Smooth-Pursuit Eye-Movement Deficits With Chemical Lesions in Macaque Nucleus Reticularis Tegmenti Pontis

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Suzuki, David A., Tetsuto Yamada, Rebecca Hoedema, and Robert D. Yee. Smooth-pursuit eye-movement deficits with chemical lesions in Macaque nucleus reticularis tegmenti pontis. J. Neurophysiol. 82: 1178–1186, 1999. Anatomic and neuronal recordings suggest that the nucleus reticularis tegmenti pontis (NRTP) of macaques may be a major pontine component of a cortico-ponto-cerebellar pathway that subserves the control of smooth-pursuit eye movements. The existence of such a pathway was implicated by the lack of permanent pursuit impairment after bilateral lesions in the dorsolateral pontine nucleus. To provide more direct evidence that NRTP is involved with regulating smooth-pursuit eye movements, chemical lesions were made in macaque NRTP by injecting either lidocaine or ibotenic acid. Injection sites first were identified by the recording of smooth-pursuit-related modulations in neuronal activity. The resulting lesions caused significant deficits in both the maintenance and the initiation of smooth-pursuit eye movements. After lesion formation, the gain of constant-velocity, maintained smooth-pursuit eye movements decreased, on the average, by 44%. Recovery of the ability to maintain smooth-pursuit eye movements occurred over ~3 days when maintained pursuit gains attained normal values. The step-ramp, “Rashbass” task was used to investigate the effects of the lesions on the initiation of smooth-pursuit eye movements. Eye accelerations averaged over the initial 80 ms of pursuit initiation were determined and found to be decremented, on the average, by 48% after the administration of ibotenic acid. Impairments in the initiation and maintenance of smooth-pursuit eye movements were directional in nature. Upward pursuit seemed to be the most vulnerable and was impaired in all cases independent of lesioning agent and type of pursuit investigated. Downward smooth pursuit seemed more resistant to the effects of chemical lesions in NRTP. Impairments in horizontal tracking were observed with examples of deficits in ipsilaterally and contralaterally directed pursuit. The results provide behavioral support for the physiologically based and anatomic conclusion that NRTP is a component of a cortico-ponto-cerebellar circuit that presumably involves the pursuit area of the frontal eye field (FEF) and projects to ocular motor-related areas of the cerebellum. This FEF-NRTP-cerebellum path would be involved in regulating smooth-pursuit eye movements. The results implicate rNRTP in both the initiation and the maintenance of smooth-pursuit eye movements.

INTRODUCTION

The regulation of smooth-pursuit eye movements involves cortico-ponto-cerebellar circuits, the nature of which has become clearer during the past several years. Two precerebellar pontine structures of import are the dorsolateral pontine nucleus (DLPN) and the nucleus reticularis tegmenti pontis (NRTP). Physiological and anatomic results indicate that DLPN is a major processor of visual motion and pursuit-related signals presumably from the middle temporal and superior temporal areas of the cerebral cortex (Mustari et al. 1988; Suzuki and Keller 1984; Suzuki et al. 1990a; Thier et al. 1988). Unilateral, chemical lesions made in DLPN resulted in significant deficits in both the initiation and the maintenance of smooth-pursuit eye movements (May et al. 1988). However, bilateral lesions to DLPN, while creating significant deficits in tracking performance, did not permanently impair the smooth-pursuit eye-movement system (May and Keller 1988). The lack of long-lasting effects may have been due to a parallel cortico-ponto-cerebellar pathway that complements the route involving DLPN. Because the frontal eye field contains pursuit-related cells (Gottlieb et al. 1993, 1994; MacAvoy et al. 1991) and is known to project to NRTP (Huerta et al. 1986; Künzle and Akert 1977; Leichnetz et al. 1984; Stanton et al. 1988), it has been hypothesized that such a parallel smooth-pursuit pathway could be composed of projections from the pursuit cells of the frontal eye field to NRTP (Suzuki et al. 1990b). Completion of the cortico-ponto-cerebellar pathway would involve the NRTP projections to vermal lobules-VI, VII, the floculus, and the fastigial nucleus (Brodal 1980, 1982; Thier and Thier 1993; Yamada and Noda 1987), cerebellar structures that are involved with smooth-pursuit eye movements (e.g., Fuchs et al. 1994; Lisberger and Fuchs 1978; Miles and Fuller 1975; Noda and Suzuki 1979; Suzuki and Keller 1988a,b). Recent findings support the notion that NRTP and specifically the rostral portion of NRTP (rNRTP) is involved with smooth-pursuit eye movement control. Single-cell activity that is modulated during smooth-pursuit eye movements has been observed (Suzuki et al. 1991) and slow, pursuit-like eye movements have been evoked with microstimulation of rNRTP (Yamada et al. 1996). This study was initiated to provide behavioral support for the physiologically based conclusion that rNRTP is involved with the regulation of smooth-pursuit eye movements. Pharmacological lesions in rNRTP consistently resulted in significantly impaired smooth-pursuit eye movements. The results implicate rNRTP involvement in both the initiation and the maintenance of smooth-pursuit eye movements.

