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Dynamic visualization of the developing nervous system of the bullfrog *Rana catesbeiana*

Seth S. Horowitz^{a,b} and Andrea Megela Simmons^{a,b}

^aDept. of Psychology, Brown University, Providence, RI 02912

^bDept. of Neuroscience, Brown University, Providence, RI 02912

Abstract

Anuran amphibians undergo a rapid and dramatic process of metamorphosis featuring widespread structural reorganization of the central nervous system. Although morphological changes during embryonic stages of anuran development have been well-documented, much less information is available describing structural changes in the brain during larval (tadpole) stages. Using still images from cresyl violet stained material, we present an adaptation of the digital image and video manipulation technique of morphing that allows these images to be compiled in such a manner as to highlight key periods in tadpole brain development in a dynamic fashion. We present three morphed video data sets from ranid tadpoles that facilitate the identification of developmental changes in nuclear boundaries at different levels of the neuraxis. The use of animation allows dynamic examination of anatomical changes across long developmental spans without requiring additional anatomical preparations or specialized expensive equipment.

Section: Structural Organization of the Brain

Keywords

anuran; plasticity; image analyses; morphing; development; atlas

1. Introduction

The construction of brain atlases is an essential tool in neuroanatomy, both for understanding processes of brain development and for characterizing structural organization in the adult. Atlases have commonly been presented as series of two-dimensional static images, with resolution limited by analog reproduction. Advances in imaging technology and the accessibility of the Internet have led to the introduction of digital brain atlases, which allow better temporal and spatial resolution and the ability to integrate various data sets (Maye et al., 2006). Many of these digital atlases are comprised of images derived from magnetic resonance imaging, a technique that requires special expertise and equipment not readily accessible to all investigators (van Essen, 2002). Other imaging techniques such as timelapse video recordings (Harris et al., 1987), time lapse confocal microscopy (Kulesa and Fraser, 2000; Wu and Cline, 2003) and two-photon imaging (Steetdler et al., 2006) are also valuable in visualizing structural organization, but these techniques are typically limited to a small volume of tissue and again

Corresponding author: Seth S. Horowitz, Dept. of Psychology, Box 1853, Brown University, Providence, RI 02912, Phone: 401-863-3012, Fax: 401-863-1300, Email: Seth_Horowitz@BROWN.EDU.

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require specialized equipment. The technical and practical limitations in acquiring images by these newer techniques have to date restricted their applicability to a small number of laboratories and a small number of species.

Anuran amphibians (frogs and toads) are important models for the study of brain development, because their nervous systems undergo rapid and dramatic structural changes as part of normal development. These changes underlie the process of metamorphosis, and they culminate in the transformation of the animal from a herbivorous, limbless larva (tadpole) to a carnivorous, four-limbed adult. All sensory and motor systems undergo transformation during metamorphosis. Metamorphic changes are particularly dramatic in frogs such as the American bullfrog (*Rana catesbeiana*), which transforms from a wholly aquatic to an amphibious animal at metamorphic climax, but are also apparent in the African clawed toad, *Xenopus laevis*, which remains wholly aquatic after completion of climax. Most anatomical imaging work on anurans has focused on *Xenopus laevis*, and then on events transpiring during embryonic stages (e.g., Harris et al., 1987; Papan et al., 2006). There are fewer descriptions of neuroanatomical changes occurring during tadpole stages in any anuran species, and the research that is available primarily focuses on the development of a particular neural system (visual system: Chahoud et al., 1996; olfactory system: Gaudin and Gascuel, 2005; auditory system: Horowitz et al., 2007), or examines anatomical organization of a single brain nucleus or group of neurons within a limited time span of larval development (e.g., Wu and Cline, 2003). As a consequence, there are no atlases available on developing anurans that provide information on the structural organization of the brain as a whole during post-embryonic larval development and during the transformation to the adult form.

