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Effect of Pharmacological Inactivation of Nucleus Reticularis Tegmenti Pontis on Saccadic Eye Movements in the Monkey

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Abstract

The superior colliculus (SC) provides signals for the generation of saccades via a direct pathway to the brain stem burst generator (BG). In addition, it sends saccade-related activity to the BG indirectly through the cerebellum via a relay in the nucleus reticularis tegmenti pontis (NRTP). Lesions of the oculomotor vermis, lobules VIc and VII, and inactivation of the caudal fastigial nucleus, the cerebellar output nucleus to which it projects, produce saccade dysmetria but have little effect on saccade peak velocity and duration. We expected similar deficits from inactivation of the NRTP. Instead, injections as small as 80 nl into the NRTP first slowed ipsiversive saccades and then gradually reduced their amplitudes. Postinjection saccades had slower peak velocities and longer durations than preinjection saccades with similar amplitudes. Contraversive saccades retained their normal kinematics. When the gains of ipsiversive saccades to 10° target steps had fallen to their lowest values (0.28 ± 0.19 ; mean $\pm$ SD; $n = 10$ experiments), the gains of contraversive saccades to 10° target steps had decreased very little (0.82 ± 0.11). Eventually, ipsiversive saccades did not exceed 5°, even to 20° target steps. Moreover, these small remaining saccades apparently were made with considerable difficulty because their latencies increased substantially. When ipsiversive saccade gain was at its lowest, the gain and kinematics of vertical saccades to 10° target steps exhibited inconsistent changes. We argue that our injections did not compromise the direct SC pathway. Therefore these data suggest that the cerebellar saccade pathway does not simply modulate BG activity but is required for horizontal saccades to occur at all.

INTRODUCTION

The oculomotor system employs eye movements called saccades to shift the direction of gaze rapidly from one object to another. For many years, saccadic movements have been characterized nicely by models that employ only the superior colliculus (SC) and a brain stem burst generator (BG) in the pons and mesencephalon (Robinson 1975). In these models, the SC drives the BG with a signal proportional to desired saccade size. The BG is configured as a local feedback circuit, which compares the desired eye displacement with a signal proportional to the evolving actual eye displacement during the saccade. The feedback circuit serves to produce a burst of spikes the duration of which is proportional to that of the saccade and hence its amplitude (Becker 1989; Scudder et al. 2002). This BG burst, in turn, provides the phasic drive to ocular motor neurons that is required to generate the saccade. Robinson's simple yet elegant model accurately simulated the metrics of saccades and their accuracy.

At about the same time that Robinson proposed his model, however, others began showing that the oculomotor cerebellum, which includes lobules VIc and VII of the vermis and the caudal fastigial nucleus (CFN) to which it projects (Noda et al. 1990), was important for the

generation of accurate saccades. Saccade dysmetria occurred after permanent lesions that included the oculomotor vermis (Barash et al. 1999; Ritchie 1976; Takagi et al. 1998; Vilis and Hore 1981), reversible (Iwamoto and Yoshida 2002; Robinson et al. 1993), or electrolytic (Goldberg et al. 1993) lesions of the CFN and removal of the entire midline cerebellum (Optican and Robinson 1980). These studies indicated that the oculomotor cerebellum also is an important element of the subcortical circuitry involved with saccade generation.

The behavior and projections of CFN neurons, the output of the oculomotor cerebellum, appear appropriate to help shape the burst in the BG. CFN neurons discharge a burst of spikes for saccades in all directions, but the timing of the burst varies with the direction of the saccade and its amplitude (Fuchs et al. 1993; Inaba et al. 2003; Ohtsuka and Noda 1991; Scudder and McGee 2003). Both anatomical tracer (Langer and Kaneko 1990; Noda et al. 1990) and electrophysiological studies (Scudder and McGee 2000) indicate that the CFN projects directly to elements in the burst generator for horizontal saccades. Indeed, electrical stimulation of the CFN elicits saccades at latencies consistent with a disynaptic connection (Noda et al. 1988). The signs of the connections and the relation of CFN burst timing with the onset and end of the saccade lead to a plausible model in which CFN activity helps to terminate and possibly also to initiate the premotoneuronal burst in the BG (Fuchs et al. 1993).

The principal input to the CFN originates in the oculomotor vermis, which receives the majority of its afferents from the nucleus reticularis tegmenti pontis (NRTP) (Brodal 1980; Thielert and Thier 1993; Yamada and Noda 1987). The NRTP also projects directly to the CFN (Gonzalo-Ruiz and Leichnetz 1990; Noda et al. 1990). The NRTP, in turn, is a major recipient of projections from the SC (Harting 1977; Kawamura et al. 1974; Scudder et al. 1996a). Therefore in addition to its direct influence on the BG, the SC also gains access to the BG via a cerebellar loop that includes the NRTP. This side branch appears to help shape the BG burst and thereby, the amplitude of the saccade.

If the cerebellum forms a side branch from the SC that tunes up the saccade burst, we would expect that inactivating its major input, the NRTP, would compromise saccades in the same way that inactivation of the cerebellar part of the pathway does. Accordingly, we predicted that saccades would be dysmetric. Moreover, lesions of the CFN have either no (Vilis and Hore 1981) or a relatively modest (Robinson et al. 1993) effect on saccade metrics, i.e., the tight relations between saccade peak velocity and duration and saccade amplitude [the so-called main sequence relations (Bahill et al. 1975; Becker 1989)]. Therefore we also predicted that NRTP lesions would have a modest influence, at most, on saccade dynamics. In this scenario, the NRTP input to the oculomotor cerebellum is necessary only to make saccades accurate. Thus if they become inaccurate due to NRTP inactivation, saccades still would have relatively normal main sequence relations. On the other hand, if the NRTP were part of the direct pathway from the SC to the BG, its inactivation would presumably eliminate saccades completely (Goebel et al. 1971; Hepp and Henn 1984) or at least affect saccade metrics (Barton et al. 2003; Scudder 1997). We report here the unexpected finding that a functional NRTP appears to be required for ipsiversive saccades to occur at all.

A preliminary description of these results has appeared in abstract form (Kaneko and Fuchs 2004).

