Evidence for Brainstem Structures Participating in Oculomotor Integration

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The cerebellar flocculus has been implicated in vestibulo-oculomotor control. One major central input to this structure originates from brainstem cells in the paramedian tract (PMT), whose function is unknown. Here it is reported that PMT cells in the pons carry vestibular and eye movement signals and their pharmacological inactivation produces a leaky integrator combined with vestibular imbalance. The results suggest that PMT cells provide the cerebellum with sensory and motor signals that are essential for velocity-to-position integration, a common premotor process that is required in all motor systems.

The neuronal machinery that creates a final output signal for eye movements, located in the brainstem, is one of the best understood premotor circuits. To work properly, this brainstem mechanism needs assistance from the cerebellum, another brain structure that has long been implicated in fine movement control. In particular, the flocculus is a major cerebellar region that is intimately involved in oculomotor function. Among other things, ablation of the primate flocculus impairs smooth tracking of visual targets and maintenance of eccentric gaze (*1*). It is generally believed that the cerebellum receives both sensory and motor information and sends out a signal that ensures normal operation of the premotor circuitry.

For gaze maintenance, the velocity-encoded motor command must be integrated to produce an appropriate eye position command by a neural circuit called the oculomotor neural integrator (*2*). Cerebellar-brainstem interaction is necessary for this neural integration process (*1*, *3*, *4*). The cell groups of the PMT, several cell clusters located along the midline of the pons and medulla, are one possible neuronal substrate that participate in this interaction (*5*, *6*). They project to the flocculus and receive direct projections from brainstem areas containing preoculomotor neurons. However, little is known about the exact role of PMT cells in controlling eye movements. We studied one subgroup of PMT cells in the pons at the level immediately rostral to the abducens nucleus.

We searched the pontine PMT region for neurons discharging in relation to eye movements. In the ventral part of this region, we encountered many omnipause neurons (OPNs), which paused for saccades in all directions. In the medial longitudinal fasciculus (MLF) dorsal to this OPN area, there was a concentration of units with vertical eye movement–related activity. All these unit spikes were judged to be somatic on the basis of the criteria described in (*7*). Because these vertical eye movement–related neurons increased their firing rate for upward eye movements and showed a burst-tonic firing pattern, we call them up-BT neurons. The location of 35 up-BT neurons collected in this study is shown in Fig. 1A. OPNs are shown for comparison. Most up-BT neurons were located in a region 0 to 2.0 mm rostral to the rostral pole of the abducens nucleus and within the boundary of the MLF.

The behavior of an up-BT neuron during saccades is shown in Fig. 1B. During intersaccadic intervals, the cell exhibits tonic firing rates that increase linearly with upward eye position (Fig. 1C). The correlation was highly significant for all 35 up-BT neurons examined ($r = 0.78$ to 0.97; $P < 0.001$). The slope of the regression lines representing eye-position sensitivity ranged from 1.4 to 8.9, with a mean of 3.8 ± 1.6 (spikes/s)/°. Up-BT neurons exhibited a burst of spikes for every upward saccade (Fig. 1B). There is also a linear relationship between the number of spikes in the burst component and the amplitude of upward saccades (Fig. 1D). The slope of regression lines representing saccadic sensitivity ranged from 0.31 to 6.90, with a mean of 2.70 ± 1.41 spikes/°. These results indicate that up-BT cells encode both eye position and velocity with high fidelity.

