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8. *P. falciparum* was maintained under standard conditions (*28*) with human O^+ erythrocytes in complete medium (RPMI 1640 medium with 10% human AB^+ serum and 40 μg/ml gentamicin). Human cell lines were cultured as described (*29*).
9. Supplementary material showing in vitro G25 activities, all data on G25-treated monkeys, detailed G25 clinical effects, animal study protocols, VB5 accumulation assays, and the chemical syntheses of VB5-T and VB5-H are available on *Science* Online at www.sciencemag.org/cgi/content/full/295/5558/1311/DC1.
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REPORTS

Midbrain Control of Three-Dimensional Head Orientation

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Little is known about the neural mechanisms controlling head posture and why they fail in clinical syndromes like torticollis. It is well established, however, that the brain controls eye position by integrating eye velocity commands. By electrically stimulating and reversibly inactivating midbrain sites in the head-free (nonimmobilized) monkey, we found that the interstitial nucleus of Cajal functions as a neural integrator for head posture. We suggest that a bilateral imbalance in this structure, through either direct damage or inappropriate input, could be one of the mechanisms underlying torticollis.

The head participates in the control of visual gaze (*1*, *2*) and in several stabilizing/righting reflexes (*3*, *4*). However, some people have a clinical disorder called torticollis (literally meaning "twisted neck") that causes their heads to become locked in inappropriate positions (*5*, *6*). Although these abnormal positions may have various horizontal and vertical components, the head is usually deviated torsionally (i.e., rolled about an axis running between the nose and the back of the head) (*6*). Unfortunately, little is known about the neural mechanisms of either normal or abnormal head posture. For example, it is not known if head posture is an emergent property of distributed reflex systems or if there is one common path that sets the desired level of neck muscle activation (*6*).

One clue might be drawn from the oculomotor system, which is closely associated with head control during gaze shifts (*1*, *7*) but is much better understood. Eye orientation is held by a neural integrator that converts velocity-like movement commands into tonic position signals for the eye muscles (*8*). The pons and medulla have circuitry for horizontal eye velocity integration (*9*), whereas the midbrain interstitial nucleus of Cajal (INC) participates in integrating vertical and torsional eye movement signals, with clockwise (CW) and counterclockwise (CCW) torsion controlled on opposite sides of the midline (*10*, *11*). Anatomical and physiological evidence also implicate the INC in the control of head motion because some of its output neurons participate in the interstitio-spinal tract, which controls neck muscles (*12*). Thus, the INC might also have a neural integrator for head control (*13*, *14*).

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We recorded three-dimensional (3D) orientations of the eye and head in four alert and behaving monkeys (*Macaca fascicularis*) (*15*, *16*). The 3D motor behavior and brainstem physiology of these monkeys are nearly identical to that of humans (*17*, *18*). Electrical stimulations were delivered to the INC and surrounding regions during periods of motionless gaze fixation (*19*). In all, 93 putative INC sites were stimulated in monkey 1 (M1), 19 sites in M2, 9 sites in M3, and 8 sites in M4 in light, dim light, and complete darkness.

Figure 1 shows simulated head caricatures accurately depicting final 3D head orientations measured after stimulations in either the left or right INC. These stimulations produced mainly torsional deviations in head position, with final head postures resembling those seen in torticollis. The same stimulations produced small or variable vertical movements (perhaps because up and down vertical signals are intermingled in the INC so that they cancel during stimulation) and even smaller systematic horizontal movements (Fig. 1, B and C). Stimulation of the right side (from midline) always produced CW rotations of the head (77 sites), whereas leftward stimulations always produced CCW head rotations (52 sites), from the subject's viewpoint (*20*).

These observations suggest that the INC is involved in the active control of head orientation. To understand the nature of its control signals, we looked at the time course of the evoked torsional movements (Fig. 1, B and C). These trajectories initially showed a delayed and sluggish response after stimulation onset, as expected with a high-inertia system like the head. But once in motion, the head moved with a constant velocity "ramp" until stimulation offset. Then, after a brief delay, the head stopped moving and held all or most of its induced torsional position (*21*). This is the sort of time course expected if one charges up an integrator with a constant input and then

sends the output signal to a high-inertia system like the head.

We then reversibly inactivated small regions of neural tissue by injecting the γ-aminobutyric acid ($GABA_A$) receptor agonist muscimol into several of the same midbrain sites. The injections were performed with the animals in complete darkness to prevent contamination by visual feedback. A total of 22 such injections were performed—15 in M1, 5 in M2, and 2 in M3. If the INC has a neural integrator for head position, as it does for eye position, one would expect the muscimol injections to disrupt this circuit such that the animals can no longer hold their final head orientations at the end of each gaze shift.

