Gaze shifts evoked by electrical stimulation of the superior colliculus in the head-unrestrained cat. I. Effect of the locus and of the parameters of stimulation

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Abstract

Several studies have suggested that the pattern of neuronal activity in the superior colliculus (SC) interacts with the well-known topographical coding of saccades (motor map). To further describe this interaction, we recorded gaze saccades evoked by electrical microstimulation of SC deeper layers in the head-unrestrained cat and systematically varied the collicular locus (25 sites) and parameters (intensity, frequency) of the stimulation. Long stimulation trains were used to avoid saccade truncation. We found that the direction and amplitude of evoked gaze shifts were related to the stimulation locus, describing a gaze shift map. For 18 out of 20 sites the amplitude, but not the direction, also strongly depended on stimulation strength. Indeed, gaze amplitude continuously increased when raising current intensity up to several times the threshold value \( T \), whereas varying pulse frequency from 150 to 750 pulses per second (p.p.s.) revealed an optimal frequency range (300 and 500 p.p.s.) eliciting the largest gaze shifts. Moreover, the intensity effect on amplitude increased in an orderly fashion with the rostro-caudal stimulation locus. Gaze shift amplitude was not related to the number of delivered stimulation pulses. Concerning movement initiation, increasing either intensity or frequency led to an exponential decrease in gaze latency until minimal values near 30 ms were reached, but the number of pulses delivered during the corresponding latency period remained constant within a 300–500 p.p.s. frequency range. These findings indicate that the pattern of collicular discharge evoked by electrical stimulation strongly interacts with the gaze shift map and provide evidence for a summation of collicular activities by downstream premotor neurons.

Introduction

Several neural processes contributing to the production of rapid (saccadic) displacements of the visual axis gaze toward a visual target have been identified. However, the mechanisms involved in the transformation of visual information about target position into motor commands driving the ocular and neck muscles are still not completely elucidated. Due to its organization in superimposed sensory and motor layers, the superior colliculus (SC) has long been thought to play a key role in these sensory–motor transformations (see for reviews Sparks & Mays, 1990; Guitton, 1991; Stein & Meredith, 1993). Based on unit recording, inactivation and electrical stimulation data, numerous models of how these transformations take place in the SC have been proposed (e.g. Lee et al., 1988; Van Opstal & Van Gisbergen, 1989; Tweed & Vilis, 1990; Munoz et al., 1991; Waitzman et al., 1991; Lefèvre & Galiana, 1992; Van Opstal & Kappen, 1993; Arai et al., 1994; Optican, 1995; Bozis & Moschovakis, 1998).

Electrical stimulation in the deeper layers of the SC produce saccadic gaze displacements which closely resemble natural gaze shifts. Although these artificially triggered movements are not controlled in the same way as natural ones (Goldberg et al., 1993; Melis & Van Gisbergen, 1996; Coimbra et al., 2000), systematic tests of the effects of the electrical stimulation parameters and of the stimulated collicular locus on evoked movements provide fruitful information about the coding of saccadic signals in the motor SC layers. Early stimulation studies delineated the existence of a map of saccadic eye movements (Robinson, 1972; see also McIlwain, 1990 in the cat) in close spatial register with the visual map (Schiller & Stryker, 1972). Numerous investigations have complemented these pioneering studies by showing that the electrical stimulation parameters also affect the metrics and dynamics of evoked saccades. Nevertheless, the following issues are still debated or unexplored.

The first issue relates to the fact that only a few studies have investigated the combined eye/head gaze shifts evoked by SC stimulation (Roucoux et al., 1980; Munoz et al., 1991; Paré et al., 1994; Freedman et al., 1996) and that a systematic exploration of the SC motor map in the head-unrestrained condition is still lacking. The second issue concerns the effect of stimulation train duration on the amplitude of the evoked movements, leading to truncation of saccades with stimulation trains which are too short (Guitton et al., 1980; Paré et al., 1994; Freedman et al., 1996; Stanford et al., 1996), contrasting with the initial proposition of Robinson (1972) and Schiller & Stryker (1972). This implies that train duration should be carefully controlled when studying the effect of other stimulation parameters (intensity, frequency and site) on the gaze shift amplitude.

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Thirdly, the strength of the electrical stimulation (current intensity and/or pulse frequency) has also been shown to influence the amplitude of evoked movements (eye saccades in the cat, Straschill & Rieger, 1973; Stein et al., 1976; Grantyn et al., 1996; eye saccades in the monkey, Schiller & Sandell, 1983; Sparks & Mays, 1983; Van Opstal et al., 1990; head movements in the barn owl, du Lac & Knudsen, 1990) but to very different degrees among studies. Methodological differences such as the use of short or long stimulation trains (see previous point) could account for these differences but other potential factors such as the location of the stimulation sites could not be appreciated in these past studies. We note also that variations of current intensity have rarely been expressed as a function of the threshold value (see, however, Grantyn et al., 1996).

The main objective of our study was to gain further insight into the collicular coding of saccadic gaze shifts. To this end, we investigated the characteristics of gaze saccades evoked by stimulation of the SC in the head-unrestrained cat and chose to (i) use stimulation trains which outlasted the evoked gaze shift, (ii) test the effect of varying the other stimulation parameters (current intensity and pulse frequency) and analyse the current intensity effect with respect to a threshold value determined for each site, and (iii) systematically vary the electrode location along a large rostro-caudal portion of the SC motor map. This work has already been presented in abstract form (Pélisson & Guillaume, 2000).

