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# A Monoclonal Antibody That Recognizes Somatic Motor Neurons in the Mature Rat Nervous System

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**In order to obtain markers selective for motor neurons, an *in vitro* immunization was carried out using a crude homogenate of embryonic rat ventral spinal cord. We have generated a monoclonal antibody, MO-1, that binds selectively to the cell bodies of somatic motor neurons in the brain stem and spinal cord of the adult rat nervous system. In a survey of both peripheral and central nervous systems, intense labeling by MO-1 appears exclusive to this class of cholinergic neuron. Immunoreactivity is predominantly intracellular and is detectable within the somata as well as the proximal regions of processes but is absent along fiber tracts and at neuromuscular junctions. This staining pattern indicates that MO-1 does not recognize other molecules known to be present in motor neurons, such as choline acetyltransferase, acetylcholinesterase, agrin, or the calcitonin-gene-related-peptide. In the spinal cord, antibody binding begins to be detectable in motor neurons late in development, during the second postnatal week. Thus, MO-1 appears to recognize a novel cellular component that accumulates in somatic motor neurons during terminal stages of differentiation.**

Studies on neuronal development have traditionally focused on morphological and physiological changes as well as on the expression of neurotransmitters and transmitter synthesizing enzymes. In recent years, investigations on cell lineage and differentiation have been greatly facilitated by the use of markers that identify select cell types and cells at different stages of development. The use of antibodies that define specific populations has now permitted detailed analyses of progenitor-progeny relationships in glial (Lillien et al., 1988; Miller et al., 1989) and sympathoadrenal lineages (Carnahan and Patterson, 1988; Anderson, 1989).

The aim of our experiments was to obtain monoclonal antibodies that can selectively distinguish somatic motor neurons from other neuronal populations. Such markers are desirable for 2 reasons. First, *in vitro* studies of motor neurons have been hampered by difficulties in identifying this class of neuron (for example, see O'Brien and Fischbach, 1986; Smith et al., 1986). Currently available markers that are known to recognize motor neurons also bind to other types of neurons (see below) and therefore cannot be used to identify motor neurons unambiguously. Second, antibodies that recognize molecules and epitopes unique to motor neurons will be important in studies on the biochemical characterization and differentiation of this cell type. Motor neurons are among the best studied of neuronal populations. Their patterns of birth (Nornes and Das, 1974; Phelps et al., 1988), death (Hamburger, 1975; Williams et al., 1987; for review, see Oppenheim, 1981), axonal growth (for review, see Landmesser, 1980), and synapse formation (for review, see Dennis et al., 1981) have been extensively investigated in a number of vertebrate species, both *in vivo* and *in vitro*. However, little is known about how and when motor neurons become biochemically specialized because relatively few molecules unique to these cells have been characterized.

Of the molecules known to be present in motor neurons, the best studied is the transmitter synthesizing enzyme, choline acetyltransferase (CAT). Since motor neurons use ACh as a neurotransmitter, they produce CAT and immunoreactivity to the enzyme is detectable in rat spinal cord by the 13th day of gestation (Phelps et al., 1989). In addition, motor neurons bear a number of other molecules known to be present in cholinergic neurons including acetylcholinesterase, and a group of cholinergic-specific gangliosides (Richardson et al., 1982; Obrocki and Borroni, 1988). However, all of these molecules are common to, and therefore do not distinguish between, different groups of cholinergic neurons.

A second group of molecules includes cell surface, cytoplasmic, and cytoskeletal antigens shared by motor neurons and certain noncholinergic populations (Miller and Benzer, 1983; Tanaka and Obata, 1984; Hinton et al., 1988; Stephenson and Kushner, 1988; Zaremba et al., 1989). Polyclonal antibodies to the calcitonin-gene related peptide (CGRP) react strongly with motor neurons in both cranial motor nuclei (Takami et al., 1985) and spinal cord in mammals (Gibson et al., 1984) and in avians (New and Mudge, 1986; Villar et al., 1988). In addition, anti-CGRP stains small sensory neurons within the dorsal root ganglion as well as other populations of neurons in the brain (Kruger et al., 1988). A neuron-specific, 57 kDa intermediate filament protein has also been described in somatic motor neurons of cranial motor nuclei and spinal cord and in sensory neurons of dorsal root ganglia (Leonard et al., 1988; Parysek and Goldman, 1988). Finally, an endogenous lactose-binding lectin, RL14.5, is selectively expressed in spinal motor neurons and sensory neurons (Regan et al., 1986). While these markers are all present in motor neurons, none are exclusive for this class of neuron.

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The results of the above studies indicate that antibodies showing a strict specificity for somatic motor neurons are rare. McMahan and colleagues have generated antibodies against agrin, a molecule that is present at the neuromuscular junction (Reist et al., 1987) and can induce the aggregation of a number of postsynaptic molecules in cultured myotubes (Nitkin et al., 1987). Anti-agrin recognizes only motor neurons in sections of *Torpedo,* frog, and chick spinal cord (Magill-Solc and McMahan, 1988). However, it is not currently known if any other neuronal population outside the spinal cord also bears immunoreactivity for agrin.

Since motor neurons are among the first neurons to be born and to differentiate within the spinal cord (Nornes and Das, 1974; Phelps et al., 1988, 1989), we used a crude homogenate of the ventral half of embryonic spinal cord to raise monoclonal antibodies selective for motor neurons. In the present study, we describe a monoclonal antibody, MO-1, that binds to somatic motor neurons in the adult rat brain and spinal cord but does not recognize other cholinergic neurons. In fact, with the exception of light immunoreactivity seen in neurons of the red nucleus, MO-1 recognizes an epitope that is detectable only in somatic motor neurons and demonstrates that motor neurons are biochemically distinct from all other neuronal populations.

## Materials and Methods

*Animals.* Adult Sprague-Dawley rats of both sexes, weighing 250–350 gm, and pregnant females were obtained from Charles River Laboratories (Wilmington, MA). Animals were sacrificed with an overdose of carbon dioxide, and their tissues and embryos were removed and used for preparation of the antigen mixture or immunohistochemical analyses. No detectable difference was found between male and female animals in immunohistochemistry.

