Horizontal Smooth Pursuit Adaptation in Macaques After Muscimol Inactivation of the Dorsolateral Pontine Nucleus (DLPN)

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Submitted 2 February 2007; accepted in final form 30 August 2007

Ono S, Mustari MJ. Horizontal smooth pursuit adaptation in macaques after muscimol inactivation of the dorsolateral pontine nucleus (DLPN). J Neurophysiol 98: 2918–2932, 2007. First published September 5, 2007; doi:10.1152/jn.00115.2007. The smooth pursuit (SP) system can adapt its response to developmental changes, injury, and behavioral context. Previous lesion and single-unit recording studies show that the macaque cerebellum plays a role in SP initiation, maintenance, and adaptation. The aim of this study was to determine the potential role of the DLPN in SP adaptation. The DLPN receives inputs from the cortical SP system and delivers eye and visual motion information to the dorsal/ventral paraflocculus and vermis of the cerebellum. We studied SP adaptation in two juvenile rhesus monkeys (Macaca mulatta), using double steps of target speed that step-up (10–30°/s) or step-down (25–5°/s). We used microinjection of muscimol (2%; 0.15 μl) to reversibly inactivate the DLPN, unilaterally. After DLPN inactivation, initial ipsilesional SP acceleration (first 100 ms) was significantly reduced by 47–74% (P < 0.01; unpaired t-test) of control values in the single-speed step- ramp paradigm. Similarly, ipsilesional steady-state SP velocity was also reduced by 59–78% (P < 0.01; unpaired t-test) of control values. Contralesional SP was not impaired after DLPN inactivation. Control testing showed significant adaptive changes of initial SP eye acceleration after 100 trials in either step-up or step-down paradigms. After inactivation, during ipsilesional SP, adaptation was impaired in the step-up but not in the step-down paradigm. In contrast, during contralateral tracking, adaptive capability remained in the step-down but not in the step-up paradigm. Therefore SP adaptation could depend, in part, on direction sensitive eye/visual motion information provided by DLPN neurons to cerebellum.

INTRODUCTION

The smooth pursuit system is able to adapt to changes associated with development, aging, or injury (for review see Leigh and Zee 2006). Previous studies have documented adaptive capability in the smooth pursuit system using a double step of target speed in monkey (e.g., Kahlon and Lisberger 1996; Takagi et al. 2000) and human subjects (Fukushima et al. 1996; Ogawa and Fujita 1997). These studies have demonstrated that significant adaptive changes of initial eye acceleration (first 100 ms) occur during a double-step smooth pursuit paradigm where the target begins moving at one speed for the first 100 ms and then changes to either a higher or lower speed. Kahlon and Lisberger (1996) have provided evidence that adaptive changes of initial smooth pursuit during a double-step paradigm are influenced by both retinal image motion and eye motion. In subsequent studies, these authors found that some Purkinje cells in the floccular complex showed changes in simple and complex spike activity during smooth pursuit adaptation (Kahlon and Lisberger 2000). Other regions of the cerebellum also play a role in smooth pursuit adaptation. For example, smooth pursuit adaptation was severely impaired after lesions to the oculomotor vermis (Takagi et al. 2000). The actual source of signals essential for driving pursuit–related adaptation remains to be fully identified.

The dorsolateral pontine nucleus (DLPN) is a major source of visual and oculomotor inputs to the cerebellum (Glickstein et al. 1994; for review see Thier and Möck 2006). The DLPN region of the basilar pons is known to receive cortical inputs from the extrastriate cortex (Distler et al. 2002; Giolli et al. 2001; Glickstein et al. 1980, 1994; May and Andersen 1986; Tusa and Ungerleider 1988), including middle temporal (MT), medial superior temporal (MST), and frontal eye field (FEF) areas, which are specialized for visual motion processing and smooth pursuit (e.g., Keating 1991; Komatsu and Wurtz 1988; Maunsell and Newsome 1987; for review see Lynch and Tian 2006). Slightly different nomenclatures (e.g., Nyby and Jansen 1951; for review see Thier and Möck 2006) have been used to indicate subregions of the basilar pons and the location of the DLPN (see DISCUSSION). The DLPN sends mossy fiber projections primarily to the contralateral ventral paraflocculus and dorsal paraflocculus (Glickstein et al. 1994; Nagao et al. 1997) and also projects to vermal lobules VI and VII (Brodal 1979, 1982; Langer 1985; Thiebert and Thier 1993). Single-unit recording (Mustari et al. 1988; Suzuki and Keller 1984; Suzuki et al. 1990; Thier et al. 1988) and lesion studies (May et al. 1988; Ono et al. 2003) demonstrate that DLPN neurons carry appropriate signals to play a role in the initiation and maintenance of smooth pursuit. Unilateral DLPN inactivation using muscimol produces consistent deficits in the ability to generate and maintain smooth pursuit in the ipsilesional direction. However, DLPN lesions do not completely eliminate smooth pursuit.

Recently, we used multiple-linear regression modeling to demonstrate that a large proportion of DLPN smooth pursuit neurons encode mostly smooth pursuit eye motion with only small contributions from retinal error motion (Ono et al. 2004, 2005). Other DLPN neurons with sensitivity to large-field visual motion also provide visual input to the ventral and dorsal paraflocculus and vermis. However, such large-field visual motion responses are not involved in foveal smooth pursuit or in smooth pursuit adaptation in double-step paradigms, where
target motion is over a dark background. It is unknown whether DLPN neurons, which primarily provide eye motion (velocity) signals to the cerebellum (Mustari et al. 1988; Ono et al. 2004, 2005), could also play a role in smooth pursuit adaptation. In this study, we inactivated the DLPN with muscimol to test its potential role in adaptive changes of horizontal smooth pursuit during double steps of target speed. Our results indicate that the DLPN plays a role in smooth pursuit adaptation but other pathways may also make important contributions.

