**Functional Restoration of Acoustic Units and Adult-Generated Neurons after Hypothalamic Lesion**

Mei-Fang Cheng,1 Jing-Pian Peng,3 Gang Chen,1 Jeffrey P. Gardner,4 Edward M. Bonder2

1 Department of Psychology, Rutgers University, Newark, New Jersey 07102

2 Department of Biological Sciences, Rutgers University, Newark, New Jersey 07102

3 Institute of Zoology, Chinese Academy of Sciences, Beijing 100080, PR China

4 Hypertension Research Center and Department of Pediatrics, University of New Jersey, Newark, New Jersey 07102

Received 5 June 2003; accepted 10 November 2003

**ABSTRACT:** The hypothalamus of the adult ring dove contains acoustic units that respond to species-specific coo vocalization. Loss of nest coo leads to unsuccessful breeding. However, the recovery of nest coo in some doves suggests that these units are capable of self-renewal. We have previously shown that lesioning the hypothalamus generates the addition of new neurons at the lesioned area. In this study, we sought to determine whether lesion-induced new neurons are involved in the recovery of coo-responsive units. We systematically recorded electrical activity in the ventromedial nucleus (VMN) of the hypothalamus, before and after lesion, for varying periods up to 3 months. Recordings were made when the birds were at rest (spontaneous discharge) and when the birds were exposed to acoustic stimulations (evoked discharge). Concurrently, the lesioned area was monitored for changes in cell types by using bromodeoxyuridine (BrdU) to label newly divided cells and NeuN to identify mature neurons. For 1 month after lesion, there was no sign of electrical activity, and only BrdU-labeled cells were present. When the first electrical activity occurred, it displayed abnormal spontaneous bursting patterns. The mature discharge patterns (both spontaneous and evoked) occurred after detection of BrdU+/NeuN+ double-labeled cells 2–3 months postlesion and were similar to those found in intact and sham-lesioned birds. Double-labeled cells bore morphologic characteristics of a neuron and were confirmed with z-stack analysis using confocal laser scanning microscopy. Moreover, double-labeled cells were not stained for glial fibrillary acidic protein (GFAP), suggesting that they were neurons. The number of coo-responsive units was significantly correlated with that of BrdU+/NeuN+ cells. Furthermore, the marker for recording sites revealed that coo-responsive units were colocalized with BrdU+/NeuN+ cells. Taken together, the evidence strongly suggests that lesion-induced addition of new neurons promotes the functional recovery of the adult hypothalamus.

© 2004 Wiley Periodicals, Inc. J Neurobiol 00: 000 – 000, 2004

**Keywords:** hypothalamus; adult neurogenesis; brain injury; coo-responsive units; recovery of function

---

**INTRODUCTION**

Studies have shown that recovery from brain damage can take several forms, including re-establishment of function within the remaining tissue (Rose et al., 1997), tissue transplantation (Dunnett, 1994; Freed et al., 1995; Borlongan et al., 1997), and reorganization of the undamaged area (Luria et al., 1975; Robertson and Murre, 1999; Dijkhuizen et al., 2003). More recently, the potential use of stem cells for brain repair has emerged as a promising area for research (for review: Gage, 2000; Horner and Gage, 2000; Macklis, 2001; Song et al., 2002a). Self-repair in the adult brain after cell loss has been shown in a few brain regions, including the hippocampus of rats (Gould and Cameron, 1996; Cameron and McKay, 1999), the high vocal center (HVC) of songbirds (Kirn and Nottebohm, 1993; Kirn et al., 1994).
Scharff et al., 2000), the neocortex of mice (Magavi et al., 2000), and the hypothalamus of ring doves (Cao et al., 2002). In the HVC, the addition of newly formed neurons was associated with the recovery of singing behavior (Scharff et al., 2000; Li et al., 2000). In the hippocampus, Shors et al. (2001) used a toxin (MAM) that caused proliferating cells to diminish adult-generated cells and found that dentate neurogenesis is associated with the formation of memory trace. A study by Feng et al. (2001) using presenilin-1 knockout mice showed, however, that dentate neurogenesis is not required for memory formation but may play a role in the periodic clearance of outdated hippocampal memory traces. Thus, the functionality of induced adult neurogenesis remains an unresolved issue. Specifically, do newly induced adult neurons assume the functional properties of the damaged tissue?

To address this question, we systematically recorded, from a specific damaged brain area, electrical activities before and after bilateral electrolytic lesions of the ventromedial nucleus (VMN) of the hypothalamus in the adult ring dove. The hypothalamus in the adult ring dove contains special acoustic units that respond only to species-specific nest coo auditory stimulation (Cheng et al., 1998). Fully restored electrical activity at the lesioned area is expected to reestablish responses to nest coo stimulation. We recorded electrical activity in the VMN when the bird was at rest (spontaneous firing: Experiment I) and when the bird was exposed to biologically relevant auditory stimulation (evoked discharge: Experiment II) before and after lesion on a weekly basis for up to 3 months. Concurrently, in each experiment, we monitored changes in cell types at the lesioned area by using antibody against bromodeoxyuridine (BrdU) for newborn cells and antibody against NeuN for mature neurons. The BrdU+ and NeuN+ double-labeled cells, namely the newly generated neurons, were further validated with antibody against glial fibrillary acidic protein (GFAP) for astrocytes and z-stack analysis using laser scanning confocal imaging.

If induced new neurons found at the lesion site assume the function of the lost cells, the following results are expected: the restoration of electrical signals at the lesioned area will coincide with the detection of new neurons; and fully restored electrical activity should reflect electrical properties characteristic of the region before lesion.

MATERIALS AND METHODS

Animals

Ring doves (Streptopelia risoria) were bred in the indoor colony at the USDA-certified Rutgers University Animal Facilities at the Newark campus and kept on a 12:12 h light/dark cycle. A total of 122 birds (57 male, 65 female) were used in the study (61 birds in each experiment). The age of the birds ranged from 7 months to 1 year old (weight 160–180 g). After electrolytic lesioning, 36 birds were returned to cages with their original mates. Other birds (n = 86) were returned to stock holding cages of six to eight birds of mixed sexes. The birds were divided into 15 groups based on the survival time postlesion (1, 8, 15, and weekly until 99 days postlesion). In each experiment, 16 birds were used as control birds. Control I (sham lesion) birds (n = 16) were injected with BrdU and received electrode insertion with no current; Control II birds (n = 4) received electrolytic lesion but no BrdU; and Control III (intact) birds (n = 4) received neither BrdU nor electrolytic lesion. In Control I, the sham birds were divided into five groups of different survival time postlesion, perfused at the same time as the corresponding experimental group, and allowed to survive for the same length of time. In addition, five birds were used for confocal image analysis of double-labeled cells.

