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CLONAL ORGANIZATION OF THE CENTRAL NERVOUS SYSTEM OF THE FROG

II. Clones Stemming from Individual Blastomeres of the 32- and 64-Cell Stages[1]

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Abstract

Horseradish peroxidase injected into individual blastomeres of 32- and 64-cell embryos of *Xenopus laevis* was identified in cells of the central nervous system (CNS) at larval stages 31 to 39. The CNS received contributions from 24 blastomeres of the 32-cell stage and 38 blastomeres of the 64-cell stage. The region of CNS in which all of the labeled descendants of a single blastomere were dispersed is called a clonal domain. Mingling of labeled and unlabeled cells always occurred in a clonal domain, but boundaries were seen between such a labeled region and completely unlabeled regions. These boundaries occurred at various places in the CNS but were most frequently seen in the transverse plane at the level of the isthmus between mesencephalon and rhombencephalon and in the horizontal plane between dorsal and ventral regions of the CNS. When the maternal clonal domain was partitioned between the descendants of the daughter cells, the partitioning occurred only at one or both of those boundaries. The constant relationship between the position of each of the initially labeled blastomeres and the final spatial distribution of the labeled descendants in the CNS provided a detailed fate map of the main regions of the CNS in the 32- and 64-cell embryo.

In an earlier publication (Hirose and Jacobson, 1979), we showed that horseradish peroxidase (HRP) injected into individual blastomeres in a series of embryos of *Xenopus* at the 2- to 16-cell stages was conserved in the descendants of the injected blastomeres and those labeled descendants could be seen in the central nervous system at later stages of development when characteristic components of the adult CNS were recognizable and many types of labeled nerve cells could be identified. We observed that labeled cells occupied characteristic regions of the CNS correlated with the position, size, and shape of the initially labeled blastomere. The region of the CNS in which all of the labeled descendants of a single blastomere were dispersed was termed a clonal domain.

In this publication, we have extended the clonal analysis to the 32- and 64-cell embryonic stages. Our main aim was to determine the spatial distribution in the CNS of the cells that descended from individual blastomeres of the 32- and 64-cell stages, that is, to map their clonal domains in the well developed central nervous system. In our previous study (Hirose and Jacobson, 1979), clonal analysis was done on embryos selected for their regular and symmetrical pattern of cleavage. In this study, we have also selected such embryos at 32- and 64-cell stages in order to be able to identify a particular blastomere in a series of embryos and to compare the spatial distribution of clones arising from blastomeres of the 8- and 16-cell stages with those arising from blastomeres of the 32- and 64-cell stages.

Materials and Methods

HRP injection. Xenopus laevis embryos were obtained from matings induced by injecting chorionic gonadotropin. Embryos were selected with well marked pigment gradients in the animal-vegetal and dorsal-ventral axes and embryos with regular and symmetrical patterns of cleavage were selected for injection at 32- and 64-cell stages. The percentage of such embryos varied between matings from 0 to more than 50%. For ease of description, we have designated the injected blastomeres at 32- and 64-cell stages as shown in Figure 1 and defined in Hirose and Jacobson (1979). An intracellular injection of horseradish peroxidase (HRP) solution (type IX; Sigma; 20% in Steinberg solution, 1 to 2 nl) was given by pressure lasting a few seconds into a selected blastomere by means of a glass micropipette with a tip diameter of about 5 μm.

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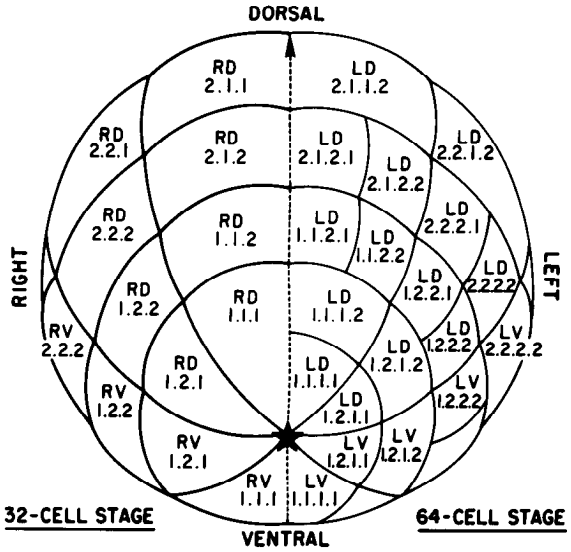

Figure 1. Blastomere positions and designations at 32-cell stage (*right side* of embryo) and 64-cell stage (*left side* of embryo). The embryo is shown in anterior (animal) dorsal view; the *star* is at the animal pole, the *arrow* is in the dorsal midline pointing at the vegetal pole, which is not visible. Diameter of the embryo is about 1.3 mm.

In order to avoid the passage of HRP from the injected cell to its sister cell, the injection was made only after cleavage had been fully completed and shortly before the onset of the succeeding cleavage. The position, shape, and size of the injected blastomere was recorded photographically or by accurate drawing. In several cases, the position of the injected blastomere and its descendants for three or four generations was monitored by videotape recording for several hours from the time of injection until the 512- or 1000-cell blastula stage. Fifteen embryos were allowed to develop after the injection for 6 to 12 hr at 22°C until they reached stages 10 to 12½ of Nieuwkoop and Faber (1967) and then were fixed, sectioned, and processed as described below in order to determine the amount of dispersal of labeled cells from the initial site of injection and the amount of mingling of labeled and unlabeled cells. Another 210 injected animals were killed 30 to 50 hr after injection when they had reached larval stages 31 to 39 of Nieuwkoop and Faber (1967) and then they were processed as described below.

Histochemistry. Embryos were fixed for 6 hr at 4°C in 0.5% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, and washed twice in sucrose solution (5% for 6 hr and 15% for 2 hr, both in 0.1 M phosphate buffer, pH 7.4, at 4°C). Frozen sections were cut at 24 μm, mounted on subbed slides, and soaked for 15 min in 0.1 M phosphate buffer, pH 7.4, containing 12.5 mg of diaminobenzidine tetrahydrochloride/100 ml of solution, after which 0.3 ml of 0.3% hydrogen peroxide was added and the reaction was allowed to proceed for 5 to 10 min to form the brown reaction product which will be referred to as the "label" (Graham and Karnofsky, 1966).

Topological analysis. Although all embryos were studied histologically, in only 52 cases were camera lucida drawings made of the spatial distribution of all labeled cells in a complete series of sections through the CNS. These drawings were used for making three-dimensional reconstructions and for mapping the distribution of labeled cells by the graphical reconstruction procedure termed topological analysis (Nieuwenhuys, 1974; Opdam et al., 1976). In that method, the distance on the ventricular surface was measured from ventral midline to dorsal midline on each side in each section through the CNS, the distances of each labeled cell from the ventral midline of the brain and spinal cord were measured accurately by means of a Numonics graphics calculator, and the labeled cells then were projected onto the ventricular surface. When these data were plotted, the ventricular surface was depicted as it would appear if the CNS had been slit open longitudinally at the dorsal midline and opened out like a book with the ventral midline acting as the hinge. Labeled cells were depicted as if projected onto the plane of the opened-out ventricular surface. Each labeled cell thus was projected to a position at or close to that from which it had originated in the ventricular germinal zone.

Control experiments. These were the same as detailed in Jacobson and Hirose (1978) and Hirose and Jacobson (1979).

Results

Controls and their results were the same as reported earlier (Jacobson and Hirose, 1978; Hirose and Jacobson, 1979).

Videotape recording of the injected blastomere and its descendants showed that the daughter cells cohered so that, at the 512- and 1000-cell blastula stages, all the descendants that were visible at the surface remained at the place formerly occupied by the initially injected blastomere. This is consistent with the earlier report that coherent cell proliferation occurs during cleavage in the blastula of the axolotl (Hara, 1977). However, when the embryos which had been labeled at 32- or 64-cell stages were killed at gastrula stages 6 to 12 hr after the injection, the labeled cells were found to have dispersed from the injection site and to have mingled with unlabeled cells (M. Jacobson and K. Liu, manuscript in preparation). Slight mingling and dispersal was seen in the presumptive ectodermal layer in embryos killed at the initial gastrula stage 9, about 6 hr after injection. The amount of cell mingling and dispersal increased progressively during gastrulation and at stage 12½, when the ecto-, meso-, and endodermal layers were clearly delimited, labeled cells dispersed among unlabeled cells were observed in all three germ layers.

Of 224 embryos injected with HRP and allowed to develop beyond gastrulation, 14 died before reaching stage 31, another 21 developed normally but failed to process properly, and the remaining 189 embryos, all of which appeared normal at the time of fixation, were successfully processed for histological examination of the distribution of labeled cells. In all specimens killed at larval stages 31 to 39, labeled cells were seen in various tissues outside the CNS, but only the pattern of CNS labeling will be described here.

Most labeled cells were at early stages of development and one could not say whether they would later differ-

entiate as neurons or as glial cells. However, by stage 31, the Rohon-Beard cells were easily seen (Figs. 5 and 6) and by stage 39, the Mauthner cells were easily recognizable. In many such cases, the label extended into the neurites from the cell body and labeled axons could be traced for long distances. Only the positions of labeled cell bodies and not their long processes were taken into account in mapping the configurations of clonal domains. The distribution of labeled cell bodies in the CNS was recorded in relation to well known and easily recognizable anatomical landmarks of the embryonic CNS, such as cranial nerve nuclei and other cell masses as well as ventricular grooves and other features of the ventricular system, especially the floor plate and sulcus limitans.

Clonal domains stemming from blastomeres of the 32-cell stage

In this section, each clonal domain is designated according to the blastomere from which it had arisen (Fig. 1). However, as no significant differences were found between the clonal domains on the left and right sides, the designation L or R is omitted in the following account of the results. The number of successfully processed cases is given in parentheses following the blastomere designation. Only those blastomeres that contributed descendants to the CNS are considered. In all cases, the clonal domains contained mixtures of labeled and unlabeled cells.

