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Research report

Social and spatial changes induce multiple survival regimes for new neurons in two regions of the adult brain: An anatomical representation of time?

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Abstract

Male zebra finches reared in family groups were housed initially in small indoors cages with three other companions. At 4–5 months of age these birds were treated with [^{3}H]-thymidine and then placed in large outdoors aviaries by themselves or with other zebra finches. Counts of new neurons were made 40, 60 and 150 days after the change in housing. Recruitment of new neurons in nidopallium caudale (NC) was higher than in the hippocampal complex (HC); but in both brain regions it was higher in communally housed birds than in birds housed singly, suggesting that the complexity of the social setting affects new neuron survival. In addition, the new neurons lived longer in rostral NC than in its caudal counterpart, and neuronal turnover was faster and more significant in NC than in HC. Albeit indirect, this may be the first suggestion that different parts of the brain upgrade memories at different time intervals, yielding an anatomical representation of time.

Keywords: Neurogenesis; Neuronal replacement; Avian brain; Chronological representation; Memory; Recruitment

1. Introduction

New neurons continue to be added to the brain of adult birds and mammals [reviewed in 2,7,9,12,23,24,26]. Since their brain does not continue to grow, these new neurons are thought to replace older ones that have died and in one study this has been confirmed experimentally [27]. Circumstantial evidence from the song system of birds suggests this replacement is related to the acquisition of new information [13,23]. Direct evidence of a causal link between replacement of a particular type of neuron and learning remains, however, elusive because no experiment has tested how the selective blockage of such a replacement affects learning. However, it has been shown that systemic treatment with a toxin that blocks cell proliferation and therefore recruitment of any new cells interferes with some types of hippocampal-dependent learning [30,31]. The present report does not change this balance of facts and hypotheses, but it adds new information about an aspect of adult neurogenesis, the survival of the new neurons.

We here report on the survival of new neurons in two regions of the adult zebra finch brain, the nidopallium caudale (NC) and the hippocampal complex (HC). There is some information about what these two parts of the brain do. The NC includes various auditory relays that probably play a role in vocal communication as well as in the processing of other types of auditory input [34,20]. The HC of birds has been studied particularly in species that cache food items and later must remember where they put them [29]. The HC is particularly large in species that conceal and retrieve food in this manner and therefore it seems likely that, as is the case for the mammalian hippocampus, it plays a role in the acquisition and retrieval of spatial information [15]. There is no prior information from birds that links the HC to auditory or social variables. We still know little about the diversity of cell types present in NC and HC and their connections.

An earlier report described the impact that differences in social setting have on the recruitment of new NC neurons in the adult zebra finch. That report noted that when neurons born immediately before a change in social setting were counted 40 days later, these counts were higher when the birds in the new setting were housed communally than when they were housed singly or in pairs [18]. The present report extends these ear-

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lier observations by including birds that survived 40, 60 or 150 days after the end of [^{3}H]-thymidine treatment. Our experimental design allowed us to compare how social setting, part of brain and survival time affected new neuron counts. We use the information derived from this design to propose a new theory of time representation in the brain.

2. Materials and methods

2.1. General

The present study is an extension of a previous one that also used zebra finches and employed the same methods as the present one [18]. Two outdoor breeding colonies, at The Meier Segals Garden for Zoological Research at Tel-Aviv University, Israel, provided our subjects. Juvenile zebra finches were banded at fledging for individual identification using a randomly chosen unique color combination of four plastic rings, two on each leg. These juveniles were kept in their breeding colony until the age of 45–60 days, when they became independent and could also be sexed by their plumage. Only males were used for our experiments, as described below.

2.2. Experimental design

At 45–60 days, the juvenile males were removed from their native colony. Each one of them was put in a standard cage (65 cm × 35 cm × 45 cm), together with three other unrelated individuals, to avoid stress that, in this very sociable species, might result from isolation. The three strangers consisted of another juvenile (same age, opposite gender), and two adults (a female and a male). Each such cage was visually isolated from the outside environment, and placed by itself, so that the experimental bird that was kept in it, could only hear or see the three zebra finches with which it was housed. Cages and aviaries were exposed to natural illumination conditions (10.1–14.7 h of light per day) that changed seasonally. The range of mean daily temperatures was from 12 to 30 °C. Our birds are able to breed any time of year under these conditions. For this reason and because individuals for each of the experimental groups were obtained at all times of the year, seasonal changes in temperature and photoperiod were unlikely to have affected the outcome of our study.

The birds we used in our experiments were kept under the above conditions until the age of 4–5 months, well after male zebra finches normally reach sexual maturity (80 days). At that time each male was treated with the cell birth marker [^{3}H]-thymidine (see below) and two hours later was placed in a large outdoor aviary (1.5 m × 1.5 m × 2 m). The walls of these aviaries were covered with burlap and the aviary had a roof of opaque plastic. Distance between aviaries was 50 m. Because of all these conditions birds in any given aviary could not hear nor see those in other aviaries. The interior arrangement – perches, food and water dishes – was the same for all aviaries.

When the birds were moved to the outside aviaries, they encountered either of two social environments—they were housed singly or communally. Males held in each of these two settings were killed 40, 60 and 150 days after [^{3}H]-thymidine treatment (5–7 birds for each survival). The 40-day survival period allowed enough time for neurons born at the time of injection to migrate to their final destination and go through final anatomical differentiation [3,14]. During this survival period there also was enough time for some of the new neurons to be culled [3,13]. It is possible, too, that neurogenic stem cells labeled with [^{3}H]-thymidine before the change in social setting divided again later, at various times after the change in social setting. We refer to the number of neurons present at 40-day survival as number of neurons "recruited". Our previous work [18], which used a 40-day survival period, showed that differences in social setting affected the number of new neuron recruitment: birds housed communally had significantly more new neurons in some forebrain regions than birds kept singly. In order to investigate whether social setting might also affect the turnover of these new neurons after day 40, we added 60-day and 150-day survival groups. Thus, our experimental design yielded a total of six groups that differed in social environment and/or survival time, as follows.

2.2.1. Simple social environment: Groups 1–3

Males housed singly could not see or hear other zebra finches. Isolation is probably not likely to occur in nature in this social species. However, our previous work [18] indicated that new neuron recruitment was very similar in isolates and in birds housed as male–female pairs, so in the present experiment isolates represent the simplest possible social setting. We compared new neuron numbers in birds housed singly with those in birds housed with many companions. Below we indicate the nature of each experimental group and number of birds in it. There were three groups of birds housed singly, as follows.

- Group 1—Birds killed 40 days after end of [^{3}H]-thymidine treatment (n = 5).
- Group 2—Birds killed 60 days after end of [^{3}H]-thymidine treatment (n = 6).
- Group 3—Birds killed 150 days after end of [^{3}H]-thymidine treatment (n = 5).

2.2.2. Complex social environment: Groups 4–6

In this case, males were introduced into an aviary which housed a pre-existing group of 40–45 adult male and female zebra finches, all of which were strangers to the experimental birds. There were three such large groups and experimental birds were added to them as they became available. No birds were removed from these large groups before the experimental birds themselves were taken out. No breeding boxes were placed in any of the aviaries, so birds held in them did not engage in nest building, egg-laying, incubation and rearing of young. As for the isolates, the three complex social environment groups differed in the survival time from treatment until they were killed.

- Group 4—Birds killed 40 days after end of [^{3}H]-thymidine treatment (n = 5).
- Group 5—Birds killed 60 days after end of [^{3}H]-thymidine treatment (n = 6).
- Group 6—Birds killed 150 days after end of [^{3}H]-thymidine treatment (n = 7).

