DHTP: 39.8 ± 8.0 filled cells; $P > 0.40$, matched-pairs t-test]. The ratio of motoneurons innervating muscle fibers is similar in the two groups and cannot explain the unusual condition of the DHTP-treated females.

On one feature, the anatomical location of the HRP-filled motoneurons, DHTP and TP females were drastically different. In adult females given TP prenatally, most motoneurons innervating the BC were found in the same location as in males: the medial motoneuronal column normally occupied by the SNB (Figs. 1 and 2). However, the BC of females treated with DHTP was innervated by motoneurons in an anomalous, lateral location—the DLN motoneuronal column 500 μm away (Fig. 2). HRP injections into male BC virtually never labeled lateral motoneurons (Fig. 2). Thus, TP produces females with BC muscles primarily innervated by the same motoneuron column as in males, the medial SNB pool. But DHTP produces females with muscles innervated by an anatomically different and, relative to males, abnormal motoneuronal pool.

Vertebrate motoneurons innervating a given muscle are organized in rostrocaudally oriented columns occupying the same spinal location with consistency across individuals (*8*), making the neuromuscular system a valuable model of the specificity of connections in the nervous system (*9*). However, to my knowledge, no one has completely accounted for the development of this specificity. These results represent an example of the effect of manipulation of an identified, naturally occurring developmental cue (androgen) on the pattern of neuromuscular connectivity. Testosterone is the principal androgen produced by rat testes, and dihydrotestosterone is the major active metabolite in the periphery (*10*). The different configurations of innervation produced by the two androgens suggest that the normal pattern of connectivity in the SNB system may be determined by relative contributions of the two hormones, which is determined by the activity of 5α-reductase (E.C.1.3.99.5), the enzyme controlling the irreversible conversion of testosterone to 5α-dihydrotestosterone (*10*). The activity of 5α-reductase varies considerably across rat brain regions (*11*), and there is evidence of variable reductase concentrations in motoneuronal nuclei of the rat lumbar spinal cord (*12*). Development of the normal innervation pattern by the SNB may require the regional inhibition of testosterone's conversion to dihydrotestosterone, which would be circumvented by the direct administration of DHTP. Prenatal DHTP treatment could accomplish this unique innervation pattern by altering the migration, death, or axonal outgrowth of developing motoneurons. By whichever mechanism such disparate results are accomplished, they represent a unique example of alterations in the pattern of motoneuron-to-muscle innervation through the use of normally relevant developmental cues. An understanding of how this anomalous pattern comes about may elucidate mechanisms by which normal innervation patterns are achieved.

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Expression of Myelin-Associated Glycoprotein by Small Neurons of the Dorsal Root Ganglion in Chickens

Abstract. *Biochemical and immunocytochemical investigations have shown that myelin-associated glycoprotein (MAG) is exclusively related to myelin and myelin-forming cells in mammals. In the present study it was found that dorsal root ganglia in young chickens display MAG-immunoreactive material in most small sensory neurons. The presence of MAG at the surface of small sensory neurons raises the question of whether this glycoprotein acts as a cell adhesion molecule in lower vertebrates.*

Myelin-associated glycoprotein (MAG), an intrinsic glycoprotein of high molecular weight, is specifically located in myelinating oligodendrocytes but not in neurons, astrocytes, or other cells of the central nervous system in mammals (*1–3*). In rat peripheral nerves antiserum to MAG stains myelinating Schwann cells and the periaxonal regions of myelinated nerve fibers but not trigeminal ganglion cells (*3*). Hence it has been postulated that MAG may play a key role in starting myelinogenesis before compaction of the lamellae (*1–3*).

We used a rabbit antiserum raised against MAG purified from rat myelin (*4*) as an immunocytochemical probe of the dorsal root ganglia (DRG) of 5- to 7-day-old chickens. A first series of experiments was carried out with light microscopic immunocytochemical methods (*5*). Except for the expected reaction in myelinating Schwann cells (Fig. 1A), small ganglion cell bodies located mainly in the mediodorsal region of the DRG displayed the strongest immunostaining (Fig. 1A). Most of the small ganglion cells reacted with antiserum to MAG, whereas the large ganglion cells were free of any staining (Fig. 1B). In the small neurons the immunoprecipitate extended throughout the whole perikaryon, with strong accumulation in cytoplasmic areas suspected to correspond to the Golgi apparatus (Fig. 1B). This location was confirmed by electron microscopy (Fig. 1C) with a preembedding immunocytochemical procedure (*5*). The immunoreactive precipitates were related to cisternal membranes of the rough endoplasmic reticulum and concentrated in the saccules of the Golgi apparatus and in a few vesicles (Fig. 1C). Furthermore, reactive products were located at the cell surface of small ganglion cells (Fig. 1, B and D). The presence of MAG-immuno-