METHODS

We performed these experiments on three juvenile Rhesus (*Macaca mulatta*) monkeys A–C. Each animal underwent an initial sterile surgery in which we implanted a scleral search coil to measure eye movements and attached stabilization lugs to the skull, which were held during the experiment to prevent the head from turning (Soetedjo et al. 2002b). We measured head-

fixed eye movements with an electromagnetic technique that had a sensitivity of 15 min of arc and an accuracy, in a well-trained monkey, of $\pm 0.5^\circ$ (Fuchs and Robinson 1966; Robinson 1963). The monkey was rewarded for following with its eyes a laser spot that appeared on a flat tangent screen at a distance of 68 cm. The spot subtended an angle of 0.4° . Before the spot reached the screen, it was deflected by mirrors mounted on two orthogonal galvanometers, which were driven by a Macintosh G4 computer through a National Instruments interface. The computer-controlled galvanometers placed the spot anywhere within $\pm 37^\circ$ horizontally and $\pm 22^\circ$ vertically of the primary direction of gaze. If the monkeys maintained their eyes within a $\pm 2^\circ$ window surrounding the target for ~ 2 s, they were rewarded with a small amount of applesauce. The accuracy criterion was not enforced during the saccadic reaction time. Because muscimol inactivation produced hypometric initial saccades so that several corrective saccades were required to reach the target, we reduced the time-on-target criterion to 300–500 ms after the injections.

Localization of NRTP and the injection procedure

After the animals had been trained to make accurate saccadic responses to the jumping laser spot, we implanted a stainless steel recording cylinder that was aimed at the NRTP in the rostral pons. The cylinder, which was placed over a hole trephined in the posterior cranium and centered on the midline, was tilted backward 30° from the vertical and directed 1–3 mm anterior to stereotaxic zero. After the animal had recovered from the surgery for at least a week, we slowly drove either sharpened tungsten recording microelectrodes or injection pipettes protected by a short cannula through the cylinder and into the brain by means of a hydraulic microdrive.

Because the pons contains many elements of the saccade burst generator, we took several precautions to ensure that our injections were centered on the NRTP and that they avoided other saccade-related structures. First, as mentioned in the previous paragraph, our approach to the NRTP was tilted backward to avoid driving electrodes through the SC. An added advantage of this approach was that any wicking backward along the injection cannula would not involve a saccade-related structure. Second, in all monkeys, we first localized the NRTP on the basis of its characteristic single-neuron activity (Crandall and Keller 1985; Soetedjo et al. 2002a; Takeichi et al. 2005). Saccade-related NRTP neurons all had broadly tuned movement fields with a variety of optimal directions, had either closed or open movement fields, and were concentrated near the midline. Based on both single and multiple unit activity, we then mapped the boundaries of the NRTP in as few tracks as possible. Third, we located the omnipause neurons (OPNs) (Büttner-Ennever et al. 1988; Langer and Kaneko 1990) and determined the distance of their rostral border from the NRTP. Fourth, we determined the locations of neurons with discharge patterns characteristic of excitatory burst neurons (EBNs) (for review, see Scudder et al. 2002). Before each injection, we identified the NRTP either on the basis of an isolated saccade-related burst neuron or by the presence of unresolved saccade-related bursts within the boundaries of the previously mapped NRTP. We then replaced the recording electrode with an injection pipette at the same location and lowered it to the depth of the previously recorded saccade-related activity. We used pneumatic pressure to deliver muscimol (2 mg/ml in phosphate buffer, pH 7.4) with an accuracy of ~ 10 nl via the pipette (Kaneko 1996, 1997; Soetedjo et al. 2002b). In 9 of the 10 experiments, we made single injections ranging from 80 to 520 nl. In the remaining experiment (A2), when the initial small injection had not produced a substantial saccade slowing or hypometria, we injected another 100 nl.

We developed our experimental procedures on *monkey A*, which had been used for other brain-stem recordings. First, we confirmed that our identification of the NRTP was indeed accurate by reconstructing the electrode penetrations and the injection sites made on the basis of NRTP-

like activity. Figure 1A shows the gliosis produced by one of the injections (red arrow) and by a more medial electrode penetration, both of which traversed the left NRTP (below dashed line). In Fig. 1B, we have reconstructed neighboring transverse sections of the pons caudal to the NRTP to locate the nearest elements of the saccade burst generator. In this monkey, the location of the omnipause neurons (nucleus raphe interpositus, rip) (Büttner-Ennever et al. 1988) lay ≥ 2.5 mm caudal of our most caudal NRTP injections. Most of the EBNs that we recorded were clustered just lateral and dorsal to rip. Reconstruction of electrode penetrations and injection tracks in *monkey B* (*monkey C* is still active in other experiments) revealed a similar if not greater separation of BG neurons from our NRTP injection sites.

Second, we optimized the injection conditions to have the best chance of pharmacologically affecting only the NRTP. First, in *monkey A*, we determined that injections of as little as 80 – 100 nl could produce a demonstrable deficit. For these volumes, ipsiversive saccades exhibited very subtle decreases first in velocity and then amplitude, deficits that, as we shall document below, are characteristic of inactivation of the NRTP. Moreover, although we used injections ranging from 80 to 520 nl in *monkeys B* and *C*, their effect on saccadic eye movements did not vary in any systematic way with the volume of the injection. We saw slowing with injections as small as 80 nl, which can be expected to affect neurons at a distance of up to approximately ± 300 μ m (e.g., Martin 1991). Our average 250-nl injection would affect a volume of ~ 1.12 mm in diameter (800 μ m $\times$ 1.4 effective spread). This observation suggests that, even when larger volumes were employed, the deficits were not due to encroachment on other elements of the brain stem burst generator. Second, we chose muscimol, a GABA_A agonist, to affect only NRTP cell somata and not any axons from the burst generator, which might be passing through or near the region

Experimental protocol

In most experiments, we were able to compare saccades to the same target steps before and during inactivation. Prior to the injection, we collected two blocks of normal data. First, we usually required the monkey to track horizontal target jumps of 5, 10, 15, and 20° from pseudorandom initial positions within $\pm 25^\circ$ of the primary direction of gaze. Between 10 and 15 saccades were collected for each size and direction of target jump. Second, we required the monkey to track 10° target jumps that were initiated from straight ahead and landed either along the horizontal or vertical meridians or along axes located halfway in between, i.e., every 45° from zero (rightward) through 360°. We collected between 10 and 15 saccades for each of these eight directions.