*Production of monoclonal antibodies.* The immunogen was prepared from spinal cords dissected from embryos at 14–15 d of gestation (E14–15). The ventral halves of embryonic spinal cords were harvested and homogenized in ice-cold PBS (154 mM sodium chloride, 10 mM sodium phosphate, pH 7.4) with phenylmethylsulfonyl fluoride (Sigma, St. Louis, MO); then stored at −20°C until used. *In vitro* immunization was performed according to the method of Boss (1986). Briefly, spleen cells were collected from naive BALB/c mice and cultivated at $1 \times 10^7$ cells/ml with the immunogen mixture at a concentration of 14 μg protein/ml in RPMI Medium 1640 (Irvine Scientific, Santa Ana, CA) containing 20% fetal calf serum, 20 μg/ml *N*-acetylmuramyl-L-alanyl-D-isoglutamine (Calbiochem-Behring, San Diego, CA), $5 \times 10^{-5}$ M 2-mercaptoethanol (Sigma, St. Louis, MO), 2 mM glutamine (Irvine Scientific, Santa Ana, CA), and 1 mM sodium pyruvate (Irvine Scientific, Santa Ana, CA). After 4 d of incubation at 37°C, the spleen cells were harvested and fused with SP2/0 myeloma cells with 50% polyethleneglycol 5000 in 75 mM HEPES, pH 7.4 (Boehringer Mannheim, Indianapolis, IN). Fused cells were plated into 96 well plates and grown in HAT medium constituted in RPMI. Culture supernatants were screened by immunohistochemical staining of adult and embryonic spinal cord sections, and hybridomas of interest were subcloned by limiting dilution.

*Immunohistochemistry.* Adult and embryonic tissues were frozen in OCT embedding medium (Miles, Elkhart, IN) on dry ice and stored at −80°C until cut. Sections 10 μm thick were cut on a cryostat at −15°C, picked up on gelatin-coated slides and air-dried for at least 30 min. Sections were often stored for up to 6 months in dessicated slide boxes at −20°C until used.

Fresh-frozen, 10-μm-thick sections of E14 and adult spinal cords were directly incubated with culture media for screening hybridoma supernatants. For studies on MO-1, the sections were briefly dipped (5–10 min) in acetone at room temperature for better preservation of tissues, then rinsed for 30 min in PBS containing 10% normal goat serum prior to incubation with antibodies.

Tissue sections were first incubated with primary antibodies for 1–2 hr at room temperature or overnight at 4°C and then given three 10 min rinses in PBS. Three different secondary antibodies were employed to visualize sites of monoclonal antibody-binding: hi-fluorescent goat anti-mouse Ig (Antibodies Inc., Davis, CA) for screening hybridoma supernatants and goat anti-mouse IgM conjugated with FITC or with HRP (Chemicon, El Segundo, CA) for MO-1 and MO-2 experiments. To reduce binding to nonspecific rat epitopes, secondary antibodies were diluted and preincubated for 1 hr at room temperature with PBS containing 10% normal rat serum, then centrifuged at 50,000 rpm in a Beckman TL-100 ultracentrifuge for 20 min prior to use. Sections were next incubated with secondary antibodies for 1 hr at room temperature and rinsed in PBS. Fluorescein-labeled sections were mounted with 0.2% *p*-phenylenediamine (Sigma, St. Louis, MO) in 100 mM sodium bicarbonate, pH 9, and 50% glycerol to reduce quenching (Valnes and Brandtzaug, 1985). When HRP-conjugated secondary antibodies were used, sections were incubated in diaminobenzidine tetrahydrochloride (DAB, Dojin Chemicals, Japan; 150 μg/ml in 50 mM Tris, pH 7.4, 0.03% $H_2O_2$) for 2–5 min to visualize HRP-labeled sites. The reaction was terminated by rinsing in distilled water before mounting in Permount (Fisher Scientific, Springfield, NJ).

For CAT immunohistochemistry (Houser et al., 1983), freshly cut sections were immediately fixed in 4% paraformaldehyde (Polysciences, Inc., Warrington, PA) in 120 mM phosphate buffer, pH 7.3, for 1 min, followed by a 30 min rinse in PBS. A second rinse in 100 mM Tris with 240 mM sodium chloride, pH 7.3 containing 10% normal goat serum (TBS-NGS) for 30 min blocked nonspecific binding sites. The sections were then incubated with purified 1E6, a monoclonal antibody against choline acetyltransferase (10 μg/ml in TBS-NGS; Crawford et al., 1982) for 2 hr at room temperature or overnight at 4°C. Slides were next incubated with goat anti-mouse IgG (1:120 in TBS-NGS; American Qualex, Inc., La Mirada, CA) for 1 hr followed by mouse antiperoxidase-HRP complex (PAP, 1:120 in TBS-NGS; Sternberger-Meyer Immunochemicals, Inc., Jarretsville, MD) for another hour. To increase the intensity of the signal, the goat anti-mouse antibody and the PAP steps were repeated. Finally, the sections were reacted with DAB and mounted for viewing. To reduce nonspecific binding, TBS containing 0.1% BSA (Sigma, St. Louis, MO) was used for rinsing in between incubation steps.

We referred to the Atlas of Rat Brain (Paxinos and Watson, 1986) for identification of structures within the rat brain and spinal cord.

## Results

### *MO-1 recognizes somatic motor neurons in the spinal cord and brain stem*

The fusion produced 576 hybridomas that secreted antibodies, and these were subsequently screened for immunoreactivity on fresh frozen sections of adult and embryonic spinal cord. One clone, MO-1, produced antibodies of the IgM class that reacted with the somata of large neurons in the ventral horn in adult rat spinal cords (Fig. 1*B*). Immunoreactivity was detectable only in the cell bodies and proximal region of processes and was excluded from nuclei. The size and location of these cells indicated that they were somatic motor neurons. This was confirmed when the same ventral horn neuronal population, in an adjacent section, was stained with 1E6, a monoclonal antibody to the ACh synthesizing enzyme, CAT (Fig. 1*C*). A second population of CAT-positive cells was found in the intermediolateral columns of thoracic spinal cord (Fig. 1*C*) and consists of preganglionic neurons that innervate sympathetic ganglia in the peripheral nervous system (Barber et al., 1984). These cholinergic neurons were not recognized by MO-1 (Fig. 1*B*).

Motor nuclei in the adult rat brain stem were also examined for MO-1 reactivity. In 10 μm coronal sections of the midbrain, neurons of the oculomotor nucleus (motor nucleus III, Fig. 2) were strongly immunoreactive for MO-1. This motor nucleus innervates the extrinsic muscles of the eye. In the same field, neurons of the red nucleus showed low levels of staining. In more caudal sections, neurons of the trigeminal motor nucleus (motor nucleus V, Fig. 3, *A–C*), the abducens nucleus (motor nucleus VI, Fig. 3, *D–F*) and the motor facial nucleus (motor nucleus VII) all displayed immunoreactivity for MO-1 and CAT. Motor nucleus V innervates the striated muscles controlling mastication. A small population of noradrenergic neurons (Fig.

