borda and F. Nottebohm, unpublished observations) the survival of new HVC neurons, an effect achieved, apparently, through a rise in the production of brain-derived neurotrophic factor (Rasika et al., 1994, 1999; Li et al., 2000). This mechanism

This review draws on work that benefited from very generous support of the National Institutes of Mental Health (Public Health Service Grant MH 18343), the Mary Flagler Cary Charitable Trust, Howard Phipps, and the late Herbert Singer. My wife, Marta Nottebohm, edited the text and made many helpful suggestions.

Correspondence should be addressed to Dr. Nottebohm at the above address. E-mail: nottebo@mail.rockefeller.edu.

 0270-6474/02/220624-05$15.00/0

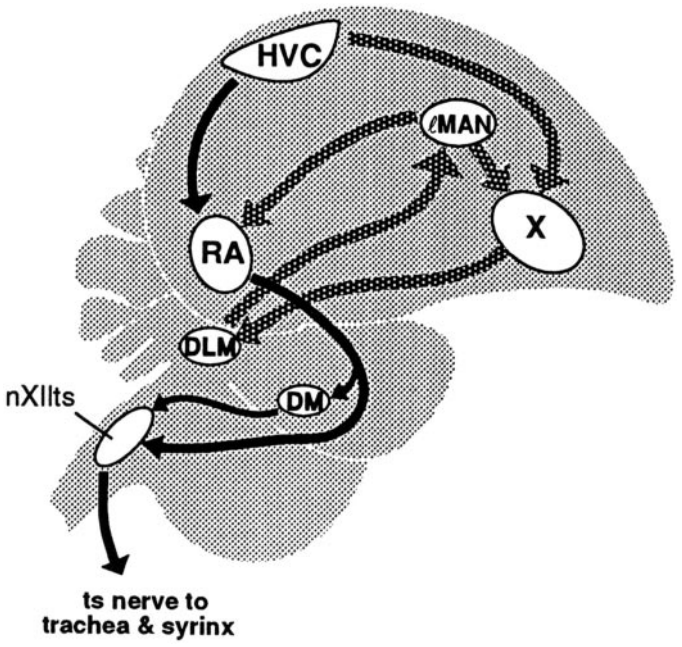

Figure 1. Sagittal section of oscine songbird's brain with schematic drawing of song system (Nottebohm, 1999), including some of the major nuclei and connecting pathways. The motor pathway necessary for production of learned song is shown by *black arrows*. The anterior forebrain pathway necessary for acquisition of learned song, but not for production, is shown by *stippled arrows*. Notice that the HVC is the source of both pathways. HVC neurons that project to the RA are replaceable; those that project to area X (*X*) are not replaceable. All the connections shown are ipsilateral. *lMAN*, Lateral magnocellular nucleus of anterior neostriatum; *DLM*, dorsolateral thalamic nucleus; *DM*, dorsomedial nucleus of the intercollicular complex; *nXIIts,* tracheosyringeal portion of the hypoglossal nucleus.

(Fig. 2) will tend to eliminate replaceable neurons that are underused, while promoting the survival of replaceable neurons that are much in use. This process may also result in the culling of some memories and the promotion of others. Allotment of new neurons to various circuits and/or survival of the new neurons may shift as seasonal behaviors such as reproduction, territorial defense, food caching, and migration are activated and then suppressed. Data on hand support this view for behaviors as disparate as singing (Kirn et al., 1994; Tramontin and Brenowitz, 1999), food caching (Barnea and Nottebohm, 1994), and colonial social interactions (Lipkind et al., 2001).

Caveats to bear in mind when sampling for new neurons

As more scientists study adult neurogenesis and neuronal replacement, it will be important that some minimum standards of evidence be accepted. Comments follow that address this issue.

Behavioral context

Recruitment of new neurons is affected in birds (Barnea and Nottebohm, 1994, 1996; Li et al., 2000) and mammals (Kempermann et al., 1997, 1998; Gould et al., 1998, 1999; Galea and McEwen, 1999; van Praag et al., 1999; Boonstra et al., 2001; Shors et al., 2001) by environmental and behavioral variables. For this reason, it might be a good idea to include in the study of any one species, gender, and age group a sample of individuals trapped in the wild, injected with a birth date marker, and released again. If these individuals are recaptured weeks or months later, the data obtained from them will reveal the extent to which new neurons are added to their adult brain under normal, free-ranging conditions. This is a very feasible experiment (Barnea and Nottebohm, 1994; Boonstra et al., 2001), and there is no good excuse for bypassing it. Negative results of adult neurogenesis based solely on data from animals kept in simple laboratory settings should be eyed with suspicion. We have known for some years that captivity (Barnea and Nottebohm, 1994) and the attendant environmental simplicity (Kempermann et al., 1997), physical inactivity (von Praag et el., 1999), and often social stress (Gould et al., 1998), can inhibit the recruitment of new neurons in adult brain.