Using natural and electrical stimuli, we examined a possible vestibular input to up-BT neurons. We recorded responses to rotations in two vertical canal planes. Typical responses for one neuron are shown in Fig. 2. The spike discharge of the neuron is well modulated during rotation in the contralateral anterior–ipsilateral posterior canal (c-ac/i-pc) plane (Fig. 2A). The modulation is approximately in-phase with nose-down head velocity, which excites the contralateral anterior semicircular canal. In contrast, response to rotation in the ipsilateral anterior–contralateral posterior canal (i-ac/c-pc) plane was much weaker (Fig. 2B). Up-BT neurons exhibited clear excitatory responses to electrical stimulation of the contralateral vestibular nerve (Fig. 2C) with latencies ranging from 1.44 to 2.60 ms, which suggests that the short-

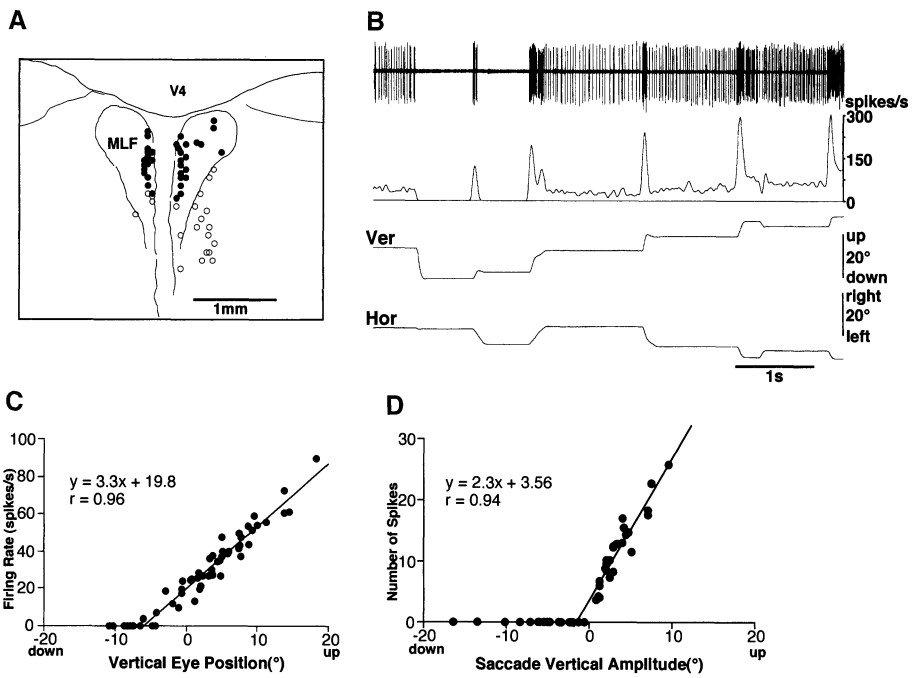

Fig. 1. Location (**A**) and firing characteristics (**B** through **D**) of up-BT neurons. Filled and open circles in (A) represent up-BT neurons and OPNs, respectively. V4, fourth ventricle; MLF, medial longitudinal fasciculus. Traces in (B) are, from top, spike activity, firing rate, and vertical (Ver) and horizontal (Hor) eye positions.

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est pathway was disynaptic. Thus, up-BT neuron responses to electrical and natural vestibular stimulation suggest that they receive direct excitatory input from secondary vestibular neurons mediating signals from the contralateral anterior semicircular canal. The result is consistent with anatomical findings that the PMT region receives projections from the vestibular nucleus (*8*).

We reversibly inactivated up-BT cells by injecting muscimol at sites where these cells were recorded. After muscimol injection, a consistent symptom was downbeat nystagmus, which was best evoked on looking downward. Unlike simple vestibular nystagmus, vertical gaze holding was impaired, as indicated by an exponential time course of slow phases (gaze-evoked nystagmus) (Fig. 3). Similar but milder symptoms were often seen for horizontal movements. The oculomotor deficit began within about 5 min, was maximal in about 30 min, and recovered several hours after injections of muscimol.

To measure the extent of damage in the velocity-to-position integration process, we estimated the time constant (TC) of the exponential drift of the slow phases. The null position and the TC were measured for vertical eye movements after each of 11 injections. In a given injection, TCs varied across slow phases so the averaged TC during maximal deficits was taken as a representative value for the injection. TCs ranged from 0.31 to 1.02 s, with a mean of 0.57 s (n = 11). The null position was found to depend on the initial eye position of the slow phase: the more downward the initial position, the less upward the null position. For each of 11 injections, there was a linear relationship between the two parameters (r = 0.77–0.99, $P < 0.001$). Although the null was distributed in a large range of eye position, there was a point above which the direction of the slow phase was downward. This reversal point ranged from about 15° to 25° upward, well above the primary position, resulting in slow phases with upward directions in a broad range of vertical eye positions.