After the muscimol had been delivered, we monitored its efficacy by examining the accuracy of horizontal saccades to 10° and sometimes 20° target steps. As the muscimol began to take effect, ipsiversive saccades began to slow and then gradually fell short of the target. Once slowing or dysmetria was noticeable, we collected alternating blocks of saccades in the eight directions and horizontal saccades to several target amplitudes for as long as the monkey continued to work. Because the time course of the effect of the injection varied widely from experiment to experiment (see RESULTS), we were unable to collect exactly the same data in all experiments.

Data analysis

We recorded the horizontal and vertical positions of the eye and target and stored them on hard drives, digital video-tape backup, and optical disks for off-line analysis (see Kaneko and Fukushima 1998 for details). The metrics of each primary and corrective saccade to a target step were determined off-line by means of homemade interactive programs. Briefly, saccades were detected when eye velocity exceeded 50°/s, and their onset and offset were defined as the time at which the velocity rose above and fell below 10°/s, respectively. Once a saccade

was detected, the program determined saccade onset and offset and the time of peak velocity of each saccade component. In a few cases (<5%), artifacts (e.g., noise, blinks, etc.) caused incorrect computer markings, which the second author adjusted by hand. Our homemade program then determined the amplitude, duration, and peak velocity of saccades, the saccade latency relative to the target step and the saccade gain (eye movement amplitude/target movement amplitude). Because in some experiments, especially in *monkey B*, contraversive saccades sometimes were launched before a staircase of ipsiversive saccades had reached the target displacement (see RESULTS), all target movement amplitudes were taken as the difference between target eccentricity and the actual starting position of the saccade. All accepted saccades were stored and analyzed by other homemade or commercial (Igor, WaveMetrics, Lake Oswego, OR; Matlab, Mathworks, Natick, MA) software programs to compare the metrics of saccades before or immediately after the injection with those at various times after the injection. The Student's *t*-test was used for statistical analysis with $P < 0.05$ indicating statistical significance, unless otherwise stated. Averages are presented as means with their SDs.

The main sequence relations between saccade duration or peak velocity and amplitude were fit with the following equations (Becker 1989): $Dur = Dur_0 + k_1 A$; $PkVel = k_2 [A/(k_3 + A)]$ where *Dur*, *PkVel* and *A* are saccade duration, peak velocity, and amplitude, respectively. To determine whether the fits of the main sequence data before and after the injection were different, we tested two models. The first model, the null hypothesis, was that one curve fit all the data points, both pre- and postinjection. The second model, the alternative hypothesis, was that the pre- and postadaptation data sets were better fit by different curves. If the addition of the sum of squares for the two different curves was less than the sum of squares for the curve describing the combined data, then individual fits were better and the injection had produced a statistically significant effect (Motulsky and Christopoulos 2004).

The Animal Care and Use Committee at the University of Washington approved all the surgical and training procedures. The veterinary staff of the Regional Primate Research Center cared for the animals. The animals were housed under conditions that comply with National Institutes of Health standards as stated in the *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* and the recommendations from the Institution of Laboratory Resources and the American Association for Accreditation of Laboratory Care International.

RESULTS

Data set

We injected muscimol into the NRTP in 10 experiments on two monkeys. *Monkey B* received six injections (B1–B6) and *monkey C* received four injections (C1–C4).

The injection was ≥ 1 mm off the midline in all six experiments in *monkey B* and in one experiment in *monkey C*. In all those seven, the injection severely impaired ipsiversive saccades. The remaining three injections in *monkey C* were deliberately placed closer to the midline but, based on the deficits they produced, were more effective at inactivating one side than the other. In two, the effect was primarily on rightward (ipsiversive) saccades. In the remaining injection, both ipsiversive and contraversive saccades were affected but the effect on ipsiversive saccades was slightly greater.

General deficits

All muscimol injections into the NRTP eventually produced the same qualitative deficits in saccadic eye movements. At variable amounts of time after the different injections, there was

an initial slowing of saccades ipsiversive to the injection site followed almost immediately by a progressive ipsiversive hypometria. Once the hypometria began, there was a steady reduction in the gain of ipsiversive saccades to average values <0.1 . Eventually, the animals appeared to find it extremely difficult to elicit ipsiversive saccades at all, and those saccades that were elicited often were preceded by an unusually long latency and/or had a large vertical component. In contrast with the dramatic hypometria and slowing of ipsiversive saccades, contraversive and vertical saccades showed relatively modest changes in their accuracy and metrics.

In some experiments, the reduction in ipsiversive saccade amplitude occurred very rapidly and/or the animal eventually became frustrated with its inability to reach the target. Thus it was not possible to document the observations on saccades of all sizes and directions for every injection. Therefore in what follows, data from some saccade conditions will not have been obtained in every one of the 10 experimental injections. Table 1 identifies which of the 10 experiments contributed to the quantitative documentation of each observation (attribute) in the various saccade analyses performed below. Injections were made with roughly equal frequency into the right and left NRTP and the size of the injection ranged from 80 to 520 nl (avg. 250 nl).

Ipsiversive hypometria

Figure 2, *A* and *B*, demonstrates the effect of muscimol injections on saccades in various directions for representative experiments in *monkeys C* and *B*, respectively. In this figure, both monkeys made saccades to 10° target steps that originated from straight ahead and were directed randomly in one of eight directions along either the horizontal or vertical axes, or along axes half way (at every 45°) between. Prior to the injection (red squares), the amplitude of saccades in all directions was very similar to that of the target step that evoked them (black asterisks). By 46–49 min after the injection in *monkey C*, almost all horizontal saccades ipsiversive to the injection site were reduced in amplitude (blue diamonds). By 60–73 min after the injection, most ipsiversive horizontal saccades were $<5^\circ$ (green circles). As can be seen in the data for ipsiversive oblique saccades, the hypometria appeared to occur almost solely in the horizontal component.

Even after the amplitude of ipsiversive horizontal saccades had decreased by $\sim 50\%$, contraversive saccades were only slightly hypometric. As was the case for ipsiversive saccades, the modest reduction in the amplitude of contraversive saccades occurred largely in the horizontal component as can be seen clearly for the oblique saccades. Finally, for *monkey C*, inactivation of the NRTP had little effect on vertical saccades. For this injection, neither the amplitude of pure vertical saccades nor that of the vertical component of oblique ipsiversive saccades showed a consistent change.

