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Research Note

Selective retrograde transneuronal transport of wheat germ agglutinin-conjugated horseradish peroxidase in the oculomotor system*

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Summary. The fate of wheat germ agglutinin-conjugated horseradish peroxidase (WGA/HRP) subsequent to its uptake and retrograde axonal transport in abducens motoneurons of the monkey was studied using histochemical localization of WGA/HRP reaction product and light microscopy. Injections of WGA/HRP into monkey lateral rectus muscles produced a pattern of labelled motoneurons like that obtained with native HRP. In contrast to the native HRP data, WGA/HRP injections consistently labelled additional neuronal populations in the ipsilateral medial vestibular nucleus and contralateral dorsal medullary reticular formation. These regions correspond to those containing neurons known to make inhibitory synaptic contact with abducens motoneurons. No labelled neurons were observed in regions which contain excitatory premotor neurons. These data are consistent with the notion of retrograde transneuronal transport of WGA/HRP to premotor neurons. The specificity of the transneuronal exchange is indicated by the finding that only certain populations of premotor neurons were labelled. The precise manner by which preferential transneuronal transport of WGA/HRP is attained remains to be determined.

Key words: Transneuronal transport – Oculomotor system – Wheat germ agglutinin-conjugated horseradish peroxidase – Abducens nucleus – Monkey

Brainstem afferents to the abducens nucleus have been identified by means of retrograde and anterograde tracer techniques (Maciewicz et al.1977; Büttner-Ennever and Henn 1976; Gacek 1979; McCrea et al. 1979; Baker and Spencer 1981). Typically, neuroanatomical tracer techniques allow visualization of only those neurons having direct connections with the region of interest. Afferent or efferent neuronal connections, however, also have been demonstrated through *transneuronal transport* of various neuroanatomcial tracers (Büttner-Ennever et al. 1981; Ruda and Coulter 1982; Spencer et al. 1982; Correia et al. 1983; Carleton and Carpenter 1984). Some of the substances (e.g., amino acids) previously shown to undergo transneuronal transport are of low molecular weight and are transferred in a non-specific manner as evident by both neuronal and glial labelling. Several studies (Gerfan et al. 1982; Ruda and Coulter 1982; Spencer et al. 1982; LaVail et al. 1983), however, have implicated WGA/HRP in specific (i.e., transsynaptic) *anterograde* transneuronal transport following peripheral injections. Conflicting reports exist (Schwab et al. 1979; Harrison et al. 1984) as to whether WGA/HRP undergoes transsynaptic transfer subsequent to *retrograde* axonal transport.

The present study has examined regions known to project to the abducens nucleus following injections of WGA/HRP into the lateral rectus muscle. In contrast to earlier studies which used native HRP, these injectons labelled not only motoneurons but also selective populations of premotor neurons which are known to be inhibitory upon abducens motoneurons. Taken together, these data are consistent with the notion of retrograde transneuronal transport of WGA/HRP following initial uptake by motoneurons.

Five adult monkeys (2 *Macaca mulatta* and 3 *M. arctoides*) were anesthetized with intramuscular Ketamine hydrochloride and intravenous diabutal sodium and prepared for unilateral injection of the lateral rectus muscle. Extraocular muscles were exposed by dissection, as described previously

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(Spencer and Porter 1981; Porter et al. 1983), and the lateral rectus muscle was injected with 20 μl of 1% (w/v) WGA/HRP (Sigma) in 0.1 M tris buffer (pH 7.6). An additional monkey received a unilateral injection of the lateral rectus muscle with 40 μl of 10% (w/v) native HRP (Sigma VI). This animal, and those from previous studies (Spencer and Porter 1981; Porter et al. 1983), formed the basis for comparison of animals receiving native HRP injections with those injected with WGA/HRP. After a 48-h survival period, each animal was anesthetized and perfused intracardially with physiological saline followed by 3,000 ml of 1% paraformaldehyde-1.25% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The brainstem was then blocked in situ in the Horsley-Clarke stereotaxic plane, briefly postfixed (1–2 h), and washed in 0.1 M phosphate buffer. Serial 50 μm sections through the brainstem were obtained with either a freezing microtome or a Vibratome and were processed for the histochemical demonstration of WGA/HRP using the tetramethylbenzidine chromagen (Mesulam 1978). Sections were mounted on slides pretreated with chrom alum/gelatin and were counterstained with 1% neurtral red for light microscopic examination using brightfield and darkfield illumination. The distributions of WGA/HRP-labelled neurons were charted with reference to nuclear boundaries using a microscope equipped with a drawing attachment. Neuronal counts and soma diameter data were obtained from 3 of the WGA/HRP animals.

