Three-Dimensional Eye–Head Coordination After Injection of Muscimol Into the Interstitial Nucleus of Cajal (INC)

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Three-dimensional eye–head coordination after injection of muscimol into the interstitial nucleus of Cajal (INC). J Neurophysiol 97: 2322–2338, 2007. First published January 17, 2007; doi:10.1152/jn.00752.2006. The interstitial nucleus of Cajal (INC) is thought to be the “neural integrator” for torsional/vertical eye position and head posture. Here, we investigated the coordination of eye and head movements after reversible INC inactivation. Three-dimensional (3-D) eye–head movements were recorded in three head-unrestrained monkeys using search coils. INC sites were identified by unit recording/electrical stimulation and then reversibly inactivated by 0.3 µl of 0.05% muscimol injection into 26 INC sites. After muscimol injection, the eye and head tilted torsionally CW/CCW after left/right INC inactivation. Three-dimensional (3-D) eye–head movements were recorded in three head-unrestrained monkeys using search coils. INC sites were identified by unit recording/electrical stimulation and then reversibly inactivated by 0.3 µl of 0.05% muscimol injection into 26 INC sites. After muscimol injection, the eye and head tilted torsionally CW/CCW after left/right INC inactivation. Two) the eye and head tilted torsionally CW/CCW after left/right INC inactivation, respectively. Horizontal gaze/head drifts were inconsistently present and did not result in considerable position offsets. Vertical eye drift was dependent on both vertical eye position and the magnitude of the previous vertical saccade, as in head-fixed condition. This correlation was smaller for gaze and head drift, suggesting that the gaze and head deficits could not be explained by a first-order integrator model. Ocular counterroll (OC) was completely disrupted. The gain of torsional vestibuloocular reflex (VOR) during spontaneous eye and head movements was reduced by 22% in both CW/CCW directions after either left or right INC inactivation. Our results suggest a complex interdependence of eye and head deficits after INC inactivation during fixation, gaze shifts, and VOR. Some of our results resemble the symptoms of spasmodic torticollis (ST).

INTRODUCTION

Gaze shifts are composed of coordinated eye and head movements (Freedman and Sparks 1997a; Guitton 1992; Phillips et al. 1995; Tomlinson 1990). To fixate visual targets, the eye and head must hold their position fixed after every gaze shift. Robinson (1968) proposed a model for saccadic eye movements that included a phasic velocity signal that drives the eyes from their initial position toward that of the target (King and Fuchs 1979; Luschei and Fuchs 1972) and a steplike tonic position signal that keeps the eyes directed in their final postmovement position until the next movement occurs (otherwise the eyes would drift back toward their resting position) (Cannon and Robinson 1987; Cheron and Godaux 1987; Crawford et al. 1991). The position signal is thought to be computed as a mathematical integration of the velocity signal in a structure called the neural integrator. It was recently proposed that there is a similar integrator for head orientation (Freedman and Sparks 2000; Klier et al. 2002a).

The nucleus prepositus hypoglossi (NPH) was previously shown to function as the neural integrator for horizontal eye movements (Cannon and Robinson 1987; Godaux et al. 1993; McFarland and Fuchs 1992). A number of studies showed that the mesencephalic interstitial nucleus of Cajal (INC) is the neural integrator for vertical and torsional components of eye movements (Crawford 1994; Crawford and Vilis 1993; Crawford et al. 1991; Fukushima 1987; Fukushima et al. 1990; Heldmen et al. 1998; King et al. 1981; Ranalli et al. 1988). Similarly, single-unit recordings of the INC neurons showed saccade-related burst activity for up/down and torsional quick eye rotations (Helmchen et al. 1996). These studies suggest that intermingled up/down integrators are located in both left and right INC, whereas the right and left INC control clockwise (CW) and counterclockwise (CCW) eye position, respectively, in a canal-like coordinated system that is aligned with Listing’s plane of the eye (Crawford 1994). To support such a scheme the INC receives the requisite anatomic inputs (Fukushima et al. 1980; King et al. 1980) and projects to the ocular motoneurons (Dalezios et al. 1998; Steiger and Buttnar-Ennever 1979; Szentagothai 1943). In addition, the INC also projects to the cervical spinal cord segments for neck control (Fukushima et al. 1980; Kohnstamm 1900; Nyberg-Hansen 1966; Probst 1900). Unilateral stimulation of the INC was previously shown to produce ipsitorsional eye movements that hold their final position until the next movement occurs (Crawford et al. 1991). After pharmacological inactivation of the INC, ocular torsion is offset in the opposite direction (Crawford and Vilis 1993; Helmchen et al. 1998). Moreover, animals are still able to make eye movements in all directions but there is a failure in holding up and down and torsional eye positions, manifested as an ocular drift that depends on both the eye position and the previous saccade amplitude (Crawford and Vilis 1993; Helmchen et al. 1998).

It has long been known that the INC is also responsible for head movements (Hassler and Dieckmann 1970) and recent investigations suggest that the INC also functions as the neural integrator for vertical and torsional head movements (Klier and Crawford 2003; Klier et al. 2002a). Similar observations described above for the eye were made for the head after
unilateral stimulation and pharmacological inactivation of the INC (Klier et al. 2002a). Neural integrators for CW and CCW head position also appear to be located in the right and left INC, respectively. However, in contrast to the eye, these are arranged in a Fick-like coordinate system, like the behavioral constraint observed in the head (Klier et al. 2007). Normally head torsion is kept near zero in Fick coordinates, where the head rotates about a body-fixed vertical axis and a head-fixed horizontal axis (Glenn and Vilis 1992). However, unilateral stimulation of the INC produces torsional head rotations (about the Fick coordinate for torsion) that violate this constraint (Klier et al. 2002a, 2007.

To date, the role of the INC as the neural integrator for torsional and vertical position holding was mainly studied separately for the eye and head (Crawford 1994; Crawford and Vilis 1993; Crawford et al. 1991; Fukushima 1987; Fukushima et al. 1990; Helmchen et al. 1998; King et al. 1981; Klier and Crawford 2003; Klier et al. 2002a). No one simultaneously studied both eye and head movements and specifically eye–head coordination during INC inactivation in the monkey. Note that gaze control is not a trivial addition of the eye and head observations because there are complex interactions between the eye and head during gaze saccades (Freedman et al. 1996; Gandhi and Sparks 2001; Guitton 1992). In addition, the head influences the eye through ocular counterroll (OC) and the vestibulocular reflex (VOR). Recent studies suggest that OC is degraded after INC inactivation (Crawford et al. 2003; Glasauer et al. 1998), whereas others report that OC is independent of INC function (Helmchen et al. 1998). It is also expected that INC failure should alter vertical and torsional VOR gain because the neural integrator forms the “indirect path” of the VOR (Cannon and Robinson 1987).

To study eye–head coordination after INC inactivation, we compared three-dimensional (3-D) aspects of head-unrestrained horizontal head position and the eye-in-head (eye) during the fixation period before and after pharmacological inactivation of the INC. We looked at the pattern of eye and head torsional offsets in the head-unrestrained monkey and their development over time. We also tested whether INC inactivation produces horizontal and vertical offsets in eye or head position and whether the contribution of head position to gaze position changes over time. To investigate the hypothesis that the INC is a neural integrator, we studied all components of eye and head for position-holding deficits (drift) and their dependency on position and the kinematics of the velocity input and compared our results to previous head-fixed studies (Crawford and Vilis 1993; Helmchen et al. 1998). We also compared the gain of the torsional, vertical, and horizontal angular VOR and functionality of OC before and after INC inactivation. Some of these results were previously presented in preliminary form (Farshadmanesh et al. 2005).