Although the saccade data for *monkey B* (Fig. 2*B*) tended to be more variable, it showed similar deficits but on a different time scale. After only 0–8 min, some ipsiversive leftward saccades showed reduced amplitudes (blue diamonds), and after 16–24 min (green circles) the amplitudes of many ipsiversive saccades to 10° horizontal target steps and the ipsiversive component of oblique saccades were $\sim 3^\circ$. Note that the data were obtained in 8-min blocks during which time the effect of the muscimol was increasing. As we will see later, once the muscimol took effect, the decrease in saccade amplitude was rather rapid in this monkey so some of the variability in ipsiversive saccade amplitudes in Fig. 2*B* is the result of the evolution of the effect during the data block. After this injection, contraversive saccades (rightward) to horizontal target steps and the contraversive components of oblique saccades also experienced some reductions, which were the largest seen in any experiment. Again, the vertical component of ipsiversive oblique saccades was modestly affected, if at all, as were upward saccades to vertical target steps.

Deficits similar to those revealed in the direction data illustrated in Fig. 2 were seen for all the injections with sufficient data, i.e., four experiments in *monkey B* and four in *monkey C* (Table 1, direction tuning).

Time course of amplitude deficits in horizontal saccades

IPSIVERSIVE SACCADSES—The data in Fig. 2 suggest that the injection begins to take effect at different times after the muscimol is infused. Comparable decreases in the amplitudes of ipsiversive saccades were noticed after 60–73 min in the data illustrated Fig. 2A, but after only 16 min in the data illustrated in Fig. 2B. In Figs. 3 and 4, we document the effects of the injections over the time course of each of five experiments in *monkey B* and four in *monkey C* (Table 1, horizontal amplitude time course) by tracking the gain of horizontal saccades to 10° target steps. In the remaining injection in B4, we collected only oblique saccades, but the horizontal component of those saccades decreased with a time course like that of the data for B6.

For the injections in *monkey B* (Fig. 3), the decrease in size of ipsiversive saccades began sooner than it did in *monkey C* (Fig. 4). However, for individual experiments on the same monkey, the time course of the developing ipsiversive hypometria also varied widely. The time to reach a gain of 0.5 after the completion of the injection took from ~6.5 min to ~36 min in *monkey B* and from ~25 to ~62 min in *monkey C*. Moreover, once it began, the fall from the highest to lowest ipsiversive gain generally was more rapid after the injections in *monkey B* than after those in *monkey C*. Finally, the lowest gains reached in *monkey B* often were <0.2; the average of the lowest five ipsiversive gains for each experiment in *monkey B* ranged from 0.03 to 0.10 with an overall average of 0.06. In contrast, the lowest ipsiversive gains reached in *monkey C* never were so low; the average of the lowest five ipsiversive gains for experiments C1 to C3 ranged from 0.25 to 0.48 with an overall average of 0.35.

Both monkeys apparently found it difficult to generate ipsiversive saccades when the inactivation was having its maximal effect. This difficulty was manifest in several ways. First, ipsiversive saccades late in the experiment occurred at longer latencies than did those early in the injection. Figure 5 compares the ipsiversive saccade latencies at the lowest gains reached by each monkey (≤ 0.2 for *monkey B* and ≤ 0.4 for *monkey C*) with those early in the injection when the gains were between 0.9 and 1.1. Because ipsiversive saccades were infrequent at the lowest gains, we combined all of the relevant data in all of the experiments for each monkey, i.e., all the data from the five experiments in *monkey B* (Fig. 3A) and the data from the three experiments with unilateral deficits in *monkey C* (Fig. 4A). For both *monkeys B* and *C* (Fig. 5, ■), the vast majority of saccade latencies prior to any reduction in the gain of ipsiversive saccades to 10° target steps (gains between 0.9 and 1.1) were <250 ms (average of 174 and 157 ms, respectively). After the muscimol injections had reduced the ipsiversive saccade gain to low values, the latencies of the few saccades that still could be elicited were almost all in excess of 250 ms (■, average of 775 and 462 ms, respectively for *monkeys B* and *C*). Second, some ipsiversive target steps had elicited no saccade at all by the time that the next target step occurred (from 1 to 4 s later). Third, some ipsiversive target steps elicited ipsiversive saccades only if they occurred as the horizontal component of an oblique saccade with a large vertical component.

After the gains of ipsiversive saccades had decreased, the animal often made a staircase of ipsiversive saccades to an ipsiversive target step. Figure 6, which illustrates responses from both *monkeys B* and *C*, shows that not only does the initial saccade to the target step tend to have a long latency, but the spacing of successive saccades also appears unusually long. This can be seen in Fig. 6A where the interval between the two saccades to the contraversive target step is substantially shorter than any of those between the previous ipsiversive saccades. Although we collected data mostly on saccades to 10° target steps, Fig. 6B shows that the

ipsiversive hypometria demonstrated for 10° saccades in Figs. 3 and 4 also occurred for saccades to larger and smaller target steps.

Finally, even after multiple saccade attempts, the eye sometimes did not reach the target before it had jumped to a new contraversive location. This was especially true for *monkey B*, which began saccades to contraversive target steps from an eye position that was offset by an average of $2.20 \pm 1.39^\circ$ toward the target (data from B1, 2, 3, 5, and 6); the contraversive offset preceding ipsiversive saccades was only $0.29 \pm 0.15^\circ$. Consequently, some of the amplitude reduction of contraversive saccades seen in Fig. 2B was due to the contraversive offset, which reduced the actual contraversive target displacement relative to the eye. Perhaps because his ipsiversive gain did not drop as far, *monkey C* exhibited a much more modest average contraversive offset of $0.18 \pm 0.41^\circ$ preceding ipsiversive saccades and an ipsiversive offset of $0.17 \pm 0.16^\circ$ preceding contraversive saccades. As we point out in METHODS, all of the gains in Figs. 3 and 4 were calculated with the actual target displacement relative to the eye position at saccade onset, i.e., the true target eccentricity.

These offsets appear to resemble those that occur after inactivation of the caudal fastigial nucleus (Goffart et al. 2003; Robinson et al. 1993). However, our target stepped every few seconds or less so it is unclear whether the monkey could have reached the target eventually given enough time.