The effective injection sites for producing vertical gaze-holding impairment in one animal are summarized in Fig. 4. These sites corresponded to the region where most pontine up-BT neurons were recorded (Figs. 1A and 4B). Results were similar in two other animals. Injections of saline at effective PMT sites did not impair gaze holding, which indicates that the effect of muscimol injection was not due to damage to MLF fibers.

Inactivation of PMT cells had an effect on the integrator function similar to that found in previous experiments lesioning the flocculus, vestibular nucleus, nucleus prepositus hypoglossi, or the interstitial nucleus of Cajal (*1, 9–11*). This implies that the integrity of all related structures is important for integration. Silencing PMT cells, probably by altering the activity in the flocculus, indirectly affected premotor eye position signals in these brainstem structures, leading to the gaze-holding impairment. Clinically, this experiment raises a possibility that gaze-evoked nystagmus in human MLF lesions may be partly due to damage to PMT cells.

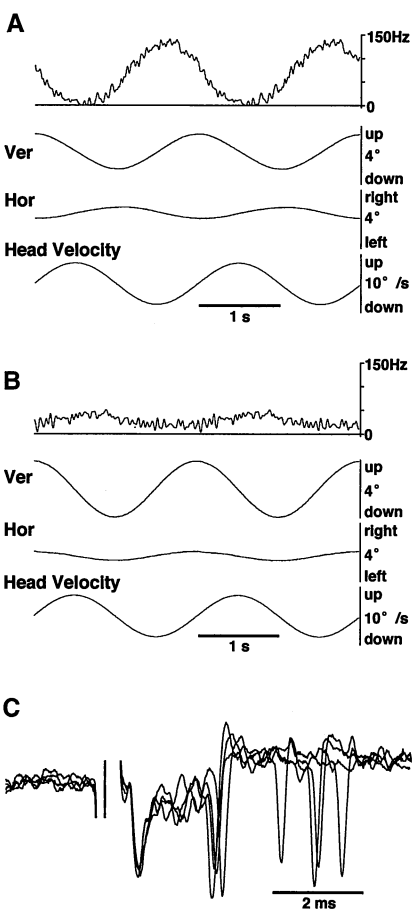

Fig. 2. Responses of up-BT neurons to natural (**A** and **B**) and electrical (**C**) vestibular stimuli. Averaged responses during sinusoidal rotation at 0.5 Hz are shown in (A) and (B).