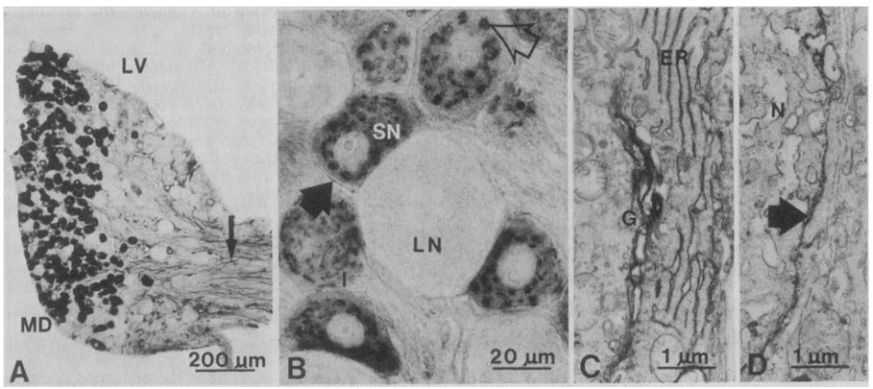

Fig. 1. Localization of MAG-immunoreactive material in small sensory neurons of chicken DRG. (A) Immunostained small ganglion cell bodies are clustered mainly in the mediodorsal region (MD), whereas the unstained large sensory neurons occupy mainly the lateroventral region (LV). As expected, myelinating Schwann cells and myelinated nerve fibers display MAG immunostaining (arrow). (B) The small sensory neurons (SN) accumulate the MAG-immunoreactive material in the Golgi apparatus (open arrow). Beside the faint staining of the outer cytoplasm, the reaction located at the neuronal cell surface (closed arrow) may occasionally be seen. The large neurons (LN) remained unstained. (C and D) Electron microscopic immunocytochemistry confirms the high MAG density of immunoprecipitates in cisternal membranes of the rough endoplasmic reticulum (ER) and stacks of Golgi saccules (G). Note the immunostaining coating the cell surface (arrows in B and D) of the neuron (N).

reactive components at the surface of small ganglion cells could create new conditions of interaction between these neurons and their cellular environment.

The specificity of the immunostaining is indicated by the following: (i) no immunoreaction occurred in DRG when the MAG antiserum was substituted by normal rabbit serum, (ii) after treatment of tissue with chloroform-methanol to extract gangliosides and other glycolipids (*4*) the immunoreaction with MAG antiserum was unchanged, and (iii) in mice DRG stained with MAG antiserum the reaction occurred in myelinating Schwann cells but not in ganglion cells. Immunoblotting (*6*) was used to analyze chicken DRG extracts. The immunoblots were compared with those of rat brain and rat and chicken sciatic nerve (Fig. 2, A to C). MAG–cross-reacting material recovered in chicken DRG (Fig. 2D) comigrated with the same apparent molecular weight (130 kD) as in chicken sciatic nerve (Fig. 2C). Hence chicken DRG contain MAG-immunoreactive macromolecules similar to those in chicken peripheral myelinated nerve fibers but different from those in rat nervous tissues. In other words, both neurons and myelinating Schwann cells in the chicken may synthesize identical MAG-immunoreactive macromolecules. These macromolecules could correspond to MAG or to closely related molecules sharing with MAG some of its antigenic sites.

The neuronal origin of the MAG-immunoreactive material coating the cell surface of small ganglion cell bodies was assessed by the presence of immunoprecipitates in the rough endoplasmic reticulum and Golgi apparatus. The unexpected presence of plasma membrane–bound MAG-immunoreactive material in small sensory neurons of chickens but not of rats (*3*) and mice raises the question of whether MAG could accomplish functions other than myelinogenesis in birds. In the avian peripheral nervous system MAG could be an ancestral molecule still expressed in different cell types, while the mammalian MAG could be a novel form whose role is restricted to myelinogenesis. The location of MAG-immunoreactive macromolecules at the cell surface could limit cell movements and stop the migration of small neurons in the mediodorsal region of the chicken DRG. If MAG acts as a "cell-adhesion molecule" (*8*) in the chicken, it could prevent the mixing of ganglion cells that occurs in mammals. The finding that some clusters of cells displaying MAG immunoreactivity are also found in other neural crest derivatives, such as sympathetic ganglia and adrenal medulla (*9*), further supports this hypothesis.

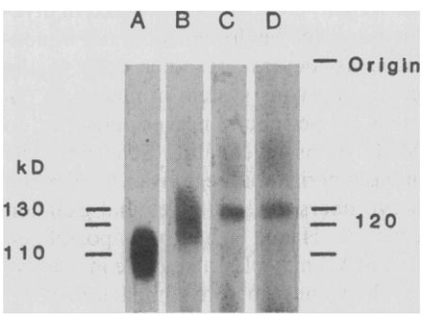

Fig. 2. Autoradiograph of an immunoblot obtained with the same antiserum to MAG (*4*). The radioactive spot visualizes the polypeptide band-binding MAG antiserum. In rat brain myelin (A) and rat sciatic nerve (B) the band is located at a molecular weight corresponding to 110 and 120 kD, respectively (*7*). In the chicken a 130-kD band is found in the sciatic nerve (C). The chicken DRG (D) display a pattern of MAG-binding which is identical to that observed in the sciatic nerve.

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5. Chickens 5 to 7 days old were fixed by perfusion with $HgCl_2$ and formaldehyde (*3*). Lumbosacral DRG were sectioned with a Vibratome. The 20-µm thick sections (Fig. 1, A and B) were stained with 1:250 MAG antiserum by the unlabeled peroxidase-antiperoxidase method [L. A. Sternberger, P. H. Hardy, Jr., J. J. Cuculis, H. G. Meyer, *J. Histochem. Cytochem.* **18**, 315 (1970); N. H. Sternberger, Y. Itoyama, M. W. Kies, H. deF. Webster, *J. Neurocytol.* **7**, 25 (1978)]. Electron microscopic immunocytochemistry was performed on slightly teased DRG after perfusion with 0.5 percent formaldehyde and 0.002 percent glutaraldehyde in phosphate buffer. After immunostaining, small pieces of ganglia were embedded in Epon.
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