Farshad Farshadmanesh, Pengfei Chang, Hongying Wang, Xiaogang Yan, Brian D. Corneil and J. Douglas Crawford

You might find this additional information useful...

Supplemental material for this article can be found at:
http://jn.physiology.org/cgi/content/full/90363.2008/DC1

This article cites 49 articles, 17 of which you can access free at:
http://jn.physiology.org/cgi/content/full/100/3/1677#BIBL

Updated information and services including high-resolution figures, can be found at:
http://jn.physiology.org/cgi/content/full/100/3/1677

Additional material and information about *Journal of Neurophysiology* can be found at:
http://www.the-aps.org/publications/jn

This information is current as of September 17, 2009 .

Report

Neck Muscle Synergies During Stimulation and Inactivation of the Interstitial Nucleus of Cajal (INC)

Farshad Farshadmanesh,[1] Pengfei Chang,[1,3] Hongying Wang,[1] Xiaogang Yan,[1] Brian D. Corneil,[2] and J. Douglas Crawford[1]

[1]*York Center for Vision Research, Canadian Institutes of Health Research Group for Action and Perception, Departments of Psychology, Biology, and Kinesiology and Health Sciences, York University, Toronto;* [2]*Departments of Physiology and Pharmacology and Psychology, University of Western Ontario, London, Ontario, Canada; and* [3]*Neuroscience Center, Yuquan Hospital, Tsinghua University, Beijing, China*

Farshadmanesh F, Chang P, Wang H, Yan X, Corneil BD, Crawford JD. Neck muscle synergies during stimulation and inactivation of the interstitial nucleus of Cajal (INC). *J Neurophysiol* 100: 1677–1685, 2008. First published June 25, 2008; doi:10.1152/jn.90363.2008. The interstitial nucleus of Cajal (INC) is thought to control torsional and vertical head posture. Unilateral microstimulation of the INC evokes torsional head rotation to positions that are maintained until stimulation offset. Unilateral INC inactivation evokes head position-holding deficits with the head tilted in the opposite direction. However, the underlying muscle synergies for these opposite behavioral effects are unknown. Here, we examined neck muscle activity in head-unrestrained monkeys before and during stimulation (50 μA, 200 ms, 300 Hz) and inactivation (injection of 0.3 μl of 0.05% muscimol) of the same INC sites. Three-dimensional eye and head movements were recorded simultaneously with electromyographic (EMG) activity in six bilateral neck muscles: sternocleidomastoid (SCM), splenius capitis (SP), rectus capitis posterior major (RCPmaj.), occipital capitis inferior (OCI), complexus (COM), and biventer cervicis (BC). INC stimulation evoked a phasic, short-latency (~5–10 ms) facilitation and later (~100–200 ms) a more tonic facilitation in the activity of ipsi-SCM, ipsi-SP, ipsi-COM, ipsi-BC, contra-RCPmaj., and contra-OCI. Unilateral INC inactivation led to an increase in the activity of contra-SCM, ipsi-SP, ipsi-RCPmaj., and ipsi-OCI and a decrease in the activity of contra-RCPmaj. and contra-OCI. Thus the influence of INC stimulation and inactivation were opposite on some muscles (i.e., contra-OCI and contra-RCPmaj.), but the comparative influences on other neck muscles were more variable. These results show that the relationship between the neck muscle responses during INC stimulation and inactivation is much more complex than the relationship between the overt behaviors.

INTRODUCTION

The position signal for controlling torsional and vertical eye and head posture is thought to be generated in the interstitial nucleus of Cajal (INC) (Crawford et al. 1991; Fukushima 1987; Helmchen et al. 1998; Klier and Crawford 2003; Klier et al. 2002). Unilateral INC stimulation evokes characteristic ipsi-torsional (i.e., relative to a nasooccipital axis) head rotations (Fig. 1*A*). On the other hand, unilateral INC inactivation evokes head position-holding deficits with the head titled in the opposite direction (i.e., relative to the inactivated INC; Fig. 1*B*) (Farshadmanesh et al. 2007; Fukushima et al. 1985; Klier et al.

2002). In other words, head postures immediately following *right* INC stimulation resemble head postures during *left* INC inactivation and vice versa. However, the underlying muscle synergies that are responsible for these behavioral effects are not known. Given the highly redundant organization of neck muscles (i.e., in theory, any one head posture could be produced by many combinations of muscle activity), it cannot be assumed that these synergies would also be opposite.

The primary purpose of the current study was to test the relationship between neck electromyographic (EMG) activity during stimulation and inactivation of the INC. To our knowledge, no previous EMG study in any species has made a systematic site-by-site comparison of the neck muscle synergies related to stimulation and inactivation of the INC or any other brain region. Moreover, we aimed to test this question in monkeys. The monkey has been the experimental model for many studies of INC function (Crawford et al. 1991; Farshadmanesh et al. 2007; Helmchen et al. 1998; Klier et al. 2002), but none of these studies incorporated EMG recordings. The cat has also been an important experimental model for testing INC function (Anderson 1981; Chimoto et al. 1999; Fukushima 1987; Fukushima and Kato 1985; Fukushima et al. 1990; Markham et al. 1966; Roste and Dietrichs 1988; Zuk et al. 1983) and several of these studies did look at the EMG activity of neck muscles during the inactivation of the INC. For example, Fukushima et al. (1987) observed that the torted head posture following unilateral INC inactivation was associated with an increase in the activity of all ipsilateral and some of the dorsal contralateral neck muscles that they recorded. However, there are no guarantees that the patterns of EMG recruitment following INC inactivation in the cat will be observed in the monkey, given the substantial differences in the musculoskeletal organization of the head–neck system in these animals (Richmond et al. 1999a, 2001). The current study thus provides the first opportunity to confirm some of these important basic observations derived from the cat in a primate experimental model.

The current study was designed to establish the pattern of EMG activity in six bilaterally recorded neck muscles following unilateral stimulation and inactivation of the INC in primates. We selected muscles that were accessible and likely to be involved in the patterns of head rotation/tilt observed in
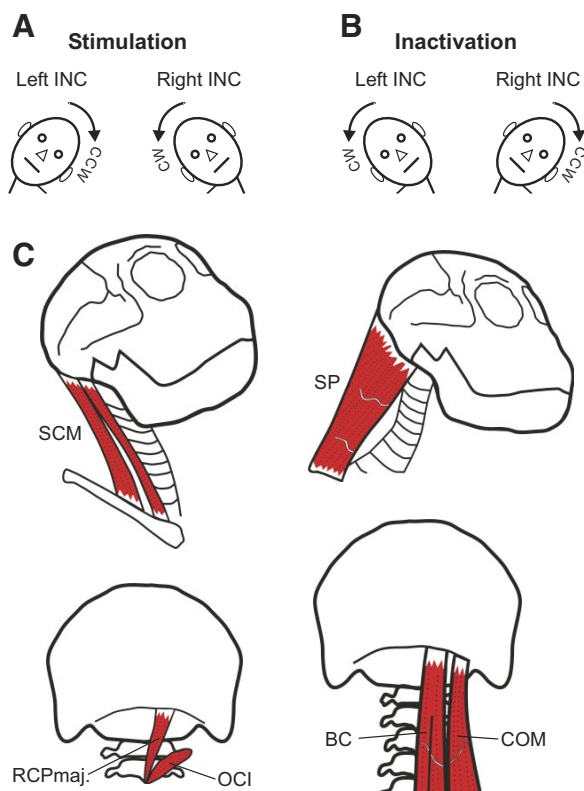