BrdU Injections

All birds except the intact control birds (Control III) received two intramuscular BrdU injections at 40 mg/kg body weight before surgery and additional injections beginning 4 days after surgery. Injections were given at 4 day intervals thereafter. The total number of injections birds received after lesion depended on survival time; for example, birds surviving 24 h received two injections whereas birds surviving 92 or 99 days received 24 injections in total.

Preparatory Surgery

Two days before electrolytic lesion and electrophysiologic recording, birds were anesthetized with chloropent (0.24 ml/100 g, intramuscularly) and placed in a Kopf small animal stereotaxic device. Two small holes were drilled over the VMN of the right and left hemispheres. Two stainless steel hollow posts (O.D. = 0.8 mm, I.D. = 0.6 mm, length = 3.5 mm) were installed and secured with acrylic glue and dental cement, one each in the right and left hemispheres. After the stainless steel hollow posts were positioned, a metal post (14 mm, O.D. = 3.5 with a 5 mm diameter at base) was affixed just behind the intersections of the sagittal and coronal sutures. The wound was closed with Neosporin ointment, and the birds were allowed to recover on a heating pad. Once the birds recovered (2–3 h), they were returned to their cages.

Electrolytic Lesion

Electrolytic lesion was made by inserting an insulated 0.4 mm O.D. tungsten electrode with exposed tip (diameter 0.15–0.2 mm; Micro Probe, Inc.) into the VMN area (ventral, 8.2–8.5 mm from the dura to VMN) via the stain-
Acoustic Stimuli

Before the experiment, coo vocalizations were recorded from birds placed in a small recording chamber; the behavioral context of each recorded coo (nest coo versus bow coo) was noted. Manipulation and analysis of the vocalizations was done using a computerized analysis and re-synthesis system previously described (Margoliash, 2000). The recorded nest coos were edited and digitized with SIGNAL sound analysis software (Engineering Design, Belmont, MA).

Reversed coos were created using the “reverse time buffer” command. Computer generated 500 ms white noise bursts, 2/s at a 75 dB sound pressure level (SPL), with a bandwidth of 0–11 kHz were used. Nest coos were edited to contain a high number of coos without noise or other artifacts. Each session lasted 280 s, with a pause of 60 s between sessions. An acoustic stimuli set included: female nest coo and reversed female nest coo; male nest coo and reversed male nest coo; and white noise. All vocalizations were standardized to an intercall interval of 2 s and maximum amplitude of 75 ± 2 dB SPL. We created a composite tape or floppy disk consisting of three parts in the following order: white noise (100 s), female nest coo (280 s), and reversed female nest coo (100 s) with a 60 s interval between parts. A tape or floppy disk of the male nest coo was similarly edited (Cheng et al., 1998).

Sounds were presented through a Yamaha NS 10M speaker positioned 50 cm directly in front of the birds. The bird’s head was immobilized with a screw to the metal post of a device at a 45° angle without ear bars. The speaker was calibrated in decibels SPL using a calibrated condenser microphone (Bruel-Kjaer 4135) placed to duplicate the position of the bird’s head in the stereotaxic instrument. The peak amplitude of the sound stimulus was set to 70–75 dB SPL with a manual attenuator. The outputs of different acoustic stimuli were monitored using a storage oscilloscope.

Recordings

Methods used for recording electrical activity in the VMN area were essentially the same as those previously reported (Cheng et al., 1998). In brief, the electrical activity of a single cell was recorded by using glass micropipettes (tip, 1–2 µm; 8–20 MΩ impedance by BL-1000 microelectrode tester) filled with 2% potamine sky blue (PSB; Biomedical, Cleveland, OH) in 3 M NaCl. Recording microelectrodes were lowered into the VMN via the hollow post through the dura by using a Burleigh Piezoelectric Microstepper under the visual guidance of a Zeiss microscope.

Micropipettes were connected to a preamplifier by a 0.12 mm wire of platinum lead. Single unit signals were amplified by a Neurodata IR-283 preamplifier, monitored with a Marantz 420 audio monitor, displayed together with acoustic stimuli on a Tektronix 5110 storage oscilloscope, and stored on a personal computer. A Nicolet 4094 C digital oscilloscope was linked to a Macintosh computer via a National Instrument NB-GPIB board for online collection in the form of spike shapes, firing patterns, and peri-event histogram records.

Recording areas ranged from 6.5–9.0 mm deep, starting from the nonlesioned area (V = 6.5 mm), and recording advanced 80–100 µm at a time until normal discharge reappeared at the ventral boundary of the VMN (V = 8.8–9.5 mm). If no activity was registered for 3 min, the recording electrode was lowered another 80–100 µm deeper into the recording area. There was no electrical activity of any kind in all the recording sites for the first 29 days after lesion; hence they were registered as sites without activity during this period. Recordings were carried out for all experimental birds at an interval of 7 days until the end of the survival time postlesion. Before perfusion, the precise position of each recording electrode was marked by iontophoresis of PSB dye by passing 5–10 µA of negative current through the recording electrodes for 10–20 min or by application of PSB dyes using a pneumatic pressure ejection system. Recording sites were localized by examination of 8–10 µm frozen sections.

Immunohistochemistry

Just before the birds were sacrificed, the anesthetized birds were perfused intracardially with 0.9% NaCl solution, followed by cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brains were removed and fixed in 4% paraformaldehyde—0.1 phosphate-buffered saline (PBS) overnight at 4°C, then in 25% sucrose-PBS solution overnight at 4°C. Coronal sections were cut on an HM350 cryostat microtome (Micro) at 10 µm thickness. The slides were stored at −80°C until use. The distribution of the new cells that incorporated BrdU was determined by using an immunohistochemical method. The sections were immersed in 2 N HCl for 30 min at 37°C then in 0.1 M Borate buffer at a pH of 8.5 for 10 min. This pretreatment, which partially hydrolyzes the DNA to produce single-stranded DNA, is necessary because the mouse-generated primary antibody is directed against single-stranded DNA containing BrdU. Subsequently, sections were washed with 0.2 M PBS three times for 5 min (3 × 5) at room temperature. After rinsing, the tissue was incubated with 4% normal horse serum for 1 h, then in the anti-BrdU antibody (Boehringer Mannheim; diluted 1:200 with PBS, pH 7.4) at 4°C overnight in a humidified chamber, followed by incubation with the biotinylated secondary antibody (antimouse IgG; Vector; diluted 1:200 in PBS, pH 7.4) for 60–90 min. The slides were washed in PBS (3 × 5 min) and
then processed according to the manufacturer’s instructions (Vector).