**Methods**

**Surgical procedures**

A detailed description of our surgical procedures can be found in earlier publications (Mustari et al. 1988, 1997, 2001; Ono et al. 2005). Behavioral and single-unit data were collected from two normal juvenile rhesus monkeys (*Macaca mulatta*), weighing 4–5 kg. Surgical procedures were carried out under aseptic conditions in a dedicated surgical suite using isoflurane anesthesia (1.25–2.5%). Vital signs including blood pressure, heart rate, blood oxygenation, body temperature, and CO2 in expired air were monitored with a SurgiVet instrument (Waukesha, WI). All values were maintained within normal physiological limits. Postsurgical analgesia (buprenorphine, 0.01 mg/kg, q6 h) and antiinflammatory (Banamine, 1.0 mg/kg, q6 h) treatment were used for several days, as indicated. We used stereotaxic methods to implant a titanium head stabilization post and titanium or Cilux recording chambers (Crist Instrument, Hagerstown, MD). In the same surgery a scleral search coil for measuring eye movements (Fuchs and Robinson 1966) was implanted underneath the conjunctiva of one eye using the technique of Judge et al. (1980). DLPN recording chambers were stereotaxically implanted (anterior = 2 mm; lateral = 1 mm; tilted 20° away from midline) and aimed such that a track located in the center of the chamber intersected a point near the oculomotor nucleus. All surgical procedures were performed in strict compliance with National Institutes of Health guidelines and the protocols were reviewed and approved by the Institutional Animal Care and Use Committee at Emory University.

**Localization of DLPN and muscimol injection**

To accurately locate the DLPN region, we first mapped the oculomotor nucleus (OMN) and the anatomical midline, which is defined by finding OMN neurons with rightward or leftward on directions on either side of the midline. Next, we localized the DLPN by its stereotaxic location relative to the oculomotor nucleus and by finding neurons that were modulated for motion of either a large-field (75 × 75°) visual stimulus or during smooth pursuit of a small-diameter (0.2°) target spot moving (± 10°; 0.1–0.75 Hz) over a dark background. We found that most of our DLPN smooth pursuit neurons were located 3–5 mm lateral and 4–6 mm deeper than oculomotor burst tonic neurons. The sites of our smooth pursuit or visual motion–related neurons in DLPN are consistent with those reported in previous single-unit recording studies that included histological verification (Kawano et al. 1992; Mustari et al. 1988; Ono et al. 2004, 2005 Suzuki et al. 1990) (Fig. 1). We used Nissl-stained sections from earlier studies (Ono et al. 2004) to create the representative line drawing in Fig. 1A. We used magnetic resonance imaging (MRI, T1-weighted, fast spin echo; Siemens, 3T magnet) to verify that our injection locations were in the DLPN region (Fig. 1B). The monkeys from the current study are still subjects in other studies, so histological confirmation is not available.

In muscimol injection experiments (Table 1), we used a small-diameter (<50 μm) micropipette whose tip was positioned at the depth where smooth pursuit–related neurons in DLPN were recorded. Injections were delivered using a picoliter pump (WPI-PV830) connected to the micropipette to provide timed pressure pulses, allowing a gradual delivery of muscimol over several minutes (Mustari et al. 2001; Ono et al. 2003). Based on MRI images, our injection sites correspond to those of previous muscimol injection studies (May et al. 1988; Ono et al. 2003), which could involve parts of the lateral dorsal basilar pontine gray immediately adjacent to the DLPN (Dicie et al. 2004).

The efficacy of muscimol injections was confirmed by measuring the gain of horizontal smooth pursuit during sinusoidal and step-ramp tracking (May et al. 1988; Ono et al. 2003). We always used unilateral muscimol injections to inactivate the DLPN (Table 1). Smooth pursuit measurements were taken 15 min after the injection and in some experiments also at the conclusion of gain modification experiments (≥2 h postinjection). We estimate that our injections blocked most of the DLPN based on the volume and concentration of muscimol in our injections compared with those in published studies (Arikan et al. 2002). For example, Arikan and colleagues found that a 1-μl muscimol injection produced a block in neural activity 4 mm in diameter. Based on relative volumes, our injections were substantially smaller (0.2 μl), consistent with a likely effective block of 1- to 2-mm diameter. We confirmed that the muscimol we injected in the DLPN did not spread to distant basilar pontine nuclei ([e.g., nucleus reticularis tegmenti pontis (NRTP) or medial dorsal pontine nucleus (MDPN)]) known to be involved in smooth pursuit. We did this by conducting simultaneous single-unit recording in the NRTP during DLPN muscimol inactivation. We found that NRTP neurons remained active...
2°) reward window around current target position. Motion of the was rewarded with juice for keeping its eye position within a small target at known horizontal and vertical eccentricities. The monkey calibrated by requiring the monkey to fixate a small-diameter (0.2°) stimuli were rear projected onto a tangent screen 57 cm distant. All of were conducted in a sound-attenuated and light-tight room. Visual taxic plane. Behavioral testing and single-unit recording experiments testing after muscimol inactivation of the DLPN as postlesion. Track-Throughout the presentation of our results, we refer to smooth pursuit each monkey, which produced no deficits in smooth pursuit (Table 1). Control studies using physiological saline injections in the DLPN of each monkey, which produced no deficits in smooth pursuit (Table 1). Throughout the presentation of our results, we refer to smooth pursuit testing after muscimol inactivation of the DLPN as postlesion. Tracking toward the side of DLPN inactivation is referred to as ipsilesional.

