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BRAIN RESEARCH

Research Report

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

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ARTICLE INFO

Article history:
Accepted 17 April 2007
Available online 6 May 2007

Keywords:
Anuran
Plasticity
Image analyses
Morphing
Development
Atlas

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.

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 time-lapse video recordings (Harris et al., 1987), time-lapse confocal microscopy (Kulesa and Fraser, 2000; Wu and Cline, 2003) and two-photon imaging (Stettler et al., 2006) are also valuable in visualizing structural organization, but these techniques are typically limited to a small volume of tissue and again require specialized equipment. The technical and practical limitations in acquiring images by

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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; VIIm, seventh motor nucleus; VLd,v, ventral lateral thalamic nucleus, dorsal and ventral; VM, ventromedial thalamic nucleus; VTeg, ventral tegmentum

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doi:10.1016/j.brainres.2007.04.078

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 *X. laevis* and then on events transpiring during embryonic stages (e.g., Harris et al., 1987; Papan et al., 2007). 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 postembryonic 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 postembryonic 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 bright-field 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 Rubinson, 1983; Templin and Simmons, 2005), they are highlighted in the medullary morph video (Supplementary Video 1). 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