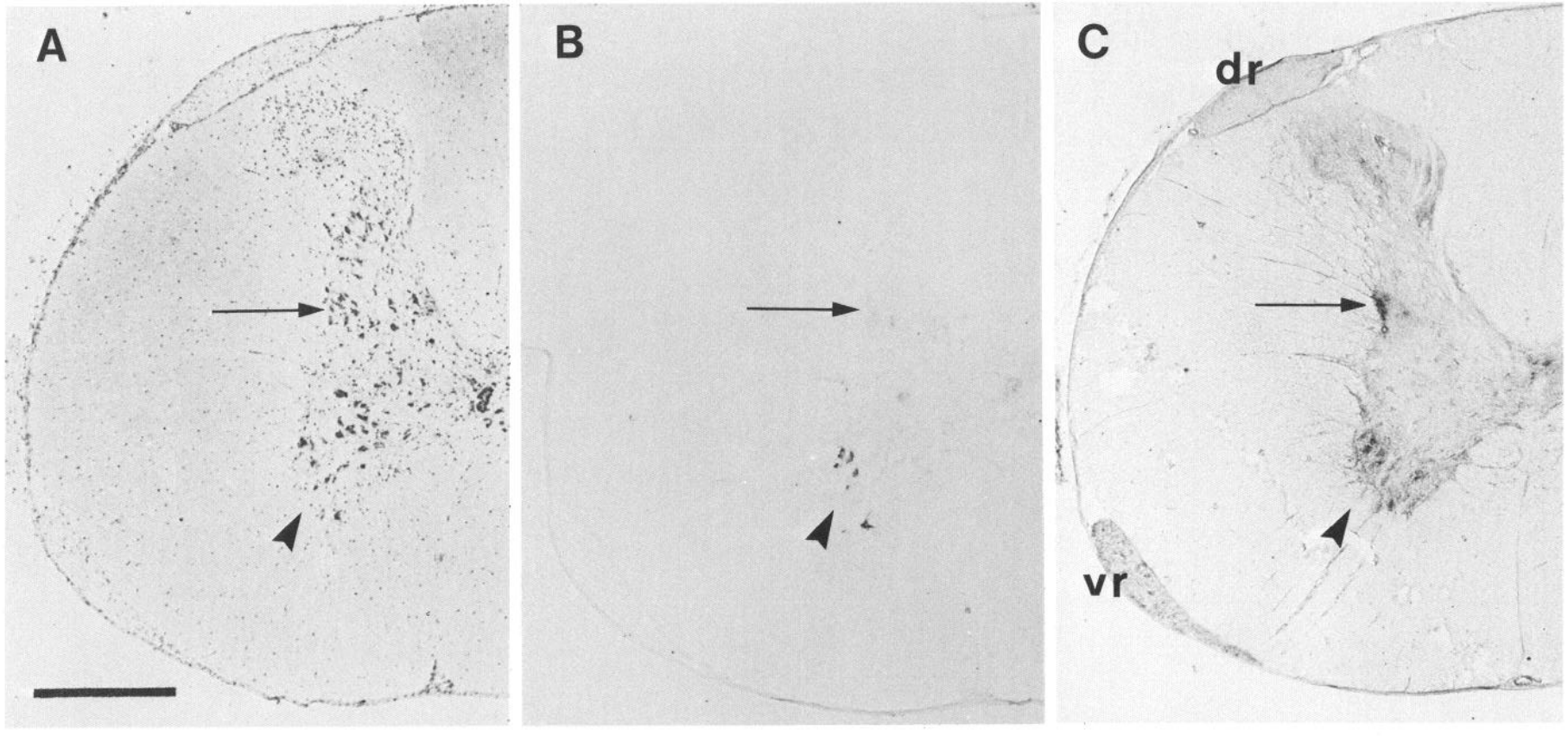


*Figure 1.* MO-1 recognizes somatic motor neurons but not preganglionic neurons in thoracic spinal cord. Consecutive cryostat sections were Nissl-stained (*A*) or reacted with monoclonal antibodies MO-1 (*B*) or 1E6 (*C*) and peroxidase-labeled secondary antibodies. Somatic motor neurons (*arrowhead*) in the ventral lateral column are stained by both antibodies (*B, C*). Cholinergic preganglionic neurons in the intermediolateral column (*arrow*) only react with anti-CAT (*C*). The ventral root (*vr*), containing motor neuronal axons, is also stained by 1E6, but the dorsal root (*dr*) is not. Scale bar, 500 $\mu$m.

3A) located at the lateral margin of this nucleus was not recognized by either MO-1 or 1E6 (Fig. 3, B, C). The mesencephalic trigeminal nucleus, which receives proprioceptive signals from muscle spindles in the masticatory muscles, was also not stained by MO-1 (not shown). Motor nucleus VI provides innervation of the lateral rectus muscle of the eye, whereas neurons in motor nucleus VII synapse upon the mimetic muscles of the face. In each motor nucleus, the somatic motor neurons were CAT-positive, and appeared strongly and selectively stained by monoclonal antibody MO-1. Fiber tracts of cholinergic neurons, such as the facial nerve traversing lateral to motor nucleus VI, were also revealed by antibodies to CAT but were not stained with MO-1 (Fig. 3, E, F).

Sections containing both motor nuclei X and XII (the dorsal motor nucleus of the vagus and the hypoglossal nucleus, respectively) were also examined for sites of 1E6 and MO-1 binding. Motor nucleus X consists of preganglionic neurons that innervate the peripheral parasympathetic nervous system. Motor nucleus XII, on the other hand, is made up of motor neurons that directly innervate the tongue musculature. Although both populations of motor neurons are cholinergic and reacted with 1E6 (Fig. 4C), MO-1 only recognized the hypoglossal motor neurons (Fig. 4B). At this level of the brain stem, some neurons in the nucleus ambiguus were also found to be strongly immunoreactive for both MO-1 and CAT (not shown). This small, laterally located population of motor neurons synapses on the striated muscles of the pharnyx, larnyx, and esophagus.