Neuronal identification

Rigorous criteria should be used to ensure that cells called new neurons are neurons. There are several ways to identify neurons, and it is a good idea to use as many of them as possible. Simple morphological evidence can be obtained with a Nissl stain such as cresyl violet, which reveals the relatively large, clear nuclei, with one or two nucleoli, typical of many neurons (Goldman and Nottebohm, 1983). A closer look at anatomy can be obtained by ultrastructural analysis, using electron microscopy (Goldman and Nottebohm, 1983; Burd and Nottebohm, 1985). In addition, if new cells can be impaled *in vivo* with a hollow glass electrode, then this electrode can be used to record neurophysiological responses to natural stimuli and to fill the cell with a die that reveals its processes, including dendritic spines (Paton and Nottebohm, 1984). If the new cells are projection neurons, it should be also possible to backfill them by injection of a retrograde tracer into their innervation target (Kirn et al., 1991). All the above methods, as indicated by the corresponding references, were used to confirm the neuronal identity of new HVC cells in adult canaries.

Proof of origin

As part of the effort to avoid false claims of adult neurogenesis, it is important to provide proof of where the new neuron was born, identity of the cell that gave birth to it, and evidence of how the new neuron migrated from birth site to work site. Conclusive information on these three matters is not yet available for HVC neurons born in adulthood, but is available for other neurons added to the forebrain of adult canaries. In these birds, the same cells, radial cells also known as radial glia, that guide the migration of young neurons also give birth to the young neurons (Alvarez-Buylla and Nottebohm, 1988; Alvarez-Buylla et al., 1990).

How reliable are neuronal markers?

Recently, several authors have deemed it sufficient to show that cells thought to be neurons are positive to specific neuronal markers. However, the reliability of these markers depends on the marker occurring only on neurons and in no other type of cell found in the CNS; moreover, it depends on antibodies that will only recognize the marker and will not cross-react with other markers. Because conclusive evidence about these two conditions is hard to come by, it is important to provide direct evidence of origin, anatomy, connectivity, and functional status of cells claimed as new neurons.

Choosing the right survival time

Deciding on the length of survival time after injection of the birth-date marker is critical. It can take anywhere from 1 week to $\geq$20 d for new cells in songbird telencephalon to be found in

