Neurogenesis in the Vocalization Pathway of *Xenopus laevis*

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ABSTRACT

We examined possible contributions of neurogenesis to sex differences in the vocalization pathway of the South African clawed frog, *Xenopus laevis*. Birthdates of neurons were obtained from autoradiograms of animals receiving tritiated thymidine from gastrulation through 1 month after metamorphosis. Thymidine availability studies showed that 80% of the [3H]-thymidine injected into embryos and tadpoles was incorporated into the DNA of dividing cells within 3 hours. We observed 3 patterns of neurogenesis: late-short, a short burst of proliferation occurred late in development in the anterior preoptic area, the ventromedial nucleus of the thalamus, and the pretrigeminal nucleus of the dorsal segmental area of the medulla; protracted-bimodal, a prolonged period of proliferation with an early and a late peak in the number of labeled cells occurred in the ventral striatum and in the ventrolateral and posterior nuclei of the thalamus; protracted-unimodal, a prolonged period of proliferation with a single early peak occurred in the inferior reticular formation and in the medial and lateral nuclei IX–X (containing laryngeal motor neurons). There were no differences between sexes in the number of tritiated thymidine labeled cells in any nucleus. The difference in nucleus IX–X neuron number in adults does not appear to result from sex differences in the proliferation of these cells during development. Since neurons in the vocalization pathway do not exhibit androgen receptors until after neurogenesis is complete, we also conclude that androgen probably does not regulate the genesis of these cells.

Key words: cell birthdate, [3H]-thymidine, horseradish peroxidase, nucleus ambiguus, steroid hormone-target neurons

The vocalizations of *Xenopus laevis*, the South African clawed frog, are sexually dimorphic. Only adult males produce the mate call, a sound that attracts sexually receptive females (Russell, '54; Wetzel and Kelley, '83; Pickers, '83). In the adult male, mate calling is under the control of gonadal androgen (Kelley and Pfaff, '76; Wetzel and Kelley, '83). Although dominant females have moderate levels of circulating androgen, they do not give male-typical mate calls nor can they be induced to do so by treatment with exogenous androgen (Hannigan and Kelley, '86). The effector organ for mate calling, the larynx (Ridewood, 1898; Yager, '82; Tobias and Kelley, '85), is sexually dimorphic in that it is larger, has more muscle fibers, and higher levels of androgen receptor in males than in females (Segil et al., '83; Kelley, '86). A neural pathway for vocalization in *X. laevis* has been described (Wetzel et al., '85). The larynx is innervated by motor neurons located in the caudal medulla in the motor nucleus of cranial nerves IX and X (n.IX–X: Kelley, '80). Cells in n.IX–X receive afferent input from the adjacent reticular formation and from nucleus DTAM (pretrigeminal nucleus of the dorsal segmental area of the medulla). Nucleus DTAM is innervated by striatal, preoptic, and thalamic neurons as well as by collaterals of neurons in n.IX–X. These nuclei thus are some of those contributing to the generation of vocalization and are referred to as the "calling circuit.”

A striking sex difference in the calling circuit is seen in n.IX–X, which contains the motor neurons that innervate the glottal and binar muscles (m. dilator laryngis) of the larynx. Adult males have approximately 1,200 n.IX–X neurons, while adult females have about 750 (Hannigan and Kelley, '81). The mean size of neuronal cell bodies is

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larger in males; males have more large neurons and females have more small ones (Hennigan and Kelley, '83; Simpson et al., '86). In addition, the dendrites of male n.IX-X neurons are as much as 2.7 times longer than those of females (Kelley and Fenstermaker, '83). Neuroanatomical tracing studies with horseradish peroxidase (HRP) (Wetzel et al., '85) suggest two differences in connectivity between vocal pathway nuclei of males and females: both the projection from the preoptic area to DTAM and the collateral projection from n.IX-X to DTAM are more robust in males. All of the nuclei in the vocalization pathway contain neurons that concentrate testosterone or its metabolites (Kelley et al., '75; Kelley, '81). We are interested in determining how steroid hormones interact with neural systems during development to produce the structural basis for sexually dimorphic behavior patterns. In particular, we chose to determine the developmental basis for sex differences in the CNS pathway for vocalization. We address three questions: 1) What is the developmental pattern of neurogenesis in the vocalization pathway? 2) Are sex differences in the number of n.IX-X neurons a result of differences in the number of cells produced in males and females? 3) What is the relationship between the timing of neurogenesis and the onset of androgen sensitivity in nuclei of the vocalization pathway? The primary experimental approach in this paper is [3H]thymidine autoradiography (Sidman, '70) used to determine the time of final DNA replication ("birthdates") in neurons located in each of the vocalization pathway nuclei. In addition, we combined HRP histochemistry with thymidine autoradiography to identify the position of larval motor neurons during late stages of tadpole development.

METHODS

Cell birthdates

The ontogeny of neurons in the calling pathway of *X. laevis* was studied during development from gastrulation (stage 11; Nieuwkoop and Faber, '56) through 1 month after metamorphosis. At 22-24°C, developing *X. laevis* reaching stage 11 at about 12 hr after fertilization, stage 20 at about 22 hr, stage 30 at about 1 1/2 days, stage 40 at about 2 3/4 days, stage 50 at about 15 days, stage 60 at about 46 days, and stage 66 (metamorphosis) at about 58 days (Nieuwkoop and Faber, '56). Embryos and tadpoles from stages 11 to 50 were obtained by breeding HCG-injected adults. Stage 51 to 66 (metamorphic climax) tadpoles and 1 week, 2 week, and 1 month postmetamorphic (PM) juveniles were obtained from Nasco Co. (Fl. Atkinson, Wisconsin).

Calibrated microelectrodes (tip diameter = 20-30 μm) were used to inject each animal with a single pulse of [3H]thymidine (New England Nuclear #NET-027, specific activity = 6.7 Ci/mmol). Animals older than stage 25 were anesthetized by hypothermia prior to injection. Embryos (prior to stage 35/36) were manually dejellied before injection and were maintained in Nu-Twitty solution (Rugh, '78) until stage 37/38, at which time they were transferred through dilute Nu-Twitty solution into aged tap water. All postembryonic animals were reared in aged tap water. [3H]thymidine was injected into the yolk plug, gut endoderm, or peritoneal cavity. Stages 11-29 animals received 30-50 nCi of [3H]thymidine, stages 30-40 received 60-100 nCi, stages 41-49 received 90-150 nCi, stages 50-66 received 500 nCi-1 μCi, and PM animals received 1 μCi/g body weight.

