Generation of Torsional and Vertical Eye Position Signals by the Interstitial Nucleus of Cajal

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The neural integrator, which converts eye velocity signals into position signals, is central to oculomotor theory. Similar integrators are probably necessary in any neural system that changes and maintains muscular tension. The integrator for horizontal eye position is in the pons, but the locations of the vertical and torsional integrators have not been clearly defined. Recording three-dimensional eye movements in alert monkeys during microstimulation and pharmacological inactivation of midbrain sites showed that the interstitial nucleus of Cajal generates both the torsional and vertical eye position signals. Up and down signals are linked with clockwise signals in the right brain and counterclockwise signals in the left brain. This three-dimensional coordinate system achieves orthogonality and bilateral symmetry without redundancy and optimizes energy efficiency for horizontal visual scanning.

MOST CONTROL SYSTEMS FOR EYE movement produce patterns of neural activity that resemble velocity signals. These signals alone will move the eye to the desired position but will not prevent the eye muscles from relaxing and pulling the eye back to a resting position. Because this eye relaxation does not occur, it was proposed that these oculomotor systems share a common integrator that converts phasic velocity signals into a tonic position signal for both eyes (*1*).

The integrator for horizontal eye rotations appears to be located in the nucleus prepositus hypoglossi region of the pons (*2*). Pharmacological inactivation of this site produced a profound deficit in the ability to hold horizontal eye positions without affecting rapid eye movements. However, much of the vertical position signal remained intact, and torsional eye rotations, which we define as rotation about a head-fixed axis approximately perpendicular to the face, were not examined. Eye movement systems require a three-dimensional velocity-to-position transformation, including horizontal, vertical, and torsional integrators (*3*). When the head is rotated torsionally, for example, right ear toward the right shoulder, the eyes rotate in the opposite direction and hold their final position. Furthermore, integration in all three dimensions is necessary even during simple horizontal rotations (*4*).

Where then are the vertical and torsional integrators? There is some evidence that the vertical integrator is in the midbrain interstitial nucleus of Cajal (INC). First, neural firing patterns in the cat and monkey INC had a tonic component resembling a vertical eye position signal (*5*). Second, electrolytic lesioning of the cells and fibers in the INC region of the cat produced a deficit in the ability to hold vertical eye positions, although the rate of drift was not rapid enough to indicate complete integrator failure (*6*). These experiments did not examine torsional eye position; however, the INC receives input from structures involved in both vertical and torsional eye rotations (midbrain burst neurons and vertical semicircular canals) and projects to motoneurons that rotate the eye both vertically and torsionally (Fig. 1) (*7, 8*). We therefore tested whether the INC is involved in generating both the vertical and torsional eye position signals.

Three-dimensional eye movements were recorded in five monkeys, *Macaca fascicularis*. Two wire search coils were implanted beneath the conjunctiva of one eye during sodium pentobarbitol anesthesia (29 mg per kilogram of body weight, intraperitoneal) (*9*). In two monkeys, coils were implanted in both eyes for simultaneous binocular recording. The INC and surrounding region were explored with a combination of single-unit recording, electrical microstimulation, and drug microinjection at multiple sites while alert animals made voluntary rapid eye movements (saccades) to visual targets. The anatomical locations of recording sites were confirmed by histological examination of an electrolytic lesion.

Single-unit recordings identified the INC region between the more anterior-lateral burst region related to saccades and the more posterior-medial ocular motoneurons (Fig. 1). Recordings confirmed that INC neuron firing frequencies were correlated to vertical eye position (*5*). Neurons that increased their activity either during upward or downward eye positions were intermingled within the INC, and most of these showed a burst of activity during saccades in these directions. Activity in the left INC was indistinguishable from that in the right INC, which superficially suggests redundancy. However, we were unable to correlate changes in neural activity to changes in ocular torsion, because saccades keep torsion very near zero (*10*).

To determine if the two sides of the INC are functionally distinct, we used unilateral microstimulation with monopolar electrodes (20-μA, 0.5-ms cathodal pulses; 200 Hz for 300 to 600 ms). INC stimulation produced similar stereotypical eye movements in all animals (Fig. 2). Speed of rotation was constant during stimulation, and the final eye position was maintained, indicating that the stimulus input had been