After the injection of muscimol into the INC, the animals were still able to generate torsional, vertical, and horizontal head movements during gaze shifts (dotted curves) (Fig. 2). However, after these movements, they were unable to hold a stable head posture. Instead, the head appeared to drift toward some resting position (solid curves). This pattern occurred immediately after injections made directly into the INC but failed to occur for more lateral, posterior, or anterior injections.

To quantify this effect, we computed the time constant of drift in the torsional, vertical, and horizontal dimensions (*22*). This is the indicator of "position holding" used to quantify integrator failure in the oculomotor system (*10*). During normal gaze fixations, time constants were 20.96 ± 5.60 s (mean ± SE) for torsional head position, 19.64 ± 5.25 s for vertical head position, and 12.75 ± 3.41 s for horizontal head position, indicating very stable holding of the head. However, after INC muscimol injections, time constants (averaged across the 11 experiments) dropped to 0.68 ± 0.05 s (mean ± SE) for torsional head position, 0.72 ± 0.07 s for vertical head position, and 4.73 ± 1.09 s for horizontal head position. This indicates a severe deficit in holding torsional and vertical head components, with a more moderate effect on the horizontal component. According to neural integrator theory (*8*), this suggests that the central neural integrators for torsional and vertical head position were incapacitated, leaving the head to drift at a rate determined by its own mechanical properties.

What might these results have to do with torticollis? We have described the initial response to a pharmacological lesion, whereas clinical torticollis is presumably the final response to some damage or dysfunction. Figure 3 illustrates the typical development of head deficits, in the torsional dimension, after muscimol injection. Initially, the monkeys developed a torsional head nystagmus. This term, taken from the oculomotor literature, refers to a pattern of slow drifting movements (solid curve) interspersed with more rapid resetting movements (dotted curve). However, as the injection-induced deficit progressed, the animals stopped producing the rapid corrective components. Without these resetting components, the head settled to a torsionally shifted range. This shifting effect usually began immediately after muscimol injection (in the dark) and peaked after 30 min, with deviations in torsional head posture of up to 85°.

To characterize these final head postures, we simulated head caricatures (*23*) that accurately described the final 3D head orientations of the three monkeys (while they looked straight ahead). Compared with controls, left INC injections consistently caused CW deviations in head posture, whereas right INC injections caused CCW head deviations (as well as variable horizontal and vertical offsets), i.e., opposite to positions produced by stimulating the same sites (Fig. 1). When we quantified the full range of these final head orientations across all gaze directions (*24*, *25*), we found the pattern to be identical to that observed in torticollis patients (i.e., torsionally shifted, but with the same shape and torsional variance as controls) (*6*). Further quantitative studies will be required to compare the horizontal and vertical offsets in these animals with those seen in humans.

We interpret these data to suggest that there is a neural integrator for the purpose of holding final head posture. Specifically, the INC plays a vital role in the neural circuit for the integration of torsional head movement commands, like its role in eye movement control (*10*, *11*). And, as in the oculomotor system (*10*), CW and CCW head torsion is controlled on opposite sides of the midbrain. This does not necessarily mean that exactly the same neurons serve this function for both the eye and head integrators; very likely different neurons are required to control these two structures.

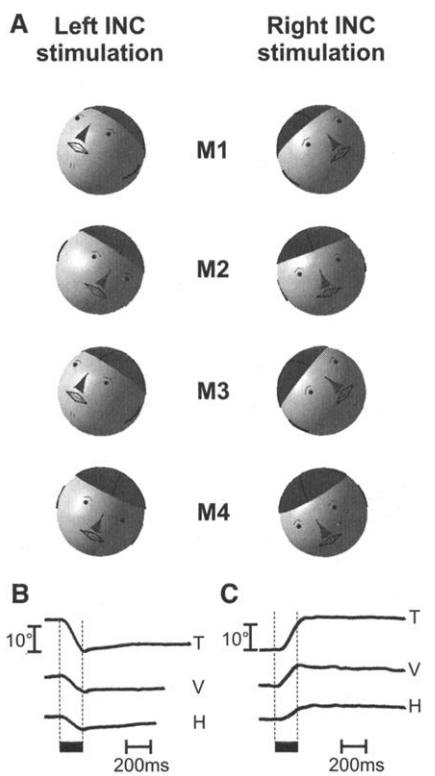

Fig. 1. 3D head positions elicited by INC stimulation. (**A**) M1 to M4. CCW head rotations were elicited after stimulations of the left INC (left column), whereas CW head rotations were elicited after stimulations of the right INC (right column). (**B** and **C**) Torsional, vertical, and horizontal components of head movement are plotted against time. Upward directions in these plots indicate CW, upward, and leftward movements. The area between dashed vertical lines indicates the period of stimulation. Elicited gaze (eye-in-space) movements were in the same direction as the elicited head movements but were somewhat larger in magnitude because of additional eye-in-head movements.