Materials and methods

Animal preparation

A detailed description of animal preparation, experimental set-up and data recording can be found in a previous paper (Goffart & Pélisson, 1998). Two cats were prepared for the experiments under general anaesthesia and aseptic conditions following the guidelines from the French Ministry of Agriculture (87/848) and from the European Community (86/609/EEC). Two coils were implanted to allow recording of gaze and head positions by the search-coil-in-magnetic-field technique (Robinson, 1963). Two trephine holes were made in the skull and two recording chambers centred on the midline were stereotaxically implanted, one over the SC and one over the cerebellum (the cerebellum chamber permitted collection of data, reported in the companion paper (Guillaume & Pélisson, 2001), on the effect of the inactivation of the caudal fastigial nucleus on collicular-evoked gaze shifts). Finally, a U-shaped plastic piece was cemented to the skull with dental cement to allow the painless restraint of the animal’s head during some experimental phases.

Experimental setup and training

The animal, lying in a hammock that gently restrained the body without constraint on natural movements of the head, was placed inside a 1-m coil frame (CNC Engineering, Seattle, USA) with the head situated at the centre of the frame. An opaque screen covering 19° of visual angle was situated in a fronto-parallel plane at a distance of 41 cm. Cats were trained to orient gaze toward a visual target (a spoon of food purée) when suddenly presented to either side of the screen and were rewarded directly from the spoon after each correct orienting gaze shift.

Experimental paradigm

The cat’s head was first restrained and an electrode was lowered toward the intermediate/deep layers of the SC through a micro-manipulator (Narishige MO900) attached to the recording chamber. The initial phase of electrode lowering was based on stereotaxic data, and the electrode’s entrance into the SC superficial layers was easily and precisely identified by the strong visual activity recorded at this level. From this point, the electrode was further lowered by 2 mm, and sometimes adjusted by ±0.25 mm around this depth to search for a lower threshold site. Then, the larger part of the Narishige manipulator was removed, leaving only a light, 2.5-cm high, piece holding the electrode in place; the animal’s head was freed and recordings started. To keep the animal in a good motivational and arousal state, electrical stimulation was applied while the cat was waiting for the target presentation and was randomly intermixed with visual trials. In these last trials, the visual target was presented randomly on either side of the opaque screen and the animal was rewarded after orienting to the target. Both stimulation and visual trials (= 50% each) were recorded for subsequent analysis, until ≈ 200 stimulation trials were made. We then proceeded with the second part of the study described in our companion paper (Guillaume & Pélisson, 2001).

Electrical stimulation

Home-made tungsten electrodes with an impedance of 0.2–1 MΩ were used (Merrill & Ainsworth, 1972). These electrodes were glass-coated for the last 20 mm and were thus quite rigid. Electrical stimulation consisted of trains of 0.5-ms cathodal pulses delivered by a S88 Grass stimulator and a PSIU6 isolation unit. For 24 stimulation sites, trains of 300-ms duration and 300 pulses per second (p.p.s.) pulse frequency (500 p.p.s. for the 25th site) were first used to determine the threshold current intensity (T) defined as the intensity which evoked a gaze shift in > 75% of stimulation trials (mean T = 7.9 ± 5.1 μA, n = 24 sites). Then, several current intensities which were multiples of T were tested at a frequency of 300 p.p.s. For nearly half of these sites, frequencies of 150 and/or 500 p.p.s. were also tested (fixed current intensity) and for two sites a wider range of frequencies was used (see Results). Table 1 lists all current intensities and pulse frequencies tested. Train duration was generally kept at 300 ms or sometimes reduced to avoid the triggering of secondary movements which would reach mechanical limits. Great care was taken not to truncate the primary gaze shifts. Each response in which the time of the easily identifiable onset of the deceleration phase lagged behind stimulation termination (see Results for examples) was considered potentially truncated and was rejected from analysis. For the last 2–4 experiments in each cat, a small marking lesion was performed at the stimulation site by injecting a constant current (30 μA, 15 s) through the tip of the electrode. These lesions served to reconstruct the stimulation sites by conventional postmortem histological procedures (Fig. 1).

Data recording and analysis

Search coils signals were linearized and scaled on-line by a computer program and then recorded (sampling rate 500 Hz) on a second PC running a commercial data acquisition software (DataWave Technologies, Longmont, CO, USA). The envelop of the stimulation train (stimulation trials) or the position of the visual target (visual trials) were also recorded in the same data file.

Off-line analysis was performed by software developed in the laboratory. Gaze and head signals were digitally filtered (FIR filter, 70 Hz cutoff frequency), differentiated and eye signals were obtained by subtracting head signals from gaze signals. The onset and termination of gaze shifts and of head movements were automatically detected based on a velocity criterion (30°/s). All trials were then displayed in order to check this automatic detection and if necessary
Modified gazes were needed mostly when a fast drift followed the gaze shift, which occurred with large current intensities and/or caudally located stimulation sites. In these cases, the termination of the gaze saccade was clearly identified on the velocity records as the sharp transition between the strong velocity reduction phase (deceleration phase) and the constant velocity phase related to the gaze drift. Several raw spatial and temporal parameters were extracted for each trial and processed by a spreadsheet program (StatSoft, Inc., Tulsa, OK.

Electrode penetrations were aimed at the horizontal meridian of the collicular map in order to focus on nearly horizontally directed gaze shifts. Locations of stimulation sites along the antero-posterior axis of the SC were expressed in two ways. First, we used the stereotaxic coordinate of the microelectrode track to estimate the location of the desired stimulation site. Second, because the possibility of slight deviation of the electrode from its intended vertical trajectory cannot be entirely ruled out, we used for the rest of the analysis the mean amplitude of gaze shifts evoked by a standard stimulation (2 × T current intensity and 300 p.p.s. pulse frequency) as a behavioral measure of the site antero-posterior position (for five sites for which 2 × T current intensity was not tested, we performed a linear interpolation from the two closest intensity values). On the whole, sites in left and right SC were equally represented (12 and 13, respectively). For the sake of simplicity and comparison, results obtained from the right SC have been mirrored such that all data are represented as rightward responses (positive displacements) obtained from a single putative left SC.

