THE LOCAL LOOP OF THE SACCADIC SYSTEM CLOSSES DOWNSTREAM OF THE SUPERIOR COLLICULUS

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Abstract—Models of the saccadic system differ in several respects including the signals fed back to their comparators, as well as the location and identity of the units that could serve as comparators. Some models place the comparator in the superior colliculus while others assign this role to the reticular formation. To test the plausibility of reticular models we stimulated electrically efferent fibers of the superior colliculus (SC) of alert cats along their course through the pons, in the predorsal bundle (PDB). Our data demonstrate that electrical stimulation of the PDB evokes saccades, even with stimuli of relatively low frequency (100 Hz), which are often accompanied by slow drifts. The velocity and latency of saccades are influenced by the intensity and frequency of stimulation while their amplitude depends on the intensity of stimulation and the initial position of the eyes. The dynamics of evoked saccades are comparable to those of natural, self-generated saccades of the cat and to those evoked in response to the electrical stimulation of the SC. We also show that PDB-evoked saccades are not abolished by lesions of the SC and that therefore antidromic activation of the SC is not needed for their generation. Our data clearly demonstrate that the burst generator of the horizontal saccadic system is located downstream of the SC. If it is configured as a local loop controller, as assumed by most models of the saccadic system, our data also demonstrate that its comparator is located beyond the decussation of SC efferent fibers, in the pons. © 2006 IBRO. Published by Elsevier Ltd. All rights reserved.

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Saccades are stereotyped rapid eye movements generated by the coordinated activation of many cell classes distributed throughout the brain (Moschovakis et al., 1996). During saccades in their on-direction, extraocular motoneurons (MNs) emit bursts of discharge followed by tonic discharges proportional to the position reached by the eyes. MN bursts are generated by neural circuits, the burst generators, that once triggered run a preprogrammed course (Ron and Robinson, 1973). As proposed early on, the ability of these circuits to generate bursts and ensure saccadic accuracy is due to their configuration as feedback loop controllers (Robinson, 1973). The closed loop configuration of the burst generator is supported by considerable evidence. Firstly, some saccades (e.g. the slow ones produced by patients suffering from spinocerebellar atrophy) can be modified in mid-flight (Zee et al., 1976). Moreover, saccades pause in mid-flight in response to electrical stimulation of the pontine region containing omnipause neurons (OPNs) but resume their course and can acquire their targets after the end of the stimulation (King and Fuchs, 1977). Also, saccades executed toward a stationary target can vary considerably in terms of duration, yet their size is held within a very limited range of values. This phenomenon is common in normal subjects, and is particularly pronounced after i.v. injections of small doses of valium (Becker et al., 1981; Jürgens et al., 1981). Finally, lidocaine injection into the paramedian pontine reticular formation (PPRF) severely reduces the peak velocity of horizontal saccades, sometimes by as much as 50% (Barton et al., 2003). Their size, however, reduces by much less (10–20%) due to proportional increases in duration, and their accuracy is thus only moderately compromised.

Although there is general agreement regarding the closed loop configuration of the burst generator, the debate is still continuing as regards the signals it uses for its feedback and the location and identity of its comparator (reviewed in Sparks, 2002). Focusing on this latter issue, it is instructive to recall the model proposed by Robinson and his colleagues (van Gisbergen et al., 1981), who assumed that its feedback loop closes locally in the reticular formation, at the level of the medium lead burst (MLB) neurons (Fig. 1A). MLB neurons emit bursts encoding saccade size and they project to MNs, both directly and through the neural integrator (NI). The latter is composed of networks (subdivided into those that prefer horizontal movements and are housed in the nucleus prepositus hypoglossi (PH) (Navarro-López et al., 2004) and those that prefer vertical movements and are housed in the interstitial nucleus of Cajal (Dalezios et al., 1998)) that create the tonic eye position (E) related discharge also encountered in MNs. Additionally, the NI is thought to supply MLBs with an E feedback signal that they compare with a command signal of desired eye position (E*); equal to target position with...
A. Robinson’s model

![Diagram of Robinson’s bang-bang model]

B. Trans-collicular model

![Diagram of trans-collicular model]

Fig. 1. Schematic illustration of models of the local loop of the saccadic system. (A) Robinson’s bang-bang model. (B) Trans-collicular model. Solid and dashed lines symbolize excitatory and inhibitory connections, respectively. Dots indicate a series of intervening transformation steps. See text for further explanations.

respect to the head) supplied by higher order structures. The instantaneous difference between \( E' \) and \( E \) is the motor error signal (Me) encountered in MLB neurons. This drives the eyes until \( E \) matches \( E' \), whereupon Me is reduced to zero, MLB firing stops and the eyes land on target. Neither the intensity nor the duration of MLB bursts needs to be preprogrammed in this model. Instead, they are automatically adjusted for the desired saccade size through the operation of the feedback loop. Although other models of the saccadic system rely on feedback signals of eye displacement (\( \Delta E \)) to adjust the size of MLB bursts and they use long lead burst (LLB) neurons rather than MLB neurons as comparators (Scudder, 1988; Moschovakis, 1994), they likewise assume that the feedback loop closes locally in the reticular formation.

Fig. 1B illustrates an alternative model, which includes the superior colliculus (SC) in the feedback loop of the saccadic system. It was motivated by the observation that the decline of the instantaneous firing frequency curve of a particular class of collicular saccade-related burst neurons (“clipped” cells) is tightly related to the dynamic Me during saccades (Waitzman et al., 1991). This model assumes that the input to the saccadic system is a tonic signal that specifies the eye position error (EPe, equal to the distance between initial and \( E' \)). During a saccade, a corollary discharge of instantaneous distance from the initial eye position (\( \Delta E \)) provided by a resettable integrator (RI) is subtracted from the EPe signal and the result is conveyed by “clipped” cells to their target neurons in the midbrain and the pons. When \( \Delta E \) equals EPe, firing rate becomes zero, and the saccade terminates. Evidence in support of this scheme was obtained with the help of electrical stimulation of the OPN area. As predicted by the trans-collicular model, this prolongs the duration of the discharge of saccade related SC neurons till after the end of the stimulation and the acquisition of the target (Keller and Edelman, 1994). This configuration was maintained in a number of more recent models (e.g., Lefèvre and Galiana, 1992; Arai et al., 1994; Grossberg et al., 1997; Arai and Keller, 2005). Such single loop trans-collicular models suggest that the circuitry downstream of the SC would generate normal saccades even if it operated in an open loop configuration (Das et al., 1995). Implicitly, this means that all feedback signals necessary for saccade generation pass through the SC, and any local feedback loop in the lower brainstem is redundant or even nonexistent.

It should be possible to decide whether a saccade generating local loop exists in the pons by studying eye movements evoked in response to electrical stimulation of brain stem sites lying downstream of the putative comparator in the SC. If the comparator of the saccadic system is downstream of the SC, eye movements evoked by electrical stimulation of SC efferents should display the kinematic properties of normal saccades. If, on the other hand, a pontine comparator does not exist, electrical stimulation anywhere downstream of the SC should cause the eyes to move for the duration of the stimulus. Further, eye movements evoked in response to stimuli delivered as frequency steps, should be dominated by the dynamics of the oculomotor plant and of structures intervening between the point of stimulation and the plant (such as the NI). Their time course should therefore resemble ramps and/or exponential slow drifts rather than saccades.

To test the existence of a postcollicular comparator we electrically stimulated the major tectofugal fiber bundle, the predorsal bundle (PDB in Fig. 1), which conveys the output of the SC to the PPRF. Electrical stimulation of the pontine tegmentum has been long known to evoke eye movements (Bender, 1964) but, surprisingly, there is no conclusive evidence that saccades can be evoked from it including its most medial aspect, i.e. the PPRF (Cohen and Komatsu-zaki, 1972). Our results demonstrate that electrical stimulation of the PDB can generate saccades whose dynamics are well in the range of normal saccades in the cat and remain so after chemical inactivation or lesion of the SC. A preliminary version of our results has been presented in an abstract (Moschovakis et al., 2005).