In this paper, we present atlas-like images of the brain (from hindbrain to thalamus) of developing bullfrogs over the developmental span from the youngest post-embryonic animals through early postmetamorphic froglets. To compile these images, we applied the technique of morphing, a common video manipulation method that allows dynamic mapping of anatomical changes at a variety of resolutions for time spans of any length based on the tissue samples available. The morphing technique requires no specialized equipment, and software is easily available through the Internet. We present three data sets, derived from brightfield Nissl-stained sections of the hindbrain, midbrain and thalamus. These data sets not only highlight, in still images, important structural changes in the tadpole's central nervous system over development, but they also emphasize the utility of morph videos in examining dynamic aspects of these changes.

2. Results

In individual frames from the cresyl violet stained medullary sections (Fig. 1A) of a stage 22 tadpole (left) and a postmetamorphic froglet (right), several developmental trends can be detected. First, there is a qualitative increase in the overall size of the medulla and in relative cell number. Second, the dorsal medullary nucleus (DMN, the amphibian homolog of the mammalian cochlear nucleus; upper right black box in Fig. 1A) migrates from a more lateral position at the entry point of the eighth nerve to a more medial region previously occupied by the lateral line neuropil (LLnp, site of termination of fibers from the anterior lateral line nerve). Third, the superior olivary nucleus (SON, lower right black box) appears to remain stable in position across development, only increasing in cell number.

Although some of these changes have been previously reported (Jacoby and Robinson, 1983; Templin and Simmons, 2005), they are highlighted in the medullary morph video (Online supplemental materials, MedullaCVmorph.avi). Moreover, the video shows developmental trends in other medullary areas that have not been previously described. There is a change in the overall shape of the medulla from stage 22 to froglet stages, concomitant with an increase

in its longitudinal extent and a widening and then contracting of the fourth ventricle. There is also a progressive change in cell density around the ventricular zone. This area, primarily composed of the reticular gray, shows changes in formation of cellular lamina that are not immediately obvious from examination of still images. From stages 22 to 25, there is substantial overall growth, the central cell-rich region around the ventricular zone spreads and differentiates from the white fiber regions into a clear reticular gray, and the DMN and SON both increase in cell number and form more discrete nuclear boundaries. From stages 25 to 36, the DMN enlarges proportionally but cell bodies increase in number in a series of compression and expansion steps, remaining approximately in the same lateral position. Around stage 37, the DMN enlarges and shifts medially. This dorsomedial migration continues throughout the remainder of larval development and by the froglet stage, the DMN is much larger and more medial. Thus, the translocation of the DMN across development is not a simple linear shift in position, but rather a series of compression/translocation steps.

As the medulla enlarges, the SON also enlarges, while still remaining in relatively the same position. Half-way through the video, the centroid of cell density shifts slightly medially, then at stage 33, the medial portion itself enlarges, pushing the centroid of the SON back laterally at stage 36. The SON ends up larger and about in the same relative position as at stage 25. Moreover, at about stage 30, a gap in cell density appears at approximately the ventrodorsal midpoint of the SON, and remains for the rest of the larval period. While examination of individual images does not show this as an obvious feature (Fig. 1A), in the video the division is quite clear as a relative decrease in density of cells at a specific point while the adjacent regions increase in number. This may indicate that there are two subnuclei or subpopulations within the SON as a whole. It also highlights the basic nature of using a video for examination of development in that complex features become more apparent when they are in motion; the pattern of cell emergence and redistribution is substantially clearer due to the apparent movement induced by the morph. While certain regions may look distorted when the morph video is frozen on a single frame, by using distortion cues, it is possible to identify regions where cellular reorganization is taking place and provide targets for examination of developmental shifts.