FIG. 1. Schematic representation of head posture after stimulation of interstitial nucleus of Cajal (INC, *A*) and INC inactivation (*B*). CW, clockwise; CCW, counterclockwise. *C*: schematic drawings of examined neck muscles (right side of the neck): SCM, sternocleidomastoid; SP, splenius capitis; RCPmaj., rectus capitis posterior major; OCI, occipital capitis inferior; BC, biventer cervicis; COM, complexus.

previous behavioral experiments. In addition to describing the basic synergies of muscle activity that we observed during stimulation and activation, we provide a site-by-site quantitative comparison of the effects of stimulation versus inactivation. Specifically, we tested whether the changes in neck muscle activity after INC inactivation are simply opposite to those evoked by INC stimulation (as one might predict from the opposite behavioral patterns) or whether the underlying muscular relationships are more complex than their behavioral counterparts might seem to predict. The answer to this question could have important implications for understanding the relationship between INC physiology and pathology.

METHODS

Surgical procedures

The data were collected in two female *Macaca mulata* monkeys (M1 and M2). 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. Each animal underwent two surgeries. During the first surgery, we implanted two eye coils in one eye of each animal to record eye movements and an acrylic skull cap equipped with a head stage to allow access to the INC during the experiment. Prior to each experiment session, two more coils were fastened onto a plastic platform on the skull cap to record head movements. Three-dimensional eye and

head movements were recorded using methods described previously (Crawford et al. 1999).

Two weeks later, in a second surgery, chronically indwelling bipolar hook electrodes were implanted in six neck muscles bilaterally (12 electrodes total): sternocleidomastoid (SCM), splenius capitis (SP), rectus capitis posterior major (RCPmaj.), occipital capitis inferior (OCI), biventer cervicis (BC), and complexus (COM) (Fig. 1*C*). Surgical details have been published elsewhere (Elsley et al. 2007). We selected these muscles because they are accessible and possibly associated with neck torsion. The SCM, SP, RCPmaj., and OCI muscles mainly function as head turners, whereas COM and BC muscles are known to be neck extensors (Corneil et al. 2001; Richmond et al. 2001). Among head turners, the suboccipital ones (RCPmaj. and OCI) are adjacent to the cervical vertebrae and are rich in properioceptors (Bakker and Richmond 1982; Richmond et al. 1999b). In this study, the muscles are referred to as being ipsi- or contralateral relative to the site of stimulation or injection. The wires of all electrodes were connected to an EMG connector embedded within the acrylic skull cap.

Experimental procedures

Before each experiment, a potential INC location along anterioposterior and mediolateral coordinates was selected for penetration using a method described previously (Farshadmanesh et al. 2007). Before the task began, animals were required to initially fixate a cross, back-projected at the center of a screen in front of them in a dark room. Animals were trained on a task requiring them to make a gaze shift to one of nine targets that appeared on the screen (one center; four up, down, left, and right, each 30° from center; and four in between, each 42° from center). A juice reward was given only when the animal's gaze *and* head positions were within computer-controlled fixation windows (around every target; gaze: 10° diameter; head: 20° diameter) and after the head movement was complete (i.e., where head velocity was <10°/s).

For stimulation trials, the preselected *penetration* location was electrically stimulated at different vertical depths (*sites*) using monophasic cathodal stimulations of 50 μA with pulse widths of 0.5 ms and frequency of 300 Hz, and pulse trains of 200 ms (Klier et al. 2007). The minimum separation between the sites was 500 μm. While performing the task, the stimulation was delivered 1,000 ms after the monkey's gaze and head entered the fixation window (for every target) and head velocity was <10°/s. For each INC site, we ran at least one block of the task. Stimulation was delivered randomly three times for each target location within the block.

After stimulating several sites within each INC location, we returned to the site where stimulation evoked the largest head rotation for that particular INC location. For inactivation trials, 0.3 μl of 0.05% muscimol solution was injected into the upper and lateral region of the selected INC site (Farshadmanesh et al. 2007). Eye and head coil and EMG data were recorded for a minimum of 40 min after muscimol injection while the monkey continued to perform the task. This encouraged the animal to continue to make head movements, enabling us to obtain a range of *initial* two-dimensional (horizontal and vertical) head positions similar to that we had during the stimulation. Each experimental session was conducted every other day to allow enough time for recovery from a performed injection. The locations of the injections were further confirmed by postmortem histological analysis. The details are published elsewhere (Farshadmanesh et al. 2007; Klier et al. 2007). The entire region of each INC was explored for each monkey in an orderly stereotaxic pattern. However, to provide a direct site-by-site comparison, here we describe data only from sites that were explored using both stimulation and inactivation.
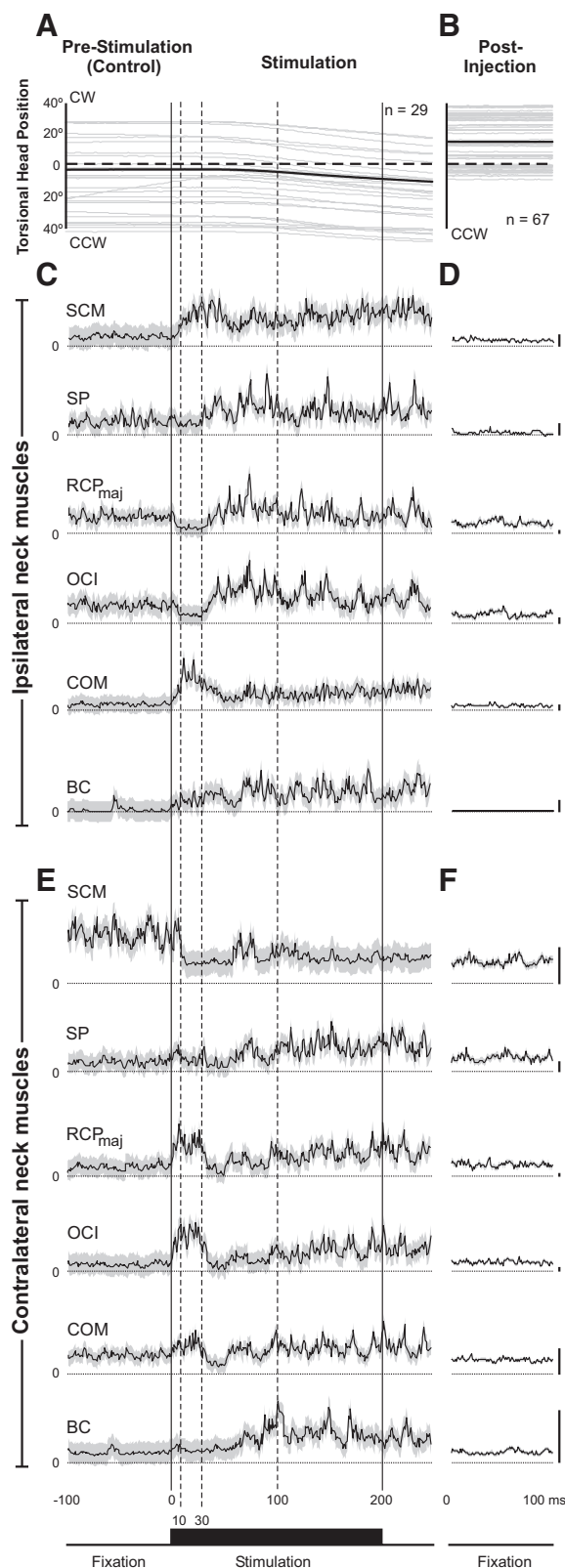
Data collection and analysis