**EXPERIMENTAL PROCEDURES**

We used four purpose-bred adult cats (code named E, F, G and PU), weighing 3–4 kg, complying with European Union regulations on biosafety and the use of live animals in research (directive 86/609), as well as with the “Principles of laboratory animal care” (NIH publication No. 86–23, revised 1985) and were approved by the institutional ethics committee. Ethical considerations obliged us to keep to a minimum the number of animals we employed and their suffering. They were surgically prepared for chronic recording and stimulation experiments under pentobarbital anesthesia (induction with 40 mg/kg, i.p., supplemented with 5 mg/kg/h, i.v.) and sterile conditions. Stainless steel bolts were cemented to the skull for painless head fixation, and three turns of Teflon insulated stainless steel wire were passed under the insertions of the extraocular muscles to form a search coil. For craniotomy, the skull was exposed from the bregma to the occipital crest and the center of the desired opening was marked on the occipital bone at the stereotaxic coordinate P13–P16, depending on the size of the skull. Using this mark we drew a circular contour (diameter 12 mm) around which the bone was removed with a dental drill paying particular attention to avoid any local damage to the dura. After verifying that the 10 mm chamber fits the opening, the dura was excised along its perimeter. Using a specially designed carrier the chamber was gently advanced and placed in touch with
the surface of the cerebellar vermis. The chamber was oriented in rostro-ventral direction, with an inclination of 27° relative to the stereotaxic coronal plane. When extrapolated to the dorso-ventral level of the rhombencephalon, its axis crossed the brainstem surface at the stereotaxic coordinate P3–P4, thus permitting the exploration of the pons from the abducens nucleus caudally (P6) to the pontomesencephalic junction rostrally. The narrow space between the chamber’s outer wall and the edge of the bone was then closed with small pieces of the Gelfoam before applying a small quantity of the fast-curing dental cement (Unifar, GC Corporation, Tokyo, Japan) all around the base of the outer wall of the chamber. The outside of the chamber (10 mm in height) was completely embedded from the bottom to the top with a slow-curing dental cement (Resivy, Prodont-Holliger S.A., Vence, France) which produces less heat during polymerization. After the cement became hard, the carrier head, which occupied the inner space of the chamber, was removed, the cerebellar surface and the inner walls of the chamber were thoroughly rinsed with sterile Ringer, followed by 1% hydrogen peroxide, and the chamber was filled with a gel containing dexamethasone and antibiotic (neomycin) and capped. In four experiments in one cat (cat E) we recorded responses to PDB stimulation after chemical inactivation and/or lesion of the SC. To gain access to the superior colliculi, openings were made bilaterally in the parietal bones at a later stage of the chronic experiment, under ketamine (20 mg/kg, i.m.) anesthesia. Between experiments, the openings were hermetically closed with bi-layer paraffin/plastic sheets after applying the dexamethasone–neomycin gel. The sheets were fixed by exerting cally closed with bi-layer paraffin/plastic sheets after applying the paraffin filled pressure on the lateral edges of the upward facing plastic with dexamethasone–neomycin gel. The sheets were fixed by exerting cally closed with bi-layer paraffin/plastic sheets after applying the paraffin filled pressure on the lateral edges of the upward facing plastic with dexamethasone–neomycin gel. The sheets were fixed by exerting cally closed with bi-layer paraffin/plastic sheets after applying the paraffin filled pressure on the lateral edges of the upward facing plastic with dexamethasone–neomycin gel. The sheets were fixed by exerting. For each stimulation frequency, we defined current threshold (T) as the lowest intensity at which movements were evoked by about one third of the total number of stimulus trains. At the highest frequency we used T varied between 4 and 35 μA (mean±S.D.: 12.9±5.8 μA, median: 10.0 μA). Since responses at intensities Addition of <5 μA were exceptional, we routinely started stimulat open each site by setting the intensity at 5 μA, and then increased it in steps of 5 μA. To avoid excessive stimulation we did not attempt a true threshold straddling with the use of still smaller steps. If no response was evoked at 40 μA, the site was classified as negative (no response). Stimulus trains were applied at intervals of 5 s for about 3–10 min, thus allowing us to collect data from 35–120 trials at each site, with a particular combination of stimulus parameters. During each block of trials we attracted the cat’s gaze to several ipsi- and contralateral locations so that evoked movements would start from a wide range of initial Es.

We used stereotaxic coordinates to guide stimulation electrodes toward the brain stem through the cerebellum. In each cat, we first recorded the discharge of single MNs to map the location of the abducens nucleus. In addition, we recorded the activity of OPNs to verify the location of the midline. The location of stimulation sites relative to anatomical landmarks was verified histologically. For this, we used biocytin-filled micropipettes (4% solution in 0.75 M NaCl buffered by 0.05 M Tris) in the last experiment in each cat. Biocytin was iontophoresed (−5 μA, pulse duration 0.6 s, repetition rate 1 Hz) at four or five points, 0.5 mm from the estimated midline and separated rostro-caudally by 0.8–1.0 mm. All injections were made at the same depth with respect to the stereotaxic reference point. Biocytin pipettes followed the trajectory of some of the tracks explored during the stimulation experiments. The biocytin reaction product was visualized in 50 μm serial sections, which were cut parallel to the electrode tracks and processed using conventional procedures (e.g., Moschovakis et al., 1998b). Biocytin deposits had dense cores, 0.2–0.3 mm in diameter. The 3-D coordinates of their centers were used to correct the stereotaxically estimated medio-lateral position and the depth of stimulation points. In this report we shall present the data collected in the rostral half of the pontine tegmentum, roughly corresponding to the antero-posterior extents of the nuclei reticularis pontis oralis (RPO) and reticularis tegmenti pontis (NRTP).

Evoked eye movements were sampled at a rate of 500 Hz. Their horizontal components were analyzed off-line using the Spike2 (Cambridge Electronic Design Ltd., Cambridge, UK) software. The onset of the stimulus trains as well as the onset and offset of saccades was marked as described before (e.g., Grantyn et al., 1996). The onset of horizontal saccade components was determined by velocity threshold 20°/s. The threshold was decreased to 15°/s for saccades smaller than 3° and correspondingly low peak velocities (in the range 20–45°/s). Unless they were followed by fast (>20°/s) postsaccadic drifts, we used the same velocity threshold to determine the end of saccades. In the former cases, we used the discontinuity of velocity traces at the transition between the rapid deceleration phase of saccades and the slow deceleration or fixation drifts.

In four experiments in one cat (cat E) we recorded responses to PDB stimulation after chemical inactivation and/or lesion of the contralateral SC. Injections of lidocaine (5% in 0.9% NaCl) were made in three experiments separated from each other by one week. Muscimol (0.3% in 0.9% NaCl) was injected in the fourth experiment. The injection barrel of three-barreled glass pipettes was connected to a 10-μl syringe through a needle embedded in its shaft. An adjacent barrel contained an electrolytically thinned tungsten wire. Its 50 μm diameter end protruded beyond the glass...
pipette for 150 μm. A sharp tip was formed by additional etching after fixing and insulating the wire inside the pipette. The third barrel was added to facilitate manufacture of straight, rigid shanks with parallel walls over lengths of 20–25 mm. It was filled with isotonic NaCl and was not used in our experiments. The overall diameter of our pipettes was 0.2–0.25 mm. To lower a pipette toward the SC the back end of its embedded needle was fixed to the syringe carried by a micromanipulator.

Tungsten electrodes were used to detect the collicular surface by monitoring visually evoked multiunit activity, to search minimal threshold depth for stimulation-induced saccades and to evaluate the effectiveness of the injection. We injected drug solutions by pressure in large volumes (3.5–5 μl, see Table 2) and over relatively short injection times (2–4 min). The first step of the experimental protocol consisted in recording spontaneous eye movements. During the second step we searched for PDB sites suitable for control records of evoked saccades. This search was guided by PDB mapping during the preceding eight experiments on the same animal, so that one track was enough to find the bundle. After recording control responses to stimulation of a selected PDB site, the electrode was left in the same position till the end of the experiment. Similarly, the positioning of injection pipette in the SC during the third step of the protocol was guided by mapping during preceding experiments and by the detection of visual activity in the superficial layers. Mapping in depth of T for eliciting saccades was accomplished on the borders of the PDB (Fig. 2), as delineated by the location, at the rostral limit of the pons, is marked with an asterisk in Fig. 2 (plot PU 4.3). The T was 8 μA at 330 Hz, it increased to 15 μA at 200 Hz and to 20 μA at 100 Hz. Saccadic components were clearly identifiable at all three frequencies and they always terminated long before the end of the stimulus train. Whenever E remained stationary after saccades (Fig. 3A), we could use the 20°/s velocity threshold to determine unambiguously saccade onset and offset. In such cases, saccade amplitude could be exactly measured and used to calculate the velocity–amplitude relationship. Evoked movements changed qualitatively with increasing pulse rate; low velocity ramp- or exponential-like movements lasting till the end of the stimulus train often followed saccades. Such movements resemble slow drifts evoked in response to the electrical stimulation of the SC (Grantyn et al., 1996). They have been attributed to relatively direct projections from the SC to extraocular MNs that largely bypass the burst generators (see Grantyn et al., 1996) and (Moschovakis et al., 1998a) for a list of fibers and neurons likely to mediate them. In the example shown in Fig. 3, postsaccadic slow movements (drifts) appeared first at 200 Hz (Fig. 3D, E) and their amplitude increased at 330 Hz (Fig. 3G, H). In such cases, we could not always use the same 20°/s velocity threshold to determine the end of saccadic components. Instead, we used the transition (inflection) from rapid (saccade offset) to slow (drift onset) deceleration on the velocity trace. In the absence of such inflections, the main sequence could not be determined. Such movements were considered not to conform to the kinematic characteristics of saccades. On the basis of this criterion, four PDB sites were judged not to evoke saccades.

