(5 ml) was added gradually to this solution while vortexing. The solution was vortexed an additional 2 min and then passed through a 0.45-μm pore size syringe filter (Gelman Scientific); 50 to 150 μl was added to 10 ml of ASW in a 60-mm petri dish and several drops of a concentrated embryo suspension were added. The dish was placed in the dark for 1 hour, then the embryos were washed several times over 30 min with ASW and placed in microinjection chambers (*11*). In separate chambers, PMCs of unlabeled recipient embryos were removed by directing a gentle flow of ASW into the blastocoel through a micropipette (*9*). RITC-labeled PMCs were removed from donor embryos and 50 to 60 cells were microinjected into each PMC-depleted recipient with a beveled siliconized microneedle attached to a pressure injection apparatus (*11*). Previous studies have shown that 50 PMCs are a sufficient number to completely suppress SMC skeletogenesis (*9*). After the microsurgery (a 1/2- to 1-hour procedure), embryos were placed in depression slides in humid dishes and development continued. At intervals, the embryos were collected with a mouth pipette, placed again in microinjection chambers, and then irradiated (2 to 4 min) (100-watt mercury arc lamp of Nikon Diaphot epifluorescence microscope; Nikon G-1B cube, with 546/10 nm excitation filter, 580 nm dichroic mirror, and 590 nm barrier filter). In each trial, 15 to 30 PMC-depleted embryos were allowed to develop in parallel with embryos that had received PMC transplants. Some of these PMC-depleted embryos were fixed and processed for indirect immunofluorescence at the time of photoablations. These embryos served as an internal control to confirm that conversion as monitored by MAb 6a9 immunoreactivity had not begun at the time of photoablation.

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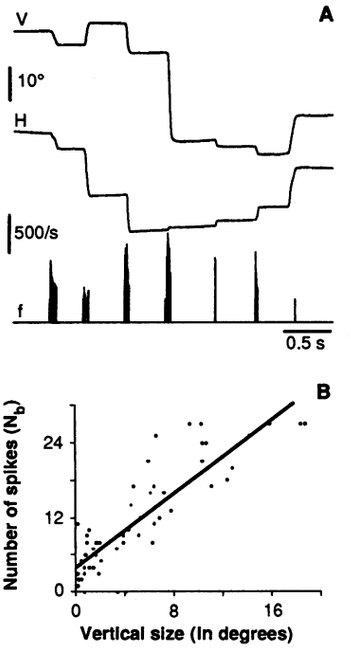

Fig. 1. (**A**) Discharge pattern of one typical VMLB; f, firing rate; H, instantaneous horizontal eye position; V, instantaneous vertical eye position. (**B**) The number of spikes in the burst of one VMLB (n_b) as a function of amplitude of downward components of saccades or vertical size (v). A straight line described by the equation $n_b = 1.49v + 4.04$ was fitted through points that represent downward saccades ($r = 0.86$).

A Structural Basis for Hering's Law: Projections to Extraocular Motoneurons

ADONIS K. MOSCHOVAKIS, CHARLES A. SCUDDER,* STEVEN M. HIGHSTEIN

Conjugate eye movements are executed through the concurrent activation of several muscles in both eyes. The neural mechanisms that underlie such synergistic muscle activations have been a matter of considerable experimentation and debate. In order to investigate this issue, the projections of a class of primate premotoneuronal cells were studied, namely, the vertical medium-lead burst neurons (VMLBs), which drive vertical rapid eye movements. Axons of upward VMLBs ramify bilaterally within motoneuron pools that supply the superior rectus and inferior oblique muscles of both eyes. Axons of downward VMLBs ramify ipsilaterally in the inferior rectus portion of the oculomotor nucleus and in the trochlear nucleus. Thus, VMLBs can drive vertical motoneuron pools of both eyes during conjugate vertical rapid eye movements; these data support Hering's law.