METH ODS

Animals and surgery

Three adult female monkeys (two Macaca fascicularis and one Macaca mulata) were each implanted with a skullcap composed of dental acrylic. The latter was fitted with a stainless steel chamber (mounted over a trephine hole in the skull and centered at 5 mm anterior and 0 mm lateral in stereotaxic coordinates) (Paxinos et al. 2000; Shantha et al. 1968) that allowed access to the INC. A stainless steel cylinder was attached to the front of the skullcap to immobilize the head when necessary. Two 5-mm-diameter scleral search coils were surgically implanted into one eye of each animal, placed so that the two were not parallel (one coil was placed in the nasal-superior quadrant, whereas the second was located in the nasal-inferior) to record 3-D eye movements (Crawford et al. 1999; Tweed et al. 1990). Surgery was performed under aseptic conditions and general anesthesia (isoflurane, 0.8–1.2%). After surgery, animals were medicated with an analgesic and prophylactic antibiotics. All protocols were in accordance with the Canadian Council on Animal Care guidelines on the use of laboratory animals and were preapproved by the York University Animal Care Committee.

We modified a Crist Instruments primate chair by removing the top plate and replacing it with a canvas cloth that buckled snugly at the back (Crawford et al. 1999) to ensure that the monkey’s head and neck were able to move freely. To prevent the upper body (up to the shoulders) from rotating about the vertical axis, we placed plastic jackets with one coil was placed in the nasal-superior first quadrant, whereas the second was located in the nasal-inferior) to record 3-D eye movements (Crawford et al. 1999; Tweed et al. 1990). During each experiment, the implanted stainless steel cylinder was released by a clamp allowing the head to move freely (Crawford et al. 1999).

Experimental procedures

Identification of the INC. Before each experiment began, we lowered a tungsten microelectrode (0.5–1.5 MΩ impedance; FHC) through a guide cannula at a preselected location using a hydraulic microdrive (model MO-90S; Narishige), while the animal’s head was restrained. Neuronal activity was then monitored on an audio monitor to identify burst-tonic neurons characteristic of the INC (Fukushima et al. 1990; King et al. 1981). If the tonic neural activity correlated with vertical eye positions, then the site was deemed to be a potential INC site. The rostral interstitial nucleus of the medial longitudinal fasciculus (rMLF), which is located anterior and lateral and also above the INC, showed similar burst activity during vertical movements, but was completely inactive during fixations (see Crawford and Vilis 1992; King and Fuchs 1979). To differentiate potential INC sites from the ventromedial third cranial nucleus region, we observed eye movements elicited in response to electrical microstimulation in head-fixed and head-unrestrained conditions [monophasic cathodal stimulations of 50 μA with pulse widths of 0.5 ms (300 Hz), and pulse trains of 200 ms]. If torsional eye and head movements were elicited after stimulation in the same direction and if the eye positions were maintained after electrical stimulation (Crawford et al. 1991), then we classified the site as likely lying within INC and examined it further with the injection of muscimol (see next section). The INC was further identified by a characteristic pattern of ocular drift, observed immediately after INC inactivation (see next section for details). In this way the entire region was explored for each monkey in an orderly stereotaxic pattern (every 0.5 mm in both anterior–posterior and medial–lateral directions). The temporary localized inactivation of the INC sites in this study was further confirmed by histological analysis (using tissue fixation, slicing the brain every 80 μm, staining the slides with 0.05% cresyl violet) and then observing under a light microscope to compare with primate brain atlas (Paxinos et al. 2000) to be within the anatomical INC region.

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INACTIVATION OF THE INC. After identifying the INC by stimulation, without disturbing the guide cannula or the microdrive, a Hamilton syringe (10 μl) was inserted into the cannula and lowered down to a depth determined during the stimulation phase (see preceding section), usually in the upper and lateral 0.5-mm region of the preidentified INC site (we did not inject muscimol into all the INC sites we explored during stimulation to avoid completely destroying the nucleus). The syringe was used to inject 0.3 μl of 0.05% muscimol solution (Sigma–Aldrich) into the region. Muscimol is known to have a strong reversible agonistic effect on neurons with γ-aminobutyric acid type A (GABA <sub>A</sub>) receptors (Hollis and Boyd 2003). Injecting the muscimol directly into the INC can produce immediate oculomotor deficits (Crawford and Vilis 1993). To allow enough time for recovery from a performed injection, experiments were conducted only every other day (because the effects of muscimol can last for 8 to 9 h). Injecting saline into two INC sites that previously evoked position-holding deficits after the injection of muscimol into them did not produce any position-holding deficits.

DATA ACQUISITION. Eye and head coil signals were monitored on-line during experimentation and were recorded at a 100 Hz sampling rate for the first two animals and at 1,000 Hz for the third monkey for further off-line analysis with MATLAB software (The MathWorks). Data were recorded in 100 s files in both dark and dim conditions. Data recording began immediately after the injection and continued for 40–60 min. Peak effects were observed after 30–40 min after muscimol injection. Therefore to compare early and late effects of muscimol injection, 15 and 60 min postinjection data were chosen. Animals were never required to make saccades with the head fixed for extended periods of time nor were they trained in any way that would have influenced their normal pattern of eye–head coordination. During stimulations and injections, the monkeys moved their eyes and heads at will but an experimenter presented novel visual objects (in dim light) or novel sounds (in the dark) from behind a barrier to encourage the monkey to use its entire eye–head motor ranges (Guitton et al. 1990; Klier et al. 2003). At the beginning of each experiment and before the injection of muscimol, a similar random paradigm was run as a control for the injection data.

From a total of 38 muscimol injection sites investigated in three monkeys, we were able to obtain 3-D eye-movement recordings from 26 sites before one of the eye coils broke. The remaining 12 sites (not analyzed here) were explored and used for analysis in other experiments that required measurement of head orientation only (Klier et al. 2002a, 2007). The 26 sites included here all met the following criteria: 1) torsional eye and head movements were consistently evoked in the same vertical and torsional directions during the stimulation phase and 2) torsional and/or vertical ocular drift was observed within 15 min of muscimol injection. In the first animal (M1), two sites were obtained in the right INC and one site in the left INC. In animal 2 (M2), six sites were obtained in the right INC and three sites in the left INC. In the third animal (M3), seven sites in the right INC and seven sites in the left INC were obtained.

Data analysis

QUANTIFICATION OF COIL SIGNALS. Before every experiment, each animal was required to fixate a target presented directly in front of the monkey at a distance of 40 cm from the monkey’s eyes. Coil signals were measured at this straightahead position to obtain an initial reference position for the eye and head in space coordinates. We then used this reference position to compute quaternions that represent rotations of the eye and head using a method described previously (Crawford et al. 1999; Tweed et al. 1990). For a more intuitive comparison between eye and head data and for statistical analysis, the quaternions were sometimes transformed into linear angular measures of 3-D eye position (Crawford and Guitton 1997). Using this method, any final eye or head orientation could be described as a rotation vector from the initial reference eye/head position, respectively. Angular velocity vectors were computed from quaternions using a method described previously. Detailed discussions of mathematics of quaternions are available elsewhere (Crawford and Vilis 1991; Tweed and Vilis 1987; Tweed et al. 1990; Westheimer 1957).