Still frames from cresyl violet stained sections of the midbrain (Fig. 1B) from a stage 22 tadpole (left) and a froglet (right) also display several obvious developmental changes. In the stage 22 animal, cells are densely concentrated, without any clear laminar arrangement, around the midline dorsally and ventrally to the optic ventricle. Moreover, the optic ventricle is continuous with the aqueduct of Sylvius. In the froglet, the optic ventricle and the aqueduct are discrete structures, and there is a clear laminar arrangement of the optic tectum (homolog to the mammalian superior colliculus) and the laminar and principal regions of the torus semicircularis (TS, homolog to the mammalian inferior colliculus). The video morph (Online supplemental materials, MidbrainCVmorph.avi) provides further insights into the nuclear organization of the developing midbrain. In general, the midbrain as a whole enlarges primarily along the dorsoventral axis, with the TS swelling upward through stage 25. The earliest post-embryonic developmental range (from stages 22 to 26) shows some of the most dynamic anatomical changes as the optic tectum develops its basic laminated structure and the TS emerges from the periventricular cell mass. From stages 26-30, the TS stabilizes in size although the rest of the midbrain enlarges, again mostly in the dorsoventral dimension. From stages 30-37, the TS still remains the same while the rest of the midbrain expands, primarily due to the widening of the ventricles. At stage 37, the dorso-medial TS begins growing again, pushing upward until stage 40. At stage 40, the ventral TS and underlying tegmental nuclei expand dorsally, and expanding the left and right TS both medially and dorsally, closing off the connection between the optic ventricle and the aqueduct of Sylvius, as previously described (Kumaresan et al., 1998). The morph video demonstrates a probable mechanism underlying this separation, as it becomes clear that there is substantial increase in density and overall

growth in a ventro-dorsal direction of the TS and underlying tegmental regions between stages 40 and 43. This identifies this area as a possible region for investigation of cell division and/or differentiation. By stage 43, a small darker region forms at the medial ventricular margin where the two newly joined TS structures meet to form the intercollicular nucleus. At the same time, the TS ceases its differential expansion and grows at approximately the same rate as the rest of the brain.

The video also shows that the layered arrangement of the optic tectum emerges between stages 22 and 26. During this time span, the developing tectal laminations extend laterally around the optic ventricle to the laminar nucleus of the TS. After stage 26, the layered organization of the optic tectum remains stable, with further development showing primarily changes in width and cell density in certain layers. In contrast, the laminar nucleus and underlying principal nucleus of the TS undergo substantial changes in cell density and number of cell layers, with the principal nucleus not showing a clear laminar arrangement until stage 40. These comparisons suggest that major anatomical changes in the visual midbrain occur during a more limited span of larval development than those in the auditory midbrain. Although the emergence of laminations in the tadpole optic tectum (Senn, 1972) and TS (Kumaresan et al., 1998) have been described previously, the morph video allows direct comparison of the different time courses of maturation of these two nuclei.

Cresyl violet stained sections of the thalamus (Fig. 1C) from a stage 22 tadpole (left) and a froglet (right) show that this brain region exhibits fewer changes in overall organization than occurs in medullary and midbrain regions, while still undergoing dramatic changes in size. In both the stage 22 tadpole and the froglet, cell density is greatest around the third ventricle, with a laminar arrangement apparent in the ventral and central thalamic nuclei. The appearance of laminations in the earliest larval stages suggests that, by this marker, these thalamic nuclei seem to more closely parallel optic tectum development than TS development, where lamination is a later-developing feature.

The thalamus morph highlights the stability as well as the differentiation and growth of target zones. In the stage 22 tadpole, the two halves of the thalamus are joined at the dorsal midline, not to separate until stage 30. Overall, the thalamus grows by progressively widening and expansion of this dorsal region. The more ventral region undergoes its greatest widening around stage 35. The cell dense posterior region of the preoptic area extends more posteriorly between stages 22-30, and is visible in more caudal sections beginning around stage 35. While this could be explained by a bias slice in the coronal plane, examination of multiple sections from different animals have shown that this is a consistent result. Between stages 22-30, there is an increase in horizontal extension and an increase in the number of laminations in thalamic nuclei, with clear laminations appearing in the anterior nucleus by stage 30. The transition to late larval (stage 35) stages highlights the growth of the dorsal region and the corresponding expansion of the anterior, central and lateral thalamic nuclei to cover a significant portion of the dorsal region. Increased segregations of laminae throughout the nuclear regions and the disappearance of the preoptic cells from this region are also observed. From stage 35 to stage 40, there is an increase in size and cell density, but no major reorganization. From stage 43 (climax) onward, laminations seem stable, and the modest increase in size appears correlated with an expansion of extranuclear regions, possibly indicating expansion of fiber pathways, particularly in the lateralmost areas during the transition to the postmetamorphic froglet stage. The enteropeduncular nucleus and the suprachiasmatic nucleus remain stable in position and relative density across development.