Sites evoking movements with saccade-like velocity profiles were subjected to a further selection taking into account the relationship between the peak velocity and the amplitude of their horizontal components. These had to obey the main sequence relationship with minimal slopes defined individually from the spontaneous saccades of each cat. The values calculated from samples of spontaneous saccades were equal to 9.6, 7.9, 10.8 and 14.1°/s/° in cats E, G, F and PU, respectively. For evoked saccades we accepted values as small as 5.2 (cat E), 5.3 (cat G), 6.1 (cat F) and 7.7 (cat PU) °/s/°, which correspond to the slope of the main sequence curve of self-generated saccades with the slowest horizontal velocity (20% of the total sample). Regression slopes of evoked saccades depended on stimulus intensity and frequency. Increasing the stimulus intensity (by 1.25 to two times) while keeping the frequency constant (330 Hz) resulted in steeper slopes (two-tailed paired t-test: t = 5.88; df = 14; P < 0.0001; mean difference = 3.28°/s/°). The effect of changing the frequency of stimulation is illustrated for one PDB site from

**RESULTS**

Fig. 2 provides an overview of the region we explored with the aim to stimulate collicular axons descending in the PDB. Because stimulation near the midline might activate OPNs present throughout the caudal pontine reticular nucleus we limited the analysis to stimulus sites located in either side of the rostral pons, at the level of RPo (2.0–5.0 mm anterior to the abducens nucleus). Here we describe the effects of stimulation applied to points inside or on the borders of the PDB (n = 64), as delineated by the presence of its compact longitudinal fascicles. Responses from points located in the reticular core, 1.3–2.0 mm from the midline (PPRF points) were analyzed as a separate group. Deeper points, beginning with the dorsal border of the NRTP were discarded. We also discarded points inside the dorsal extension of the NRTP, which could have activated its dispersed cell groups invading the paramedian tracts.

** Movements evoked by PDB stimulation **

Movements evoked from PDB sites were always ipsiver- 

sive and usually consisted of a combination of fast and slow components. Fig. 3 illustrates examples of the horizontal component of eye movements evoked in alert cats in response to the electrical stimulation of one PDB site. Its location, at the rostral limit of the pons, is marked with an asterisk in Fig. 2 (plot PU 4.3). The T was 8 μA at 330 Hz, it increased to 15 μA at 200 Hz and to 20 μA at 100 Hz. Saccadic components were clearly identifiable at all three frequencies and they always terminated long before the end of the stimulus train. Whenever E remained stationary after saccades (Fig. 3A), we could use the 20°/s velocity threshold to determine unambiguously saccade onset and offset. In such cases, saccade amplitude could be exactly measured and used to calculate the velocity–amplitude relationship. Evoked movements changed qualitatively with increasing pulse rate; low velocity ramp- or exponential-like movements lasting till the end of the stimulus train often followed saccades. Such movements resemble slow drifts evoked in response to the electrical stimulation of the SC (Grantyn et al., 1996). They have been attributed to relatively direct projections from the SC to extraocular MNs that largely bypass the burst generators (see Grantyn et al., 1996) and (Moschovakis et al., 1998a) for a list of fibers and neurons likely to mediate them. In the example shown in Fig. 3, postsaccadic slow movements (drifts) appeared first at 200 Hz (Fig. 3D, E) and their amplitude increased at 330 Hz (Fig. 3G, H). In such cases, we could not always use the same 20°/s velocity threshold to determine the end of saccadic components. Instead, we used the transition (inflection) from rapid (saccade offset) to slow (drift onset) deceleration on the velocity trace. In the absence of such inflections, the main sequence could not be determined. Such movements were considered not to conform to the kinematic characteristics of saccades. On the basis of this criterion, four PDB sites were judged not to evoke saccades.

Sites evoking movements with saccade-like velocity profiles were subjected to a further selection taking into account the relationship between the peak velocity and the amplitude of their horizontal components. These had to obey the main sequence relationship with minimal slopes defined individually from the spontaneous saccades of each cat. The values calculated from samples of spontaneous saccades were equal to 9.6, 7.9, 10.8 and 14.1°/s/° in cats E, G, F and PU, respectively. For evoked saccades we accepted values as small as 5.2 (cat E), 5.3 (cat G), 6.1 (cat F) and 7.7 (cat PU) °/s/°, which correspond to the slope of the main sequence curve of self-generated saccades with the slowest horizontal velocity (20% of the total sample). Regression slopes of evoked saccades depended on stimulus intensity and frequency. Increasing the stimulus intensity (by 1.25 to two times) while keeping the frequency constant (330 Hz) resulted in steeper slopes (two-tailed paired t-test: t = 5.88; df = 14; P < 0.0001; mean difference = 3.28°/s/°). The effect of changing the frequency of stimulation is illustrated for one PDB site from
cat PU in Fig. 3. The slope of the main sequence curve was equal to 8.1°/s at the stimulation frequency of 100 Hz (Fig. 3C) and it increased to 10.3 at 200 Hz (Fig. 3F) and 13.7°/s at 330 Hz (Fig. 3I). These values satisfied well the criterion of the minimal main sequence slope adopted for cat PU (7.7°/s). Stimulus sites at which fast movements did not attain this main sequence criterion were considered as “non-saccadic.” On the basis of this criterion, another
two PDB sites were judged not to evoke saccades. Overall, the slopes of the main sequence curves of saccades evoked with optimal combinations of stimulus intensity and pulse rate ranged from 5.9–19.8°/s/° (mean ± S.D.: 11.9 ± 3.0°/s/°) and values greater than 9°/s/° were observed in 80% (46/58) of the effective PDB stimulation sites. Thus, the majority of our PDB sites evoked saccades with main sequence slopes in the range previously reported for spontaneous saccades in the cat (9°/s/°; Evanger and Fuchs, 1978; Guitton et al., 1984). Frequency dependent increases of the slope of the main sequence curve such as those illustrated in Fig. 3 were significant in all sites of cat PU and other cats from which enough data were collected to evaluate them statistically (e.g. paired t-test: t = 3.03, df = 14, P = 0.009, mean difference = 1.74°/s/°, when the frequency was increased from 100 to 330 Hz).

Having shown that stimulation of the PDB evokes saccades we wished to compare them with saccades evoked from the SC. To this end we used data obtained from the PDB for the purposes of this report (15 sites in cats E and PU) as well as data obtained from the SC in cat E and in previous experiments (22 sites in nine animals) using identical stimulation parameters (200 Hz and 2×T). Table 1 provides a summary description of the main sequence curves of the saccades evoked from all these sites. As shown here, the sample of saccades evoked from the PDB is well matched to that of saccades evoked from the SC in that the size of the characteristic vectors was equal to 8.87° (S.D.: 2.92) when averaged across PDB sites and 7.70° (S.D.: 3.26) across SC sites. Table 1 also documents the only noticeable difference we found between PDB- and SC-evoked saccades, namely that the slope of the main sequence curve of saccades evoked from the SC (mean ± S.D.: 13.2 ± 2.70°/s/°) was, on the average, steeper (P < 0.05, Student’s t-test) than that of saccades evoked from the PDB (mean ± S.D.: 11.3 ± 2.3°/s/°).
Table 1. Comparison of saccades evoked in response to stimulation of the SC and of the PDB with stimulation at pulse rate 200 Hz and relative intensity 2×T