Behavioral paradigms

During all experiments, monkeys were seated in a primate chair (Crist Instrument) with their head stabilized in the horizontal stereotaxic plane. Behavioral testing and single-unit recording experiments were conducted in a sound-attenuated and light-tight room. Visual stimuli were rear projected onto a tangent screen 57 cm distant. All of our monkeys were extensively trained to perform a fixation task and track a small-diameter (0.2°) target spot moving in sinusoidal or step-ramp trajectories. Eye position data (see following text) were calculated by requiring the monkey to fixate a small-diameter (0.2°) target at known horizontal and vertical eccentricities. The monkey was rewarded with juice for keeping its eye position within a small (±2°) reward window around current target position. Motion of the target spot was produced by a computer-controlled two-axis mirror galvanometer setup (General Scanning, Watertown, MA). We used the initial acceleration and steady-state smooth pursuit velocity during horizontal step-ramp tracking (velocity of 10–40°/s) to characterize the quality of smooth pursuit. The size of the step was adjusted so that smooth pursuit was initiated without initial saccades (Rashbass 1961). Adaptive changes of horizontal smooth pursuit were produced by double steps of target speed that step-up (10–30°/s) or step-down (25–5°/s). In the step-up paradigm, the target begins moving at 10°/s for step-up and 25°/s for step-down paradigm) were used before and after adaptation paradigms. We conducted one set of adaptation trials in a given experimental session. Therefore the animals received at least four adaptation paradigms (step-up rightward, step-up leftward, step-down rightward, step-down leftward) before lesion on different days. On a few days, adaptation and control directions were exchanged with long time intervals (≥30 min) between the first and second sessions as reported in other studies (Kahlon and Lisberger 1996). The data associated with muscimol inactivation studies (postlesion) were collected on different days.

Data collection and analysis

Eye movements were detected using standard electromagnetic methods (Fuchs and Robinson 1966) using precision hardware (CNC Electronics, Seattle, WA). Eye and target position feedback signals were processed with antialiasing filters at 200 Hz using six-pole Bessel filters before digitization at 1 kHz with 16-bit precision using CED-Power1401 hardware (Cambridge Electronic Designs, Cambridge, UK). Velocity data were generated by digital differentiation of the position arrays using a central-difference algorithm in Matlab (The MathWorks, Natick, MA). Saccades were marked with a cursor on eye velocity traces and were removed. After desaccading, the missing eye data were replaced with a linear fit connecting the pre- and postsaccadic regions of data using custom Matlab routines (The MathWorks). Pursuit initiation during step-ramp tracking was taken as the time that average eye speed reached ≥3SD above the pretrial values during fixation. Initial acceleration was calculated as the average eye acceleration in the first 100-ms period of pursuit. Average steady-state velocity was defined as the region where eye velocity reached a plateau, typically taken between 200 and 300 ms after pursuit initiation. At least ten trials of rightward or leftward step-ramp tracking were averaged to quantify initial acceleration and steady-state velocity. We have used the convention of representing rightward eye position as positive values in our plots, unless stated otherwise.

Results

Horizontal smooth pursuit after unilateral DLPN inactivation

After unilateral injection of muscimol in DLPN, we observed consistent deficits in the monkey’s ability to generate and maintain smooth pursuit of a target moving toward the side of injection (ipsilesional). However, monkeys did continue to produce smooth pursuit at lower gains. Unilateral inactivation of the DLPN did not produce deficits in fixation or saccadic tracking (not illustrated). Figure 2 illustrates representative sinusoidal smooth pursuit eye position (Fig. 2A) and velocity (Fig. 2B) in control testing and postlesion of the left DLPN. In postlesion testing, we found that the monkey used a combination of smooth pursuit and catch-up saccades to keep his eyes on the target during leftward (ipsilesional) tracking. In contrast, contralesional (rightward) smooth pursuit was indistinguishable from control testing (Fig. 2A). We measured peak eye