Thus, in surveying different populations of somatic motor neurons in the brain stem and spinal cord of adult rats, we find that MO-1 consistently reacted with this class of cholinergic neurons. Furthermore, when we compared consecutive sections stained by the Nissl method, with MO-1 and 1E6, virtually all somatic motor neurons appeared to be immunoreactive for MO-1.

### *Selectivity of MO-1 for somatic motor neurons*

Since MO-1 appeared to bind selectively to somatic motor neurons in every motor neuronal pool examined within the adult rat nervous system, the question then arose as to whether it is an exclusive marker for this class of neurons. Although all MO-1-positive cells are also cholinergic, not all cholinergic neurons were recognized by MO-1. Preganglionic neurons innervating the peripheral autonomic nervous system, such as those in the intermediolateral column of the spinal cord (Fig. 1) and the dorsal motor nucleus of the vagus (Fig. 4), were stained by 1E6 but not MO-1. Other populations of cholinergic neurons in the CNS, including those in the basal forebrain, were also not recognized by MO-1. In the PNS, parasympathetic postganglionic neurons are known to use ACh as a neurotransmitter. Cryostat sections of the rat ciliary ganglion showed no immunoreactivity for MO-1. Similarly, a subset of peripheral sympathetic neurons originating from the stellate ganglia innervate the sweat glands of the rat footpads and acquire a cholinergic phenotype during development (Landis et al., 1988). However, no immunoreactive cells were ever found in sections of adult stellate ganglia stained with MO-1.

Another monoclonal antibody of the IgM class, MO-2, stained motor neurons and peripheral neurons (Urakami and Chiu, unpublished observations). When neighboring sections of the superior cervical ganglion were reacted with MO-1 and MO-2, only the latter revealed immunoreactive cells (Fig. 5B). MO-1 showed no staining above background (Fig. 5A). Similarly, MO-1 did not react with neurons in dorsal root ganglia. We have not found any neurons in the PNS that are immunoreactive for MO-1.

Since somatic motor neurons generally have large cell bodies, and such cells seem to be especially susceptible to spurious immunocytochemical staining, we examined other populations of large neurons in the rat nervous system for MO-1 immunoreactivity. Purkinje cells of the cerebellum are known to be immunoreactive to many antibodies, but they were not recognized by MO-1. Neither were neurons of the deep cerebellar nuclei. Sensory neurons, such as those of the mesencephalic trigeminal nucleus or the dorsal root ganglion, also possess large somata, but MO-1 did not react with these neurons. Large cells in the red nucleus did show low levels of MO-1 immunoreactivity that appeared higher than background, but this was qualitatively different from the intense staining seen in somatic motor neurons (Fig. 2A). Thus, MO-1 appears to be very selective for somatic motor neurons in the adult rat nervous system.

### *Developmental appearance of MO-1 immunoreactivity in the rat spinal cord*

In order to find out where and when MO-1 immunoreactivity first appears during development, sections of embryonic and neonatal rat spinal cords were examined. Somatic motor neurons were the only cells recognized by this antibody in the developing spinal cord. Antibody binding was first detectable late in development, on postnatal day 8, in sections taken at the brachial level of the spinal cord (Fig. 6D). Staining was very faint at this stage but increased in intensity with age so that by postnatal day 28, motor neuronal staining had risen to adult levels. Thus, in the spinal cord, MO-1 retains its specificity for motor neurons throughout development. Furthermore, this epi-

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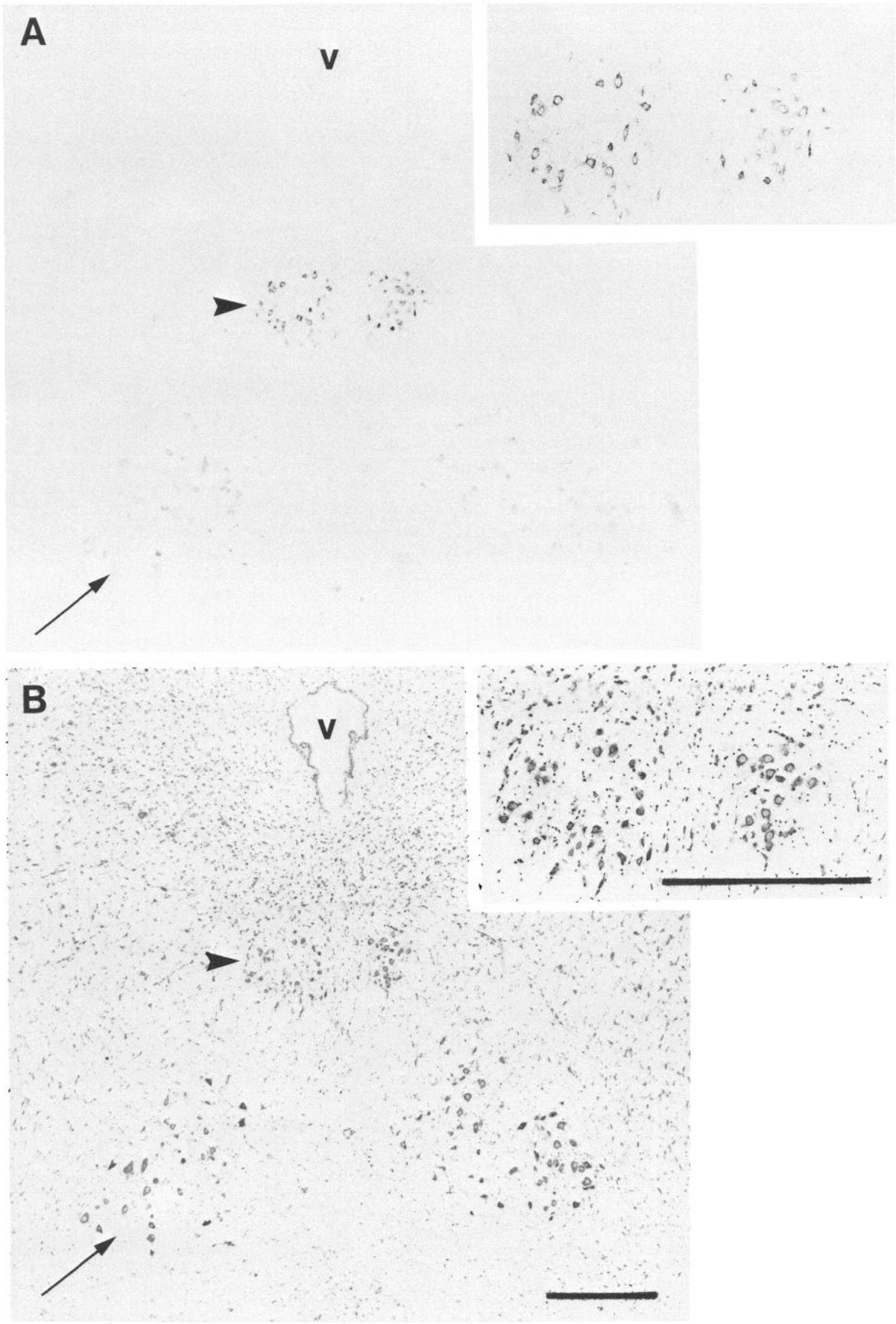
*Figure 2.* MO-1 stains motor neurons much more strongly than neurons of the red nucleus. Adjacent 10 μm coronal sections of the rat midbrain stained with MO-1 (*A*) or by the Nissl method (*B*) revealed that neurons of the oculomotor nucleus (motor nucleus III, *arrowhead*) are more intensely stained by MO-1 than those of the red nucleus (*arrow*). *Inserts* show the oculomotor nuclei at higher magnification. *v*, ventricular aqueduct. Scale bars, 500 μm.