To assess the effects of varying stimulation parameters and stimulation sites on gaze shifts with similar initial conditions, we selected for analysis only gaze shifts initiated from the central 6° horizontal position range, leading to the rejection of 28.7% of the total number of responses.

Results

**Gaze metrics**

**Position of stimulation site**

Figure 2 presents the effect of the stimulated site within the SC on the metrics of the evoked gaze shifts. Panel A shows

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**Table 1. Parameters of the electrical stimuli used at each site**

<table>
<thead>
<tr>
<th>Site</th>
<th>Frequency (p.p.s.)</th>
<th>Threshold (μA)</th>
<th>Intensity (× T)</th>
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<tr>
<td>O01</td>
<td>150</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>225</td>
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<td>2</td>
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<td>750</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
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<td></td>
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<td>1, 1.5, 2, 3</td>
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</tr>
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<td></td>
<td>120</td>
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<td></td>
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<td>7.0</td>
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<td></td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
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<td>500</td>
<td>1, 2, 3, 8</td>
<td></td>
</tr>
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<td>5.0</td>
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<tr>
<td></td>
<td>300</td>
<td>1, 1.5, 2</td>
<td></td>
</tr>
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</tr>
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<tr>
<td></td>
<td>500</td>
<td>1, 1.2, 3.3, 4</td>
<td></td>
</tr>
<tr>
<td>L20</td>
<td>300</td>
<td>15.0</td>
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<td>L21*</td>
<td>300</td>
<td>12.5</td>
<td>1, 1.2, 2.4, 3.2</td>
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<td></td>
<td>500</td>
<td>1, 1.6, 2.4, 3.2</td>
<td></td>
</tr>
<tr>
<td>L22</td>
<td>500</td>
<td>4.0</td>
<td>1, 2</td>
</tr>
<tr>
<td>L23</td>
<td>300</td>
<td>4.0</td>
<td>1, 2</td>
</tr>
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</table>

A threshold intensity was determined for each site with a pulse frequency of 300 p.p.s. (except for site L22, 500 p.p.s.). Other tested frequencies and intensities (expressed relative to the threshold, T) are also listed. Sites marked with an asterisk are those for which the intensity of 2 × T at 300 p.p.s. was not available. See text for further details.

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Fig. 1. Frontal sections of the SC containing the four marking lesions performed in cat L. The section shown in the upper part was more rostral than the one shown in the lower part. Labels identify each site.
individual gaze shifts evoked by the stimulation of three different SC sites (antero-posterior stereotaxic coordinates 3.3, 2.3 and 1.4 mm for left, middle and right columns, respectively) with a near-constant current intensity (1.8, 2 and 2 T, respectively) and a pulse frequency of 300 p.p.s. The three rows consist, from top to bottom, of horizontal position profiles (gaze, head and eye), horizontal velocity profiles and spatial trajectories of the primary gaze shifts. Notice the strong increase of the horizontal component of evoked gaze shifts and associated head movements as the stimulation location was moved caudally. The data shown in the leftmost column are from the stimulation site which produced the smallest and largest gaze amplitude, respectively; middle column shows data from an ‘intermediate’ site. Top, middle and bottom rows show, respectively, the time course of horizontal positions (gaze, head and eye), the corresponding velocity profiles and the X-Y trajectories of the primary gaze shifts (delimited by vertical lines). Gray bars at the top of the velocity row indicate SC stimulation. (B) Plot of the mean or calculated (five sites) gaze tangential amplitude (intensity 2 T and frequency 300 p.p.s., except for site L22: frequency 500 p.p.s.) as a function of the antero-posterior stereotaxic position of the stimulated SC site. Labels identify the stimulation sites in cat L and O. The grey curve indicates the exponential function fitted to the data [equation, gaze tangential amplitude = 89.5 × exp(-0.47 × stereotaxic coordinate)]. The two grey bars on the X axis correspond to the rostral (left) and caudal (right) ends of the intermediate/deep layers of the SC (mean estimation from the two cats). These boundaries have been reconstructed from the electrolytic marking lesions (see Materials and methods).
Finally, the response shown in the middle column was recorded by stimulating a site which led to gaze shifts of an intermediate horizontal amplitude (mean, 30.9 ± 3.2°, n = 12).

Panel B shows plotted, for the entire sample of stimulation sites, the relationship between the tangential amplitude of gaze shifts evoked at a 2 × T intensity and the antero-posterior stereotaxic coordinate of the stimulating electrode. The stimulation frequency was 300 p.p.s. (except for site L22, frequency 500 p.p.s.). There is some ordering of the amplitude of evoked gaze shift which discloses the SC motor map. Indeed, as the stimulation site was progressively more caudal in the SC, the gaze tangential amplitude correspondingly increased. In agreement with several previous studies, this relationship was nonlinear and could be fitted with the following exponential function: gaze tangential amplitude = 89.5 × exp(-0.47 × stereotaxic coordinate). Note that the variability of the data is quite large, as the amplitude from individual experiments (sites) could differ by up to 125% (site L21) from the fitting curve (mean, 32 ± 29%). This variability could partly be related to some uncertainty about the actual location of the stimulation sites in the SC, especially for the caudal region (a deviation of only 500 μm of the electrode track from its intended trajectory would translate into an amplitude variation of ~15°). Thus a functional characterization of site positions was used for the rest of the paper (see Materials and methods).