<table>
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<tr>
<th>Experiment Number</th>
<th>Concentration</th>
<th>Volume, ml</th>
<th>Side</th>
<th>Ipsi</th>
<th>Contra</th>
<th>Adaptive Change Postlesion, % [Late – Early]/Early] × 100</th>
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<tr>
<td>W1</td>
<td>5 mg/250 µl (2%)</td>
<td>150</td>
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<td>70.9°**</td>
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<tr>
<td>W2</td>
<td>5 mg/2.5 ml (0.2%)</td>
<td>200</td>
<td>Left</td>
<td>74.1°</td>
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<td>20.3</td>
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<tr>
<td>W3</td>
<td>5 mg/1.25 ml (0.4%)</td>
<td>200</td>
<td>Left</td>
<td>65.0°**</td>
<td>98.6</td>
<td>0.7</td>
</tr>
<tr>
<td>W4</td>
<td>5 mg/1.25 ml (0.4%)</td>
<td>200</td>
<td>Left</td>
<td>60.2°**</td>
<td>88.7</td>
<td>—</td>
</tr>
<tr>
<td>W5</td>
<td>5 mg/1.25 ml (0.4%)</td>
<td>200</td>
<td>Left</td>
<td>46.6°**</td>
<td>95.4</td>
<td>—</td>
</tr>
<tr>
<td>W6</td>
<td>Saline (0.9%)</td>
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<td>Left</td>
<td>97.4</td>
<td>98.1</td>
<td>127.0°**</td>
</tr>
<tr>
<td>H1</td>
<td>5 mg/1.25 ml (0.4%)</td>
<td>200</td>
<td>Right</td>
<td>63.1°</td>
<td>74.9°</td>
<td>1.2</td>
</tr>
<tr>
<td>H2</td>
<td>5 mg/1.25 ml (0.4%)</td>
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<td>Right</td>
<td>71.5°</td>
<td>105.7</td>
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<td>200</td>
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<td>58.6°</td>
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<td>62.7°</td>
<td>85.2</td>
<td>—</td>
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<tr>
<td>H5</td>
<td>Saline (0.9%)</td>
<td>200</td>
<td>Right</td>
<td>107.5</td>
<td>101.2</td>
<td>89.0°**</td>
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Percentage adaptive change was calculated as the ratio of eye acceleration early and late in adaptation. Significance: *P < 0.05, **P < 0.01, unpaired t-test.
velocity for each cycle of ipsi- and contralesional pursuit. Smooth pursuit peak velocity in the ipsilesional direction was significantly reduced compared with control values (13.5 ± 0.53°/s, control; 10.2 ± 0.24°/s, postlesion; P < 0.001, unpaired t-test). There was no significant deficit in eye velocity during contralesional pursuit (13.1 ± 0.57°/s, control; 13.3 ± 0.3°/s, postlesion) (Fig. 2B). We measured the initial acceleration and steady-state smooth pursuit velocity to characterize the quality of ipsilesional smooth pursuit during step-ramp tracking. Figure 3 shows representative eye velocity (top panels) and acceleration (bottom panels) traces for different ramp speeds (10–40°/s) in the ipsilesional direction. After unilateral DLPN inactivation (Fig. 3B), both smooth pursuit velocity and initial acceleration were reduced for each target velocity condition. Mean steady-state eye velocity and initial acceleration (first 100 ms) for two monkeys are shown in Fig. 4. Before inactivation, steady-state eye velocity was close to target velocity (e.g., mean pursuit gain = 0.94 ± 0.08 for monkey 1). After unilateral DLPN inactivation, ipsilesional steady-state velocity was significantly reduced by 59–78% (P < 0.01; unpaired t-test) of control values. Similarly, initial acceleration during ipsilesional tracking was reduced by 47–74% (P < 0.01; unpaired t-test) of control values. Note that even 1–2 h after muscimol injection, there were still similar deficits in eye acceleration and velocity (dotted lines). These findings indicate that pursuit performance after muscimol injection did not progressively decline even after smooth pursuit adaptation testing was complete. For all of our muscimol injections (Table 1), initial ipsilesional smooth pursuit acceleration was significantly reduced, whereas there were no consistent deficits in contralesional tracking (Table 1). The deficits we observed are comparable to those reported in previous DLPN lesion/inactivation studies (May et al. 1988; Ono et al. 2003).

**Smooth pursuit adaptation: step-up paradigm**

We studied the effects of unilateral DLPN inactivation on smooth pursuit adaptation in a double-step paradigm. Figure 5 shows representative eye position traces during single-step control trials (10°/s, ramp speed) and during the step-up trials (10–30°/s) for control and postlesional tracking in the ipsilesional direction. The step-up paradigm is designed to increase initial eye acceleration during smooth pursuit. During early adaptation (first 10 trials) control testing, the monkey used catch-up saccades after the target speed increased to maintain his eye on target (Fig. 5A, second panel). Later in adaptation (last 10 of 100 trials), the monkey followed the target smoothly even after the speed was increased to 30°/s (Fig. 5A, third panel). After adaptation, single-step control trials at 10°/s (Fig. 5A, bottom panel) show that eye position overshoots the target, revealing an adapted smooth pursuit response. In contrast, after DLPN inactivation the monkey showed little evidence of adaptation even after 100 trials (Fig. 5B, third panel). In fact, the monkey still needed a catch-up saccade to maintain his eyes on target after the second step of target speed. During single-step control trials after adaptation testing, eye position did not overshoot the target (Fig. 5B, bottom).

We used eye velocity traces to compare behavioral responses in different smooth pursuit adaptation conditions. Figure 6 shows average eye velocity traces obtained in single-step control trials before and after adaptation (top panels) and for early and late adaptation trials (bottom panels). We found that there were significant adaptive changes during the initial part of smooth pursuit, control testing (Fig. 6A). In contrast, during postlesion testing we did not observe significant differences in eye velocity between early and late adaptation trials (Fig. 6B).

To quantitatively estimate smooth pursuit adaptation, we calculated initial acceleration as the average eye acceleration in the first 100 ms of smooth pursuit. Individual initial eye accelerations during adaptation across 200 trials are plotted in Fig. 7. We used the absolute value of acceleration in these plots to produce positively sloped fit lines to gain increases, regardless of the direction of smooth pursuit. Control testing (Fig. 7A) showed significant adaptive changes of initial eye acceleration after 100 trials (90.8 ± 19.5°/s², first 10 trials; 228 ± 21.3°/s², last 10 trials during adaptation; P < 0.001, unpaired t-test). After DLPN inactivation (Fig. 7B), ipsilesional smooth pursuit adaptation was significantly impaired, as measured after 100 trials (78.2 ± 21.7°/s², first 10 trials; 90.4 ± 20.9°/s², last 10 trials during adaptation; P = 0.22, unpaired t-test). This was the case even though unilateral DLPN inactivation still allowed >150°/s² of ipsilesional initial eye acceleration (Figs. 3 and 4). Note that even 1–2 h after muscimol injection, there were still
similar deficits in eye acceleration and velocity (Fig. 4, dotted lines). In contrast to the impairment in adaptation for ipsilesional tracking, adaptation for contralateral tracking was similar to that found in control testing (e.g., 91.6 ± 16°/s², first 10 trials; 186.7 ± 22°/s², last 10 trials during adaptation; \( P < 0.001 \), unpaired \( t \)-test; see Fig. 11A).