ORDINARY MOTOR ACTIVITY depends on the coactivation of synergistic muscle groups. A special case of this phenomenon is encountered in the oculomotor system where extraocular muscles of both eyes must be coactivated if the resulting eye movements are to be conjugate (that is, for both eyes to move simultaneously in the same direction and by the same amount). Over a century ago, Hering proposed a mechanism to account for eye conjugacy known as Hering's Law of Equal Innervation (*1*). According to this law, the two eyes move in a conjugate manner because they receive identical signals from the brain (equal innervation). Hering suggested that there was equal outflow in the nerves innervating the separate extraocular muscles. Because it is now known that each motoneuron pool innervates only one muscle (*2*), the issue is whether, and how, the motoneuron pools that innervate the separate muscles receive equal innervation. The known synaptic organization of the oculomotor system has not thus far offered a satisfying validation of Hering's principle. A stronger validation would be obtained if branches from a single neuron to separate motoneuron pools could be demonstrated.

A. K. Moschovakis, Laboratory of Neural Control, National Institute of Neurological Diseases and Stroke, Bethesda, MD 20892.
C. A. Scudder and S. M. Highstein, Department of Otolaryngology, Washington University School of Medicine, St. Louis, MO 63110.

*To whom correspondence should be addressed.

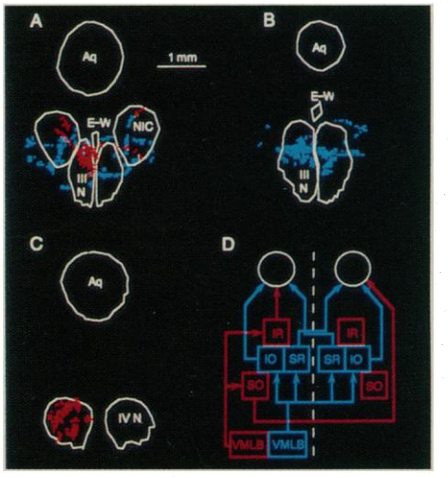

Fig. 2. (**A** through **C**) Frontal plots of the terminal fields distributed in the rostral (A) and caudal (B) half of the oculomotor nucleus and in the trochlear (C) nucleus by one upward (blue) and one downward (red) VMLB that were intraaxonally injected with HRP in alert, behaving primates. (**D**) Bilateral schematic diagram of the organization of the vertical saccadic system (eyes indicated by open circles, the midline by dashes). Arrows indicate the projections of up (blue) and down (red) VMLBs, as well as up (blue) and down (red) motoneurons. III N, oculomotor nucleus; IV N, trochlear nucleus; Aq, aqueduct; E-W, Edinger-Westphal nucleus; NIC, interstitial nucleus of Cajal; IO, inferior oblique; IR, inferior rectus; SO, superior oblique; SR, superior rectus.

With the emergence of the technique of intracellular recording and dye injection in alert animals (*3*), it is now possible to test directly whether such an anatomical substrate for Hering's law exists. While monitoring the instantaneous position of the eyes (*4*) of alert, behaving squirrel monkeys, we intraaxonally recorded the activity of VMLBs, that is, the premotoneurons that are responsible for the execution of vertical rapid eye movements (*5*). Recordings were made with glass micropipettes filled with a 10% solution of horseradish peroxidase (HRP) in 50 mM tris buffer (pH 7.4). After functional characterization, axons of VMLBs were injected with HRP. The animals were deeply anesthetized with pentobarbitol (40 mg/kg) and perfused 36 to 50 hours after intracellular labeling, and the brain tissue was histochemically processed for visualization of the injected VMLBs (*6*). The synaptic boutons (7) of 18 neurons that are typical of the 40 VMLBs that we have encountered were plotted from serial frontal sections through a computer-based reconstruction system (*8*). Parameters of neuronal discharge and eye movements were analyzed off-line on a PDP-11/73 computer (Digital Equipment Corp., Waltham, Massachusetts) (*9*).