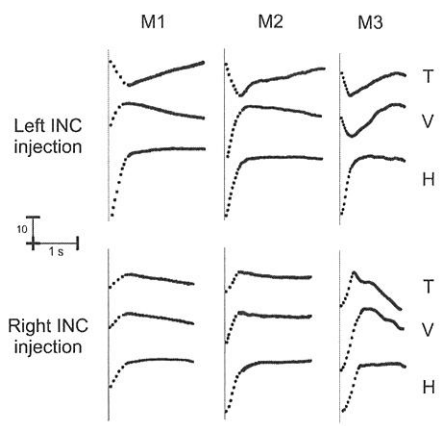

Fig. 2. Head holding deficits brought about by INC inactivation. Torsional (T), vertical (V), and horizontal (H) components of typical head movements after INC inactivation are plotted against time. Examples are shown for each of three monkeys (M1, M2, and M3) for left (upper row) and right (lower row) INC inactivation.

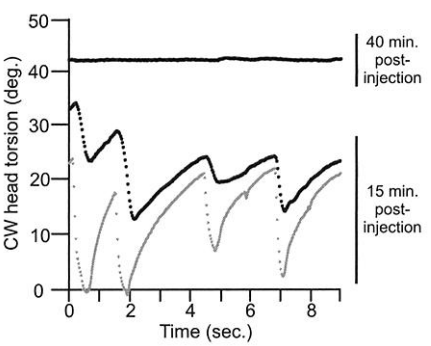

Fig. 3. Time course of changes in torsional head posture after left INC muscimol injection. Torsional head position (black) is plotted against time. Corresponding gaze (eye-in-space) trajectories (gray) followed a similar pattern of movement but were slightly larger in amplitude because gaze is composed of both head and eye-in-head movements (note that head and gaze were not always so tightly coupled).

These results and conclusions might also provide insights into the neural mechanism for human torticollis. Whereas previously this disorder was often thought to be a secondary response to problems in eye control, here we show that it can result from damage to the primary mesencephalic circuits for head motor control. Midbrain damage in humans is known to produce similar symptoms of torticollis (*26*, *27*). According to our interpretation, torticollis can result from a bilateral imbalance between the two sides of the INC, which control opposite directions of torsion. This could result from direct damage to the INC neural integrator or from an input imbalance to the INC (*28*, *29*).

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15. This method requires three orthogonal magnetic fields generating three signals in each of two search coils per eye/head. The resulting signals are converted into quaternions, which give the torsional, vertical, and horizontal components of rotation from reference position to any other position.
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19. Tungsten microelectrodes were used to delivered pulse trains of (50 μA, 200 Hz) 300 ms.
20. The largest head responses were evoked along the midline oculomotor region. Head responses were also evoked in an additional 34 midline sites within the more anterior riMLF (rostral interstitial nucleus of the medial longitudinal fasciculus) region. riMLF stimulation produced similar effects but had different unit responses and little or no head nystagmus during muscimol injections.
21. This torsion was corrected by subsequent self-induced head movements, which included a torsional component that brought the head back to a natural position.
22. We first computed the velocity of drift as a function of head position along each dimension. The inverse of these values was then taken to obtain the time constant of drift.
23. Web fig. 1 is available on *Science* Online at www.sciencemag.org/cgi/content/full/295/5558/1314/DC1.
24. To quantify the orientation ranges of the head, we used the same method applied previously to torticollis patients. Second-order surface fits were made to head quaternions with the following formula for a quaternion (q): $q_1 = a_1 + a_2q_2 + a_3q_3 + a_4(q_2)^2 + 2a_5q_2q_3 + a_6(q_3)^2$, where q_1 is the torsional, q_2 is the vertical, and q_3 is the horizontal component. The thicknesses of these surfaces are then computed as the torsional standard deviation of the individual data points from the fit. The final torsional scatter of the head orientation ranges from their ideal fits was not significantly different in the postinjection data (4.19° ± 0.38° SE) as compared with preinjection behavioral controls (4.59° ± 0.36° SE) ($P > 0.519$). In addition, the characteristic twists of these surfaces (a_5) were not significantly different between controls and either M1 ($P > 0.507$), M2 ($P > 0.523$), or M3 ($P > 0.945$). These results are identical to those reported for torticollis patients.
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28. For example, damage to other centers implicated in torticollis, such as the basal ganglia, could produce torticollis by failing to send properly balanced inputs to the INC. If so, we would predict that damage to these areas would produce torticollis without the drift or nystagmus observed here.
29. An imbalance in a horizontal head position integrator, presumably centralized in some other brainstem structure, could similarly explain the stronger horizontal deviations seen in some patients.

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