Smooth pursuit adaptation: step-down paradigm

We also examined the effects of unilateral DLPN inactivation on smooth pursuit adaptation in the ipsilesional direction during the step-down paradigm (25°–5°/s). This paradigm is designed to decrease initial eye acceleration during smooth pursuit. The top panel of Fig. 8A illustrates saccade-free smooth pursuit in the single-step control paradigm (25°/s target motion). In the middle two panels smooth pursuit early and late in adaptation are illustrated. In the early trials of control testing, eye position overshoots the target position after the decrease in initial target speed (Fig. 8A, second panel). Late in adaptation, eye position nearly matches target position after the decrease in target speed to 5°/s (Fig. 8A, third panel). We found similar adaptive capability postlesion in the step-down paradigm (Fig. 8B, third panel). This was the case even though pursuit gain was decreased postlesion compared with control testing (Fig. 8B, top). These adaptive changes are best illustrated using average eye velocity traces as described earlier. The top panel of Fig. 9 illustrates control and postlesion testing during single-step control trials before and after adaptation. Eye velocity shows significant adaptation in step-down testing, even though DLPN inactivation alone produced a decreased smooth pursuit gain (Fig. 9B, top). During step-down testing a clear progression in smooth pursuit adaptation occurs from early to late trials both control and postlesion testing (Fig. 9B, bottom).

We calculated trial-by-trial initial acceleration for step-down testing. Individual initial eye acceleration values during the step-down paradigm are plotted as a function of trial number in Fig. 10. Control testing (Fig. 10A) illustrates that significant adaptive changes of initial eye acceleration occur continuously during the 100 trials (153.9 ± 9.3°/s², first 10 trials; 93.7 ± 18.6°/s², last 10 trials during adaptation; \( P < 0.001 \), unpaired \( t \)-test). After DLPN inactivation (Fig. 10B), adaptation was clearly present in the step-down paradigm (106.1 ± 10.4°/s², first 10 trials; 67.2 ± 20.1°/s², last 10 trials during adaptation; \( P < 0.001 \), unpaired \( t \)-test). We also tested smooth pursuit in the contralateral direction. We found no significant adaptive changes of initial eye acceleration during contralateral smooth pursuit after DLPN inactivation (122.8 ± 25.6°/s², first 10 trials; 105 ± 19.4°/s², last 10 trials during adaptation; \( P = 0.10 \), unpaired \( t \)-test, Fig. 11B). This was the case even though there was no significant deficit in smooth pursuit in the contralateral direction during single-step control tracking postinactivation (Table 1).

Comparison of adaptive capability between step-up and step-down paradigms

The results of each adaptation experiment across 100–200 trials for step-up and step-down paradigms in our two monkeys are shown in Fig. 11. Before DLPN inactivation, smooth pursuit adaptation occurred within the first 100 trials in both step-up and step-down paradigms (Fig. 11, filled symbols). After DLPN inactivation, initial eye acceleration during the step-up paradigm did not show adaptive change even after 200 trials for each experiment (Fig. 11A, open symbols). In contrast, step-down trials showed significant adaptive changes of initial eye acceleration, even though the relative gains were lower than control values (Fig. 11B, open symbols). Further
Comparison of eye acceleration early and late in adaptation postlesion showed that there were no significant adaptive changes in the step-up paradigm (Fig. 12A, left column). However, there were significant (P < 0.01; unpaired t-test) adaptive changes in the step-down paradigm (Fig. 12B). We found that relative adaptive changes, defined as the percentage difference between early and late trials in step-down testing, were similar to those observed in control testing (Table 1). In contrast, relative adaptive changes during step-up testing were low. Adaptive capability was defined as the percentage change between control and postlesion values. Adaptive capabilities were significantly (P < 0.001) different between step-up (52 ± 10.8%) and step-down (98.8 ± 8.4%) paradigms, postlesion. This was the case even though postlesion initial eye acceleration had similar values in step-up (68.8 ± 5.9%) and step-down (58.8 ± 13.4%) paradigms for pursuit in the ipsilesional direction. There was no correlation between ipsilesional pursuit capability and adaptive capability postlesion for step-up (r² = 0.44, P = 0.15) and step-down paradigms (r² = 0.01, P = 0.85).

DISCUSSION

The smooth pursuit system has the capability to adapt to changes associated with development, injury, and new behavioral demands (for review see Leigh and Zee 2006). The nature and source of signals that support smooth pursuit adaptation are incompletely understood, but could involve smooth pursuit–related parts of specific cortical, brain stem, and cerebellar regions (for review see Büttner and Büttner-Ennever 2006). We hypothesized that the DLPN region might be a source of signals essential for adaptation because of its well-known role in normal smooth pursuit (Mustari et al. 1988; Ono et al. 2005; Suzuki and Keller 1984; Suzuki et al. 1990; Thier et al. 1988). In all of these published single-unit studies the DLPN was described as a region in the basilar pons located lateral to fascicles of the cerebral peduncle and at the medial aspect of the...
smooth pursuit adaptation during the step-up paradigm. Representative eye and target position traces in the ipsilesional direction are shown before and after adaptation in control testing (A, left column) and postlesion (B, right column). Target began moving leftward at 10°/s for the first 100 ms and stepped 30°/s. From top to bottom, panels show single-step control data before adaptation, double-step data early in adaptation (first 10 trials), late in adaptation (last 10 trials of 100 trials), and single-step control data after adaptation.

FIG. 5. Smooth pursuit adaptation during the step-up paradigm. Representative eye and target position traces in the ipsilesional direction are shown before and after adaptation in control testing (A, left column) and postlesion (B, right column). Target began moving leftward at 10°/s for the first 100 ms and stepped 30°/s. From top to bottom, panels show single-step control data before adaptation, double-step data early in adaptation (first 10 trials), late in adaptation (last 10 trials of 100 trials), and single-step control data after adaptation.