2.3. [^{3}H]-thymidine treatment, histology and autoradiography

New neurons were labeled by intramuscular injection of a radioactive form of thymidine ([^{3}H]-thymidine), which is a marker of DNA synthesis and therefore of cell birth (for details, see [18]). Each bird received one injection per day (6.7 Ci/mM; 50 μCi) on each of six consecutive days, into the pectoral muscle; birds were injected in the morning, between 10 and 12 a.m. Two hours after the last [^{3}H]-thymidine dose, birds were introduced to their new environments, where they stayed for the various survivals. At the end of the survival period, birds were weighed, killed with an overdose of anesthesia, and perfused with 20 ml of saline followed by 50 ml of 4% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.4 (PB). Brains were removed, placed in the same fixative, and after a minimum of 7 h in it, were embedded in polyethylene glycol, blocked transversely and cut at 6-μm intervals. Serial sections (every 10th, at intervals of 60 μm) were collected in PB and mounted on chromalum-coated slides. The sections were then delipidized in xylene/ethanol and coated with nuclear track emulsion NTB2 (Kodak). After 4 weeks of incubation at 4 °C the emulsion was developed and the sections were stained with 0.5% cresyl violet and coverslipped with Accu-Mount mounting medium (Baxter Scientific Products, McGraw Park, IL).

2.4. Identification of neurons using cresyl violet and immunohistochemistry, and use of Neu-N marker to validate cresyl counts of new neurons

Cells were identified as neurons by the size, shape and staining properties of their nuclei. Our criteria for neuronal identification were that the nucleus had to be larger than 5 μm in diameter, round, with clear nucleoplasm and one or two darkly staining nucleoli; all these features are readily apparent in tissue stained with cresyl violet, a Nissl stain. The reliability of cresyl violet-based identifications of the new neurons in songbirds has been confirmed by electron microscopy in HVC [5,8]. In addition, HVC [^{3}H]-labeled cells with neuronal morphology have been identified as neurons also by intracellular neurophysiological recordings [25]. A neuron was considered to be [^{3}H]-labeled if, as a result of autoradiography, the number of exposed (black) silver grains over its nucleus was 20 times or higher than that of the background level, measured over the neuropil; this criterion usually required that a labeled neuron have a minimum of five grains over its nucleus, as in [18].

At the time of the work reported here, the use of neuronal markers for the identification of [^{3}H]-labeled neurons was not yet common in Israel, where the work was done. Therefore, as in our previous publication [18], we wanted to test whether counts of cells identified as [^{3}H]-labeled neurons were similar when using cresyl violet or a specific neuronal marker. If the answer was yes, then we would take it as evidence that our counts using cresyl violet-stained material were reliable. For this, four adult male birds that were not part of our experimental groups were treated with [^{3}H]-thymidine as the experimental birds and killed 40 days after the last injection. Their brains were fixed and cut as described in the previous section. One set of brain sections was stained with cresyl violet, as described earlier, and another adjacent set was stained with the neuron-specific marker anti-Neu-N (mouse monoclonal anti Neu-N, Chemicon International, Temecula, CA), that has been used successfully in mammals [21,35] and birds [17]. In our material the Neu-N marker stained the nucleus and the cytoplasm of post-migratory neurons.

The protocol for the sections stained with the Neu-N marker was as follows. Sections were incubated in 0.01 M citrate buffer at 90 °C for 10 min, washed with 0.01 M phosphate buffer (PB) five times, for 10 min each, and then incubated in blocking buffer (PB containing 10% normal horse serum (NHS) and 0.3% Triton X100), at room temperature (RT) for 30 min. Then, sections were incubated in primary antibody (anti-Neu-N, 1:200 in PB) with 0.3% Triton and 2% NHS, at 4 °C for 48 h, washed with PB with 0.1% Triton for 10 min, and followed by three additional washes with PB, 10 min each. After washing, sections were incubated in secondary antibody (biotinylated anti-mouse IgG, Vector Laboratories, Burlingame, CA; 1:200 in PB) with 0.3% Triton and 2% NHS, for 2 h at RT, and washed three times with PB for 5 min each. Sections were then exposed to avidin biotin peroxidase reaction (Elite-ABC kit, Vector Laboratories, Burlingame, CA; 1:100 in PB), for 90 min, then washed three times with PB for 5 min each, followed by 3,3′-diaminobenzidine tetrahydrochloride (DAB, Sigma, Israel) reaction (in PB containing 16% DAB and 0.01% H_2O_2), at RT, for 3–8 min. Finally, sections were washed three times with PB, for 5 min each and then processed for autoradiography [4]. We also performed a control, where we omitted the primary antibody. Control sections showed no staining. In addition, in order to confirm the neuronal specificity of the anti-Neu-N marker in our material, we prepared a few sections as for immunohistochemical staining and then counterstained these sections with either cresyl violet of methylene blue, which allowed us to see cell somata and the staining pattern of their nuclei. This allowed us to see the size, shape and nuclear staining of Neu-N-negative as well as of Neu-N-positive cells. Areas free of neuronal somata (the ventricular zone and the outer layer of the optic tectum) were examined and found to contain no Neu-N positive cells. We also examined the size, shape and nuclear staining in areas which contain both neurons and non-neuronal cells (nidopallium caudale and nucleus isthmi pars magnocellularis) and found that Neu-N negative cells had nuclei that did not meet our neuronal criteria, while cells with nuclei that met our neuronal criteria were all Neu-N positive. In addition to these visual, confirmatory inspections, we counted in adjacent NC sections from four birds the number of [^{3}H]-labeled neurons when the cells were stained with cresyl violet or with Neu-N. This way of confirming that our counts of neurons based on cresyl violet staining were reliable is the same as the one that was used in our previous work [18], and for details see Fig. 2 there. It should be noted here, that the production of neurons in adult avian telencephalon has been described and confirmed in a number of ways and the phenomenon itself has ceased to be controversial [23].

2.5. Mapping and quantification

We focused our interest on two brain regions: nidopallium caudale (NC [11]) and the hippocampal complex (HC). NC was chosen because it is known to include auditory projections [34] and regions that are activated by playbacks of conspecific song [19]; these auditory representations are likely to play a role in vocal communication and so would have been more active in birds held communally than in birds held singly. Moreover, in an earlier study, new neuron recruitment in NC was shown to be sensitive to social manipulation [18]. For its part, HC is known to be involved in the processing of spatial information [15,28], with no evidence that auditory inputs reach it. For these, admittedly incomplete reasons, we speculated that neuronal recruitment and survival might differ between these two brain regions.

Both NC and HC have boundaries that are easy to recognize in transverse sections: for NC, we followed the criteria described in [18]: the wall of the lateral ventricle and the surface of the brain define its medial, dorsal and lateral limits of NC; the lamina archistriatalis dorsalis (LAD) provides its ventral boundary. For HC, we followed the criteria described in [4]. Briefly, the dorsal, ventral, and medial boundaries of HC are the surface of the brain, the lateral ventricle (V) and the midline, respectively. Its lateral boundary is defined by an increase in cell density and a change in cell type from larger neurons to a mixture of both large and small neurons. Both NC and HC extend rostrally and caudally beyond the region we sampled; therefore, the volume of NC and HC that we report refers only to the tissue bracketed between the arbitrary anterior and posterior limits that we set for our sampling, defined by distance from point zero in the rostrocaudal axis. Point zero, when looking at the whole brain from above, is at the medal confluence of the two hemispheres and the rostral tip of the cerebellum, see Fig. 1A.

The rostro-caudal extent of our sampling in NC and HC is shown in Fig. 1. Ideally, one would want to sample as many sections as possible, but given the number of birds involved and time limitations, we settled, as previously [18] for a spacing of 600 μm between tissue sections sampled. In three of the groups, sampling in NC was at 300 μm (see below).