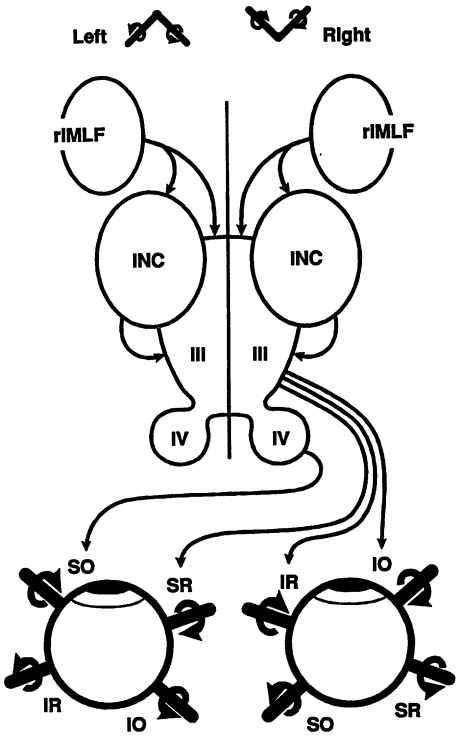

Fig. 1. Schematic diagram of the midbrain structures involved in generating vertical and torsional saccades. Brain structures and eyes are viewed from above the subject. Only excitatory connections are illustrated. During a saccade, neurons in the rostral interstitial nucleus of the medial longitudinal fasciculus (riMLF) send a burst of activity directly to ipsilateral motoneurons of the third and fourth cranial nerve nuclei and to the ipsilateral INC. The current investigation suggests that the INC converts this burst into a position signal, which tonically excites the ipsilateral motoneurons. Motoneurons of the right brain innervate eye muscles that rotate the eyes clockwise and vertically, whereas motoneurons of the left brain innervate the counterclockwise-vertical muscles. Thick lines projecting from the eyes show the axes and direction of rotation produced by the individual muscles. The small axes at the top of the figure summarize the rotations controlled by neurons on each side. III, oculomotor nucleus; IV, trochlear nucleus; SO, superior oblique muscle; SR, superior rectus; IR, inferior rectus; IO, inferior oblique.

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integrated. Both eyes rotated with the same direction and magnitude. In contrast, stimulation of the adjacent motoneurons produced exponential monocular rotation that was not maintained.

The direction of eye rotation was always clockwise during right INC stimulation and counterclockwise during left stimulation. Torsional velocities were as great as 50° per second during stimulation 1 mm lateral of brain midline but dissipated at 2 mm lateral. Vertical rotation was occasionally almost as large as the torsional rotation (Fig. 2B) but was usually much smaller (Fig. 2A) with a variable direction. Thus, intermingled "up" and "down" neurons appeared to cancel each other's effects. Very little horizontal rotation was observed. One interpretation of these results is that (i) the INC integrates its inputs for motoneurons of both eyes, and (ii) the INC, like the adjacent midbrain oculomotor structures, is organized into clockwise-up and clockwise-down neurons on the right side, and counterclockwise-up and counterclockwise-down neurons on the left side.

This interpretation was confirmed by chemical inactivation of neurons originating in the INC (*11*). The electrode was withdrawn from its guide tube, and a cannula was lowered to the previously identified site. We then injected 0.3 μl of a 0.05% muscimol solution, a drug that reversibly inactivates neuron cell bodies by selectively binding to receptors for the inhibitory neurotransmitter γ-aminobutyric acid (GABA). Injection of GABA produced similar effects but for a shorter time period. Inactivation of structures adjacent to the INC produced saccade deficits without positional drift. However, unilateral injection of muscimol into the INC resulted in an immediate failure to hold eye positions without profoundly affecting saccades. The position deficit reached a peak within half an hour and recovered after several hours.

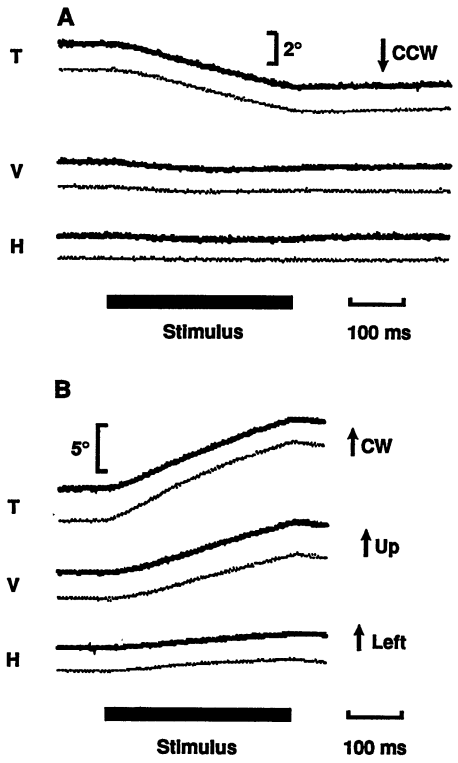

Fig. 2. Eye position during unilateral microstimulation of the (**A**) left and (**B**) right INC. Position of left eye (thick line) and right eye (thin line) are plotted as a function of time. The standard stimulus was delivered during the time indicated by the bar. The ramplike change in position indicates relatively constant velocity. Eye position traces are truncated at the beginning of the first saccade. CW, clockwise; CCW, counterclockwise; T, torsional eye position; V, vertical; H, horizontal.