D1.1.1 (n = 20). The clonal domain extended longitudinally from the lamina terminalis to the caudal end of spinal cord (Figs. 2*A* and 14). Its rostral portion extended bilaterally across the ventral midline and included the lamina terminalis, ventral telencephalon, ventral diencephalon, and ventral mesencephalon. The majority of labeled cells were located in the ventral parts of the telencephalon and diencephalon extending across the ventral midline to both sides of the brain. The ventral halves of both olfactory placodes were well labeled in all cases. The caudal portion of the clonal domain was entirely on the side of the injection and included the ventral half of the rhombencephalon and regions of the spinal cord ventral to the sulcus limitans. In most cases, the sulcus limitans formed a dorsal boundary to the clonal domain. Labeled cell bodies were located in the ventral parts of both retinae. All cellular layers of the retina contained labeled cells. Other types of clearly identifiable structures in this clonal domain were neurons in the motor nuclei of cranial nerves III, IV, VI, VII, IX, and X and motoneurons in the ventral and ventrolateral regions of the spinal cord.

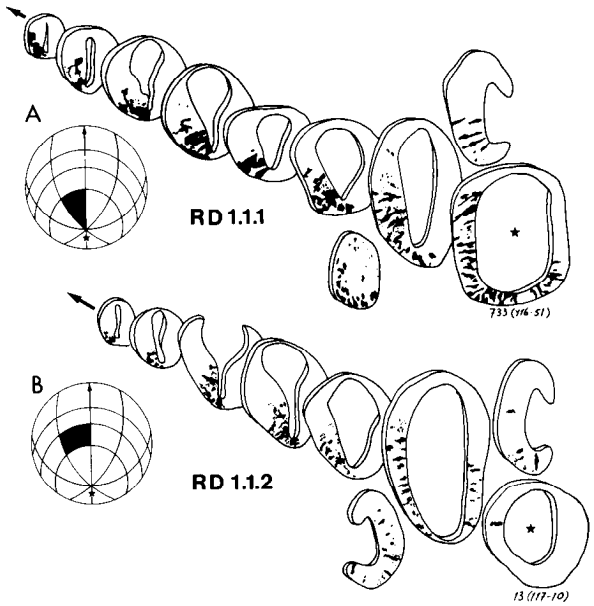

Figure 2. Serial section reconstructions made from camera lucida drawings of every 10th section through the CNS of *Xenopus* larvae at stage 39. Each specimen had received an injection of HRP into a single blastomere at the 32-cell stage as shown in diagrams of the embryos. *A*, Blastomere RD 1.1.1; *B*, blastomere RD 1.1.2. Labeled cells are shown in *black*, unlabeled regions in *white*. The *star* is at the animal pole of the embryo and the rostral pole of the CNS. The *arrow* is in the dorsal midline pointing at the vegetal pole of the embryo and at the caudal pole of the CNS.

D1.1.2 (n = 9). This is similar to D1.1.1 except that in six cases of D1.1.2, labeling was absent or sparse in the telencephalon and diencephalon and the olfactory placode was not labeled. The clonal domain had a portion extending bilaterally in the ventral regions of telencephalon, diencephalon, and mesencephalon in three cases and in all cases, a caudal portion of the clonal domain was confined ipsilaterally to the regions of rhombencephalon and spinal cord ventral to the sulcus limitans (Figs. 2*B* and 14). Some cells in the floor plate were labeled. The ventral parts of both retinae were labeled. The types of CNS cells labeled in D1.1.2 were the same as those labeled in D1.1.1.

D1.2.1 (n = 10). The entire clonal domain was on the injected side and was confined to the dorsal regions of the CNS extending from the lamina terminalis to the caudal end of the spinal cord (Figs. 3, 4*A*, and 14). The dorsal half of the olfactory placode and the dorsal region of the ipsilateral retina were labeled in all cases. In the diencephalon, the label was confined to the ipsilateral epithalamus and even in the epiphysis, the cells were labeled on the ipsilateral side only. Labeling occurred in the mesencephalic tectum, rhombic lip, and in cranial nuclei V, VII, and VIII. Some cells were labeled in the ganglia of cranial nerves V and VIII. The dorsal half of the otic vesicle was labeled in five cases. The majority of labeled cells were in the telencephalon, diencephalon, and mesencephalon.

D1.2.2 (n = 7). Labeled cells were mainly confined to the dorsal half of the CNS extending from the telencephalon, where labeling was sparse, to the spinal cord (Figs. 4*B* and 14). In addition, some labeled cells were found in the ventral mesencephalon in two cases and in the ventral diencephalon and ventral mesencephalon in two cases. The majority of labeled cells were in the dorsal parts of the mesencephalon and rhombencephalon including some cells in principal nucleus V and nucleus VIII. In the spinal cord, the label occupied the dorsolateral region and included some cells whose axons, filled with label, could be seen projecting across the ventral commissure to the lateral spinal funiculi on the opposite side.

D2.2.1 (n = 3). A few widely scattered labeled cells

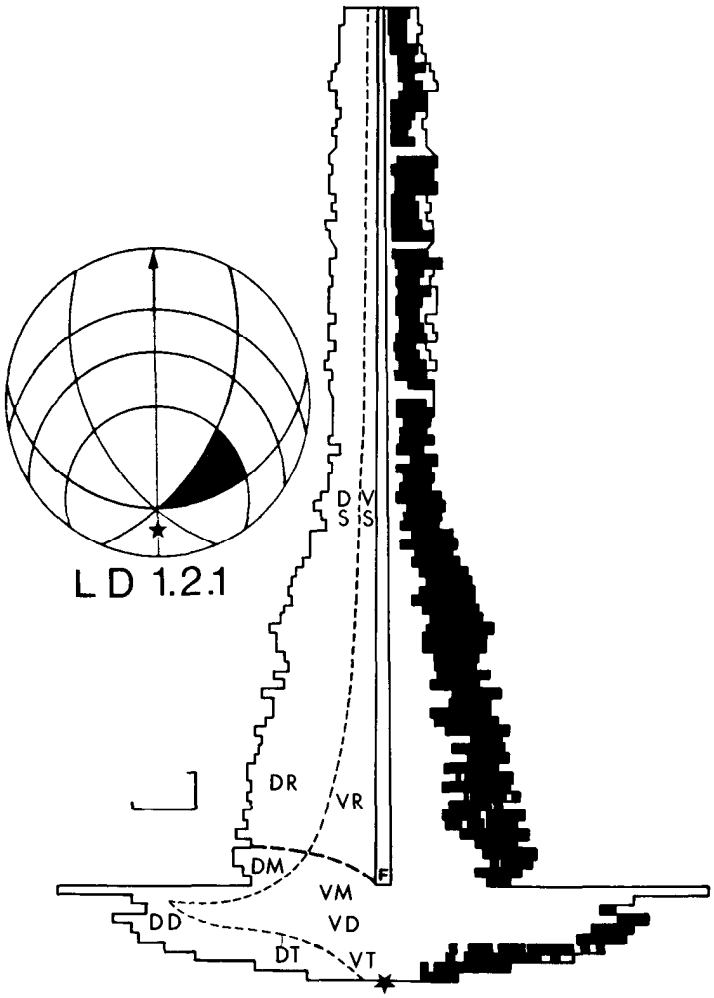

Figure 3.[4] Reconstruction of the CNS of *Xenopus* at stage 39 that had received an injection into blastomere LD 1.2.1 at the 32-cell stage. The labeled regions (*black*) and unlabeled regions (*white*) are shown in 104 consecutive serial sections extending from the lamina terminalis (*star*) to near the caudal end of the spinal cord. The CNS is shown as if opened along the dorsal midline and folded out with the ventral midline forming the axis of symmetry. The *light dashed line* shows the approximate division between dorsal and ventral regions of the CNS. The *heavy dashed line* shows the approximate position of the boundary between mesencephalon and rhombencephalon. *Scale* = 100 μm; note difference of scale in rostrocaudal and in dorsoventral axes.

were located in the spinal cord on both sides of the sulcus limitans (Fig. 15).

D2.2.2 (n = 9). The entire clonal domain was located in the rhombencephalon and spinal cord (Fig. 15). The labeled cells were located throughout the rhombencephalon and spinal cord, both dorsal and ventral to the sulcus limitans. A few labeled cells were seen in ganglia of cranial nerves VIII, IX, and X and in the nucleus of fasciculus solitarius in some specimens. Mauthner's neuron was labeled on the injected side in seven cases and was present but unlabeled in two cases.

D2.1.2 (n = 8). There was very sparse labeling of a few cells in the ventral and ventrolateral spinal cord, including the floor plate, in four specimens (Fig. 15), but no labeling of the CNS was found in four specimens.

D2.1.1 (n = 5). Very few cells were labeled in the ventral spinal cord and floor plate in three specimens (Fig. 15). No labeling was seen in the CNS in two specimens.

V1.1.1 (n = 5). No labeling in the CNS occurred in three cases. There was sparse labeling in the olfactory placode in all cases. In two cases, a few labeled cells, including Rohon-Beard cells, were scattered along the entire length of the spinal cord and some cells were labeled in the dorsal spinal ganglia.

V1.2.1 (n = 9). Labeling was ipsilateral and entirely caudal to the isthmus and was confined to the dorsal regions of the rhombencephalon and the entire length of the spinal cord in seven specimens (Figs. 5 and 6*A*). In two specimens, there were a few labeled cells in the dorsal parts of the telencephalon, diencephalon, and mesencephalon on the injected side only (Fig. 16). In all cases, there were considerable numbers of labeled cells in the spinal dorsal root ganglia on the injected side. The otic vesicle on the injected side was labeled throughout. Some extramedullary neurons were labeled and located outside the spinal cord and dorsal to it. Most labeled extramedullary neurons were ipsilateral, but a few were

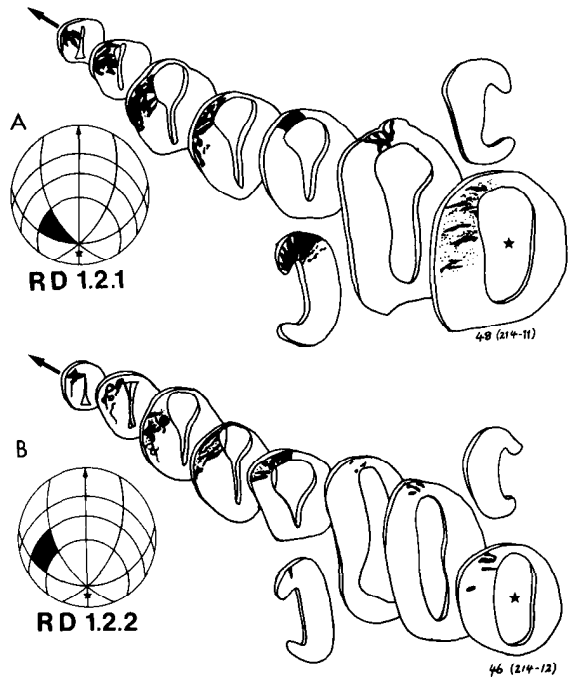

Figure 4. Serial section reconstructions made from camera lucida drawings of every 10th section through the CNS of *Xenopus* larvae at stage 34. Each specimen had received an injection of HRP at the 32-cell stage. *A*, Blastomere RD 1.2.1; *B*, blastomere RD 1.2.2. Labeled cells are shown in *black*, unlabeled regions in *white*. Other conventions as in Figure 2.