We used a computerized brain mapping system (Neurolucida; Stereo Investigator, from MicroBrightField Inc.) to draw the boundaries of NC and HC in each tissue section sampled, enter the position of each labeled neuron, count neurons and quantify other neuronal parameters, as described below. All mapping was done "blind" as to the experimental conditions. NC and HC were sampled in all brains from the six experimental groups. Preliminary mapping in both areas showed no hemispheric differences in the number of labeled neurons per mm^3. Therefore, for purposes of characterizing neuronal survival in each of the six experimental groups we mapped sections only from the left hemisphere.

The following measurements were taken.

Estimate of the NC and HC volume sampled. This volume was defined by the position of the rostralmost and caudalmost sections used for counting [^{3}H]-labeled neurons and therefore it does not correspond to the volume of the whole NC or whole HC. It was estimated for the NC and HC of each bird by measuring the area of NC and HC in each of the sections used for counting [^{3}H]-labeled neurons (3 or 5, see below), adding these areas and multiplying the sum by the sampling interval.

Number of [^{3}H]-labeled neurons per mm^3. All [^{3}H]-thymidine labeled neurons were mapped and counted in the sections sampled, using a 63× objective. In NC we sampled at least three sections, as in our previous study [18], and in HC, five sections. These sections, also used to estimate the NC and HC volume sampled, were spaced at 600 μm intervals. Each section was completely scanned using the Neurolucida meander scan probe. NC is a large brain region not only rostro-caudally, but also dorso-ventrally, with a mean area in the sections sampled of 5.6 mm^2. Accordingly, the total average NC area sampled per brain was 16.8 mm^2. In Groups 4–6 we sampled two additional sections (for details see next paragraph), bringing the mean total area of the five sections sampled to 28 mm^2. In HC, the average area per section was 0.5 mm^2, and the average total area sampled per brain was 2.5 mm^2. We also measured nuclear diameters of [^{3}H]-labeled neurons in NC and in HC (see below). From the knowledge of section thickness and nuclear diameter of labeled neurons, using the Abercrombie stereological correction equation [10], we could estimate for each of the levels sampled in NC and HC, the number of [^{3}H]-labeled neurons per unit volume at that level.

Search for rostro-caudal differences in new neuron recruitment. The rostro-caudal extent of HC sampling was similar to that of our earlier sampling of this region in black-capped chickadees, in a study that revealed rostro-caudal differences in new neuron recruitment under conditions of enriched spatial cues [4]. The rostro-caudal extent of NC sampling was determined by our earlier study [18], which the present one extends. Unpublished data from that earlier study suggested there was in communally housed birds a tendency (though not significant) for an increase in number of labeled neurons per unit volume in the caudal reaches of the NC region sampled; these birds had been killed 40 days after [^{3}H]-thymidine treatment. Since NC is a large brain area, this tendency raised the possibility that NC might be composed, rostrocaudally, of successive domains that respond differently to the social variable. We wanted to test this possibility in the NC of communally housed birds (Groups 4–6), so in addition to the three sections described above, two more interleafed between the prior three were mapped, so that a total of five sections, spaced at 300 μm intervals, were sampled in these birds along the rostro-caudal axis of NC. The rostralmost,

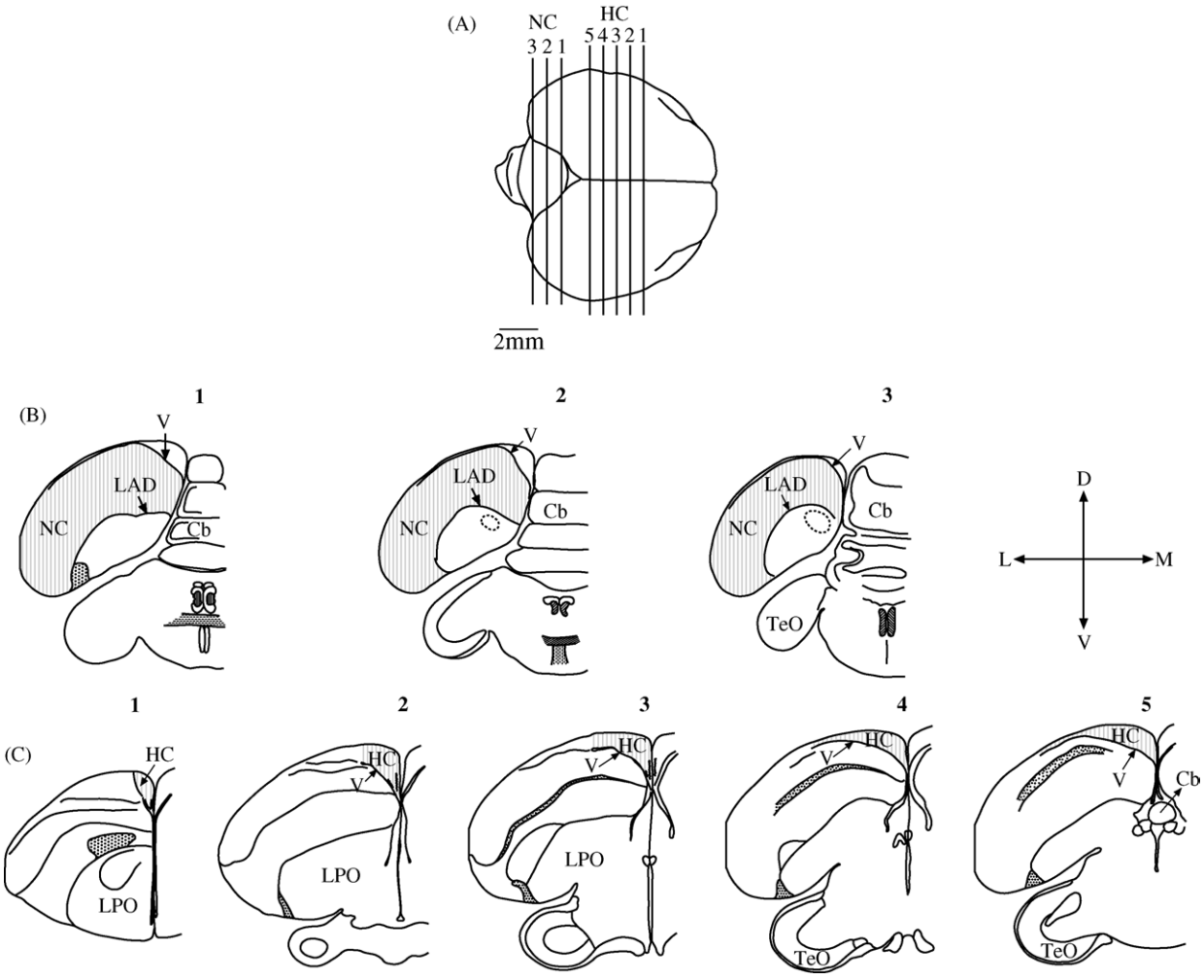

Fig. 1. (A) Top schematic view of the brain of an adult zebra finch male; anterior is to the right, posterior to the left. We indicate the position of the frontal sections taken from nidopallium caudale (NC) and from the hippocampal complex (HC). Distance between sections is 600 μm. (B) The three levels at which NC (shaded) was sampled in birds from all six groups are the same as in [18]; in addition, two more sections were taken (see text) in the communally housed birds; these sections were interleafed between the caudal most (#3) and middle one (#2) and between the rostral most (#1) and middle one. (C) The five levels of HC (stippled) that were sampled. Abbreviations: Cb, cerebellum; LAD, lamina archistriatalis dorsalis; TeO, optic tectum; V, lateral ventricle. These anatomical landmarks should suffice to relate these sections to the richer anatomical detail present in the canary atlas [33], from which the terminology is borrowed.

middle and caudalmost sections were, in isolates and communally housed birds in the same respective positions.