*Figure 3.* Cholinergic neurons in motor nuclei V and VI are immunoreactive for MO-1. Neighboring sections of the trigeminal motor nucleus (motor nucleus V, *A–C*) and the abducens nucleus (motor nucleus VI, *D–F*) were Nissl stained (*A, D*) or reacted with MO-1 (*B, E*) or 1E6 (*C, F*). Cholinergic neurons in both motor nuclei are immunopositive for MO-1 (*B, E*) and 1E6 (*C, F*). In addition, a few neurons belonging to the accessory motor nucleus of the facial nerve (*open arrow, D–F*), which innervates the digastric muscle (Szekely and Matesz, 1982), are immunopositive for both antibodies. However, a small population of noradrenergic cells at the edge of motor nucleus V is not recognized by either antibody (*arrow, A–C*). The fiber tract of the 7th nerve (*asterisk, D–F*) is strongly CAT-immunoreactive (*F*) but does not show up with Nissl stain or MO-1. *Arrowhead* (*D-F*), motor nucleus VI. Scale bars, 500 μm.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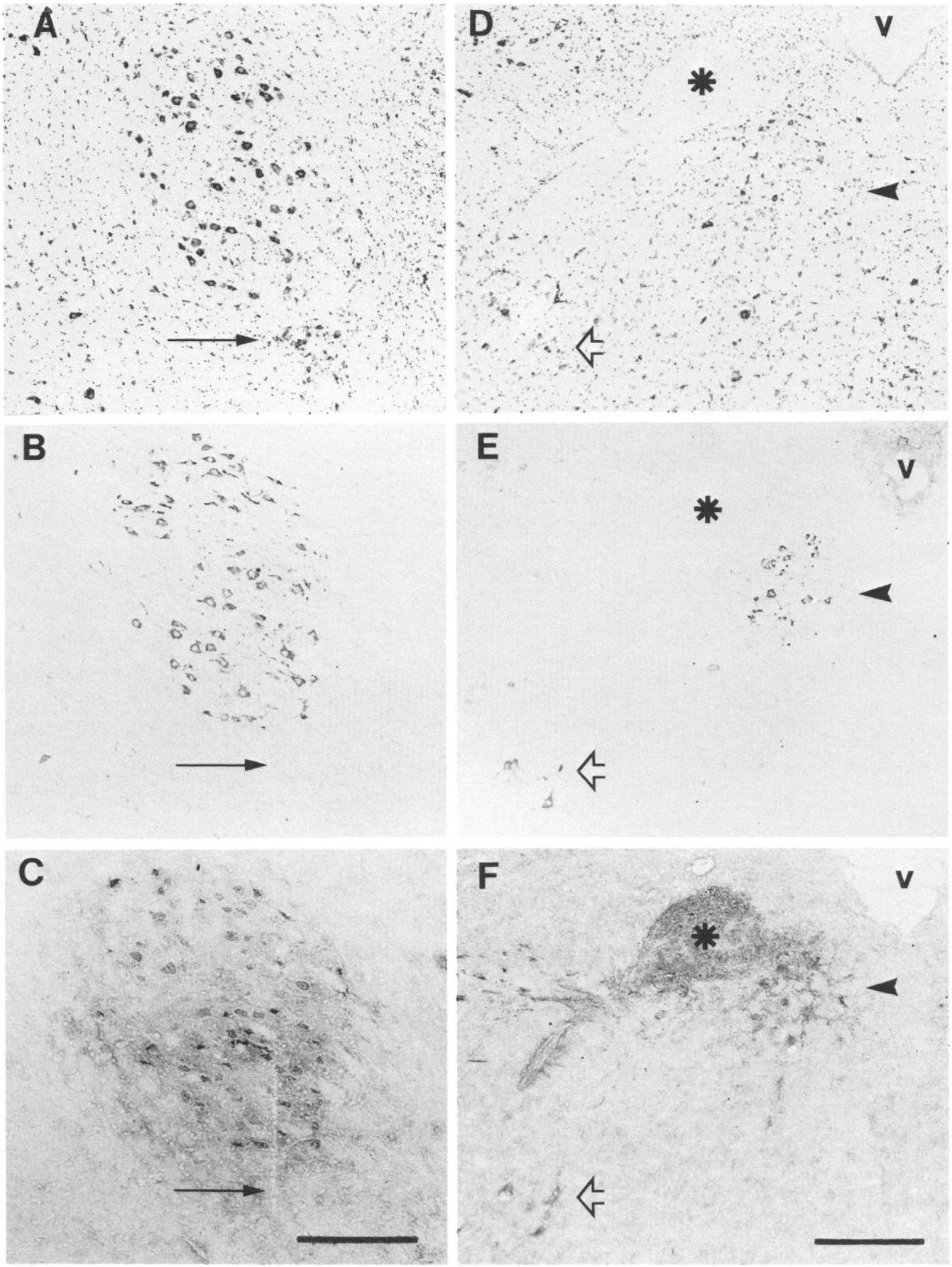
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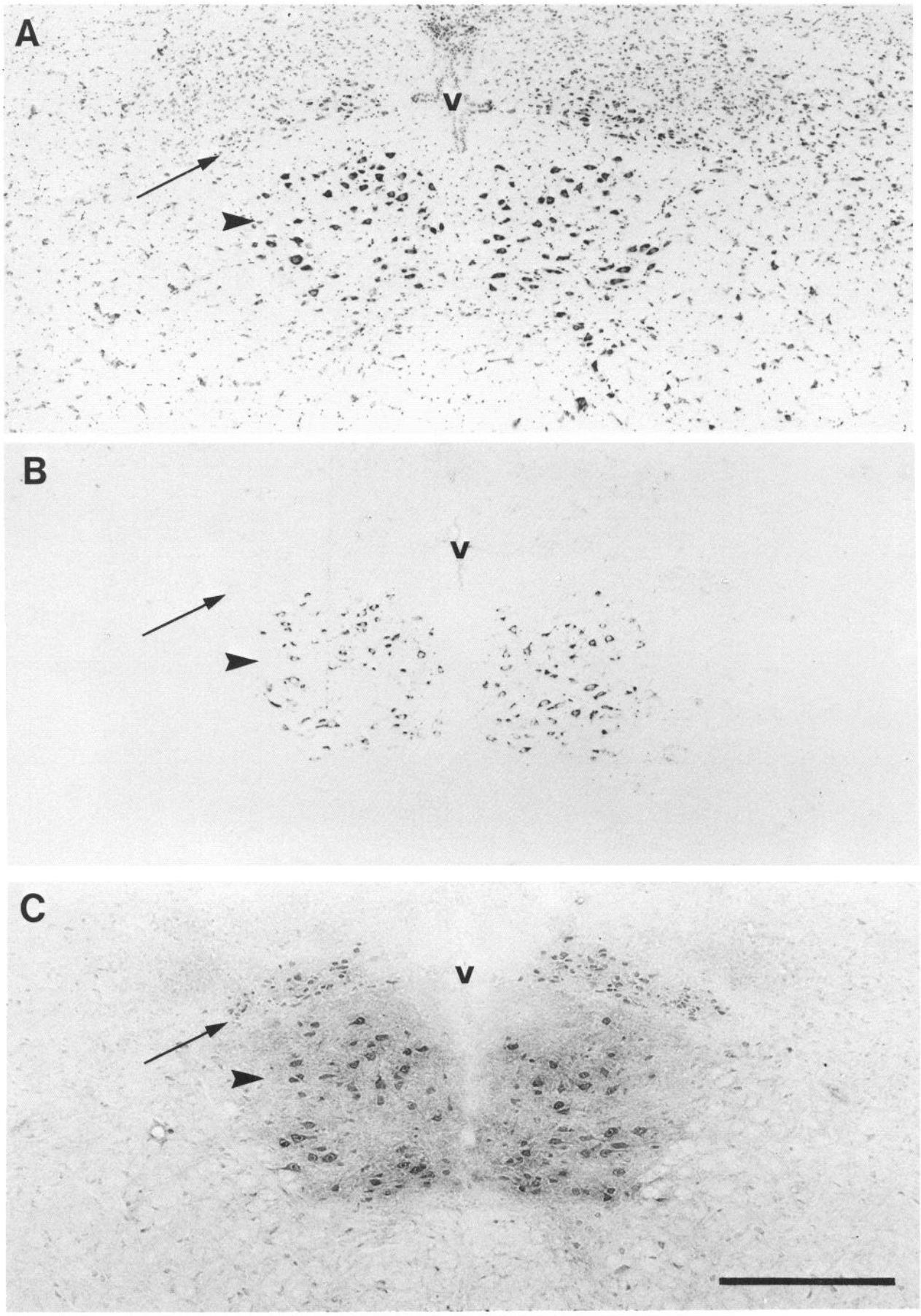
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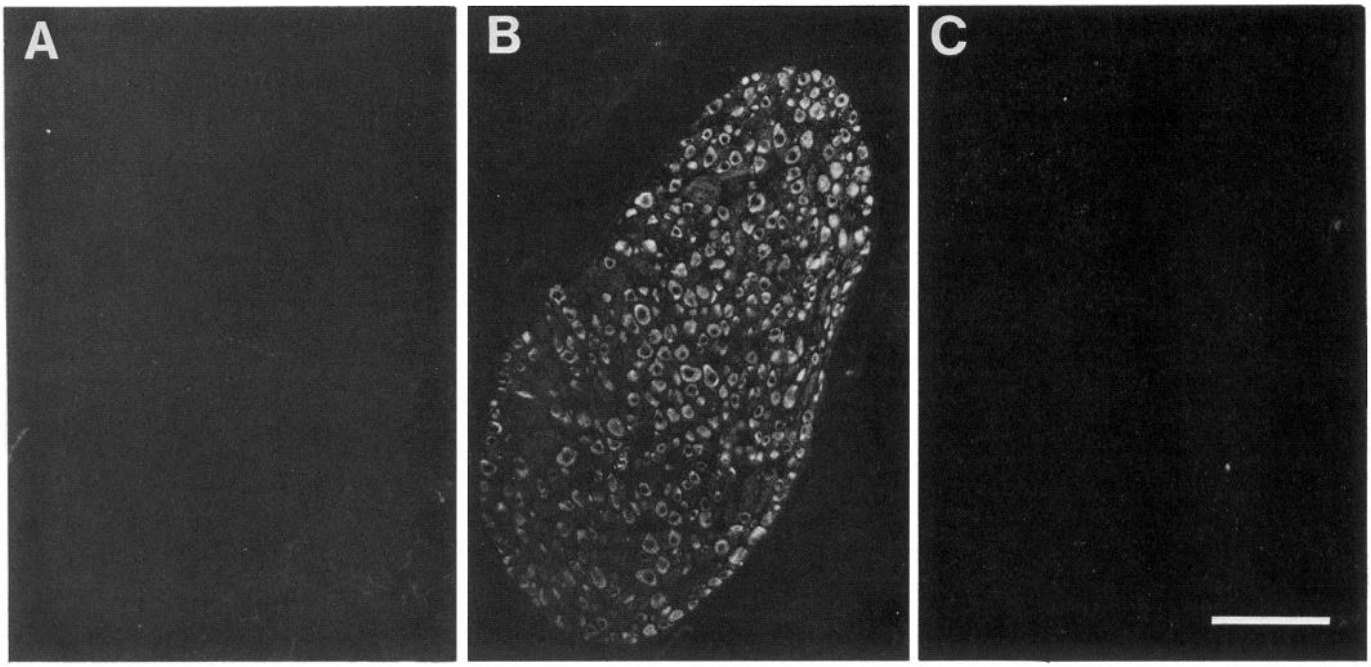