The middle cerebellar peduncle. The basilar pontine gray has been divided into subregions by Nyby and Jansen (1951) and more recently by Dicke and colleagues (2004). These pontine subdivisions provide helpful standardized references but are not tied to known patterns of differentiable cytoarchitecture. Rather, anatomical studies of cortical-pontine and ponto-cerebellar projection patterns show a patchy distribution of label, which may not be limited to one designated nucleus (for review see Thier and Möck 2006). Therefore our muscimol injections are described as targeting the DLPN region as if it were a simply defined nucleus. We placed our muscimol injections in the midst of smooth pursuit–related neurons after we had extensively mapped the region in single-unit recording studies (e.g., Fig. 1). Muscimol injections placed in this manner produce consistent deficits in smooth pursuit. We used unilateral muscimol injections to inactivate the DLPN region. We verified that our injections were effective by showing that smooth pursuit gain and initial acceleration in the ipsilesional direction were significantly impaired, as reported in earlier studies (May et al. 1988; Ono et al. 2003). We then studied the effects of unilateral DLPN inactivation on smooth pursuit adaptation in a double-step paradigm. Before muscimol injection, we found significant adaptive changes of smooth pursuit initial eye acceleration after 100–200 trials in either step-up or step-down paradigms, as previously reported by other investigators (Fukushima et al. 1996; Kahlon and Lisberger 1996, 2000; Ogawa and Fujita 1997; Takagi et al. 2000). After unilateral DLPN inactivation, smooth pursuit adaptation was significantly impaired in the step-up paradigm for ipsilesional tracking and the step-down paradigm for contralesional tracking.

FIG. 5. Smooth pursuit adaptation during the step-up paradigm. Representative eye and target position traces in the ipsilesional direction are shown before and after adaptation in control testing (A, left column) and postlesion (B, right column). Target began moving leftward at 10°/s for the first 100 ms and stepped 30°/s. From top to bottom, panels show single-step control data before adaptation, double-step data early in adaptation (first 10 trials), late in adaptation (last 10 trials of 100 trials), and single-step control data after adaptation.
contrast, adaptive capability remained in the step-down paradigm for ipsilesional tracking and the step-up paradigm for contralesional tracking. Here we discuss the implications of our results for potential mechanisms and pathways that could support smooth pursuit adaptation.

Role of the DLPN in smooth pursuit adaptation

Anatomical studies show that the DLPN is one of the main sources of mossy fiber input to the dorsal/ventral paraflocculus of the cerebellum (Glickstein et al. 1994; Nagao et al. 1997; for review see Thier and Möck 2006). The DLPN also projects to vermal lobules VI and VII (Brodal 1979, 1982; Langer 1985; Thielert and Thier 1993). Both the floccular complex (Kahlon and Lisberger 2000) and oculomotor vermis (Takagi et al. 2000) have been shown to be involved in adaptation of smooth pursuit.

Earlier single-unit recording studies showed that majority of smooth pursuit–related neurons in DLPN continued their discharge when the target was extinguished briefly (100–400 ms) during maintained tracking, indicating that these neurons carry eye motion or extraretinal signals (Mustari et al. 1988; Thier et al. 1988). Furthermore, recent multiple linear-regression modeling studies indicate that the neuronal responses of a large proportion of DLPN smooth pursuit–related neurons were highly dependent on eye position and eye velocity with only...
relatively small contributions from retinal error motion (Ono et al. 2004, 2005). The DLPN does contain neurons with visual motion sensitivity for both small- and large-field visual stimuli (Dicke et al. 2004; Kawano et al. 1992; Mustari et al. 1988; Ono et al. 2004, 2005; Suzuki and Keller 1984; Suzuki et al. 1990; Thier et al. 1988). However, DLPN neurons with large-field–dependent visual responses are not modulated during smooth pursuit tracking of a small foveal target spot moving over a dark background, which constitutes the actual conditions used in double-step smooth pursuit adaptation experiments. During smooth pursuit, DLPN neurons provide eye motion signals (e.g., eye velocity) and foveal/parafoveal visual signals to the contralateral ventral/dorsal paraflocculus and vermis over mossy fiber pathways. The role of the dorsal paraflocculus in smooth pursuit has not been defined.

FIG. 8. Smooth pursuit adaptation during the step-down paradigm. Eye and target position traces for ipsilesional tracking are shown in control (A, left column) and postlesion (B, right column) testing. Target began moving leftward at 25°/s for the first 100 ms and stepped down to 5°/s. From top to bottom, panels show single-step control trials before adaptation, double-step data early in adaptation (first 10 trials), late in adaptation (last 10 trials of 100 trials), and single-step control trials after adaptation.

The source of pursuit-related signals in the DLPN is known to be derived, in part, from the cortical smooth pursuit system, which includes middle temporal (MT), medial superior temporal (MST), and frontal eye field (FEF) and supplementary eye field (SEF) regions of cortex (Distler et al. 2001; Giolli et al. 2001; Glickstein et al. 1985; for review see Möck and Thier 2006). Early studies showed that MT neurons with foveal/parafoveal visual receptive fields were modulated during smooth pursuit (Komatsu and Wurtz 1988; Newsome et al. 1988). The response of these MT neurons was shown to be visually contingent because neuronal response dropped when retinal image motion was reduced by target stabilization or blinking during pursuit. Later studies showed that MT neurons had appropriate responses to support pursuit dynamics including eye acceleration during step-ramp tracking. However, MT
neurons evinced little visual motion acceleration sensitivity per se (Lisberger and Movshon 1999; Price et al. 2005). Neurons in neighboring area MST were also modulated during smooth pursuit and were shown to carry visual motion and extraretinal signals. Extraretinal signals are revealed as continued neuronal response during pursuit when the target was briefly extinguished (Newsome et al. 1988) or when tracking an imaginary target (Ilg and Thier 2003). Extraretinal signals were also demonstrated in the dorsal part of MST (MSTd) neurons of macaques performing smooth pursuit in the frontal plane or in depth (Akao et al. 2005). It still remains to be determined what information is represented in the extraretinal signals of MST neurons. We recently provided evidence that MSTd neurons carry extraretinal signals related to volitional smooth pursuit but not to comparable smooth eye movements driven by vestibular ocular reflex pathways (Ono and Mustari 2006). It remains uncertain whether MST extraretinal signals play a role in smooth pursuit adaptation.