COORDINATE SYSTEMS. Initially we recorded the raw eye/head coil signals in an earth-fixed, orthogonal coordinate system defined by the magnetic fields, which we call space coordinates. We then used these coil signals to calculate quaternions that represented eye orientations in space (gaze) and head orientation in space (head). Eye orientation relative to the head (eye) was then computed from both the eye and head coil signals by dividing the gaze quaternion by the head quaternion (Glenn and Vilis 1992) as follows

\[ \text{Eye} = \text{Gaze}(\text{Head})^{-1} \]

The components of angular velocity are in the same coordinate system as the quaternions from which they were derived. To calculate VOR gain, it was necessary to have eye velocity and head velocity in the same coordinate system. For example, with the head tilted 90° CW (from the subject’s perspective; i.e., when the top of the subject’s head is tilted toward its right shoulder, this is considered to be a CW tilt), a leftward head rotation in space would give rise to an upward VOR in head coordinates. We calculated head velocities in head coordinates using the following formula

\[ \omega_{\text{head}} = q_{\text{head}}^{-1} \frac{d}{dt} q_{\text{head}} \]

In this formula, \( \omega_{\text{head}} \) is the angular velocity of the head relative to head coordinates, \( q_{\text{head}}^{-1} \) is the angular velocity of the head relative to space coordinates, \( q_{\text{head}}^{-1} \) represents the orientation of the head in space, and \( q_{\text{head}}^{-1} \) is its inverse. The operation between these variables is quaternion multiplication and the output \( \omega_{\text{head}} \) could be directly compared with eye-in-head velocity to compute the VOR gain.

SELECTION OF EYE AND HEAD FIXATION RANGES. We defined the fixation range to be the range that included those eye and head positions where the velocity of gaze was <10°/s (control data). For analysis, we subdivided the fixation data into the following two phases: 1) VOR phase, in which the eye is driven in an equal and opposite direction to the head; and 2) non-VOR, which starts when the VOR phase is completed and continues until the next gaze shift. These phases were chosen manually from position versus time traces of gaze, head, and eye because this method was found to be more reliable than using automatic selection software (see Klier et al. 2003). To select VOR and non-VOR ranges of gaze, head, and eye positions during the fixation, gaze was used as the primary guide. If the gaze was held perfectly (i.e., before INC inactivation) then VOR and non-VOR subranges were chosen based on the positions at which the head completed its movement (i.e., at the end of the VOR-related movement of the eye). In the case of a position-holding deficit during gaze fixation (i.e., after injecting muscimol into the INC), distinguishing between the VOR and non-VOR subranges was not always precisely possible because of the presence of eye drift. However, despite a reduction in angular VOR gain (see Fig. 8), it was still possible to distinguish between eye-position–dependent drift and head-velocity–dependent VOR both through visual inspection and through our multiple-regression analysis (see Fig. 7 and accompanying text).

SURFACE FITS. To quantitatively analyze the position ranges of gaze, head, and eye, we generated second-order surface fits of gaze, head, and eye quaternions using a previously described procedure (see Fig. 4) (Glenn and Vilis 1992; Radau et al. 1994; Tweed et al. 1990). Second-order fits were previously shown to provide the most useful description of primate data without becoming overly complex (Crawford et al. 1999; Glenn and Vilis 1992; Klier et al. 2003; Radau et al. 2002a, 2007). The 26 sites included here all met the following criteria: 1) VOR phase, in which the eye is driven in an equal and opposite direction to the head; and 2) non-VOR, which starts when the VOR phase is completed and continues until the next gaze shift. These phases were chosen manually from position versus time traces of gaze, head, and eye because this method was found to be more reliable than using automatic selection software (see Klier et al. 2003). To select VOR and non-VOR ranges of gaze, head, and eye positions during the fixation, gaze was used as the primary guide. If the gaze was held perfectly (i.e., before INC inactivation) then VOR and non-VOR subranges were chosen based on the positions at which the head completed its movement (i.e., at the end of the VOR-related movement of the eye). In the case of a position-holding deficit during gaze fixation (i.e., after injecting muscimol into the INC), distinguishing between the VOR and non-VOR subranges was not always precisely possible because of the presence of eye drift. However, despite a reduction in angular VOR gain (see Fig. 8), it was still possible to distinguish between eye-position–dependent drift and head-velocity–dependent VOR both through visual inspection and through our multiple-regression analysis (see Fig. 7 and accompanying text).
showed a nystagmus-like pattern. This was produced by a 15 min after injection of muscimol into the right INC (Fig. 4 and Supplementary Fig. 2). The coefficient \(a_0\) in the preceding equation, which shows the torsional offset of data points from the initial reference position, was used to assess the progress of torsional position-holding deficit across time after the injection of muscimol into the INC (Ceylan et al. 2003). The other coefficients describe additional tilts and curvature of the range. All of the data analysis was performed using custom-programmed functions in MATLAB (The MathWorks) software and statistics were performed using the SPSS (SPSS, Chicago, IL) program running on a personal computer.

RESULTS

Overview

This paper describes the results of 26 muscimol injections in three monkeys, 15 aimed toward the right INC and 11 aimed toward the left INC. Each site met the criteria described in METHODS (i.e., stimulation produced ipsitorsional rotations of the eyes and head and muscimol injection produced ocular drift within 15 min). Although we intended to inject the muscimol into the INC unilaterally, it is possible that in some cases the muscimol spread across the midline into the opposite INC.

In general, our results were consistent with previous observations that unilateral injection of muscimol into the INC produces torsional offsets in both eye and head orientation and a failure to hold both eye and head orientations, primarily in the torsional and vertical dimensions (Klier et al. 2002a). The main effects were most evident in the torsional components of gaze, eye, and head, plotted as a function of time in Fig. 1 for selected periods from one typical experiment. The right side of Fig. 1 shows 3-D reconstructions of head orientation at a selected point from the data (\(\uparrow\)) to give a qualitative feel for the head posture (Medendorp et al. 1999).

During control recordings (Fig. 1A) torsional gaze remained steady except for minor “blips” around the time of gaze shifts. This shows that both the torsional VOR and fixation systems were functioning normally. Eye torsion (relative to the head) also remained steady, except for small torsional quick-phase components associated with head-unrestrained saccades, followed by equal and opposite torsional slow phases (Klier et al. 2003). Head orientation also remained relatively steady during fixations and showed small torsional deviations that were distributed about zero torsion. The latter deviations occurred because head torsion is constrained in Fick coordinates (Crawford et al. 1999; Glenn and Vilis 1992), whereas the data are plotted in Cartesian coordinates.

During the early phases of the muscimol deficit (Fig. 1B), here 15 min after injection of muscimol into the right INC torsional gaze no longer held steady during fixation, but instead showed a nystagmus-like pattern. This was produced by a torsional nystagmus of the eye, often accompanied by drifting movements of the head in the same direction (for example, right after \(\uparrow\)). In addition, head (and thus gaze) torsion began to show offsets that are only transiently corrected, so that the head takes on a tilted appearance (right). During the later effects (Fig. 1C, 60 min after injection) the torsional eye and head nystagmus often subsided, coinciding with a reduction in corrective torsional quick phases toward zero torsion (although position-dependent vertical drift, not shown, usually peaked at this time). The most pronounced effect at this time was a small torsional offset in eye orientation accompanied by a much larger torsional offset in head orientation (summing to even larger offsets in torsional gaze). At this time the head often assumed dramatically tilted orientations (right). These and other deficits in eye–head coordination are explained in detail in the following sections.
**Horizontal and vertical gaze, head, and eye position ranges**

To illustrate the effect of INC inactivation on vertical and horizontal positions of gaze, the head, and the eye during the gaze fixation periods where the velocity of gaze was \(<10^6/s\) (i.e., during fixations between two consecutive movements) before and after injecting muscimol into the right INC. The results are plotted for 15- and 60-min postinjection periods to show the early and late deficit data. Horizontal position is plotted along the horizontal axis and vertical position along the vertical axis. Circles (●) denote the end of fixation period with control data in the first column.

**CONTROL DATA.** Before muscimol injection (Fig. 2, *left column*), the eye and the head were able to maintain their positions along vertical and horizontal axes without evidence of drift. This is seen by the fact that the beginning and end of the fixation period overlap (i.e., no change in the position during the fixation period). The eye position range extended more vertically, whereas the head range did so more horizontally, as observed in previous studies (Freedman and Sparks 1997b). As expected, the gaze range of positions (Fig. 2A) was a sum of both the eye and head positions and appeared to cover the visual field more equally along horizontal and vertical axes.