After vertical saccades, eye position drifted exponentially toward a central vertical null value (Fig. 3A), either upward or downward, depending on initial position (*12*). Horizontal positions did not exhibit centripetal drift. The eye also exhibited an immediate torsional drift (Fig. 3, B and C) away from the plane of zero torsion that is normally maintained by saccades (*10*). These results were observed in both eyes. Thus, the animals could only maintain a line of horizontal positions at the intersection of the torsional and vertical null values. The vertical position of this line was usually above the center of the eyes range of motion, so upward drift was most prevalent. The null line was almost always shifted clockwise during left INC inactivation (Fig. 3B) and counterclockwise during right INC inactivation (Fig. 3C). Thus, the torsional drift produced by unilateral INC inactivation was in the direction opposite to the rotation produced by comparable unilateral stimulation.

To quantify the severity of this position deficit, we computed the time constants of drift. Figure 4A illustrates the exponential nature of the drift. After a saccade, vertical and torsional positions drifted toward the null line at a rate proportional to their distance from this line. The time constant is defined as the time required to drift two-thirds of this distance. Complete failure of the integrator should result in drift with the intrinsic time constant of the eye, approximately 200 ms (*13*). The average time constants for vertical and torsional drift were indicative of total integrator failure in four out of five animals (Fig. 4B) (*14*).

These results show that the INC is an essential component of the oculomotor integrator. Earlier work suggests that integration is distributed between several regions, including the vestibular nuclei (*3, 6*). As a simplification, we can divide the brainstem oculomotor circuitry into two groups, each consisting of a saccade burst generator, an integrator, and motoneurons. The pontine group consists of neurons that drive the eye horizontally toward the side of the brain activated (*2, 15*). The midbrain group is organized so that each neuron influences both vertical and torsional eye position (Fig. 1) (*8*). Our data show that the integrator for this group is the INC. These circuits utilize the same organization that is observed in the vestibular canals, which provide a velocity signal to the integrator for the vestibulo-ocular reflex (*4, 16*).

The oculomotor and vestibular systems may have paired torsional and vertical rotations in order to maintain symmetry across the midsagittal plane (a vertical plane bisect-

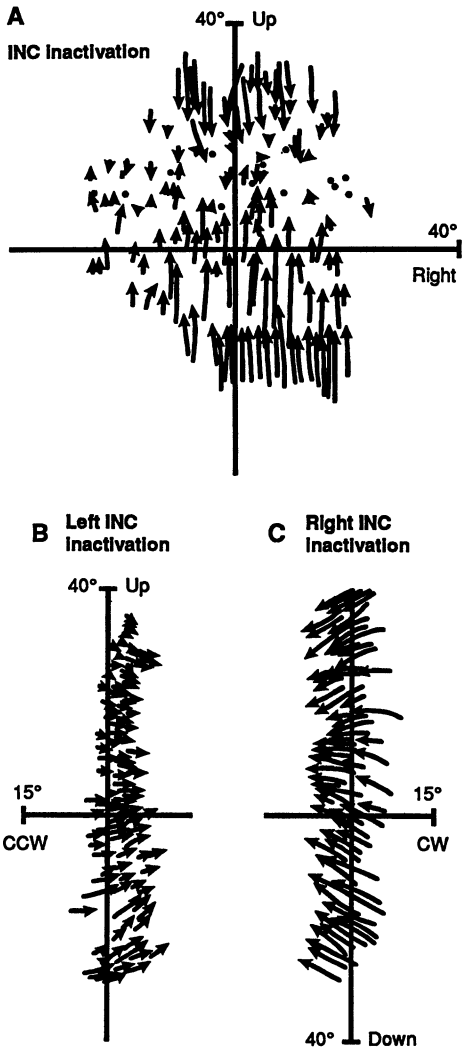

Fig. 3. Direction of eye position drift during unilateral INC inactivation. Only positions between voluntary eye movements are shown, with arrows indicating direction of drift. (**A**) Horizontal and vertical plot of eye position 48 min after muscimol injection. Torsional drift is not visible in this view. (**B** and **C**): Torsional and vertical plots of eye position immediately after muscimol injection. This direction pattern is later obscured as muscimol spreads to other structures, producing abnormal torsional saccades.