A preliminary report of these results has appeared in abstract form (Suzuki et al. 1990b).

METHODS

Preparation

Three monkeys (1 Macaca fascicularis and 2 M. nemestrina) were prepared with scleral eye coils and head implant hardware under aseptic conditions and anesthesia. Pentobarbital sodium anesthesia
was used for the *M. fasciculans* and isoflurane gas anesthesia for the two *M. nemestrina*. The eye coils (Teflon-covered stainless steel wire) were implanted surgically under the conjunctiva at the junctions of the eye muscle insertions of the right eye (Judge et al. 1980). A stainless steel chamber (17 mm diam), centered at stereotaxic anterior 6.5 mm with a dorsolateral-to-ventromedial 20° off vertical angle in the coro-
nal plane, was attached to the skull with dental acrylic cement. To immobilize the head relative to the primate chair, two small-diameter transverse, stainless steel cylinders also were attached to the skull with
dental acrylic cement. All sutured incisions were treated with antibi-
otic ointments. During the postsurgical recovery period, buprenex was
administered for analgesia and penicillin or cefazolin was adminis-
trated for prophylaxis.

The monkeys were trained, before surgery, to enter a primate chair from their home cages. After recovery from the surgical procedure, the monkeys voluntarily entered the primate chair and were placed in the
experimental setup on a daily basis. The monkeys were returned to
their home cages for the remainder of the day after each daily
recording session. The cages were provided with swings, “toys,” and
a foraging board. To ensure the good health of the monkeys, param-
eters that included weight, liquid and food intake, agility, and general
level of activity were monitored daily. Periodic physical examinations
were conducted by the veterinarian staff of the animal facilities. The experimental and surgical protocols were approved by the Indiana
University School of Medicine Animal Care and Use Committee and
complied with U. S. Public Health Service policy concerning the care
and use of laboratory animals.

**Recording and delivery of pharmacological agents**

For recording, tungsten microelectrodes insulated with Isolated-31
were lowered within and together with a 23-gauge stainless steel
guide tube to a point 10 mm dorsal to the oculomotor (IIId) nucleus.
The microelectrode was hydraulically driven out of the guide tube by a
stepper-motor, microdrive system (Kopf 650). The location of the
IIId nucleus first was determined to establish a stereotaxic reference
structure that aided the subsequent localization of NRTP. Tracks
aimed at NRTP-traversed paths that were lateral and ventral to the
oculomotor nucleus.

Before delivery of lidocaine or ibotenic acid, NRTP sites were
identified by the presence of modulations in neuronal discharge rate
that were correlated with smooth-pursuit eye movements. The micro-
electrode then was withdrawn and replaced with an injection cannula
(28-gauge stainless steel hypodermic tubing), which was positioned
with the microdrive system. To avoid effects on structures ventral to
NRTP and to minimize damage to fibers of passage, the cannula tip
was driven to a depth just at the dorsal edge of the region where the
smooth-pursuit-related unit responses previously were observed. The
cannula was connected to a 10-µl syringe (Hamilton) with the syringe,
connecting tubing, and cannula previously filled with lidocaine or
ibotenic acid. Sterile, 4% lidocaine hydrochloride (5 µl) was em-
ployed to induce reversible functional lesions (May et al. 1988). The
lidocaine was infused at a rate of 0.5 µl/min.