CONTRAVERSIVE SACCADDES—Except for a single injection in *monkey C*, NRTP inactivation had a much greater effect on ipsiversive than contraversive saccades. As can be seen in Figs. 3 and 4, at the time after the injection when the gain of 10° ipsiversive saccades had reached its nadir (<0.2 for *monkey B* and <0.4 for *monkey C*), the gain of 10° contraversive saccades in the same experiment was still quite high. To facilitate this comparison, we estimated by eye the approximate time after the injection at which the ipsiversive gain in individual experiments in Fig. 3A and Fig. 4A appeared to cross 0.2 and 0.4, respectively, and drew downward arrows from those points to indicate qualitatively the corresponding contraversive gains. To compare ipsiversive and contraversive gains quantitatively as late in each experiment as possible, we took the latest time interval after the injection in which ≥ 6 and as many as 16 ipsiversive and a comparable number of contraversive saccades had occurred. For all five experiments with sufficient data in *monkey B*, when the average ipsiversive gain had dropped to 0.16 ± 0.09 , the average contraversive gain was 0.78 ± 0.14 . For each individual injection, the differences in ipsi- and contraversive gains were highly significant ($P < 0.000$ for the worst correlation; 2 sample *t*-test assuming unequal variances). Similarly, when the average ipsiversive gain had dropped to 0.52 ± 0.06 in *monkey C*, the average contraversive gain was 0.90 ± 0.05 ($n = 3$). Again, the differences in ipsi- and contraversive gains for each individual experiment were highly significant ($P < 0.000$ for the worst correlation; 2 sample *t*-test assuming unequal variances).

Effect on vertical saccades

The two representative experiments shown in Fig. 2, A and B, suggest that even at a time when substantial reduction in the size of ipsiversive saccades had occurred, there was little effect on the amplitudes of 10° vertical saccades. To quantify the effect of NRTP inactivation on vertical saccades, we compared the metrics of control saccades elicited by 10° up- and downward target steps with those of vertical saccades during the latest time interval in which there were sufficient saccades (range of 7–13 and 7–20 saccades in *monkeys B* and *C*, respectively). Control saccades were obtained either before the injection or early after the injection but before there was an effect on ipsiversive saccades.

For the four experiments on *monkey B* and the three on *monkey C* (Table 1, vertical 10° saccades) where vertical saccades were tested, only two in *B* showed significant changes in

vertical amplitude ($P < 0.05$, 2-sample t -test assuming unequal variances). One showed an increase in both up- and downward amplitude and the other an increase in upward amplitude only. Across all four experiments in *monkey B*, the average gains for up and down saccades were 1.07 and 1.02, respectively, at the time in the experiment when the gain of ipsiversive saccades had been reduced to an average of 0.17 (range: 0.06 – 0.28). In *monkey C*, the gain of ipsiversive saccades at the time when there was no significant change in the amplitudes of 10° vertical saccades was 0.52 (0.46 – 0.57).

In all four experiments on *monkey B* and two of three on *monkey C*, there was a significant increase in downward saccade duration ($P < 0.05$). In all but one experiment, there were no significant associated changes in downward amplitude, and in only two of those were there concomitant significant decreases in downward peak velocity.

In summary, there was an inconsistent change in the amplitude of 10° vertical saccades (at least at $P < 0.05$) at a time when ipsiversive saccades were displaying highly significant hypometria. The most consistent deficit across all experiments was an increase in the duration of downward saccades to 10° target steps. Because all of these data were obtained at the end of the injections, it is possible that the slowing of the downward saccades could have been due to fatigue. However, contraversive saccades showed no such effect (see following text).

Effect on horizontal saccade metrics

SLOWING OF IPSIVERSIVE SACCADSES—Even before an NRTP injection caused a measurable decrease in the amplitude of ipsiversive saccades, they became slower. This slowing was especially noticeable for early postinjection saccades to 20° target steps. Figure 7A compares saccades to 20° target steps made 0–2 min after the end of the injection (red traces) in experiment B1 to preinjection saccades (green traces) selected to have a similar average amplitude. Although the selected saccades before and after the injection were roughly of the same amplitude ($21.38 \pm 1.03^\circ$ SD and $20.77 \pm 0.64^\circ$, respectively), the postinjection saccades had much longer durations (72.67 ± 6.58 vs. 47.12 ± 2.67 ms) and substantially reduced peak velocities (444.42 ± 48.96 vs. $816.36 \pm 57.81^\circ/\text{s}$). Figure 7B shows a more subtle slowing for saccades to 20° target steps recorded 19–23 min after the end of the injection C1. In this experiment, peak saccade velocity again decreased from 603.53 ± 30.52 to $459.15 \pm 58.51^\circ/\text{s}$ and duration increased from 62.67 ± 3.14 to 74.83 ± 7.62 ms. In this data set, there was a slight decrease in average saccade amplitude from 18.35 ± 0.48 to $17.69 \pm 0.59^\circ$.

In both experiments, saccade slowing already was manifest shortly after movement onset. Although there was a slight increase in the duration of the acceleration phase (time to peak velocity) of the saccade, the most dramatic changes in the velocity profile occurred after peak saccade velocity. The increase in this deceleration phase accounted for the bulk of the increase in saccade duration.

The slowing of ipsiversive saccades of about the same amplitude occurred gradually. Figure 8 shows the change in the peak velocity of saccades with gains between 0.9 and 1.1 to 10° target steps as the effect of the inactivation progressed for three experiments. For experiments B3 and B5 (Fig. 8A), the peak velocity of $\sim 10^\circ$ saccades decreased gradually (over 20 and 30 min, respectively) from ~ 400 to $200^\circ/\text{s}$ and for experiment C3 (Fig. 8B) from ~ 524 to $375^\circ/\text{s}$ (in ~ 43 min). Over the times when there was a gradual reduction in the peak velocities of ipsiversive saccades, peak contraversive saccade velocities changed little.

EFFECT ON MAIN SEQUENCE RELATIONS—Although slowing was usually first noticeable for larger saccades, some slowing occurred for most ipsiversive saccades to all target steps tested. The slowing is clearly revealed in the main sequence relations (Bahill et al. 1975) that plot peak eye velocity and duration as a function of saccade amplitude. Figure 9,

which presents representative data from experiments B1 (Fig. 9, *A* and *C*) and C2 (*B* and *D*), respectively, show these relations before the injections (blue squares), at a time after the injection when ipsiversive saccades still had reasonable gains (red diamonds, within the 1st 8 min in B1 and from the 19–23 min in C2) and late in the experiment when almost all ipsiversive saccades were $<10^\circ$ (green circles, from 8 to 24 min in B1 and 31 to 34 min in C2). The fits of data with the functions described in methods revealed that after the injection, the entire main sequence relation for ipsiversive saccades, except for the very smallest, was shifted toward lower peak velocities and longer durations. In contrast there was little effect on the dynamics of contraversive saccades in either experiment.