Tadpoles injected prior to stage 57 were allowed to survive until stage 62 when the brain has developed an essentially adult morphology. Animals injected at or after stage 57 were allowed to survive for 2 weeks, deeply anesthetized with MS-222 and chilled in crushed ice. Brains were removed, fixed in phosphate buffered (pH = 7.4) 10% formalin for a minimum of 48 hours, and then processed for paraffin histology (10 μm horizontal serial sections). Sections were dipped in Kodak NTB-2 nuclear-track emulsion, exposed for 30 days at 4°C in sealed light-proof boxes containing desiccant, and developed for 3 minutes in D-19 at 16°C. The resulting autoradiograms were counterstained with cresyl violet and examined at 250X under a Zeiss compound microscope for the presence of heavily labeled cells, which were defined as cells having a grain count over the nucleus of at least 10X background. Out of 500 animals processed, approximately 300 had heavily labeled cells. The best 100 of these animals, in terms of good tissue morphology and low background grain level, were chosen for quantification. In 2 to 3 animals injected at each stage, the heavily labeled cells on one side of the brain were counted in each of the brain nuclei in the calling pathway: the ventral striatum, anterior preoptic area, ventromedial, ventrolateral, and posterior nuclei of the thalamus, DTAM, nucleus retilcarius inferior, and n.IX-X. The labeled cells in 3 brain areas not in the calling circuit, the posterior preoptic area, secondary visceral nuclei, and motor nucleus of cranial nerve VII, were also counted. Brain nuclei were identified on the basis of the anatomical descriptions of Kelley ('81), Kelley et al. ('75), Marrell et al. ('75), Nikundiwe and Nieuwenhuys ('83), Neary and Northcutt ('83), Simpson et al. ('86), and Gorlick and Kelley ('86). The mean cell size in each of these areas was obtained by measuring 10 cell nuclei in the center of each nucleus in 5 stage 62 animals. A correction for double counts due to cell splitting was applied by using the Abercrombie method (Abercrombie, '46; Konigsmark, '70).

The sex of PM animals was determined by visual inspection of the gonads in situ with a dissection microscope. The gonads, fat bodies, and suprarenal glands of premetamorphic animals were removed at the time the animals were sacrificed, fixed in 10% buffered formalin, processed for paraffin histology, cut into 10 μm transverse sections, and stained with hematoxylin-eosin. Sex was identified by the morphology of the gonads and the presence of immature spermatozoa or oocytes. The number of heavily labeled cells found in each calling pathway nucleus was compared in males and females at stages 11-14 (n = 3 males, 4 females), 24-28 (n = 6 males, 4 females), 33-37 (n = 3 males, 4 females), 41-45 (n = 4 males, 6 females), and 53-54 (n = 4 males, 4 females) by Mann-Whitney U tests (Siegel, '56). The additional animals counted at these stages to increase sample size were not included in the analysis of cell birthdate data.

[3H]thymidine availability

A critical point affecting interpretation of the autoradiographic birthdate experiments is the length of time that labeled thymidine is available to cells undergoing DNA synthesis. This was determined for 4 different developmental stages using the method of Heathcote and Sargent ('74).

Tadpoles of stages 10/11, 19/20, 29/30, and 40 were injected with a single pulse of [3H]thymidine (New England Nuclear #027Z, specific activity = 80 Ci/mmol). Stages 10/11 and 19/20 animals received 60-100 nCi of [3H]thymi-
dine, stage 29/30 received 90–150 nCi, and stage 40 re-
cived 150–250 nCi. Each injected animal was placed into
an individual container filled with a large volume of Nu-
Twitty solution. At intervals from 5 minutes to 48 hours
after injection, tadpoles were anesthetized by hypothermia
and were homogenized in an equal volume of 100 nl to 1 ml
of 2% SDS. Stages 10/11, 19/20, 29/30, and 40 were pooled
in groups of 11, 10, 9, and 6 respectively; 2 to 3 replicates of
each time interval were prepared. Twenty microliter ali-
quots of homogenate were applied to each of two 1 cm²
squares of 3 MM Whatman filter paper and allowed to air
dry. One filter was counted in PPO-PPOP-toluene scintilla-
tion cocktail to provide a measure of total [³H]thyminide
uptake. The other filter was placed in 5% trichloroacetic
acid (TCA) at 0°C and was gently agitated for 30 min. The
filter was then washed twice in 5% TCA at 0°C, and 3
times each in 100% ethanol and in acetone at room temper-
ature, and dried. The radioactivity in the unwashed filters
was corrected to account for a 5% loss in counts that occurs when labeled DNA is counted in the prescence of tissue homogenates (Hatchwell and Sanger, '84). Duplicates of each sample were prepared and samples were counted for 20 min or until counting error was
less than 2%.

A control for non-DNA specific binding was conducted.
Ten stages 40 tadpoles were homogenized in 100 µl of 2%
SDS; 10 µCi of [³H]thyminide was added and the mixture
was then vortexed. Fifteen microliter aliquots of the
homogenate were applied to each of two 3 MM Whatman filter
papers, and the filters were treated as above to determine
radioactivity bound to [³H]thyminide. Less than 0.2% of the
[³H]thyminide was bound to the filters; thus, the possibility
of non-DNA specific binding was less than 0.2%.

RESULTS

Cell birthdates

Analysis of the cell count data revealed 3 overall patterns
of neuronal birthdates in the calling pathway (see Fig. 2).

1. Neurogenesis in the anterior preoptic area (APOA),
ventromedial nucleus (VM), and pretrigeminal nucleus of
the dorsal tegmental area of the medulla (DTAM) occurred
over a short period late in development (between stages 50
and 62).

2. Neurogenesis in the ventral striatum (VS), and the
ventrolateral (VL) and posterior (P) nuclei of the thalamus
occurred over a protracted period of development from
stage 11/12 to stages 57–65. The frequency of labeled cells
was bimodally distributed with an early peak at stage 11/12
to 20–22, and a later peak at stages 38–40 to 52–58.

3. Neurogenesis in the inferior reticular formation (RI)
and in neurons of cranial nerve IX and X (both medial
and lateral cells, see below) also occurred over a protracted
period of development, but showed a unimodal distribution
of labeled cells.