The ibotenic acid (Sigma Chemical) was dissolved in 0.1 M sodium
phosphate buffer to yield a final concentration of 15 µg/µl. To
facilitate the dissolving of the ibotenic acid, small drops of 1. O N
sodium hydroxide were added to make the solution slightly basic.
Approximately 1.2 µl of ibotenic acid were slowly infused during 30
min. The injection cannula was left in place for an additional 20 min
before it was slowly withdrawn and the animal returned to its cage.
Eye movement recording sessions began 18–20 h later. No side
effects e.g., poor balance, paresis or paresthesia of a limb, were
observed.

**Paradigms**

The monkeys were trained with standard conditioning techniques to
fixate and track a 0.25°, back-projected, laser-generated red light spot
that was moved with an X,Y-mirror galvanometer system. The 90 × 90°
tangent screen was located ~60 cm in front of the monkeys in a
dark room. An Apple Macintosh IIIfx computer (programmed with
LabView) controlled visual target presentation, monitored behavior,
and rewarded correct task performance. Liquid intake was controlled
for 5 days each week and freely available on weekends. During the
experimental sessions, the extent of liquid intake was self-determined
by the monkeys because they received performance-dependent liquid
rewards until they were satiated. Reward delivery was contingent on
eye position being within a small electronic “window” surrounding
the position of the red fixation spot. Window size was dependent on
target speed and was usually set for 2–4°. After chemical lesions were
made, larger spatial window sizes were employed to accommodate
poor eye movement performance. Window size was varied with the
degree of task difficulty and the magnitude of the pursuit deficit. It
was set large enough not to frustrate a motivated animal that had
difficulty initiating or maintaining pursuit and to a size small enough
to maintain motivated task performance. The lesion-induced changes
in pursuit gain were not thought to be due to changes in window size
because the deficits were often direction specific. Eye position was
determined with the magnetic-field search-coil method (Robinson
1963).

Monkeys were required to both initiate and maintain smooth-
pursuit eye movements. For pursuit initiation, a “step-ramp” or Rash-
bass (Rashbass 1961)-type task was employed, whereby the target first
stepped in the direction opposite to the direction of subsequent con-
stant velocity target movement. This minimized the occurrence of
corrective saccades during the initiation period. Maintained smooth-
pursuit eye movements were studied by requiring the monkeys to
track periodic target movements of 10–20° amplitudes and 0.2- to
1.0-Hz frequencies. Target motions were usually constant velocity but
sometimes sinusoidal.

**Recording and data analysis**

Analogue signals were filtered at 202 Hz (Frequency Devices,
8-pole Bessel) and sampled at 1 kHz per channel. Horizontal and
vertical eye and target positions were stored on a magneto-optical disk
(Pinnacle Micro REO-650) for later off-line analysis. Data was ana-
lyzed with a 486 microcomputer employing interactive, analytic soft-
ware that was developed with ASYST (Keithley). For eye velocity
traces such as those in Fig. 5, eye position signals were differentiated
with an ASYST supplied algorithm that interpolated a second-degree
polynomial through consecutive (eye position) data points and then
differentiated the polynomial (Blahut 1984). The resulting eye ve-
locity signal was smoothed with an ASYST algorithm that employed
a Blackman window (Blackman and Tukey 1958). The smoothing
resulted in an 11-ms advance in the velocity signal relative to the eye
position signal. This did not affect determination of eye speeds, which
were quantified in a different manner described in the following text.

The vector term “velocity” was used when referring to differenti-
te eye position traces, whereas eye “speed” was specifically applied
to the measured eye velocity amplitudes. Eye speed was determined
by employing an interactive program that allowed the user to select
segments of the constant velocity smooth-pursuit eye position signal
for analysis. These pursuit segments were selected from instances
when a monkey appeared to be exerting its best effort. The results,
therefore represent a conservative quantification of the behavioral
deficits. The duration of the selected segments were ≥100 ms and
routinely >200 ms. Eye position values within a selected segment
were fitted with a linear regression line and the slope taken as eye
speed. At least 5 and typically >10 pursuit segments were averaged
to obtain a time-weighted, average eye speed for specific pursuit
parameter settings.