Additional measurements. As in our previous study [18], we took some additional measurements from the most caudal sampling sections of NC and HC of all brains. This section was chosen because in that previous study it was found to be the most sensitive to the experimental manipulation. It was our assumption (untested), that parameters quantified in this most caudal section were representative of the entire region (NC or HC) sampled. The additional parameters were:

1. *Estimates of neuronal density*. In all brains, neurons (labeled and unlabeled) were counted in the most caudal NC and HC sections. In NC sections, these counts were made in eight squares each with an area 0.02 mm^2 (140 μm × 140 μm), and in HC counts were made in four such squares. Neurons were counted only if their nuclei completely appeared within the boundaries of the squares. The position of the squares was randomly chosen by the mapping software, using the Neurolucida fractionator probe, and yielded 97–227 NC neurons and 82–147 HC neurons per bird. Neurons were packed more densely in HC than in NC. As explained above for the labeled neurons, data from the sampling squares, along with the knowledge of the tissue thickness and data on the nuclear diameter of NC and HC neurons (see below), were used to estimate, using the Abercrombie stereological correction, the total number of NC and HC neurons per unit volume.
2. *Nuclear diameters of all neurons*. In all brains, four of the eight samples taken in NC were used for measuring the nuclear diameters of labeled and unlabeled neurons, yielding measurements on 37–112 neurons per brain; the same was done in all of the HC sampling squares, yielding measurements on 82–147 neurons per brain. From these measurements we calculated, for each brain, the mean neuronal nuclear diameter in NC and in HC. This variable is important for comparing neuronal counts, because neurons with larger nuclei would tend to be over-represented in such counts. Therefore, as for the previous section, we used this variable for the stereological correction in order to accurately estimate numbers of total neurons per mm^3.
3. *Nuclear diameters of [^{3}H]-labeled neurons*. Nuclear diameters of [^{3}H]-labeled neurons were also measured in all brains, for both brain regions. In NC, these measurements were taken from the most caudal section, in ten 0.02 mm^2 squares, randomly chosen by the software (using the Neurolucida fractionator probe). However, as we shall see in Section 3, some of the experimental groups had few [^{3}H]-labeled neurons, and therefore in these cases, the sample had to be enlarged. For this, we revisited sections in which less than ten labeled neurons had been measured, and this time scanned the whole section, by using the Neurolucida meander scan probe, which yielded 6–18

labeled neurons per brain. In HC, where the overall density of labeled neurons was low (see Section 3), we maximized our sampling by measuring the nuclear diameters of all [^{3}H]-labeled neurons that we encountered in the five sections mapped in each brain, not only the most caudal one. This yielded 1–22 labeled HC neurons per brain. From these measurements we estimated for each bird the mean nuclear diameter of HC and NC [^{3}H]-labeled neurons. As for the previous section, we used this variable for the stereological correction in order to accurately estimate numbers of [^{3}H]-labeled neurons per mm^3.

4. *Number of exposed silver grains per cell nucleus*. The number of silver grains per neuronal nucleus, both in NC and in HC, was counted in each of the [^{3}H]-labeled neurons whose diameter was measured and whose [^{3}H]-labeling met our criterion for a labeled cell. From these counts we arrived, for each brain region, at a mean number of grains per [^{3}H]-labeled neuronal nucleus.

As explained above, some of the measurements from the most caudal section were used for stereological corrections in all sections which were sampled, under the assumption (untested) that these parameters did not differ rostral-caudally. Measurements of nuclear diameters of labeled HC neurons, which were taken throughout the HC did not show systematic rostral-caudal differences.

2.6. Percentage of labeled neurons

Our estimates, for each bird, of total (labeled and unlabeled) neurons per unit volume (obtained from the most caudal section of that brain area), and the number of [^{3}H]-labeled neurons, allowed us to derive an estimate of the percentage of labeled NC and HC neurons in each individual and in each of the six experimental groups.

2.7. Validation of neuronal counts using the Neu-N marker

The procedure involved was described earlier. The intention was to satisfy ourselves that neuronal counts based on cresyl violet staining were reliable. The goal was to compare the counts of [^{3}H]-labeled neurons using cresyl violet staining versus Neu-N-staining.

2.8. Statistical analysis

The following data were subjected to statistical analysis: body mass; number of [^{3}H]-labeled HC and NC neurons per mm^3; number of all neurons (labeled and unlabeled) per mm^3; mean nuclear diameter of all (labeled and unlabeled) HC and NC neurons; mean nuclear diameter of labeled neurons only; and mean number of silver grains per neuronal nucleus of [^{3}H]-labeled neurons. Data that were expressed as number of cells per mm^3 were transformed, prior to the statistical analysis, by using the square root transformation [32]. Throughout the statistical analysis $P \leq 0.05$ was considered significant. For comparing numbers of labeled neurons per mm^3 obtained from several section levels, analysis of variance was performed using ANOVA (repeated measures). Other data were analyzed by two-way ANOVA or *t*-tests (two-tailed). Post hoc comparisons were carried out by the Tukey (HSD for unequal *N*) method.

3. Results

Our results will be described separately for each of the two brain regions, NC and HC, on which we focused our attention.

3.1. Nidopallium caudale (NC)

3.1.1. Volume of region sampled

There was no significant difference in NC volume sampled between experimental groups, as follows—Group 1: $8.92 \pm 1.37\,\text{mm}^3$; Group 2: $8.98 \pm 1.25\,\text{mm}^3$; Group 3: $9.85 \pm 1.34\,\text{mm}^3$; Group 4: $8.41 \pm 0.66\,\text{mm}^3$; Group 5: $9.64 \pm 1.34\,\text{mm}^3$; Group 6: $10.36 \pm 1.68\,\text{mm}^3$.

3.1.2. Nuclear diameters of all NC neurons and of just [^{3}H]-labeled neurons

There were no significant differences in mean neuronal nuclear diameter that could be attributed to the manner in which the birds were housed or to an interaction between social setting and survival. This absence of a difference applied both to the "total" sample of [^{3}H]-labeled and unlabeled NC neurons (range: 9.3–10.4 μm) as well as to the sample of just [^{3}H]-labeled NC neurons (range: 10.2–12.0 μm). Assuming that this information, obtained from the most caudal NC sampling section, was representative of the entire NC volume sampled, it meant that comparisons of total neuron number and [^{3}H]-labeled neuron number per unit area of NC could be made directly between housing conditions and for the various survival times without the need to modify our counts using stereological corrections. It meant, too, that comparisons of total neuron number and of number of [^{3}H]-labeled neurons would be very similar between experimental groups whether we used numbers per unit area or estimates per unit volume and that the magnitude and direction of these comparisons would not be affected by stereological corrections which, nonetheless, we did.

3.1.3. Number of total neurons per mm^3

Estimates of mean neuron number per mm^3 did not differ significantly between our six experimental groups, as follows—Group 1: $59{,}905 \pm 9278$; Group 2: $56{,}845 \pm 7204$; Group 3: $59{,}623 \pm 23{,}594$; Group 4: $77{,}686 \pm 15{,}776$; Group 5: $58{,}749 \pm 19{,}657$; Group 6: $54{,}815 \pm 7908$.

3.1.4. Number of [^{3}H]-labeled neurons per mm^3

We wanted to know whether social setting affected the recruitment and survival of new NC neurons per mm^3. We assumed that recovery of [^{3}H]-label was efficient and that therefore all neurons with enough label to meet criterion were counted. However, since our data on number of [^{3}H]-labeled neurons per mm^3 in each brain were collected from several sections, we first had to establish whether we could pool the results for all sections.

In the three groups of birds housed by themselves (Groups 1–3), for which we counted [^{3}H]-labeled neurons per mm^3 in three sections, there was a significant difference in number of [^{3}H]-labeled neurons per mm^3 between sections ($F_{(2,24)} = 4.40$, $P = 0.024$) and a significant interaction between section position and experimental group ($F_{(4,24)} = 3.67$, $P = 0.018$). In the three groups of birds housed communally (Groups 4–6), for which we counted [^{3}H]-labeled neurons per mm^3 in five sections there was no significant difference in number of [^{3}H]-labeled neurons per mm^3 between sections but there was a significant interaction between section position and experimental group ($F_{(8,44)} = 2.43$, $P = 0.029$). Because of these differences we did not pool the data from all sections, and therefore we present, for all experimental groups, our estimates of [^{3}H]-labeled neurons per mm^3 for each section (Fig. 2A and B).