3. Discussion

Computer-based video morphing allows examination of anatomical image data sets from any level of resolution over potentially very long temporal spans, and it provides an intuitive tool for determination of regions of interest across defined developmental periods. Dynamic visualization of data sets from cresyl violet stained sections of developing bullfrog brains greatly help in elucidating changes in brain compartmentalization, not restricted to any particular sensory or motor system. As such, they provide an overall gestalt view of brain organization and reorganization over metamorphosis, and they facilitate assessment of relative maturational changes in different neural systems. Analyses of both the still images and the videos indicate that, although the brain as a whole increases in size and lateral extent over development, different levels of the brain appear to undergo maximal anatomical restructuring at different time periods. In particular, the optic tectum and the thalamus both are observed to exhibit their most dramatic changes between hatchling and early larval stages, while the DMN and the TS undergo structural reorganization over a more extended time period. These data suggest that the auditory midbrain is less mature during larval development than the visual midbrain, but they also suggest that auditory nuclei in the thalamus may not undergo extended morphological changes, other than changes in cell density, reflecting the reorganization of brainstem auditory nuclei. The comparison of nuclei at different levels of the brain and using two imaging technique thus can provide interesting insights into the comparative patterning of development of different neural systems. Moreover, dynamic visualization of changes in brain areas between different stage groups can pinpoint the time periods of most dramatic change for further, more detailed analysis.

3.1. Technical considerations

Morph videos are simple to set up and can be developed from existing data sets, but can require substantial time for initial planning and then for refinement, particularly if using sections which undergo major conformational or labeling changes. One of the primary applications of morph videos is to use them to guide future anatomical work; however, the virtual temporal “resolution” is delimited by the number of sections used to generate the final video. If too few sections are used across a specific developmental range, while the video may visually progress smoothly, its utility will be minimal. Sections with damage or distortion due to slicing, staining or mounting artifacts can induce false distortion regions. Furthermore, care must be taken regarding interpretation of changes in position of nuclear boundaries. While morphing can increase the ease of visualization of movement of a specific group of cells and thus facilitate formulation of new hypotheses regarding brain maturation, it is not possible to determine from the videos alone whether factors such as active cell migration or simply displacement secondary to gross size changes are the cause of such movement.

Our data sets are based on cresyl violet stained material imaged with brightfield microscopy. Other types of section staining and illumination, such as fiber label and darkfield, can also be used for morph video application. Images of sections labeled with fluorescent dyes can also be used for morphing, but non-ratiometric fluorophores are intrinsically more difficult to work with than brightfield or darkfield sections as they can show significant intra-section changes in background fluorescence, brightness and other factors.

Another difficulty in the use of morph videos is the need to observe them repeatedly and/or pause and replay segments in order to use them for distortion analysis. The dynamic aspect of morph video makes identifying changes more intuitive to the viewer, but requires more viewing time for identification of discrete regions for further analyses. It should be noted that this is not an issue when using morph videos for presentation or pedagogical applications.

3.2. Utility of dynamic image analyses

Obtaining better spatial and temporal resolution images of anatomical structures both in vivo and in vitro is the basis of a significant segment of biomedical and bioengineering research. Much of the progress in the field has emerged from development of more flexible software tools (Fischl et al., 2001; Bors et al., 2004; Guetat et al., 2006). In comparison to point-by-point comparison of static specimens, video morphing provides an intuitive means for identifying regions of anatomical change across long developmental spans, as well as providing a teaching tool that highlights the temporally dynamic aspects of brain reorganization in a way not otherwise possible. The current state of the art of off-the-shelf computer graphics software allows a significant degree of semi-automation for image standardization which can substantially reduce preparation time. Furthermore, if morph development is integrated into the early stages of a project's planning, provision of registration points during tissue processing can speed up alignment and placement issues otherwise carried out in graphics software. Wider adoption of this technique will undoubtedly lead to substantial improvements and automation of the process, and lead to the development of forms that can be used in a quantitative fashion.