There were no differences between sexes in the number of
heavily labeled cells in any nucleus at any of the devel-

HRP histochemistry and [³H]thyminide autoradiography

One interesting result of the birthdate experiments was
that although n.IX–X neurons were lightly labeled by [³H]-
thymidine after an injection at any developmental stages,
we never saw heavy label in these cells. However, cells that
appeared to be motor neurons (i.e., large multipolar cell
bodies with larger nuclei and prominent nucleiol located
at the lateral edge of the central gray of the medulla just
medial to n.IX–X neurons) were always heavily labeled in
animals injected with [³H]thyminid between stages 11
and 50. We tentatively identified these heavily labeled cells
as n.IX–X M (= medial), but wanted to know if they indeed
were laryngeal motor neurons. We thus used HRP histo-
chemistry in conjunction with [³H]thyminide autoradio-
graphy to determine whether these medially located labeled
neurons innervated the laryngeal musculature.

Stages 25–30 tadpoles were injected with [³H]thyminide
(specific activity = 6.7 Ci/mmol) as described above. Birth-
date experiments showed that many medial cells are
undergoing final DNA synthesis at this time. These ani-
mals were allowed to develop until stages 61–65. They
were then chilled in crushed ice, and glass microtropettes
were used to inject HRP (0.01% solution of Sigma type I
HRP in 4% DMSO) into 1 binipinnate muscle of the larynx.
The muscle was injected by opening the mouth and inject-
ing HRP into the binipinnate, which is visible just lateral
and caudal to the glottis. Animals were allowed to sur-

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NEUROGENESIS IN XENOPUS

Opisthodermal stages examined (P > 0.05 by Mann-Whitney U test). The pattern of neurogenesis in each brain nucleus will be described in a rostrocaudal sequence. Anatomical descriptions and cell size measurements refer to stage 62 tadpole brains in the horizontal plane of section.

**Ventral striatum.** The ventral striatum (VS) consists of medium sized cells (mean diameter of cell body = 9.5 μm, range = 8–12 μm) that occupy the ventral third of the posterior and posterolateral margins of the paired first ventricles in the telencephalon (Fig. 1). Heavily labeled cells (see Fig. 3) were found in VS between stages 11/12 and 65 (see Fig. 2). The highest numbers of labeled cells were seen between stages 11/12 and 12. A second, slightly lower peak of proliferation occurred between stages 37/38 and 58. All labeled cells were in the lateral 1/4 of VS from stage 11/12 through about stage 45 when labeled cells began to appear more medially. By stage 50, labeled cells occupied the lateral 1/2 of the nucleus. During stage 53/54, labeled cells were seen throughout the mediolateral extent of VS, but were confined to the medial 1/2 of the nucleus from stage 57 to the end of proliferation at stage 66. Whereas labelled medial cells were found throughout the dorsoventral extent of VS, the vast majority of labeled lateral cells were located in the dorsal half of the nucleus.

**Anterior preoptic area.** The anterior preoptic area (APOA) is an arrowhead-shaped nucleus that surrounds the anterior half of the preoptic recess in the anterior diencephalon (Fig. 1). It is comprised of densely packed medium sized cells (mean diameter = 9.1 μm, range = 8–11 μm). Heavily labeled cells (Fig. 3) were seen throughout APOA from stage 51 to stage 65 (Fig. 2), rising in frequency to a peak at stage 58–60 and then falling rapidly to a low level by stage 62.

**Posterior preoptic area.** The posterior preoptic area (PPOA) surrounds the posterior half of the preoptic recess.

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**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
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<tr>
<td>APOA</td>
<td>anterior preoptic area</td>
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<tr>
<td>DTAM</td>
<td>pretectal nucleus of the dorsal tegmental area of the medulla</td>
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<tr>
<td>n.VII</td>
<td>motor neuron of cranial nerve nucleus VII</td>
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<tr>
<td>n.IX-X L</td>
<td>lateral neuron of cranial nerve nucleus IX-X</td>
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<tr>
<td>n.IX-X M</td>
<td>medial neuron of cranial nerve nucleus IX-X</td>
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<tr>
<td>P</td>
<td>posterior nucleus</td>
</tr>
<tr>
<td>PPOA</td>
<td>posterior preoptic area</td>
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<tr>
<td>Ri</td>
<td>nucleus reticularis inferior</td>
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<tr>
<td>VL</td>
<td>ventrolateral nucleus</td>
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<tr>
<td>VM</td>
<td>ventromedial nucleus</td>
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<td>VS</td>
<td>ventral striatum</td>
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<tr>
<td>2°VIS</td>
<td>secondary visceral nucleus</td>
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<tr>
<td>IX-X</td>
<td>fourth root of cranial nerve IX-X</td>
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![Fig 1. Drawings of representative horizontal 10 μm sections through the brain of a stage 62 X. laevis tadpole. Sections are presented from dorsal to ventral, and section A is about midway through the brain. Major cell groups seen after cresyl violet staining are outlined. A. The posterior nucleus (P) of the thalamus and the pretectal nucleus of the dorsal tegmental area of the medulla (DTAM) are in the visualization pathway. The number of [3H]thymidine labeled cells in the secondary visceral nucleus (2°VIS) was counted to provide a comparison with DTAM. B. The ventromedial (VM) and ventral lateral (VL) nuclei of the ventral thalamus are in the visualization pathway. The motor nucleus of cranial nerve VII (n.VII) was compared to n.IX-X. C. The ventral striatum (VS), inferior reticular formation (Ri), lateral neurons of cranial nerve nucleus IX-X (n.IX-X L), and medial neurons of cranial nerve nucleus IX-X (n.IX-X M) are all in the visualization pathway; n.IX-X M neurons are labeled by black dots at the lateral margin of Ri. D. The anterior preoptic area (APOA) is in the visualization pathway. The posterior preoptic area (PPOA) was compared to APOA. Scale bar = 1 mm.](image-url)
It is located just posterior to APOA and is separated from it by a narrow acellular space (Fig. 1). The PPOA has not yet been implicated in the calling pathway and does not contain steroid-hormone concentrating cells. This nucleus was counted to provide a comparison with APOA. Heavily labeled cells were seen in PPOA from stage 51 to stage 65, with a peak in frequency at stage 55 (Fig. 2).