**INC INACTIVATION DATA: A. POSITION DRIFT.** Fifteen minutes after the injection into the right INC, the animal was able to make eye–head gaze shifts in all horizontal and vertical directions throughout the entire visual field, but was no longer able to hold the eye and the head steady between gaze shifts (Fig. 2, *middle column*). Note that the direction of torsional drift (not shown here) was consistently CCW in this experiment (see Supplementary Fig. 1 for details). The contribution of the head to vertical gaze increased significantly \(P < 0.005\) after the INC inactivation. This probably occurred because the vertical eye position was drifting more rapidly toward the “null” resting position (Crawford and Vilis 1993) than the head (see *Temporal characteristics of the drift*). The contribution of the head to horizontal gaze did not change significantly after INC inactivation \((P = 0.39)\).

**Torsional gaze, head, and eye position ranges**

Torsional ranges of gaze, head, and eye positions during the gaze fixation periods, before and after INC inactivation, are shown in Fig. 3. Control and right INC data are the same as in Fig. 2. The *middle column* (Fig. 3, *C, H*, and *M*) shows control data, whereas the *left* and *right columns* illustrate the data after the inactivation of left and right INC, respectively. In each panel, position vectors appear from a side view, which represents torsional position about the torsional axis (i.e., the abscissa) and horizontal position about the vertical axis (i.e., the ordinate). A right-hand convention is used to identify the

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**Fig. 2.** Vertical position of gaze, the head, and the eye plotted as a function of horizontal position recorded over 100-s intervals. Only positions during fixation periods between consecutive movements are shown from a behind view. Control data are plotted in the *first column* (*A, D, G*). *B, E*, and *H*: early deficit during a typical right INC experiment (15 min after muscimol injection). *C, F*, and *I*: late deficit in the same INC site after 60 min. Data coordinate axes are aligned with magnetic field directions of the recording system. Solid circles (●) denote the endpoint of each drift sequence. Animal: M3.
direction of rotation about each axis (see caricature). For example, holding the thumb of the right hand aligned with the axis of rotation, the fingers will curl in the direction of rotation. Each circle (●) represents the end of one position vector, emanating from the origin. These same conventions were used in previous studies of 3-D gaze, head, and eye orientation (Crawford et al. 1999; Glenn and Vilis 1992). Before the INC was inactivated, the eye and head were able to hold their position between any two consecutive movements. Therefore, we did not find any correlation between theINC site and the severity of the head torsional tilt usually increased over time.

Figure 3 also suggests that the null resting ranges of gaze, head, and eye shift torsionally, CCW during right INC inactivation and CW during left INC inactivation. This finding is consistent with previous studies that studied eye and head deficits separately after reversible INC inactivation (Crawford et al. 1991; Klier et al. 2002a). Often, but not always, the head began to tilt torsionally before the eye (Fig. 3, G and L). However, we did not find any correlation between the INC site being inactivated and this observation. The severity of the head torsional tilt usually increased over time.

Figure 4 represents the second-order fit surfaces made to the range of postdrift positions (i.e., immediately before the next gaze shift begins; see METHODS) of the fixation period data averaged across animals. Surface fits are seen from a side view and the coordinate system is the same as in Fig. 3. Each horizontal and vertical line within every surface represents 10°. Solid lines show the front edge of the surface fit. A look at the control behavior data shows that gaze and head fits resemble a twisted “Fick” surface (Fig. 4, C and H, respectively), whereas a surface fit of eye data (Fig. 4M) is better aligned with Listing’s plane. This is typical of results reported in many previous studies (Crawford et al. 1999; Glenn and Vilis 1992), where the twisted Fick surface results from rotating the head about a body-fixed vertical axis and a head-fixed horizontal axis. During the early deficit (15 min after injecting the muscimol), gaze and head fits shifted away from the “null”
resting position along the torsional axis (Fig. 4, B, D, G, and J). Similarly, after left INC inactivation, the fit of eye data moved in the same direction as of the head (Fig. 4N). However, there was no shift in the fit of eye data after right INC inactivation (Fig. 4L). During the late deficit (i.e., 60 min after injecting the muscimol) the shift clearly increased over time (Fig. 4).

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The complete coefficients of surface fits for gaze, head, and eye data during the postdrift period, before (control) and 15 and 60 min after (early and late deficits, respectively) the inactivation of left/right INC, are included in Supplementary Table 1 for all three animals. The most significant changes in shape were generally observed for gaze, which is not surprising considering it as a combination of both the eye and head. The average across all animals of coefficient \(a_1\), which quantifies the torsional deviation of position range relative to a reference position (torsional offset), was measured (mean ± SD) before and 60 min after left and right INC inactivation (see Table 1A). The magnitude of \(a_1\) increased significantly for gaze [left INC, \(t(9) = -3.94; P < 0.005\); right INC, \(t(14) = 6.13; P < 0.001\)], the head [left INC, \(t(9) = -2.98; P < 0.05\); right INC, \(t(14) = 5.43; P < 0.001\)], and the eye [right INC, \(t(14) = 2.81; P < 0.05\)] across all animals.

The twist score \(a_5\), which quantifies the amount of “Fick twist” of the surface, also changed after the inactivation of either left or right INCs. The \(a_5\) value (mean ± SD) before and 60 min after left and right INC inactivation, averaged across all three animals, is shown in Table 1B. Reduction in the \(a_5\) was significant in both gaze \([t(14) = -3.12; P < 0.01]\) and the head \([t(14) = -3.51; P < 0.005]\) after right INC inactivation.

**TABLE 1.** Surface-fit coefficients \(a_1\) and \(a_5\) for gaze, head, and eye.

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 25)</th>
<th>Left INC (n = 10)</th>
<th>Right INC (n = 15)</th>
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<tbody>
<tr>
<td><strong>A. Coefficient (a_1), averaged across all animals</strong></td>
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<tr>
<td>Gaze</td>
<td>0.02 ± 0.05</td>
<td>0.22 ± 0.18</td>
<td>-0.30 ± 0.20</td>
</tr>
<tr>
<td>Head</td>
<td>0.02 ± 0.05</td>
<td>0.18 ± 0.18</td>
<td>-0.26 ± 0.21</td>
</tr>
<tr>
<td>Eye</td>
<td>0.00 ± 0.02</td>
<td>0.01 ± 0.07</td>
<td>-0.03 ± 0.03</td>
</tr>
<tr>
<td><strong>B. Coefficient (a_5), averaged across all animals</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gaze</td>
<td>-0.84 ± 0.25</td>
<td>-0.44 ± 0.70</td>
<td>-0.37 ± 0.40</td>
</tr>
<tr>
<td>Head</td>
<td>-0.73 ± 0.32</td>
<td>-0.20 ± 0.67</td>
<td>-0.11 ± 0.47</td>
</tr>
<tr>
<td>Eye</td>
<td>-0.17 ± 0.77</td>
<td>-0.79 ± 1.88</td>
<td>0.10 ± 1.00</td>
</tr>
</tbody>
</table>

Values are means across three animals ± SE for control and left and right INC inactivation.
but only in the head \([r(9) = -3.18; P < 0.05]\) after left INC inactivation. Despite the small reduction in the Fick twist it was not eliminated after INC inactivation.