Eye acceleration was calculated by first differentiating the eye
position signals. From the display of the eye velocity trace, the
operator marked the beginning of the smooth-pursuit eye movement,
and a linear regression line then was fitted through the eye velocity points during the 80 ms after the designated onset time of pursuit. The slope of the regression line was taken as the average eye acceleration during the 80 ms. This duration corresponds to the open-loop portion of pursuit initiation that has been well studied by Lisberger and his colleagues (e.g., Lisberger and Westbrook 1985). At least 5 and typically ≥10 episodes of pursuit initiation per parameter were analyzed and the results averaged. The effects of the lesions on corrective saccade metrics and predictive pursuit were not investigated.

**Histology**

The monkeys were anesthetized deeply and perfused rapidly through the left ventricle with 1 l of buffered isotonic saline followed by a rapid perfusion of 2 l then slow perfusion of two additional liters of buffered 10% formalin. The brains were blocked, frozen, and cut in the coronal plane in 40-μm sections. The resulting sections were stained with cresyl violet. Ibotenic acid lesion sites were identified by a decrease in the number of stained neuronal cells and an increase in number of glial cells.

**RESULTS**

The effects on smooth-pursuit eye movements of lidocaine and ibotenic acid were studied in three monkeys. In one monkey, only lidocaine was administered to study the effects of transient functional lesions. In the following text, this site is referred to as site 1. In a second monkey, both lidocaine (site 2L) and ibotenic acid (site 2) injections were made. The ibotenic acid lesion was made 4 days after the lidocaine injection at the same NRTP site. The third monkey received ibotenic acid injected at two different NRTP locations (sites 3 and 4). The two ibotenic acid injections were made 5 days apart. Injection site 4 was 0.8 mm anterior to site 3 and in the same medial-lateral location.

**Location of lesions**

Histological examination indicated that ibotenic acid site 2 was in the right rNRTP and sites 3 and 4 were in the left rNRTP (Fig. 1). There was some uncertainty as to the exact borders of the lesions, which were not as clear as the edges of the hatched areas in Fig. 1 would seem to imply. However, the center of all three lesion sites were located within the rostral end of NRTP, anterior to ≥2 mm of normal-appearing portions of NRTP. Because the ibotenic acid injections were made just dorsal to observed pursuit-related unit activity, the core of the lesions were within NRTP. To a limited degree, the edges of the affected region may have intruded ventrally into the dorsal and dorsomedial pontine nuclei. Results obtained for site 4 (Fig. 1, bottom), which was centered on the dorsal aspect of rNRTP and seemed to have excluded involvement of neighboring pontine nuclei, appeared similar to those obtained from sites 2 and 3. This would support the notion that the observed results can be attributed primarily to lesions of rNRTP. Although it was impossible to reconstruct the extent of the functional lesion created by lidocaine, both right and left rNRTP were probably affected. This presumption is based on estimations of injection site location and volume of lidocaine injected.

**Effects of NRTP lesions on maintained smooth-pursuit eye movements**

Transient functional lesions created by the injection of lidocaine into rNRTP resulted in clear deficits in the ability to maintain smooth-pursuit eye movements as shown in Fig. 2.
Normally, a monkey can maintain a fairly high gain for pursuit over the 13–38°/s target velocity range associated with the pursuit epochs in Fig. 2, left. When 5 μl lidocaine was infused into NRTP, the ability to maintain smooth-pursuit eye movements was significantly degraded as exemplified by the pursuit epochs in Fig. 2, right. In this case, upward smooth pursuit was severely affected, though downward pursuit gain remained fairly high. To be sure, there were instances where downward pursuit was less than optimal, but these instances might have been due to the influence of the large retinal errors that existed at the turnabout points after the poor upward pursuit. In contrast, the first and third half-cycles of downward pursuit at 0.4 Hz appeared to be close to normal, suggesting that the ability to maintain downward smooth-pursuit eye movements remained relatively unimpaired by the lesion.

For the same lidocaine session represented by the results in Fig. 2, both ipsilaterally (rightward) and contralaterally (leftward) directed maintained pursuit also were impaired. The deficit for contralaterally directed pursuit was greater than for maintained pursuit in the ipsilateral direction (Fig. 8A). With respect to these lidocaine results, the terms “ipsilateral” and “contralateral” must be viewed with caution. Although the injection site was known to be in the right NRTP, the exact borders of the lidocaine induced lesion were not known.