Color development was carried out in PBS solution containing 0.025% diaminobenzidine (DAB), 0.01% H_2O_2 for 6–10 min at room temperature. Anti-NeuN, which recognizes a neuron-specific nuclear protein (Mullen et al., 1992), was used to detect the mature neurons. After BrdU staining, the slides were incubated in 1:100 anti-NeuN (Chemicon) in PBS containing 4% normal horse serum and 0.2% Triton X-100 at 4°C overnight. We used the same procedure here as in the BrdU immunostaining except that the DAB concentration was 0.05%. A control slide, to which the primary antibody was not added, was included in each experiment for control of background staining.

For confocal images, birds were subjected to the identical surgery and injection procedure as in Experiment II, excluding recording sessions. The tissues were stained using indirect immunofluorescence followed by analysis using a BioRad MRC 1024 laser scanning confocal microscope. The sections were pretreated as described above and then incubated in PBS with a cocktail of primary antibody of rat anti-BrdU (Accurate; 1:250) and mouse anti-NeuN (Chemicon; 1:200) overnight at 4°C. After several rinses, the slices were incubated in a pooled solution of biotinylated goat anti-rat IgG (Jackson ImmunoResearch Lab; 1:250) and Alexa 488-conjugated donkey anti-rat IgG for 2 h followed by streptavidin-conjugated Alexa 568 (all from Molecular Probes; 1:250) for 2 h. A few slides were used to control for specificity of the antibody by omitting the primary antibody. Immunofluorescent triple staining for BrdU, NeuN, and GFAP was also performed to determine whether NeuN^+ cells were also glial cells. For triple labeling, the cocktail of primary antibodies included rat anti-BrdU, mouse anti-NeuN, and rabbit anti-GFAP (Dako; 1:100), and the pooled secondary antibody solution included biotinylated donkey anti-rat IgG (Jackson ImmunoResearch Lab; 1:250), Alexa 488-conjugated donkey antimouse IgG, and Cy5-conjugated donkey anti-rabbit IgG (Jackson ImmunoResearch Lab; 1:250). The confocal images were collected using the Kalman average method. The z step was set at 1 μm or less for the z-series analysis.

**Data Acquisition and Analysis**

The neurons responding to acoustic stimuli were studied and classified by a poststimulus and peristimulus time histogram. The number of spikes evoked by each stimulus was recorded for the duration of the stimulus, and the series was repeated at 75 ± 2 dB SPL for each recording site. The spikes (including spontaneous firing) were counted digitally and analyzed by MaLab software and Scope software (courtesy of J. Tepper, Rutgers University). A response was considered excitatory when the firing rate during an acoustic stimulus was at least 15% higher than the control levels of background firing. A response was considered inhibitory when a spontaneous discharge was at least 15% lower than control levels (Cheng et al., 1998). The firing frequency (spontaneous or before and during acoustic stimulation of each unit) was analyzed with a computer system.

Analysis of variance (ANOVA) was used to examine statistical effects in the data. Repeated-measures ANOVA was used to examine overall group differences followed by Newman-Keuls multiple comparison tests. Differences in activity sites before and after lesion were analyzed with Student’s t test. Statistical significance was accepted if p values were less than or equal to 0.05.

For each brain processed for BrdU or NeuN immunoreaction, the number of BrdU^+ or BrdU^+ NeuN^+ double-labeled cells was counted in the lesioned VMN area and nonlesioned area. As shown in Figure 1(A), a lesioned area is defined as the area covering the entire lesion site including the border adjacent to the intact area. The size of the lesioned area ranged from 0.8 to 1 mm. To avoid bias in cell counts and physiologic recordings, the following procedure was adopted. For each experimental bird, in addition to the number that was assigned at birth, a new number (a scramble of all experimental birds) was assigned and used during the electrophysiologic recording session. The tissue slides bearing the bird’s original number contained no other information regarding the bird. The list containing the matching numbers was not made available until the time of the statistical analysis. In each brain, at least six sections (proximal to the lesion) where the lesion was present and five sections where the lesion was absent (distal to the lesion) were analyzed. Sections that were considered distal to the lesion were located dorsal to the VMN or in a few cases at the anterior medial hypothalamus. Means were determined for each side for each bird, and the data were analyzed with Student’s t test.

**RESULTS**

**Recording Sites in Relation to Morphologic Changes at the Lesioned Area**

Figure 1 shows the locations of recording areas within the VMN and the areas dorsal and ventral to the VMN area [Fig. 1(A)]. The morphologic landscape of the lesioned area from which recordings were made changed as survival time increased [Fig. 1(B)]. Immediately after electrolytic lesion, NeuN^+ cells were notably absent at the lesioned area. However, the following three distinct terrains can be discerned [Fig. 1(B), Top: left panel]: at the center of the lesioned area, the necrotic area was sometimes filled with single BrdU^+ cells; the periphery was populated by single BrdU^+ cells; and a region filled with nongranular materials was sandwiched between these two regions. We divided postlesion recordings into three groups, short (postlesion 1–36 days), medium (postlesion 42–71 days), and long (78–99 days) survival time. During the short survival period, recordings were made from the center of the lesioned area as well as the periphery. During the medium survival period,
the nongranular region had shrunk considerably [Fig. 1(B), Top: middle panel], and recordings were mostly from the peripheral area that had infiltrated into a formerly semiopaque area. During the long survival period, the semiopaque area had virtually resolved, and in some cases the necrosis also diminished in size or healed completely [Fig. 1(B), Top: right panel]. Recordings in this period included sites formerly filled with nongranular materials. Figure 1(B) (bottom panel) shows the representative distribution of recordings and double-labeled cells in the three survival periods. As survival time increased, the number of double-labeled cells that merged with responsive sites increased. The sites that responded to coo stimulation during the medium survival time exhibited mostly abnormal discharge patterns and began normal discharge patterns during the long survival time.

Electrical Signals at the Lesioned Area: Silence to Abnormal to Normal Spontaneous Firing

Experiment 1 was designed to record changes in spontaneous electrical activity after VMN lesion. We recorded single-cell discharge from birds at rest before lesion and at weekly intervals after lesion. A subgroup of these birds was killed at different survival times to provide data points for immunohistochemical identi-
ification of cell types in the VMN. To ensure that each recording was made with birds in a comparably normal state, electrical activity from 214 sites ($n = 45$) in the area dorsal to the VMN ($V = 6.0–7.2$ mm; Karten and Hodos, 1967) was recorded for control reference. We found that 84 sites exhibited spontaneous firing at an average of $3.7 \pm 0.8$ pulses/s. At the end of each recording from VMN, the electrode was lowered to $V = 8.8–9.2$ mm, an area ventral to the VMN. Thirty-six (29.5%) of 122 sites exhibited spontaneous firing at an average of $5.9 \pm 1.2$ pulses/s.

