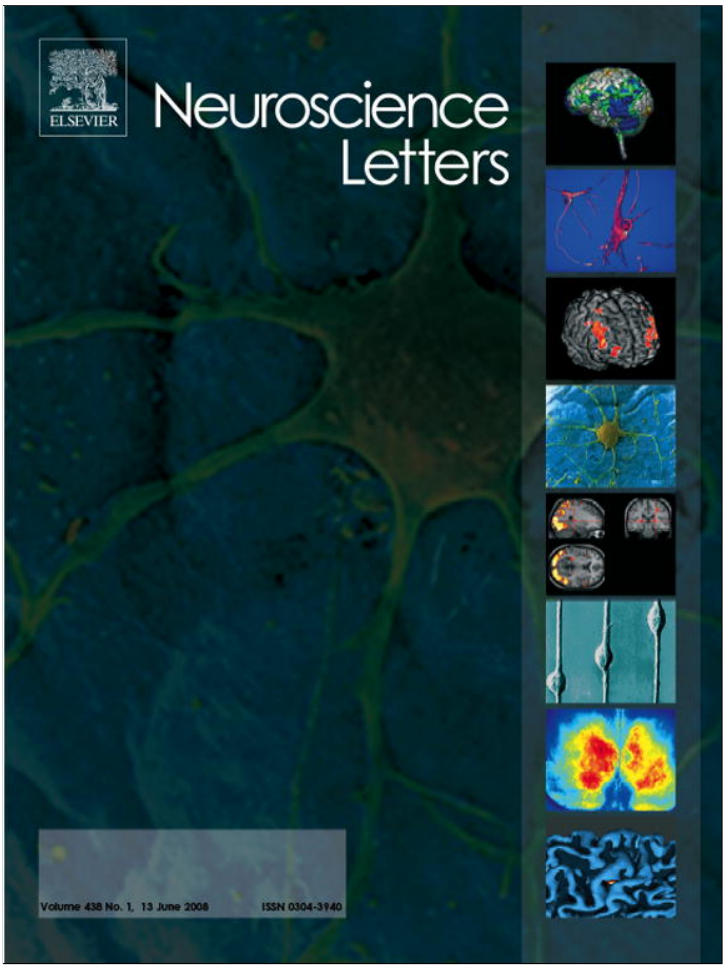

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High-resolution paraventricular nucleus serial section model constructed within a traditional rat brain atlas

Donna M. Simmons, Larry W. Swanson*

Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089-2520, USA

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ABSTRACT

As a starting point for constructing a high-resolution, resliceable computer graphics model for the extraction, quantitative analysis, display, and modeling of neuroanatomical data the outer border and the boundaries of inner divisions and parts of the paraventricular nucleus have been drawn for all 39 serial histological sections prepared for a published reference atlas of the rat brain. This careful parceling revealed three new features of paraventricular nucleus topography: the full rostral extent of the anterior parvicellular part, the caudal end of the medial magnocellular part, and a thin rostrolateral extension of the dorsal medial parvicellular part composed at least in part of neurons expressing corticotropin-releasing hormone. The vector graphics drawings were aligned using the already established alignment of nine consecutive, relevant Atlas Levels, and then contours were smoothed to eliminate nonlinear distortions associated with histological mounting. This dataset was then used to create three-dimensional contour and surface models of the paraventricular nucleus, as well as two-dimensional horizontal and sagittal projections of its outer border. The computer graphics files containing raw and smoothed drawings for all 39 serial sections are supplied for use by researchers interested in developing new or better computer graphics analysis tools involving the paraventricular nucleus. This work may also stimulate the long range goal of creating a high-resolution, resliceable, computer graphics model of the whole brain, and eventually the whole nervous system, that is useful for quantitative analysis and topological transformation.