In both social settings, no consistent or obvious rostro-caudal gradients were observed in number of [^{3}H]-labeled neurons per mm^3. However, when comparing the sections at the two ends of the rostro-caudal distance sampled – most rostral and most

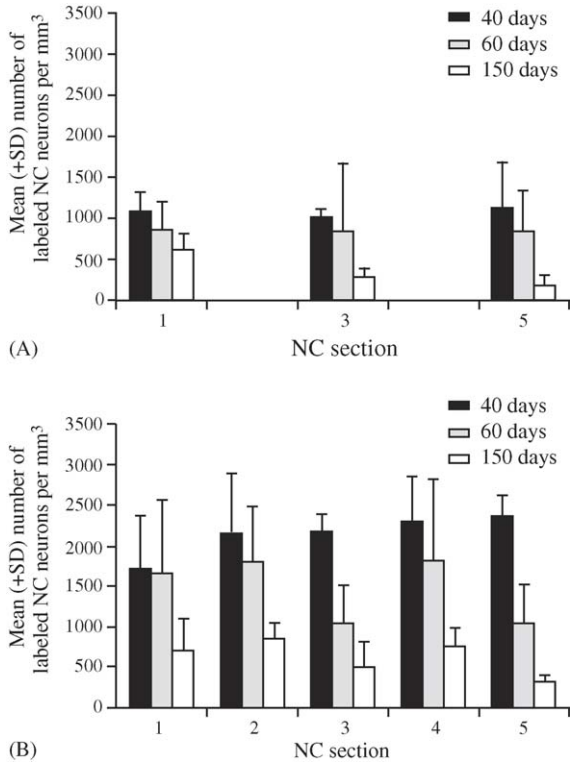

Fig. 2. (A) Mean (±S.D.) number of [^{3}H]-labeled neurons per mm^3 in the three NC sections of birds housed singly (Groups 1–3). (B) Mean (±S.D.) number of [^{3}H]-labeled neurons per mm^3 in the five NC sections of birds housed communally (Groups 4–6). In both panels section 1 is the most rostral one and section 5 the most caudal one. Shading key indicates the three survival groups (40, 60 and 150 days).

caudal – we found, in both social settings, that while at 40 day survivals there was no significant difference in the number of [^{3}H]-labeled neurons per mm^3 between the two sections, at 150 days survivals the fraction of new neurons surviving was significantly lower in the most caudal section (Table 1). In both social settings, about half of the number of [^{3}H]-labeled neurons present at 40 day survival were still present at 150 days in the rostral section, while only about 15% were present in the most caudal one. Fig. 2 shows, too, for both social settings and for all NC sections, that the number of [^{3}H]-labeled neurons present at day 40 had dropped by day 150.

When focusing on the most caudal NC section, analysis of the number of [^{3}H]-labeled neurons per mm^3 revealed significant differences between social environments ($F_{(1,27)} = 6.47$, $P = 0.0169$) and between survival times (40, 60 and 150 days; $F_{(2,27)} = 33.68$, $P \leq 0.0001$). No interaction was found between social setting and survival time. Fig. 3 shows the extent of the differences. Three points are worth noting.

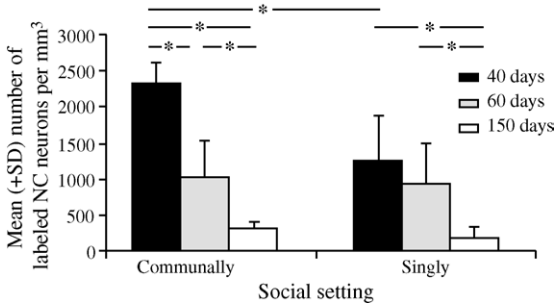

Fig. 3. Mean (±S.D.) number of [^{3}H]-labeled neurons per mm^3 in the most caudal NC section of birds that were housed communally or singly and killed 40, 60 and 150 days after [^{3}H]-treatment (* indicates $P \leq 0.05$).

- *Social setting affects neuronal recruitment*: when birds are exposed to a new large heterosexual group and tested 40 days later, significantly more new neurons are recruited in their NC than in the NC of birds that are kept singly and tested at the same time. This observation repeats an earlier finding (Lipkind et al., 2002).
- *Neuronal turnover occurs in both social settings:* in both social settings numbers of new neurons decrease with time, so that 150 days after treatment significantly fewer new neurons are found in NC than observed at 40 days. The low numbers at 150 days are similar in both groups. If we assume that overall neuron numbers in this part of the brain remain constant, then neuronal turnover occurs both in birds housed singly as well as in those housed in large groups.
- *Social setting affects short-term neuronal turnover*: 74% of the [^{3}H]-labeled NC neurons present on day 40 were still present at day 60 in the birds housed singly, but this fraction was 44% in the birds housed communally (Fig. 4). However, at 150 days the percentage of [^{3}H]-labeled neurons still present was similar in both social settings.

Though the differences between survival groups in the singly housed and communally housed birds were maximal when focusing just on the last caudal section of the NC region sampled,

Table 1
Mean (±S.D.) number of [^{3}H]-labeled neurons per mm^3 in the two NC sections mapped that were most distant of each other in each of the survival groups of birds housed communally or singly

Birds housed	Survival (days)	Number of [^{3}H]-labeled neurons per mm^3 in		*P*-value
		Most rostral section (#1 in Fig. 1B)	Most caudal section (#3 in Fig. 1B)	
Communally	40	1724.1 ± 663.7	2355.9 ± 283.9	ns
	60	1664.0 ± 881.6	1039.1 ± 499.6	0.01
	150	727.6 ± 377.0	324.8 ± 83.5	0.02
Singly	40	1228.2 ± 249.7	1273.4 ± 635.2	ns
	60	966.9 ± 395.1	938.1 ± 564.8	ns
	150	683.1 ± 233.8	201.0 ± 146.2	0.01

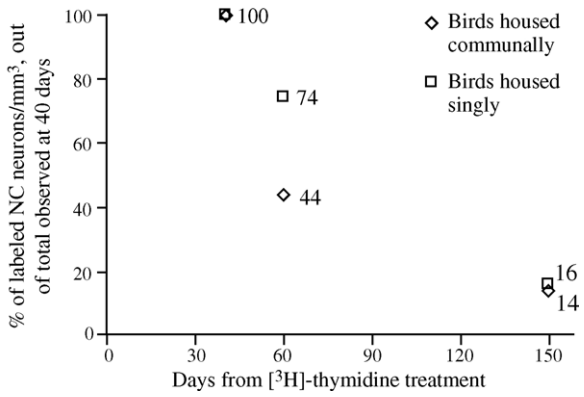

Fig. 4. Percentages of [^{3}H]-labeled neurons which were observed at three time survivals in the most caudal NC section of birds housed singly or communally.

a similar trend was also seen when we averaged for each bird the values for the same three section levels (1, 2 and 3 in the singly housed birds and 1, 3 and 5 in the communally housed ones). Fig. 5 shows the results of this comparison. It can be seen that the results are very similar to those shown in Fig. 3. In both cases there is a marked and significant disappearance, by day 150, of a majority of the [^{3}H]-labeled cells counted on survival day 40.