Donders’ law states that for any unique gaze direction, regardless of how the eye reaches its target, the eye always assumes the same orientation in 3-D. Conversely, if the eye does not obey Donders’ law, for each gaze direction, eye-in-space (gaze) would have a different torsional orientation and this would result in a thicker position range. To investigate how well the Donders’ law is obeyed after INC inactivation, we calculated the torsional thickness (SD of the fit surface) of the gaze, head, and eye ranges. Because the torsional thicknesses from left and right INC experiments were not significantly different, we pooled these results together for the averages shown in Supplementary Fig. 2. This was done separately for the postgaze shift (pre-drift) data range (Supplementary Fig. 2A), which should be determined by the gaze saccade generator; for the post-drift data (Supplementary Fig. 2B), which should be determined by characteristics of the integrator failure; and for the overall combination of these two (Supplementary Fig. 2C). The latter was analyzed to characterize the complete distribution of torsional gaze, head, and eye positions. In each case the torsional SD increased. The increase in torsional thickness was significant for pre-, post-, and pre- + post-drift position of gaze, head, and eye data \((P < 0.05)\) except for the eye post-drift position (paired \(t\)-test). All injection sites produced deficits in both the eye and head orientation (see Supplementary Table 1). Thus inactivation of the INC produced a breakdown in Donders’ law both in the torsional shift and torsional variance of eye and head positions.

**Temporal characteristics of the drift**

To examine the characteristics of the drift period in more detail, Fig. 5 shows gaze (left columns), head (center columns), and eye (right columns) positions (ordinate) during fixation periods plotted against time (abscissa). Torsional (top row), vertical (middle row), and horizontal (bottom row) components are shown separately before (control) and after the inactivation (here, 60 min after injecting the muscimol into the right INC). Each panel represents the data of one file recorded in the dark for 100 s. The beginning of each fixation trace was aligned to the left in each panel. Before injection, there was no change in eye or head position during fixation periods. In other words, the control traces resembled flat horizontal lines on the figure, independent of initial position. This was true for all the components of 3-D gaze, head, and eye position.

**FIG. 5.** Gaze, head, and eye positions during the fixation period are shown before and 60 min after the injection of muscimol into one right INC site. Data are plotted separately for torsional, vertical, and horizontal components in the 1st, 2nd, and 3rd rows, respectively. Insets in panels \(C_2\), \(F_2\), and \(I_2\) represent the eye data scaled up 3 times along the \(y\)-axis. Fixation traces are aligned to the left. Animal: M3.
TORSION. Integrator failure is expected to cause an exponential, position-dependent decay in eye and head position. Consistent with this, the torsional position of gaze, the head, and the eye after INC inactivation shows the drift (Fig. 5, top row). In this example (right INC inactivation), gaze, head, and eye position drifted mainly in the CCW direction, although the eye and head occasionally exhibited CW drifts. The time course of torsional eye and head drifts was exponential, similar to what was previously reported in the eye in head-fixed studies (Cannon and Robinson 1987; Kaneko 1997).

Immediately after INC inactivation (not shown in Fig. 5) there was no obvious relationship between the magnitude and the rate of the torsional drift and postmovement torsional position (i.e., suggesting a long time constant of drift with a large offset in the null resting position). However, during the late deficit period, torsional eye drifts (Fig. 5C2) tended to have a larger magnitude at extreme torsional positions in the opposite direction to the drift. In addition, their rate appeared to be determined by the eccentricity of eye position, similar to the results of previous head-fixed studies (Crawford and Vilis 1993). This clear relationship between torsional drift magnitude and postmovement torsional position was not observed for either gaze or head drifts.

VERTICAL. In this example (Fig. 5, middle row), gaze drifts appeared to be mostly downward, whereas the head and eye drifts were observed in both upward and downward directions. Similar to torsional drifts, larger vertical eye drifts were seen at larger vertical positions during the late deficit period. A mixture of gaze and head drifts with different magnitudes was observed at different vertical positions and again, in contrast to previous head-fixed reports on vertical eye drifts, there was no clear relationship between the postmovement gaze/head position and the magnitude and/or the rate of gaze/head drift. Moreover, the frequency of vertical drift generally increased as the lesion progressed. Again, vertical eye and head drifts had an exponential time course similar to that of torsional drift. However, as reported previously in head-fixed oculomotor studies (Crawford and Vilis 1993; Helmchen et al. 1998), position drift did not drift toward a single null position.

HORIZONTAL. Horizontal head position did not hold steady after the injection (Fig. 5H2), but overall horizontal gaze was better held after muscimol injection comparing to torsional and vertical gaze. (i.e., fewer gaze drifts were observed; Fig. 5G2). This could be explained by a more functional horizontal VOR, meaning that the eye was still capable of compensating for head movement by rotating horizontally in the opposite direction much better than torsionally and vertically. There was no clear relationship between horizontal drift magnitude and horizontal position of gaze, the head, and the eye (see next section for quantification). As suggested in Fig. 2B, some of this horizontal drift could be accounted for if gaze and head torsion drifted about a gaze- or head-fixed axis, which would produce a cross-talk pattern with horizontal and vertical drift patterns plotted in these space-fixed coordinates.

QUANTIFICATION OF VERTICAL DRIFT. In this section we quantify how the rate of drift depends on initial position and the metrics of the previous movement, using a method similar to that reported previously for the head-fixed monkey (Crawford and Vilis 1993). As in the previous study, vertical drift was analyzed because it had a wider range of initial positions than torsional drift and was more consistent than horizontal drift. Figure 6 shows example regression plots taken from typical control and INC inactivation data. Figure 6A shows the average velocity of vertical drift (V) plotted as a function of initial (postmovement) vertical position (P) and Fig. 6B shows V plotted as a function of the magnitude of vertical component of the previous movement (M). Technically, V is the derivative of eye orientation, computed by subtracting the final position from the initial position and dividing by time for each drift segment, done for the first 100 ms of the postmovement period for gaze and the eye and for the first 200 ms for the head. Gaze, head, and eye regressions are plotted separately. As in the head-fixed condition (Crawford and Vilis 1993), vertical drift was dependent on both initial eye position (P) and previous saccade magnitude (M) (Fig. 6, A and B, last rows). To quantify this, we performed a multiple regression analysis to further examine the relationship between these variables (Bevington 1969). We also included head velocity as an independent variable in the ocular drift analysis to account for the influence of the VOR. In this analysis the time constant of the drift is equal to the inverse of the slope (V/P). Thus high V/P slopes indicate low time constants and a significant position-dependent drift.

The summary of quantitative analysis, averaged across left and right INC sites of all three animals, is shown in Fig. 7. Bars illustrate the average statistical values for control (white bars) and late deficit data (black bars). Figure 7, A, B, and C represents eye, head, and gaze data, respectively. The two-way correlation coefficients (r) are shown for the relationship between initial postmovement vertical position and vertical drift average angular velocity (r[pv]), vertical movement magnitude and vertical drift average angular velocity (r[mv]), and two independent variables, initial postmovement vertical position and vertical movement magnitude (r[pml]). The slopes (m) of vertical drift angular velocity versus vertical position for a constant vertical movement magnitude (m[pv]) and vertical drift angular velocity versus vertical movement magnitude for a constant vertical position (m[mv]) were also calculated from the multiple regression analysis.