The spontaneous discharge in the VMN exhibited three patterns before lesion: a slow and irregular pattern averaging $4.4 \pm 1.2$ impulses/s [Fig. 2(A)]; a phasic discharge [Fig. 2(B)]; and a fast, regular pattern averaging $8.6 \pm 2.3$ pulses/s [Fig. 2(C)]. In stark contrast to prelesion recordings, 24 h after lesion, of 458 sites ($V = 7.6–8.6$ mm) from 45 birds, none showed any spontaneous electrical activity. In fact, no electrical signal was detected in the lesioned group until day 29 postlesion. Similarly, Control group II (lesion but no BrdU) also showed no activity ($n = 2$, 35 units). Conversely, Control groups I (sham lesion) and III (intact) had normal spontaneous firing patterns for all data points (recordings at 8, 43, 64, 85, and 99 days postlesion). Hence, a combined total ($n = 16$) of 72 of 175 (41%) sites exhibited a mean firing frequency of $4.7 \pm 0.8$ pulses/s. On days 29 and 36 postlesion, 2 of 214 sites and 3 of 188 sites, respectively, exhibited discharge patterns that were marked by three to five clusters of incomplete bursting of variable amplitude ($1.1 \pm 0.5$ mV) and frequency ($2.5–6.5$ “spikes”)/s [Fig. 2(D,E)]. These discharges were not normal neuronal activity in that they were characteristically unstable. For example: the waveform and duration of discharge fluctuated over time; the magnitude of amplitude and the length of the incubation period (the latency between stimulation and onset of action potential) varied over time; and both hyperpolarization potential and depolarization potential changed over time. Could these abnormal electrical patterns be attributable to a higher amplifier gain in the lesioned area than in the control area? Although background noise was higher in the lesioned area than in the nonlesioned area (control area), the amplifier gains were comparable as the electrode descended from control to lesioned to control area. In other words, the electrical discharge pattern was not due to a difference in amplifier gain between recordings from the control and lesioned areas. Also, we monitored possible changes in electrical impedance associated with the broken tip of an electrode and discarded data generated by broken electrodes. The abnormal discharges, therefore, most likely reflect the electrical properties of the sites at the time of recording.

Figure 2 Samples of spontaneous electrical activity in VMN before and after lesions. (A), (B), and (C) were recorded from VMN before lesions: (A) A slow and irregular discharge pattern; (B) a phasic firing pattern; (C) a fast firing pattern. (D–H) were recorded after lesions: (D) was recorded at 42 days postlesion; (E) was recorded 64 days postlesion. Note that (D) and (E) lack complete form of a discharge characteristic of an extracellular recording. (E) and (F) exhibit both normal and abnormal forms of discharge; (G) and (H) exhibit normal forms of discharge from birds that survived 99 days.

In sum, during the short survival period, in birds that survived up to 36 days postlesion, there was no evidence of normal spontaneous firings (Table 1). During the medium survival period, an increasing number of sites exhibited abnormal electrical activities similar to those shown in Figure 2(D) and 2(E). These abnormal patterns persisted until day 64 postlesion, when 2 of 14 discharge sites of 167 sites from 21 birds exhibited normal (i.e., prelesion) neuronal patterns. Sites exhibiting normal discharge patterns continued to increase in number as the survival period lengthened. By 99 days postlesion, 39 of 156 sites showed spontaneous discharge, of which 11 sites ex-
hibited normal firing patterns. Figure 2(G,H) shows such sites from two birds. Consistent with the lesion groups, Control group II (lesion but no BrdU) exhibited spontaneous abnormal firing during the medium survival period and normal firing patterns only in the long survival period (Table 1).

In sum, sham lesioning did not appear to affect the spontaneous discharge. The number of sites exhibiting normal spontaneous discharge remained constant throughout the experiment. The corresponding sites in the lesioned birds, however, were not activated until 9 weeks after lesion and increased gradually thereafter. At the conclusion of the experiment (3 months after bilateral lesions), the percentage of sites that had normal discharge was still only a fraction of that observed in the sham birds; the vast majority of electrical signals in the lesioned birds consisted of the abnormal firing pattern (Fig. 3: compare gray and black bars).

### Nest Coo-Responsive Units at the Lesioned Area: Silence to Abnormal to Normal Response

In Experiment II, birds were given experimental and control treatments identical to that in Experiment I except that acoustic stimulation (male nest coo, female nest coo, reversed male nest coo, reversed female nest coo, and white noise) was introduced during the recording session to determine whether spontaneously firing sites assume the function that is characteristic of the VMN, namely, sites exhibiting selective responsiveness to coo stimulation. In total, 294 sites were recorded. Female nest coo evoked an excitatory

---

### Table 1 Characteristics of Experimental Birds, Development of Spontaneous Firing Units, and Double-Labeled Cells at the Lesion Site