To quantify the effect of NRTP inactivation on saccade dynamics, we fit main sequence plots like those in Fig. 9 for all the experiments with sufficient data (Table 1, horizontal main sequence). As postinjection data sets, we used those data obtained early enough after the injection that ipsiversive saccades still had a reasonable range of amplitudes (e.g., the red diamonds in Fig. 9). Figure 10, *A* and *B*, shows the fits for all the appropriate experiments on *monkeys B* and *C*, respectively with peak velocities plotted here as absolute values. We have identified pre- (solid lines) and postinjection (dashed lines) fits for each experiment with the same colors. For ipsiversive saccades, the peak velocity relation of the postinjection fit always was separate from and lower than the preinjection fit for the same experiment. The duration relation of the postinjection fit always was separate from and higher than the preinjection fit. For contraversive saccades, in contrast, the pre- and postinjection fits for both the peak velocity and duration relations tended to lie very close together for the same experiment. The one exception was experiment C4 (black fits in Fig. 10*B*), a juxtamidline injection that affected both rightward (taken as ipsiversive because the deficit was greater) and leftward (contraversive) saccades.

For all seven experiments with unilateral injections (4 in *monkey B* and 3 in *monkey C*), ipsiversive saccades showed a highly significant decrease in peak velocity ($P < 0.000$) and an increase in duration ($P < 0.004$). The effect on the dynamics of contraversive saccades was somewhat more variable. For six experiments, the injection produced no change in the peak velocity versus amplitude relation ($P > 0.17$). For four of the previous six, there also was no effect on the duration versus amplitude relation ($P > 0.21$). In the seventh experiment, the injection caused both a significant decrease in the peak velocity and an increase in the duration of contraversive saccades. Therefore for all seven experiments taken together, 10 of the 14 main sequence relations for contraversive saccades showed no statistically significant change after the injection of muscimol into the NRTP. For the eighth experiment, the juxtamidline injection C4, both rightward and leftward saccades were significantly slower.

Persistence of the dysmetria

Another study that injected muscimol into the NRTP reported that gaze became deviated toward the ipsiversive direction and that saccades were confined to a 15° square surrounding the deviated position. Also, contraversive saccades never crossed the center of gaze. Moreover, these saccade deficits lasted for > 3 wk (Suzuki et al. 2000).

Our saccade deficits not only were markedly different, but they had largely dissipated by the following day. We documented the persisting deficits quantitatively in experiments B4 (10° target steps) and B5 (5 – 20° target steps) in which the muscimol injection had decreased the ipsiversive gains to 0.19 ± 0.10 ($n = 13$) and 0.23 ± 0.17 ($n = 12$), respectively. The next day the ipsiversive gains had recovered to 0.92 ± 0.05 ($n = 13$) and 0.97 ± 0.08 ($n = 13$), respectively. These recovered gains were either barely significantly less than ($P = 0.04$) or not significantly different from ($P = 0.33$) the preinjection gains on the day of the injection [1.00 ± 0.07 (6) and 0.94 ± 0.05 (7), respectively]. It should be mentioned that our injections averaged 250 nl, whereas theirs were 1200 nl with 7.5 times the concentration of muscimol.

DISCUSSION

Our data show that injections of the GABA_A agonist muscimol centered on the saccade portion of the NRTP affect both the amplitude and peak velocity of ipsiversive saccades. In all 10 injections, ipsiversive saccades initially exhibited some slowing (Figs. 7 and 8) and soon thereafter began to fall short of ipsiversive target steps (Fig. 2). Saccade size continued to decrease over the next 10–20 min in different injections (Figs. 3A and 4A) until at the peak of the effect, saccades to large ipsiversive target steps of 15° or more often were <5° (Fig. 6). At this point in time, there was only a modest reduction, if any, in the amplitude and velocity of contraversive saccades (Figs. 3B and 4B) and no consistent change in the metrics of 10° vertical saccades.

The effects of our inactivations imply that the medial portion of the NRTP has a primary role in the generation of horizontal saccades. The medial NRTP does not appear to be involved in the generation of vertical saccades although it does contain long-lead burst neurons that discharge best for saccades with vertical directions (Kaneko 2006; Takeichi et al. 2005). A mesencephalic nucleus may play a similar role for vertical saccades.

Our data differ from those reported by two other studies that examined saccades after muscimol inactivation of NRTP. We already have considered the differences between the results of our study and those reported in an abstract by Suzuki et al. (2000). In the other study, van Opstal et al. (1996) examined the effect of NRTP inactivation on the three-dimensional metrics of spontaneous saccades. Unlike our study, they did not report any ipsiversive hypometria although it would have been difficult to discern without knowledge of the saccades' targets. Moreover, their injection sites appear to have been much more lateral in the NRTP than were ours.

Differences between monkeys and injections

It is unclear why the muscimol injections drove the ipsiversive gains lower in *monkey B* than in *monkey C*. In both animals, we collected data for as long as the animal worked. However, *monkey C*'s deficits appeared at longer times after the injection so perhaps he was already sated with the reward. If so, he may not have been as motivated to continue the tracking task long enough to reveal the lower gains. However, the examples in Fig. 6 indicate that at least in those experiments illustrated, the monkeys continued to try to reach the target even when their gains were quite low.

We cannot account for the differences in the time course and final value of the ipsiversive gain after different injections. The differences might have been due to both the amount of the injection and its location in the NRTP. For example, larger injections placed more laterally might be expected to cause deficits at about the same time after the injection as smaller injections placed more medially where NRTP neurons are more numerous. We saw no trend in the time course or final value of the ipsiversive dysmetria with either the size or laterality of the injection. However, all of our injections were fairly closely spaced within ± 1.5 mm of the functionally identified midline and most of our injection volumes were between 160 and 260 nl (4 of 5 in Fig. 3 and 3 of 4 in Fig. 4). Therefore it would be problematic to draw any conclusions on the basis of these small differences in only 10 experiments. Perhaps the slower time course of changes in ipsiversive gain and the relatively smaller decrease in gain in *monkey C*'s experiments (compared with those in *monkey B*) was due to less electrode damage of a very busy area in that monkey. Indeed our first injection in *monkey B* was made on track 30, whereas the first injection in *monkey C* was made on track 12. However, this observation is hardly definitive. Therefore we cannot account for the differences in the course and amount of the gain changes to 10° target steps seen in Figs. 3 and 4, either for the same monkey or across

monkeys. However, all 10 experiments in both monkeys consistently showed the same qualitative impairment of horizontal saccades.