4. Experimental procedures

Images for the morphing procedure may be derived from any source format including brightfield, darkfield, fluorescent or confocal microscopy. The description below is based on three data sets compiled from tissue sections from the medulla, midbrain and thalamus of *Rana catesbeiana* tadpoles and early postmetamorphic froglets.

4.1. Histological procedures

Animal procedures were reviewed and approved by the Brown University Institutional Animal Care and Use Committee, and are consistent with federal guidelines. Tadpoles were staged according to the staging tables developed by Gosner (1960), which are based on examination of external morphology (primarily state of differentiation of the limbs). They were then further classified into one of five metamorphic groups as described by McDiarmid and Altig (1999). These groups are hatchlings (stages 21-25, the earliest freely-living postembryonic animals); early larval stages (stages 26-30, with undifferentiated hindlimb buds and no forelimbs); late larval stages (stages 31-41, progressive differentiation of hindlimbs, internal forelimb development); and metamorphic climax (stages 42-46, with fully-developed hindlimbs and a range of forelimb development, from initial external emergence to full development). In addition we included sections from postmetamorphic froglets (immature frogs that have completed metamorphic development but with snout vent lengths of less than 5.5 cm; Boatright-Horowitz and Simmons, 1995). On the basis of functional and anatomical data collected in our laboratory (Boatright-Horowitz and Simmons, 1997; Horowitz et al., 2007), we further divided the late larval group into two groups, one comprising stages 31-37 (we which term the late larval period) and the other comprising stages 38-41 (which we term the deaf period). Postmetamorphic froglets (snoutvent lengths <5.5 cm, 1-90 days after completion of climax) were also used. Since the Gosner and other developmental staging techniques (e.g., *Xenopus laevis*: Nieuwkoop and Faber, 1956; mouse, Theiler, 1989) are based on external morphological embryonic or postembryonic features, there may be poor correlation between these stage labels and anatomical or molecular changes in the brain. For this reason, we included at least one, and often two, images per stage group to identify any within-stage group changes (because of limitations of file size for uploading the morph videos, more images could not be included). Use of external stages rather than temporal measures such as embryonic day is a necessity with ranids due to their highly variable and environmentally dependent development rates (Corse and Metter, 1980). Images are derived from the following specimens: medulla, 8 animals [1 hatchling (stage 22), 2 early larval (stages 25, 33), 2 late larval (stages 36, 37), 1 deaf period (stage 40), 1 climax (stage 43), 1 froglet]; midbrain, 7 animals [1 hatchling (stage

22), 2 early larval (stages 25, 30), 1 late larval (stage 37), 1 deaf period (stage 40), 1 climax (stage 43), 1 froglet]; thalamus, 7 animals (1 hatchling (stage 22), 1 early larval (stage 30), 1 late larval (stage 35), 1 deaf period (stage 40), 2 climax (stage 43, 45) 1 froglet]. The particular stages chosen were based on the availability of sections that were not cut on a bias or otherwise damaged, and on preliminary work showing that these stages were representative examples of the kinds of structural changes that occur in the developing brain. For data sets in which exact time courses of developmental change are unknown, as in ranid tadpoles, it is necessary to perform considerable preliminary work to allow good choice of stages. Without limitations of file size, more images can be included.

Animals were terminally anesthetized in 0.15% MS-222 (tricaine methanesulfonate, pH 8.0, Sigma, St. Louis MO), then transcardially perfused with heparinized 0.9% saline followed by 4% paraformaldehyde (pH 7.4). Brains were removed and postfixed overnight at 4°C in 4% paraformaldehyde. Overlying meninges were removed and brains were embedded in agarose (5% in saline) and sliced by vibratome (50 µm coronal sections). Sections were placed in 0.5% (w/v) cresyl violet acetate staining solution (pH to 3.7 using glacial acetic acid) for 10 min, washed, and dehydrated in graded ethanol series. Background staining was removed with Citrisolv (Fisher Scientific, Pittsburgh PA) followed by a 30 sec wash in xylenes (Fisher Scientific). Sections were mounted using Cytoseal acrylic mounting medium (VWR, Bridgeport NJ).