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Birds</th>
<th>Survival Time after Lesion</th>
<th>BrdU Injs.</th>
<th>Recordings after Lesion</th>
<th>Spontaneous Firing Unit</th>
<th>BrdU-Labeled Cells</th>
<th>BrdU-NeuN-Labeled Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Number of Birds</td>
<td>Survival Time after Lesion</td>
<td>BrdU Injs.</td>
<td>Number of Birds</td>
<td>BrdU-Labeled Cells</td>
<td>BrdU-NeuN-Labeled Cells</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Experiment I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>24 h</td>
<td>2</td>
<td>45</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>8 days</td>
<td>3</td>
<td>42</td>
<td>0</td>
<td>381.8 ± 5.7</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>15 days</td>
<td>3</td>
<td>39</td>
<td>0</td>
<td>442.2 ± 7.8</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>22 days</td>
<td>5</td>
<td>37</td>
<td>0</td>
<td>357.4 ± 6.7</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>29 days</td>
<td>6</td>
<td>34</td>
<td>2*</td>
<td>321.7 ± 8.2</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>36 days</td>
<td>8</td>
<td>31</td>
<td>3*</td>
<td>289.5 ± 4.2</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>43 days</td>
<td>12</td>
<td>29</td>
<td>7*</td>
<td>223.8 ± 6.9</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>50 days</td>
<td>12</td>
<td>26</td>
<td>14*</td>
<td>219.3 ± 7.9</td>
<td>3.6 ± 1.4</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>57 days</td>
<td>13</td>
<td>24</td>
<td>23*</td>
<td>221.2 ± 6.4</td>
<td>5.5 ± 1.1</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>64 days</td>
<td>15</td>
<td>21</td>
<td>2 + 12*</td>
<td>198.4 ± 5.4</td>
<td>22.4 ± 2.5</td>
</tr>
<tr>
<td>11</td>
<td>4</td>
<td>71 days</td>
<td>19</td>
<td>18</td>
<td>5 + 46*</td>
<td>156.5 ± 6.6</td>
<td>36.1 ± 1.8</td>
</tr>
<tr>
<td>12</td>
<td>3</td>
<td>78 days</td>
<td>19</td>
<td>14</td>
<td>7 + 40*</td>
<td>167.8 ± 5.1</td>
<td>51.2 ± 3.3</td>
</tr>
<tr>
<td>13</td>
<td>4</td>
<td>85 days</td>
<td>20</td>
<td>11</td>
<td>12 + 32*</td>
<td>142.9 ± 4.6</td>
<td>87.5 ± 6.2</td>
</tr>
<tr>
<td>14</td>
<td>3</td>
<td>92 days</td>
<td>24</td>
<td>7</td>
<td>9 + 36*</td>
<td>156.3 ± 6.5</td>
<td>112.8 ± 5.3</td>
</tr>
<tr>
<td>15</td>
<td>4</td>
<td>99 days</td>
<td>24</td>
<td>4</td>
<td>11 + 28*</td>
<td>134.4 ± 6.1</td>
<td>96.2 ± 4.7</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIa</td>
<td>1</td>
<td>8 days</td>
<td>3</td>
<td>8</td>
<td>78</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CIIa</td>
<td>2</td>
<td>43 days</td>
<td>12</td>
<td>7</td>
<td>59</td>
<td>3.4 ± 1.5</td>
<td>0</td>
</tr>
<tr>
<td>CIIIa</td>
<td>2</td>
<td>64 days</td>
<td>15</td>
<td>5</td>
<td>47</td>
<td>2.4 ± 1.1</td>
<td>0</td>
</tr>
<tr>
<td>CIVa</td>
<td>1</td>
<td>85 days</td>
<td>20</td>
<td>3</td>
<td>38</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CIVb</td>
<td>2</td>
<td>99 days</td>
<td>24</td>
<td>2</td>
<td>26</td>
<td>3.6 ± 1.4</td>
<td>0</td>
</tr>
<tr>
<td>CIVc</td>
<td>2</td>
<td>50 days</td>
<td>12</td>
<td>4</td>
<td>8*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CIVd</td>
<td>2</td>
<td>99 days</td>
<td>24</td>
<td>2</td>
<td>3 + 4*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CIVe</td>
<td>2</td>
<td>64 days</td>
<td>15</td>
<td>4</td>
<td>57</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CIVf</td>
<td>2</td>
<td>92 days</td>
<td>24</td>
<td>2</td>
<td>42</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Suffix a, b, c, and d refer to survival days of the control groups. For Control I groups, birds were perfused on day 8 (CIa), 43 (CIIa), 64 (CIIIa), 85 (CIVa), or 99 (CIVb) after BrdU injections. For Control II groups, birds were perfused on day 50 (CIIa) or 99 (CIVb) after the VMN lesion. For Control III groups, birds were perfused on 64 (CIIIa) or 92 (CIIIb).

* Refers to the units displaying abnormal electrical activity. Numbers of BrdU- and NeuN-positive double-labeled cells are group mean, errors are standard deviation, and for each bird six slides with 250 μm interval between slides were analyzed.
response in 28 sites and an inhibitory response in six sites. Male nest coo evoked an excitatory response in nine sites and an inhibitory response in 12 sites. The remaining 239 sites showed no response. Among the 55 responsive sites, none responded to white noise or reversed versions of nest coos. Recordings from the dorsal area to the VMN did not show any response to acoustic stimulation.

During the short survival period, none of the sites in lesioned birds were responsive to coo stimulation, whereas some sites in Control group I (sham lesion) were responsive as early as 24 h after surgery. It was not until the medium survival period (42–71 days postlesion) that excitatory and inhibitory responses to coo stimulation began to emerge, albeit in abnormal discharge patterns. On day 42 postlesion, 2 of 132 sites showed evoked discharge in response to female nest coo stimulation similar to the abnormal firing pattern shown in Figure 2(D). The number of responsive sites increased with each day postlesion. For example, on day 72 postlesion, 11 of 127 sites responded to coo stimulation, although these evoked responses did not have a normal pattern (Table 2).

During the long survival period (78–99 days postlesion), the evoked electrical response to female nest coo stimulation began to exhibit normal discharge patterns. Of 156 sites recorded, 16 responded to coo stimulation (four inhibitory and 12 excitatory). Six of the excitatory sites responded only to female nest coo stimulation and not to any other acoustic stimulation, including the reversed female nest coo. The ratio of normal to abnormal discharge in response to coo stimulation was reduced to 1:1. On days 92 and 102 postlesion, 14 and eight sites, respectively, exhibited a nest coo evoked response. Six of these 22 sites responded only to female nest coo (Table 2). In contrast to the sham birds [Fig. 4(A)], some lesioned birds showed abnormal “spikes” in evoked discharge [Fig. 4(B)], and some showed normal discharge [Fig. 4(C)]. Notice that the abnormal discharge pattern contained three abnormal spikes, and each spike was followed by intense hyperpolarization [Fig. 4(B)]. The normal discharge pattern of the lesioned birds is indistinguishable from that of the sham birds [Fig. 4: compare (A) and (C)]. Henceforth, we designate the sites that exhibit normal discharge in response to coo stimulation as the coo-responsive units. Interestingly, the coo-responsive units were found only among males housed with a mate (female) after lesion.

Figure 5 shows a typical female nest coo-specific unit. The unit was relatively silent at rest and in response to playback of reversed male coo, reversed female coo, or white noise stimulation, but responded intensely to female nest coo and only mildly to male nest coo. The unit, therefore, selectively responds to female nest coo stimulation. The male nest coo-specific units also exhibited similarly selective responses. Female nest coo-specific units also did not respond to the male bow coo stimulation (not shown), thus cor-

---

**Figure 3** A histogram showing changes in spontaneous discharge at different survival times after VMN lesion. Black bars, sham lesion, displaying firing pattern shown in Figure 2 (A–C); white bars, bilateral lesion, displaying abnormal firing pattern shown in Figure 2 (D–E); gray bars, bilateral lesion, displaying normal firing pattern as in sham lesion.
roborating our earlier findings of the selective nature of the female nest coo-specific units in the VMN (Cheng et al., 1998).