WGA/HRP injections of the monkey lateral rectus muscle resulted in a pattern of labelled motoneurons similar to that obtained with native HRP (present findings; Spencer and Porter 1981; Porter et al. 1983). Motoneurons labelled following muscle injections were localized to the ipsilateral abducens and ventral abducens nuclei (Fig. 1A). Use of the WGA/HRP tracer resulted in enhanced visualization of the motoneuron soma/dendritic tree relative to that obtained with native HRP. Only an occasional labelled neuron was observed in brainstem motor nuclei (e.g. facial) other than the ipsilateral abducens nucleus, thereby indicating that diffusion of WGA/HRP from the injection site was minimal.

In contrast to data obtained with native HRP, WGA/HRP injections of the lateral rectus muscle consistently labelled, though less densely than motoneurons, a population of neurons in the rostral portion of the contralateral dorsal medullary reticular formation (i.e., dorsomedial gigantocellular field, Fig. 1A). Labelled neurons were 21.1 μm in mean diameter (range 15.2–30.5 μm) and formed a well-circumscribed cluster immediately ventromedial to the contralateral abducens nucleus. These neurons were situated within 1 mm of the midline and extended longitudinally from the rostral pole of the abducens nucleus, where the sixth nerve begins its descent to the pontomedullary junction, to the caudal pole of the nucleus (Fig. 2). The largest concentration of labelled neurons in this region of the reticular formation was located in the vicinity of the central third of the abducens nucleus (Fig. 2D and 2E). The mean number of labelled neurons was 225 (range 196–240). Besides the ventral abducens motoneurons which were separated from the principal abducens nucleus (see Spencer and Porter 1981), no labelled neurons were observed in the ipsilateral medullary or pontine reticular formation.

A second population of lightly labelled neurons, which also was unique to animals injected with WGA/HRP, was located in the ipsilateral medial vestibular nucleus (Figs. 1B and 2). These labelled neurons were distributed along the ventromedial border of the medial vestibular nucleus (see Fig. 2A and 2B) and were found primarily in its rostral portion. Like those neurons observed in the contralateral medullary reticular formation, the labelling density of these vestibular neurons was considerably less than that of abducens motoneurons. The mean number of labelled neurons was 257 (range 236–282) and mean soma diameter was 23.7 μm (range 17.1–32.4 μm). No labelled neurons were observed in the contralateral vestibular complex. Other regions known to contain neurons which synapse upon abducens motoneurons (e.g., oculomotor and prepositus hypoglossi nuclei) were conspicuously devoid of labelled neurons.

Consistent with previous data (Porter et al. 1983), first-order sensory neurons innervating the lateral rectus muscle were labelled in all animals and were found to be restricted to the ipsilateral trigeminal ganglion. Although central terminal fields of such sensory neurons, representing sites of synaptic termi-

Fig. 1A and B. Light photomicrographs of neurons labelled following injection of WGA/HRP into the left lateral rectus muscle. **A** Darkfield photomicrograph of transneuronally labelled neurons in the contralateral dorsal medullary reticular formation. Magnification: 293 ×. Inset: Labelled motoneurons in the left abducens nucleus; arrow indicates the position of labelled neurons in the contralateral reticular formation (brightfield illumination). Magnification: 10 ×. **B** Darkfield photomicrograph of transneuronally labelled neurons in the ipsilateral medial vestibular nucleus. Magnification: 293 ×. Inset: Brightfield photomicrograph illustrating the position of labelled neurons in the ventromedial portion of the medial vestibular nucleus. Magnification: 20 ×