To quantify the effects of NRTP lesions on maintained smooth-pursuit eye movements, pursuit gains were calculated as the ratio of pursuit eye speed to target speed and plotted as a function of target speed as shown in Fig. 3. Control eye movements were elicited without the placement of the injection cannula. The pursuit epochs shown in Fig. 2 were included in the analysis for Fig. 3A. In addition, gain values were determined after the injection of saline into NRTP and are shown in Fig. 3, A and B. For a similar volume (5 μl) of saline injected into about the same site as the lidocaine injection, no significant differences in maintained pursuit were observed between the cannula-less control conditions (○) and following the saline injection (□). This indicates that the lidocaine-induced pursuit deficits were not due to nonspecific mechanical stress effects on pontine structures nor to potential tissue damage that may have resulted from the injection of the volumes employed. The results can more specifically be attributed to an effect on a smooth-pursuit-related component structure. Furthermore effects on pursuit of the injection cannula also could be ruled out, indicating that data obtained in the absence of the injection cannula could be used for controls. As noted above, but not shown in Fig. 2, maintained pursuit in the ipsilateral and contralateral directions also were impaired by lidocaine. The magnitudes of these impairments are shown in Fig. 3B. Standard errors for most points in Fig. 3, A–D, were within the vertical dimensions of the symbols used in the graphs and were omitted for clarity.

Deficits in maintained smooth-pursuit eye movements also were observed after the administration of ibotenic acid, a compound that specifically destroys cell somata while sparing fibers passing through the region (Schwarcz et al. 1979). Because lidocaine affects both cell bodies and fibers passing through the region, the use of ibotenic acid was necessitated to more definitively argue that the observed impairment of maintained pursuit was specifically due to lesioning of cells in rNRTP. As shown in Fig. 3C, an ibotenic-acid-induced rNRTP lesion did result in deficits in maintained smooth-pursuit eye movements. For this site, upward maintained smooth-pursuit was significantly impaired (●). Downward, ipsilateral, and contralateral maintained pursuit appeared normal (not shown). The normality of maintained smooth-pursuit eye movements in directions other than up, indicates that the deficits in upward

![FIG. 2. Effects of lidocaine on maintained smooth-pursuit eye movements. Monkey was required to track a target moving at constant velocities associated with 16° amplitude oscillations at 3 frequencies. Control behavior is represented by the 3 eye-position traces (left). Examples of smooth-pursuit eye movements elicited after the injection of lidocaine are shown on the right. *, blinks riding on the vertical eye position signal; 20° amplitude scale applies to all panels. Applicable time scales shown beneath each panel. Site 1.](image)
tracking were specific and not due to any global effects on the motor system or pontine functions.

Another indication of the ability of the pursuit system to match eye to target velocity is the plateau in eye velocity observed during responses to step-ramp target motion (e.g., Fig. 5). For comparison, pursuit gains calculated from such plateau values of eye velocity (divided by target velocity) are presented in Fig. 3D. For site 4, comparable decrements in pursuit gains were observed for the eye velocity-plateau of pursuit initiation (●) and for pursuit maintained for periodic target motion (□). When there were difficulties in initiating pursuit, i.e., when the initial eye acceleration was less than normal, there were always decrements in the amplitude of the eye velocity plateau. However, problems with initiating pursuit could affect the initial velocity plateau without affecting subsequent maintained pursuit after the first cycle (cf. Fig. 8, B and E). Thus periodic target motion was used to characterize the ability to maintain pursuit rather than step-ramp target motion and the associated eye velocity plateaus observed during pursuit initiation.

To obtain a representative indicator for the decrement in maintained smooth-pursuit eye movements, gains for maintained pursuit elicited to periodic, constant velocity target motion were calculated and the results presented in Fig. 4. Decrements in maintained pursuit gains were evident for all lesion sites and ranged from 38% to 53% for the direction of pursuit that was most affected at each site. The average reduction in maintained smooth-pursuit gain following a rNRTP lesion was 44%. The results for site 3 were obtained from only two cycles of pursuit for a target velocity of 16°/s because the monkey had difficulty maintaining pursuit at this target velocity. This monkey could not maintain pursuit at higher target velocities. Thus the average 44% gain reduction was a conservative minimum value reflecting the process of selecting for the best pursuit responses. The average gain reduction would have been even greater if site 3 results obtained for target velocities >16°/s were included.