**Current intensity**

Figure 3 illustrates for two typical sites (panel A, site L20; panel B, site L13) the effects of increasing the current intensity on the evoked gaze shifts (stimulation frequency, 300 p.p.s.). The data are shown in the same format as in Fig. 2A. The increase in intensity (from left to right: 1 × T, 2 × T, 4 × T for site L20 and 1 × T, 1.5 × T, 2 × T for site L13) led to a shortening of movement latency and an increase in the horizontal component of the gaze shift. X–Y trajectories illustrate that the gaze shift direction remained nearly unaffected.

Figure 4 illustrates the relationships between current intensity and gaze tangential amplitude (panels A and C) or direction (panels B and D) for three representative SC sites in cat L (upper panels) and cat O (lower panels) (stimulation frequency 300 p.p.s.). For all sites, gaze tangential amplitude increased as a function of current intensity, an effect which could be extremely large. Across all six sites shown in Fig. 3, the relative change of gaze shift amplitude between 1 × T and 2 × T intensities ranged from 153% (site L05) to 385% (site L13). Regarding gaze shift direction (Fig. 3B and D), there was no consistent effect of current intensity and the small mean values resulted from our sampling strategy emphasizing the horizontal meridian of the collicular motor map (see Materials and methods).

The effects of varying current intensity on gaze shift amplitude are summarized in Fig. 5 for the 20 sites for which at least three different intensities were tested. In panel A (cat L) and panel B (cat O) data points are mean values and each curve corresponds to a different SC site. These plots confirm the presence of a tight link between current intensity and gaze tangential amplitude and further indicate that the strength of this link varied among stimulation sites. Note that, except for a single site in cat L, no clear gaze amplitude saturation was observed within the tested range of current intensities. This is especially striking for the two sites for which current intensities of 4 × T and beyond have been tested (cat L). In addition, we emphasize that for some sites the tangential amplitude of gaze shifts elicited at the highest tested intensity was already >60°, which precluded any further increase of current intensity because of potential restrictions related to the head movement mechanical limits. Panel C further investigates the interaction of stimulation locus and current intensity on the saccade amplitude; for each site a linear regression analysis was performed and the regression line slope was plotted as a function of stimulation site position. A statistically significant correlation (P < 0.05) was found for 18 out of the 20 tested sites (filled circles). Furthermore, the strength of these positive correlations is related to the site position on the SC; the slope increased progressively as the electrode was moved toward the caudal pole of the SC. Note again that, to avoid reaching the mechanical limits, the stimulation of the caudalmost sites was restricted to a narrow intensity range (1 × T–2 × T).

**Pulse frequency**

The effects of varying stimulation pulse frequency will be first described for an intensity of 2 × T. A statistical analysis including data obtained with all tested intensities will then be provided. Figure 6A illustrates typical responses evoked in cat O at a frequency of 150, 300 and 500 p.p.s. (left, middle and right columns, respectively) in the same format as in Figs 2A and 3. Both horizontal gaze amplitude and velocity increased from 150 to 300 p.p.s. In contrast, between 300 and 500 p.p.s., horizontal gaze amplitude remained constant whereas gaze peak velocity was higher at 500 p.p.s. than at 300 p.p.s. Note also the progressive reduction of gaze (= eye) and head latency with increasing values of pulse frequency. The effects of varying the pulse frequency on gaze shift metrics are summarized in Fig. 6B and C for all sites tested with a current intensity fixed at 2 × T. The pattern of amplitude changes exemplified in panel A was confirmed; increasing the frequency from 150 to 300 p.p.s. led to an increase in gaze amplitude and a further increase of frequency to 500 p.p.s. did not affect gaze amplitude.

To quantitatively evaluate this pattern of results, we performed a one-way ANOVA with pulse frequency (150, 300 and 500 p.p.s.) as the main factor. We included in this analysis all experimental series (21 series, n = 48 values; current intensity held constant within each series) testing 300 p.p.s. and at least one other pulse frequency (150 and/or 500 p.p.s.), comprising experimental series using a 2 × T intensity but also series using other intensities. Given the effects of site position and current intensity on gaze shift amplitude (see Figs 2, 5 and 6B), we normalized the data based on the mean amplitude value obtained at 300 p.p.s. (= 100%). Mean normalized amplitudes were 54.4% and 106.2% for 150 and 500 p.p.s., respectively. The results of the ANOVA indicate a main effect of frequency on gaze shift amplitude (F_{2,45} = 31.54, P < 0.001). Post hoc comparisons (Tukey test) showed that mean values for 300 and 500 p.p.s. were not significantly different (P > 0.05), whereas the mean value at 150 p.p.s. differed significantly from the 300 p.p.s. value (P < 0.001) or from the 500 p.p.s. value (P < 0.001).

Panel B also shows that frequency values beyond 500 p.p.s. were tested for two sites and elicited gaze shifts with a smaller amplitude than those evoked by a 300- or a 500-p.p.s. frequency stimulation. The t-tests for independent samples performed on individual data revealed that the mean gaze tangential amplitude significantly decreased between 500 p.p.s. (22.2 ± 4.5°) and 650 p.p.s. (16.9 ± 1.8°) (t_{25} = 3.29, P < 0.01) for site O15 and between 450 p.p.s. (21.4 ± 4.4°) and 750 p.p.s. (13.8 ± 4.9°) (t_{45} = 4.57, P < 0.001) for site O01. In summary, gaze shift amplitude depended on
stimulation frequency in a systematic manner: gaze amplitude increased from 150 to 300 p.p.s., remained constant from 300 to 500 p.p.s. and, according to the data from two sites, decreased for frequencies >500 p.p.s. Panel C presents results concerning movement direction. As for the effect of current intensity, varying frequency did not induce any modification of movement direction.