3.1.5. Mean number of exposed silver grains per [^{3}H]-labeled NC neuronal nucleus

No significant differences were found in number of exposed silver grains per [^{3}H]-labeled NC neuronal nucleus between brains which came from different dipping batches. This indicates that possible differences in autoradiographical procedure, which might have existed between batches, did not affect the number of grains per nucleus. However, overall analysis of variance revealed a significant difference between the two social settings ($F_{(1,27)} = 9.3$, $P < 0.005$) and a significant interaction between social setting and survival time ($F_{(2,27)} = 7.65$, $P < 0.002$). Table 2 details the nature of these differences. Forty days after [^{3}H]-thymidine treatment, a comparison of birds housed singly and communally showed a difference in favor of more label in the communally held birds, but this difference was not significant. Sixty days after treatment the sign of this difference was maintained and it was now significant. This difference had disappeared by 150 days, with a significant reduction in the amount of label in the communally held birds.

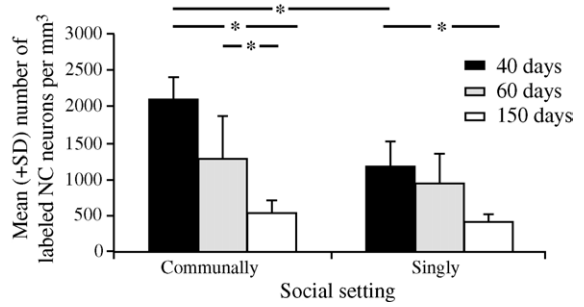

Fig. 5. Mean (±S.D.) number of [^{3}H]-labeled neurons per mm^3 of NC averaging sections 1–3 of the singly housed birds and sections 1, 3 and 5 of the communally housed ones 40, 60 and 150 days after [^{3}H]-treatment (* indicates $P \leq 0.05$).

Table 2
Mean (±S.D.) number of exposed silver grains per [^{3}H]-labeled NC neuronal nucleus in the various experimental groups (* indicates $P = 0.05$.; ** indicates $P = 0.01$)

Birds housed	Days from treatment		
	40	60	150
Singly	13.8 ± 2.5	13.1 ± 3.3 ↑ ** ↓	17.3 ± 1.6
Communally	17.1 ± 1.8	20.9 ± 3.8 ←*→	15.6 ± 3.3

Fig. 6 shows that the difference in label seen at 60-day survival resulted from a difference in the distribution of the most common intensity of label. The most common intensity was nine exposed silver grains in the birds housed singly, while birds housed communally had two peaks at, approximately, 18 and 30 grains per labeled nucleus. Over longer survival (150 days), the distribution of label in the communally housed birds shifted to the left as the total number of labeled cells declined; the labeled cells that persisted had less label than at 60 days.

3.1.6. Neu-N labeling

The numbers of [^{3}H]-labeled NC neurons per mm^3 that were counted in two adjacent sections (one reacted with cresyl violet

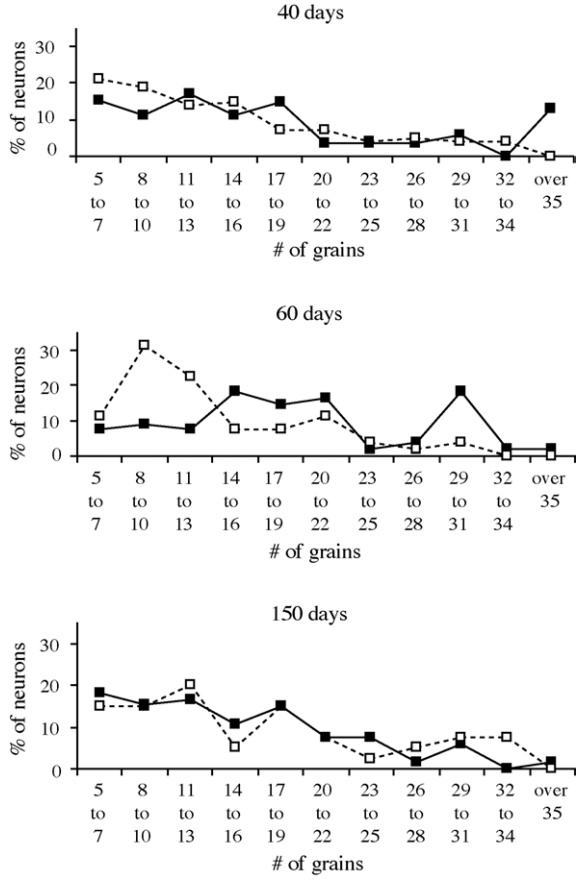

Fig. 6. Distribution of the number of exposed silver grains in [^{3}H]-labeled NC neurons, in each of the social settings and survivals. (Open squares—birds housed singly; closed squares—birds housed communally.)

and the other with anti-Neu-N antibody) of four birds were as follows: 283 versus 304; 515 versus 509; 268 versus 298; 281 versus 295. These results are very similar in all cases and were in line with the results of a similar comparison reported earlier [18]. We take this as validation of our use, for quantification purposes, of sections stained with cresyl violet in which neuronal identity was established by the size and staining pattern of nuclei—relatively large, with clear cytoplasm and just a single or double nucleolus.

3.1.7. Anatomical distribution of the [^{3}H]-labeled neurons in NC

[^{3}H]-labeled neurons occurred throughout NC with no particular clustering around obvious anatomical landmarks. As a group their nuclear diameters fell at the upper end of the distribution observed among all NC neurons. We have no information about the connectivity of these cells.

3.2. Hippocampus (HC)

3.2.1. Volume of region sampled

There was no significant difference in HC volume sampled between experimental groups, as follows—Group 1: $1.60 \pm 0.13\,mm^3$; Group 2: $1.50 \pm 0.12\,mm^3$; Group 3: $1.42 \pm 0.23\,mm^3$; Group 4: $1.50 \pm 0.10\,mm^3$; Group 5: $1.29 \pm 0.23\,mm^3$; Group 6: $1.36 \pm 0.13\,mm^3$.

3.2.2. Nuclear diameters of total (labeled and unlabeled) and neuronal densities of HC neurons

No significant group or survival differences and no significant interaction between group and survival were found in mean nuclear diameters of total ([^{3}H]-labeled and unlabeled) HC neurons, which ranged between 9.9 and 10.8 μm. Mean neuronal densities (neurons per mm^3) did not differ between groups or between survivals (Group 1: $68{,}927 \pm 5342$; Group 2: $91{,}878 \pm 27{,}807$; Group 3: $95{,}422 \pm 25{,}204$; Group 4: $87{,}034 \pm 16{,}187$; Group 5: $69{,}865 \pm 13{,}741$; Group 6: $68{,}877 \pm 3479$).

3.2.3. Nuclear diameters of HC [^{3}H]-labeled neurons and number of exposed silver grains per [^{3}H]-labeled neuronal nucleus

No significant group or survival differences and no significant interaction between group and survival were found either in mean diameters of [^{3}H]-labeled HC neuronal nuclei (which ranged between 9.5 and 10.8 μm), or in the mean number of exposed silver grains per [^{3}H]-labeled neuronal nucleus (which ranged between 11.8 and 17.4).

3.2.4. Number of [^{3}H]-labeled neurons per mm^3

The results of hippocampal [^{3}H]-labeling are shown in Fig. 7. It can be seen that the number of labeled cells was greater in the communally housed birds than in the singly housed ones. This came as a surprise, since we had not expected to find HC affected by social change. Since the data showed a tendency for greater numbers of [^{3}H]-labeled neurons per mm^3 of HC in the caudal than in the rostral part of HC, our first step in the analysis of data, as for the NC, was to test whether or not we could pool results from the five HC sections which were mapped in each brain. Since the total number of [^{3}H]-labeled neurons encountered in the HC was low (see Sections 2 and 2.5), we were aware that such a statistical analysis, of using section position as a variable, might not be strong. Therefore, at an early stage of mapping, we tested whether doubling (from 5 to 10) the number of HC sections sampled for counts of labeled neurons would significantly alter our estimated mean number of [^{3}H]-labeled neurons per mm^3 for the entire volume of HC sampled. We did this in brains from Group 4 (communally housed, 40 days survival). The results (not shown) were no different when sampling 5 or 10 sections, suggesting that the results obtained from 5 sections were not a sampling artifact but a reflection of the lower level of neuronal recruitment in HC under the conditions of our experiment. Of course, it is still possible that random variability between sections (given the small amount of tissue sampled per section), obscured real rostrocaudal trends, but we cannot address this issue.