Cytologic Transformation at the Lesioned Area: Necrosis to Addition of BrdU+/NeuN+ Cells

We scored cells as BrdU+ if they had nuclear labeling but no cytoplasmic labeling and as NeuN+ if they had cytoplasmic labeling with only light nuclear labeling. Cells were double-labeled if they had both of these attributes. Figure 6 shows changes in cell types after short and long survival times. The VMN area of the sham birds was filled only with NeuN+/cells [Fig. 6(A)]; in contrast, in the lesioned birds, BrdU+ cells also were prominently present. Newborn cells (BrdU+) initially populated primarily the center and the periphery of the lesion site [Fig. 6(B)]. From days 29–64 postlesion, BrdU+ cells began to infiltrate the semiopaque fluid region, and by 72–78 days, many of the BrdU+ cells in this region were double labeled with NeuN+. The number of BrdU+/NeuN+ cells increased as the survival time increased and it peaked at the 92–98 day range [Fig. 6(C)]. The BrdU+/NeuN+ cells were various shapes as shown in Figure 7. These cells at the lesioned area typically had a large nucleus, a distinct axon process, and dendritic spines [Fig. 7(A–D)]; the BrdU+/NeuN+ cells were absent, however, in the nonlesioned area (the control counting area) and in the sham birds.

The BrdU+/NeuN+ cells at the lesioned area were further analyzed using laser scanning confocal microscopy. The confocal images were collected using the Kalman average method (n/7–9). The z step was set at 1/9262 m or less for the z-series analysis. At least 40 double-labeled cells were identified using z-series analysis. As shown in Figure 8, colabeling of BrdU and NeuN in the VMN area was verified by rotating cells in orthogonal planes. Furthermore, as shown in Figure 9, the BrdU+/NeuN+ cells were not stained for GFAP, suggesting that these cells retained neuronal immunoreactivity distinguishable from that of glia.

Table 2 Characteristics of Experimental Birds, Development of Coo-Responsive Units, and Double-Labeled Cells at the Lesion Site

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Birds</th>
<th>Survival Time after Lesion</th>
<th>BrdU Injs.</th>
<th>Record of Responding to Acoustic Stimulus after Lesion</th>
<th>Number of Birds</th>
<th>BrdU-Labeled Cells</th>
<th>BrdU-NeuN-Labeled Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of Birds</td>
<td>Survival Time after Lesion</td>
<td>BrdU Injs.</td>
<td>Acoustic Unit</td>
<td>BrdU Injs.</td>
<td>Excitatory Unit</td>
<td>Inhibitory Unit</td>
</tr>
<tr>
<td></td>
<td>Number of Birds</td>
<td>Survival Time after Lesion</td>
<td>BrdU Injs.</td>
<td></td>
<td>BrdU Injs.</td>
<td>Excitatory Unit</td>
<td>Inhibitory Unit</td>
</tr>
<tr>
<td>Experiment II</td>
<td>1</td>
<td>4</td>
<td>24 h</td>
<td>2</td>
<td>45</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>12 days</td>
<td>2</td>
<td>41</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4</td>
<td>22 days</td>
<td>4</td>
<td>38</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3</td>
<td>32 days</td>
<td>7</td>
<td>34</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>3</td>
<td>42 days</td>
<td>12</td>
<td>31</td>
<td>2*</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>4</td>
<td>52 days</td>
<td>12</td>
<td>26</td>
<td>4*</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>4</td>
<td>62 days</td>
<td>16</td>
<td>22</td>
<td>1 + 6*</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>4</td>
<td>72 days</td>
<td>17</td>
<td>18</td>
<td>5 + 4* + 1†</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>5</td>
<td>82 days</td>
<td>20</td>
<td>14</td>
<td>6 + 3* + 3†</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>4</td>
<td>92 days</td>
<td>23</td>
<td>9</td>
<td>7 + 4†</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>5</td>
<td>102 days</td>
<td>25</td>
<td>5</td>
<td>5 + 2†</td>
<td>1</td>
</tr>
<tr>
<td>Control</td>
<td>C Ia</td>
<td>1</td>
<td>24 h</td>
<td>2</td>
<td>8</td>
<td>44</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>C Ib</td>
<td>2</td>
<td>32 days</td>
<td>7</td>
<td>7</td>
<td>36</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>C Ic</td>
<td>2</td>
<td>62 days</td>
<td>16</td>
<td>5</td>
<td>28</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>C Id</td>
<td>1</td>
<td>82 days</td>
<td>20</td>
<td>3</td>
<td>25</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>C Ie</td>
<td>2</td>
<td>102 days</td>
<td>25</td>
<td>2</td>
<td>18</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>C Ia</td>
<td>2</td>
<td>50 days</td>
<td>12</td>
<td>4</td>
<td>2*</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>C Iib</td>
<td>2</td>
<td>99 days</td>
<td>24</td>
<td>2</td>
<td>3 + 1* + 1†</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>C IIIa</td>
<td>2</td>
<td>64 days</td>
<td>15</td>
<td>4</td>
<td>24</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>C IIIb</td>
<td>2</td>
<td>92 days</td>
<td>24</td>
<td>2</td>
<td>25</td>
<td>8</td>
</tr>
</tbody>
</table>

Suffix a, b, c, and d are the same as in Table 1.
* Refers to units displaying the abnormal evoked electrical activity.
† Refers to special coo-responsive units.
Figure 4  Response of female nest coo-sensitive units in VMN of female ring doves surviving 92–102 days. (A) Normal spontaneous discharge and evoked discharge in response to female nest coo stimulation in control group (sham lesion). Top, histogram of the unit’s response. The discharge is highlighted. Middle, evoked specific response by female nest coo stimulation. Bottom, computer amplitude display of female nest coo: amplitude over time. (B) Unit 4401-57 (98 days) exhibited normal spontaneous discharge and abnormal discharge evoked by female nest coo stimulation. Top, histogram of the unit’s response. Middle, the unit’s normal spontaneous discharge and evoked response by female nest coo stimulation. Note the three pulses of abnormal discharge. Bottom, computer amplitude display of female nest coo: amplitude over time. (C) Unit 4477-18 (102 days) exhibited excitatory response to female nest coo stimulation. All histograms represent the sum of 10 repetitions of the stimulus. Bin size, 20 ms.
Locations of Nest Coo-Responsive Units and BrdU+/NeuN+ Double-Labeled Cells

We determined the number of double-labeled cells from the center of the lesioned area radiating out to the periphery. For birds surviving 99 days, the number of double-labeled cells at 100, 200, 300, 400, and 500 μm from the center of the lesioned area, was on average 6 ± 2, 18 ± 3, 35 ± 2, 12 ± 1, and 5 ± 2 cells/mm², respectively. Most of the acoustic-responsive units were also located within the periphery 100 to 500 μm from the center, suggesting that they may be colocalized.