The r[pv] before and after muscimol injection was significantly different for the eye, head, and gaze (P < 0.05), whereas the r[mv] was significant for only the eye and head (P < 0.05) (see values in Fig. 7). Moreover, the m[pv] was steeper after INC inactivation for the eye, head, and gaze, although it was significantly different for the head only (P < 0.05). On the other hand, the m[mv] was significantly higher only in the eye (P < 0.05), whereas it decreased for the head and gaze. Finally, the average multiple regression coefficients (R[pnv]) were clearly higher for the eye data after INC inactivation, a finding similar to that previously reported in head-fixed studies (Crawford and Vilis 1993). This value was smaller for the head and showed only a small increase for gaze after INC inactivation. All individual R values were significant for the eye (P < 0.05), although this was not the case for either the head or gaze data. In summary, this analysis further confirms the dependency of vertical drift of the eye on both initial vertical eye position and the magnitude of vertical component of the previous saccade after pharmacological INC inactivation. In contrast, vertical drift of the head appeared to be mainly dependent on head vertical position and was less affected by the magnitude of
3-D angular VOR

During natural gaze shifts, the eye reaches the target before the head because of the larger mass of the head and slower head rotations and then, while the head continues to rotate, gaze is maintained by the angular VOR. Figure 8 illustrates angular VOR gain during spontaneous eye and head movements before and 60 min after inactivating the left and right INC for torsional, vertical, and horizontal components. Each panel plots the average angular velocity of the eye as a function of the average angular velocity of the head for a series of VOR “slow phases” during left INC inactivation (left column), during normal behavior (center column), and during right INC inactivation (right column). Because eye data were initially calculated in head coordinates and head data in space coordinates, head data were rotated into head coordinates to avoid coordinate system mismatch (see METHODS). The slope of the linear regression of data set represents the VOR gain, which ideally should equal −1 (gray dotted line). Data points (gray circles) together with the regression (gain) of these data (thin solid line) are shown for one animal (M3). Because VOR gains from left and right INC experiments were not significantly different, we pooled them together and the average regression of the pooled data across all three animals is represented as a thick solid line (n = 26).

In looking at the torsional component of the data in Fig. 8, there is a clear decrease in angular VOR gain after the inactivation of either the left or right INCs. This was observed across all animals and the average torsional VOR gain of three animals was measured as −0.78 ± 0.13 and −0.61 ± 0.24 for the control and deficit data, respectively. The reduction in torsional VOR gain was modest but significant [t(25) = −3.68; P < 0.05]. The gain of torsional VOR was reduced in both CW and CCW directions after either left or right INC inactivation and there was no significant difference between these two
directions ($P = 0.10$ and $P = 0.80$ for left and right INC, respectively). The gain of vertical and horizontal angular VOR exhibited an inconsistent pattern between left and right INC experiments and across all data. In some cases, the gain of vertical VOR decreased, whereas in other experiments vertical VOR gain was still intact by the end of the experiment. Average vertical VOR gain was $0.85 \pm 0.10$ (control) and $0.84 \pm 0.16$ (deficit). Overall, horizontal VOR showed no change after INC inactivation (Control, $-0.70 \pm 0.13$; deficit, $-0.72 \pm 0.21$). The change in vertical and horizontal VOR gain after INC inactivation was not significant ($vertical, P = 0.67$; horizontal, $P = 0.66$). In summary, torsional VOR gain showed a modest reduction after INC inactivation, but vertical and horizontal VOR gain showed no significant reduction.

Static ocular counterroll (OC)

It is known that when the head is tilted toward either shoulder—CW or CCW about the nasooccipital axis—the eye attempts to compensate for this rotation by rotating about the same torsional axis in the opposite direction with a gain of 6–10% (Baarsma and Collewijn 1975; Collewijn et al. 1985; Ott 1992; Seidman et al. 1995). This phenomenon is known as static OC. Helmchen et al. (1998) suggested that OC was intact after INC inactivation, although Crawford et al. (2003) reported that during passive head tilts, the static OC is implemented through the neural integrator for 3-D eye and head position and is therefore disrupted after the INC inactivation. Does the same hold during head-unrestrained gaze shifts?

We examined whether OC persisted as the head progressively tilted after INC inactivation. Figures 3 and 4 suggest that when the muscimol effect progressed, the eye plane shifted in the same direction as the head rather than in the opposite direction (as predicted by OC). This is quantified in Fig. 9. There, the torsional offset ($a_t$) of the eye is plotted as a function of the torsional offset ($a_h$) of the head. Each circle represents the data from one recorded file (each file in 100 s).
All recorded files of every INC site, immediately until 60 min after injecting the muscimol, are pooled for each animal. Left and right INC data are also pooled together (separately for each animal) and shown as empty (○) and filled (●) circles, respectively. Left and right columns of Fig. 9 illustrate the fit to pre- and postdrift data (i.e., the postmuscimol-injection positions at the end of the gaze shift and before gaze shifts, respectively). Small head tilts appeared earlier in experiments, whereas larger head tilts (far left or right on abscissa) tended to be late effects. The dashed line represents the gain of the normal OC (10%), whereas the solid line shows the fit to the postinjection data. We could not measure control values of OC in these animals because they did not tilt their heads before the muscimol injection. Therefore we used an estimate of 10% gain based on the previous literature (Crawford et al. 2003).

Comparing the approximately expected normal ocular gain (dashed line) with that of inactivation data (solid line) revealed that OC was severely impaired after the INC inactivation. During lesser deficits (small head tilts), the data sometimes followed the predicted OC curve (dashed line). However, for larger head tilts (corresponding to greater deficits) the eye tended to roll in the same direction as the head. Averaged across all animals, the observed gain of the OC for pre- and postdrift data was measured as 0.08 ($R^2 = 0.18$) and 0.09 ($R^2 = 0.27$), similar in size to that predicted by normal ocular gain, but rather in the opposite direction. These slopes were statistically different from the normal estimated 0.1 (10%) gain ($P < 0.05$) and even from a slope of zero ($P < 0.05$). Thus OC was completely disrupted.

**Discussion**

This is the first study to simultaneously quantify deficits in both eye and head control in the monkey during inactivation of the INC. In general, our results support observations reported previously for the eye (Crawford et al. 1991; Fukushima et al. 1990; Helmchen et al. 1998) and the head (Klier et al. 2002a) separately, in particular the idea that the INC is involved in controlling eye and head 3-D orientation; however, the more in-depth analysis provided here for the head suggests that the INC may be more complex than a first-order integrator for position components within a Cartesian coordinate system. Our results also show that some aspects of Donders’ laws of the eye and head are disrupted during acute INC inactivation, but horizontal–vertical head orientation and contribution to gaze were intact. Finally, OC is severely affected, but torsional VOR is only moderately affected during active gaze shifts. These results and their clinical significance, will be considered below.

**Postural deficits after INC inactivation**

Injection of muscimol into the INC did not produce consistent offsets in the horizontal or vertical components of the gaze or head posture, but had a clear effect on the torsional range of both the eye and head. Generally the head tilt was larger and, in most cases, both the eyes and head tilted CW after left INC injection and CCW after right INC inactivation. However, eye and head tilt were not always tightly correlated. During some experiments the eye shift appeared before that of the head and, in other cases, the head deficit built up more rapidly (see Fig. 3G). We were unable to discern a specific anatomic pattern for these effects, although this suggests some degree of independence between the control signals for eye and head posture within the INC. Finally, the three-dimensional range, although shifted torsionally, retained its characteristic “Fick shape”, although with a reduced twist. Similar findings were reported previously during INC inactivation in the cat (Fukushima et al. 1985a) and similar contralateral head tilts were observed after reversible inactivation of the rostral mesencephalic reticular formation (MRF) in the primate (Waitzman et al. 2000b), which lies adjacent to the INC. This raises the question of whether some of the head tilt observed in our experiments was attributable to spread of muscimol to the MRF. This is possible, but is unlikely to be the only explanation for our results. First, the oculomotor deficits that occur during MRF and INC inactivation are quite different (for example, inactivation of the INC always produces integrator failure, whereas injection of muscimol into the MRF does not), so these effects are not easily confused. More important, although the early time course of head tilt after injection of muscimol into the MRF was not reported, 2–3 h after such injections head tilt was relatively small (20–30°) and occurred after only half the injections (Waitzman et al. 2000b). In our experiments head tilt was consistent, appeared fairly quickly after our injections (usually within 15 min), often exceeded 30° during our experiment, and after 2–3 h—after animals were returned to their cage—could sometimes reach values of about 90°, including tilt of the shoulders (by our visual observations). Thus the INC appears to be a “hot spot” for this effect.