**Ventromedial nucleus of the central thalamus.** The ventromedial nucleus (VM) is medial and adjacent to the third ventricle in the diencephalon (Fig. 1) and extends along the entire anterior-posterior length of the ventral thalamus. It consists of medium sized cells (mean diameter = 9.6 μm, range = 7–12 μm), arranged in a 4-5 tightly clustered laminae, each 3-6 cells wide. Heavily labeled cells occurred throughout VM from stage 50 to stage 60 with a sharp peak in frequency at stage 55 (Fig. 2).

**Ventralateral nucleus of the central thalamus.** The ventrolateral nucleus (VL) is lateral to and posterior to VM (Fig. 1), but consists of 5-7 much less densely packed laminae, each 2-4 cells wide. It is made up of medium sized cells (mean diameter = 9.5 μm, range = 6-12 μm). Heavily labeled cells (Fig. 3) were seen in VL from stage 51 to stage 58 (Fig. 2). Peaks in frequency occurred at stages 11/12 to 20 and at stages 35/36 to 50. Very few labeled cells were seen between stages 58 and 66. Labeling began in the lateral 3 laminae of cells in the posterior half of the nucleus. By stage 20, labeling had spread throughout the medial-lateral aspect of VL, but was still confined to the posterior half of the nucleus. As the second peak of proliferation began at stage 35/36, heavily labeled cells started to appear in the anterior half of VL and increased in frequency until stage 50 when most of the labeled cells were in the anterior half of the nucleus. By stage 53 all labeled cells were found in the anterior half of VL.

**Posterior nucleus of the thalamus.** Wezel et al. (85) used HRP to identify cells projecting to DTAM; some are located in the vicinity of the central nucleus of the thalamus. Identification of thalamic nuclei in horizontal sections can be quite difficult. Examination of their histological material revealed that most of these cells are in the anterior part of the adjacent posterior nucleus (P) of the thalamus. We thus counted heavily labeled cells in P. The posterior nucleus is tear-drop shaped (Fig. 1), dorsal and posterior to VM. It is classified as one of the periventricular nuclei of the dorsal thalamus (Neyy and joyells) with although it extends quite ventrally at its posterior end. The nucleus consists of medium sized cells (mean diameter = 10.4 μm, range = 8-14 μm) that are arranged in 2-3 densely packed distinct laminae medially and 3-4 loosely packed laminae laterally. Heavily labeled cells (Fig. 3) occurred in

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**Fig. 2.** Number of [%] thyroxine labeled neurons in vocalization pathways nuclei of *X. laevis* during development. Abscissa indicates stage of development when tadpoles (stage 11-60) or metamorphosing juveniles (1 week, 2 week, 4 week) received a single pulse injection of [%] thyroxine. Ordinate indicates number of [%] thyroxine labeled cells on one side of the brain. Closed circles are median values for vocalization pathways nuclei. Variations above and below these indicate ranges. Open circles are median values for control nuclei, and ranges are not shown. Each value is the median of counts from 2-3 brains.

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The number of heavily labeled neurons was counted in all nuclei, except for VT-X (Fig. 2) where only receive labeled neurons were counted (see text). Small arrows at stage 50 indicate where small, scattered, histologically competent to produce steroid hormones. Large arrows at stage 60 indicate when these vesicles occur first time lesions in a hypothalam cultured CNS. Neurons in VS and APOA (Fig. 3A, B) do not have hypothalamotelencephalic neurons.
Fig. 3. Autoradiograms of [3H]thymidine labeled cells. Horizontal 10 μm sections are from brains of tadpoles sacrificed at stages 63-64. Neuron cell bodies are counterstained with cresyl violet. Anterior is up. A. APOA in a tadpole injected with [3H]thymidine at stage 57. The optic recess is seen at the extreme right. B. P in a tadpole injected at stage 15. C. VL in a tadpole injected at stage 15. Unlabeled cells in the laminae of VM are seen in the upper right. D. VS in a stage 15 tadpole. The posterior end of the telencephalic first ventricle appears at the upper left. Scale bar = 20 μm and applies to all photomicrographs.
P between stage 10/11 and stage 57 (Fig. 2) with 2 peaks in proliferation, one at stages 11/12 to 20 and another at stages 41 to 55. All labeled cells were located in the posterior lateral half of the nucleus from stage 11/12 until stages 40–42 when anteromedial labeled cells appeared. By stage 53, nearly all of the labeled cells were in the anteromedial part of P, and by stage 55 all of the labeled cells were in the extreme anterior 1/4 of the nucleus.

Pretrigeminal nucleus of the dorsal tegmental area of the medulla. The pretrigeminal nucleus of the dorsal tegmental area (DTAM) is composed of densely packed medium sized cells (mean diameter = 9.4 μm, range = 8–12 μm) located ventral to the cerebellum in the dorsal tegmentum. It is a triangular-shaped nucleus that occupies a position lateral to the cerebral aqueduct, posterior and medial to the nucleus isthmi, and just posterior-medial and adjacent to the secondary viscerotonic nucleus (Fig. 1). Heavily labeled cells were seen in DTAM between stage 49 and stage 55, although fewer labeled cells occurred after stage 57 (Fig. 2). A peak in the number of labeled cells occurred at stage 55. The earliest heavily labeled cells were found in the postero-lateral part of DTAM (stage 49); labeled cells did not appear anteriorly until stage 53. By stage 55, nearly all heavily labeled cells were located in the anterior 1/3 of the nucleus.

Secondary visceral nucleus. The secondary visceral nucleus (2*VIS) is located just anterior and medial to DTAM (Fig. 1). The 2*VIS is a non-steroid-hormone concentrating nucleus that was counted to provide a comparison with DTAM. Heavily labeled cells occurred in 2*VIS between stages 49 and stage 57, with a peak in frequency at stage 54 (Fig. 2), although a few labeled cells were seen from stage 57 to stage 64.

Inferior reticular formation. The inferior reticular formation (RI) is a group of medium sized cells (mean diameter = 9.6 μm, range = 8–12 μm) located in the lateral half of the central gray of the medulla, medial to the column of n.IX–X neurons (Fig. 1). Heavily labeled cells (Fig. 4) were seen throughout RI from stage 11/12 to stage 56 (Fig. 2), although a few labeled cells were seen in 1 week PM and 2 week PM animals. The highest frequency of labeled RI cells occurred between stage 15 and stage 27/28.