Fig. 3. Effect of current intensity on the metrics of evoked gaze shifts. Examples of individual responses evoked at a pulse frequency of 300 p.p.s. Same organization as Fig. 2A. (A) Representative gaze shifts evoked by stimulating site L20 with a current intensity of 1 × T, 2 × T and 4 × T (left, middle and right columns, respectively). (B) Representative gaze shifts evoked by stimulating site L13 with a current intensity of 1 × T, 1.5 × T and 2 × T (left, middle and right columns, respectively). Note that, for both sites, the amplitude and peak velocity of evoked movements markedly increased as a function of current intensity whereas gaze direction was not much affected.
Gaze shift initiation

Current intensity

As reported by others we found that increasing the current intensity led to a decrease in gaze latency, particularly between $1 \times T$ and $2 \times T$ intensities. Raising stimulation current also led to a decrease of the eye-to-head delay until very small values indicating a nearly synchronous triggering of eye and head movements at a $3 \times T$ intensity.

Pulse frequency

The two variables used to investigate the gaze shift initiation mechanism are shown in Fig. 7 (inset): the latency period (in ms) and the number of stimulation pulses (NSP) delivered during the corresponding period, computed as NSP = latency $\times$ pulse frequency/1000. Two other measurements, which will be considered at the end of this section, are also illustrated in this inset: the number of stimulation pulses delivered during the movement time (NSP2 = gaze shift duration $\times$ pulse frequency/1000), and the number of stimulation pulses delivered from stimulation onset to gaze shift offset [NSP3 = (gaze latency + gaze duration) $\times$ pulse frequency/1000].

Figure 7 plots gaze latency and NSP1 as a function of pulse frequency for two representative sites (panels A and B) and for all sites tested (panels C and D). Increasing the frequency led to an exponential decrease of mean latency (panels A and C) and of its variability (panel A). Note the relatively large variability observed across the tested sites, which is to be related to the effect of site position (not shown). The plots of number of pulses NSP1 as a function of stimulation frequency (panels B and D) indicate that pulse frequency had hardly any effect on NSP1. Note further that, for the site for which the highest frequency was tested (750 p.p.s.), a marked NSP1 increase was observed simultaneously with a gaze latency increase. This observation may be paralleled to the decrease in gaze amplitude observed for frequencies larger than 500 p.p.s. (see Fig. 6B).

The effect of pulse frequency on latency and on NSP1 was tested by one-way ANOVAs. These analyses were based on the same 21 experimental series as for the analysis of the influence of pulse frequency on gaze tangential amplitude (see above). Latency and NSP1 data were also normalized with respect to the 300 p.p.s. values, leading to mean normalized latency values of 250.7 and 62.4% and mean normalized NSP1 values of 125.3 and 104.1% for 150 and 500 p.p.s., respectively. The ANOVA results indicate a main effect of frequency both on latency ($F_{2,45} = 154.24$, $P < 0.001$) and on NSP1 ($F_{2,45} = 6.02$, $P < 0.01$). Nevertheless, post hoc comparisons (Tukey test) revealed different results for the two dependent variables: concerning the latency, the three mean values were significantly different from each other ($P < 0.001$), whereas for NSP1 only two comparisons revealed statistically significant differences ($P < 0.01$ for 150 vs. 300 p.p.s., $P < 0.05$ for 150 vs. 500 p.p.s., but $P > 0.05$ for 300 vs. 500 p.p.s.). Thus, whereas both the latency and the NSP1 significantly decreased as the pulse frequency increased from 150 to 300 p.p.s., only the latency further decreased from 300 to 500 p.p.s. Note that this pattern of results for NSP1 is reminiscent of the one obtained for the frequency effect on gaze amplitude.

We also explored the relationship between the number of pulses and gaze shift amplitude. Because, as reported above, the amplitude remained constant from 300 to 500 p.p.s., it was possible to test if the number of pulses was a relevant parameter in this case. As just shown, NSP1 during time to initiation was not significantly different
between 300 and 500 p.p.s. Thus, the two NSP2 and NSP3 measures (see Fig. 7, inset) are equivalent and we chose NSP2 for simplicity. A t-test for dependent samples indicated a statistically significantly larger NSP2 at 500 than at 300 p.p.s. (difference 20.6 pulses, $t_{17} = 14.82$, $P < 0.001$. Thus the delivered NSP2 (or NSP3 if we assume that NSP1 is important in amplitude coding) was on average far greater for the 500 p.p.s. frequency although the gaze shift amplitude was not statistically different between the two conditions.

**Discussion**

**Electrical stimulation**

We first briefly discuss on the relationship between the activated SC neuronal population and the electrical stimulation parameters. The critical aspect of the duration of the stimulation train has already been underlined in the introduction. Concerning the current intensity, it is well accepted that it primarily influences the size of the recruited population (Yeomans, 1990; Tehovnik, 1996). Thus, in the present study, systematic variations in current intensity were achieved to modify the size of the recruited SC population (spatial variable) and to study its influence on the characteristics of evoked gaze shifts. Concerning the pulse frequency, Yeomans (1990) proposed that it is ‘a temporal variable that alters the number of action potentials’ (p 53). McIlwain (1982) further showed that, besides a direct excitation of neuronal elements, electrical stimulation delivered to the cat SC leads to a synaptically mediated excitation. Because this last component varies with the stimulation pulse frequency due to the temporal summation phenomenon, we shall also consider the possibility that the systematic variations of pulse frequencies used in the present study led to variations of the size (spatial variable) of the recruited population in the SC.