In Fig. 4, the control gains for sites 1, 3, and 4 were <0.9. With horizontal sinusoidal tracking, gains of >0.9 are typical. The slightly lower control gains observed for these sites may have been due to using constant velocity tracking and the fact that these gains were for upward tracking, which was usually not as good as horizontal tracking. This latter explanation may be the most valid because the data for site 2 were for contralateral, i.e., horizontal maintained smooth pursuit and yielded an average control gain of 0.95.

FIG. 3. Effects of lidocaine- and ibotenic-acid-induced lesions in NRTP on the gain of maintained smooth-pursuit eye movements. A: effects of lidocaine on the gains for up maintained smooth-pursuit eye movements over a range of target speeds. ○, control gains obtained in the absence of the injection cannula; ●, gains after saline injection. ◐, gains with lidocaine administration. Site 1. B: effects of lidocaine on the gains for horizontal maintained smooth-pursuit eye movements. ○ and ●, pursuit gains after saline injection. Corresponding ◐ and ◔ gains with lidocaine administration. Site 1. C: effects of an ibotenic acid lesion on the gains for up maintained pursuit. ○, control gains. ●, maintained pursuit gains after ibotenic acid administration. Site 4. D: ibotenic acid lesion effects on the gains, calculated as the plateau eye velocity divided by target velocity, for up pursuit to step-ramp target motion. Results from C are plotted with ... for comparison. ◐, ○, ●, and ◔, control gains. Corresponding ◔, ●, and ◐ gains for 3 ibotenic acid lesion sites.

FIG. 4. Average deficits in gains of maintained smooth-pursuit eye movements elicited to periodic, constant velocity target motion. For a general characterization of the gain reduction, gains were plotted for the directions of pursuit that were most impaired. Lidocaine was injected at site 1, whereas ibotenic acid was injected at rNRTP sites 2–4. Target velocity was 27°/s for site 1, 32°/s for sites 2 and 4, and 16°/s for site 3. Results are for rightward maintained pursuit at site 2 and upward pursuit for site 1, 3, and 4. ▼, average control gains. ●, average gains after ibotenic acid or lidocaine administration. Error bars, SE.
Effects of ibotenic acid on the initiation of smooth-pursuit eye movements

Lesions created by ibotenic acid in rNRTP resulted in significant deficits in eye acceleration during the initiation of smooth-pursuit eye movements. Representative epochs of smooth-pursuit initiation are shown in Fig. 5 for step-ramp target movements. The diverging average eye velocity traces for control versus postlesion behavior (Fig. 5, right) indicate a decrement in initial eye acceleration. Contralateral eye acceleration averaged over the first 80 ms of pursuit initiation, the open-loop portion (Lisberger and Westbrook 1985), was 251°/s² for controls and 151°/s² after lesion formation. Average upward eye acceleration decreased from 183°/s² for controls to 34°/s² for pursuit initiation after lesion formation by ibotenic acid. Ipsilateral and downward eye accelerations were also decremented after rNRTP lesion formation from 285 to 115°/s² and 187 to 112°/s², respectively. Because maintained smooth-pursuit eye movements for ipsilateral, contralateral, and downward target movements were approximately normal, at least at 16°/s, these pan-directional deficits in pursuit initiation were presumably not due to nonspecific effects on the eye-movement system.

For the same site, the effects of ibotenic acid on pursuit initiation were studied over a range of target velocities and the results are presented in Fig. 6. Smooth-pursuit eye accelerations initiated in response to step-ramp target motion were averaged during the initial 80 ms to obtain the data points in Fig. 6. Each point represents the average of ≥5 and typically 10 trials of pursuit initiation. All four cardinal directions of pursuit initiation were affected. Significant recovery was observed 2 days after the administration of ibotenic acid, and essentially normal values for eye acceleration were obtained for pursuit initiation studied 3 days postlesion.

To facilitate comparison, the effects of rNRTP lesions on the initial eye acceleration of pursuit initiation were studied at or near a target speed of 30°/s for all lesion sites. Results were obtained for 12–20 trials of pursuit initiation in the ipsilateral direction for site 2 and in the upward direction for the remaining sites. Because a representative indication of the deficit in eye acceleration was sought, the results are for the direction of pursuit initiation that was most impacted for a given site. Initiation-related pursuit eye accelerations were normalized relative to control values which were set equal to one. The results are presented in Fig. 7 as the ratio of postlesion initial eye accelerations to prelesion control values.