Eye and head position and EMG data were recorded simultaneously before, during INC stimulation, and after INC inactivation. Eye and head coil signals were digitized at 10 kHz and then downsampled off-line by a factor of 10 to 1 kHz and converted to quaternions that represent the orientations of the eye and head, respectively (Crawford et al. 1999; Tweed et al. 1990). EMG signals were first differentially amplified at a headstage plugged directly on the EMG connector ($\times 20$ gain) and then band-pass filtered (20 Hz to 17 kHz). The conditioned EMG signals were then fed into a signal processing unit (Plexon; $\times 50$ gain; bandwidth, 100 Hz to 4 kHz) and were digitized at 10 kHz for later off-line analysis.

EMG signals were rectified and integrated into 1-ms time bins, using a method described previously (Bak and Loeb 1979; Corneil et al. 2001). For stimulation data we analyzed the data during both *early* (10–30 ms) and *late* (100–200 ms) periods following stimulation onset (recall stimulation duration was 200 ms). Analysis of the early period has the advantage of showing a more direct phasic effect before the evoked head rotation since this range of data is likely to involve activation produced by direct interstitiospinal tract connections (Isa and Sasaki 2002). On the other hand, analysis of the late period examines more tonic neck muscle activity during the actual head rotation (see Fig. 2A). Since the main goal of the current study was to compare stimulation with inactivation (which induces a tonic head tilt), we present the late stimulation results in the main text but for comparison we also provide the early stimulation results as supplementary data.[1] The general observations were similar in both cases.

From a behavioral point of view, we observed inactivation-evoked head tilts with similar and comparable magnitude to head rotations evoked by INC stimulation only 30–40 min after injecting the muscimol. Therefore to analyze EMG data during INC inactivation, we selected data collected in a range of 30–40 min after the injection of muscimol into the INC. We took the mean level of neck EMG activity in the first 100 ms of a segment during which the head was held stable, as a representative of inactivation data. For baseline activity, we selected the 100-ms segments before the application of each stimulation pulse. Stimulation and inactivation data of each INC site were then normalized relative to the baseline activity of that site. Note that the baseline, stimulation, and inactivation data presented in the main text report the mean level of EMG activity averaged over a 100-ms interval.

The main purpose of the current study was to compare neck muscle activity during INC stimulation and inactivation. It has been shown that neck muscle activity in primate varies with head position (Bizzi et al. 1971; Corneil et al. 2001; Lestienne et al. 1995, 2000). Here, we did not want to report position-dependent effects. Instead, we at-

tempted to account for head position effect and remove any variance in EMG activity related to horizontal and vertical head position. This way, we were able to isolate the EMG activity at the straight-ahead position and compare only the effects that were consequent to our manipulation, rather than the noncentral head position. To do this, rather than simply averaging all data across different initial head

FIG. 2. Electromyographic activity of 12 recorded muscles for a typical left INC site of animal M2. Torsional head position is plotted for prestimulation (control), stimulation (A), and postinjection (B) of the *same* INC site. In A and B, individual trials are shown as thin gray lines, whereas the thick black line represents the averaged data. Injection data represent the first 100 ms of each fixation period in a file recorded for 100 s, 35 min after injecting the muscimol. The solid vertical lines indicate the stimulation onset and offset, respectively. The dashed vertical lines represent 10, 30, and 100 ms of poststimulation, respectively. The quantitative analysis of stimulation data examined the intervals 10–30 and 100–200 ms after stimulation onset (early and late periods, respectively). We present the results from the late stimulation period in the main text. Corresponding electromyographic (EMG) activity of ipsilateral neck muscles is shown in C and D, whereas the EMG activity of contralateral neck muscles is shown in E and F. The muscles are referred to as being ipsi- or contralateral relative to the site of stimulation or injection. Here, the thick black line represents the average EMG activity for each muscle. The $\pm$SE is plotted as a gray patch for each muscle. Vertical scale bars on the right side of the EMG traces denote 20 μV. Stimulation onset and offset are shown as a solid black bar in the bottom. CW, clockwise; CCW, counterclockwise.

---

[1] The online version of this article contains supplemental data.

positions, for each muscle at each INC site, we first calculated the EMG activity, initial horizontal, and initial vertical head positions for our baseline, stimulation, or inactivation data. We then performed a multiple regression analysis between initial horizontal and vertical head position values (independent variables) and neck EMG activity (dependent variable) for each muscle, and fit a plane to these data (blue plane in Fig. 3A). The means ± SD goodness-of-fit values for all muscles across both animals are shown in Table 1. The distance of this fitted plane from zero at the center head position (where both horizontal and vertical head position values are zero) was calculated as the characteristic EMG activity of that particular muscle, independent of variations in initial head position. This method is similar to what we used previously to compute the characteristic gaze displacement vector (Klier et al. 2001).

RESULTS

For the current study, we analyzed data from INC sites only where stimulation produced a head rotation ≥10°. Because in this study we compare stimulation and inactivation data on a site-by-site basis, we have also constrained our data to the one stimulation site within each vertical electrode penetration that most closely matched the depth of the subsequent injection site. In most cases (13 of 18) the sites were identical and in 3 and 2 of 18 cases the stimulation and injection sites colocalized within 0.5 and 1 mm, respectively. In all, we present the data from 8 (4 left, 4 right) INC sites in animal M1 and 10 (5 left, 5 right) INC sites in animal M2. Averaged across all sites, stimulation produced a final head torsional offset of 17.22 ± 8.67° (mean ± SD), very similar to the average torsional offset of the head for the inactivation data reported here (17.92 ± 12.17°; mean ± SD). However, as expected, these offsets were in the opposite direction. Across sites, stimulation and inactivation offsets were negatively correlated in both M1 ($r = -0.92$) and M2 ($r = -0.80$). See Klier et al. (2007) and Farshadmanesh et al. (2007) for a detailed kinematic analysis of head movements and postures in nearly identical experiments; here we focus on the patterns of EMG.

Typical patterns of EMG activity of neck muscles

CONTROL AND STIMULATION DATA. We first describe raw and averaged position and EMG data for an experiment that illustrates the general trends. Figure 2 shows the results of a *left* INC experiment (Supplemental Fig. S1 shows a *right* INC experiment where stimulation and inactivation rotate the head in the opposite direction, but the muscle EMG data look very similar when plotted with respect to their ipsilateral/contralateral location relative to the INC site). The patterns shown in these plots were typical of the population data (Fig. 3B and Supplemental Fig. S2A) in most respects, but each individual experiment had its own EMG idiosyncrasies.