[4] The abbreviations used on the figures are: D, diencephalon; DD, dorsal diencephalon; DM, dorsal mesencephalon; DR, dorsal rhombencephalon; DS, dorsal spinal cord; DT, dorsal telencephalon; F, floor plate; M, mesencephalon; R, rhombencephalon; S, spinal cord; T, telencephalon; VD, ventral diencephalon; VM, ventral mesencephalon; VR, ventral rhombencephalon; VS, ventral spinal cord; VT, ventral telencephalon.

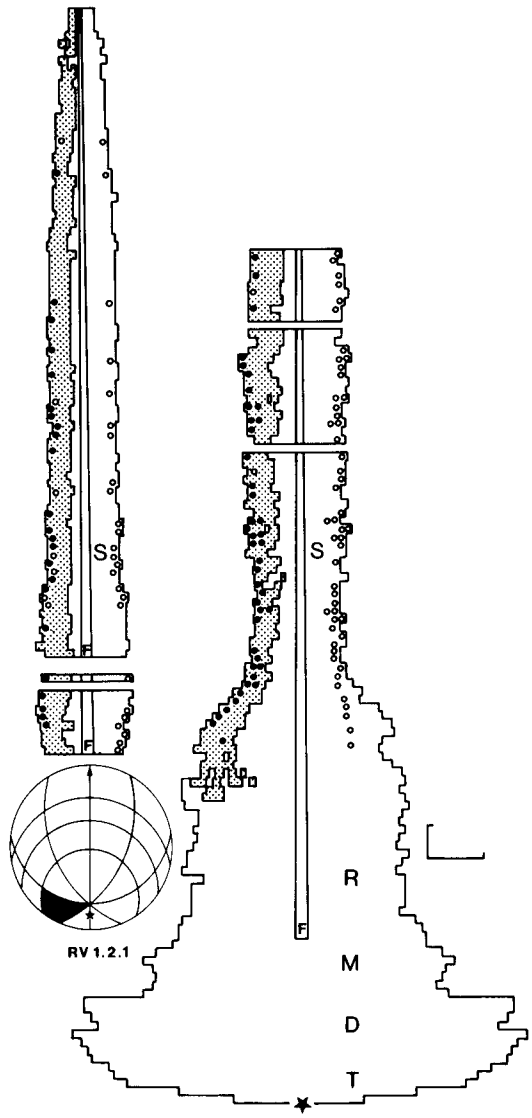

Figure 5. Reconstruction of the entire CNS of a *Xenopus* larva at stage 39 that had received an injection of HRP into blastomere RV 1.2.1 at the 32-cell stage. The CNS is shown as if opened along the dorsal midline and folded out with the ventral midline forming the axis of symmetry. Labeled regions are *stippled*; unlabeled regions are *white*. Each *black dot* indicates the position of a labeled Rohon-Beard cell. Each *white dot* shows the position of an unlabeled Rohon-Beard cell. The continuity of the reconstruction is broken by the absence of six sections, and to fit the entire CNS in the diagram, the caudal end of the spinal cord is shown separately on the *left*. The *star* is at the rostral pole of the CNS and at the animal pole on the diagram of the 32-cell embryo. *Scale* = 100 μm.

labeled on the opposite side. Labeled Rohon-Beard cells were present on the injected side only and were mingled with unlabeled Rohon-Beard cells, apparently at random, along the length of the spinal cord (Fig. 5). Well labeled neurons in the dorsolateral rhombencephalon and spinal cord were frequently seen extending their labeled axons across the ventral commissure to enter the ventrolateral funiculus on the opposite side (Fig. 6*A*).

V1.2.2 (n = 9). The clonal domain was coextensive in rhombencephalon and spinal cord with that of V1.2.1 (Fig. 6*B*) and the same types of cells were labeled in both. However, the otic vesicle was either lightly labeled or not labeled in V1.2.2. Labeled cells were found in the dorsal mesencephalon in two cases.

V2.2.2 (n = 3). Very few labeled cells were seen. They were scattered in the dorsal part of the spinal cord on the injected side and included a few labeled Rohon-Beard cells.

Clonal domains stemming from blastomeres of the 64-cell stage

D1.1.1.1 (n = 8). All of the labeled descendants of D1.1.1.1 were located entirely rostral to the isthmus with a well marked boundary between the mesencephalon and rhombencephalon (Figs. 7, 8, and 14). Labeled cell bodies were located on both sides of the ventral midline in the ventral regions of the mesencephalon including the mesencephalic peduncle, the ventral diencephalon including hypothalamus, and ventral thalamus, and labeled cells were seen in the ventral parts of the telencephalon including ventral regions of both olfactory bulbs. Label extended from ventral diencephalon into optic stalks and ventral parts of optic vesicles bilaterally in specimens at stage 32. By stage 39, the label was seen in the ventral half to two-thirds of both retinae. Cells were labeled in all three cellular layers of the retina.

D1.1.1.2 (n = 8). The clonal domain extended from the telencephalon to the caudal end of the spinal cord (Fig. 14). Label in the CNS was coextensive with that of D1.1.1.1 bilaterally in the ventral parts of the telencephalon, diencephalon, mesencephalon, and retinae. The olfactory bulbs were not labeled. In the rhombencephalon and spinal cord, the labeled cell bodies were confined to regions ventral to the sulcus limitans including many motor neurons in the somatic motor column.

D1.1.2.1 (n = 2). Sparse labeling was seen in the ventral regions of the rhombencephalon and spinal cord on the injected side (Figs. 9 and 14). The floor plate was well labeled. The notochord was lightly labeled.

D1.1.2.2 (n = 3). Labeled cells were scattered throughout the ventral regions of the CNS from the lamina terminalis to the caudal regions of the spinal cord (Fig. 14). Most labeled cells were in the mesencephalon and isthmus. The ventral part of the retina was labeled on the injected side (Fig. 9).

D1.2.1.1 (n = 3). Labeled cells were confined to the dorsal regions of the telencephalon, diencephalon, and mesencephalon, dorsal tip of the retina, and the olfactory placode on the injected side only (Fig. 10). The caudal boundary of the clonal domain was at the junction between mesencephalon and rhombencephalon (Fig. 14).

D1.2.1.2 (n = 3). The clonal domain extended from the telencephalon to the caudal spinal cord, with the majority of labeled cells in the telencephalon, diencephalon, and mesencephalon (Fig. 14). The entire clonal domain was ipsilateral and in the dorsal half of the CNS. A few labeled cells located in the dorsal part of the ipsilateral otic vesicle.

D1.2.2.1 (n = 4). Labeled cells were scattered in the dorsal telencephalon and throughout dorsal and ventral parts of the diencephalon and mesencephalon. Some labeled cells were seen in the dorsal and ventral rhomb-

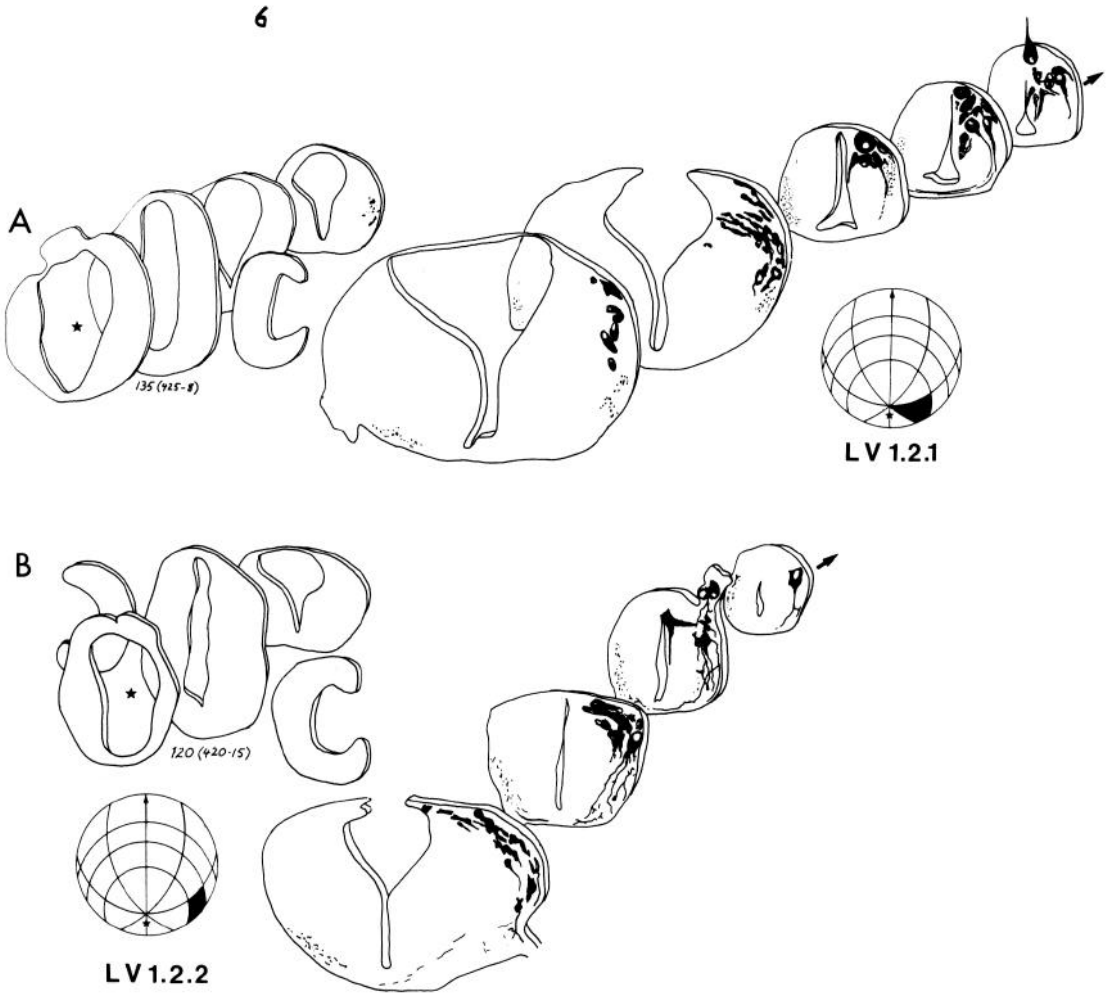

Figure 6. Serial section reconstructions made from camera lucida drawings of every 10th section through the CNS of *Xenopus* larvae at stage 39. The sections of the telencephalon, diencephalon, and mesencephalon are drawn to half the scale of the sections of rhombencephalon and spinal cord. Labeled cells are shown in *black*, unlabeled regions in *white*. Other conventions as in Figure 2. Figure 7*A* shows the labeled cells in the CNS at stage 39 after injection of blastomere LV 1.2.1 at the 32-cell stage. Figure 7*B* shows the labeled cells in the CNS at stage 39 after injection of blastomere LV 1.2.2 at the 32-cell stage.