![Fig. 5. Effects of a rNRTP lesion on the initiation of smooth-pursuit eye movements. Target speed was 30°/s in all cases. Control panels, 5 overlaid trials for each direction of prelesion smooth-pursuit eye movements initiated to step-ramp target motions. Lesion panels, 5 trials per direction of smooth-pursuit eye movements initiated 1 day after the administration of ibotenic acid. Eye velocity panels, the average eye velocity traces for smooth-pursuit initiation under control and lesioned conditions. 5–12 individual eye velocity traces were aligned on the beginning of pursuit eye movements before averaging. . . , target position. Amplitude and time calibration bars apply to all eye position traces. Site 3.](http://jn.physiology.org/lookup/doi/10.1152/jn.00721.2003)
accelerations. Decrements in eye acceleration ranged from 30 to 82% and averaged 48%.

**Directional effects**

Maintained smooth-pursuit eye movements in the upward direction appeared to be the most susceptible to lesions made in rNRTP as shown in Fig. 8. In contrast, downward maintained smooth pursuit seemed to be relatively resistant, though not impervious, to the effects of rNRTP lesions. Deficits in horizontal maintained pursuit were observed with the presumably large lesions associated with lidocaine injections (Fig. 8A) but not always with the more circumscribed ibotenic acid lesions (Fig. 8C). For ibotenic acid lesion site 3 (Fig. 8B), horizontal pursuit was normal for a target velocity of 16°/s but could not be maintained for the higher target velocity of 30°/s commonly used for constructing other panels in this figure. Thus a more profound and multi-directional deficit in maintained pursuit was actually observed for site 3, even though only measured results were plotted in Fig. 8B. Testing of vertical maintained pursuit behavior inadvertently was missed for the lesion at site 2 because attention was focused on collecting pursuit initiation data.

The results for initiating smooth-pursuit eye movements are qualitatively similar to those for maintaining pursuit. The directional effects of ibotenic acid on average eye acceleration during the initial 80 ms of pursuit initiation were plotted in Fig. 8, D–F. Upward pursuit initiation seemed to be the most consistently impaired, whereas downward pursuit initiation seemed to be more resistant to the effects of lesion formation. For site 3, it already has been noted that the monkey was unable to maintain pursuit for targets moving at 30°/s, yet the monkey was able to initiate pursuit at this target velocity (Fig. 8E). Although pursuit could be initiated, it is clear that there was a severe deficit in the initial eye acceleration.

**DISCUSSION**

The results provide behavioral support for the conclusion that rNRTP is part of the neural substrate that regulates smooth-pursuit eye movements. Chemical lesions, induced by lidocaine or due to ibotenic acid excitotoxicity, caused deficits in both the maintenance and the initiation of smooth-pursuit eye movements (Figs. 2 and 5). The functional implications of these deficits complement the observation of smooth-pursuit-related neuronal responses in rNRTP (Suzuki et al. 1991). Presumably rNRTP receives smooth-pursuit-related signals from, at least, the frontal eye field where pursuit-related activity has been recorded (Gottlieb et al. 1993, 1994; MacAvoy et al. 1991) and lesions resulted in impaired...
Effects on Maintained Pursuit
A

Effects on Pursuit Initiation
D

FIG. 8. Directional effects of lidocaine and ibotenic acid on maintained, smooth-pursuit eye movements and on the initiation of smooth pursuit. A–C: effects on maintained pursuit. Normalized gains were calculated as the ratio of postlesion gains to control gains. For specific horizontal or vertical directions, the normalized gains obtained after lidocaine or ibotenic acid application were plotted at the corners of the internal, white quadrangles. Control gains were normalized to 1 and represented at the corners of the external quadrangles. Results in A are for a lidocaine lesion. Note that reference to laterality must be viewed with care because the extent of the spread of lidocaine could not be determined. Results in B and C are for ibotenic-acid-induced lesions. D–F: ibotenic acid lesion effects on the initiation of smooth pursuit. Plotted values are derived from eye acceleration averaged during the initial 80 ms of pursuit. Normalized postlesion eye accelerations were plotted at the corners of the internal white quadrangles and normalized control values were plotted at the corners of the external quadrangles. Direction labels Up, Down, Contra(lateral), and Ipsi(lateral) shown in A apply to all cases. Calibration of the gain axes indicated in B applies to all cases. Target speed was 30°/s in all cases except B and D, where target speed was 16 and 32°/s, respectively.