In Fig. 2A (*left* INC stimulation) the head began to rotate torsionally in a counterclockwise (CCW) direction 50 ms after stimulation onset (left solid vertical line), reaching an average torsional rotation of 17° just before stimulation offset (i.e., 200 ms after stimulation onset, right solid vertical line). The activity of several ipsi- and contralateral neck muscles (relative to the side of stimulation) increased about 5–10 ms after stimulation onset. Such short-latency facilitation is seen in ipsi-SCM, ipsi-COM (Fig. 2C), contra-RCPmaj., contra-OCI, and contra-COM muscles (Fig. 2E). Such facilitation peaked about
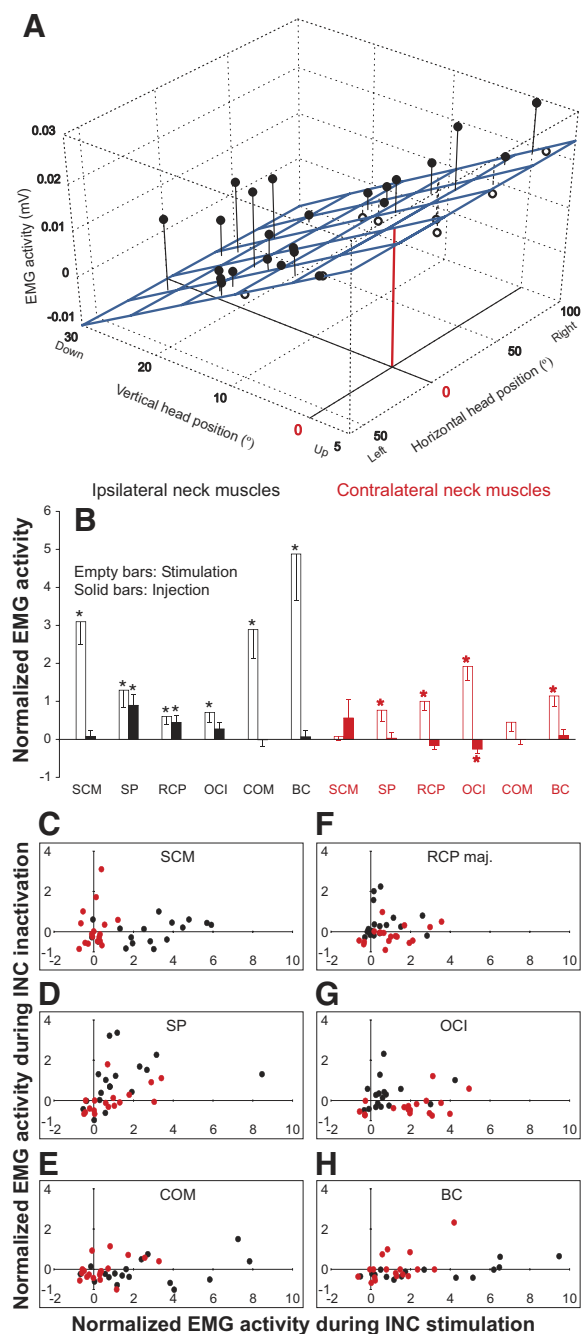

FIG. 3. *A*: an example of performed multiple regression analysis (see METHODS). Right SCM, animal M2, left INC; solid circles, data above the blue fit plane; empty circles, data below the fit plane. The vertical red line indicates the distance of the fitted plane from the straight-ahead position, where both horizontal and vertical head position values are zero, as the characteristic EMG activity. *B*: pooled and normalized EMG values and SEs (see METHODS) from all the unique sites recorded in both animals ($n = 18$ except in ipsi-RCPmaj., ipsi-OCI, and contra-SP, where $n = 17$). Normalized EMG values were subtracted from one so that here, zero means no change in EMG activity (relative to baseline), a positive value an increase, and a negative value a decrease. Empty bars represent stimulation data (100–200 ms); solid bars represent the inactivation. Black and red colors represent ipsi- and contralateral neck muscles, respectively. Asterisks indicate a significant effect relative to the control. *C–H*: normalized inactivation data (*y*-axis) of individual INC sites recorded in both animals is plotted as a function of normalized stimulation data (*x*-axis) separately for every muscle. Black and red colors represent ipsi- and contralateral neck muscles, respectively.

TABLE 1. *Goodness-of-fit (R^2) values of the multidimensional linear fits*

	L-SCM	L-SP	L-RCP maj.	L-OCI	L-COM	L-BC
Control	0.65 ± 0.18 (18)	0.36 ± 0.19 (17)	0.43 ± 0.22 (17)	0.31 ± 0.18 (16)	0.40 ± 0.17 (17)	0.15 ± 0.14 (16)
Stimulation (10–30 ms)	0.44 ± 0.23 (17)	0.27 ± 0.16 (17)	0.25 ± 0.18 (18)	0.24 ± 0.17 (17)	0.29 ± 0.12 (18)	0.15 ± 0.10 (17)
Stimulation (100–200 ms)	0.52 ± 0.23 (18)	0.47 ± 0.23 (18)	0.55 ± 0.26 (18)	0.50 ± 0.24 (18)	0.42 ± 0.26 (18)	0.23 ± 0.22 (17)
Inactivation	0.52 ± 0.29 (16)	0.48 ± 0.20 (17)	0.61 ± 0.27 (18)	0.56 ± 0.27 (18)	0.51 ± 0.21 (17)	0.25 ± 0.26 (17)

	R-SCM	R-SP	R-RCP maj.	R-OCI	R-COM	R-BC
Control	0.40 ± 0.22 (18)	0.32 ± 0.16 (18)	0.44 ± 0.12 (17)	0.38 ± 0.15 (16)	0.34 ± 0.18 (16)	0.15 ± 0.10 (16)
Stimulation (10–30 ms)	0.37 ± 0.17 (18)	0.31 ± 0.19 (18)	0.35 ± 0.25 (18)	0.31 ± 0.22 (18)	0.30 ± 0.21 (18)	0.24 ± 0.19 (18)
Stimulation (100–200 ms)	0.40 ± 0.20 (18)	0.46 ± 0.26 (18)	0.60 ± 0.23 (18)	0.56 ± 0.22 (18)	0.29 ± 0.23 (18)	0.31 ± 0.16 (18)
Inactivation	0.45 ± 0.26 (18)	0.54 ± 0.22 (16)	0.68 ± 0.13 (17)	0.65 ± 0.16 (17)	0.40 ± 0.20 (16)	0.34 ± 0.21 (17)

Values are means ± SD across animals M1 and M2 for left (L-) and right (R-) neck muscles ($n = 18$). The numbers in parentheses represent the number of sites in which the values were statistically significant ($P < 0.05$).

10–30 ms after stimulation onset in most muscles and this peak was followed by a short suppression in EMG activity that persisted for about 10 ms (i.e., see contra-RCPmaj. and contra-OCI). These short-latency facilitations were accompanied by approximately synchronized decreases in the activity of ipsi-SP, ipsi-RCPmaj., ipsi-OCI, and contra-SCM about 10 ms after stimulation onset. Such suppressive responses persisted until about 30 ms after stimulation onset.