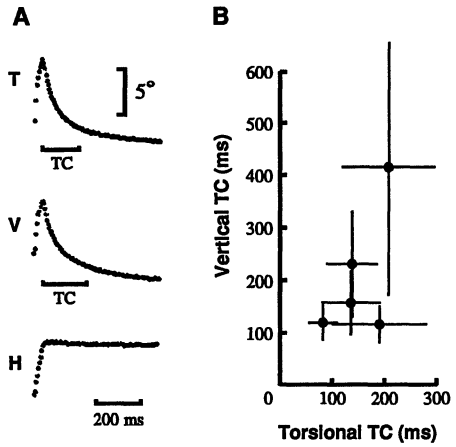

Fig. 4. Time constants (TCs) of exponential drift during unilateral INC inactivation. (**A**) Eye position is plotted as a function of time to illustrate an example of the exponential drift that followed saccades. TCs are illustrated for reference. (**B**) Vertical and torsional time constants of drift from all five animals. Solid circles, mean ±SD for one animal. To obtain this data, we selected an experiment from each animal in which a severe eye position deficit was present ~30 min after muscimol injection. TCs of vertical and torsional drift were then computed for a series of individual examples and averaged.

ing the head front to back). The left-right and clockwise-counterclockwise rotation pairs are mirror images across this plane, but up and down are not (*17*). To achieve bilateral symmetry, up and down must be represented equally on both sides of the brain. This would introduce redundancy, unless the up-down pair is combined with one of the other pairs. Vertical could be combined with horizontal to give two diagonal signals on each side and an independent torsional system. With this system, production of horizontal eye rotations would require coactivation of two muscles per eye, so that their vertical components would cancel out. In the other choice, vertical could be combined with torsional; this combination leaves an independent horizontal system that produces horizontal eye rotations without muscle coactivation. The latter choice is more energy efficient, because in normal orienting behavior, horizontal head and eye rotations are largest and most important, whereas torsional rotations are smallest and are selectively minimized (*4, 10*). Therefore, the oculomotor and vestibular systems appear to utilize the unique coordinate system that optimizes orthogonality, bilateral symmetry, nonredundancy, and energy efficiency.

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10. D. Tweed and T. Vilis, *ibid.*, p. 111. According to Listing's law, saccades confine eye position to a plane that is orthogonal to the torsional axis (the line of gaze at primary position).
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12. Vestibular imbalance produces unidirectional constant velocity drift.
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Mediation of the Attachment or Fusion Step in Vesicular Transport by the GTP-Binding Ypt1 Protein

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The function of the guanosine triphosphate (GTP)–binding protein Ypt1 in regulating vesicular traffic was studied in a cell-free system that reconstitutes transport from the endoplasmic reticulum to the Golgi. Blocking the Ypt1 protein activity resulted in accumulation of vesicles that act as an intermediate passing between the two compartments. The Ypt1 protein was found on the outer side of these vesicles. The transport process is completed by fusion of these vesicles with the acceptor compartment, and Ypt1 protein activity was needed for this step. Thus, a specific GTP-binding protein is required for either attachment or fusion (or both) of secretory vesicles with the acceptor compartment during protein secretion.

SECRETION OF PROTEINS FROM CELLS requires the orderly progression of those proteins through a series of membranous compartments (*1*). Each step in the transport pathway appears to be mediated by intermediate vesicles that form at the surface of the donor compartment and fuse specifically with the acceptor compartment. As a result, the contents of the vesicles are transported to the acceptor compartment (*2*). The basis for the specificity of fusion of vesicles with the correct acceptor compartment is not known, but multiple GTP-binding proteins may regulate this process at different steps of the pathway (*3*).

Studies of two proteins from yeast, Sec4 and Ypt1, indicate that GTP-binding proteins function in protein transport. Sec4 participates in the last step of secretion. It is localized to the plasma membrane and secretory vesicles in transit to the cell surface and is a GTP-binding protein (*4*). Ypt1 is a 23-kD GTP-binding protein (*5*) that is highly conserved; the yeast and the mammalian proteins share 70% identity (*6*). By genetic and immunolocalization analyses, Ypt1 has been shown to function in transport of proteins at the beginning of the secretory pathway in yeast and in mammalian cells (*3*). The Ypt1 protein is required for protein transport in vitro (*7, 8*). Small GTP-binding proteins have been identified in yeast and mammalian cells (*9*), and five of them have been localized to five different endocytic and secretory organelles (*10*). Association of uncharacterized small GTP-binding proteins with secretory vesicles has also been reported (*11*).

The non-hydrolyzable analog GTPγS blocks protein transport in yeast and mam-

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