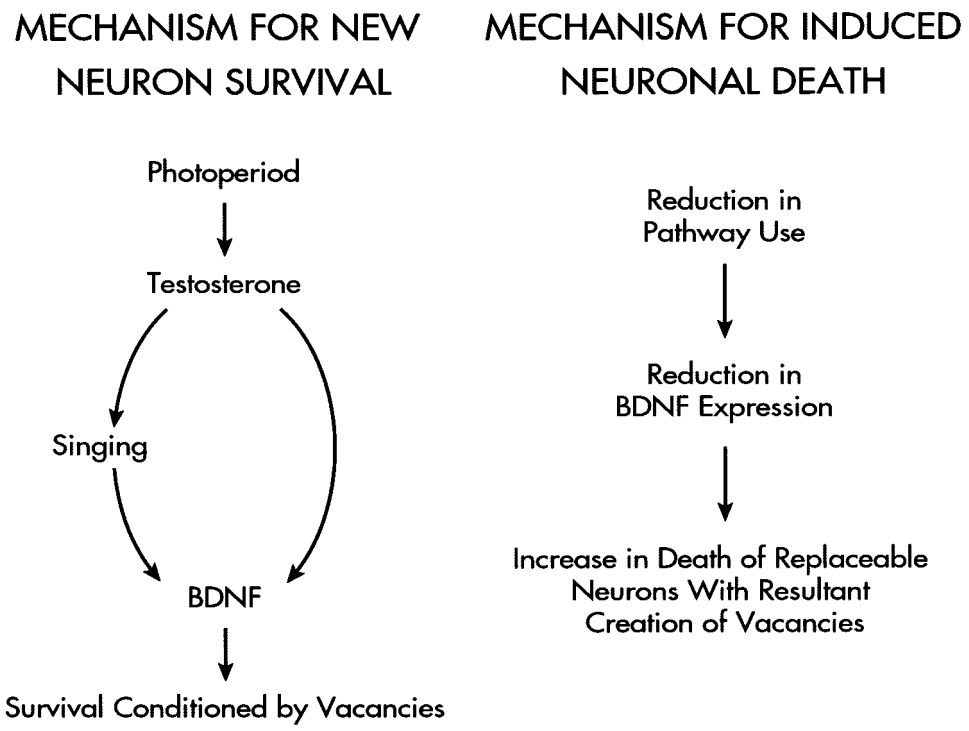

Figure 2. Chain of events that promote (*left panel*) or suppress (*right panel*) new neuron survival in the high vocal system of adult songbirds. Although the role of BDNF in promoting new neuron survival has been shown, other neurotrophins may also act in this manner. *Arrows* indicate the temporal order in which events are driven. Notice that the events that induce neuronal death, with the consequent creation of "vacancies," will also affect the survival of new neurons.

place, showing a post-migratory neuronal phenotype (Alvarez-Buylla and Nottebohm, 1988; Kirn et al., 1999). This time may be considerably longer in animals with larger brains and for destinations farther removed from neurogenic regions. Conversely, because so many of the neurons produced in adulthood survive for only a period of weeks or months, sampling should not be left until too late, when most of the new cells may have died. For these reasons, it is safest to sample over a range of survival times before deciding whether new neurons have been recruited into a particular part of the brain.

Choice of birth marker and birth marker dose

Choice of birth marker and amount injected may also affect our results and their interpretation. For example, if systemic injections of a birth date marker such as BrdU or ^{3}H-thymidine are used, injecting too little might give false negatives, whereas injecting too much might give false positives. There are, to my knowledge, no systematic studies on this. A false positive could occur, for example, if the marker used were to increase the levels of ongoing DNA repair, enhancing the marker's incorporation into cell nuclei. This could be a problem in protocols that call for a series of successive birth marker injections. I am not aware of any study that compares birth date markers and marker doses and that recommends the best protocol to reveal the number of cells born at any one time.

Quantification of birth marker evidence

Choice of markers can be important in other ways. For example, BrdU, usually visualized with an antibody, does not lend itself well to quantification of label (i.e., a cell is judged to be labeled or not). ^{3}H-thymidine, however, is well suited for quantifying the amount of label. It is possible to count the number of silver grains exposed during autoradiography, and from this infer, for example, whether a new cell resulted from one or two mitotic events after label administration. Numbers of exposed silver grains can preserve that kind of information. A recent experiment (Lipkind et al., 2001) looked at the effect of social change on new neuron recruitment. Adult zebra finches kept in groups received systemic injections of ^{3}H-thymidine once daily for 6 consecutive days; 2 hr after the last injection, individual males and females were placed in aviaries of the same size by themselves or in the company of another adult of the opposite sex. When these birds were killed 40 d later, the number of ^{3}H-labeled neurons was comparable in both groups. However, the number of exposed silver grains was 35% higher in the birds housed as pairs than in the isolates. Maybe birds in both groups recruited, initially, a similar number of new neurons, but in the isolates many of the cells in this first wave died and were replaced by new ones; the daughter cells of this second wave were born from the same set of originally labeled stem cells but, as a result of the two divisions, had only half as much label. This inference remains to be tested.

Neuronal death may hold the key

Experiments that test for variables that affect the recruitment of new neurons tend to assume a direct positive relation between the variable manipulated and an increase, for example, in the number of new cells. Yet, as mentioned earlier, the recruitment of new neurons in adult brain may always be, with few exceptions, part of a process of replacement. In such a scenario, changes in stimulation or behavior might first lead to the demise of existing neurons, and the resulting vacancies may then encourage new neuron recruitment (Scharff et al., 2000). This may seem counterintuitive, yet if much of juvenile and adult neurogenesis occurs within a frame of constant neuron numbers, then death must precede recruitment. The delay between death and replacement could be a matter of weeks (Kirn et al., 1994) or hours. Migrating neuroblasts could sidle up to existing neurons, induce their death, and then take up their position. No one has tested for this latter possibility.