<table>
<thead>
<tr>
<th>Subject</th>
<th>Site</th>
<th>(S_{X})</th>
<th>MS Slope</th>
<th>(r^2)</th>
<th>MS Y-int</th>
<th>Staircases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC</td>
<td>NB2</td>
<td>6.2</td>
<td>13.9</td>
<td>0.99</td>
<td>8.0</td>
<td>75</td>
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<tr>
<td></td>
<td>NB3</td>
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<td>15.4</td>
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<td>12.3</td>
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<tr>
<td></td>
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<td>63</td>
</tr>
<tr>
<td></td>
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<td>14.6</td>
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<td></td>
<td>STAN</td>
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<td>4.5</td>
<td>14.4</td>
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<td></td>
<td>BN2</td>
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<td></td>
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</tr>
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<td></td>
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<td>0</td>
</tr>
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<td>11.4</td>
<td>0.94</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
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<td>14-1-1</td>
<td>9.4</td>
<td>12.4</td>
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<td>5.6</td>
</tr>
<tr>
<td></td>
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<td>16-1-1</td>
<td>11.5</td>
<td>11.5</td>
<td>0.93</td>
<td>14.1</td>
</tr>
<tr>
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<td></td>
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<td>16.6</td>
</tr>
<tr>
<td>PDB</td>
<td>E</td>
<td>4-3-3</td>
<td>8.5</td>
<td>15.1</td>
<td>0.97</td>
<td>0.1</td>
</tr>
<tr>
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<tr>
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<td>13.5</td>
<td>0.96</td>
<td>24.4</td>
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<tr>
<td></td>
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<td>12.8</td>
<td>0.98</td>
<td>2.7</td>
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<td></td>
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<td>11.4</td>
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<td>9.1</td>
</tr>
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<td>11.2</td>
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<td>9.4</td>
<td>0.93</td>
<td>15.7</td>
</tr>
<tr>
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<td>7.1</td>
<td>12.5</td>
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<td>9.0</td>
<td>0.89</td>
<td>20.1</td>
</tr>
<tr>
<td></td>
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<td>9.9</td>
<td>0.96</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
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<td>8-1-5</td>
<td>12.0</td>
<td>13.7</td>
<td>0.98</td>
<td>4.5</td>
</tr>
</tbody>
</table>

Columns contain the following data (from left to right): 1, code name of experimental animals; 2, code names of stimulation sites; 3, characteristic vector \((S_{X})\) of the horizontal component of saccades; 4, slope of the main sequence curve (MS-Slope, \(h^2\)); 5, goodness of fit \((r^2)\) of the linear regression describing the main sequence; 6, intercept of the main sequence curve (MS Y-int, deg); 7, percentage of saccadic staircases (Staircases \%) encountered in stimulation trials evoking saccades.

Measurements of latency also provide a criterion for comparing PDB- and SC-evoked saccades. It should be noted that the latency of PDB evoked saccades depended on the intensity and frequency of stimulation. For example, the mean latency dropped by 32 ms (paired \(t\)-test: \(P<0.0001\), \(t=7.09\), \(df=14\)) when the intensity of 300 Hz stimuli was augmented by factors ranging \(\times1.25\) to \(\times2.0\). Similar drops of latency accompanied increments of intensity tested at 100 Hz and 200 Hz. They also accompanied increments of frequency; for example, mean latency dropped by 43.8 ms (paired \(t\)-test: \(P<0.0001\), \(t=9.48\), \(df=14\)) when the frequency of stimuli of the same intensities \((1.15–2.0\times T)\) was raised from 100 to 330 Hz. When saccades evoked in response to identical stimulation parameters are considered (frequency: 200 Hz, intensity: \(2\times T\)), the mean latency of PDB-evoked saccades was statistically indistinguishable from the one we previously reported for SC-evoked saccades (mean±S.D.: 50±19 ms; Grantyn et al., 1996).

As with SC-evoked ones, saccades evoked from the PDB were often oblique probably due to the activation of the ventral ascending (Av) branches of tectofugal axons which bifurcate before and, less frequently, after crossing to the opposite side in Meynert’s decussation (Grantyn and Grantyn, 1982; Moschovakis and Karabelas, 1985; Moschovakis et al., 1988a,b). Rostrally, these branches reach the fields of Forel, including its medial portion that is homologous to the rostral interstitial nucleus of the medial longitudinal fasciculus (rMLF) of primates (Harling et al., 1980). This nucleus houses both upward and downward pre-MNs (Moschovakis et al., 1991a,b) in both sides of the brain. Because we could not ensure the selective activation of axons whose Av collaterals project to one or the other antagonistic group, we did not expect that our PDB stimulation would evoke saccades with an obvious vertical directional preference. Indeed, vertical components satisfying our saccade identification criteria were observed in only 22 of 58 stimulation sites. Their characteristic vectors were small (range: 0.01–4.6°; mean±S.D.: 1.6±1.19°; \(n=22\)) and inconsistent, tending to drive the eyes to vertical zero. Accordingly, we did not feel that stimulation of the PDB is suitable for evaluating the contribution of post-collicular structures to the generation of vertical saccade components, and we thus did not study them in any quantitative detail.

Electrical stimulation of the SC with long trains of pulses is known to evoke sequences of similar saccades which follow closely the one after the other and are for this reason called saccadic staircases (Robinson, 1972). Fig. 4 demonstrates that staircases of saccades can be evoked in response to stimulation of the PDB as well. However, it was more difficult to evoke such staircases from the PDB than it is to evoke them from the SC. Table 1 includes information about the frequency with which they were observed in response to stimulation of the same 15 PDB sites and 21 of the 22 SC sites tested, expressed in terms of the number of trains evoking staircases over the total number of trains that evoked primary saccades. As shown here, a significant percentage of staircases (\(>10\%\)) could be evoked from about half of the...
SC sites tested (12/21) but only from two of the 15 PDB sites. The lower efficacy of the PDB stimulation was also confirmed by counts in records from another 39 PDB sites, tested with various combinations of stimulation parameters. In this sample, seven sites were effective in inducing saccadic staircases with probabilities of 12–57%.

** Movements evoked by PPRF stimulation **

Although we did not attempt a systematic analysis of the oculomotor responses evoked from the reticular core, we collected data from 28 PPRF sites separated from the paramedian tracks or from the dorsal border of the NRTP by at least 0.5 mm (Fig. 2) along 10 tracks we made at the same antero-posterior levels as those used to explore the effects of PDB stimulation, and at distances 1.3–2.1 mm from the midline. No saccade-like movements could be evoked from 18 of these 28 PPRF sites. As shown in Fig. 5C–F, eye movements evoked from these sites lasted for the duration of the stimulus train. The time course of E followed ramp-like (E, F) or exponential (C, D) trajectories. In the case of exponential movements, peak velocities could reach values as high as 50°/s but ramp-like movements rarely reached 20°/s. In no such case was it possible to detect a discontinuity during the decremental phase of the velocity profiles. Discontinuities were sometimes seen in the responses obtained from the remaining 10 reticular sites and were analyzed to reveal the presence of saccadic components. At a stimulation frequency of 330 Hz, saccade-like velocity profiles (e.g., Fig. 5A, B) were observed in four sites only (all in cat PU). In two cases, the
incidence ratios (IR, see below for definition) and hence the number of such responses were too low for a quantitative analysis \((n=7 \text{ and } n=10 \text{ at a stimulus intensity of } 30 \text{ and } 20 \mu A, \text{ respectively})\). Movements with saccade-like velocity profiles were evoked, somewhat more frequently \((n=18 \text{ and } n=15 \text{ at } 25 \mu A)\), from the other two sites. The slopes of the regression lines relating peak velocity to amplitude were 6.74 and 8.36°/s/°, only the latter (examples of which are shown in Fig. 5A, B) passing the criterion adopted in cat PU (7.7°/s/°). The amplitudes of saccades evoked from these two sites depended on initial \(E\) (\(k_{\mu}=0.39, 0.40; \text{ see Eq. 1 below for definition} \)) and had characteristic vectors equal to 2.25 and 4.71°. Because all four of the reticular sites from which we could evoke saccade-like movements were separated from the PDB by only 0.5–0.6 mm and because test currents ranged from 20 to 30 μA, stimulus spread to the PDB cannot be excluded.

At a stimulation frequency of 100 Hz, movements with saccade-like velocity profiles were evoked from six PPRF sites (four in cat G and two in cat PU). IR were low \((IR=0.22–0.59)\), so that regression analysis could be applied to the data from four sites only. The slopes of their main sequence curves were shallow \((5.82–6.61°/s/°)\) and passed the criterion in cat G (two sites) but not in cat PU. In addition, responses evoked from these four reticular sites were characterized by high thresholds \((25–35 \mu A)\) and small characteristic vectors \((0.44–2.35°)\). As with the sites tested at 330 Hz, and because of the proximity to the PDB and the intensity of the current used \((30–40 \mu A)\) its spread to the PDB cannot be excluded, except for one low efficacy site at a distance of 1.0 mm from the PDB (Fig. 2, E 3.25).