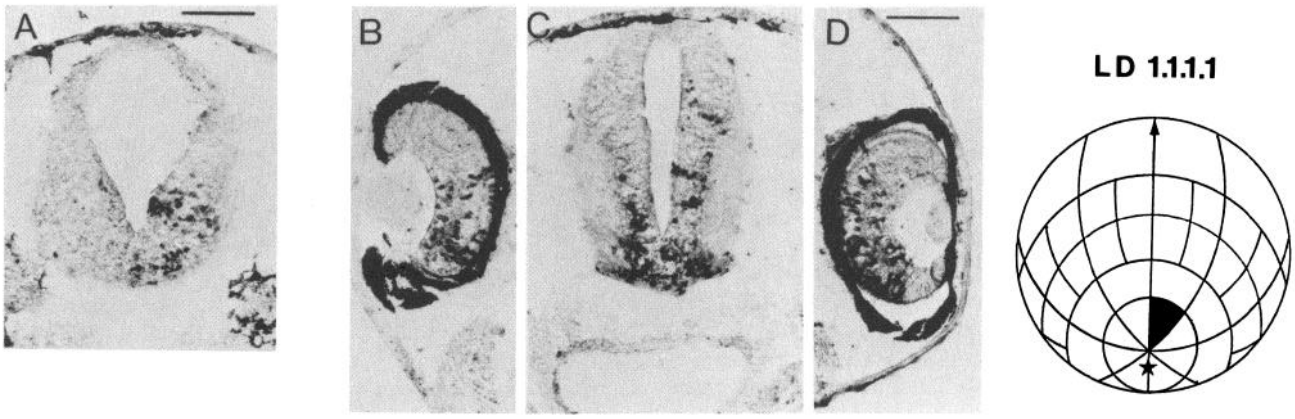

Figure 7. Photographs of sections through the telencephalon (*A*), right retina (*B*), diencephalon (*C*), and left retina (*D*) of larval *Xenopus* at stage 37 that had received an injection of HRP into blastomere LD 1.1.1.1 at the 64-cell stage. Each *bar* is 100 μm.

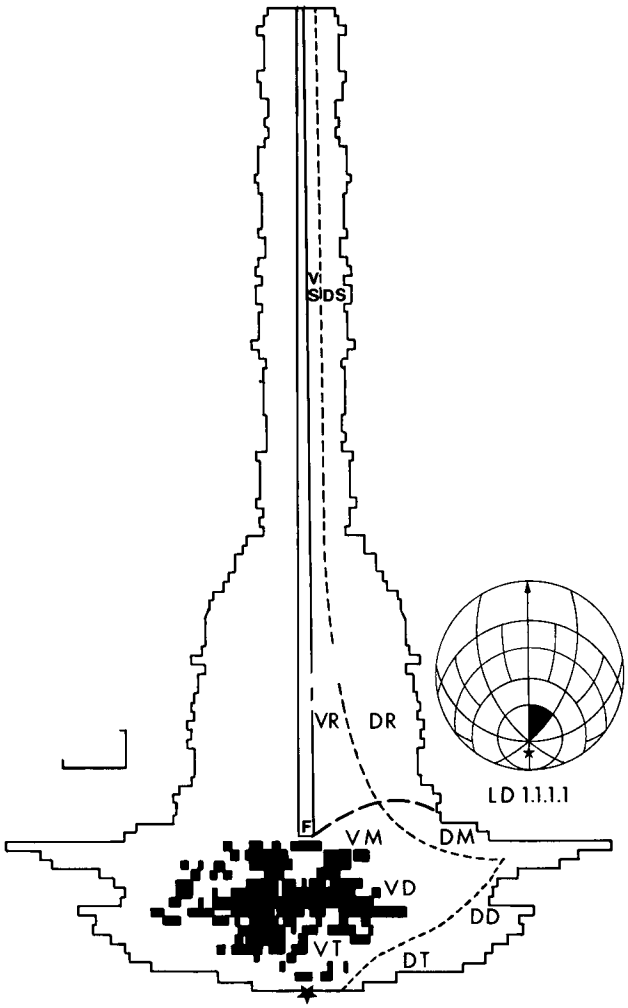

Figure 8. Reconstruction of the CNS of larval *Xenopus* at stage 37 that had received an injection of HRP into blastomere LD 1.1.1.1 at the 64-cell stage. *Black regions* are labeled, *white regions* unlabeled. The conventions are the same as in Figure 3.

encephalon in two cases. A few cells near the dorsal edge of the ipsilateral retina were labeled.

D1.2.2.2 (n = 3). Labeled cells were confined to the dorsal regions of diencephalon, mesencephalon, rhombencephalon, and spinal cord (Figs. 11 and 14). Some cells were labeled in cranial nerve ganglia V and VII and in cranial nerve nuclei V, VII, and VIII.

D2.1.1.1 (n = 3). No labeling was seen in the CNS.

D2.1.1.2 (n = 3). Very few labeled cells were located ipsilaterally in the ventral part of the spinal cord and in the floor plate (Fig. 15).

D2.1.2.1 (n = 3). Sparse labeling of cells was observed in the ventral spinal cord and floor plate ipsilaterally (Fig. 15).

D2.1.2.2 (n = 3). Labeled cells were restricted to ventral regions of spinal cord and rhombencephalon ipsilaterally (Fig. 15).

D2.2.1.2 (n = 2). Very sparse labeling was seen in dorsal and ventral regions of spinal cord ipsilaterally (Fig. 15).

D2.2.2.1 (n = 10). Labeled cells were scattered widely, dorsal and ventral to the sulcus limitans in the rhombencephalon and spinal cord on the injected side (Fig. 15). Mauthner's neuron was labeled in three specimens on the injected side, was unlabeled in five cases, and could not be found in two cases.

D2.2.2.2 (n = 11). This was similar to D2.2.2.1, but most labeled cells were dorsal to the sulcus limitans. Mauthner's neuron was not labeled in nine cases and could not be identified in two cases. Some labeled cells were seen in ganglia of cranial nerves VIII, IX, and X on the injected side.

V1.1.1.1 (n = 4). No labeling was seen in the CNS in two specimens. In the others, there were sparsely scattered labeled cells in the dorsal part of the spinal cord on the injected side, including some labeled Rohon-Beard cells.

V1.2.1.1 (n = 6). Labeled cells in the CNS were found in the rhombencephalon and spinal cord on the injected side and all labeled cells were located dorsal to the sulcus limitans in all cases and were restricted to those regions in four cases. However, in two cases, there were also a few labeled cells in the dorsal regions of the mesencephalon, diencephalon, and telencephalon on the injected side (Fig. 16). In all specimens, a large percentage of Rohon-Beard cells were labeled and those were distributed along the length of the spinal cord, interspersed with unlabeled Rohon-Beard cells and with other types of labeled and unlabeled cells in the dorsal part of the spinal cord.

V1.2.1.2 (n = 6). V1.2.1.2 was similar to V1.2.1.1, except for labeling of otic vesicle in this case. All labeled cells were caudal to the isthmus. The distribution of labeled Rohon-Beard cells was similar to that seen in V1.2.1.1.

V1.2.2.2 (n = 4). Labeled cells in the CNS were found only in the dorsal part of the rhombencephalon and spinal cord in three cases, but some cells were labeled in the dorsal mesencephalon in one case. (Figs. 12 and 16). There were some labeled cells in cranial nuclei VIII, IX, and X and a small percentage of Rohon-Beard cells were labeled.

V2.2.2.2 (n = 3). A few labeled cells were seen in the dorsal part of the spinal cord, scattered along its entire length and mingled with unlabeled cells. A few Rohon-Beard cells were labeled.

Discussion

Contributions to the CNS were made by descendants of 24 blastomeres of the 32-cell embryo and 38 blastomeres of the 64-cell embryo (Figs. 1 and 13).