Directional pursuit deficits after rNRTP lesions
The average 44% decrease in the gain of maintained pursuit indicated that lesions in rNRTP can have a profound effect on smooth-pursuit performance. The observed deficits always involved upward pursuit and sometimes involved other directions of smooth-pursuit eye movements (Fig. 8). The seemingly greater susceptibility of upward pursuit to rNRTP lesions is consistent with microstimulation results. When microstimulation was delivered to rNRTP, slow pursuit-like eye movements were evoked in the upward direction in 89% of the cases (Yamada et al. 1996). Upward eye movements were evoked even when the microelectrode recorded down smooth-pursuit unit responses at the same site just before microstimulation.

Although microstimulation and lesion results indicated that upward eye movements are especially affected, it should not be concluded that rNRTP is solely involved with vertical eye movement control. There was ample evidence that rNRTP also is involved with horizontal ocular motility. Deficits in some type of horizontal pursuit eye movements were observed for all sites. Pursuit in both horizontal directions were affected at site 1 (Figs. 3B and 8A), site 2 (Figs. 4 and 8D), and site 3 (Fig. 6 and 8E). For site 4 (Fig. 8F), deficits in ipsilateral pursuit initiation were observed. In contrast with vertical pursuit, definitive conclusions could not be made concerning which direction of horizontal pursuit was more consistently or severely affected by rNRTP lesions. The specific proportion of deficits in ipsilateral versus contralateral tracking may have been more dependent on the specific location and extent of the rNRTP lesion.

Recovery of pursuit behavior
The fairly rapid recovery of tracking ability after the administration of ibotenic acid may be due to functional compensation attributable to the surviving cells in rNRTP. No attempt was made to totally eliminate rNRTP, even unilaterally, thus the effects of complete loss of rNRTP remain undetermined. Because sites 3 and 4 were in the same animal, it was reasonable to expect a greater deficit after the lesion at site 4. Inexplicably, just the opposite was the case. In addition to the possible functional compensation by surviving cells in rNRTP, greater reliance on the cortico-ponto-cerebellar pathway mediated by the dorsolateral pontine nucleus may have contributed to the recovery of pursuit behavior. Bilaterally lesioning both the dorsolateral pontine nucleus and rNRTP would be expected to drastically and possibly permanently impair pursuit, but this remains to be determined.

Relation to cerebellum
The suggested participation of rNRTP in controlling smooth-pursuit eye movements is consistent with our increased understanding of cerebellar roles in the regulation of pursuit eye movement. Although involvement of floccular areas with smooth-pursuit functions has been known for a number of years (e.g., Lisberger and Fuchs 1978; Miles and Fuller 1975; Noda and Suzuki 1979), the involvement with pursuit functions of the oculomotor vermis and its target structure, the fastigial nucleus, has only recently become fully appreciated (Fuchs et al. 1994; Robinson et al. 1997; Suzuki and Keller 1988a,b; Suzuki et al. 1981). NRTP projects to floccular areas, oculomotor vermis, and fastigial nucleus making it, together with the dorsolateral pontine nucleus, an important source of pursuit-related information destined for the cerebellum (Brodal 1980, 1982; Gonzalo-Ruiz and Leichnetz 1990; Gonzalo-Ruiz et al. 1988; Thielert and Thier 1993; Yamada and Noda 1987). In summary, the pursuit deficits observed in this study are consistent with the conclusion that the rNRTP is part of the neural substrate for the smooth-pursuit eye movement system. Presumably rNRTP is part of a cortico-ponto-cerebellar pathway that is composed of the frontal eye field, rNRTP, and the oculomotor vermis, fastigial nucleus, and floccular areas. This cortico-ponto-cerebellar pathway parallels an MT/MST to dorsolateral pontine nucleus to oculomotor cerebellum path. Both
the initiation and the maintenance of smooth-pursuit eye movements are dependent on nRTP.

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REFERENCES


KEATING, E. G. AND GOOLY, S. G. Saccadic disorders caused by cooling the superior colliculus or the frontal eye field, or from combined lesions of both structures. Brain Res. 438: 247–255, 1988.