To summarize, we encountered considerable difficulty in evoking saccades from the reticular formation with the prolonged stimuli we employed. In most sites that evoked eye movements satisfying the criteria we adopted for identification of saccades, current spread to the PDB could not be excluded. Sites located further away, consistently gave rise to either slow, clearly non-saccadic, movements or to unreliable saccade-like responses of low peak velocities.

**Location of stimulation sites and their relative efficacy**

As shown in previous studies, the size of saccades evoked by electrical stimulation of the feline SC depends on the initial position of the eyes (e.g., McIlwain, 1986; Grantyn et al., 1996). In studies, such as the present one, which are limited to their horizontal components, this relationship obtains the form,

\[
\Delta H = S_{\mu} - k_{\mu} H_1
\]

where \(\Delta H\) is the amplitude of the horizontal component of saccades, \(H_1\) is the initial horizontal position of the eyes,
S_H is the horizontal component of the “characteristic” vector (McIlwain, 1986) and k_H is a coefficient characterizing their position sensitivity. As shown in Fig. 6, the size of the horizontal component of saccades evoked in response to stimulation of the PDB also depended on the initial horizontal E. In this particular case, S_H was equal to 8.16°, k_H to 0.65 and 94% of the variance of the dependent variable can be accounted for by changes of initial E. Except for one site in which the regression was not statistically significant, similar expressions describe the position sensitivity of saccades evoked from all our PDB sites, albeit with different S_H and k_H values. Absolute values of S_H ranged between 1.4 and 12.8°, and therefore correspond to the characteristic vectors of saccades evoked from the rostral half of the SC (Grantyn et al., 1996). The amplitude of evoked saccades depended on the intensity of stimulation as indicated by the fact that mean S_H was 2.9° bigger (paired t-test: P<0.0001, t=7.2, df=14) when relative intensities were increased 1.25-two times while the frequency was kept constant at 330 Hz. Also consistent with the analysis of responses to SC stimulation (Moschovakis et al., 1998a), values of k_H tended to increase together with the size of the horizontal component of characteristic vectors (k_H=0.39+0.03S_H, r^2=0.26, P<0.0001) and ranged between 0.28 and 0.88. The ratio S_H/k_H defines the initial position beyond which the direction of saccades should reverse (the x-intercept of the plot illustrated in Fig. 6). These initial positions of expected reversals could be as small as 2° in our sample of PDB sites. Although we explored sufficiently large and symmetrical ranges of H, we never observed overt reversals of saccade directions, but the probability to evoke saccades decreased as H, approached the midline, and in some cases we were unable to evoke centrifugal saccades when the eyes were initially deviated into the ipsilateral half of the oculomotor range (Fig. 3A, D). Accordingly, to compare the efficacy of different PDB stimulation sites we first calculated the IR, defined as the ratio of the number of movements we could accept as saccadic (based on their velocity profile and the slope of their main sequence curve) and starting from H, in the contralateral half of the oculomotor range over the total number of stimulation trials used to test a certain combination of stimulus parameters at each site and limited to the same range of H. The values IR obtained with site-specific optimal combinations of stimulus parameters ranged from 0.13–1.0 and over half of them (53.4%) surpassed 0.9. Typically, IR increased at higher stimulation intensity and frequency. For example, it increased from 0.86 at 100 Hz to 1.0 at 200 and 330 Hz for the site that gave rise to the movements illustrated in Fig. 3.

We felt that IR would not suffice to characterize the efficacy of a site because it disregards the site’s threshold for saccades (T) and the relative intensity (I/T) at which its optimal IR was obtained. Accordingly, we decided to correct it by weighing with a coefficient (K), calculated as K=(40–T)/(I/T), where I is intensity (in μA) at which the optimal IR was obtained and 40 corresponds to the upper limit of T at which a site was still accepted as an effective one (40 μA, see Experimental Procedures). The numerator of the formula we used to calculate K weighs IR in favor of sites with low threshold (e.g. it will be equal to 36 for T=4 μA, and 5 for T=35 μA). Its denominator penalizes sites where the optimal IR was obtained at higher relative intensities (I/T). We normalized K to obtain a new estimate of the sites’ efficacy, the efficacy index, EI=IR×K/28.8, where 28.8 is the highest value of K we encountered. Fig. 2 shows (as symbols of size proportional to EI) the EI values we obtained in all PDB and PPRF sites we studied, thus reflecting their efficacy in generating saccades. Of the 64 PDB sites tested, six were assigned a value equal to 0 (Fig. 2, open circles). They include points from where no responses could be evoked (n=2) or which evoked slow drifts only (another two sites). While movements with velocity profiles corresponding to saccades could be induced from the remaining two sites, the slope of their main sequence curve was too shallow to classify them as saccades (cat PU: 4.2 and 6.2°/is/°). Fifty percent of the EI values we encountered were confined to a range of 0.46–0.59, and values equal to or above 0.7 were exceptional (five of 58 sites). In the one site where we encountered a value of EI equal to 1.0, T was equal to 4 μA, and IR obtained a value of 1.0 already at a current intensity of 5 μA (1.25×T). The main effect of using EI rather than IR was to reduce the number of sites with maximal efficacy rating (1.0). Based on IR such high rating would be given to 41.4% of the sites we studied. In contrast, the EI values of these sites were distributed between 0.4 and 1.0. Thus, taking in consideration the threshold and the relative intensity usually moderated our judgment of a site’s efficacy, as illustrated by the example of a site in which T=15 μA, and IR=1.0 was obtained at 30 μA. Because of its fairly high threshold and the high relative intensity (2×T) we employed, the rating of its efficacy was reduced to 0.43 with the use of EI. On the other hand, the rating of the least effective sites (IR<0.5) remained low when EI was used instead (0.056–0.25).
Deactivation of the SC

In the monkey, most of the SC efferent fibers entering the PDB deploy recurrent collaterals near the cell body they originate from in the SC (Moschovakis et al., 1988a,b). Although this is much less frequently the case in the cat, PDB fibers emitting recurrent collaterals have been encountered in this species as well (Grantyn and Grantyn, 1982; Moschovakis and Karabelas, 1985). Because PDB fibers are glutamatergic, and are thus likely to exert excitatory influence on their targets (Mooney et al., 1990; Büttner-Ennever and Horn, 1994) it might be argued that the saccades that we evoked from the PDB are in fact due to the antidromic activation of the SC. If this were the case, evoked saccades might not be due to bursts generated by a pontine comparator receiving step signals (e.g. the pulse trains we employed) orthodromically propagated by PDB axons. Instead, they might be due to bursts generated in the SC due to the antidromic activation of recurrent collaterals. Furthermore, in case the feedback loop from the pontine saccade generator includes the SC, the latter could be activated by the PDB stimulation. The effect of axon reflex or other feedback signals on the collicular circuits could be mediated by output neurons at the subliminal fringe of the stimulation focus, i.e. those escaping the occlusion by antidromic invasion. To address this concern, we chemically inactivated the SC to test if, after this, saccades could still be evoked from the PDB and if so whether their dynamics and metrics would be altered.