The paraventricular nucleus of the hypothalamus (PVH) is best known as a critical node in neural circuitry that coordinates appropriate neuroendocrine, autonomic, and behavioral responses underlying energy balance and body water regulation, and as the brain's final common pathway for the stress response involving pituitary ACTH secretion and then adrenal glucocorticoid secretion [14–19]. The rat PVH has been studied most extensively. It contains on the order of 10^4 neurons in a wing-shaped volume of about 0.5 mm^3 on either side of the third ventricle, and experimental neuroanatomical results indicate that its neurons fall into at least three major structure-function classes: magnocellular neurosecretory with axonal projections to the posterior pituitary, parvicellular neurosecretory with projections to the median eminence's neurohemal zone, and descending with axonal output to brainstem and spinal regions controlling autonomic responses and somatomotor behavior [24,27]. Furthermore, these classes are clearly though not completely segregated spatially into magnocellular, parvicellular, and descending divisions, each of which is further segregated, so that 11 subdivisions are recognized in the spatial parceling scheme we now use [22].

This highly differentiated structural organization of the rat PVH has been utilized extensively to help define the distribution of extrinsic axonal inputs from various other parts of the brain, as well as a wide range of immunocytochemical and in situ hybridization staining patterns—in terms of specific neuron classes and types. However, because there are so many structural subdivisions in such a relatively small volume, the appearance of the rat PVH shows much variation depending on the histological plane of section, which is different in every brain. This effect is usually apparent in histological sections as an asymmetric appearance of the nucleus on the right and left side of the third ventricle.

To aid in the traditional histological analysis of PVH structure-function organization, and to encourage application of more quantitative and topological approaches potentially afforded by three-dimensional (3D) computer graphics applications, we have prepared a serial section miniatlas and 3D rendering of the nucleus, and have made the graphics files available online for

* Corresponding author at: Department of Biological Sciences, University of Southern California, Building MC 2520, Room 428 3641 Watt Way, Los Angeles, CA 90089-2520, USA. Tel.: +1 213 740 5892; fax: +1 213 741 0561.
E-mail address: lswanson@usc.edu (L.W. Swanson).

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general use. This is a practical though relatively fine-grained testbed for eventually creating a very high-resolution 3D computer graphics model of the entire rat brain that can be resliced in any plane, based on *Brain Maps: Structure of the Rat Brain* [22].

In the current atlas [22], seven relatively widely spaced, whole-brain sections through the level of the PVH were chosen for illustration (Atlas Levels 21–27; Fig. 1). To generate a high-resolution PVH miniatlas, every thionin-stained serial section through the nucleus was examined (37 sections in total, numbers 251–287). As previously described [22], they are 40 μm-thick transverse sections of a celloidin-embedded brain from an adult male rat (Sprague–Dawley strain, 315 g). PVH parceling of each section was based on the scheme adopted for *Brain Maps* [22]. First, a digital photograph of the PVH region in each section was obtained with a Spot Jr™ camera and 10× objective (Leica™ DMRE microscope). Then the various PVH subdivisions were outlined on digital images of all 37 serial sections in Adobe PhotoShop™, using the seven previously parceled Atlas Levels as starting points. Exact parceling was aided by viewing the actual sections under a microscope next to the computer monitor. The drawn outlines for each photo/section were then imported into separate layers in Adobe Illustrator™, giving a complete stack of 37 serial drawings, arranged from top (rostral, section 251) to bottom (caudal, section 287) in the Layers panel (Figs. S1, S2). Then the drawings were aligned and smoothed.

For PVH miniatlas alignment, the original alignment of the seven Atlas Levels from *Brain Maps* [22] was used unaltered as a starting point and primary reference (Fig. 1). Then, the remaining (intervening) 30 miniatlas levels were aligned with them, and with the rostrally and caudally adjacent Atlas Levels (20 and 28), using manual adjustments agreed on by both authors that pro-

Fig. 1. The paraventricular nucleus (PVH) in the context of a transverse slab of the rat brain (left half), as viewed in a contour model with the rostralmost section in front. The PVH magnocellular neurosecretory division is green, parvicellular neurosecretory division red, and descending division blue. Thicker lines are used for Atlas Level brain outlines (see ventrally, near the scale bar). Abbreviations: ac, anterior commissure; fx, fornix; IVF, interventricular foramen; rf, rhinal fissure; V3, third ventricle; VL, lateral ventricle.