4.2. Image preparation

Sections were imaged using an Olympus BX-60 research microscope (Melville NY) with a Magnafire (Optronics, Goleta CA) CCD camera linked to a Dell Pentium 4 computer using an IEEE-1394 PCI frame grabber (Texas Instruments) and saved as 24-bit RGB TIFF files. Images from the area of interest at each developmental stage were then selected for inclusion in the morph. Only sections that were parallel in sectioning plane (not cut on a bias) were included, in order to avoid artifacts. In a series of sections that may include areas changing in geometry or size, it is important to select sections that are not torn or damaged, and that show uniformity of stain across multiple sections. If the brains of interest have not been imaged with registration marks, it is also important to select areas of interest based on a fixed feature, such as the entry point for a cranial nerve or a regional boundary that is presumed not to change radically over development, such as the ventral margin of a ventricle. For medullary sections, sections were selected just anterior to the seventh motor nucleus at the point of the widest mediolateral extent of the SON. For the midbrain, we selected sections with the widest optic tectum extent that included the interpeduncular nucleus. For the thalamus, we selected sections just anterior to the habenular region at approximately the middle of the rostral/caudal axis, a region which showed the maximum extent of the dorsal region thalamic nuclei (lateral, central, anterior) and of the suprachiasmatic nucleus. Anatomical boundaries and terminology (Fig. 1) are consistent with those described for developing (Senn, 1972; Jacoby and Robinson, 1983; Fritzsche et al., 1988; Kumaresan et al., 1998; Horowitz et al., 2007) and adult (Opdam et al., 1976; Wilczynski and Endepols, 2006) ranid brains.

Image preparation was carried out with Corel Photo Paint (Corel, Ontario Canada), although numerous other commercial and freeware packages will serve just as well (e.g., Adobe Photoshop CS or ImageJ, respectively). While this paper does not intend to provide complete tutorials on image manipulation techniques, we will describe basic guidelines for creating image sets and region of interest selection for generating useful morphing videos for temporal anatomical analyses. In our data sets, images of tissue sections are centered horizontally in the image plane and cropped to include the full region from the largest section in the series (in this case, the oldest postmetamorphic specimen). While maintaining the same relative magnification, image frames are resized and/or resampled to match the final desired format

for the video; 720 pixels $\times$ 480 pixels is the standard size for DV video (readable in DV AVI, AVI and Quicktime players), although frame sizes smaller than these dimensions can be selected. It is also important to equalize brightness and contrast between sections by choosing standard light (e.g., intercellular unstained regions) and darkly stained cellular features to prevent large intensity difference artifacts during the morphing process, using either standardized tone curve or sample-target balance tools.

4.3. Morphing preparation

Procedures for setting up morphing videos are described based on MorphMan 4.0 (Stoik Imaging, Moscow, Russia); however, similar procedures are used in other morphing packages (e.g., WinMorph, which is a freeware program for Windows available as a standalone program or plugin for Final Cut and Adobe Premier). Morphing is a video procedure that allows a smooth transition from one digital image to another based on three basic steps: 1) The original image is distorted over time based on defined regions of interest to match the final image; 2) The final image begins as a distorted version which matches regions of interest of the original and undistorts over time; and 3) transparency of the two images change over time to cross fade from the original to the source. A series of images is chosen to be originals and finals in a continuous change across the developmental or other temporal span of interest (Fig. 2). The number of sections used for a developmental series should be sufficient to bracket the temporal range in which a particular change is presumed to occur to prevent excessive image distortion. We selected representative sections of the same region from each developmental stage group, as defined above, on the assumption that little change would occur within a stage group. It should be noted that the actual number of generated video frames between raw sections does not match any specific developmental time scale due to the variable rate of development of ranid tadpoles (see 4.1 above). Instead, we have chosen to use fixed time separations between sections throughout metamorphic development and a twice as long period for the climax to froglet transition. This strategy prevents excessive computation time in morphing between representative stages during which little feature reorganization is expected to occur, but may also introduce artifacts from morphing between sections too widely spaced in developmental time. This problem can be minimized by selecting multiple sections within a developmental group, but then file sizes can become very large.