Injection sites in the left SC (Exps. 1L and 2L; see Table 2 for results) were verified histologically from the glial reaction surrounding the pipette tracks. In our last experiment (4M), injection in the right SC was followed by a hemorrhage that destroyed a large portion of the nucleus (see below). The two sites in the right SC (3L and 4M) were

Table 2. Effects of unilateral inactivation of the SC on spontaneous saccades and on saccades evoked by stimulation of the PDB

<table>
<thead>
<tr>
<th>Variable</th>
<th>Exp 1L</th>
<th>Exp 2L</th>
<th>Exp 3L</th>
<th>Exp 4M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Side of SC inactivation</td>
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<td>Left</td>
<td>Right</td>
<td>Right</td>
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<tr>
<td>Drug used</td>
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<td>Lidocaine</td>
<td>Lidocaine</td>
<td>Muscimol*</td>
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<td>Injected volume (μl)</td>
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<td>5.0</td>
<td>5.0</td>
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Spontaneous saccades

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<th>Test</th>
<th>Control</th>
<th>Test</th>
<th>Control</th>
<th>Test</th>
<th>Control</th>
<th>Test</th>
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<tbody>
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<td>Time after injection end (min)</td>
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<td>1–3.5</td>
<td>3–4.5</td>
<td>5–6.5</td>
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<tr>
<td>Main sequenceb</td>
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<td>7.06</td>
<td>9.52</td>
<td>5.91</td>
<td>11.48</td>
<td>5.47</td>
<td>11.11</td>
<td>x*</td>
</tr>
<tr>
<td>R²</td>
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<td>0.89</td>
<td>0.77</td>
<td>0.66</td>
<td>0.75</td>
<td>0.90</td>
<td>0.79</td>
<td>x*</td>
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<td>0.0367</td>
<td>0.03295</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saccade frequencyb (1/min)</td>
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<td>3.6</td>
<td>24.6</td>
<td>7.8</td>
<td>26.4</td>
<td>9.0</td>
<td>21.6</td>
<td>0</td>
</tr>
<tr>
<td>Mean E shiftd (°)</td>
<td>–4.7</td>
<td>–3.6</td>
<td>4.9</td>
<td>15.4</td>
<td></td>
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Saccades evoked by stimulation of the PDB

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<th>Control</th>
<th>Test</th>
<th>Control</th>
<th>Test</th>
<th>Control</th>
<th>Test</th>
<th>Control</th>
<th>Test</th>
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<td>Right</td>
<td>Left</td>
<td>Left</td>
<td></td>
<td></td>
<td></td>
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<td>100</td>
<td>200</td>
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<tr>
<td>Intensity (μA)</td>
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<td>9</td>
<td>18</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intensity (×T)</td>
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<td>×1.5</td>
<td>×2.0</td>
<td>×1.7</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Time after injection end (min)</td>
<td>3–10</td>
<td>4–9</td>
<td>5–10</td>
<td>7–14</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Incidence ratioa</td>
<td>0.88</td>
<td>0.94</td>
<td>0.98</td>
<td>1.00</td>
<td>0.83</td>
<td>0.88</td>
<td>0.66</td>
<td>0.83</td>
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<tr>
<td>Mean latency (ms)</td>
<td>75.1</td>
<td>65.8</td>
<td>82.3</td>
<td>63.0</td>
<td>86.6</td>
<td>84.5</td>
<td>84.0</td>
<td>94.8</td>
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<tr>
<td>Difference of latencies (P)</td>
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<td>&lt;0.0001</td>
<td>0.7140</td>
<td>0.0149</td>
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<td>Characteristic vector Sx (°)</td>
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<td>6.43</td>
<td>6.81</td>
<td>–4.79</td>
<td>–3.69</td>
<td>–5.08</td>
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<tr>
<td>Difference of Sx (P)</td>
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<td>xx*</td>
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</tr>
<tr>
<td>Main sequenceb</td>
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<td>10.24</td>
<td>9.59</td>
<td>10.94</td>
<td>8.78</td>
<td>6.93</td>
<td>10.81</td>
<td>7.67</td>
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<td>R²</td>
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<td>0.74</td>
<td>0.90</td>
<td>0.95</td>
<td>0.80</td>
<td>0.83</td>
<td>0.88</td>
<td>0.76</td>
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<td>Difference of slopes (P)</td>
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<td>0.00095</td>
<td>0.00727</td>
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<td></td>
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</tr>
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</table>

a Muscimol injection was accompanied by hemorrhage and large lesion.

b Included are only data on spontaneous saccades in the same direction as that of saccades evoked by PDB stimulation, in corresponding SC-inactivation experiments.

c During the indicated recording time the cat was unable to generate leftward (contra-lesional) spontaneous saccades.

d Difference between mean horizontal E during post- and pre-injection records. Negative shifts are to left, positive to right.

e Incidence ratio is calculated by dividing the number of evoked saccades by the number of stimulus trains when initial horizontal Es are in the hemifield opposite to the side of stimulation.

f Statistical significance of the difference between control and test Sx cannot be tested because the slopes of regression lines relating amplitude to the initial eye position differ significantly (experiment 2L: P<0.0001; experiment 3L: P=0.0019).
therefore reconstructed from known positions of stereotaxically placed electrolytic lesions. In projections on the horizontal plane, two sites (Exp. 2L and 3L) were located near the center of the SC. The medio-lateral locations of the other two sites (Exps. 1L and 4M) were also in the middle of the SC but they were placed more caudally, at the border between the rostral two thirds and the caudal third of the SC. In Exps. 1L and 3L, injections were centered on a point in the lower half of the intermediate gray layer, and in Exps. 2L and 4 M in the deep gray layer near its border with the midbrain reticular formation. Histological examination of the left SC, 3–4 weeks after the injection revealed dense glial reaction and disappearance of neurons in zones surrounding the calculated positions of pipette tips. In Exp. 1L and 2L the diameters of these zones measured 2.0 and 1.3 mm medio-laterally and 2.4 and 3.0 mm dorso-ventrally, respectively. Clearly, injections of large volumes of liquid resulted in local damage of the SC. Not surprisingly, electrical stimulation of nearby SC sites in three lidocaine experiments did not evoke any response immediately after the end of the injection, even when tested with high currents (50–60 μA) and no recovery was seen at a later time (after 10–30 min). In the muscimol experiment, local SC stimulation became ineffective during the adjustment of the final position of the pipette, due either to drug leakage or to mechanical lesion. Histological evaluation of the material two weeks after this experiment showed extensive hemorrhage that destroyed all layers of the caudal two thirds of the SC, as well as the dorsal mesencephalic reticular formation laterally to the central gray.

To judge the severity of collicular inactivation we analyzed the spontaneous eye movements evoked 1–4.5 min after injection of lidocaine. These served as a control for the responses to PDB stimulation that followed immediately afterward (Table 2). Soon after lidocaine injection, the mean horizontal E shifted by 4–5° to the side of the injected SC. Fixation at greater eccentricities in the ipsilesional hemifield became unstable. It was always accompanied by slow centripetal drifts and occasionally by nystagmus-like patterns with ipsilesional quick phases. Contra-lesional saccades could cross the midline but terminated at small eccentricities (<5°). Fixations in the contra-lesional hemifield were of brief duration and sometimes unstable, showing centripetal drifts. The total time of fixation in the contra-lesional field occupied only 17–21% of the total duration of the records, in contrast to the roughly symmetrical occupancy of both hemifields prior to injection. Effects on spontaneous saccades in the same direction as those evoked by PDB stimulation, i.e. contraversive to the inactivated SC, are summarized in Table 2. In spite of our active efforts to provoke orienting movements, the frequency of saccades was substantially reduced. The ranges of saccade amplitudes and peak velocities decreased, as did the slope of the main sequence curve (by 35–52%). Unexpectedly, ipsilesional saccades were depressed in a roughly similar manner. In two lidocaine experiments, no signs of recovery of spontaneous saccades were seen 15–25 min after the injection, but it was complete 1 h later. In the third experiment (Exp. 3L, Table 2) recovery was almost complete 20 min after the injection. Accordingly, we used the earliest post-injection responses to PDB stimulation (3–10 min) to evaluate the effect of SC-inactivation. It should be noted that none of our lidocaine experiments resulted in long-term changes of the cat's posture and oculomotor behavior.

As noted above, muscimol injection (Exp. 4M, Table 2) was accompanied by vascular damage and a large lesion of the SC and subjacent tegmentum. The effect on spontaneous eye movements was devastating. Mean horizontal E shifted by more than 15° into the ipsilesional hemifield, and remained there for the duration of the experiment (2 h). Contra-lesional saccades disappeared soon (5–6.5 min) after the injection, and the few ipsilesional ones were of small amplitude (<5°) and low velocity (<40°/s). Slow centripetal drifts and short periods of nystagmoid movements rendered stable fixation impossible. These symptoms improved little when the animal was examined at later times. For example, contra-lesional saccades were seen again 45 min after the injection but only very infrequently. Starting from far eccentric Es, they never crossed the midline, so that all eye movements were made between about 5 and 20° of eccentricity in the ipsilesional hemifield. After release from the experimental setup the cat displayed curving of the body toward the lesioned side and intermittent circling in the same direction. These symptoms disappeared about 20 h later but neglect of contra-lesional space persisted for at least three days, and recovery was incomplete during the remaining two weeks of observation.