Clones that arose from sister cells of the 64-cell stage mingled in the clonal domain of their maternal cell of the 32-cell stage (Figs. 14 to 16). However, the clonal domains of sister cells were not always completely congruent or equal in extent. In some cases, only one daughter cell contributed descendants to the CNS. Thus, D2.2.1.2, D2.1.1.2, and V1.2.2.2 each contributed descendants to the CNS, but their respective sister cells D2.2.1.1, D2.1.1.1, and V1.2.2.1 did not (Figs. 15 and 16). An example of inequality of the clonal domains of two sister cells is shown in Figures 9 and 14 where the sister cells LD 1.1.2.1 and LD 1.1.2.2 shared a clonal domain located

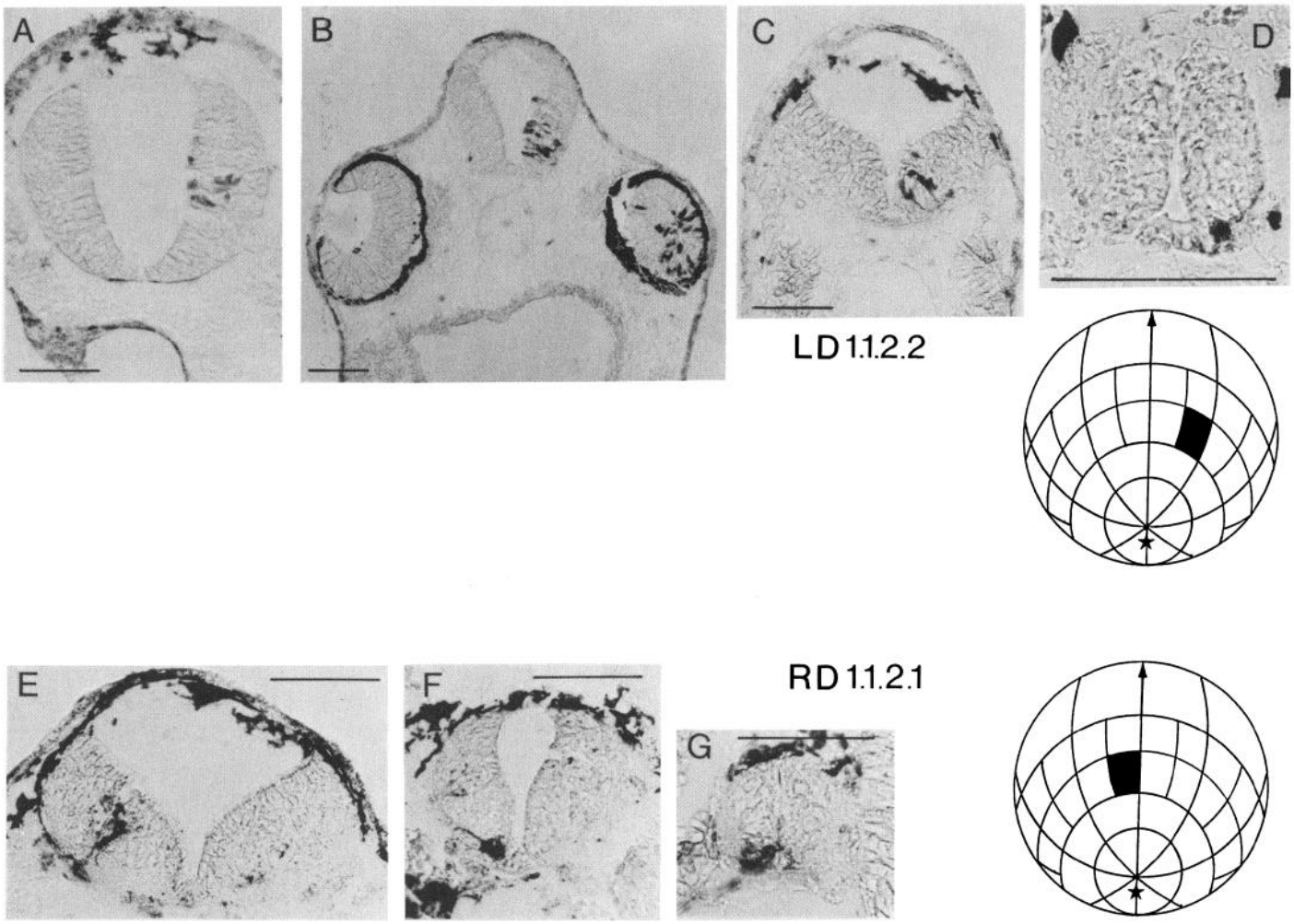

Figure 9. Photographs of serial sections through the CNS of two larval *Xenopus* at stage 34 that had each received an injection of HRP into a single blastomere at the 64-cell stage. In one embryo, the injection was given to blastomere LD 1.1.2.2 and sections are shown through the telencephalon (*A*), mesencephalon and retinae (*B*), rhombencephalon (*C*), and spinal cord (*D*) to show the labeled descendants. In the other embryo, the injection into RD 1.1.2.1 resulted in labeled descendants in the rhombencephalon (*E* and *F*) and spinal cord (*G*), but there was no labeling in the telencephalon, diencephalon, mesencephalon, or retinae. The *bars* are 100 μm.

in the ventral parts of the rhombencephalon and spinal cord, but only LD 1.1.2.2 had part of its clonal domain in the brain rostral to the rhombencephalon. Similar partition of the maternal clonal domain between the descendants of the daughter cells was seen in the cases of D1.1.1.1 and D1.1.1.2 (Fig. 14) as well as partition of D1.2.1 clonal domain between D1.2.1.1 and D1.2.1.2 (Fig. 14). The descendants of a blastomere at the 64-cell stage thus respected boundaries that were not respected by all the descendants of their maternal blastomere of the 32-cell embryo. Such boundaries were observed at two positions in the CNS: in the transverse plane at the level of the isthmus between the mesencephalon and rhombencephalon and in the horizontal plane at the level of the sulcus limitans between the dorsal and ventral parts of the CNS. The transverse boundary between the mesencephalon and rhombencephalon is illustrated in Figures 8 and 10. The horizontal boundary between the dorsal and ventral regions of the CNS is shown in Figures 10 to 12.

The following 26 clones arising from blastomeres of the 64-cell stage were entirely on one or the other side of a boundary in the transverse (coronal) plane in the isthmus region between the mesencephalon and rhombencephalon (Figs. 14 to 16). A or P shows whether the clonal domain was restricted to the anterior (rostral) or posterior (caudal) side of the isthmus: on the right side RD 1.1.1.1 (A); RD 1.1.2.1 (P); RD 1.2.1.1 (A); RD 1.2.2.1 (A, in some cases); RD 2.1.1.2 (P); RD 2.2.1.2 (P); RD 2.2.2.1 (P); RD 2.2.2.2 (P); RV 1.1.1.1 (P); RV 1.2.1.1 (P); RV 1.2.1.2 (P); RV 1.2.2.2 (P); RV 2.2.2.2 (P); and the clones descended from corresponding blastomeres on the left side. By contrast, the following 10 blastomeres of the 64-cell stage gave rise to clones that were widely distributed across the A/P boundary described above: on the right side, RD 1.1.1.2; RD 1.1.2.2; RD 1.2.1.2; RD 1.2.2.1 (some cases); RD 1.2.2.2; and clones descended from corresponding blastomeres on the left side.

All clones descended from blastomeres of the 64-cell stage, except LD/RD 1.2.2.1, 2.2.1.2, 2.2.2.1, and 2.2.2.2,

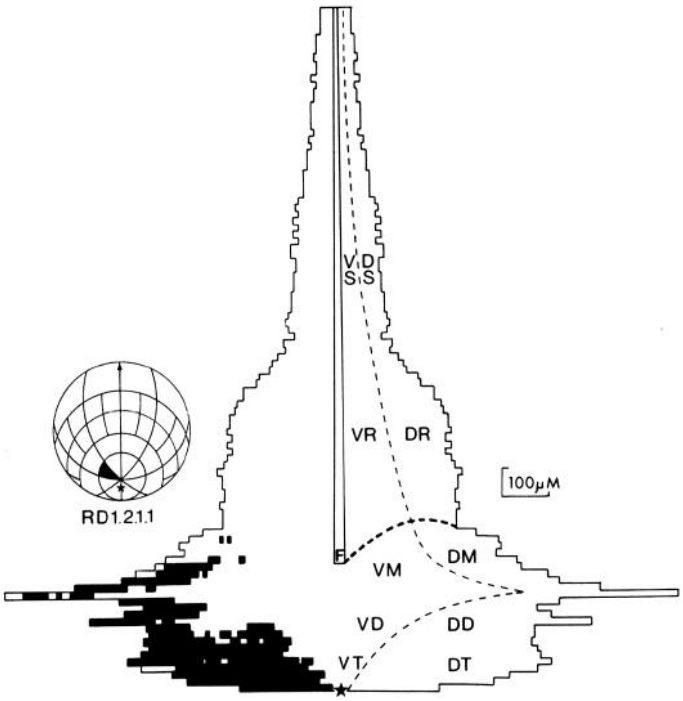

Figure 10. Reconstruction of the CNS of a larval *Xenopus* at stage 39 that had received an injection of HRP into blastomere RD 1.2.1.1 at the 64-cell stage. Labeled cells are shown in *black*; unlabeled regions are *white*. The conventions are the same as in Figure 3.

were found to be restricted in their dispersal by a well defined boundary in the horizontal plane between dorsal and ventral regions of the CNS (Figs. 10 to 12 and 14 to 16).

A fate map could be deduced from the positions of labeled cells in the CNS that originated from single labeled blastomeres in the early embryo. We have applied the following rules for construction of the fate map: (1) Regions of the CNS were represented on the external surface of the blastomere if labeling that blastomere resulted in labeling of cells in those regions of the CNS; (2) where possible, the representation of brain regions was depicted in normal order on each blastomere and continuity of the representation was preserved as the map extended over several blastomeres. Such a fate map is shown in Figure 13.

This is the most detailed fate map of the vertebrate CNS that has yet been obtained. The advance has been made possible by the advantages of the HRP technique over the vital staining methods (Jacobson and Hirose, 1978; Weisblatt et al., 1978; Hirose and Jacobson, 1979). The HRP technique does not suffer from the limitations of the vital staining methods such as difficulty of application to a single cell, diffusion from the site of application, and fading of the stain. Because of these limitations the vital staining method is capable, at best, of showing large cohesive masses of stained cells while small clumps and isolated cells labeled with vital stain are easily overlooked or are undetectable. Therefore, there is a tendency