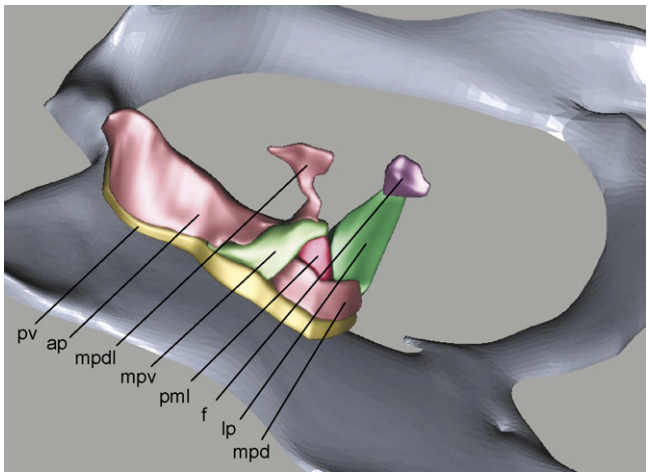

Fig. 2. A 3D PVH surface model viewed from below and the side, with rostral to the left and dorsal to the top. The gray "circle" or torus is the third ventricle, with the central hole representing the midline thalamus (see Fig. 3A). PVH parts: ap, anterior parvicellular; f, forniceal; lp, lateral parvicellular; mpd, dorsal zone of medial parvicellular; mpdl, lateral wing of mpd; mpv, ventral zone of medial parvicellular; pml, lateral zone of posterior magnocellular.

duced the smoothest visual appearance. To place the results in better spatial context, several familiar landmarks were also drawn onto the 37 serial section drawings of the atlas now in the Adobe Illustrator™ Layers panel. These fiducial markers included the outer surface of the brain, ventricular system, and fornix (Fig. 1). Finally, because each tissue section undergoes unique nonlinear distortion during the process of mounting onto a glass slide [21], with a magnitude of up to about 100 μm (unpublished observations, for the sections used here), the contours of each vector drawing were manually adjusted in Adobe Illustrator™ (Fig. S3). This step is necessary to generate smooth surfaces for 2D and 3D models.

A simple 3D contour model of the PVH as viewed in Adobe Illustrator™ layers is shown in Fig. 1, and a surface model rendered in Autodesk Maya™ is shown in Fig. 2. These renderings supercede the qualitative "artist's rendering" published in 1986 [25]. The serial section PVH parcellations can also be used to generate simple though accurate 2D outline projections of the nucleus, for example, as viewed from the top (a dorsal or horizontal projection) or from the side (a lateral or sagittal projection). The results of such an exercise are illustrated in Fig. 3, alone and in the context of a midsagittal view of the rat brain, and they supercede the corresponding qualitative sketches published in 1980 [23].

The exercise of carefully parceling every section through the PVH yielded three modifications of the scheme published in the second edition of *Brain Maps* [20]. First, what had been labeled the caudal tip of the parastrial nucleus on Atlas Level 21 is now identified instead as the rostrolateral tip of the PVH, anterior parvicellular part (PVHap) [22]. Second, the caudal end of the medial magnocellular part could be identified in Atlas Level 24. And third, a very distinctive lateral extension of the medial parvicellular part, dorsal zone (PVHmpd) was identified and labeled the lateral wing of the PVHmpd (PVHmpdl; Fig. 2). Previously [22] it had been assigned to the posterior magnocellular part, lateral zone (PVHpml, Atlas Level 25). However, it does not contain oxytocin- or vasopressin-immunoreactive neurons, but does contain corticotropin-releasing hormone (CRH)-immunoreactive neurons [16], consistent with assignment to the PVHmpd [26]. This thin extension can be traced laterally and rostrally from the main mass of the PVHmpd in eight serial sections. It appears to be innervated by hindbrain noradrenergic fibers (Ref. [11], their Fig. 4C).