*Figure 5.* Lack of MO-1 immunoreactivity in the superior cervical ganglion. Sections were reacted with MO-1 (*A*) or MO-2 (*B*) or without primary antibodies (*C*), followed with fluoresceinated secondary antibodies for visualization. Scale bar, 200 μm.

tope appears to be acquired late in the maturation of motor neurons and is then maintained at high levels in all somatic motor neurons in the adult animal.

## Discussion

Studies on the organization and development of nervous systems have been greatly facilitated by monoclonal antibody technology. This technique has produced monospecific antibody reagents that permit select populations of neurons to be distinguished from neighbors or other related neurons by the presence of some unique antigen or epitope (for review, see Valentino et al., 1985). Yet monoclonal antibodies specific for a single morphological or functional class of neurons have rarely been encountered. In this study, we report on MO-1, a monoclonal antibody that almost exclusively stains one functional class of rat CNS neurons. This antibody intensely labels somatic motor neurons in every cranial motor nucleus examined (motor nuclei III, IV, V, VI, VII, XII, nucleus ambiguus, and accessory nuclei V, VI, and VII), as well as in the spinal cord. In contrast, cholinergic systems that are not composed of somatic motor neurons—including those in the basal forebrain, brain stem, and spinal cord, such as preganglionic autonomic motor neurons and cholinergic interneurons (Barber et al., 1984)—are not immunoreactive. We find that MO-1 stains all somatic motor neurons examined, but no other cells except possibly neurons in the red nucleus. Thus, while other markers that recognize motor neurons have been described (see introductory remarks), none show the specificity seen with MO-1.

Our observations on cryostat sections do not permit us to determine if the epitope is present on cell surfaces. However, within immunoreactive neurons, staining by MO-1 is only seen in the perinuclear regions and along the initial segments of processes but is not detectable along the entire length of processes or within the synaptic terminals of these cells. Many cytoskeletal elements, such as microtubule-associated proteins and neurofilaments, show compartmentalization within neurons (Bloom et al., 1984; Hirokawa et al., 1984; Peng et al., 1986; Riederer et al., 1986; Lee et al., 1987; Shirao et al., 1987; Sato-Yoshitake et al., 1989). Cytoplasmic components, such as CAT, are also most readily detected by immunohistochemistry in somata and in proximal processes. In the case of CAT, detection in distal processes and especially in terminals is enhanced by enzymatic pretreatment of the tissue (Phelps and Vaughn, personal communication), which suggests a lack of accessibility of the epitope to immunoreagents in some parts of the cell. At present, we do not know if the MO-1 epitope is present only in cell bodies or merely undetectable in other parts of the motor neuron due either to low concentration or to masking of the epitope.

The apparent late appearance of the MO-1 epitope in motor neurons during development is interesting for 2 reasons. First, it is surprising that MO-1 was generated using tissue from embryonic spinal cord, yet no immunoreactivity was detected in sections of embryonic spinal cord. One possibility is that the levels of antigen present early in development are below detection by immunohistochemistry. Alternatively, the embryonic form of the molecule bearing the MO-1 epitope may be labile and readily lost in unfixed or acetone-fixed tissue. We could

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*Figure 4.* MO-1 distinguishes between 2 populations of cholinergic neurons in the brain stem. Consecutive sections containing motor nucleus X (*arrow*) and motor nucleus XII (*arrowhead*) were Nissl-stained (*A*) to reveal all somata or reacted with MO-1 (*B*) or 1E6 (*C*). Neurons in X innervate parasympathetic neurons of the vagus, while those in XII synapse with the muscles of the tongue. Both populations are stained by antibodies to CAT (*C*), but MO-1 only recognizes the population of somatic motor neurons (*arrowhead, B*). *v,* ventricle. Scale bar, 500 μm.