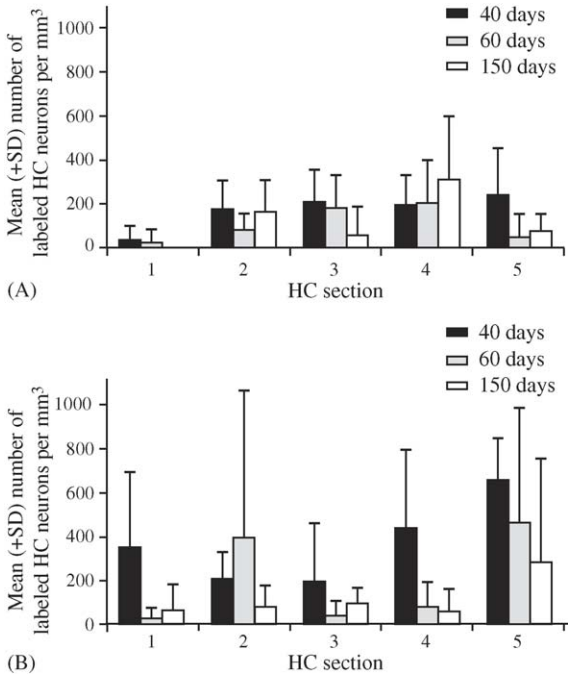

Fig. 7. (A) Mean (±S.D.) number of [^{3}H]-labeled neurons per mm^3 in the five HC sections of birds housed singly (Groups 1–3). (B) Mean (±S.D.) number of [^{3}H]-labeled neurons per mm^3 in the five HC sections of birds housed communally (Groups 4–6). In both panels section 1 is the most rostral one and section 5 the most caudal one. Shading key indicates the three survival groups (40, 60 and 150 days).

In the three groups of the birds housed singly (Groups 1–3), an analysis that preserved section identity revealed a significant difference between sections ($F_{(4,32)} = 3.59$, $P = 0.016$) and no interaction between section position and experimental group. When testing the two most remote sections in each of these three survival groups, we found, in all groups, a trend for more labeling in the most caudal section, but this trend was not significant. A same analysis for the communally housed birds (Groups 4–6) revealed no significant difference between sections and no interaction between section position and experimental group.

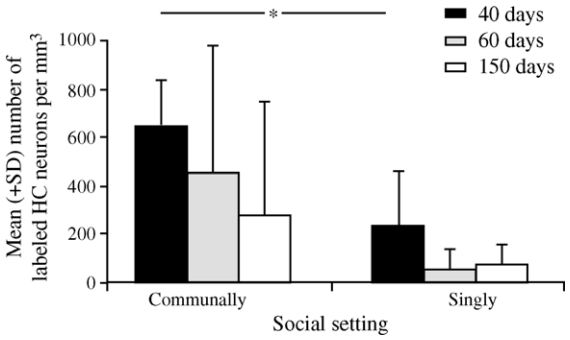

Fig. 8. Mean (±S.D.) number of [^{3}H]-labeled neurons per mm^3 in the most caudal HC section of birds that were housed communally or singly and killed 40, 60 and 150 days after [^{3}H]-treatment (* indicates $P \leq 0.05$).

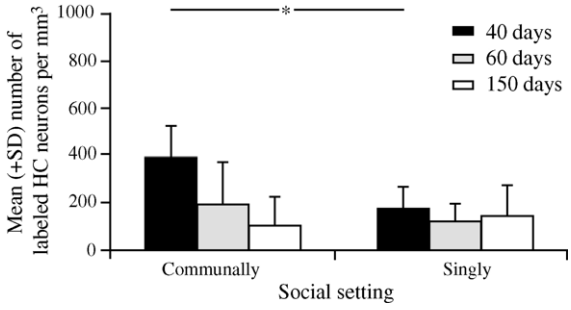

Fig. 9. Mean (±S.D.) number of [^{3}H]-labeled neurons per mm^3 of HC averaging sections 1–5 of the communally and singly housed birds, respectively, 40, 60 and 150 days after [^{3}H]-treatment (* indicates $P \leq 0.05$).

Similarly to the birds housed singly, a trend for more labeling in the most caudal section than in the most rostral one was also seen here, but again, this trend was not significant.

Because the above analysis revealed a significant difference between sections in the birds housed singly, and because there was a tendency (though not significant) for the caudal-most HC section in all groups to recruit more neurons than the rostral one, we first focused our comparison of singly and communally housed birds on this caudal section (Fig. 8). We found a significant difference between birds housed communally and birds housed singly ($F_{(1,18)} = 6.16$, $P = 0.023$), with no significant differences between survival times within each social setting, and no interaction between social setting and survival. When looking at the caudal-most section in the two brain regions – NC and HC (Figs. 3 and 8, respectively) – three points are worth noting.

- *In HC, as in NC, social setting affects neuronal recruitment*: at 40 day survivals more new neurons were recruited in HC when birds were housed communally than when birds were housed singly (652 ± 188 labeled neurons per mm^3 versus 238 ± 221, respectively; $t_{(8)} = 2.78$; $P = 0.012$).
- *HC recruits fewer new neurons than NC in both social settings*: in birds housed communally and killed 40 days after [^{3}H]-treatment, 0.75% of HC neurons were labeled versus 3% in the NC. A similar situation existed in the birds housed singly: 0.35 versus 2.1%, respectively.
- *A suggestion that in neither social setting there was much turnover of new HC neurons*: no significant differences were found between the three tested survivals (40, 60 and 150 days) in both social settings, in number of [^{3}H]-labeled HC neurons per mm^3. As already noted above, the analysis for HC was based on relatively few [^{3}H]-labeled neurons per brain. However, since the proportion of HC and NC volume sampled was similar, we suggest that our results for HC are real and different from the results obtained for NC, where in both social settings the number of new neurons per mm^3 decreased with time.

If we average the numbers of [^{3}H]-labeled neurons per unit volume found in all five HC sections sampled and shown in Fig. 7, and compare these averaged numbers in singly housed and communally housed birds (Fig. 9), the overall differences are similar to those found for the most caudal section. Social setting affects neuronal recruitment. Significantly more new HC neurons are recruited in birds that were housed communally and tested after 40 days, than in birds that are housed singly; HC recruits less new neurons than NC in both social settings; and in both social settings there is less neuronal turnover in HC than in NC (Fig. 5).

3.2.5. Anatomical distribution of the new HC neurons

The anatomical distribution of the [^{3}H]-labeled neurons in HC was very similar to that observed in the HC of black-capped chickadees (Barnea and Nottebohm, 1994). As in that earlier account it was mostly restricted to a band of relatively large cells close to the wall of the lateral ventricle (see there, Fig. 1). We have no information on the connectivity of these cells.

3.3. Body mass

No significant differences were found in body mass (measured before birds were killed) between experimental groups (mean body weight was 13.5 ± 1.48 g; $N = 34$).

4. Discussion

We have extended our earlier observation that addition of new neurons to the nidopallium caudale (NC) of adult zebra finches is significantly higher in birds housed communally than in those housed singly [18]. In that study, all birds were killed 40 days after the last injection of the birthdate marker [^{3}H]-thymidine. We wondered what might be the fate of the new neurons after day 40—e.g. at days 60 and 150. The present study fills this gap.