The DLPN and other regions of the basilar pontine gray also receive inputs from the FEF and SEF regions (for review see Möck and Thier 2006). Smooth pursuit–related responses of neurons in the FEF are related to initiation and maintenance of smooth pursuit (Gottleib et al. 1994; for review see Fukushima 2003). Pursuit-related responses of neurons in the SEF have been shown to play a role in predictive aspects of smooth pursuit and other cognitive components of tracking (Missal and Heinen 2004). Therefore the DLPN receives projections from cortical pursuit regions that carry signals related to the metrics of smooth pursuit including position, velocity, acceleration, and gain control. In addition, other signals related to cognitive components of volitional smooth pursuit behavior (e.g., prediction, attention, and target selection) could reach the basilar

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**FIG. 9.** Mean eye velocity for the step-down adaptation paradigm in control (A, left column) and postlesion (B, right column) testing for a representative experiment (H3, Table 1). Top: traces from control trials before (solid lines) and after adaptation (dashed lines). Bottom: traces from early (first 10 trials; solid lines) and late (last 10 trials of 100 trials; dashed lines) in adaptation. Eye velocity traces were desaccaded. Dotted lines indicate target velocities. Eye velocity showed significant adaptive change postlesion in the step-down paradigm.

**FIG. 10.** Average eye acceleration during ipsilesional tracking for the first 100 ms of tracking shown as a function of trial number in control (A) and postlesion (B) testing for a representative experiment (H3, Table 1). Single-step control trials (25°/s) are shown before and after adaptation. Eye acceleration showed significant adaptive change postlesion even though smooth pursuit had relatively lower gain than in control testing.
pontine nuclei. Whether such top-down signals play a role in smooth pursuit adaptation in the double-step paradigms used here is unknown. All of these results are consistent with the suggestion that direction-selective visual and eye motion signals traveling in cortical-DLPN-cerebellar pathways carry appropriate information to play complementary roles in smooth pursuit and pursuit adaptation. The cortical pursuit system also projects to the rostral NRTP (rNRTP), which is known to play a role in smooth pursuit initiation, maintenance, and gaze pursuit (Ono et al. 2004, 2005; Suzuki et al. 2003). The NRTP projects to pursuit-related regions of the cerebellum including the oculomotor vermis, which has been shown to play an essential role in smooth pursuit adaptation (see following text). Both DLPN and rNRTP could provide eye and visual motion information to regions of the cerebellum (e.g., ventral paraflocculus and vermis) known to play a role in visually guided oculomotor learning. Specific roles of NRTP and other basilar pontine nuclei (e.g., MDPN) in pursuit adaptation have not been tested.

Whether smooth pursuit adaptation during step-up or step-down paradigms depends on eye motion information provided, at least in part, by DLPN neurons that project to ventral paraflocculus and vermis is uncertain. We found a significant deficit in the step-up but not in the step-down paradigm for ipsilesional smooth pursuit. The opposite effects were found during contralesional smooth pursuit testing. Because unilateral DLPN lesions produce asymmetric deficits in smooth pursuit initiation and maintenance, it seems likely that differential effects on smooth adaptation revealed in step-up and step-down paradigms are due, in part, to asymmetry of smooth pursuit capability for ipsi- and contralesional pursuit. Therefore we would expect that bilateral DLPN lesions might produce symmetric deficits in pursuit adaptation during both step-up and step-down paradigms.

Recent lesion studies have demonstrated that the cerebellar vermis plays an essential role in smooth pursuit adaptation in a double-step paradigm (Takagi et al. 2000). In those studies bilateral vermal lesions resulted in deficits in smooth pursuit initial acceleration and smooth pursuit adaptation in both step-up and step-down paradigms. The symmetry of the reported deficits in step-up and step-down paradigms may have depended on the bilaterality of the vermal lesions. This is because unilateral lesions of the caudal fastigial nucleus, which provides the cerebellar output from the oculomotor vermis, produce asymmetric smooth pursuit deficits (Robinson et al. 1997). These results, along with our current findings, support the suggestion that unilateral vermal lesions would produce

FIG. 11. Average eye acceleration during the first 100 ms of tracking for 10 trials shown at 50-trial intervals for both monkeys. Control testing and postlesion results for step-up (A) and step-down (B) adaptation paradigms are shown. Filled circles and triangles indicate control testing and saline injection results, respectively. Open circles indicate postlesion results for the ipsilesional direction. Open circles, triangles, and squares indicate different adaptation sessions during muscimol experiments. Gray circles indicate postlesion results for contralesional (control) direction.
asymmetric deficits in pursuit adaptation during step-up and step-down paradigms.