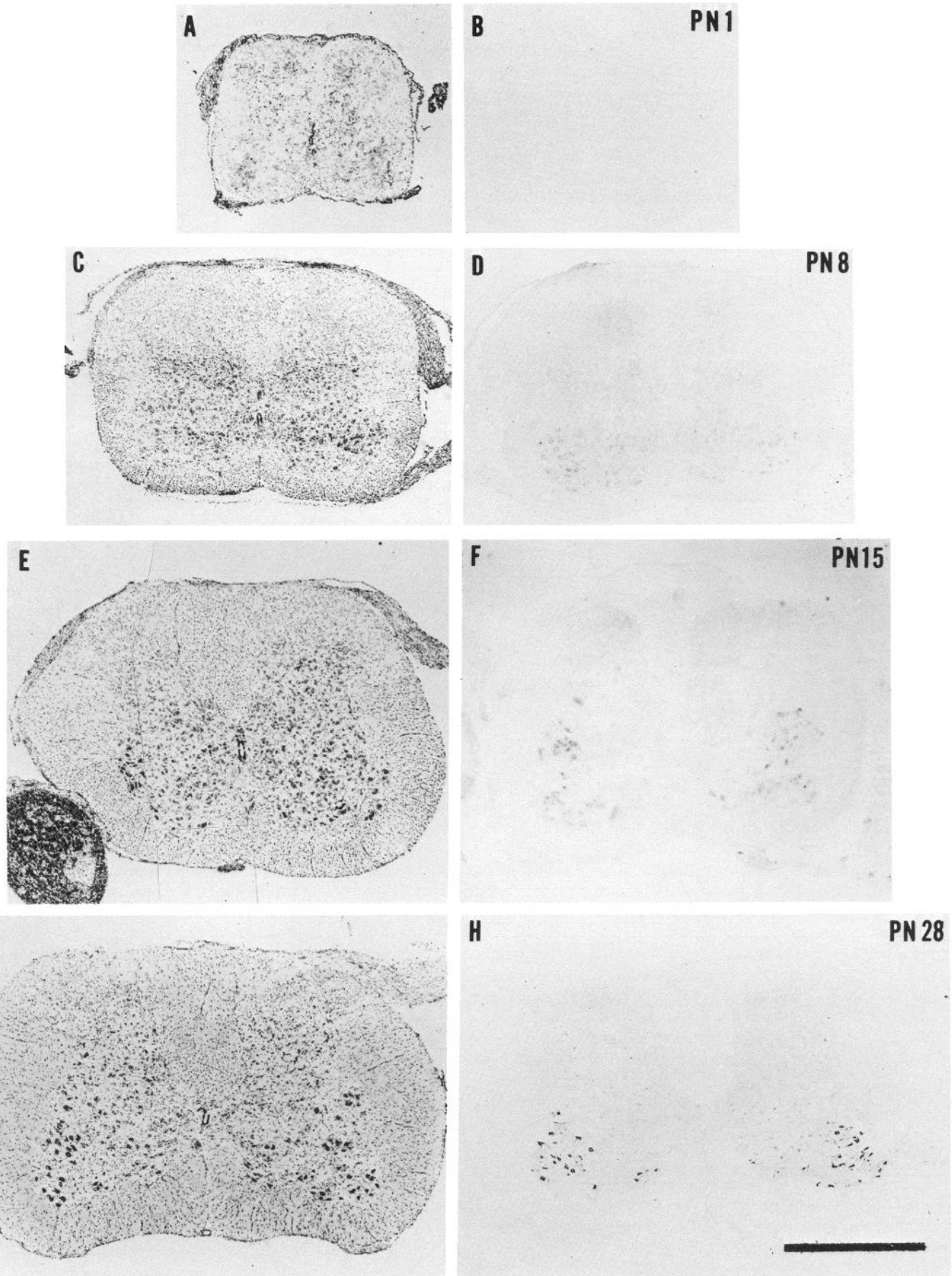


*Figure 6.* Appearance of MO-1 immunoreactivity in brachial spinal cord during postnatal development. Ten micron sections, taken from rats at postnatal days 1 (*A, B*), 8 (*C, D*), 15 (*E, F*), and 28 (*G, H*), were Nissl-stained (*A, C, E,* and *G*) or reacted with MO-1 (*B, D, F,* and *H*). Immunoreactivity of somatic motor neurons is absent on postnatal day 1 (*B*) and barely detectable at postnatal day 8 (*D*) but rises in intensity with age. Scale bar, 1 mm.

detect no staining in adult or embryonic tissue fixed with aldehydes prior to antibody binding. Thus, the epitope is resistant to acetone treatment but appears labile to stronger forms of fixation. Biochemical characterization and comparison of the immunoreactive molecule(s) in embryonic and adult spinal cord may shed light on this paradox.

A second point of interest is the dramatic increase in MO-1 immunoreactivity in motor neurons between the second and fourth weeks of postnatal life. In rats, spinal motor neurons undergo their final mitosis between embryonic days 11 and 13 (Nornes and Das, 1974; Phelps et al., 1988), and CAT enzyme activity as well as immunoreactivity are reliably detected in the spinal cord as early as E13 (Phelps et al., 1989), 10 d before birth. Synaptic contacts with skeletal muscles begin to be made around E14–E15 and continue for at least a week. By 2 weeks after birth, all the known major events in synaptic development have taken place (for review, see Dennis et al., 1981). Each muscle fiber has lost its juvenile polyneuronal innervation and is now innervated by a single motor neuron (Brown et al., 1976), and neuromuscular junctions appear fully differentiated (Chiu and Sanes, 1984). Yet at this late stage of maturation, we find that motor neurons begin to accumulate a novel cellular component, the MO-1 antigen.

In studying target dependence, Snider and Thanedar (1989) found that motor neurons in young rats showed a much greater dependence on their target muscles than those in older animals. In 1-week-old rats, up to 60% of somatic motor neurons died when chronically deprived of their target. In contrast, in 1-month-old or adult rats, only 30% of motor neurons were lost due to acute axotomy. The time course of this loss of target dependence correlates well with the period of rise in MO-1 immunoreactivity in somatic motor neurons. It is possible that acquisition of sufficient levels of the MO-1 antigen renders motor neurons independent of their postsynaptic target for survival.

The rise in MO-1 immunoreactivity during postnatal life could be caused by an increase in synthesis or a decrease in degradation within motor neurons, or by the uptake and accumulation of the antigen from exogenous sources. Because motor neurons project to targets out in the periphery, they are exposed to a wider range of molecules than most other CNS neurons and are known to take up, retrogradely transport, and accumulate nerve growth factor (Yan et al., 1988; Wayne and Heaton, 1988), as well as serum protein from the periphery (Yamamoto et al., 1987). In fact, polyclonal antibodies generated against rat serum albumin or rat immunoglobulins selectively stain somatic motor neurons in rats (Yamamoto et al., 1987). In immunodot assays, MO-1 does not recognize any component present in rat sera (Urakami and Chiu, unpublished observations). However, it is possible that the MO-1 antigen is produced by non-neuronal cells and selectively accumulated by motor neurons.

The source, identity, and function of the MO-1 antigen are at present unknown. However, the distribution of MO-1 immunoreactivity in the rat nervous system permits us to consider several possibilities. Since MO-1 does not stain all cholinergic populations, it cannot be recognizing CAT or AChE. Polyclonal antibodies to CGRP bind to somatic motor neurons in cranial motor nuclei (Takami et al., 1985) and spinal cord in mammals (Gibson et al., 1984). This distribution is similar to that seen with MO-1. Unlike MO-1, anti-CGRP also stains dorsal root ganglion neurons and a number of other populations in the rat brain (Kruger et al., 1988). However, this does not completely rule out the possibility that MO-1 might be binding to a CGRP-like molecule. Thus, if the polyclonal antibodies to CGRP are recognizing several different CGRP-immunoreactive molecules, MO-1 might be binding to only one of them.

McMahan and colleagues have purified a component, agrin, that can induce the aggregation of ACh receptors on chick myotubes in culture (Nitkin et al., 1987). Monoclonal antibodies that can neutralize this aggregating activity stain the somata of motor neurons as well as the synaptic basal lamina at chick neuromuscular junctions (Reist et al., 1987; Magill-Solc and McMahan, 1988). These observations support the hypothesis that agrin is produced by motor neurons and deposited at the synaptic cleft, where it acts on myotubes to recruit ACh receptors during synaptogenesis. Two observations rule against the possibility that MO-1 recognizes the mammalian form of agrin. First, unlike the monoclonal antibodies against agrin, MO-1 does not stain neuromuscular junctions. Second, the very late appearance of MO-1 immunoreactivity in motor neurons suggests that the antigen is unlikely to play a role in early events in the formation of the neuromuscular junction, such as the aggregation of ACh receptors. Future experiments on the biochemical identity of the MO-1 antigen will be important in order to understand its unique role in mature motor neurons.

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