The relation between neurogenesis and learning: need for a new theory of long-term memory

The debate about whether neurogenesis occurs in the brain of adult, warm-blooded vertebrates is over: it does, and the work done on songbirds has proven this beyond any reasonable doubt. Conceptually, this persistence of an embryonic trait does not force us to reconsider the way we think about the brain. What forces a reconsideration is the observation that neurons belonging to specific neuronal classes and present in otherwise healthy brains are constantly replaced.

Why does this neuronal replacement occur? That is the important question. It seems unlikely that this trait evolved to help animals recover from brain disease or lesion. Animals in nature, unprotected by a caring society, may seldom have the time to recover from brain dysfunction. It also seems unlikely that neuronal replacement evolved as a response to normal wear and tear (except, perhaps, in the olfactory epithelium?), because the anatomy of its occurrence bears no obvious relation to areas of greater or lesser use or exposure to physical damage. Moreover, spontaneous neuronal replacement was not predicted by those that have spent much of their lives studying the neurobiology of learning and whose theories would have been most affected by the occurrence of neuronal replacement. To those investigators, the discovery of constant, spontaneous neuronal replacement in adult brain came as a surprise. This surprise is not about mechanistic minutiae to which our understanding can quickly adjust, but about a phenomenon with profound implications for any overarching logic of brain function. I suggest that the significance of neuronal replacement must be addressed in the context of what dictates the limits of a brain's capacity to learn.

Until very recently, an answer to this latter question would have relied heavily on explanations involving synapses: synaptic number, efficacy, and plasticity. It has been argued that the vast number of synapses formed on each neuron and by each neuron provides the flexibility for a constant and virtually limitless updating of information. Proof of the importance correctly attributed to synapses are the two recent Nobel prizes in medicine, conferred to Paul Greengard and Erich Kandel. Yet, a theory of learning based on synapses did not predict a need for replacing healthy adult neurons by other neurons of the same kind, and so something basic must have been missing. If synaptic plasticity did not predict neuronal replacement, how must we reconstitute our logic of brain function so that both phenomena, synaptic plasticity and spontaneous neuronal replacement, can coexist, conceptually, in a harmonious manner? It is in this context that I first explored the need for a new theory of long-term memory, with facts that at the time were incomplete (Nottebohm, 1984, 1985).

Let us assume that a strong causal link between learning and neuronal replacement has been established (not the case yet), and that we observe that neurons that hold key positions in the acquisition of new long-term memories are replaced at a particularly high rate before an expected increase in memory load, e.g., seasonal song learning and food caching in birds. Why would this be advantageous? I suggest that it is advantageous because at those times, learned neurons block further learning and forgetting, and their replacement by freshly minted ones may enable new learning.

Synapses are wonderful instruments for learning, but by themselves may not be reliable repositories for long-term memory (Bailey and Kandel, 1993). Permanent changes in gene expression may be better suited to determine in a permanent manner, the number and properties of the synapses of a neuron and thereby the way in which that neuron interacts with others. After such a change in gene expression, much of the enormous plasticity normally afforded by synapses may come to an end. That cell, now a reliable repository of information, may have lost the ability to acquire new long-term memories. According to this outlook, acquisition of a long-term memory is akin to an irreversible step in cell differentiation, leading to a lasting change in circuit properties. For some kinds of neurons, but not necessarily for all, the acquisition of a long-term memory may be a one time thing, previous learning standing in the way of new learning. Those neurons, and not their individual synapses, would be the unit of long-term memory, and the number of neurons that can be modified in this manner would determine how much can be learned and remembered. For neurons that behave in this manner, the greater the number modified by long-term memory, the fewer the number still available to this end. If this hypothesis is correct, then with the passing of time, the ability to master new information and skills will suffer, and this may be a particularly acute problem in long-lived animals. I suggest that the replacement of older neurons by new ones that are, in turn, transient and replaceable, offers a vehicle for the constant and spontaneous rejuvenation of key brain circuits. This hypothesis is strong and falsifiable. It will be of particular interest to see how it fits with what is known about the role of hippocampus in learning. If the hypothesis is supported, it will provide a logic for neuronal replacement, so we can predict when and where it occurs, under what circumstances, and with what consequences.