Bilateral inactivation of the INC was previously reported to cause additional vertical offsets in head posture (Fukushima et
al. 1985b) and to limit the vertical oculomotor range (Bhida-yasiri et al. 2000; Helmchen et al. 1998), probably because of severe damage to the ability to both move and hold the eye in the vertical dimension. We did not observe such deficits during our unilateral inactivations, probably because there was still enough integrating capacity left in the INC to at least partially hold vertical eye and head positions. It was previously observed that during partial inactivation of the INC, the vertical oculomotor integrator behaves as if the remaining portions are somewhat independent of the damage and can be “charged up” with repeated movements to hold more eccentric positions (Crawford and Vilis 1993; Helmchen et al. 1998). In general, these observations are consistent with previous proposals that the INC is divided into bilaterally yoked populations of neurons that control directions similar to those of the eye muscles, with up/down signals intermingled on each side but CW/CCW organized on the right/left, respectively (Fukushima et al. 1990; King et al. 1981). This organization appears to be similar for both eye and head movements (Klier et al. 2002b), except that the coordinates for eye control appear to be aligned with Listing’s plane (Crawford 1994; Crawford and Vilis 1991; Suzuki et al. 2000), whereas the INC coordinates for head control appear to align with Fick coordinates (Klier et al. 2007). In each case, this is the natural coordinate system to match the control signals with the motor behavior of the controlled object (Crawford et al. 1999; Medendorp et al. 1998; Misslisch et al. 1998; Radau et al. 1994; Theeuwen et al. 1993; Tweed et al. 1995).

In addition to the torsional offsets that we observed, the torsional range of gaze, eye, and head orientation also increased (i.e., a breakdown in Donders’ law). In theory, the orientations of the eyes and head at their gaze-associated movements are determined by the burst generator, whereas their orientations after drift (when allowed to come to rest) are determined by the “null range” of the neural integrator (Cannon and Robinson 1987; Crawford and Vilis 1993). The null range, in turn, is determined by the level of damage to the neural integrator and imbalances in tonic vestibular input to this integrator (Crawford 1994).

**Integrator failure and drift characteristics**

In general, our data are consistent with the idea that the INC forms the neural integrator that controls the torsional and vertical components of both eye position (Crawford et al. 1991; Fukushima et al. 1990; Helmchen et al. 1998; King et al. 1981) and head position (Klier et al. 2002). After muscimol injection, the eye drifted torsionally (usually in the contralateral direction—i.e., top of the eye away from the injection site—with ipsilesional quick phases initially, but often reversing over time) and vertically (in general centripetally toward the center) and showed little consistent horizontal drift. Similar observations were made for head drift. These patterns tend to further confirm the idea that up and down signals are intermingled in each INC for the eye vertical position (Fukushima et al. 1990; King et al. 1981) and support the same hypothesis about the head (Klier et al. 2002a).

These deficits were qualitatively similar for the eye and head, but were sometimes dissociated. For example, the ocular drift generally appeared before head drift. As with the torsional offsets, we were not able to establish a consistent anatomic pattern to areas of the INC that were more sensitive for eye or head drift, but these dissociations again suggest some degree of independence between the eye and head integrators (as is obviously necessary to separately control eye and head position in normal behavior). Alternatively, this dissociation could be the result of different types of feedback control from sensory apparatus within the eye and neck muscles (Perlmutter et al. 1999; Peterson 2004; Richmond et al. 1976) or feedback to different regions of the rostral midbrain.

The temporal characteristics of drift showed an exponential pattern consistent with a progressive integrator failure. Time constants and overall characteristics were comparable with previous reports for the eye (Anderson et al. 1979; Crawford et al. 1991; Fukushima and Fukushima 1992; King and Leigh 1982; King et al. 1981) and the head (Klier et al. 2002a). However, we found that the relationship between the average angular velocity of the drift and initial postmovement vertical position or the magnitude of vertical component of the previous movement was not as clear as reported in head-fixed studies (Crawford and Vilis 1993). We found that the average drift velocity of the eye was dependent on both the magnitude of vertical component of the previous saccade and the initial vertical postmovement position of the eye, although in contrast to head-fixed experiments (Crawford and Vilis 1993), the average drift velocity of the eye had a greater dependency on the magnitude of vertical component of the previous movement. On the other hand, vertical drifts of the head appeared to be more dependent on the initial postmovement position of the head. Despite this, the correlation between initial head position and the rate of the drift was never as high as one would expect from damage to a first-order integrator with a single time constant and null position. This also might be explained by the fact that other structures beyond the INC are involved in controlling head posture.

INC inactivation does not produce horizontal oculomotor drift in head-fixed experiments, at least when the data are aligned in Listing’s coordinates (Crawford 1994). Slow horizontal eye movements during the head-unrestrained deficit are probably just VOR slow phases, which are difficult to visually disentangle from real drift. However, it is harder to discount the horizontal drift that we observed in head position. This drift showed no correlation to initial horizontal head position. One possibility is that this drift is really cross talk from torsional and vertical drift defined in a physiological coordinate system that is different from the space-fixed coordinates in which we expressed our data. This is not unexpected if the head is controlled in Fick coordinates (Klier et al. 2007), where both the horizontal and torsional axes rotate with respect to the vertical axis for horizontal head rotation. With these coordinates, head-fixed vertical drift will start to look like horizontal drift (in space-fixed Cartesian data coordinates) when the head tilts torsionally, whereas head-fixed torsional drift will appear to take on either vertical or horizontal components at different head positions. Consistent with the latter, gaze and head drift sometimes showed the “circling” pattern in horizontal–vertical components predicted by drift about a Fick torsional axis.

Also, given the rich proprioceptive feedback from the neck muscles and vestibular apparatus to the brain (Abrahams et al. 1974; Fukushima et al. 1983) and the head’s capacity for reflexive and voluntary movements independent of gaze (Collins and Barnes 1999), the possibility cannot be dismissed that...
much of our apparent drift data are contaminated by slow, self-generated corrective movements of some type.

With the exclusion of these caveats, our data are consistent with the idea that the torsional and vertical neural integrators—or some more complex variation of a neural integrator—for both the eye and head depend critically on INC function. Moreover, the INC could possibly be part of a distributed circuit, including the vestibular nuclei and the nucleus prepositus hypoglossi (Blazquez et al. 2006; Kokkoroyannis et al. 1996; Moschovakis 1997), that implements the neural integration process.

Role of the INC in vestibuloocular function

In theory, a healthy neural integrator is required for the VOR, especially at low frequencies of rotation (Cannon and Robinson 1987). The role of the INC and its surrounding reticular formation region in the temporal conversion of vertical canal signals during vertical VOR was previously shown during passive head rotations in the cat (Fukushima 1987). Lesions in the INC result in an increased VOR phase lead for vertical eye movements (King et al. 1981). Previous lesion studies in cats reported a relationship between the INC inactivation and the quick phase of the vertical VOR (Fukushima et al. 1994; King and Leigh 1982).

In the current study we found that after INC inactivation and during spontaneous eye and head movements, only the gain of the torsional VOR was reduced during active head rotations and this reduction was modest. Thus it disagrees with a previous study that reported no or only a mild decrease in torsional VOR gain after INC inactivation (Helmcnen et al. 1998), perhaps because more neurons were inactivated in our study. We did not observe any asymmetry in the torsional VOR during unilateral INC inactivation, which is consistent with previous proposals that the left and right sides of brain stem oculomotor integrators do not function independently, but rather integrate through interdependent commissural loops (Cannon and Robinson 1987; Crawford and Vilis 1993; Partsalis et al. 1994).