Lateral neurons of cranial nerve nucleus IX and X. The motor nucleus of cranial nerve n.IX–X is made up of large cells (mean diameter = 13.4 μm, range = 10–17 μm) that extend posteriorly from the insertion of the fourth root of cranial nerves IX and X at the level of the obex until a point just rostral to the motor columns of the spinal cord (Fig. 1). Most of the cells in this nucleus are located in a long column in the white matter of the caudal medulla. These lateral neurons (n.IX–X L) innervate the glottal and bipinnate muscles of the larynx in adult X. laevis (Kelley, '80; Simpson et al., '80). We never saw heavy labeling in these neurons. However, 1–18 lightly labeled cells (grain counts of 3–5X background, Fig. 4) were seen in virtually

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Fig. 4. Autoradiogram of [3H]-thymidine labeled cells in n.IX–X and RI of a tadpole injected at stage 15 and sacrificed at stage 63. Plane of 10 μm section (2*VIS). A asterisk is to the left and lateral is up. Neuron cell bodies are counterstained with cresyl-violet. Asterisks indicate 3 heavily labeled n.IX–X M neurons located at the edge of the medullary gray. Two lightly labeled n.IX–X L neurons are indicated by large open arrows. We never observed n. IX–X L cells with heavier label. Small closed arrows indicate 2 heavily labeled RI cells. Scale bar = 20 μm.
every animal injected between stage 11/12 and stage 35/36, and then remained low at 1–4 cells per animal until proliferation ceased at stage 62 (Fig. 2).

**Medial neurons of cranial nerve nucleus IX and X.** Heavily labeled, large (mean diameter = 14.0 μm, range = 10–17 μm) cells were always seen in the lateral margin of the medullary gray, medial to n.IX–X L in stage 11/12 to stage 53 animals (Fig. 2). These cells were ovoid or elongate in shape and resembled adult female laryngeal motor neurons in their morphology and medial location (Simpson et al., '86). However, there were many more of them in stage 62 tadpole brains than are seen in adult female X. laevis; they were larger in comparison to surrounding reticular cells and also extended further posteriorly than in adult females. We surmised that these heavily labeled large medial cells might be laryngeal motor neurons. HRP experiments (see below) revealed that motor neurons innervating the larynx were, indeed, located in this area in stage 62 tadpoles.

**Motor neurons of cranial nerve nucleus VII.** The motor nucleus of cranial nerve VII (n.VII) has not yet been implicated in the calling pathway nor does it contain steroid hormone concentrating cells. It is located in the white matter of the medulla, lateral to the central gray, although the most ventral of its cells are within the lateral edge of the gray matter. It is posterior and ventral to the motor nucleus of cranial nerve V and is anterior to the rostral end of n.IX–X L (Fig. 1). Counts of labeled cells in n.VII provided a comparison for data from n.IX–X. Heavily labeled cells were seen in n.VII from stage 11/12 to stage 49, although very few labeled cells occurred after stage 37/38 (Fig. 2). The highest frequency of labeled cells was between stage 13 and stage 20.

**[3H]-thymidine availability**

The percentage of [3H]-thymidine incorporated into DNA increased rapidly from 5–20% 5 minutes after injection to 80% by 1–3 hours after injection (Fig. 5). By 6 hours, essentially all of the [3H]-thymidine was incorporated into DNA in every stage examined (stages 10/11, 19/20, 29/30, and 40).

**HRP histochemistry and [3H]-thymidine autoradiography**

Eight of the 10 animals whose bipinnate muscles were injected with HRP had numerous HRP-labeled cells in both n.IX–X L and n.IX–X M (Fig. 6). One animal had no label in the brain, indicating an unsuccessful injection, and one had labeled cells scattered throughout the brain, indicating that HRP had gained access to the circulatory system. Of the 8 animals with successful HRP injections, 5 produced good autoradiograms with heavy [3H]-thymidine labeled cells and low background levels. Double labeled (HRP + [3H]-thymidine, Fig. 7) n.IX–X M cells were found in 3 of these animals: 2 in a stage 65 male, 1 in a stage 64 female, and 1 in a stage 62 male.

**DISCUSSION**

Temporal resolution of the [3H]-thymidine method

In order to pinpoint the development stage when [3H]-thymidine is incorporated into the DNA of cells undergoing their final mitosis, one must know how long the label remains available in the animal. This is especially important during early Xenopus development, when embryological changes occur quickly and interstage intervals may be as short as 30 min (Nieuwoop and Faber, '56). Vargas-Lizaridi and Lyser ('74) and Lamborghini ('80) used a modified
Schmitt-Tannhauser procedure to assay the kinetics of $[^3]H$-thymidine availability in gastrula (stages 10–12 1/2) and early tailbud (stages 21–26) X. laevis embryos. Vargas-Lizardi and Lyser (’74) reported that the effective labeling period was at least 6 hr in both stages: 75% of $[^3]H$-thymidine was incorporated into the DNA of stage 12/12 1/2 embryos 8 1/2 hr after injection; 6 hr were required for 75% uptake in stages 21–24 embryos. Lamborghini (’80), however, concluded that the effective labeling period was at least 24 hr and appeared to increase with the stage of the animal; whereas 80% uptake occurred in 18 hr in stages 10–12 embryos, 51 hr were required for 80% uptake in stages 22–26 embryos.

We used the method of Heathcote and Sargent (’84) to determine thymidine availability. This method is similar to the Schmitt-Tannhauser procedure in that both techniques depend on the isolation of DNA bound $[^3]H$-thymidine by acid treatment. However, the method used by Heathcote and Sargent (’84) is gentler on tissue since it does not require KOH digestion of tissue homogenates or repeated pelleting and washing as does the Schmitt-Tannhauser procedure (see Vargas-Lizardi and Lyser, ’74). Sequenching of non-DNA bound $[^3]H$-thymidine in pellets protected from trichloroacetic acid washes, or mechanical
and/or chemical separation of $^{3} \text{H}$-thymidine already bound to DNA might account for the different results obtained by Vargas-Lizardi and Lyser ('74) and Lamborghini ('80).

Our results indicate that $^{3} \text{H}$-thymidine is rapidly incorporated into the DNA of dividing X. laevis embryos and tadpoles. An 80% incorporation occurred in 3 hr or less in stages 10/11 and 19/20 embryos, and in about 1 hr in stages 29/30 and 40 tadpoles. Since the interval time between stage 10 and stage 25 ranges from 3/4 to 1 1/2 hr (Nieuwkoop and Faber, '56), our birthdate results during early development are accurate to 2–4 stages. Our confidence level increases to a single stage in animals of stage 26 and older, when interstage intervals are much longer than 1 hr.