In general, the later (100–200 ms) tonic activity mirrored the facilitation responses seen earlier. In the example shown in Fig. 2, after the short-latency increase and the subsequent, brief suppression in EMG activity of ipsi-SCM, ipsi-COM, contra-RCPmaj., contra-OCI, and contra-COM muscles, their activity returned to a higher-than-baseline level that persisted until stimulation offset. Similarly, after the suppression in the activity of ipsi-SP, ipsi-RCPmaj., and ipsi-OCI muscles, their activity increased to a level higher than the baseline until stimulation offset. We compare the early and late responses more quantitatively in the next section.

Overall, Fig. 2, *C* and *E* suggests that after stimulating this particular INC site, during early stimulation, the changes in the EMG activity of ipsi-SCM, ipsi-RCPmaj., and ipsi-OCI muscles are opposite relative to their contralateral counterparts, whereas the activity in both ipsi- and contralateral SP, COM, and BC muscles increases, albeit with different temporal characteristics.

INACTIVATION DATA. In Fig. 2, *B*, *D*, and *F*, we show the results of injecting muscimol into the same left INC site. By 35 min after muscimol injection, the animal's head was tilted about 17° in the clockwise (CW) direction (Fig. 2*B*), i.e., opposite to the torsional movement produced by stimulation. Compared with the baseline level of neck EMG activity in the prestimulation interval, we also observed a clear decrease in EMG activity of all ipsilateral muscles, as well as in contra-SCM and contra-COM muscles (i.e., compare the levels in Fig. 2, *D* and *F* to the level of prestimulation activity in Fig. 2, *C* and *E*, respectively). For this particular INC site, any change in the EMG activity of contra-SP, contra-RCPmaj., contra-OCI, and contra-BC muscles was negligible. Again, similar results were obtained for muscle EMG plotted ipsilateral/contralateral to a right INC inactivation site (Supplemental Fig. S1) except that the direction of head torsion was opposite (in other words, the right and left INC results were symmetric across the midline). Although relatively large changes in the activity of some muscles were observed after inactivation, from these

plots it is difficult to know whether these changes were related to torsion or to other changes in head posture. Therefore our subsequent analysis references all neck EMG activity to a straight-ahead head posture (in the horizontal and vertical dimensions).

Normalized effects of INC stimulation/inactivation

EMG activity of neck muscles can be modulated by head position. To account for this and to examine the overall effect of INC stimulation and INC inactivation on neck muscle synergies across our sample of sites, we estimated the EMG activity of each muscle at the straight-ahead position using a multiple regression analysis (see METHODS for details). This was done on data collected before stimulation (behavioral controls), during INC stimulation (both early and late periods), and after INC inactivation. Figure 3*A* provides an example of this analysis for a set of control (prestimulation) behavioral data. Note how these analyses derived the torsional intercept value at the straight-ahead position from a variety of different head positions, thereby getting rid of position-dependent effects that could contaminate a simple average.

We then normalized the stimulation and inactivation data relative to the baseline (prestimulation) values. Next we subtracted the results from one so that a value of zero means no change in EMG activity relative to baseline. Similarly, a positive value means an increase in EMG activity and a negative value a decrease. Finally, we pooled the normalized data from all the unique sites recorded in both animals ($n = 18$ except in ipsi-RCPmaj., ipsi-OCI, and contra-SP, where $n = 17$). A site-by-site correlation between the early and late stimulation data showed that these values were similar, but not identical, for all muscles. Averaged across all muscles, the correlation coefficient (r) between early and late stimulation data was 0.66. Therefore we compared both epochs to the muscimol data, but in the main text we focus on the late stimulation comparison (Fig. 3) because the head postures and averaging duration were more comparable to the muscimol data (early stimulation–inactivation comparison is provided in Supplemental Fig. S2).

STIMULATION AND INACTIVATION DATA. In Fig. 3*B*, empty bars represent stimulation data, whereas the solid bars represent the inactivation data. Black and red colors represent ipsi- and contralateral neck muscles, respectively. Positive values indicate a facilitation in muscle activity, whereas negative values

indicate a suppression. During late stimulation, muscle activity increased in all muscles (Fig. 3*B*, empty bars). Significant increases in EMG activity were observed in all except contra-SCM and contra-COM muscles (*t*-test, $P < 0.05$). This generally agrees with the example described earlier. Unilateral INC inactivation also induced large changes in EMG activity, but these were not consistent in all muscles. By 35 min after muscimol injection (Fig. 3*B*, solid bars), the only significant effects across experiments were increases in the activity of ipsi-SP and ipsi-RCPmaj. (*t*-test, $P < 0.05$) and a significant decrease in the activity of contra-OCI (*t*-test, $P < 0.05$). For the other muscles, we observed changes in EMG activity for a given site, but such changes were not significant across all sites. Figure 3*B* also suggests that INC inactivation produced opposite synergies in ipsi- versus contralateral pairs of RCPmaj. and OCI muscles.

Overall, we observed large and consistently significant changes in EMG activity of most recorded neck muscles during both INC stimulation and inactivation. However, as explained in the next section, the relationship between stimulation and inactivation was not consistent. Moreover, we expected to see opposite (ipsilateral vs. contralateral) muscle synergies in both stimulation and inactivation data, but such patterns were evident only in some muscles. This is examined more quantitatively in the next section.

COMPARISON BETWEEN STIMULATION AND INACTIVATION DATA. Kinematic measurements show the direction of torsional head tilt observed after INC inactivation is opposite to the direction of rotation produced by stimulation of the same site (Klier et al. 2002). For example, if the left INC is stimulated, the head rotates in a CCW direction, whereas inactivating the left INC evokes a CW head tilt. Are the underlying muscle synergies also opposite?

Comparing the EMG activity of ipsilateral neck muscles during late INC stimulation to the activity of the same muscles after INC inactivation in Fig. 3*B* suggests that stimulation and inactivation of the INC did not evoke opposite effects in any of the ipsilateral muscles. Further, both stimulation and inactivation evoked significant facilitation in ipsi-SP.

Among contralateral neck muscles, opposite patterns were observed during INC stimulation (late period) compared with INC inactivation in RCPmaj. and OCI muscles. In both contra-RCPmaj. and contra-OCI muscles, stimulation and inactivation produced facilitation and suppression, respectively.

Thus stimulation and inactivation did not consistently evoke opposite muscle synergies when data were averaged across experiments, although it is possible that such patterns become evident only when the data are examined on a site-by-site basis. In Fig. 3, *C–H*, we have plotted inactivation data as a function of late stimulation data for all individual experiments in each panel. These are the same data used to generate the average data in Fig. 3*B*. The *x*-axis represents the normalized INC stimulation value, whereas the normalized INC inactivation value is plotted along the *y*-axis. Similar to Fig. 3*B*, black and red colors represent ipsi- and contralateral neck muscles, respectively. If similar to behavioral findings, inactivation effects would be opposite to stimulation effects; one would expect to see a negative correlation coefficient (*r*) between stimulation and inactivation data (with data falling only within the *top left* and *bottom right* quadrants of each panel).