To assess whether BrdU+/NeuN+ double-labeled cells colocalized with coo-responsive units and thereby were responsible for the recovery of coo-responsive evoked discharge, we examined the location of the tip of the recording electrodes of coo-responsive units by using PSB dye. In total, there were 108 ± 8.6 cells expressing the BrdU+/NeuN+/PSB phenotype. Figure 10 shows the location of a BrdU+/NeuN+ double-labeled cell that merged with the PSB dye for recording site (A) and another such cell at high magnification (B).

Double-Labeled Cells and Time Course of the Recovery of Coo-Responsive Units

As shown in Figure 11, the appearance of BrdU+/NeuN+ double-labeled cells coincides with the ap-
pearance of coo-responsive units at 2–3 months postlesion (Top panel, white bars; bottom panel, black bars). Closer inspection revealed further that the first appearance of some new neurons was not accompanied by normal electrical activity. For example, the first appearance of normal spontaneous firing occurred on day 64, which was 14 days after the first double-labeled cells were detected (Table 1, #8 vs. #10). Normal discharge in response to coo stimulation was first recorded on day 72, which was 20 days after double-labeled cells were detected (Table 2, #6 vs. #8). Most labeled cells were single-labeled with BrdU (Top panel: black bar), and most recorded electrical activity consisted of abnormal discharge patterns (Bottom panel: white bars). However, as the number of double-labeled cells increased, the number of single-labeled cells decreased. This observation is consistent with the schematic distribution of double-labeled cells and coo-responsive units over various survival times [Fig. 1(B): bottom panel].

**DISCUSSION**

**Electrical Signal after Tissue Damage**

In the present study, electrical activity was used as a diagnostic tool to assess the functional recovery of neural tissue after injury. Specifically, changes in electrophysiologic activity in the VMN of the hypothalamus were monitored during recovery. The recording method took advantage of the unique features of VMN neurons in the adult ring dove. These neurons exhibit selective response to the species-specific coo vocalization of the courtship repertoire (Cheng et al., 1998). We identified these units systematically by starting recordings from a control location above the VMN and continuing through the VMN, which contains these units, to another control location below the VMN. This procedure was used in all experimental groups before and after lesion at regular intervals over 3 months. We detected the following three phases of electrical activity at the lesioned area: a silent phase where there was no detectable electrical activity during the initial 1 month period (the first detectable electrical signals were characterized as abnormal spontaneous firings); an abnormal discharge phase, which consisted of both spontaneous discharge and evoked discharge, the latter in response to acoustic stimulation and having abnormal patterns lacking the normal rapid depolarization and brief hyperpolarization typical of noninjured tissue; and a normal discharge phase where the lesioned area started to regain the electrical properties of control tissues. This phase was detected after 9 weeks of recovery. Moreover, spontaneous firing was detected 1 week prior to detection of coo-responsive units.

The female nest coo-specific units, which have been previously documented in female adult ring doves, are involved in the release of gonadotrophin-releasing hormones that cascade into ovulation (Cheng, 1992; Cheng et al., 1998). Although a similar endocrine effect has not yet been demonstrated in male doves, a role for specific nest coo-responsive units in the male has been suggested by other observations. In a previous study, we showed that a VMN-lesioned male would be unsuccessful in breeding primarily because of the protracted loss of nest coo display, unless housed with a mate (Bernstein et al., 1993). Presumably, this loss of nest coo display after lesion is a result of the loss of nest coo-responsive units in the VMN (present finding), which normally feeds information to the midbrain vocal motor neurons in the DM (the dorsomedial nuclei) via the VMN→DM pathway (Cheng et al., 1987; Chen et al., 2003).

**BrdU+/NeuN+ Double-Labeled Cells at the Lesioned Area**

Cells that were double labeled for BrdU and NeuN had the characteristic morphology of neurons, namely, the presence of a large nucleus, single axon, and multiple dendritic spines (Fig. 7). The staining of BrdU+/NeuN+ cells for GFAP confirmed that BrdU+/NeuN+ cells were newborn neurons distinguishable from glial cells (Fig. 9). To further characterize and analyze the identity of double-labeled cells, we subjected BrdU+/NeuN+ cells (five sections from each of five birds) to confocal z-stack analysis to determine the three-dimensional staining patterns of the cells (Fig. 8). From at least 40 cells confirmed by z-stack analysis, we found that there was lesion-induced addition of new cells and that these cells possessed both morphologic and immunocytochemical characteristics similar to neurons.

**BrdU+/NeuN+ Double-Labeled Cells and Restoration of Electrical Signal**

We have previously shown that a bilateral VMN lesion promotes immediate proliferative activity in the subventricular zone and subsequent migration and addition of new neurons (as identified by 3H-thymidine and Hu or NeuN double labeling) in the adult hypothalamus (Cao et al., 2002). In the present study, we monitored the cellular changes at the lesioned area concurrent with electrophysiologic recordings. Anal-
ysis of the results suggests that adult-generated neurons at the lesioned area give rise to functional recovery of physiologic properties characteristic of the VMN region. The following two lines of evidence support this claim: the coestablishment of adult-generated neurons and coo-responsive units after a similar timeline; and specific colocalization of adult-generated neurons and coo-responsive units.

Further, there is a remarkable parallel between onset of units exhibiting functional electrical activity and the detection of BrdU+/NeuN+ double-labeled cells at the lesioned area. Both reached their highest number near the end of the second month after lesion. Indeed, the correlation coefficient between the number of BrdU+/NeuN+ cells and the number of coo-responsive units (units responding specifically to male nest coo or female nest coo stimulation) is statistically significant ($r = 0.9876$, $p < 0.01$). Interestingly, early in recovery the presence of double-labeled cells (e.g., those detected on day 50) did not immediately correlate with a manifest normal firing pattern; the first recorded normal firing was 2 weeks later. This observation underscores the limitation of NeuN as a neuron-specific marker because it does not provide information regarding the functional status of the neuron. More importantly, colocalization of BrdU+/NeuN+ cells and the marker for coo-responsive units suggest that at least some of the coo-responsive units were recorded from the adult-generated cells. Consistent with this claim is the observation that both BrdU+/NeuN+ cells and coo-responsive units were located within the periphery between 100 and 500 μm from the center of the lesioned area, with the peak at about 300 μm. Henceforth, we designate this region as the recovery zone. Interestingly, massive apoptosis as a result of the VMN lesion was also found in the recovery zone (Cao et al., 2002). Given that apoptosis is thought to trigger neuronal replacement in the HVC of the adult songbird (Scharff et al., 2000) and the neocortex of adult mice (Wang et al., 1998; Magavi et al., 2000), lesion-induced apoptosis in the hypothalamus may be similarly involved in lesion-induced neuronal recruitment in the hypothalamus of the adult ring dove.