**Influence of the locus of stimulation on gaze shift metrics**

A large amount of data derived from experiments in the head-restrained condition supports the existence of a map of saccadic eye movements in the deep layers of the mammalian SC. In the head-unrestrained condition, only two previous studies have systematically tested the relationship between the amplitude of electrically evoked saccadic gaze shifts and the position of the stimulated site in the SC (Paré et al., 1994; Freedman et al., 1996). They reported data in favor of a map of saccadic gaze shifts in the SC. In the present study, the threshold stimulation intensity was determined for each site in order to allow comparison between different sites and different animals. Our results confirmed that the amplitude of the gaze shift increased continuously as the activated area was located more caudally in the SC. The range of gaze shift amplitude was large (7–77°) and gaze shifts elicted from the most caudal sites could even exceed the largest visually triggered gaze shifts which can be recorded in the cat with unrestrained head (but body restrained).
Because of time constraints, we did not record from premotor neurons at the stimulated loci (see Materials and methods); thus it is not possible to assess the spatial register between the topographical organization of evoked gaze shift amplitude and the topographical organization of premotor neuron movement fields. Based on a fewer number of stimulations sites, Paré et al. (1994) previously showed that the two topographical organizations are indeed in general register. Note, however, that the strong influence on gaze shift amplitude both of the strength of the stimulation (see below) and of the initial gaze location (Coimbra et al., 2000) renders this registering dependent on the stimulation conditions.

**Influence of the strength of the stimulation on gaze shift metrics**

The direction of evoked gaze shifts was not influenced by varying the current intensity or the pulse frequency of the electrical stimulation. This result is in agreement with previous studies (du Lac & Knudsen, 1990; Van Opstal et al., 1990; Paré et al., 1994; Stanford et al., 1996; Salas et al., 1997).

**Effect of current intensity on amplitude of gaze shifts**

An influence of the current intensity on movement amplitude has been observed in numerous previous studies. A commonly observed
relationship between amplitude and current intensity comprizes a first range of amplitude increase which can be distinguished from a second range of higher intensities where saccade amplitude saturates (Straschill & Rieger, 1973; Sparks & Mays, 1983; du Lac & Knudsen, 1990; Van Opstal et al., 1990; Salas et al., 1997; Herrero et al., 1998). In the present work, for the vast majority of stimulation sites (18 out of 20), increasing the current intensity led to a gradual and statistically significant increase in tangential amplitude of evoked gaze shifts without any clear tendency toward saturation. Note that, because direction remained constant, both horizontal and vertical components of oblique gaze shifts simultaneously increased. The absence of any clear saturation is at variance with the previously cited studies. Two non-exclusive explanations can be proposed.

First, the fact that previous studies used stimulation train of rather short duration could explain the presence of a saturation; beyond a certain intensity, the number of stimulation pulses delivered during this period (NSP1 = 19.8 pulses), the number of stimulation pulses delivered during the gaze shift and during the period from stimulation onset to gaze shift end (NSP2 and NSP3, respectively). (A and B) Quantitative data of gaze latency and NSP1, at a 2 × T current intensity, plotted as a function of pulse frequency for two typical sites (L13 and O15). Symbols are means ± SD. (C and D) The same plots have been superimposed for all data obtained in the two cats at a current intensity of 2 × T. Symbols are mean values which have been connected by lines for each site. Note that the latency decreased exponentially as pulse frequency was increased. NSP1 was, in the most studied frequency range (150–500 p.p.s.), almost constant. See text for statistical analysis.

Second, current intensities tested in the present study were not sufficiently strong and thus the saturation range of the relationship was not reached. Although this second possibility cannot be rejected based on the results of the present study, we present two observations which do not support it. First, for the caudal-most sites it was difficult to further increase the current intensity as the amplitude of evoked gaze shifts was already extremely large (> 60° starting from central position). Second, for more rostral sites, no clear saturation was observed even if the current intensity was increased up to 6 × T (e.g. 90 μA). Nevertheless, keeping the presence or absence of a saturation as uncertain, we think that the most important question to answer is the following: were collicular activations induced in the present study in the same range as natural activations generated in relation with visually triggered movement? We think that the answer is yes. Indeed, current intensities were chosen exclusively on the basis of the behavioural response by systematically determining threshold values. In addition, the electrically elicited gaze shifts have dynamic characteristics similar to those of visually triggered gaze shifts (see Figs 2A, 3 and 6A). Moreover, as already mentioned, the amplitude of the gaze shifts evoked from the caudalmost SC sites were large with respect to visually triggered responses which could be elicited during the same experimental sessions. For these reasons, we believe that the observed variations of gaze shift amplitude occurred with current intensities inducing SC activation levels which are similar to natural activation levels. We conclude that the coding of the amplitude of electrically evoked gaze shifts does not exclusively

![Fig. 7. Effect of pulse frequency on the initiation of gaze shifts. The variables considered in this figure and corresponding text are shown in the inset: the gaze latency period (66 ms) and the number of stimulation pulses delivered during this period (NSP1 = 19.8 pulses), the number of stimulation pulses delivered during the gaze shift and during the period from stimulation onset to gaze shift end (NSP2 and NSP3, respectively). (A and B) Quantitative data of gaze latency and NSP1, at a 2 × T current intensity, plotted as a function of pulse frequency for two typical sites (L13 and O15). Symbols are means ± SD. (C and D) The same plots have been superimposed for all data obtained in the two cats at a current intensity of 2 × T. Symbols are mean values which have been connected by lines for each site. Note that the latency decreased exponentially as pulse frequency was increased. NSP1 was, in the most studied frequency range (150–500 p.p.s.), almost constant. See text for statistical analysis.](image-url)
relies on the well-known topographical organization of the SC but also depends on the number of the activated neuronal population (‘mixed coding’).