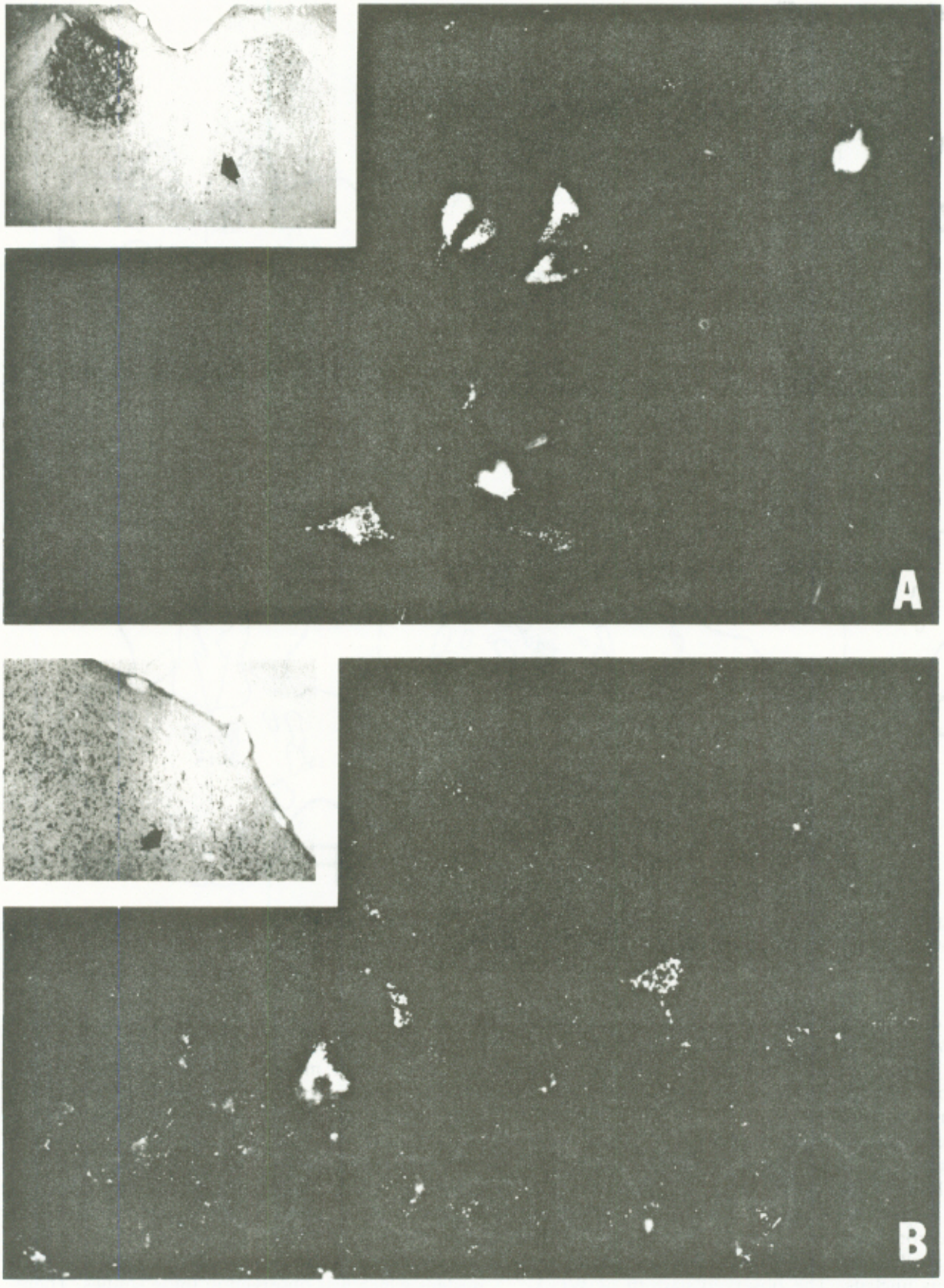

Fig. 1

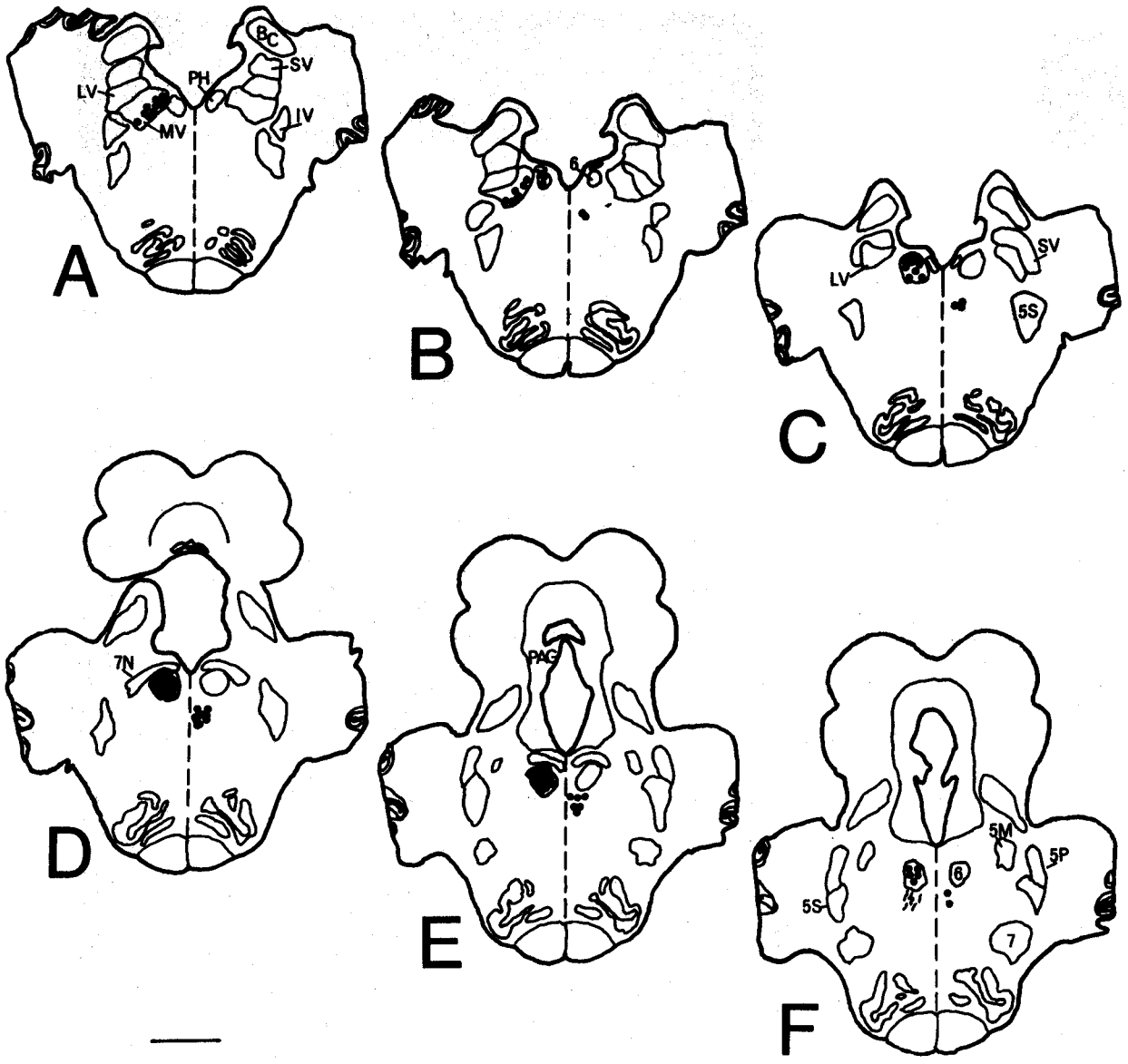

Fig. 2A–F. Chart of location of labelled neurons (filled circles) within the brainstem following injection of WGA/HRP into the left lateral rectus muscle. Sections proceed from caudal **A** to rostral **F**. Calibration bar: 2 mm

nation of ganglion cells and resulting from transganglionic WGA/HRP transport, were observed (unpublished observations), anterograde transneuronal transport of the tracer to second-order sensory neurons was not observed.

The present study has demonstrated that clear differences exist in the fate of retrograde transported WGA/HRP versus HRP after injection into the lateral rectus muscle. In addition to the anticipated motoneuron labelling, peripherally injected WGA/HRP labelled neuronal populations outside of the abducens nucleus. The observed pattern of WGA-HRP reaction product seen in vestibular and reticular nuclei could result from (a) retrograde transport to neurons in sites not previously known to innervate the extraocular muscles, (b) anterograde transneuronal transport from either motoneuron axon collaterals or the central synaptic terminals of extraocular muscle sensory neurons, or (c) retrograde transneuronal transport to neurons with make synaptic contact with abducens motoneurons. Previous studies utilizing native HRP (Spencer and Porter 1981; Porter et al. 1983) did not obtain the unique pattern of labelled neurons outside of the abducens nucleus which was observed in these studies. Therefore, in all likelihood, the labelled neurons in the