Fig. 7 compares preand post-injection (made between 4 and 9.5 min after the end of the injection) records of saccades evoked from the right PDB after lidocaine injection (5 μl) in the left SC. In this, and all other SC injection experiments we used the same stimulus parameters to obtain pre- (control) and post-injection (test) records. We did not measure thresholds after injection, assuming that their change should be reflected in the change of the IR of evoked saccades. A comparison of control (A–C) and test (D–F) responses does not reveal any systematic effect of SC-inactivation on the time course, amplitudes and velocities of PDB-evoked saccades (see also Fig. 8, 2L). If anything, the reproducibility of movements was improved in this particular example. Effects of SC-inactivation on the main sequence relationships obtained from four experiments are illustrated in Fig. 8, and Table 2 summarizes the changes of several additional parameters. Data presented in Fig. 8 permit us to qualitatively conclude that effects of lidocaine inactivation of the SC on the main sequence of PDB-evoked saccades are very weak and inconsistent. The slope of the regression line did not change in Exp. 1L, showed a slight but statistically significant (P<0.001) increase in Exp. 2L and was depressed in Exp. 3L (P<0.01). It should be noted, however, that in the latter case the slope of the main sequence relationship of spontaneous saccades was depressed by 52%, as compared with 21% for PDB-evoked saccades. The probability to evoke saccades from contralateral initial Es did not change appreciably, and laten-
Fig. 7. Horizontal components of eye movements evoked in response to the electrical stimulation of the PDB before (A–C) and after (D–F) injection of lidocaine in the left SC. Records are from experiment 2L (see Table 2 for additional quantitative data). Superimposed traces (A, B) and main sequence equation (C) are obtained from one of the two control records (n=39). Equation parameters are not identical to those in Table 2 because the latter is based on two control records (n=126). Layout and conventions as in Fig. 5.
cies either did not change or became shorter. A stronger depression of response to PDB stimulation was observed in experiment 4 M only, in which a combination of muscimol injection and an extensive vascular lesion led to the complete absence of self-generated contra-lesional saccades. Nonetheless, PDB stimulation still evoked saccades with a high probability and the depression of the slope of the main sequence curve (by 29%) was smaller than the effect on spontaneous saccades in lidocaine experiments. The E sensitivity of PDB-evoked saccades was also impervious to SC lesions since the slope of regression lines such as that illustrated in Fig. 6 was not altered appreciably by either lidocaine or muscimol injections in the contralateral SC.

**DISCUSSION**

Our data demonstrate that electrical stimulation of the PDB evokes saccades, often accompanied by slow drifts. The latency of evoked saccades is influenced by the intensity and frequency of stimulation while their amplitude depends on the intensity of stimulation and the initial position of the eyes. The dynamics of evoked saccades differ little from those of spontaneous or visually triggered saccades of the same species and from those evoked in response to the electrical stimulation of the cat SC. Since we were generally unable to evoke saccades from nearby regions of the PPRF, our data demonstrate that saccade generation relies on a specialized circuit (such as the burst generator). Our study is one of few that have combined electrical microstimulation with localized inactivation in alert behaving animals. It demonstrates that PDB-evoked saccades are not abolished by the reversible chemical inactivation of the SC and thus leads us to conclude that activation of the SC, either antidromic or orthodromic, is not needed for their generation. Our data clearly demonstrate that the burst generator of the saccadic system is located downstream of the SC. If the burst generator is configured as a local loop controller, as assumed by most models of saccadic control, our data also demonstrate that its feedback path closes downstream of the SC.

PDB-evoked saccades survive lesions of the SC

Our data demonstrate that the SC is not needed to generate saccades with normal characteristics in response to stimulation of the contralateral PDB. We shall discuss separately the results obtained with lidocaine and muscimol injections because the former is known to exert a weaker effect on saccade related SC processes (Hikosaka and Wurtz, 1985, 1986).

Lidocaine injections did not alter the likelihood of evoking saccades from the PDB and did not prolong their latencies. The slope of the velocity-amplitude relationship was significantly reduced in only one of three experiments, and the reduction was modest. Such small and poorly reproducible effects invite us to ask whether the local anesthesia involved a sufficiently large volume of the SC. This was evidently the case as shown by the elimination of responses to SC stimulation and by changes in the cat’s spontaneous oculomotor behavior shortly before testing the responses to PDB stimulation. In all lidocaine experiments we observed a strong suppression of the readiness...
to make spontaneous saccades and a restriction of their amplitude and velocity together with significantly shallower main sequence slopes. Neglect of the contra-lesional hemifield, typical of the unilateral surgical ablation of the SC (Sprague and Meikle, 1965; Flandrin and Jeannerod, 1981), was observed as well. It was expressed in the shift of the mean horizontal E to the side of the inactivated SC and in the virtual absence of orienting saccades to visual and auditory stimuli in the contra-lesional hemifield, starting from about 5° off the midline. All this indicates that a major portion of the SC was inactivated.

The muscimol experiment is of particular interest as it illustrates the effect of an unquestionably extensive inactivation. Inadvertent vascular lesion resulted in the destruction of 59% of the SC together with the underlying dorsal mesencephalic tegmentum. In this case saccades evoked by PDB stimulation showed an obvious reduction of peak velocities and shallower main sequence slopes (from 10.81–7.67°/s/°; Fig. 8 4M). However, at the same time spontaneous contra-lesional saccades were completely eliminated, mean horizontal E was strongly shifted toward the side of the lesion (15°), stable fixation was absent, and, at longer post-injection times, rare contra-lesional sac- cades failed by far (10–13°) to reach the midline. We are not able to separately estimate the effects due to the injected muscimol, tissue ablation, and the compression of adjacent regions due to bleeding. Nonetheless, this experiment demonstrates that PDB-evoked saccades can be evoked with high probability, that they obey the main se- quence relationship, and that the overall reduction of their velocity is modest in spite of the severe impairment of the SC and the inability of the animal to generate spontaneous saccades.

Comparison with previous studies

To our knowledge this is the first study to demonstrate that saccades can be evoked in response to stimulation of SC output fibers in the PDB. Cohen and Komatsuzaki (1972) stimulated the PPRF of alert monkeys, just lateral to the PDB, and the typical responses they reported consisted of constant velocity movements. They also illustrated fast movements with exponential trajectories (their “inconstant velocity” movements) but few examples of saccade-like movements. Keller (1974) as well as Sparks and col- leagues (1987, 2002) have used the PPRF stimulation paradigm and they mention only slow ramp-like evoked movements terminating only after the end of the stimulus train. In general, our observations from stimulation sites located in the core of the pontine reticular formation agree with earlier studies in that slow movements with variable time course were the predominant variety of the responses we observed. However, when electrodes were placed close to or inside the paramedian tracts, saccades could be reliably evoked.

In several respects, saccades evoked from the PDB were similar to self-generated ones of the cat and to those evoked in response to stimulation of the SC. Firstly, their velocity profiles were nearly symmetrical, and they terminated long before the end of the stimulus train. Also, their peak velocities increased together with their amplitude following the main sequence curve of the cat (Evinger and Fuchs, 1978; Guitton et al., 1984). Finally, their size depended on the initial position of the eyes, in the manner repeatedly documented for SC-evoked saccades in the same species (Guitton et al., 1980; McIlwain, 1986; Grantyn et al., 1996; Moschovakis et al., 1998a) and their latencies were indistinguishable from those of SC-evoked saccades (Grantyn et al., 1996).

There are, however, certain differences between the PDB- and the SC-evoked saccades even when the two structures are stimulated with identical parameters. Firstly, the slopes of the main sequence curve of saccades evoked from some (n=11) PDB sites were relatively shallow (<9°/s/°). Other sites were much more effective as indicated by the fact that 26% (15/58) of them displayed main sequence slopes of 14.0–19.8°/s/° which corresponds to and even exceeds the top of the ranges reported for visually triggered saccades in the cat (Guitton et al., 1984). Also, it was more difficult to evoke staircases of saccades in response to long duration stimulation of the PDB than it is to evoke them from the SC. Both could be due to differences in the efficacy with which SC stimulation and PDB stimulation activate OPNs. Excitatory SC projec- tions to OPNs are strong from the rostral pole, the so-called fixation zone, and decline toward the central and caudal locations in the SC (Paré and Guitton, 1994; Sugiu-uchi et al., 2005). Because our collicular stimulation sites were outside the rostral pole, co-activation of OPNs should be weak. This is not the case with PDB stimulation where fibers issuing from the rostral and caudal poles may have similar chances to be recruited, thus increasing the fraction of co-excited OPNs. If OPN discharge was clamped to a nonzero, even if low, value in response to PDB stimulation they could counteract the excitatory drive to burst neurons and account, at least in part, for the shallower main se- quence slopes of PDB-evoked saccades. A similar expla- nation could be proposed for the relative difficulty to evoke saccadic staircases from the PDB. During SC stimulation, the disynaptic inhibition, originating from both the rostral and the caudal SC (Yoshida et al., 2001) and maybe mediated by “latch” LLBs, apparently predominates at the beginning of the train, thus silencing OPNs and triggering the primary saccade. Secondary and higher order sac- cades would then be elicited every time the RI is reset, i.e. as soon as OPNs resume firing (Moschovakis, 1994). In view of the mixed, excitatory and inhibitory, input transmitted to OPNs by collicular axons, PDB stimulation might clamp the rate of firing of OPNs to some low value for the whole stimulus duration, thus preventing the resumption of OPN firing and disabling the resetting mechanism of the RI and the generation of saccadic staircases (Moschovakis, 1994).