Known pathways do not account for the results of NRTP inactivation

These deficits would not be expected if the NRTP serves solely as the major link between the SC and the oculomotor cerebellum, lobules VIc and VII. However, there is solid anatomical evidence for this scenario. First, the SC sends a substantial projection to the NRTP of the cat (Moschovakis et al. 1998) and the monkey (Harting 1977). Second, fills of every NRTP burst neuron labeled in the squirrel monkey showed axons diving into the brachium pontis, presumably en route to the cerebellum (Scudder et al. 1996b). In contrast, there were no axon collaterals to the vicinity of the burst generator. Third, injections into the oculomotor vermis labeled $\leq 45\%$ of the intermediate sized cells and 28% of the small cells in the mediodorsal region of the NRTP (Thielert and Thier 1993; Yamada and Noda 1987) where most of our injections were concentrated. Taken together, these studies suggest that the NRTP sends a substantial projection to the oculomotor vermis and little, if any, to the brain stem burst generator. The vermis, in turn, projects to the caudal fastigial nucleus, the efferents of which synapse on elements of the premotor burst generator for horizontal saccades (Scudder and McGee 2000; Scudder et al. 2002).

Our initial expectation therefore was that inactivation of the NRTP would produce deficits comparable with those due to lesions of the oculomotor cerebellum. However, when the oculomotor cerebellum was inactivated, the deficits were quite different from those we report here. The most prominent deficit of muscimol inactivation of the caudal fastigial nucleus was a rather large dysmetria (Goldberg et al. 1993; Iwamoto and Yoshida 2002; Robinson et al. 1993; Vilis and Hore 1981) with only a modest reduction in peak velocity and an increase, if any (Vilis and Hore 1981), in the duration of amplitude-matched saccades (Robinson et al. 1993). Lesions (Barash et al. 1999; Ritchie 1976; Takagi et al. 1998) or pharmacological inactivation (Sato and Noda 1992) of the oculomotor vermis also produced saccade dysmetria. Saccade slowing also has been reported but only after large lesions that involved not only parts of the oculomotor vermis but other vermis lobules as well (Takagi et al. 1998). Therefore inactivation of the cerebellar limb of the SC to brain stem pathway appeared to affect saccade amplitude preferentially with relatively modest effects on main-sequence relations, whereas our NRTP inactivation caused substantial deficits in both.

Comparison with inactivation of other saccade brain stem nuclei

In contrast, the saccade deficits caused by muscimol inactivation of the NRTP have certain features in common with those produced by lidocaine inactivation of the brain stem burst generator (Barton et al. 2003). Lidocaine placed among the putative excitatory burst neurons rostral to the abducens nucleus caused substantial reductions in the peak velocity ($>50\%$ estimated from data in their Tables 1 and 2) and increases in duration of ipsiversive saccades to 10 and 20° horizontal target steps. On the other hand, we estimate from their Tables 1 and 2 that the saccade gain for saccades to 10 and 20° target steps decreased by only 10–15%. Therefore their lesions had a relatively modest effect on saccade amplitude but slowed saccades considerably. Although saccade slowing was the first consequence of an NRTP injection in our monkeys, these deficits gradually escalated into a severe ipsiversive hypometria, i.e., a decrease in gain of over 80% in *monkey B* and over 60% in *monkey C*. Therefore the deficits produced by our injections differed qualitatively from those produced by local inactivations of the burst generator and could not be explained by involvement of those neurons.

Even if the effects of our NRTP muscimol injections, i.e., the slowing and eventual hypometria of ipsiversive saccades, cannot be explained by inadvertent involvement of the SC to BG to MN pathway, they also appear to be incompatible with the effects expected from impairment

of the SC to CB to MN pathway alone. Could an alternative direct pathway from the NRTP to MNs be involved? Injections of HRP into the abducens nucleus (Langer et al. 1986) or injections of tetanus toxin into the lateral rectus muscle (Horn et al. 1995) of the rhesus monkey label a small group of neurons in the dorsomedial NRTP. Moreover, anterograde label produced by injections into SC loci from which stimulation elicited small and large saccades lies in the same part of the NRTP (Büttner-Ennever et al. 1999), implying there could be a SC to NRTP to MN pathway. If damage to this pathway indeed underlies the saccade slowing produced by NRTP inactivation, it must lie outside the putative feedback loop that makes saccades accurate because the slower saccades early after the injection generally fall short of their targets (note those saccades in Fig. 8 were selected to have similar amplitudes). Some NRTP neurons could provide useful signals to abducens motoneurons because they have horizontal on directions, show a nice relation of number of spikes with saccade amplitude, and exhibit a burst that ends reliably with saccade end (Soetedjo et al. 2002a). However, it seems unlikely that elimination of this very modest pathway could have such a devastating effect on saccades if the SC to BG to MN pathway is still intact. Finally, recall that the available anatomical evidence indicates there are no projections from the NRTP to any element of the BG (Scudder et al. 1996b). Therefore it seems improbable that either the modest existing direct pathway to abducens motoneurons or the existence of a hitherto undiscovered brain stem pathway could explain the slowing of ipsiversive saccades.

If NRTP efferents do terminate mostly in the oculomotor cerebellum, how might this pathway function to explain the deficits we found? One possibility is that the NRTP provides the cerebellum with an efference copy of the desired saccade command from the SC. This signal then could be compared with feedback from the BG about the on-going saccade, i.e., the local feedback comparator would be implemented in the saccade portion of the cerebellum. A model with a cerebellar comparator successfully simulates some of the characteristics of CFN discharge (Optican 2005). If NRTP inactivation eliminates the desired saccade command reference signal, negative feedback of actual eye movement of a saccade launched by the SC input to the BG would truncate the saccade prematurely. Further experiments will be necessary to determine whether this suggested role for the NRTP has any merit.