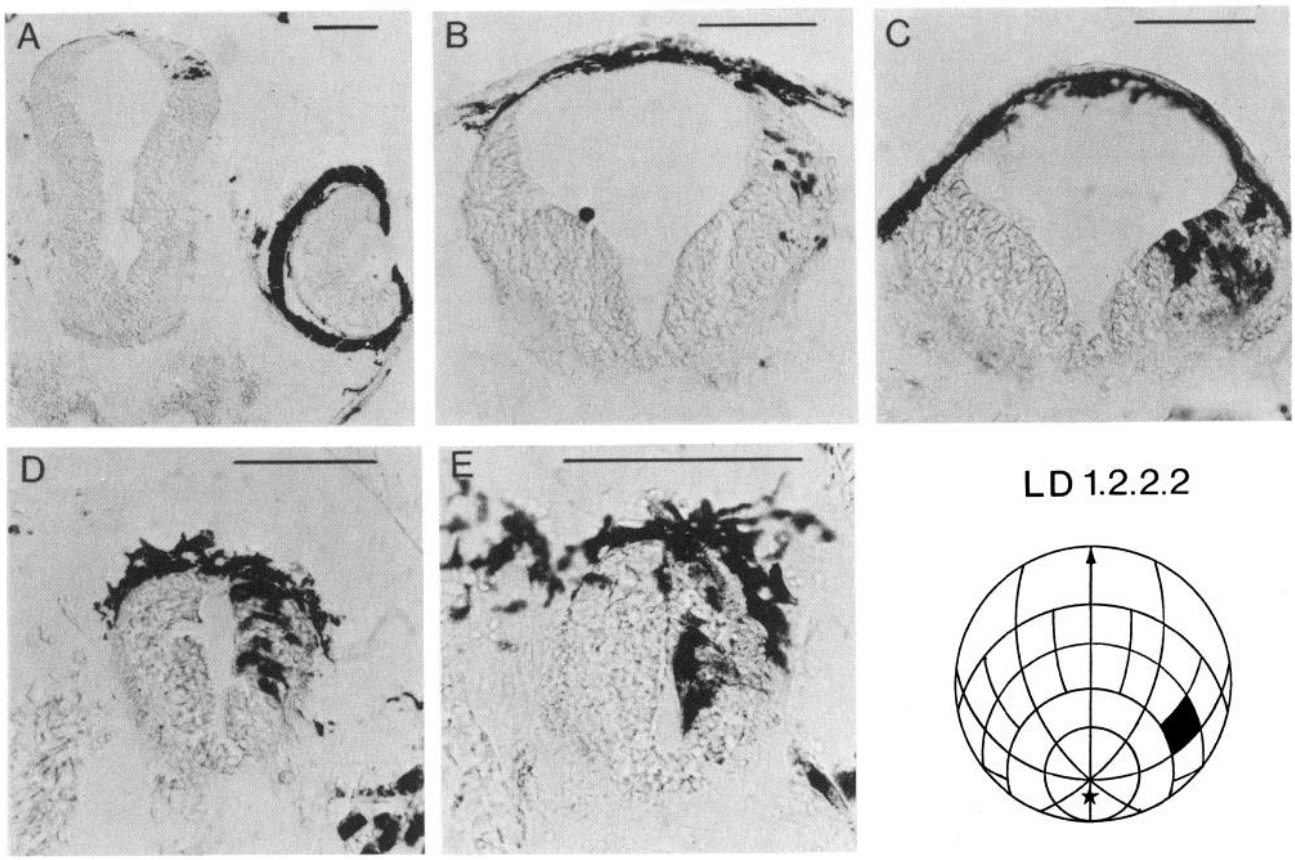

Figure 11. Photographs of sections through the CNS of larval *Xenopus* at stage 39 that had received an injection of HRP into blastomere LD 1.2.2.2 at the 64-cell stage. Labeled cells are shown in the diencephalon (*A*), caudal mesencephalon (*B*), rhombencephalon (*C*), and spinal cord (*D* and *E*). The *bars* are 100 μm.

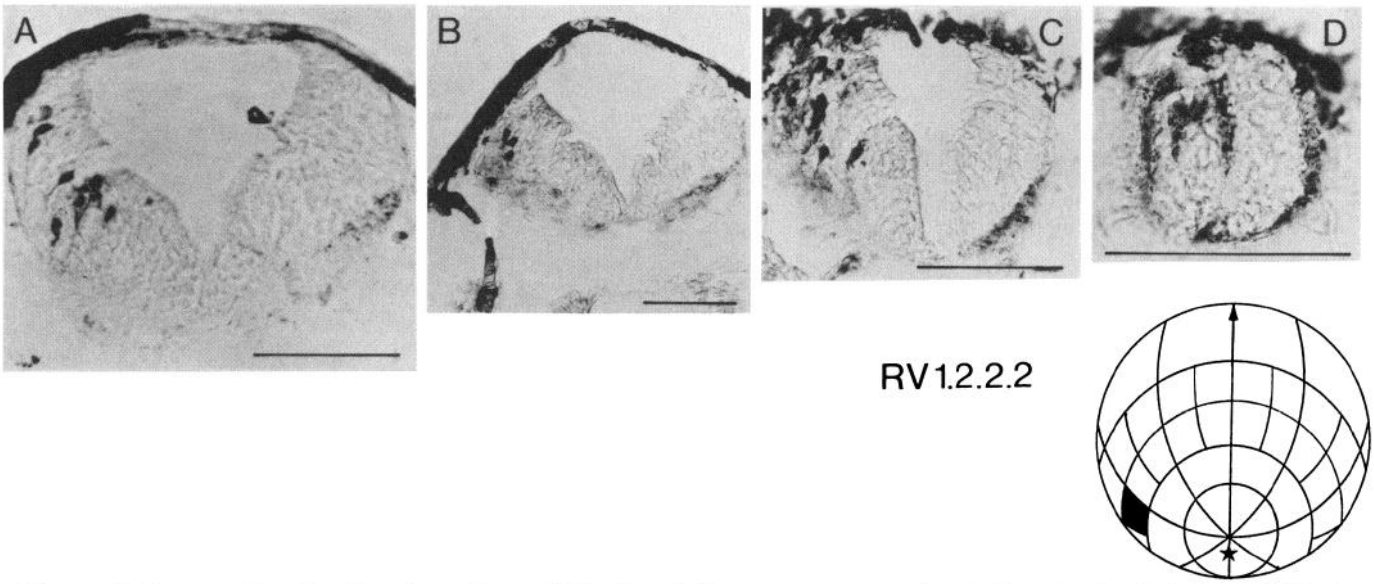

Figure 12. Photographs of sections through the CNS of larval *Xenopus* at stage 37 that had received an injection of HRP into blastomere RV 1.2.2.2 at the 64-cell stage. Labeled cells are seen in the rhombencephalon (*A* to *C*) and spinal cord (*D*). No labeling occurred in the telencephalon, diencephalon, or mesencephalon. The *bars* are 100 μm.

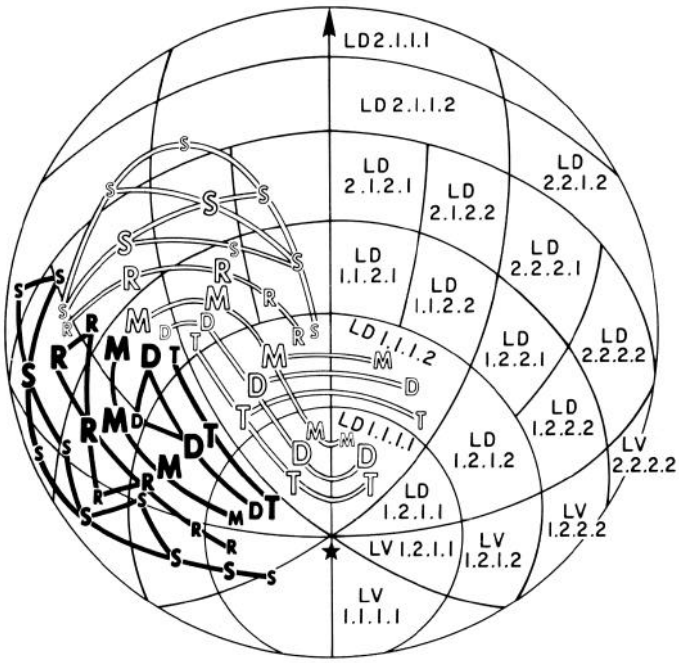

Figure 13. Fate map showing the regions of CNS populated by descendants of blastomeres of the 64-cell embryo. The embryo is viewed from the animal pole (*star*) and dorsal side (*arrow*). Blastomere designations are given on one side while on the other side are shown the regions of CNS to which each blastomere contributed descendants. *Black letters* indicate dorsal regions of the CNS; *white letters* indicate ventral regions. The *sizes* of the letters (small, medium, or large) over each blastomere show the approximate amount of labeling seen in each region of CNS after injecting that blastomere.

to underestimate the size of the prospective neural area mapped by vital staining. This underestimation is shown in the fate maps deduced by Nakamura and Kishiyama (1971) and Nakamura et al. (1978) from vital staining of individual blastomeres of *Xenopus* at the 32-cell stage. Because those authors selected embryos with regular cleavage patterns similar or identical to those used by us, their results can be compared directly with ours. They showed that only two blastomeres on each side (Ib and IIb, corresponding to our D1.1.2 and D1.2.2) contributed to the neural plate on the same side. No contribution to the CNS from blastomeres of the opposite side was reported. Three blastomeres (IIb, IIc, and IIIc, corresponding to our D1.2.2, D2.2.2, and V2.2.2) were found to contribute to the neural folds on the same side. Thus, Nakamura et al. (1978) found four blastomeres on each side at the 32-cell stage contributing to the CNS (neural plate plus neural folds), whereas we have shown that 12 blastomeres on each side at the 32-cell stage contribute descendants to the CNS. The most likely reason for the discrepancy between the fate maps obtained at the 32-cell stage by vital staining and by HRP labeling is the poor detectability of the vital stain, especially where stained and unstained cells are mingled. The discrepancies between our fate map and that of Nakamura et al. (1978) should lead to a critical re-evaluation of the results of vital staining experiments. A case in point is the failure of the vital staining method to show movement of cells across the midline of the gastrula. We have shown that this movement starts at the onset of gastrulation (M. Jacobson and K. Liu, manuscript in preparation) and that extensive interchange of cells between left and right sides of prospective forebrain and retinal areas has occurred by the end of gastrulation (stage 17) in *Xenopus* (Jacobson and Hirose, 1978). Those exchanges of cells between left and right sides of the prospective neural region of the gastrula were missed in the vital staining studies of *Xenopus* embryos (Nakamura and Kishiyama, 1971; Keller, 1975; Nakamura et al., 1978) as well as those of other anuran species (Vogt, 1929; Pasteels, 1942).