However, the actual stimulation–inactivation relationships were much more complex. Among head-turner neck muscles, late stimulation and inactivation results showed a higher correlation coefficient (*r*) in ipsi-SCM ($m = 0.18$, $r = 0.66$) than in contra-SCM muscle ($m = 1.06$, $r = 0.25$) (Fig. 3*C*). On the other hand, the correlation is higher in contra-SP ($m = 0.30$, $r = 0.56$) than in ipsi-SP muscle ($m = 0.19$, $r = 0.33$) (Fig. 3*D*); in contra-RCPmaj. ($m = 0.15$, $r = 0.36$) than in ipsi-RCPmaj. muscle ($m = 0.00$, $r = 0.00$) (Fig. 3*F*); and in contra-OCI ($m = 0.13$, $r = 0.40$) than in ipsi-OCI muscle ($m = 0.13$, $r = 0.21$) (Fig. 3*G*). In both neck extensor neck muscles, COM and BC (Fig. 3, *E* and *H*, respectively) the correlation between stimulation and inactivation data is higher in ipsilateral than in contralateral muscle (ipsi-BC, $m = 0.08$, $r = 0.66$; contra-BC, $m = 0.34$, $r = 0.58$; and ipsi-COM, $m = 0.14$, $r = 0.64$; contra-COM, $m = 0.20$, $r = 0.40$). Overall, very similar results were also obtained in the quantitative comparison between the early stimulation and the muscimol data (Supplemental Fig. S2, *B–G*). On average, slope (*m*) and correlation coefficient (*r*) values ranged from 0.36 and 0.44 to 0.24 and 0.42 for early and late stimulation data, respectively.

These results show that INC stimulation and inactivation effects were generally uncorrelated or even positively correlated. However, the intensity and significance of this correlation was different depending on the muscle. We also did not observe consistent clustering of the data in the *top left* and *bottom right* quadrants in Fig. 3, *C–H*. Therefore despite the opposite patterns observed during behavioral observations, EMG patterns of neck muscle activation after INC inactivation were not simply opposite to those observed during INC stimulation in the same sites.

DISCUSSION

This study confirms that unilateral INC stimulation and inactivation produce essentially opposite behavioral effects (Klier et al. 2002), but also tests the underlying neck muscle synergies for the first time. In general, unilateral stimulation of the INC increased neck muscle activity (at least in those we recorded), whereas unilateral INC inactivation produced more subtle effects that varied on a per muscle basis (when we controlled for the twisted head posture). Some muscle antagonists in ipsi- versus contralateral pairs showed opposite changes—for example, SCM, RCPmaj., and OCI during early INC stimulation (10–30 ms) and RCPmaj. and OCI after INC inactivation. However, the relationship between the overall patterns observed during INC stimulation and inactivation was not opposite and was generally quite complex.

Comparison between stimulation of the INC and other brain areas

EMGs of neck muscles have been studied during stimulation and inactivation of several midbrain areas including the superior colliculus (SC) in cats (Guitton et al. 1980; Hadjidimitrakis 2007; Roucoux et al. 1980) and monkeys (Corneil et al. 2002a,b); the frontal eye fields in monkeys (Elsley et al. 2007); and the prestitial nucleus in humans (Hassler et al. 1981). However, the current study is the first to demonstrate and directly compare the patterns of neck muscle activation during stimulation and inactivation of the INC in head-unrestrained

monkeys. Guitton et al. (1980) showed that stimulating different zones of the SC in cats is associated with different patterns of recruitment in biventer cervicis. For example the patterns of muscle recruitment evoked by stimulation of the anterior zone of the SC depended on the initial position of the eye in the orbit, whereas stimulation of the intermediate and posterior zones evoked more invariant patterns of recruitment. We did not find any topographic relationship between the site of INC stimulation and the evoked neck EMG responses. This is consistent with the behavioral topography observed in functional studies of the SC (Robinson 1972), as opposed to the weak or absent functional topography in the INC (Farshadmanesh et al. 2007).

There are also several more detailed but systematic differences between the EMG patterns evoked during SC and INC stimulation. For example, stimulation of the caudal SC in monkeys evokes synchronous facilitation in contra-SP, contra-RCPmaj., and contra-OCI muscles together with synchronous suppression in their ipsilateral counterparts (Corneil et al. 2002a). During early stimulation of the INC, we observed similar facilitation and suppression in RCPmaj. and OCI muscles. However, in contrast to SC stimulation, INC stimulation evoked a facilitation in both ipsi- and contra-SP muscles. Although SP does contribute to head turning, the bilateral recruitment of this muscle following INC stimulation is consistent with an accessory role for this muscle in upward head motion in the monkey (Corneil et al. 2001). Moreover, both facilitation and suppression responses during SC stimulation usually began about 10 ms after stimulation onset (Corneil et al. 2002a) whereas during INC stimulation, facilitation usually began earlier ($\sim$5–10 ms) after stimulation onset. These comparative neck EMG response latencies are consistent with known multisynaptic tectospinal pathways from the SC through the INC to the motor periphery (see Isa and Sasaki 2002 for review).

Relationship between muscle EMG and kinematics

Head movements typically observed during INC stimulation may be characterized as a combination of consistent torsional (roll) components, variable vertical (pitch) components, and small but consistent horizontal components (Klier et al. 2007). In a redundant system like the neck, not every detail of neck EMG may be related to behavior because large muscle groups might mask the effects of weaker muscle groups. However, if these muscles work together in cooperative synergies there should be a general correspondence between neck EMG activity and the components of head movement and posture during INC stimulation and inactivation.

In our experimental preparation, left and right INC stimulation generally produced CCW/right and CW/left head rotations, respectively. These movement components appear to be biomechanically consistent with our EMG results. For example, SCM, when recruited, tilts the head ipsilaterally and rotates it contralaterally. This is seen in our early stimulation data by an increase in the activity of ipsi-SCM and a decrease in contra-SCM. The contralateral head rotation can also be attributed to the increased activity in contra-RCPmaj. and contra-OCI that rotate the head mainly horizontally and in our data their facilitation was accompanied with a suppression in their ipsilateral counterparts. INC stimulation also increased

activity in bilateral BC, ipsi-COM, and ipsi-SP. These extensor muscles are thought to help hold the head upright, particularly when the animal adopts a quadrupedal posture (Richmond et al. 1999a; Vidal et al. 1986). Although it might be surprising that INC stimulation did not consistently increase the activity of ipsi-COM and ipsi-SP, these results may simply reflect the variable vertical components of head rotation observed in INC stimulation (Klier et al. 2007).

Compared with stimulation, unilateral INC inactivation produces an opposite torsional tilt pattern without consistent vertical or horizontal components (Farshadmanesh et al. 2007). When corrected for the head-position effect (Fig. 3*B*, solid bars), the tilted head posture was accompanied by a modest but significant increase in some recorded neck muscles (ipsi-SP, ipsi-RCPmaj.) and a significant decrease in contra-OCI. This is consistent with observations in the cat (Fukushima et al. 1987), where the authors found an increase in the activity of ipsi-splenius, ipsi-rectus, and contra-obliquus capitis caudalis muscles. Moreover, after the INC inactivation, there is little to no difference in the overall activation of some turner (Fig. 3*B*, ipsi-SCM, contra-SP) and extensor neck muscles (Fig. 3*B*, ipsi- and contralateral BC and COM).