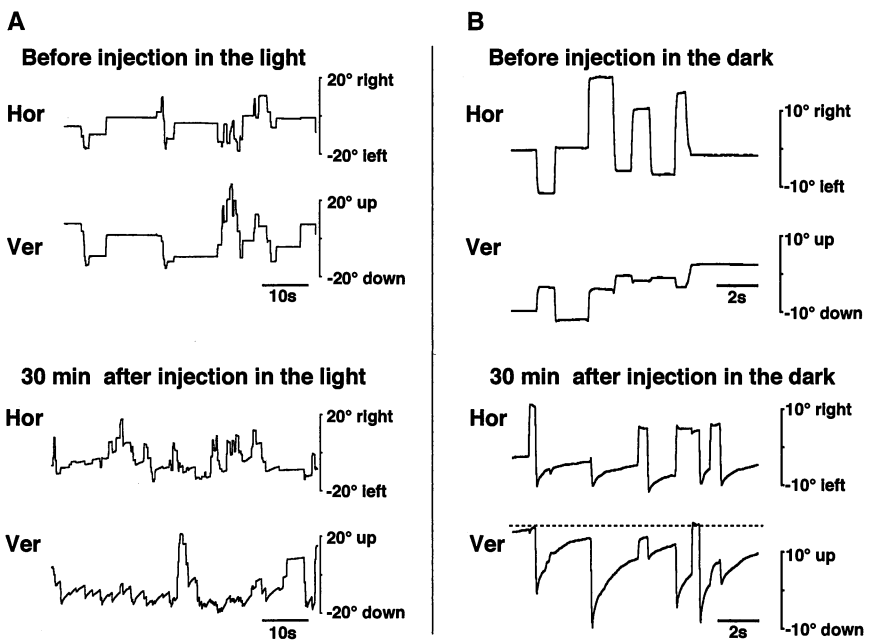

Fig. 3. Eye movements before and after muscimol injection in the light (**A**) and dark (**B**). Upper and lower traces are horizontal (Hor) and vertical (Ver) eye position, respectively. Dotted line indicates a reversal point.

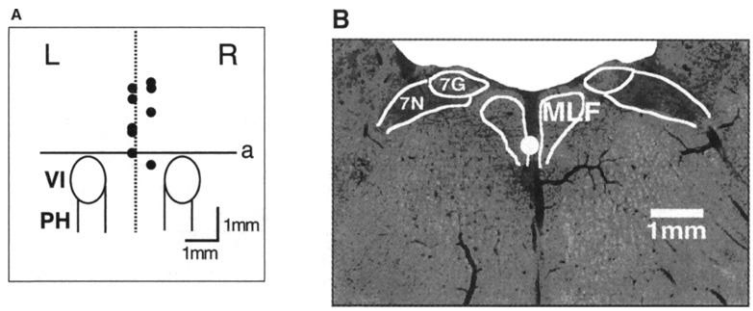

Fig. 4. Effective injection sites in the MLF shown on a horizontal (**A**) and a transverse (**B**) section. (B) Corresponds to line a in (A). 7G, genu of the facial nerve; VI, abducens nucleus; PH, nucleus prepositus hypoglossi.

A remarkable up-down asymmetry in the nystagmus, together with the strong vestibular input to up-BT cells, appears to suggest an additional vestibular imbalance. The region where we injected muscimol contained up-BT neurons and no down eye movement cells. Because up-BT neurons received excitatory inputs from the anterior semicircular canals, their inactivation might be expected to result in downward drift of the eye due to a decreased anterior canal input. Instead, the eye drifted upward in a wide oculomotor range, suggesting increased, rather than decreased, signals from the anterior canal.

What is the neural mechanism that produces the central vestibular imbalance of anterior canal dominance? Up-BT neurons probably project to the flocculus, which has a unique connection pattern with the vertical canal system. Only anterior canal–related vestibular nucleus neurons receive floccular inhibition (*12*). Therefore, inactivation of the up-BT neurons may reduce the activity of Purkinje cells, leading to disinhibition of vestibular neurons that receive inputs from the anterior canal. These vestibular neurons then exhibit increased discharge. The anterior canal input to the brainstem circuitry is increased while the posterior canal input remains unchanged. This idea is supported by a similar downbeat nystagmus after floccular lesions (*1*). This experiment thus suggests that the PMT-flocculus-vestibular nucleus pathway is important in maintaining vestibular balance. It should be noted, however, that the asymmetry of nystagmus we observed does not necessarily indicate an imbalance of vestibular inputs to the neural integrator. The PMT-flocculus pathway may be involved in an intrinsic mechanism of the integrator that sets the neutral eye position.

It is known that the cerebellum is necessary for normal operation of the brainstem neural integrators (*1, 3, 4*). The cerebellum must acquire oculomotor signals from the brainstem. This study suggests that our up-BT neurons relay eye position information to the flocculus. Furthermore, the effect of inactivation of up-BT cells indicates their importance in the oculomotor integration. The caudal pontine PMT area may be a new component of the neural integration system for vertical, and perhaps horizontal, eye movement, along with the midbrain interstitial nucleus of Cajal, vestibular nuclei, and nucleus prepositus hypoglossi.