**Effect of pulse frequency on amplitude of gaze shifts**

We constantly found the same relationship between gaze shift amplitude and pulse frequency. Increasing frequency from 150 to 300 p.p.s. led to an augmentation of gaze shift amplitude but a further increase up to 500 p.p.s. did not induce any change in amplitude. In addition, we tested two sites with frequencies beyond 500 p.p.s. and observed a decrease of movement amplitude as compared to intermediate frequencies (300–500 p.p.s.). These observations are globally consistent with the studies of Paré et al. (1994) in the head-unrestrained cat, of Freedman et al. (1996) in the head-restrained monkey (their fig. 4) and of Stanford et al. (1996) in the head-restrained monkey (fig. 12 and table 3). The only discrepancy is with the study of Stanford et al. (1996) in which no clear amplitude decrease was noted for the five sites for which frequencies > 500 p.p.s. were tested. We have no explanation for this discrepancy.

We concluded above (section ‘Effect of current intensity on amplitude of gaze shifts’) that the amplitude of evoked saccades depends on the size of the recruited population. Thus, one way to interpret the amplitude changes related to frequency variations is to consider, as mentioned in the ‘Electrical stimulation’ section, that this stimulation parameter could affect the spatial dimension of the activated collicular population. The shape of the amplitude vs. frequency relationship provides further information about this effect: it suggests that the recruited populations at 300 and 500 p.p.s. are similar, but are comparatively larger than at 150 p.p.s. and, based on the data for two sites, than at highest frequencies. The latency data, discussed below, are also consistent with a relationship between the stimulation pulse frequency and the spatial domain of recruited neurons. Further experiments are necessary to test the exact nature of this relationship, especially in the high stimulation frequency range.

**Gaze shift amplitude and stimulation pulse number**

Stanford et al. (1996) investigated, in the head-restrained monkey, the relationship between saccade amplitude and number of delivered pulses. They concluded that ‘across stimulation frequencies, the amplitude is best related to the total number of pulses in the stimulation train’ (page 3360). In contrast, we showed that in the frequency range for which the amplitude of gaze shifts remained constant (300–500 p.p.s.), the number of stimulation pulses (NSP) was statistically different. This result was obtained irrespective of whether the NSP was computed from movement duration only (NSP2) or from movement duration and latency (NSP3). In the analysis performed by Stanford et al. (1996), almost all saccades considered were deliberately truncated in order to yield a wide range of amplitude and their conclusion appears to be restricted to this special situation. Indeed, if one computes the NSP (either NSP2 or NSP3 as defined in the present study) from their data concerning nontruncated saccades (their table 3), the NSP is always different for similar amplitude saccades evoked by different pulse frequencies, which is consistent with our own findings.

**Gaze latency**

In agreement with previous studies (Straschil & Rieger, 1973; Guittton et al., 1980; Paré et al., 1994; Freedman et al., 1996; Grantyn et al., 1996; Stanford et al., 1996), we found that increasing either the pulse frequency or the current intensity led to an exponential decay of latency toward values near 30 ms. In the case of varying the pulse frequency between 300 and 500 p.p.s., the latency decrease concurred with a constant number of stimulation pulses (NSP1). Thus, variations in latency can be accounted for by the need of a roughly constant NSP1 to trigger gaze shifts. One should consider that the true number of spikes produced by the SC and driving the saccadic generator in the reticular formation depends both on NSP1 and on the number of activated collicular neurons. If the size of the recruited population is similar for 300 and 500 p.p.s., as already argued in the section on gaze metrics, a constant NSP1 is reminiscent of a fixed threshold which has to be reached to initiate a saccade. Note that a higher NSP1 was necessary to initiate a movement when the frequency was outside the 300–500 p.p.s. range. This could find an explanation if smaller populations are recruited in this case, a possibility which is again supported by metrics results. Furthermore, increasing current intensity with a fixed pulse frequency led to a reduction of latency and, therefore, of the NSP1. Because high current intensities recruit more collicular neurons and hence drive the pulse generator with a larger number of action potentials, this effect would occur because the trigger level for gaze shift initiation will be reached sooner.

Thus, these effects of stimulation frequency and current intensity on gaze shift initiation suggest that the neuronal activity driving the saccadic generator at the premotor level could be integrated both temporally across the reaction time and spatially across the collicular map until a fixed trigger level is reached. This notion of a fixed trigger level can be paralleled with the findings of Hanes & Schall (1996) who showed that visually guided saccades are initiated when the neural activity in the frontal eye field (FEF) reaches a constant threshold level for all saccades.

It is now well accepted that the saccade triggering mechanism at the premotor level is achieved by turning off the tonic inhibitory influence exerted by omnipause neurons (OPNs) on preoculomotor neurons (see for review Fuchs et al., 1985). It has recently been demonstrated that this pause in OPN activity was induced by a temporal summation of IPSPs (Yoshida et al., 1999). This study also showed that the initial phase of the OPN pause was a steep hyperpolarization with a time course which was unrelated to the dynamics of the forthcoming saccade, which is consistent with the constant trigger threshold hypothesis. In addition, a recent study showed that stimulation of the SC produces disynaptic IPSPs in OPNs (Yoshida et al., 2001). Altogether, these data suggest that SC burst activity contributes to the saccade triggering mechanisms through OPN inhibition.