Cellular parceling of the rat PVH began in 1927 with Gurdjian [7]. However, the nucleus was discovered in 1901 by Ziehen [28] in the Ring-tailed possum. He provisionally called it the *subcommissural nucleus* and described it as a medial group of large cells. It was first illustrated by Cajal [5] in the guinea pig. He described a unique, large-celled gray matter mass that he called the *subventricular nucleus* and could neither impregnate its neurons with the Golgi method nor determine their connections. The name *paraventricular nucleus* came next, from Malone's [10] description in the adult human, again of large neurons with very condensed Nissl-bodies (rough endoplasmic reticulum). Then Droogleever Fortuyn [6] mentioned it in the rabbit as the *filiform nucleus*, and a year later Nissl [12] described in this species three different parts with neurons remarkable for an internal structure resembling that of neurons in the dorsal motor nucleus of the vagus nerve [8]: a thin *sagittal cell group* adjacent to the third ventricle, a caudolateral and somewhat more dorsal *horizontal extension*, and a *body* in between. Gurdjian [7] also referred to the filiform nucleus in the rat, where he seems to have followed Nissl's basic parceling: a medial small-celled portion intimately associated with the hypothalamic periventricular gray, a lateral portion with medium-sized, closely packed neurons, and a dorsal portion that stands out because of different Nissl-staining characteristics: the neuronal nuclei display more chromatin material.

Variations on these two views (a single magnocellular part vs. three parts) were subsequently applied to a wide range of mammals (see Ref. [23]), the only real advance coming with the demonstration of a Gomori-stained "neurosecretory" projection to the posterior pituitary from large neurons in the PVH and supraoptic nucleus [3]. Then around 1980, three much more detailed parcelings were proposed. Essentially, they were based on a correlation of cytoarchitecture with chemoarchitecture, connections, Golgi impregnations, and function. One scheme with 10 parts only appeared in abstract form [9] and had little subsequent influence. But the one by Armstrong et al. [1], with seven parts, was adopted in Paxinos and Watson's atlas [13], and Swanson and Kuypers's [23] with eight parts has been refined in Swanson's atlas [22]. Both interpretations are now widely used, and supporting literature, along with subsequent modifications, have been reviewed (see Refs. [2,19]). A useful comparison of relevant parceling schemes is found in Armstrong's review [2].

The present work has three obvious implications. First, in terms of the *Brain Maps* atlas [22] it presents a very high-resolution interpretation of PVH boundaries and parceling, which can facilitate more accurate neuroanatomical mapping of experimental results (on the miniatlas) with traditional methods. Second, presentation of the raw and smoothed files (Supplementary Figures/Files S1 and S3, respectively) allows other researchers to use alternate, or develop new more powerful, computer graphics methods to create PVH reconstructions. Such methods might include automatic section alignment and smoothing, reslicing 3D models in any plane, aligning and warping experimental datasets to correspondingly sliced 3D models, 3D data construction and display, and using the 3D model as a Cartesian coordinate system in a spatially indexed database or knowledge management system [4,21]. Third, and finally, this PVH miniatlas may be used as a prototype stimulus for advancing the long term goal of producing a very high-resolution, resliceable 3D computer graphics model of the whole brain and nervous system.