vestibular and reticular nuclei lack direct projections to the extraocular muscles. Furthermore, an anterograde transneuronal route, involving transfer from a motoneuron axon collateral to a target neuron in the medial vestibular nucleus or reticular formation, is unlikely since oculomotor motoneurons either lack collaterals entirely or possess collaterals which terminate within the motor nucleus of origin (Evinger et al. 1981). Similarly, the terminal fields of extraocular muscle sensory neurons lie elsewhere in the neuraxis (unpublished observations), thereby precluding the possibility that the vestibular and reticular neurons are labelled through anterograde transneuronal transport from first-order muscle afferent neurons. Taken together, these data warrant the conclusion that the labelling of selective populations of abducens premotor neurons in the present study is the result of retrograde transneuronal transfer of the WGA/HRP tracer.

The distributions of neurons labelled by a presumed transneuronal route partially overlap that obtained subsequent to native HRP injections of the abducens nucleus proper (Maciewicz et al. 1977; Baker and Spencer 1981), thereby providing further evidence that vestibular and reticular neurons are labelled by means of a retrograde transneuronal transfer of WGA/HRP. Anatomical segregation of functional classes of premotor neurons into four distinct regions (i.e., vestibular, oculomotor, and prepositus hypoglossi nuclei and the reticular formation) allows clear determination of the cell types which were transneuronally labelled in these studies. The distributions of labelled neurons correspond to those of inhibitory vestibular (ipsilateral medial vestibular nucleus; Hikosaka et al. 1977; McCrea et al. 1980; Scudder and Fuchs 1981) and inhibitory burst (contralateral dorsal medullary reticular formation; Hikosaka et al. 1978; Scudder et al. 1982) neurons. These data suggest the existence of a specific transneuronal transport process by which only certain classes of premotor neuron are labelled.

Transneuronal transport of the WGA/HRP tracer is clearly not a random process, but rather it involves a specific transsynaptic exchange since the glial labelling which would characterize gross extrusion into the extracellular space is not obtained (Ruda and Coulter 1982; Spencer et al. 1982; LaVail et al. 1983). The manner in which a neuroanatomical tracer is handled by the motoneuron may determine whether that tracer is made available for transneuronal transfer. Retrograde transport of WGA/HRP differs from that of native HRP both in the mechanism of neuronal uptake (Schwab et al. 1978) and in the fate of the tracer once within the neuronal somata (Harper et al. 1980). By virtue of specific binding to particular membrane glycoprotein moieties, a property not exhibited by native HRP, WGA/HRP may be exteriorated through reinsertion of the WGA/HRP-glycoprotein complex into the neuronal membrane where it then becomes accessible for uptake by synaptic endings of afferent neurons.

The specificity of the transneuronal transport process suggests that properties of the premotor neuron may function in the regulation of interneuronal exchange. Since the labelled neuronal populations establish synaptic connections with many motoneurons and their synaptic endings are distributed proximal on the soma/dendritic tree (R.F. Spencer personal communication), these data may simply reflect a larger quantity of WGA/HRP "seen" by inhibitory premotor neurons. The synaptic pattern of excitatory premotor neurons (i.e., synaptic terminals placed distally on the soma/dendritic tree), therefore, may preclude their exposure to tracer, at least in an amount significant enough to be detected by histochemistry. While attractive, a theory for transneuronal transport which is based solely upon the synaptology of premotor neurons may represent an oversimplification. Evidence for anterograde transport of native HRP to some, but not all, neurons contacted by a single intracellularly-injected neuron (Baker an Grantyn 1982) suggests that the mechanism of the transcellular exchange may be related to other, as yet undetermined, intrinsic characteristics of the presynaptic and/or postsynaptic neuron.

Abbreviations

BC	Brachium conjunctivum
IV	Inferior vestibular nucleus
LV	Lateral vestibular nucleus
MV	Medial vestibular nucleus
PAG	Periaqueductal grey
PH	Nucleus prepositus hypoglossi
SV	Superior vestibular nucleus
WGA/HRP	Wheat germ agglutinin-conjugated horseradish peroxidase
5M	Motor nucleus of trigeminal nerve
5P	Principal (main sensory) nucleus of trigeminal nerve
5S	Spinal (descending) nucleus of trigeminal nerve
6	Abducens nucleus
7	Facial nucleus
7N	Facial nerve

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