Body mass did not differ significantly between experimental groups. From this we infer that all birds remained in good health. Similarly, there were no group differences in the mean diameter of all neurons or of just [^{3}H]-labeled neurons in NC and HC. Therefore, as explained in Section 3, group differences in new neuron recruitment and survival could be drawn from counts of neurons per unit area. However, it has become customary to convert the raw data into an estimate of numbers per unit volume, with the appropriate stereological correction, and this we did. These estimates of numbers assume the neuronal nuclei counted had a spherical shape and that autoradiography is an efficient way of recognizing neurons born in adulthood.

The latter assumption seems reasonable in view of the modest thickness (6 μm) of our sections. It assumes, too, that data on neuronal nuclear diameters and packing density obtained from the caudalmost NC and HC sections sampled were representative of the whole volume sampled. The same methods and assumptions applied to all groups and so we are not aware of built-in biases that would undermine our comparisons of neuronal recruitment/survival using brain region and survival time as variables.

We worried about the extent that our estimates of [^{3}H]-labeled neurons at various survival times might be contaminated by successive rounds of neuronal stem cell divisions. That is, we know that the initial labeling was, in all likelihood, the reflection of a mitotic event, but we do not know if counts of labeled neurons made thereafter reflect just initial recruitment and subsequent attrition or include, as well, new rounds of stem cell division with new generation of labeled daughter cells. We cannot exclude this possibility. However, had there been a significant new recruitment of labeled neurons after survival day 40, the mean number of exposed silver grains per labeled neuron would have decreased, yet our data (Table 2) shows no evidence of this. We infer that any divisions of labeled neuronal stem cells that contributed to our counts of labeled neurons occurred predominantly before day 40 and that the difference in number of labeled neurons observed at 60 day and 150 day survivals probably reflects just the death of a fraction of these labeled cells.

As before, the number of new NC neurons present on day 40 after [^{3}H]-thymidine treatment was significantly higher in communally housed birds than in singly housed ones, and was rather similar at all rostro-caudal levels. However, by day 150 the rate of new neuron survival was considerably higher in the rostral than in the caudal ends of the NC.

The number of exposed silver grains over the nucleus of new NC neurons was higher in communally housed birds than in birds housed singly raising the possibility that already at survival day 40, and more so by day 60, different kinds of neurons were surviving best in these two social settings. The differences in labeling could come about, for example, if these cells were born from stem cells whose S-phases of DNA synthesis differed in duration. However, it might be better not to place too much weight on group differences in number of exposed silver grains until such differences are shown to be reproducible.

One possible interpretation of the differential survival of rostral versus caudal new NC neurons is that the caudal reaches of NC are more sensitive to new environmental information, recruiting more cells when conditions change and then turning them over faster. Earlier observations have shown that new neuron survival is affected by the demise of pre-existing neurons [27] as well as by a rise in the local abundance of brain derived neurotrophic factor (BDNF; [1]). Local expression of BDNF has been shown to be activity dependent [16]. Conceivably, circumstances that promote the demise of existing neurons may also foster the survival of their replacements. If so, these two effects may be felt more strongly in caudal than in rostral NC. If the cells involved in this turnover were involved in the storage of experiential information, then updating of environmental information would occur at shorter intervals in caudal than in rostral NC. We emphasize that this interpretation is hypothetical; there is no direct evidence that NC neurons store information of any kind.

The number of [^{3}H]-labeled neurons in HC 40 days after treatment was significantly higher in communally than in singly housed birds and tended to be higher caudally than rostrally. The social effect on neuronal recruitment in the HC came as a surprise, since evidence indicates that the avian hippocampus provides spatial maps [15,28] and we did not expect that the social variable would make a significant difference—clearly our mistake. Perhaps the impact of a change in living place is much greater when accompanied by increased social complexity? The valence of physical space may be modified by social space, as individual birds chase rivals, court potential mates or avoid the aggression of rivals. The possibility that the HC provides an anatomical site for the interaction of spatial and social memories is offered with a question mark. We did not expect this outcome. However, our suggestion seems to be in line with results of earlier studies on rodent hippocampus. For example, Ergorul and Eichenbaum [6] argue that the mammalian hippocampus encodes spatial information only to the extent that space defines sequences of actions with biological significance to the animal.

An earlier experiment with free-ranging adult black-capped chickadees [4] showed that many more hippocampal neurons are added in late summer and early fall than at other times of year. During late summer and early fall these birds hide many food items throughout the forest, which they later reclaim when food becomes scarce. This peak in new neuron recruitment was markedly higher in free-ranging individuals than in others, of comparable age, housed as a group in a large outdoors aviary, in which environmental complexity was presumably lower. In that study, more neurons were added to rostral than to caudal HC, but only in the free ranging birds. The turnover of the new neurons in the free ranging birds was also greater in rostral HC than in caudal hippocampus. The observations in this earlier study were made, as in the present report, over a comparable 2 mm long stretch of HC. Taken together, these earlier observations in chickadees and our more recent ones in zebra finches suggest that different experiential variables have their maximal effect on neuronal turnover in different parts of the avian HC, which therefore may act as repositories for more or less ephemeral changes of a particular kind. Counts of [^{3}H]-labeled neurons were done in the same manner in both studies.

As already noted in Section 3, data for HC came from relatively few labeled neurons per brain. However, we believe that this sparse recruitment reflected reality and that conclusions can be drawn from these data. Accordingly, the difference in our present study between new neuron turnover in NC and HC is worth a comment. Figs. 5 and 9 show that in communally housed birds, 25% of the new NC neurons and 27% of the new HC neurons present on day 40 were still present on day 150. However, in singly housed birds these proportions were, respectively, 34 and 87%. Therefore we suggest, that in the absence of social complexity, HC responded to the physical change in housing as a one-step phenomenon, which already had been fully factored in by the changes in place by day 40; thereafter, the situation

was relatively static and so the majority of the new neurons persisted. By contrast, the social setting, which initially changed as a one-step event – from few familiar companions to many new ones – presumably continued to evolve, as the new comer established its social position in the flock; this ongoing change may have been behind the higher rate of culling of the new NC and HC neurons in the communally housed birds. Thus, the permanence of neuronal additions may depend on the permanence of the events that brought them about. Of course, it could be that the average life of new NC neurons is shorter than that of HC neurons. However, in our earlier study of free-ranging chickadees most of the new hippocampal neurons survived just a few weeks [4]; those birds lived in the wild and in a very seasonal, changing environment. The zebra finches placed in outside aviaries encountered less ongoing environmental change.

Taken together, our observations on zebra finches and black-capped chickadees – and earlier work on canaries [13] – emphasize that behavioral and environmental change are accompanied by changes in the recruitment and survival of new telencephalic neurons. The new neurons, in turn, can live for varying lengths of time, from a few weeks to many months. Those observations are compatible with existing hypotheses that link neuronal replacement to changes in memory demand [22–24]. But our results add, too, a new twist. The turnover of new NC neurons was considerably brisker in the caudal than in the rostral reaches of NC, providing the first suggestion that the updating of information – possibly by turnover of neurons that hold it – might proceed at different intervals in different parts of NC. In addition, in birds housed singly, the mean survival of new HC neurons was two-times longer than the mean survival of NC neurons. These observations raise a novel possibility: much as different kinds of sensory information have different anatomical representations in the forebrain, so too, perhaps, has calendar time. If one accepts the possibility that forebrain neurons encode – or store – information that is acquired by experience, and if one extends this possibility to the new neurons, then one form of time representation would be how long neurons that encode change survive. When these neurons are winnowed any information they might have stored will be winnowed too. Since in some parts of the brain the reduction of new neuron numbers occurs faster than in others, we suggest that the rate at which information acquired by new neurons is lost, differs between regions, thus resulting in an anatomical representation of the past. We hypothesize that those parts of the brain where neuronal replacement occurs at a brisk pace encode recent events, while parts of the brain where replacement occurs at a slower pace or not at all hold the older memories. This idea is novel enough to make us wonder if it might not offer a new way of looking at the brain, one that adds a time dimension to existing maps of function.

Acknowledgements

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