Because each DLPN provides signals mostly to the contralateral ventral paraflocculus and vermis, unilateral DLPN inactivation may have compromised the activity of smooth pursuit–related circuits on one side of the cerebellum. Recent single-unit recording studies in the ventral paraflocculus (Kahlon and Lisberger 2000) and frontal pursuit area (Chou and Lisberger 2004) provided evidence to indicate that smooth pursuit adaptation occurs downstream from cortex, possibly within the cerebellum. Therefore smooth pursuit adaptation could depend, in part, on eye/visual motion information provided by DLPN neurons to the cerebellum.

Alternative hypothesis of adaptation mechanisms for step-up and step-down paradigms

One of the main differences between the step-up and step-down paradigms is the direction of retinal error motion (calculated as the difference between target and eye motion). For example, during the step-up paradigm (Fig. 6, bottom panels), eye speed is lower than the second target speed, early in adaptation. This relatively low-gain tracking is associated with retinal error motion in the same direction as smooth pursuit. In contrast, during the step-down paradigm (Fig. 9, bottom panels), eye position overshoots the target during the second target speed, producing retinal image motion in the opposite direction to smooth pursuit. Because each DLPN carries signals representing all directions of smooth pursuit and visual motion (Kawano et al. 1992; Mustari et al. 1988; Suzuki and Keller 1984; Suzuki et al. 1990; Thier et al. 1988), the asymmetrical effects of unilateral DLPN inactivation on adaptation in step-up and step-down paradigms may be due to different sources of retinal error signals in basilar pontine or other brain regions that carry direction-selective visual information. Recent studies that coupled electrical stimulation of MT with smooth pursuit indicate that MT could provide signals appropriate for guiding smooth pursuit adaptation (Carey et al. 2005). MT could affect smooth pursuit adaptation through connections with other cortical and brain stem centers (for review see Lynch and Tian 2006). Anatomical and functional connectivity studies have demonstrated that MT sends strong inputs to the DLPN and pretectal nucleus of the optic tract (NOT) (e.g., Distler et al. 2001; Hoffmann et al. 1992; for review see Gamlin 2006).

Kahlon and Lisberger (2000) described that, at least some, ventral parafloccular Purkinje cells showed changes in simple-spike and complex-spike firing during smooth pursuit adaptation in the double-step paradigm. They found that visual complex spikes had appropriate responses to guide motor

Fig. 12. Adaptive capability in different double-step paradigms plotted as bar graphs that show the mean (SD) of initial eye acceleration in step-up (left column) and step-down (right column) paradigms for ipsilesional pursuit. Top panels: eye acceleration early and late in adaptation, control, and postlesion. Bottom panels: percentage of adaptive change. Asterisks indicate statistically significant differences (P < 0.01; unpaired t-test).
learning in the step-down but not in the step-up paradigms. Complex spikes in the floccular complex are known to have contraversive direction selectivity (Stone and Lisberger 1990). For example, during rightward pursuit in the step-down condition, the direction of retinal error is leftward. The left inferior olive provides complex spikes, signaling leftward visual error. This leftward information is provided, in large part, by the left NOT, which is known to project heavily to the left inferior olive (Büttner-Ennever et al. 1996; Mustari et al. 1994; Nagao et al. 1997; for review see Giolli et al. 2006). These findings suggest that direction selective visual complex spikes provided by the contralateral inferior olive are important for smooth pursuit adaptation. Kahlon and Lisberger (2000) also suggested that eye motion signals in mossy fiber pathways that drive simple spikes could play a role in motor learning in step-up paradigms. Therefore it seems likely that interaction of simple and complex spikes might be necessary for step-up and step-down adaptation for both pursuit directions. The main sources of mossy fiber and visual climbing fibers reaching the ventral paraflocculus are DLPN and NOT (e.g., Büttner-Ennever et al. 1996; Mustari et al. 1994; Nagao et al. 1997; for review see Giolli et al. 2006), respectively. Interactions between DLPN and NOT-derived signals may contribute to pursuit adaptation.

According to current theories of cerebellar learning, simple spike activity derived from mossy fiber input provides signals for moment-by-moment control of motor output (e.g., smooth pursuit), whereas complex spike activity from climbing fibers provides visual error signals that guide motor learning in the cerebellar cortex (e.g., Albus 1971; Ito 1972; Marr 1969; Raymond and Lisberger 1998). If smooth pursuit adaptation is supported, at least in part, by interactions between simple and complex spikes, inputs from both DLPN and NOT might be necessary for adaptation. Therefore lack of DLPN inputs might break down the interaction between the activity of simple and complex spikes in ventral paraflocculus contralateral to the inactivated DLPN. To test this possibility further, single-unit recording, electrical stimulation, and inactivation studies involving the NOT would be necessary.

Differential role of the DLPN in smooth pursuit and VOR adaptation

Smooth pursuit and vestibuloocular reflex (VOR) adaptation may be supported by similar neural mechanisms involving interactions of climbing fiber– and mossy fiber–driven activity in Purkinje cells located in different regions of the cerebellum. We recently reported that the DLPN inactivation does not interfere with short-term adaptation of VOR as tested in a visual-vestibular mismatch paradigm (Ono et al. 2003). In contrast, our current study shows that smooth pursuit adaptation in a double-step paradigm was significantly impaired after DLPN inactivation. The pathways involved in visual-vestibular plasticity and adaptation of smooth pursuit may be quite different. Because the VOR is a reflex-driven eye movement, volitional eye motion signals carried in DLPN neurons may not be necessary for plasticity of the VOR. Rather, interactions between visual climbing fiber–derived complex spikes and vestibular mossy fiber–derived simple spikes are thought to be most important (e.g., Raymond and Lisberger 1998) for VOR plasticity, especially for higher frequencies of movement. In contrast, during smooth pursuit adaptation, interactions be-


J Neurophysiol • VOL 98 • NOVEMBER 2007 • www.jn.org

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