Physiological and anatomical evidence suggests that VMLBs cause the execution of vertical rapid eye movements. The activity of one VMLB in relation to rapid eye movements is illustrated in Fig. 1A. A high-frequency burst of activity preceded downward saccades by about 5 ms. Otherwise, the neuron was silent for as long as the animal was alert, except for a few spikes during upward saccades. Parameters of the discharge of VMLBs are highly correlated with the metrics of vertical saccades. For example, the number of spikes in the bursts of Fig. 1A is highly correlated with the amplitude of downward components of saccades (Fig. 1B). Units preferring upward saccades behaved similarly. The relatively small cell bodies of VMLBs were recovered in the rostral interstitial nucleus of the medial longitudinal fasciculus (riMLF). This finding confirms descriptions based on extracellular and intracellular recording techniques (*10*). It is also consistent with the fact that lesions to this area cause vertical gaze paralysis in animal preparations and human patients (*11*).

Axons of VMLBs run caudally through the interstitial nucleus of Cajal and the MLF of the same side. Their collaterals ramify extensively within the oculomotor nucleus and the interstitial nucleus of Cajal. Boutons of VMLBs that discharge before upward saccades occur are distributed bilaterally within the ventrocaudal pole of the oculomotor nucleus (Fig. 2), within motoneuron pools that innervate mainly the superior rectus and to a lesser extent the inferior oblique muscles of both eyes (*2, 12*). Plots of the terminal fields of VMLBs that discharge before downward saccades showed a different spatial distribution (Fig. 2). Boutons of downward VMLBs are distributed ipsilaterally in both the rostrodorsal pole of the oculomotor nucleus and the trochlear nucleus. These portions of the primate oculomotor complex contain motoneurons that innervate the inferior rectus muscle of the ipsilateral eye and the superior oblique muscle of the opposite eye, respectively (*2, 12*).

Our data show that single VMLBs project to motoneuron pools that innervate yoked muscles of both eyes; this finding thereby establishes, at least qualitatively, the existence of an anatomical substrate for Hering's law in the vertical saccadic system. Because boutons deployed by each VMLB are not equally distributed among the motoneuron pools they project to and because the synaptic efficacy of each bouton is unknown, our data do not provide a quantitative proof of Hering's law. A pattern of termination in the oculomotor complex similar to that of VMLBs exists for secondary vestibular neurons that participate in the vertical vestibulo-ocular reflex (*13*). However, our data are more relevant to Hering's law because VMLBs provide motoneurons with almost all of their signal during vertical saccades (*5*), whereas the vestibular neurons provide motoneurons with only part of their signal during the vertical vestibuloocular reflex (*14*).

This stronger form of equal innervation to vertical ocular motoneurons contrasts with a weaker form implemented in the horizontal oculomotor system. Horizontal saccades are only approximately conjugate (*15*). Saccades of the abducting eye are bigger and faster than saccades of the adducting eye, resulting in a relatively large transient divergence of the two eyes. This divergence probably occurs because the motoneurons of the medial rectus muscle, contained in the oculomotor nucleus, and those of the lateral rectus muscle, contained in the abducens nucleus, are not mutually innervated by the same premotoneurons. Rather, the coordinated activation of contralateral medial rectus motoneurons is mediated by a special class of abducens "internuclear neurons" (*16*) that receive many of the same inputs as abducens motoneurons (*17*). Other examples of disconjugacy are known: the horizontal components of saccades can be very different under pathological conditions (*18*), and saccades made from a far to a near target can have unequal horizontal components (*19*) to accomplish the convergence of the eyes necessary to bring both eyes on target. Such disconjugate eye movements might yet be the result of a conjugate command, as envisioned by Hering, with a disconjugate command superimposed on it. Therefore, it might be that the weaker form of equal innervation evident in the horizontal oculomotor system allows more opportunity for the addition of disconjugate commands, whereas a stronger form is used for the vertical oculomotor system where disconjugacy is not needed.

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