VII. Materials and Methods

Embryos were obtained from timed-pregnant Sprague-Dawley rats. *The time of insemination was considered the beginning of embryonic day 0* (e0), and all times given in the literature were adjusted to this convention.

Pregnant rats were deeply anesthetized with sodium pentobarbital (120 mg / kg) and the uterus was surgically removed and quickly placed in ice-cold saline solution. The embryos were then removed from the uterus, membranes, and so on; photographed; and placed separately in a 4% solution of paraformaldehyde in phosphate buffer, with 10% sucrose; and stored a 4°C. A day or two later, the embryos (n=113) were washed briefly in saline solution and oriented in plastic containers filled with a 1 : 1 mixture of Tissue-Tek (Miles Inc., Elkhard, IN) and Immu-mount (Shandon, Pittsburgh, PA). The containers were then dipped in dry ice-cooled hexane until completely frozen. The resulting blocks were placed in air-tight plastic bottles and stored at -70°C until sectioned. Serial sections through the embryos were obtained in a cryostat and collected on poly-L-lysine-subbed slides. Most of the series consisted of 20 µm thick sections, although some were cut at 10, 15, or 30 µm.

For this atlas, serial frozen sections were obtained from 80 embryos (10-15 embryos from e10-e15, e17). In addition, a series of paraffin-embedded rat embryos (e17-e20) was available for examination (embedded using standard methods). For illustrating e10-e17, frozen sections were used because they show the appearance of the developing nervous system in the type of material most commonly employed for *in situ* hybridization and immunohistochemistry. However, a paraffin-embedded brain was used to illustrate e20 because the histology was so much better than in comparable frozen sections.

Histological sections were stained with cresyl violet (Simmons and Swanson 1993) to reveal cytoarchitectonic features. The sections were photographed with Kodachrome 25 professional film and a Wild M3Z stereomicroscope. The resulting chromes were scanned at 400 dpi with a Nikon LS-3500 SR1 film scanner, and the files imported into Adobe Photoshop 3, where they were composed. They were then placed in the bottom layer of an Adobe Illustrator 5 file, and traced to produce drawings, guided by direct microscopic observation of the histological sections.