These observations again emphasize the importance of the floor plate as a morphological landmark, the essential significance of which was first grasped by Kingsbury (1920, 1922, 1930). He recognized that, in a representative selection of vertebrates, there is a fundamental morpho-

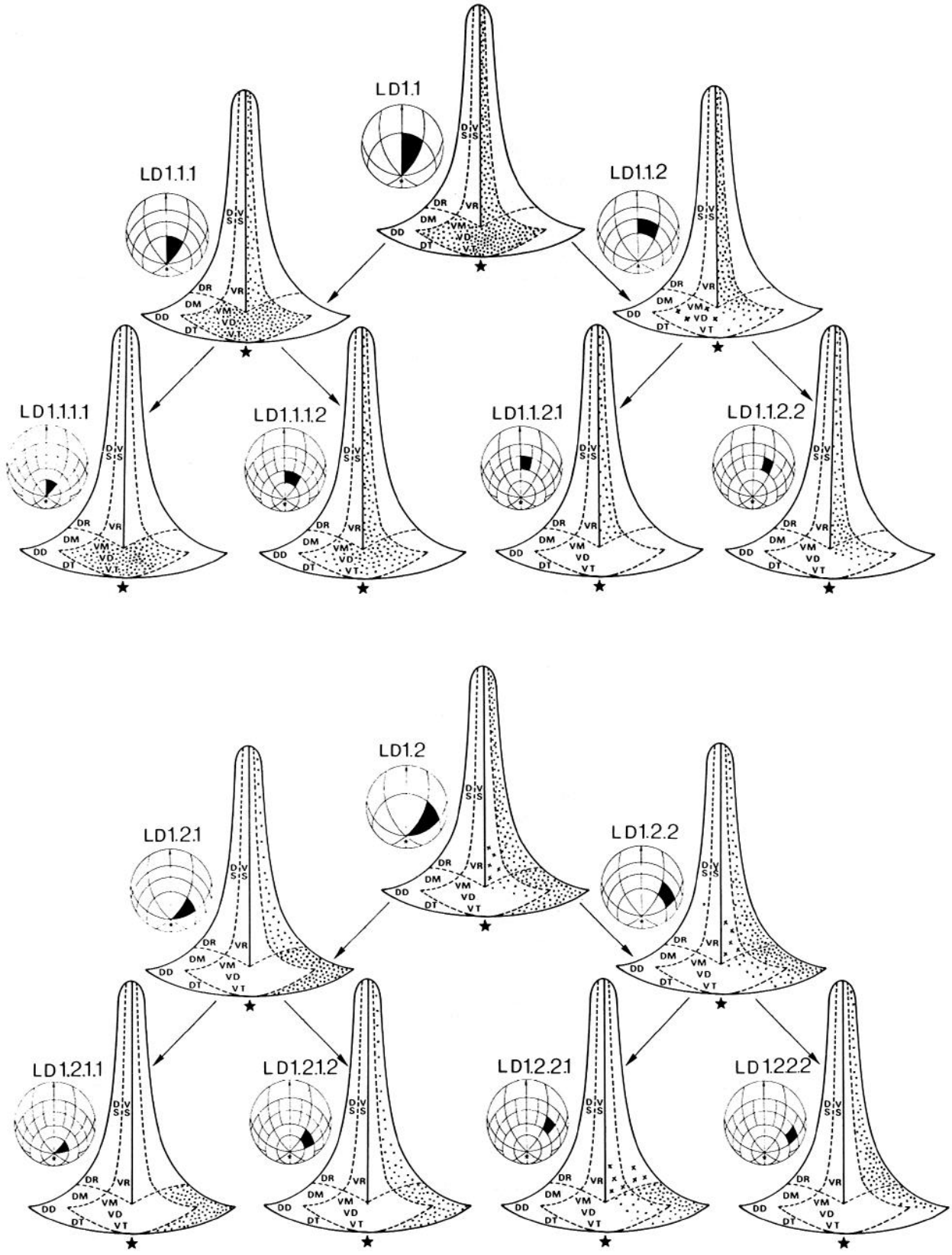

Figure 14. Diagrams of the position in the CNS of labeled cells arising from individual blastomeres of the 16-, 32-, and 64-cell stages. *Stipple* indicates the regions in which labeled cells were found in the majority of specimens and the *density of the stipple* indicates the relative density of labeled cells. *Crosses* indicate regions in which labeled cells were found in some specimens. The conventions are the same as in Figure 3.

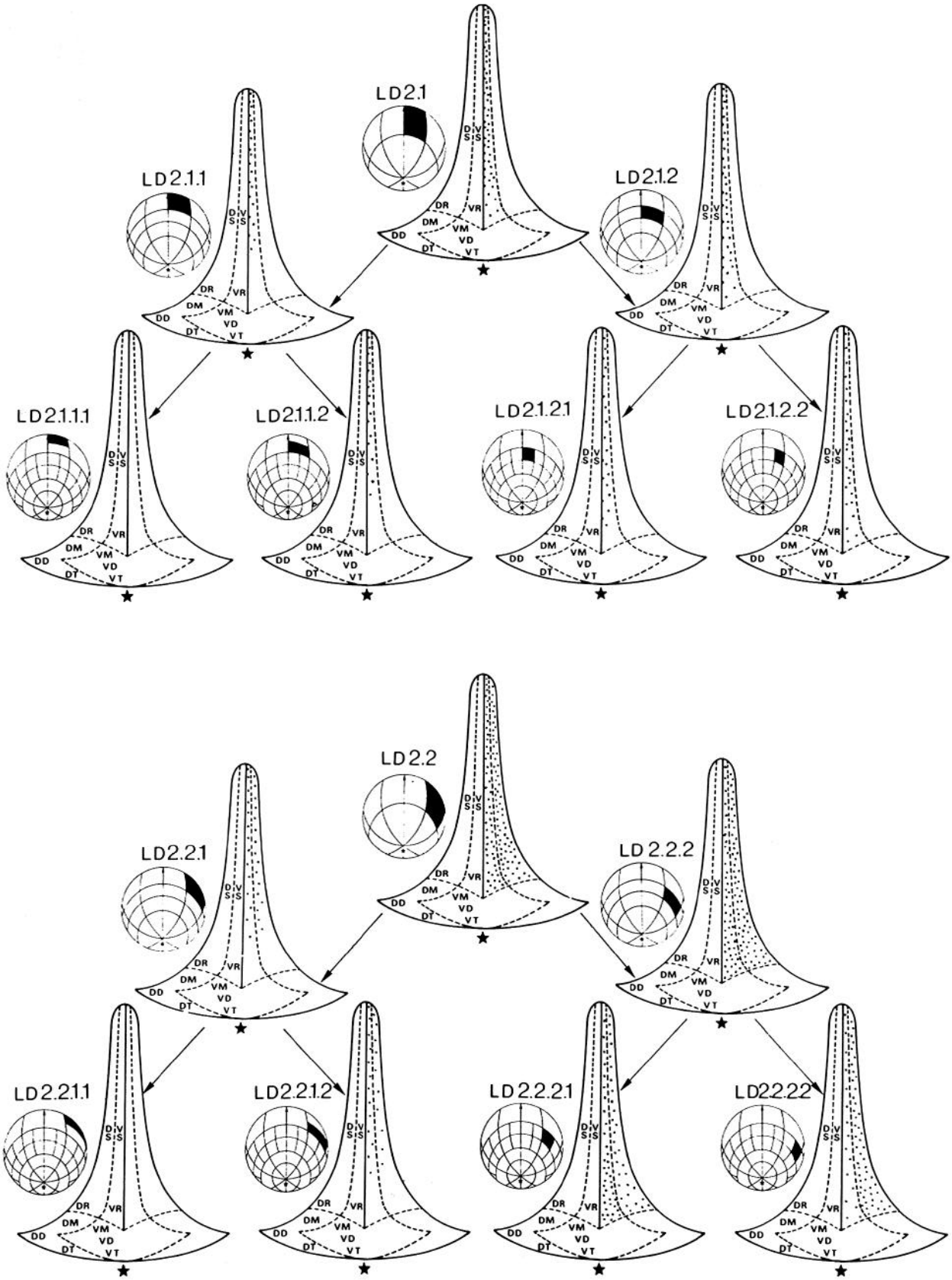

Figure 15. Diagrams of the position in the CNS of labeled cells arising from individual blastomeres of the 16-, 32-, and 64-cell stages. See the legend to Figure 14 for details.

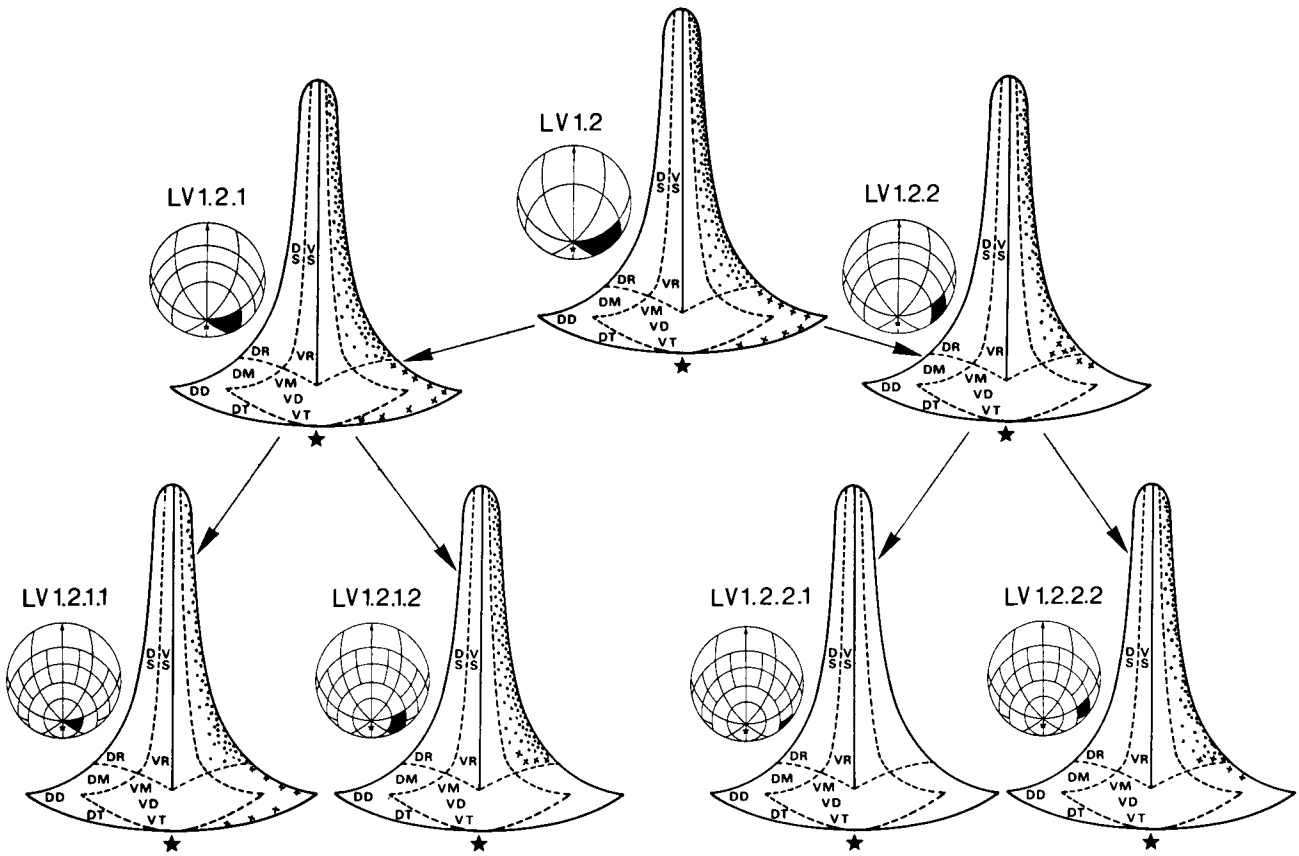

Figure 16. Diagrams of the position in the CNS of labeled cells arising from individual blastomeres of the 16-, 32-, and 64-cell stages. See the legend to Figure 14 for details.

logical difference between the regions of CNS (rhombencephalon and spinal cord) that are separated ventrally by the floor plate and the regions of the brain, ahead of the end of the floor plate, that form a continuous structure across the midline. We have shown that the regions of ventral mesencephalon, diencephalon, and telencephalon that are rostral to the end of the floor plate are formed by mingling of cell bodies across the ventral midline, whereas no cells cross the midline in the rhombencephalon and spinal cord. It remains to be shown whether translocation of cells across the midline of the prospective neural area also occurs in other anurans and whether the bilateral origins of the ventral regions of the brain rostral to the isthmus are a general characteristic of the vertebrates.