The possibility exists that the increase in neuronal cells at the lesioned area may be due to increased labeling of DNA repair, given that the birds in the long survival group also had more BrdU injections. However, this seems unlikely given the steady decline in the number of BrdU-labeled cells. The coupling of the gradual decline of BrdU+ cells on one hand and the gradual increase in BrdU+/NeuN+ cells and coo-responsive units on the other hand (Fig. 11) is best interpreted in light of the idea that as many single-
labeled BrdU cells matured and became double labeled with NeuN, the number of BrdU cells decreased. It is also conceivable that the number of damaged cells in need of repair might have been greater on the lesion periphery where we found the labeled cells and coo-responsive units. Hence, the double-labeled cells might have been repaired neurons on the periphery that moved into the lesioned area during recovery. However, given that the surviving neurons that were labeled by BrdU were dying cells that re-entered the lethal cell cycle (Yang et al., 2001; Rakic, 2002), one would expect the number of double-labeled cells to decrease as survival time increases. Instead, our data show an increase in the number of double-labeled cells. Moreover, even though cells such as macrophages are endowed with mobility, it would be a novel idea to suggest that neuronal cells can move from one region to another region of the brain. On balance, evidence of the lesion-induced addition of new neurons (Cao et al., 2002) reinforces our contention that the double-labeled cells found at the lesioned area in the present study were newly-generated neurons.

In songbirds, maximal neuronal labeling was seen at survival periods shorter than 3 months. This difference between songbirds and ring doves could be a combination of any of the following factors:

1. The distance from the lateral ventricular zone (LVZ) to the vocal control nuclei (HVC) is shorter than the distance from the LVZ to the hypothalamus. The precise distance is difficult to measure without knowing the actual migration path.

2. In contrast to the HVC in songbirds, where the mechanisms of neurogenesis are already in place, lesioned tissue in the adult ring dove hypothalamus must first undergo microenvi-
mental changes from a non-neurogenic to a neurogenic state. This transformation may involve a host of events, such as apoptotic clearance and astrocyte-radial glia transformation, as well as other cellular reactivity that may be related to the absorption of necrotic tissue, necessary for providing a neurogenesis-promoting environment for the migration to occur.

An earlier experiment (Fowler et al., 2002) suggested that social contact promotes cell proliferation in the hypothalamus of adult voles. However, we found negligible amounts of BrdU+ cells, if any, in the intact and sham control groups, which confirms both our previous findings (Ling et al., 1997; Cao et al., 2002) and that of songbirds (Nottebohm, 1985; 1989), that adult hypothalamus does not normally recruit new cells. Nonetheless, these studies suggest that adult neurogenesis is responsive to brain perturbation even in brain regions that are not normally neurogenic.

**Origin of the New Neurons**

As in songbirds (Goldman and Nottebohm, 1983; Alvarez-Buylla and Nottebohm, 1988; Alvarez-Buylla and Kirn, 1997), neurogenesis in the adult ring dove (Ling et al., 1997) originates from the LVZ, although it is remarkably sparse and limited to the telencephalon. The present study was not designed to determine whether the newly formed neurons were generated in the LVZ. In an earlier study, we showed
that VMN lesion was followed by an increase in LVZ neuronal precursor cells and their subsequent migration and differentiation. This activity suggested that addition of new neurons at the lesioned area months later originated from LVZ neuronal precursor cells (Cao et al., 2002). In the current study, detection of most of the double-labeled cells and special coo-responsive units was also made during the same time period, that is, 2–3 months after lesion (Tables 1 and 2), suggesting, therefore, that newly formed neurons at the lesioned area might have migrated from the LVZ.

**Functionality of New Neurons in the Adult Brain**

Are newly generated neurons in the adult brain recruited into the functional circuit? This question was first addressed in songbirds by Paton and Nottebohm (1984), who recorded action potentials from cells in the HVC that were labeled with H-thymidine and had neuronal morphology. In a subsequent study, combining H-thymidine autoradiography and horseradish peroxidase (HRP) retrograde labeling, these authors further confirmed that new neurons in the HVC are local interneurons (Paton et al., 1985). However, recently Scharff et al. (2000) provided evidence suggesting that induced death of a projection neuron (HVC→RA) was compensated for by new projection neurons of the same type. Progress has also been made on the hippocampus. Using hippocampal slice preparation, van Praag et al. (2002) recorded electrophysiologic properties (membrane potential and postsynaptic currents) of 1-month-old new neurons. These observations and the demonstration of synaptic neurotransmitter release and neurotransmitter receptors (Song et al., 2002b), along with connectivity data from newly formed granule cells (Stanfield and Trice, 1988), provide the clearest evidence for the functionality of adult neurogenesis in a self-renewal system.

In the present study, we show that the restoration of local interneurons (Paton et al., 1985). However, recently Scharff et al. (2000) provided evidence suggesting that induced death of a projection neuron (HVC→RA) was compensated for by new projection neurons of the same type. Progress has also been made on the hippocampus. Using hippocampal slice preparation, van Praag et al. (2002) recorded electrophysiologic properties (membrane potential and postsynaptic currents) of 1-month-old new neurons. These observations and the demonstration of synaptic neurotransmitter release and neurotransmitter receptors (Song et al., 2002b), along with connectivity data from newly formed granule cells (Stanfield and Trice, 1988), provide the clearest evidence for the functionality of adult neurogenesis in a self-renewal system.

In the present study, we show that the restoration of local interneurons (Paton et al., 1985). However, recently Scharff et al. (2000) provided evidence suggesting that induced death of a projection neuron (HVC→RA) was compensated for by new projection neurons of the same type. Progress has also been made on the hippocampus. Using hippocampal slice preparation, van Praag et al. (2002) recorded electrophysiologic properties (membrane potential and postsynaptic currents) of 1-month-old new neurons. These observations and the demonstration of synaptic neurotransmitter release and neurotransmitter receptors (Song et al., 2002b), along with connectivity data from newly formed granule cells (Stanfield and Trice, 1988), provide the clearest evidence for the functionality of adult neurogenesis in a self-renewal system.

**REFERENCES**


Functionality of Induced Adult Neurogenesis