Our values agree with those of Heathcote and Sargent ('84), who reported 95% uptake of $^{3} \text{H}$-thymidine in 1 hr for stage 35/36, and less than 2 hr for stage 50 X. laevis tadpoles. Similar values (1–4 hr) have been obtained for mammals using both autoradiographic (Blenkinsopp, '68; Hickey et al., '83) and biochemical (Spector, '80) assays. Our results do not support Lamborghini's ('80) suggestion that the effective labeling period increases with developmental stage in Xenopus. Heathcote and Sargent ('84) hypothesized that the rapid incorporation rates they observed in stage 35/36 and stage 50 tadpoles were due to an efficient circulatory system that can quickly deliver $^{3} \text{H}$-thymidine to and clear it from dividing cells. However, our results indicate that incorporation of $^{3} \text{H}$-thymidine into the DNA of dividing cells is also quite rapid in young embryos that do not yet have a circulatory system. In young embryos, the short distances over which $^{3} \text{H}$-thymidine is required to diffuse coupled to the presence of a greater proportion of dividing cells may compensate for the developmental immaturity of the circulatory system.

The length of the cell cycle relative to the duration of $^{3} \text{H}$-thymidine availability can affect the degree of labeling in dividing cells. A cell whose S-phase is short relative to the period of $^{3} \text{H}$-thymidine availability will incorporate more labeled DNA than will a cell whose S-phase is long relative to the period of availability. The S-phase in X. laevis embryonic cells, at least in endoderm, increases from 1 hr at stage 11 to 3 hr at stage 15 and to 5 hr at stage 18 (Graham and Morgan, '66). Thus, numbers of heavily labeled cells seen during the earliest stages of our study (stages 11–15) may be slightly inflated as a result of a short cell-cycle time. However, at all stages after stage 15, the number of heavily labeled cells observed is probably reduced since the cell cycle is long compared to the length of time that $^{3} \text{H}$-thymidine is available. At stage 57/58, S-phase in X. laevis retina is about 13 hr long (Beach and Jacobson, '79).

Patterns of neurogenesis in the vocalization pathway

We observed 3 patterns of neurogenesis in nuclei of the vocalization pathway (see Fig. 2): late-short, a short burst of proliferation late in development in APOA, VM, and DTAM; protracted-bimodal, a prolonged period of proliferation with two peaks (early and late) in the number of labeled cells in VS, VL, and P; protracted-unimodal, a prolonged period of proliferation with a single early peak in Ri, n.IX–X, and n.IX–X. L. Kelley and colleagues have shown that all vocalization nuclei contain steroid-hormone concentrating neurons (Kelley et al., '75; Morrell et al., '75; Kelley, '80, '81). However, the 3 patterns of neurogenesis we observed in these nuclei do not correlate with the particular hormone(s) (testosterone, dihydrotestosterone, estradiol) that each nucleus concentrates: n.IX–X, DTAM, and Ri concentrate androgens; APOA and the ventral thalamic nuclei concentrate androgens and estradiol; and VS concentrates only estradiol.

Not all cells in vocalization nuclei have steroid hormone receptors. Although large numbers of hormone concentrating cells have been reported for VS, APOA, DTAM, and n.IX–X L in adult X. laevis (Kelley et al., '75; Morrell et al., '75; Kelley, '81), only in n.IX–X L do a majority of the neurons appear to concentrate androgens (Kelley et al., '75; Kelley, '81). Our neurogenesis data thus includes birthdates of nonhormone-sensitive neurons as well as of those that do have steroid hormone receptors. It is impossible to identify proliferating cells that subsequently become hormone concentrating neurons with autoradiographic methods since $^{3} \text{H}$-thymidine and $^{3} \text{H}$-steroids both accumulate in cell nuclei. However, it should be possible to double-label such cells by combining $^{3} \text{H}$-thymidine autoradiography with immunocytochemistry, utilizing antibodies to steroid hormone receptors; antibodies to the androgen receptor, however, are not yet available.

Not all neurons in vocalization nuclei are involved in the control of vocal behavior. This is especially clear in the complex thalamus, which has extensive afferent and efferent connections with a large number of different brain areas (Neary and Northcutt, '83), and in the reticular formation of the medulla through which pass a large number of ascending and descending fibers (Nikundiwe and Nieuwenhuys, '83). It is thus possible that the prorated neurogenesis we observe in Ri and in the thalamus reflects overlapping periods of proliferation in different functional subsets of neurons. However, this is probably not the case in n.IX–X L, since neurons in this nucleus appear to be directly and exclusively involved in the production of vocal behavior (Simpson et al., '86; Kelley, '86).

Both short and protracted patterns of neurogenesis have been reported for other brain nuclei in X. laevis. Mauthner's neurons are born during an extremely short period early in development (stages 10–12 1/2, Vargas-Lizardi and Lyser, '74; Rohon-Beard cells are also proliferate as early as stage 10 1/2, but continue being born through stage 27 (Lamborghini, '80). The duration of neurogenesis in Rohon-Beard cells in similar to that seen in APOA, VM, and DTAM, but occurs earlier in development. Protracted neurogenesis in X. laevis (stages 29–50 cells observed is probably reduced since the cell cycle is long compared to the length of time that $^{3} \text{H}$-thymidine is available. At stage 57/58, S-phase in X. laevis retina is about 13 hr long (Beach and Jacobson, '79).
that we see in APOA, VM, and DTAM. The protracted neurogenesis that we observe during development in the other X. laevis vocalization nuclei has not been reported for steroid-hormone concentrating areas in other vertebrates. However, an extremely protracted capacity for neurogenesis that extends through adulthood has been reported in HVC, a hormone-concentrating forebrain nucleus in canaries that is involved in song production (Goldman and Nottebohm, '83; Paton and Nottebohm, '84; Paton et al., '85).