The significance of the regional subdivision of the fate map shown in Figure 13 and the limits of spatial resolution of the fate map require some discussion. While we have succeeded in mapping the prospective neural region of the frog embryo in more detail than has hitherto been achieved, the spatial resolution of the map is intrinsically limited by the amount of cell dispersal and cell mingling that occurs after the time of initial injection of the label. We believe we were at fault in underestimating the amount of cell mingling between the descendants of some blastomeres of the 8- and 16-cell stages (Hirose and Jacobson, 1979). The error arose from the very high density of labeled cells which obscured unlabeled cells in the clonal domains arising from blastomeres of the 8- and 16-cell stage. The regions of the CNS that are shared by the descendants of different blastomeres of the 16-cell stage are shown in Figures 14 to 16. Our results of injecting blastomeres at 8-, 16-, 32-, and 64-cell stages show that considerable mingling of labeled and unlabeled cells is present in the CNS at larval stages 31 to 39. Some of this dispersal and mingling of cells occurs during gastrulation (Keller, 1978; M. Jacobson and K. Liu, manuscript in preparation), and migration of cells in the CNS accounts for additional dispersal and mingling at later stages (Jacobson, 1978). The question thus arises how, in the face of the evidence of much cell mingling, it is possible to find the constancy that we observed between each blastomere and the regions of the CNS in which its descendants ultimately came to rest. The fact of constant relationships between regions of the CNS and particular blastomeres leads us to conclude that cell dispersal must be restricted so that the descendants of particular blastomeres are destined to occupy certain regions in the CNS. However, within each region, considerable mingling of the descendants of different blastomeres occurs (Figs. 14 to 16). Apparently, the restriction of cell mingling applies only to mingling between certain regions and not to mingling within each region.

The fate map shown in Figure 13 signifies that individual blastomeres each contribute some cells to particular regions of the CNS and that several blastomeres contribute cells to each region. It does not show that there are any constant relationships between a particular blastomere and a specific type of cell except in the trivial sense that certain types of cells are always located in certain regions of the CNS. The only test that our experiments might allow us to make of whether any commitment to

differentiation of any specific type of cell had occurred would be to determine whether a specific type of nerve cell always arose from one ancestral blastomere. This test would be of little value if coherent clonal growth occurred. However, as we know that considerable cell dispersal and mingling occurs, the test may be of value especially in the case of the types of neurons that originate earliest in the frog's CNS, namely Mauthner's neuron which originates as a postmitotic neuron at gastrulation stage 11 to 12 (Vargas-Lizardi and Lyser, 1974) and the Rohon-Beard cells, the majority of which originate before the end of gastrulation (Lamborghini, 1980).

The evidence shows that commitment to production of Mauthner's neuron, the first neuron to originate in the rhombencephalon, had not occurred at the 32- or 64-cell stages. Mauthner's neuron was labeled after injection of D2.2.2 or D2.2.2.1 only but was not labeled in all cases of injection of those blastomeres. It was labeled in seven and unlabeled in two cases after injection of D2.2.2 and after injection of D2.2.2.1 in 10 embryos, Mauthner's neuron was labeled in three cases, unlabeled in five cases, and not found in two cases. In the unlabeled cases, Mauthner's neuron had lost the label or it had descended from an uninjected blastomere. On the latter hypothesis, D2.2.2 and D2.2.2.1 merely have the highest probability of giving rise to Mauthner's neuron, and if a sufficiently large number of cases were studied, that neuron would be expected to arise from other blastomeres that contributed descendants to the rhombencephalon. Because one Mauthner's neuron normally differentiates on each side of the rhombencephalon, it can arise only from a single ancestral cell in each case, but the evidence is consistent with a probabilistic origin of Mauthner's neuron from more than one possible ancestral cell at the 64-cell stage.

The case of the Rohon-Beard cells is more complex because, like all types of neurons that arise in large numbers, although located in a specific region of the CNS, they may originate from a single stem cell or from multiple ancestors. Our results show that the Rohon-Beard cells originated from three ancestral cells on each side of the 32-cell embryo (LV or RV 1.2.1, 1.2.2, and 2.2.2) and from four ancestral cells on each side of the 64-cell embryo (LV or RV 1.2.1.1, 1.2.1.2, 1.2.2.2, and 2.2.2.2). In addition to Rohon-Beard cells, each of those blastomeres gave rise to other types of cells in the dorsal region of the rhombencephalon and spinal cord. Each ancestral cell gave rise to Rohon-Beard cells scattered along the length of the CNS interspersed with Rohon-Beard cells that had originated from other ancestral cells. This shows that, between the 64-cell stage and the time at which the Rohon-Beard cells settled down at their final relative positions, there had been considerable cell dispersal and mingling. If the cell dispersal and mingling had been completely random and unconstrained, we could not have deduced a fate map. In fact, we found that the clone that descended from any individual blastomere did not disperse beyond certain regions of the CNS so that there was a constant relationship between the position of the initially labeled blastomere and the *region* of CNS in which its descendants were finally dispersed. A fate map could be deduced at the level of resolution of regions of the CNS as shown in Figure 13. Because each region was populated by the mingled descendants of several blastomeres of the 64-cell embryo and because commitment to differentiation of types of cells in the CNS had not occurred at the 64-cell stage, the relationship between an individual blastomere and any specific type of cell in the CNS was only probabilistic.

References

Graham, R. C., and M. J. Karnofsky (1966) The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney: Ultrastructural cytochemistry by a new technique. J. Histochem. Cytochem. *14:* 271–302.

Hara, K. (1977) The cleavage pattern of the axolotl egg studied by cinematography and cell counting. Wilhelm Roux Arch. Entw. Mech. Org. *181:* 73–87.

Hirose, G., and M. Jacobson (1979) Clonal organization of the central nervous system of the frog. I. Clones stemming from individual blastomeres of the 16-cell and earlier stages. Dev. Biol. *71:* 191–202.

Jacobson, M. (1978) *Developmental Neurobiology*, Ed. 2, Plenum Press, New York.

Jacobson, M., and G. Hirose (1978) Origin of the retina from both sides of the embryonic brain: A contribution to the problem of crossing at the optic chiasma. Science *202:* 637–639.

Keller, R. E. (1975) Vital dye mapping of the gastrula and neurula of *Xenopus laevis*. I. Prospective areas and morphogenetic movements of the superficial layer. Dev. Biol. *42:* 222–241.

Keller, R. E. (1978) Time-lapse cinemicrographic analysis of superficial cell behavior during and prior to gastrulation. J. Morphol. *157:* 223–248.

Kingsbury, B. F. (1920) The extent of the floor-plate of His and its significance. J. Comp. Neurol. *32:* 113–135.

Kingsbury, B. F. (1922) The fundamental plan of the vertebrate brain. J. Comp. Neurol. *34:* 461–491.

Kingsbury, B. F. (1930) The developmental significance of the floor-plate of the brain and spinal cord. J. Comp. Neurol. *50:* 177–207.

Lamborghini, J. L. (1980) Rohon-Beard cells and other large neurons in *Xenopus* embryos originate during gastrulation. J. Comp. Neurol. *189:* 323–333.

Nakamura, O., and K. Kishiyama (1971) Prospective fates of blastomeres at the 32 cell stage of *Xenopus laevis* embryos. Proc. Jpn. Acad. *47:* 407–412.

Nakamura, O., H. Takasaki, and A. Nagata (1978) Further studies of the prospective fates of blastomeres at the 32-cell stage of *Xenopus laevis* embryos. Med. Biol. *56:* 355–360.

Nieuwenhuys, R. (1974) Topological analysis of the brain stem: A general introduction. J. Comp. Neurol. *156:* 255–276.

Nieuwkoop, P. O., and J. Faber (1967) *Normal Table of Xenopus laevis Daudin*, Ed. 2, North-Holland, Amsterdam.

Opdam, P., M. Kemali, and R. Nieuwenhuys (1976) Topological analysis of the brain stem of the frogs *Rana esculenta* and *Rana catesbeiana*. J. Comp. Neurol. *165:* 307–332.

Pasteels, J. (1942) New observations concerning the maps of presumptive areas of the young amphibian gastrula (*Amblystoma* and *Discoglossus*). J. Exp. Zool. *89:* 255–281.

Vargas-Lizardi, P., and K. M. Lyser (1974) Time of origin of Mauthner's neuron in *Xenopus laevis* embryos. Dev. Biol. *38:* 220–228.

Vogt, W. (1929) Gestaltunganalyse am Amphibienkeim mit örtlicher Vitalfärbung. II. Teil. Gastrulation und Mesodermbildung bei Urodelen und Anuren. Wilhelm Roux Arch. Entw. Mech. Org. *120:* 384–706.

Weisblatt, D. A., R. T. Sawyer, and G. S. Stent (1978) Cell lineage analysis by intracellular injection of a trace enzyme. Science *202:* 1295–1298.