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7. Five adult cats were prepared for recording of neuronal activity in the brainstem. All experiments were done with the permission of the Animal Experiment Committee of the University of Tsukuba, which is operated in accordance with Japanese Governmental Law (no. 105). A coil was implanted subconjunctivally under pentobarbital sodium anesthesia and aseptic conditions to measure eye movement by a magnetic-search coil technique (*13*). The tympanic bulla on each side was opened, and silver-ball electrodes were implanted on the round window to stimulate the vestibular nerve. After recovery from surgery, each animal was trained to accept restraining conditions without stress. A position 16.5° nose up from the stereotaxic horizontal was taken as a zero vertical position. This was near the center of the vertical oculomotor range. Glass-coated tungsten electrodes were used for extracellular recordings. Care was taken to ensure that the recording was from a cell body and not from an axon. Only negative-positive spikes with a duration (time to positive peak) of >250 μs were regarded as action potentials of the soma. These unit spikes could be recorded not only during advancement but also during withdrawal of the electrode, another indication of somatic recording. The MLF was identified physiologically by recording monosynaptic volleys from the vestibular nerves and by monosynaptic activation of secondary vestibular axons, which all exhibited initial positivity. The explored region extended rostrocaudally from 2.0 mm anterior to 1.5 mm posterior of the rostral pole of the abducens nucleus and ventrally to 3.5 mm from the floor of the fourth ventricle. The lateral limit of the region extended from the midline to about 1.5 mm. The tonic firing rate of a cell was defined as an average rate for the fixation period. The size of the burst component for a given saccade was estimated by subtracting the eye position–dependent component from the total number of spikes [see (*14*)]. For natural vestibular stimulation, the turntable was rotated sinusoidally in the light in two mutually orthogonal planes approximately coplanar with the two vertical canal pairs (45° away from the pitch-and-roll planes). The two planes are called the c-ac/i-pc plane and the i-ac/c-pc plane, depending on their relation to the side of neurons studied. Because the null position, an asymptote of the exponential drift, appeared to vary from one slow phase to another, we estimated the null and TC for each slow phase. We first determined the null as a position best linearizing the logarithm of eye displacement from that position as a function of time. At the termination of the experiments, some recording sites were marked by making small electrolytic lesions. The animals were then killed with a lethal dose of pentobarbital sodium and perfused. In three alert cats, muscimol, an inhibitory neurotransmitter, γ-aminobutyric acid type A receptor agonist (1.0 μg per microliter of saline, 0.2 to 0.6 μl), was injected in the MLF of the pons with an injection needle to suppress spike activity of somata without affecting that of MLF fibers.
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Direct Targeting of Light Signals to a Promoter Element–Bound Transcription Factor

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Light signals perceived by the phytochrome family of sensory photoreceptors are transduced to photoresponsive genes by an unknown mechanism. Here, we show that the basic helix-loop-helix transcription factor PIF3 binds specifically to a G-box DNA-sequence motif present in various light-regulated gene promoters, and that phytochrome B binds reversibly to G-box–bound PIF3 specifically upon light-triggered conversion of the photoreceptor to its biologically active conformer. We suggest that the phytochromes may function as integral light-switchable components of transcriptional regulator complexes, permitting continuous and immediate sensing of changes in this environmental signal directly at target gene promoters.

Plants use a set of sensory photoreceptors to monitor the environment for informational light signals (*1*). The phytochrome (phy) family, comprising five members (phyA to phyE) in *Arabidopsis*, track the red (R) and far red (FR) light wavelengths by virtue of their capacity for photoinduced, reversible switching between two conformers: the R-absorbing, biologically inactive Pr form and the FR-absorbing, biologically active Pfr form. Each phy molecule is a dimer of subunits that consist of a ≈125-kD polypeptide with a covalently bound tetrapyrrole chromophore that is autocatalytically attached by the apoprotein (*2*). Light-driven Pfr formation induces changes in the expression of numerous genes underlying various aspects of

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