**Functional implications of the stimulation strength vs. gaze shift amplitude relationship**

In the following, we discuss what the proposed ‘mixed coding’ for the amplitude of electrically evoked gaze shifts, i.e. coding which relies both on locus and extent of activated neuronal population, entails concerning the processing of efferent SC impulses. We next add some remarks concerning the consequences for the generation of visually triggered gaze shifts.

**Summation vs. average of SC efferent impulses**

Two types of schemes have been proposed to describe how collicular outputs are processed at the level of the reticular formation; the ‘vector sum’ hypothesis (McCowan, 1976, 1982; Sparks et al., 1976) and the ‘vector average’ hypothesis (Lee et al., 1988; Hanes & Wurtz, 2001).
Figure 8 presents the predictions of these two hypotheses regarding the effect of varying current intensity on saccade amplitude. Four different stimulation sites and three current intensities for each site are considered. These simulations are based on the fact that the amplitude of saccadic gaze shifts evoked using a fixed current intensity varies in a non-linear fashion according to the rostro-caudal location of the stimulation site on the motor map [gaze amplitude = 89.5 × \exp(-0.47 × \text{Stco})] see Fig. 2B]. We make the three following assumptions. First, the size of the recruited population at 2 × T is fixed at 1 mm of tissue. This value was estimated after McIlwain (1982) and from the mean threshold intensity observed in our study (7.9 μA). Because in the model this 1-mm space is covered by 21 cells, the contribution of each individual cell was taken to be CC = PGA/21 such that both averaging and summation hypotheses predict identical saccade amplitudes at 2 × T. Saccade amplitude corresponding to each intensity was computed either as the mean PGA of all recruited cells or as the sum of their CC in the averaging or summation hypotheses, respectively. For 3 × T and 1 × T current intensities, the recruited population size was 1.5 mm and 0.5 mm (note that the linear relation between the neural tissue recruited area and the current intensity agrees with the generally assumed proportionality between the square root of current intensity and the recruited population radius). (B) Plots of theoretical gaze amplitude vs. current intensity predicted from the averaging and summation hypotheses, respectively. Note the very slight current-related amplitude increase in the case of the averaging hypothesis (due to the nonlinearity of the map) and the strong and site-dependent relationship in the case of the summation hypothesis.

We emphasize that the scheme of vector average proposed by Lee et al. (1988) includes weighting mechanisms which cancel out the nonlinearity of collicular amplitude coding. Thus, this ‘weighted averaging’ scheme predicts no change of saccade amplitude when increasing the current intensity.

In contrast, the prediction of the vector sum hypothesis is a strong increase of saccade amplitude as a function of current intensity (panel B, lower part). In addition, this increase is larger for stimulations situated caudally in the SC motor map than in rostral stimulation sites. These two features are reminiscent of the saccade amplitude vs. current intensity relationships observed experimentally (Figs 4A and C, and 5A and B). In addition, the simulated slope values fit very well the actual values. Note that other predicted values would have been obtained with a different relationship between recruited population size and stimulation intensity, but the important point is that the slopes derived from the summation hypothesis would still be much larger than those derived from the average hypothesis, and would therefore still better match the actual data. In conclusion, the comparison between predictions and actual data indicates that the vector sum hypothesis is clearly more consistent with the experimental data than is the vector average hypothesis.

We make two additional remarks. First, because we proposed that the effect of pulse frequency on gaze amplitude could be explained in a similar way as the effect of intensity (by a variation in the size of the recruited population), the frequency data are also compatible with the vector sum hypothesis. Second, we note that this hypothesis of a summation of collicular action potentials impinging on reticular formation neurons is consistent with the actual knowledge on spatio-
temporal integration of neuronal activity. In contrast, no physiologically plausible model implementing an average scheme at the reticular formation level exists.

**Generation of visually triggered gaze shifts**

Because the summation of SC efferent impulses relies on the pattern of anatomical projections to the reticular formation, it would not depend on the origin of SC activation. This entails several consequences for the generation of visually triggered gaze shifts. (i) These movements should also rely on summation of SC efferent impulses. (ii) The collicular coding of the amplitude of these movements would also be mixed and depend on both locus and extent of activated neuronal population. (iii) Finally, the generation of visually triggered saccades of correct amplitude cannot rely exclusively on an accurate control of the site of SC activation but must also involve a precise regulation of its size through various feedback and feedforward influences on the SC motor map.

**Conclusions**

The results of the present study confirm in the head-unrestrained cat the existence of a map of saccadic gaze shifts in the deep collicular layers, and also underline the effect of the pattern of collicular activity on the characteristics of gaze shifts. This ‘mixed coding’ has important implications regarding the processing of SC efferent impulses by downstream structures. Indeed, our data are compatible with a summation of collicular saccadic activities by downstream neuronal circuits, rather than with the often-cited vector average hypothesis. Concerning gaze shift initiation, we provide evidence for a spatial (across the SC motor map) and temporal (during the latency period) integration of the neuronal activity until a fixed trigger level is reached. This again requires a summation of collicular signals by downstream neuronal elements. Finally, our data are also consistent with a separate collicular coding of saccade metrics and dynamics. Altogether, these findings suggest that the usually-observed stereotyped gaze shift behaviour for visually elicited responses requires various feedback and feedforward influences on the SC motor map which accurately control the extent and intensity of the active population of collicular output neurons.

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**Abbreviations**

NSP, number of stimulation pulses; OPN, omnipause neuron; p.p.s., pulses per second; SC, superior colliculus.

**References**


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