Adult neurogenesis, neuronal replacement, and the biology of brain stem cells are now poised for molecular reductionism and clinical applications. I have little doubt that these will come. However, the task of rejuvenating and reconstituting damaged CNSs will be difficult. As we broach it, much time may be wasted using suboptimal animal models. I suggest it would be wise to look more closely at adult neurogenesis and neuronal replacement in a diversity of free-ranging animals leading a normal life, because I believe this material furnishes the best examples of what nature can do. Spontaneous neuronal replacement is an improbable brain feature. Perhaps, before we try our wizardry, we should find out how nature uses it.

REFERENCES

Alvarez-Buylla A, Nottebohm F (1988) Migration of young neurons in adult avian brain. Nature 335:353–354.

Alvarez-Buylla A, Theelen M, Nottebohm F (1990) 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.

Bailey CH, Kandel ER (1993) Structural changes accompanying memory storage. Annu Rev Physiol 55:397–426.

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.

Boonstra R, Galea L, Matthews S, Wojtowicz JM (2001) Adult neurogenesis in natural populations. Can J Physiol Pharmacol 79:297–302.

Bottjer SW, Miesner EA, Arnold AP (1984) Forebrain lesions disrupt development but not maintenance of song in passerine birds. Science 224:901–903.

Bottjer SW, Halsema KA, Brown SA, Miesner EA (1989) Axonal connections of a forebrain nucleus involved with vocal learning in zebra finches. J Comp Neurol 279:312–326.

Burd GD, 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.

Galea LA, McEwen BS (1999) Sex and seasonal differences in the rate of cell proliferation in the dentate gyrus of adult wild meadow voles. Neuroscience 89:955–964.
Goldman SA, 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, Tanapat P, McEwen BS, Flugge G, Fuchs E (1998) Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress. Proc Natl Acad Sci USA 95:3168–3171.
Gould E, Beylin A, Tanapat P, Reeves A, Shors TJ (1999) Learning enhances adult neurogenesis in the hippocampal formation. Nat Neurosci 2:260–265.
Kempermann G, Kuhn HG, Gage FH (1997) More hippocampal neurons in adult mice living in an enriched environment. Nature 386:493–495.
Kempermann G, Kuhn HG, Gage FH (1998) Experience-induced neurogenesis in the senescent dentate gyrus. J Neurosci 18:3206–3212.
Kirn JR, Nottebohm F (1993) Direct evidence for loss and replacement of projection neurons in adult canary brain. J Neurosci 13:1654–1663.
Kirn JR, Alvarez-Buylla A, Nottebohm F (1991) Production and survival of projection neurons in a forebrain vocal center of adult male canaries. J Neurosci 11:1756–1762.
Kirn JR, O'Loughllin 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 19:7844–7848.
Kirn JR, Fishman Y, Sasportas K, Alvarez-Buylla A, 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.
Li XC, Jarvis ED, Alvarez-Borda B, Lim DA, Nottebohm F (2000) A relation between behavior, neurotrophin expression and new neuron survival. Proc Natl Acad Sci USA 97:8584–8589.
Lipkind D, Nottebohm F, Rado R, Barnea A (2001) Social change affects the survival of new neurons in the forebrain of adult songbirds. Behav Brain Res, in press.
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. Ann NY Acad Sci 457:143–161.
Nottebohm F (1999) The anatomy and timing of vocal learning in birds. In: The design of animal communication, Chap 3 (Hauser MD, Konishi M, eds), pp 63–110. Cambridge, MA: MIT Press.
Nottebohm F, Stokes TM, Leonard CM (1976) Central control of song in the canary, *Serinus canaria*. J Comp Neurol 165:457–486.
Paton JA, Nottebohm F (1984) Neurons generated in the adult brain are recruited into functional circuits. Science 225:1046–1048.
Rasika S, Nottebohm F, Alvarez-Buylla A (1994) Testosterone increases the recruitment and/or survival of new HVC 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.
Scharff CJ, 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.
Scharff C, Kirn J, Macklis J, Nottebohm F (2000) Targeted neuronal death affects neuronal replacement and vocal behavior in adult songbirds. Neuron 25:481–492.
Shors TJ, Miesegaes G, Beylin A, Zhao M, Rydel T, Gould E (2001) Neurogenesis in the adult is involved in the formation of trace memories. Nature 410:372–376.
Tramontin AD, Brenowitz EA (1999) A field study of seasonal neuronal incorporation into the song control system of a songbird that lacks adult song learning. J Neurobiol 40:316–326.
Van Praag H, Kempermann G, Gage FH (1999) Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nat Neurosci 2:266–270.