Jacobson and Gorski ('81) found that neurons in the SDN-POA were born as late as day 18 of gestation, while neurons in adjacent nonsexually dimorphic regions of the preptic area had completed neurogenesis by day 16. In addition, the adult volume of the SDN-POA has been shown to be influenced by the steroid hormone milieu during early postnatal life (Gorski et al., '78; Jacobson et al., '81). These data suggested that neurons in steroid-hormone sensitive CNS areas might be born later than neurons in adjacent nonhormone sensitive areas. We examined neurogenesis in 3 brain nuclei (POPOA, 2°VIS, and n.VII) that are neither steroid-hormone concentrating nor in the vocalization pathway, but are located adjacent to vocalization nuclei (APOA, DTAM, and n.IX-X L, respectively). The duration of neurogenesis was identical in vocalization and control nuclei in 2 cases, APOA vs POPOA and DTAM vs 2°VIS, although peak proliferation did occur slightly earlier in the nonhormone-concentrating control nuclei (POPOA and 2°VIS, see Fig. 2). In contrast, neurogenesis clearly peaked earlier and ended sooner in n.VII than it did in n.IX-X L. The hypothesis of later neurogenesis in hormone sensitive neurons thus appears to gain support in X. laevis predominantly from n.IX-X L, the vocalization nucleus with the largest proportion of steroid-hormone (androgen) concentrating neurons.

Cranial nerve nucleus IX-X contains motor neurons that innervate the vocal effector muscles, the glottis and bippinale muscles of the larynx (Kelley, '80; Simpson et al., '86). It is in these neurons that we found our most surprising results. We never saw heavy labeling in these neurons. However, lightly labeled cells were seen in almost every animal injected between stage 11/12 and stage 62. To our knowledge, this pattern of neurogenesis has not previously been reported. Such light labeling might occur if, through out development, these cells have a very long S-phase relative to the pulse length of available [3H]-thymidine.

**Is sexual dimorphism in adult n.IX-X neuron number a result of differences in cell proliferation?**

One striking sexual dimorphism in X. laevis vocalization is that adult males have approximately 1,200 n.IX-X neurons, while adult females have only about 750 (Hannigan and Kelley, '81). Three possible developmental mechanisms could account for this difference: sex differences in cell proliferation, cell death, or cell differentiation. We tested the hypothesis that cell proliferation is sexually dimorphic by comparing the number of n.IX-X neurons born in males and females at stages 11-14, 24-28, 33-37, 41-45, and 53-54. There were no significant differences between sexes at any developmental stage in the number of [3H]-thymidine labeled neurons in n.IX-X L or in any of the other vocalization pathway nuclei. Therefore, the adult differences in the number of n.IX-X neurons do not appear to result from sex differences in the numbers of these cells produced during development. The same situation occurs in the genesis of neurons in the sexually dimorphic spinal nucleus of the bullocavernous (SNB) in rats. The SNB motor neurons, which innervate the bullocavernous and levator ani muscles in male but not in female rats, are only one-third as numerous in adult females as in adult males (Breedlove and Arnold, '80). This sex difference is not a result of sex differences in the proliferation of motor neurons during development (Breedlove et al., '83). Thus, adult sex differences in cell number occur only in both X. laevis n.IX-X and rat SNB must be a result of a postmitotic process such as cell death or cell differentiation.

**Relationship between the timing of neurogenesis and the onset of androgen sensitivity**

In adult X. laevis, most of the brain nuclei that effect vocalization contain androgen-concentrating neurons. Many cells in DTAM, most of the neurons in n.IX-X, and cells in the thalamus, reticular formation, and preoptic area are labeled after [3H]-testosterone or [3H]-dihydrotestosterone administration (Kelley et al., '75; Kelley, '80, '81). Steroid hormone exposure during development is responsible for establishing sexual dimorphism in neural structures of rats (Breedlove and Arnold, '80). In birds (Gurney et al., '80). In X. laevis, androgen appears to be critical for masculine differentiation of the larynx during postemorphic development (Sassoon et al., '86; Kelley, '86). It is thus reasonable to ask whether androgens might influence the development of sexual dimorphism in the CNS. In order to evaluate the possible role of steroid hormones in the development of sexual dimorphism, it is useful to consider the relationship between neurogenesis and 2 relevant developmental benchmarks: the beginning of hormone production, and the onset of androgen-concentrating capability in vocalization nuclei. Although the levels of circulating hormone in X. laevis tadpoles are not known, the histological appearance of the developing gonads indicates that both testes and ovaries have differentiated by stage 89 (Nieuwkoop and Faber, '66). This is the stage when neurogenesis ends or is nearly over in all of the vocalization pathway nuclei (see Fig. 2) and suggests that cell proliferation ends before hormone secretion begins. The timing of the second benchmark supports this hypothesis. Androgens are presumed to act on target cells through mechanisms that involve their selective accumulation via intracellular receptor proteins (McEwen et al., '79). We used steroid hormone autoradiography to determine the onset of androgen (dihydrotestosterone) sensitivity in neurons in the vocalization nuclei (Gerlick and Kelley, '86). The adult labeling pattern does not appear until stages 62-64 (see Fig. 2), after neurogenesis is completed. It is unlikely that circulating hormone could affect cells that had not yet developed androgen receptors. We conclude that androgen does not influence the genesis of neurons in the vocalization pathway of X. laevis directly.

**Possible role of cell death and migration of n.IX-X M neurons**

We have presented evidence that the dimorphism in n.IX-X neuron number does not arise through sex differences in cell proliferation during development. This dimorphism must, then, be a result of the postmitotic processes of cell death and/or differentiation. Our data suggest sex differences in adult n.IX-X number could arise through a combination of both processes acting on n.IX-X M cells.

Ontogenetic cell death, a process in which more neurons are born than will survive, is a normally occurring devel-
neurogenesis in Xenopus

A developmental mechanism for the establishment of appropriate numbers of neurons in the CNS (Oppenheim, '81; Cunningham, '82; Hamburger and Oppenheim, '82). The survival of developing motor neurons is dependent on the presence of their target musculature (Hamburger and Levi-Montalcini, '49; Hamburger, '75). Recent work on the development of the larynx (Sassoon et al., '86) suggests that an expanding muscle-fiber pool provides an appropriate substrate for increased motor neuron survival in males. The larynx is a monomorphonon and feminine at metamorphosis; males and females both have about 4,000 muscle fibers (Sassoon et al., '85). Where no increase in fiber number occurs in females, males show rapid rates of muscle fiber addition (about 100 fibers/day) beginning at metamorphosis and continuing until the adult number of 32,000 fibers is attained at about a year (Sassoon et al., '85; Kelley, '86). Masculine differentiation of the larynx results from androgen-induced myogenesis (Sassoon et al., '86). This myogenesis provides an expanding periphery that could rescue laryngeal motor neurons in male, but not in female, frogs.