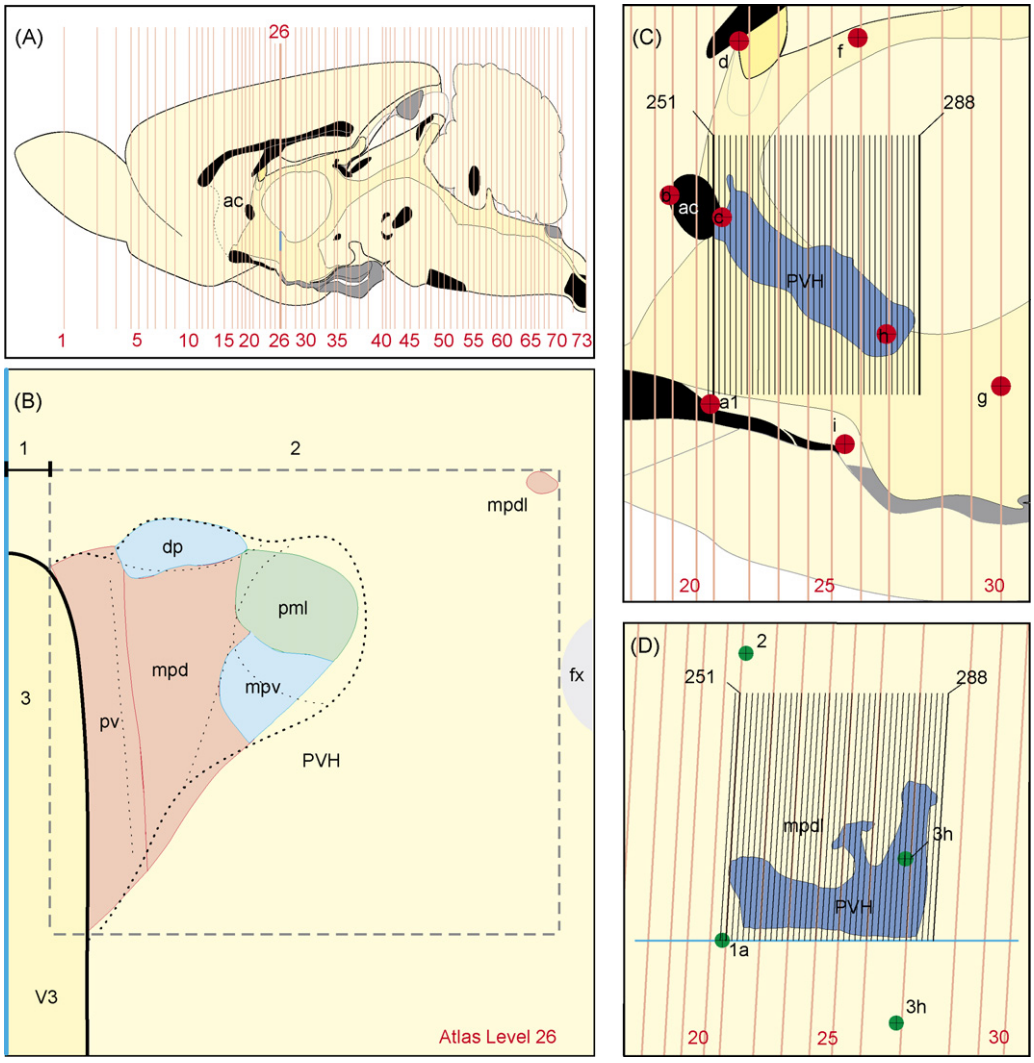

Fig. 3. Two-dimensional projections of the PVH. (A) This midsagittal view of the rat brain shows the 73 Atlas Levels of Brain Maps [22]. (B) This shows the PVH, fornix (fx), and third ventricle (V3) on "classic PVH" Atlas Level 26 (see part A). Colored outlines indicate current parceling, dashed black lines original parceling [22]. The dashed box indicates the total PVH outline from all 37 histological sections (Fig. 1); 1 is the distance from midline, 2 the total width, and 3 the total height. (C) This shows a lateral or sagittal projection of the PVH outer border (purple) as determined from the heights of 37 serial histological sections (251–288). Red dots are fiducial marks for measuring the angle of experimental brain sections as compared to this reference brain (see Ref. [22], CD-ROM). (D) This shows a dorsal or horizontal projection of the PVH outer border (purple), as determined from section widths and distance from midline, similar to part C. Note that the histological sections were not cut precisely perpendicular to the brain's longitudinal axis, but at a slight (4°) angle. Green dots are fiducial marks, as in part C. Other abbreviations: ac, anterior commissure; dp, dorsal parvicellular part; mpd, dorsal zone of medial parvicellular part; mpdl, lateral wing of mpd; mpv, ventral zone of medial parvicellular part; pml, lateral zone of posterior magnocellular part.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neulet.2008.04.057.

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