Relationship between stimulation and inactivation

Some of the differences between our stimulation and inactivation results could be related to the variable (site-to-site) vertical components seen in stimulation more than inactivation, as discussed earlier. To investigate this further a detailed site-by-site biomechanical analysis of neck EMG versus movement components is required. However, there are several other likely differences. Our experiment was designed to match previous behavioral experiments in the monkey, where stimulation produces active head *rotations*, starting from an upright head posture, whereas inactivation produces tonically tilted head *postures*. Gravitational and inertial factors would be significant, and not identical, in these two cases, depending on the point of time during stimulation. Moreover, with stimulation we were looking at effects that occurred over a time span of 200 ms, whereas to obtain comparable behavioral results we needed to analyze inactivation data at 30–40 min after muscimol injection.

Given these differences, postural offset evoked by INC inactivation could be related to activation of some muscles, inactivation of other muscles, and reactive stretch in other muscles due to the mass of the head and gravity in a tilted position (Vidal et al. 1986). Moreover, with the head tonically tilted, both cervicocollic (Peterson et al. 1985) and vestibulocollic (Schor et al. 1988) reflexes were likely activated. Finally, during prolonged INC inactivation, the sensory feedback from neck proprioceptive (Abrahams et al. 1974), the vestibular system (Fukushima et al. 1983), and vision may have evoked compensatory responses. Thus the EMG patterns reported here could be representative of both INC deficits (primary) and compensatory mechanisms (secondary) used by other brain areas. In contrast, the EMG signals associated with the early (10–30 ms) phase of stimulation are simpler to interpret since they probably represent activations related to hard-wired interstitiospinal synergies (Isa and Sasaki 2002) and precede the evoked head movement. It is not surprising that we found differences between these results and the inactivation results

(even though the magnitude of head rotation was comparable). Further, EMG recruitment during the late (100–200 ms) stimulation likely reflects additional contributions of spinal circuits and other movement-related reflexes that operate on a much shorter timescale than the compensatory mechanisms engaged following muscimol inactivation. This suggests that the main differences could relate to either *1*) the intrinsic mechanisms of stimulation versus muscimol injection or *2*) longer-term adjustments related to the recruitment of secondary compensatory mechanisms.

It is possible that earlier effects following INC inactivation (i.e., perhaps 5–10 min postinjection) are less contaminated by such compensatory mechanisms and more directly represent the deficit in the INC itself, although this data set also has much smaller behavioral effects. Another alternative approach (not possible with our stimulation electrodes) would be to compare the effects of longer-term tonic stimulation with inactivation over a comparable timeframe. However, in light of our results, it seems unlikely that one would achieve exactly opposite patterns of EMG activation—however similar the behavior—during stimulation and inactivation.

Clinical implications

Unilateral INC inactivation in primates evokes characteristic head postures similar to the symptoms of cervical dystonia (torticollis) in humans (Loher et al. 2004) and several studies suggest a causal relationship between the INC dysfunction and torticollis (Farshadmanesh et al. 2007; Malouin and Bedard 1982; Munchau and Bronstein 2001; Vasin et al. 1985). Tijssen et al. (2000) showed that the activity in SCM and SP muscles is in phase in torticollis patients but not in control subjects. In agreement with this, the results of the current study suggest that EMG patterns evoked by INC stimulation in SCM and SP muscles show a basic in-phase relationship.

EMG has been shown to be a reliable tool for predicting and confirming clinical findings in dystonia (Delval et al. 2004; Wang et al. 2006). For example, EMG has been used in both diagnosis (Vasilescu and Dieckmann 1975) and treatment follow-up of torticollis (Domzal and Tutaj 2000). Raeva et al. (1987) suggested that combining EMG examination with the use of functional motor and oculomotor tests facilitates the identification of the primarily involved groups of muscles and can help to specify these groups.

It has been suggested that unilateral INC inactivation is an experimental model for torticollis resulting directly from damage to the INC (Klier et al. 2002, 2007), whereas INC stimulation could serve as an experimental model for torticollis that originates from inappropriate inputs from upstream structures (such as the basal ganglia) to the INC (Farshadmanesh et al. 2007; Klier et al. 2002). If so, the differences in neck EMG associated with these two different sources of head tilt could aid in diagnosis and intervention.

REFERENCES

Abrahams VC, Richmond F, Rose PK. Proceedings: proprioceptive and retinal afferent projections to the superior colliculus of the cat and their connexions to the tectospinal tract. *J Physiol* 241: 101P–102P, 1974.
Anderson JH. Ocular torsion in the cat after lesions of the interstitial nucleus of Cajal. *Ann NY Acad Sci* 374: 865–871, 1981.
Bak MJ, Loeb GE. A pulsed integrator for EMG analysis. *Electroencephalogr Clin Neurophysiol* 47: 738–741, 1979.
Bakker DA, Richmond FJ. Muscle spindle complexes in muscles around upper cervical vertebrae in the cat. *J Neurophysiol* 48: 62–74, 1982.
Bizzi E, Kalil RE, Tagliasco V. Eye-head coordination in monkeys: evidence for centrally patterned organization. *Science* 173: 452–454, 1971.
Chimoto S, Iwamoto Y, Yoshida K. Projections and firing properties of down eye-movement neurons in the interstitial nucleus of Cajal in the cat. *J Neurophysiol* 81: 1199–1211, 1999.
Corneil BD, Olivier E, Munoz DP. Neck muscle responses to stimulation of monkey superior colliculus. I. Topography and manipulation of stimulation parameters. *J Neurophysiol* 88: 1980–1999, 2002a.
Corneil BD, Olivier E, Munoz DP. Neck muscle responses to stimulation of monkey superior colliculus. II. Gaze shift initiation and volitional head movements. *J Neurophysiol* 88: 2000–2018, 2002b.
Corneil BD, Olivier E, Richmond FJ, Loeb GE, Munoz DP. Neck muscles in the rhesus monkey. II. Electromyographic patterns of activation underlying postures and movements. *J Neurophysiol* 86: 1729–1749, 2001.
Crawford JD, Cadera W, Vilis T. Generation of torsional and vertical eye position signals by the interstitial nucleus of Cajal. *Science* 252: 1551–1553, 1991.
Crawford JD, Ceylan MZ, Klier EM, Guitton D. Three-dimensional eye–head coordination during gaze saccades in the primate. *J Neurophysiol* 81: 1760–1782, 1999.
Delval A, Krystkowiak P, Cassim F, Skrzypzak L, Destee A, Derambure P, Defebvre L. Usefulness and limitations of polymyographic recordings in dystonia. *Rev Neurol* (*Paris*) 160: 547–553, 2004.
Domzal TM, Tutaj A. Electromyography monitoring in the treatment of torticollis with botulinum toxin. *Pol Merkur Lekarski* 8: 474–475, 2000.
Elsley JK, Nagy B, Cushing SL, Corneil BD. Widespread presaccadic recruitment of neck muscles by stimulation of the primate frontal eye fields. *J Neurophysiol* 98: 1333–1354, 2007.
Farshadmanesh F, Klier EM, Chang P, Wang H, Crawford JD. Three-dimensional eye–head coordination after injection of muscimol into the interstitial nucleus of Cajal (INC). *J Neurophysiol* 97: 2322–2338, 2007.
Fukushima K. The interstitial nucleus of Cajal and its role in the control of movements of head and eyes. *Prog Neurobiol* 29: 107–192, 1987.
Fukushima K, Fukushima J, Harada C, Ohashi T, Kase M. Neuronal activity related to vertical eye movement in the region of the interstitial nucleus of Cajal in alert cats. *Exp Brain Res* 79: 43–64, 1990.
Fukushima K, Fukushima J, Terashima T. The pathways responsible for the characteristic head posture produced by lesions of the interstitial nucleus of Cajal in the cat. *Exp Brain Res* 68: 88–102, 1987.
Fukushima K, Kato M. The interstitial nucleus of Cajal: its connections and functional considerations. *Nippon Seirigaku Zasshi* 47: 115–129, 1985.
Fukushima K, Takahashi K, Kato M. Responses of vestibular neurons to stimulation of the interstitial nucleus of Cajal in the cat. *Exp Brain Res* 51: 1–15, 1983.
Fukushima K, Takahashi K, Kudo J, Kato M. Interstitial-vestibular interaction in the control of head posture. *Exp Brain Res* 57: 264–270, 1985.
Guitton D, Crommelinck M, Roucoux A. Stimulation of the superior colliculus in the alert cat. I. Eye movements and neck EMG activity evoked when the head is restrained. *Exp Brain Res* 39: 63–73, 1980.
Hadjidimitrakis K, Moschovakis AK, Dalezios Y, Grantyn A. Eye position modulates the electromyographic responses of neck muscles to electrical stimulation of the superior colliculus in the alert cat. *Exp Brain Res* 179: 1–16, 2007.
Hassler R, Vasilescu C, Dieckmann G. Electromyographic activity of neck muscles in patients affected by retrocollis under the influence of stimulation and coagulation of the prestitial nucleus of the midbrain. *Appl Neurophysiol* 44: 291–301, 1981.
Helmchen C, Rambold H, Fuhry L, Buttner U. Deficits in vertical and torsional eye movements after uni- and bilateral muscimol inactivation of