Earlier sections are chosen as “source” images with the later developmental section selected as a “target.” Section outlines as well as regions of interest are demarcated by control-point limited polygons in both source and target, and registration points on known landmarks (e.g., ventralmost point of a known nucleus, or outline of a ventricle) matched between them. Once the outlines and regions of interest have been created and matched, the transparency curve is edited to provide a proper crossfade at the point when the distortion of the source and undistortion of the target are at a mutual minimum. In our experience, a hyperbolic curve with the maximum transparency of the source occurring shortly before the completion of the morph provides the least visible image distortion (Fig. 3). Furthermore, while a variety of morphing algorithms are typically provided in each software package (e.g., gravity, fast gravity, triangulation and smooth triangulation), smooth triangulation for the rendering yields the least distortion. This is not to imply that morphological changes follow a hyperbolic curve between developmental stages, but rather that use of this algorithm minimizes distortion, as measured by transient smearing and pixelation, in creation of the video morph.

Video rendering and output options define the temporal resolution of the video. Selection of rendering frame rate requires a trade-off between smoothness of interframe change and file size. While a low frame rate (10-12 fps) allows creation of video suitable for presentation in online formats, it also limits the ability to recognize subtle changes from frame to frame. Videos presented in this paper use a video frame rate of 12 fps with color set to 24 bits and 7-8 source/

target pairs, in order to limit videos to 10 M in size. If there are no size limitations for presentation, frame rates of up to 30 fps create superior smoothness during transitions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

A, anterior thalamic nucleus; AS, aqueduct of Sylvius; C, central thalamic nucleus; DMN, dorsal medullary nucleus; DTeg, dorsal tegmentum; Ent, enteropeduncular nucleus; III, third ventricle; IMLF, interstitial nucleus of the medial longitudinal fasciculus; INT, interpeduncular nucleus; IV, 4th ventricle; La, lateral thalamic nucleus; LLnp, lateral line neuropil; OT, optic tectum; OV, optic ventricle; Pre, preoptic area; SCN, suprachiasmatic nucleus; SON, superior olivary nucleus; TS, torus semicircularis; TSl, laminar nucleus of the TS; TSp, principal nucleus of the TS; VIIIm, seventh motor nucleus; VLd,v, ventral lateral thalamic nucleus, dorsal and ventral; VM, ventromedial thalamic nucleus; VTeg, ventral tegmentum.

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Fig. 1.

Single frames from cresyl violet stained sections of the medulla (A), midbrain (B), and thalamus (C) of stage 22 tadpole (left) and froglet (right) animals. Images have been converted to grayscale. A) Boxes highlight shift of the DMN from a lateral to a more dorsal and medial position (top boxes) as well as the positional stability but putative split of the SON into two populations (dashed line in oval). B) Boxes highlight the area of the torus semicircularis, showing the radical growth and change in geometry of the subnuclei and the ventricular regions. The actual regions underlying these changes are more apparent in the video morphs than in the still images. C) The thalamus shows similar increase in size to other regions, but comparatively little differentiation. Upper boxes show the changes in the anterior, central and lateral nuclei,

and the lower box shows the ventral nuclei, nuclear regions of maximal increase in size and lamination across development. Scale bars = 1 mm.

Fig. 2.

Samples of original cresyl violet labeled sections from developmental groups from Stage 22 (post hatchling) through postmetamorphic froglet used in generation of the video morphs described in this paper (stage 22 and froglet images are found in Fig. 1). Images have been converted to grayscale. Development progresses from top to bottom, with source and target images formed from vertical pairs within the columns. Each target becomes the source in the subsequent morph and the morphs are edited together to create a cohesive developmental narrative. Not all section images were used in final production of morph videos. Developmental staging is based on Gosner (1960).

Fig. 3.

Sample screen from a commercial morphing program “Morph Man” (Stoik Imaging). Source and target images and region of interest editing points (top and bottom left) as well as control over transparency and project preview window (lower right) are common features in almost all morphing software.