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Fig. 1. Reconstruction of an injection site in the left nucleus reticularis tegmenti pontis (NRTP) of *monkey A*. **A:** high-power view of a transverse section through the rostral pons showing the injection site (red arrow) and a more medial recording track. Dashed line identifies the dorsal border of NRTP. *Inset:* same section at lower power. **B:** tracings of 3 low-power transverse sections that include the injection site (red arrow) and 2 sections 3.0 and 4.0 mm caudal to it. Omnipause neurons in the rip lie 4.0 mm caudal to our injection site. 4, trochlear nucleus; SC, superior colliculus; mlf, medial longitudinal fasciculus; nrtp, nucleus reticularis tegmenti pontis; Pyr, pyramidal tract; 6, rootlets of the abducens nerve; rip, nucleus raphe.

Fig. 2.

Effect of NRTP muscimol injections on the amplitude of targeting saccades in different directions for representative experiments on each monkey (C3, *A*, and B2, *B*). The graph shows the vertical and horizontal component amplitudes of saccades that were elicited by 10° target jumps that started straight ahead and were either horizontal or vertical or along vectors every 45° in between. The direction of the step was varied pseudo randomly. *A*: data from early in the experiment (between 10 and 13 min after completion of the injection when saccade accuracy still was normal, red squares), after 46 – 49 min (blue diamonds), and after 60 – 73 min (green circles) by which time the injection had its maximal effect. *B*: data from before the injection (red squares), between 0 and 8 min after the injection was complete (blue diamonds) and at 16 – 24 min (green circles), by which time the injection had its maximal effect. Ipsiversive saccades and the ipsiversive component of oblique saccades exhibited a substantial increasing hypometria, contraversive saccades a very modest increasing hypometria and vertical saccades

little change, if any. Black asterisks show target amplitudes in each direction and *not* final target positions.

Fig. 3.

Change in the gains of saccades to 10° horizontal target steps as a function of the time after a NRTP muscimol injection in 5 experiments on monkey B. *A*: ipsiversive saccades. *B*: contraversive saccades. Injection amounts were 160 nl (green circles, B6), 200 nl (yellow circles, B3), 240 nl (blue diamonds, B2), 250 nl (red squares, B1), and 520 nl (black triangles, B5). Gain reductions of ipsiversive saccades began earlier and were much greater than changes of contraversive saccades. Downward arrows show approximate times after the injection when ipsiversive gains had fallen to ~0.2.

Fig. 4.

Change in the gains of saccades to 10° horizontal target steps as a function of the time after each NRTP muscimol injection in 4 experiments on *monkey C*. *A*: ipsiversive saccades. *B*: contraversive saccades. Injection amounts were 150 nl (green triangles, C1), 250 nl (red squares, C2), 260 nl (blue diamonds, C3), and 400 nl (yellow circles, C4). Downward arrows show approximate times after the injection when ipsiversive gains had fallen to ~0.4. Note that C4 was a deliberate juxtamidline injection.

Fig. 5.

Comparison of saccade latencies just after an injection with those after ipsiversive saccade gain had dropped substantially. *A*: data from the 5 experiments illustrated in Fig. 3A. Latencies soon after the injection are taken from those trials where $0.9 < \text{gain} < 1.1$ (■). Latencies late in the experiment were taken from those trials where $\text{gain} \leq 0.2$ (▨). *B*: data from the 4 experiments illustrated in Fig. 4A. Latencies soon after the injection are taken from those trials where $0.9 < \text{gain} < 1.1$ (■ histogram). Latencies late in the experiment were taken from those trials where $\text{gain} \leq 0.4$ (▨). For both monkeys, ipsiversive saccade latencies at the lowest gains late in the experiment were much longer than those when saccades were accurate; average $\pm$ SD latencies for the normal and low gain data are shown at the top right of each histogram.

Fig. 6.

Examples of the multiple horizontal saccades to ipsiversive target steps at a time when ipsiversive saccade gain was low. Records in *A* and *B* are from experiments B1 and C2, respectively. Traces are horizontal eye (E_h ; black) and target (T ; gray) position.

Fig. 7. Demonstration of the change in velocity of ipsiversive saccades before a substantial hypometria had occurred. *A* and *B*: saccades to 20° ipsiversive target steps before (green) and soon after (red) muscimol injections into the NRTP in experiments B1 and C1, respectively. Saccades before and after the injection have been selected to have similar amplitudes. Traces in *top* and *bottom* are horizontal eye position and velocity, respectively. All traces aligned on saccade onset.

Fig. 8.

Changes in peak saccade velocity for horizontal saccades of similar sizes as a function of the time after the injection. *A*: data from experiments B3 ($\circ$) and B5 ($\bullet$). *B*: data from experiment C3. All saccades were to 10° horizontal target steps and were selected to have gains between 0.9 and 1.1.

Fig. 9.

Horizontal peak velocity and duration as a function of saccade amplitude for experiments B1 (A and C) and C2 (B and D). A and C: data from before the injection (blue squares), from the interval between the end of the injection and 8 min later (red diamonds) and from 8 to 24 min (green circles) after the injection. The trials between the vertical lines contributed to Fig. 7A. B and D: data from before the injection (blue squares), from the interval 19–23 min after the end of the injection (red diamonds) and from the interval 31–34 min after the injection (green circles). The blue and red lines are fit (see methods for equations) to the squares and diamonds, respectively. In both experiments, the slowed ipsiversive saccades fell off the preinjection main sequence fits whereas contraversive saccades largely did not.

Fig. 10.

Fits of duration and absolute peak velocity as a function of amplitude for ipsiversive and contraversive horizontal saccades for all experiments on *monkey B* (A) and *monkey C* (B). Pre- and postadaptation fits are solid and dashed lines, respectively. Postinjection data are from blocks that still displayed a range of ipsiversive amplitudes, which is indicated by the length of the fitted curves. For each monkey, fits with the same color are from the same experiment.

TABLE 1

Summary of experiments and attributes tested in each

Experiment No.	Injection		Attribute Tested				Vertical 10° Saccade
	Volume, nl	Side	General Deficits	Direction Tuning	Horizontal Amplitude Time Course	Horizontal Main Sequence	
B1	250	L					
B2	240	L	X	H only	X	X	X
B3	200	R	X	X	X	≤ 10° only	X
B4	80					H comp only	
B5	520	L	X	Obliq only	X	X	X
B6	160	L	X	X	X	X	X
C1	150	R	X	X	X	X	X
C2	250	L	X	X	X	X	X
C3	260	R	X	X	X	X	X
C4	400	R/L	X	X	X	X	X

L, left; R, right; X, if attribute tested. In B1 and B4, only horizontal and oblique saccades, respectively, were collected.