Cell death could occur in the n.IX-X L population of motor neurons. However, we believe that the medial n.IX- X M cells are more likely candidates. Retrograde labeling following HRP injection of the larynx (see Fig. 6) showed that many n.IX-X M neurons innervate the bipinnate muscles in both sexes as early as stage 62 (and perhaps beginning as early as stage 56, Avitalle and Gorlich, unpublished data). Few medial laryngeal motor neurons are present in adults (Simpson et al., '86). Possibly n.IX-X M cells are saved from death by the larger pool of laryngeal muscle fibers that develops in postmetamorphic males, and these neurons then migrate to the lateral column of n.IX-X L neurons. Lucifer yellow (Simpson et al., '86) and Golgi (Kelley and Fenstemaker, '83) studies have shown that the dendrites of adult n.IX-X L neurons are directed medially, toward the reticular formation, where n.IX-X M cells are found during development. The dendritic trees of laryngeal neurons are much longer in males than in females (Kelley and Fenstemaker, '83). The simplest mechanism for the proposed migration of n.IX-X M neurons is passive lateral movement resulting from increased growth of dendrites in males; these cells are literally “pushed” to a lateral position. Dendritic growth could be triggered by a retrograde synaptic influence (see Breedlove, '86) arising from maintained contact with laryngeal muscle fibers. However, given that steroid hormones can promote neurite outgrowth (To- ran-Allerand, '76, '84), androgen could also act directly on n.IX-X M cells to increase dendrite growth in males. Androgens can apparently regulate the length of dendrites of SNB motor neurons in adult rats (Kurt et al., '86).

Recent work suggests that cell death and migration are also involved in establishing the sexually dimorphic SNB nucleus of rats. Five days before birth, the number of SNB motor neurons is small, and this number increases in both sexes through the day before birth (Nordeen et al., '84). If HRP is injected into the perineal muscles of developing rats during this increase, labeled motor neurons are seen outside the SNB (Sengelaub and Arnold, '86). These cells are not found after birth, and Sengelaub and Arnold ('86) suggest that they are in the process of migrating into the SNB from the lateral motor column. Ontogenetic cell death occurs in the SNB during the first 10 postnatal days (Nordeen et al., '85), and androgen is critical in determining SNB motor neuron number (Breedlove and Arnold, '83a,b; Breedlove, '84; Sengelaub et al., '85). Androgen apparently acts to maintain perineal muscles in males that atrophy in females, thus providing a larger muscle target that rescues male SNB motor neurons from death (see Breedlove, '86 for review). Our findings in Xenopus and the data available from the rat SNB system suggest a general model (Fig. 8) for the development of hormone-sensitive, sexually dimorphic neumuscular systems. Androgen acts at the periphery to provide a larger amount of target (i.e., more muscle fibers) in males than in females; androgen increases cell proliferation in Xenopus bipinnate muscle and prevents muscle atrophy in the rat bulbocavernous and levator ani. The net effect of both processes is to greatly reduce the amount of ontogenetic cell death of the motor neurons that innervate these muscles in males. The cells that are spared could be recruited through migration from an ectopic pool of motor neurons that establishes connections with target musculature early in development. While the model presented here is consistent with available data, a number of

PROLIFERATION

ANDROGEN

LARYNX MUSCLE

nIX-X M SURVIVAL

DENDRITE OUTGROWTH

MIGRATION

MORE nIX-X L

Fig. 8. Hypothetical model for the development of sexual dimorphism in the number of laryngeal neurons in X. laevis. After neurogenic proliferation of n.IX-X motor neurons is completed, androgen secreted by the developing testes of male postmetamorphic juveniles stimulates the production of more muscle fibers in the androgen-sensitive bipinnate muscle of the larynx. The increasing muscle target size in males reduces ontogenetic cell death of n.IX-X M cells. The surviving n.IX-X M motor neurons then respond to high circulating androgen levels in male juveniles by elaborating dendrites. Such dendritic growth could cause migration of n.IX-X M cells to a more lateral position and account for the larger number of n.IX-X L neurons found in adult male X. laevis. Cautions to this hypothetical scenario are discussed in the text.
critical questions remain unanswered. For example, we do not yet know when cell death occurs in X.larci, or whether and when motor neurons are overproduced. Sexual dimorphism in laryngeal muscle fiber number does not exist at metamorphosis, but [1] is apparent by 3 months after metamorphosis, and masculinization of the larynx appears to be largely established during the first 6 months of postmetamorphic development (Sassoon et al., '85; Kellet, '86). Thus, these processes most likely occur during the first 6 months after metamorphosis. We also have not yet seen n.IX-X M cells in the process of migration. However, verification of this process should be possible by retrograde labeling with HRP during postmetamorphic development. If the Xenopus system is like that proposed for the rat SNB, migration should precede the period of otogenic cell death. However, there is no a priori reason to dismiss the possibility that motoneurons migrate during cell death and that only surviving motoneurons migrate.

We have already discussed the surprising observation that, while many n.IX-X M neurons were heavily labeled by [3H]-thymidine, only lightly labeled cells were seen in n.IX-X L. We may be able to exploit this situation to identify n.IX-X M cells that have migrated. If the migration hypothesis is correct, then heavily labeled cells should be seen in the lateral column of laryngeal motor neurons in animals injected with [3H]-thymidine early in development (at stages 11-30, when n.IX-X M proliferation is greatest) and sacrificed at 3 months or more after metamorphosis, when differences in laryngeal muscle fiber number are first apparent (Kelley, '86). Many more neurons should be labeled in males than in females. One caveat must be considered. Although we have suggested that light [3H]-thymidine labeling in n.IX-X L neurons could result from an especially long S-phase during neurogenesis, another explanation is possible. The existence of polyplody neurons in the CNS of developing mammals has been debated for nearly 20 years (Herman and Lapham, '66, '69; Fujita, '74; Schwartz and Bhatnagar, '81), although polyplody in human gial cells has been accepted (Mann and Yates, '73; Bohm et al., '81). Xenopus laevis has 36 chromosomes, but chromosome number stages from 20 to 108 in the genus Xenopus (Thiebau and Fischberg, '77). If n.IX-X M cells reduce ploidy by dividing without replication in conjunction with their lateral migration, a small amount of [3H]-thymidine in all n.IX-X L neurons would result. This, of course, implies that all of the lateral neurons arise from the migration of n.IX-X M cells, and, if so, would require modification of parts of our hypothetical scenario.

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LITERATURE CITED


NEUROGENESIS IN XENOPUS