Evidently, not all PDB sites were equally effective in generating saccades. Besides six PDB sites from which we were unable to evoke saccades, our sample of 58 effective sites contains five sites from which we could obtain saccades with a probability lower than 0.5, even when the starting position of the eyes was deviated con-
tralaterally. The EI of these sites was also low (<0.25) because of elevated thresholds (20–40 μA). On the other hand, we were able to evoke centripetal saccades with a probability (IR) of 1 from 41% of our effective sites with optimal combinations of stimulation parameters. Typically, such sites were characterized by low thresholds (≤10 μA); they did not need strong currents to obtain reliable responses, and obtained thus high EI values (>0.5). This leads us to conclude that the precise location of the electrode tip is a major source of the observed variability of stimulation efficacy. The probability of evoking saccades also depended on the initial position of the eyes; in some sites it proved very difficult to evoke centrifugal saccades. This could be due to the relatively small size of the characteristic vectors of evoked saccades in particular when combined with relatively high position sensitivities. The small size of the characteristic vectors could, in turn, be due to our occasional inability to recruit a large enough number of PDB fibers because of the small tips and weak stimulation currents we employed. The choice of our stimulation technique was dictated by the need to obtain the best possible spatial selectivity of our stimuli, in particular since we were targeting a narrow fiber bundle (<0.5 mm in mediolateral extent). Restricting the current range to 4–40 μA was needed to reduce the effective current spread while the upper limit was imposed by the current passing capacity of the capillary or tungsten microelectrodes we employed.

Besides saccades, electrical stimulation of the PDB was seen to generate slow drifts akin to those evoked in response to the electrical stimulation of the SC (Grantyn et al., 1996). These have been attributed to relatively direct projections from the SC to extraocular MNs that largely bypass the burst generators (Grantyn et al., 1996; Moschovakis et al., 1998a). These authors provided a substantial list of fibers and neurons likely to mediate them. Briefly, they include collicular afferents deploying terminal fields in the abducens nucleus (Grantyn and Grantyn, 1982; Grantyn and Berthoz, 1985; Olivier et al., 1993), reticulospinal neurons which receive input from the SC and project to the abducens nucleus (Grantyn et al., 1980, 1987), neurons of the PH which also receive input from the SC and project to the abducens nucleus (Grantyn and Grantyn, 1982; Grantyn and Berthoz, 1985; Olivier et al., 1993) and tectorecipient reticulospinal neurons which discharge during orienting eye–neck synergies and give substantial collateral projections to both the PH and the medial vestibular nucleus (Grantyn et al., 1980, 1987, 1992). Since, most of the tectal efferents participating in these projections pass through the PDB, their stimulation and the generation of slow drifts were unavoidable in a majority of our stimulation sites.

**Implications for models of the saccadic system**

Our experiments were designed to test the existence of a post-collicular saccade generating mechanism rather than distinguish between alternative post-collicular mechanisms. They are thus consistent with any post-collicular pulse generator and not just those that have been proposed for the brain stem. Such an alternative is a trans-cerebellar pulse generator (Quaia et al., 1999). Engagement of this circuit in our experiments is consistent with the fact that the PDB projects to the NRTP (e.g., Graham, 1977; Grantyn and Grantyn, 1982; Huerta and Harting, 1984) which then projects to the fastigial oculomotor region (FOR). It is also consistent with the well-known involvement of both the NRTP (Keller and Crandall, 1981) and the FOR (Ohtsuka and Noda, 1991; Goffart et al., 2004) in saccade generation. However, there are several reasons to doubt that the cerebellum houses the local feedback loop of the saccadic system (summarized in Scudder et al., 2002). For example, the discharge of FOR neurons is too variable to entrust them with the control of saccade metrics. More importantly, one of the predictions of a trans-cerebellar feedback loop model has been experimentally refuted. Rather than last for the duration of the stimulation (as predicted by the trans-cerebellar model of Quaia et al., 1999), SC-evoked saccades stop well before the end of the stimulation and their velocity profiles do not change markedly when the FOR is lesioned (Guillaume and Pélisson, 2001). In light of this evidence and our present findings, it is parsimonious to conclude that saccades evoked in response to the electrical stimulation of the cerebellum are due to the engagement of the same circuit that is responsible for the generation of saccades after PDB, SC and possibly cortical stimulation as well, i.e. a burst generator located downstream of all these structures in the brainstem.

Several models place the SC inside the local feedback loop of the saccadic system (Waitzman et al., 1991; Lefèvre and Galiana, 1992; van Opstal and Kappen, 1993; Arai et al., 1994; Grossberg et al., 1997). Consistent with this notion, the instantaneous firing rate of “clipped” presaccadic SC cells decreases together with instantaneous M, for a wide range of saccade sizes (Waitzman et al., 1988). Moreover, the discharge of SC neurons and the trajectory of the eyes can be disrupted in parallel. For example, saccades are known to be interrupted in mid-flight in response to OPN stimulation and to resume their course after the end of the stimulation (Keller, 1977; King and Fuchs, 1977) and the same is true of the discharge of SC burst cells (Keller and Edelman, 1994; Munoz et al., 1996). Also, the duration of the discharge of presaccadic SC cells increases together with the duration of saccades and their intensity decreases with saccade velocity following muscimol injections in the OPN area (Soetedjo et al., 2002) and when saccades are perturbed by blinks (Goosens and van Opstal, 2000).

Our experiments were not intended to test the existence of a feedback loop operating at the level of the SC, and therefore they do not disprove it. However, there are reasons to doubt that the trans-collicular feedback loop could perform all operations usually assigned to the local feedback loop of the saccadic system. For example, to satisfy the normal amplitude–duration relationships, models that place the SC inside the local loop predict that SC burst neurons firing for bigger saccades should fire longer. This is difficult to reconcile with the fact that the duration of
the bursts of SC presaccadic cells is poorly correlated to the size of the saccades they prefer (Sparks and Mays, 1990). Also, the frequency of the discharge of SC neurons is reduced considerably more than expected from the reduction of the velocity of saccades accompanied by vergence (Walton and Mays, 2003). Moreover, the discharge of SC presaccadic neurons is poorly related to dynamic Me in several circumstances, such as when saccades are executed in the absence of a precise target (e.g., to the center of a large rectangle; Edelman and Goldberg, 2003) or when they are perturbed with OPN stimuli (Keller and Edelman, 1994) or blinks (Goossens and van Opstal, 2000). The same is true for saccades slowed down following muscimol injections in the OPN area (Soetedjo et al., 2002). In all these circumstances, a local feedback loop that, consistent with our data, operates below the SC would generate saccades that follow a normal time course despite receiving SC signals that are not tightly correlated with saccade dynamics.

It might be argued that electrical stimulation of the SC suffices to decide if the local loop of the saccadic system closes through the SC or below it. This is based on the assumption that stimulation of the SC preferentially activates its output fibers, which thus carry a signal dominated by the time course of the stimulation rather than the results of computations performed by a comparator residing in it (Gnadt et al., 2001). However, incoming preterminal fibers are more likely to be activated by the electrical stimulus (Gustafsson and Jankowska, 1976). This is consistent with the preferential activation of axonal branching points (the relative density of which increases in and near axonal terminal fields) rather than cell bodies or axonal initial segments following electrical stimulation of cortical gray layers (Nowak and Bullier, 1998a,b). Accordingly, when electrical stimulation is applied to the SC, afferent pathways (providing the SC with feedforward as well as feedback information) and intrinsic SC connections are unavoidably activated in addition to SC output fibers and it is as likely to engage the input to its putative comparator as to engage its output. In contrast, the electrical stimulation of the PDB used in this study eliminates the ambiguity of intracollicular stimulation, in particular when combined with SC inactivation. Our data thus provide conclusive evidence that saccades can be readily evoked even if the output of the SC is a step function of time and, consequently, that an independent burst generator of the saccadic system is located downstream of the SC.

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