The vertical VOR may have been preserved because enough vertical integration was maintained—perhaps in parallel, independent circuits in the opposite side of the INC (Crawford and Vilis 1993)—to allow VOR function. This would be particularly true during natural head movements that mainly contain high-frequency components implemented by the direct (non-integrator) pathway of the VOR. In other words, measurements of VOR gain during slow passive head rotations probably overestimate the damage to the VOR in natural movements. Horizontal VOR gain was largely unaffected in our experiments. This is consistent with the view that the INC is mainly responsible for torsional and vertical position components.

The neural integrator was previously suggested to mediate the OC reflex, which is defined by conjugate eye rotations in the opposite direction of a head tilt during saccades and smooth-pursuit eye movements (Crawford et al. 2003; Glasauer et al. 2001; Halmagyi et al. 1990; Ohashi et al. 1998). However, some experiments reported preserved OC after INC lesions in cats (Anderson 1981) and monkeys (Helmcnen et al. 1998). Although we sometimes observed compensatory eye torsion in the early stages of head tilt, OC disappeared over the course of the experiment and was never observed in the later stages of experiments. Instead, the eye usually rotated in the same direction as the head. This differs from the compensatory eye position shifts observed during ipsiwersive head tilts produced by inactivation of the MRF (Waitzman et al. 2000a), suggesting that the latter head tilts may have resulted from an imbalance of input to the INC (integrator) without disrupting the INC itself.

Clinical implications

From a clinical perspective, after muscimol injection our animals showed symptoms of gaze-paretic nystagmus (a position-dependent drift of the eye) in the vertical and torsional components (Ranalli et al. 1988), a contralateral ocular tilt reaction (torsional tilt of the eyes) (Westheimer and Blair 1975), and rotatory spasmodic torticollis (ST) (Loher et al. 2004). All of these deficits have long been associated with midbrain damage in humans (Bertrand et al. 1978; Plant et al. 1989; Raeva et al. 1987; Vasin et al. 1985) and the physiological relationship of the INC to the oculomotor components of these disorders was previously considered in detail elsewhere (Crawford and Vilis 1993; Fukushima 1987; Helmcnen et al. 1998; Ranalli et al. 1988).

Less is known about the etiology of ST, also known as cervical dystonia. Lesions in the midbrain (as well as other structures) were previously suggested to play a major role in ST (Bertrand et al. 1978; Plant et al. 1989; Raeva et al. 1987; Vasin et al. 1985). ST may include both horizontal and rotatory (i.e., head tilts around an anteroposterior axis in which one of the ears approaches the ipsilateral shoulder) offsets in head posture (Loher et al. 2004). Other than these offsets, ST patients still show torsionally restricted position ranges with the characteristic “Fick twist” (Medendorp et al. 1999). Moreover, rotatory ST is often associated with oculomotor deficits such as ocular nystagmus (Anastasopoulos et al. 1998; Bronstein and Rudge 1986), VOR deficits (Bronstein and Rudge 1986), and deficits in ocular counterroll (Averbuch-Heller et al. 1997; Diamond et al. 1988). Our animals showed the major symptoms of rotatory ST, especially in the late effects, but not all of the typical details. The rotated posture of the head was striking and, in the late effect, showed a torsionally restricted range that still preserved the normal “Fick twist”, although to a slightly lesser degree than normal. At first glance, this small reduction appears to be at odds with the retention of this twist in ST patients relative to control subjects (Medendorp et al. 1999). However, 1) these patients may have made adjustments to their primary deficits that would not be observed in our acute experiments and 2) because these measurements cannot be made “before and after” ST in patients one cannot be certain that there is absolutely no difference between ST patients and control subjects without examining a much larger patient population. The “big picture” is that our monkeys maintained the Fick twist to an appreciable degree after muscimol injection, as observed in ST patients.

The major difference between our data and the typical ST patient was the clear and consistent oculomotor drift seen in our monkeys. There are two likely reasons for this difference. First, again we are looking at acute pharmacological damage to the INC, whereas clinical descriptions of ST are all in patients who are suffering from a chronic disorder. To develop our experimental model of ST further, one would have to look at
the long-term effects and possible partial recovery from permanent INC damage. Second, it is likely that many cases of rotatory ST do not involve direct damage to the INC, but rather result from either asymmetric activation of the circuitry for head torsion at or above the level of the INC (for example in the basal ganglia, rMLF, or MRF), which could produce an imbalance of activity within the INC without the additional ocular symptoms we observed (Klier et al. 2002a).

Finally, some earlier studies suggested that the oculomotor deficits observed in patients with ST could be secondary to the head dysfunction and vice versa (Kushner 1979; Williams et al. 1996). However, our experiment suggests that the correlation of oculomotor deficits in patients with ST may be an epiphenomenon that could be explained by 1) the proximity of oculomotor and cephalomotor integrators within the INC and 2) the complex cephalomotor, oculomotor, and vestibulococular deficits caused by their damage. Thus these symptoms could be correlated without causing each other.

Physiological conclusions: the INC as a neural integrator for both eye and head movements

Many of the results of the current study—including drift of both eye and head position, offsets in torsional eye and head position, and deficits in the VOR during INC inactivation—can be accounted for by previous proposals that the INC serves as the torsional/vertical integrators for both eye and head orientation (Fukushima 1987; Klier and Crawford 2003). The deficits reported in this study were clearly more complex, in part because of the complexity of real-world eye–head coordination, but perhaps in part because the integrator concept is a simplification. Like some previous studies (Crawford and Vilis 1993; Helmcchen et al. 1998), we found that the oculomotor position-holding deficits were more complex than that predicted by a damage to a first-order integrator (with drift depending on both saccade metrics and eye position), but this can be accounted for if either the integrator is organized into quasi-parallel modules (Cannon and Robinson 1987; Crawford and Vilis 1993) or if the integrator is dependent on sensorimotor context (Chan and Galiana 2005).

Similarly, the general deficits in head posture after INC inactivation are consistent with the general idea of integrator failure—with offsets in the null range and exponential, position-dependent drift—although the details are more complicated. The poor correlation between head position and head drift suggests that there are other factors at play. One possibility is that the neural integrator concept is too simple to describe the head controller, which must deal with much more complex dynamics and loads than the oculomotor controller.

Moreover, our analysis assumed that there was constant input to the integrator during fixation periods. However, sensory feedback about head drift and tilt from the vestibular and neck proprioceptive systems may have given rise to reflexive or centrally driven “voluntary” inputs to the integrator. To model this, one would need an integrator embedded within feedback loops and one would have to achieve a way to measure the inputs to the integrator.

A final question is whether we should consider one INC integrator with a shared signal for eye and head posture (or even a gaze integrator), or separate commingled integrators for the eye and head, sharing space because of a common evolutionary heritage and the need for similar gaze-related and vestibular inputs. The possibility of completely shared eye and head integrators is unlikely in our view because eye and head position are often dissociated under natural conditions not only in extent and timing during gaze shifts but even frequently in opposite directions during the VOR (i.e., Ceylan et al. 2000). Our data add to this by showing that the neural integrator deficit accompanied by INC inactivation can result in quantitatively different offsets and drift characteristics in the eye and head that also develop with a different time course. However, our data cannot distinguish between the possibility that there are completely independent eye and head integrators or the possibility that there is partial overlap in the circuitry of the two integrators. Even single-unit recording of INC neurons during eye–head dissociation paradigms may not answer this question because neurons can share similar inputs (and thus show correlated responses) but have completely different outputs. The answer to this question probably requires a laborious cell-by-cell analysis of physiology and anatomic connections in behavioral paradigms that clearly dissociate eye and head orientation.

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References


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