the interstitial nucleus of Cajal of the alert monkey. *Exp Brain Res* 119: 436–452, 1998.

Isa T, Sasaki S. Brainstem control of head movements during orienting; organization of the premotor circuits. *Prog Neurobiol* 66: 205–241, 2002.

Klier EM, Crawford JD. Neural control of three-dimensional eye and head posture. *Ann NY Acad Sci* 1004: 122–131, 2003.

Klier EM, Wang H, Constantin AG, Crawford JD. Midbrain control of three-dimensional head orientation. *Science* 295: 1314–1316, 2002.

Klier EM, Wang H, Crawford JD. The superior colliculus encodes gaze commands in retinal coordinates. *Nat Neurosci* 4: 627–632, 2001.

Klier EM, Wang H, Crawford JD. Interstitial nucleus of Cajal encodes three-dimensional head orientations in Fick-like coordinates. *J Neurophysiol* 97: 604–617, 2007.

Lestienne FG, Le Goff B, Liverneaux PA. Head movement trajectory in three-dimensional space during orienting behavior toward visual targets in rhesus monkeys. *Exp Brain Res* 102: 393–406, 1995.

Lestienne FG, Thullier F, Archambault P, Levin MF, Feldman AG. Multi-muscle control of head movements in monkeys: the referent configuration hypothesis. *Neurosci Lett* 283: 65–68, 2000.

Loher TJ, Pohle T, Krauss JK. Functional stereotactic surgery for treatment of cervical dystonia: review of the experience from the lesional era. *Stereotact Funct Neurosurg* 82: 1–13, 2004.

Malouin F, Bedard PJ. Frontal torticollis (head tilt) induced by electrolytic lesion and kainic acid injection in monkeys and cats. *Exp Neurol* 78: 551–560, 1982.

Markham CH, Precht W, Shimazu H. Effect of stimulation of interstitial nucleus of Cajal on vestibular unit activity in the cat. *J Neurophysiol* 29: 493–507, 1966.

Munchau A, Bronstein AM. Role of the vestibular system in the pathophysiology of spasmodic torticollis. *J Neurol Neurosurg Psychiatry* 71: 285–288, 2001.

Peterson BW, Goldberg J, Bilotto G, Fuller JH. Cervicocollic reflex: its dynamic properties and interaction with vestibular reflexes. *J Neurophysiol* 54: 90–109, 1985.

Raeva SN, Vasin N, Vainberg NA, Shabalov VA, Medzhidov MR. Electromyography in patients with spastic torticollis. *Zh Vopr Neirokhir Im N N Burdenko* 46–53, 1987.

Richmond FJ, Corneil BD, Singh K. Animal models of motor systems: cautionary tales from studies of head movement. *Prog Brain Res* 123: 411–416, 1999a.

Richmond FJ, Singh K, Corneil BD. Marked non-uniformity of fiber-type composition in the primate suboccipital muscle obliquus capitis inferior. *Exp Brain Res* 125: 14–18, 1999b.

Richmond FJ, Singh K, Corneil BD. Neck muscles in the rhesus monkey. I. Muscle morphometry and histochemistry. *J Neurophysiol* 86: 1717–1728, 2001.

Robinson DA. Eye movements evoked by collicular stimulation in the alert monkey. *Vision Res* 12: 1795–1808, 1972.

Roste GK, Dietrichs E. Demonstration of a sparse direct projection from the interstitial nucleus of Cajal to the cerebellum in the cat. *Arch Ital Biol* 126: 111–118, 1988.

Roucoux A, Guitton D, Crommelinck M. Stimulation of the superior colliculus in the alert cat. II. Eye and head movements evoked when the head is unrestrained. *Exp Brain Res* 39: 75–85, 1980.

Schor RH, Kearney RE, Dieringer N. Reflex stabilization of the head. In: *Control of Head Movement*, edited by Peterson BW, Richmond F. New York: Oxford Univ. Press, 1988, p. 141–166.

Tijssen MA, Marsden JF, Brown P. Frequency analysis of EMG activity in patients with idiopathic torticollis. *Brain* 123: 677–686, 2000.

Tweed D, Cadera W, Vilis T. Computing three-dimensional eye position quaternions and eye velocity from search coil signals. *Vision Res* 30: 97–110, 1990.

Vasilescu C, Dieckmann G. Electromyographic investigations in torticollis. *Appl Neurophysiol* 38: 153–160, 1975.

Vasin N, Medzhidov MR, Shabalov VA. Stereotaxic destruction of the interstitial nucleus of Cajal on spastic torticollis. *Zh Vopr Neirokhir Im N N Burdenko* 3–7, 1985.

Vidal PP, Graf W, Berthoz A. The orientation of the cervical vertebral column in unrestrained awake animals. I. Resting position. *Exp Brain Res* 61: 549–559, 1986.

Wang S, Liu X, Yianni J, Green AL, Joint C, Stein JF, Bain PG, Gregory R, Aziz TZ. Use of surface electromyography to assess and select patients with idiopathic dystonia for bilateral pallidal stimulation. *J Neurosurg* 105: 21–25, 2006.

Zuk A, Rutherford JG, Gwyn DG. Projections from the interstitial nucleus of Cajal to the inferior olive and to the spinal cord in cat: a retrograde fluorescent double-labeling study. *Neurosci Lett* 38: 95–101, 1983.