In Multiple-Step Gaze Shifts: Omnipause (OPNs) and Collicular Fixation Neurons Encode Gaze Position Error; OPNs Gate Saccades

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INTRODUCTION

Critical to normal perception is the ability to voluntarily fixate a target of interest for controlled periods of time without gaze being swept away by unwanted saccadic eye movements. This fundamental motor requirement is thought to be fulfilled at the brain stem reticular level by “omnipause” neurons (OPNs), which are located in the nucleus raphe interpositus and whose tonic activity during fixation inhibits the burst generator that drives saccades (reviewed by Moschovakis et al. 1996). In accordance with this gating function, OPNs in cat and monkey (head-fixed) pause for the duration of eye saccades (Cohen and Henn 1972; Everling et al. 1998; Evinger et al. 1982; Keller 1974, 1977; King and Fuchs 1977; Langer and Kaneko 1990; Luschei and Fuchs 1972). OPN gating function may extend to saccadic gaze shifts made with the head unrestrained because OPNs also pause for the duration of these movements (Paré and Guitton 1998; Sylvestre et al. 2001).

When saccades are made, descending signals appropriately gate OPN activity. Two candidate structures for this function are the frontal eye fields (FEF) and rostral superior colliculus (SC), both of which contain neurons with fixation-related discharges that are thought to project onto OPNs (SC: Büttner-Ennever et al. 1999; Gandhi and Keller 1997; Munoz and Guitton 1989, 1991; Munoz and Wurtz 1993a,b; Ohtsuka and Nagasaka 1999; Paré and Guitton 1994; FEF: reviewed in Hanes et al. 1998; Segraves 1992). In this paper we are concerned with the collicular projection.

It has been hypothesized that fixation neurons in the rostral SC (SCFNs) contribute to suppressing saccades via direct excitatory projection to both OPNs and collicular interneurons that inhibit the SC’s motor map (Munoz and Guitton 1989, 1991; Munoz and Istvan 1998; Munoz and Wurtz 1993a,b; Munoz et al. 1991). Direct projections of identified SCFNs onto OPNs have not been explicitly shown, but nevertheless, there is compelling circumstantial evidence supporting such a connection. 1) For eye saccades and single-step head-unrestrained gaze shifts, superior colliculus fixation neurons (SCFNs) recorded in the intermediate to deep layers of the rostral SC can display an activity pattern quite similar to OPNs (monkey SCFNs: Everling et al. 1998; Munoz and Wurtz 1993a; cat: Bergeron and Guit-
4) During attentive fixation, SCFNs are more active (Munoz and Wurtz 1993a), and during this behavioral state, stronger stimulation of the SC’s motor map is required to trigger a saccade (Paré et al. 1994). 5) When the SCFN region is deactivated pharmacologically, monkeys make irrepressible saccades to visual targets (Munoz and Wurtz 1993b).

Despite the anatomical and physiological evidence supporting the role of the rostral SC in modulating OPN activity, such a specialized role for SCFNs has been questioned (Everling et al. 1998; Gandhi and Keller 1999a,b; Krauzlis et al. 2000). Indeed, the tonic discharge pattern of OPNs may not, during certain tasks, be a simple replica of the SCFN discharge. For example, in monkeys performing a “gap” saccade task—wherein the fixation point is extinguished a few hundred milliseconds (the gap) before target presentation—the tonic discharge frequency of SCFNs diminishes during the gap period, whereas that of OPNs does not (Everling et al. 1998). A further difference is that OPNs pause for all small saccades, whereas SCFNs burst, or remain active, for small contraversive saccades (Krauzlis et al. 2000; Munoz and Wurtz 1993a; Peck and Baro 1997).

To probe how SCFNs might influence OPNs, we compare in this study the discharge characteristics of both cell types during horizontal multiple-step gaze shifts (Fig. 1, B and D). These movements are composed of a variable number of gaze saccades, of variable amplitude, interspersed with fixation periods of variable duration (Bergeron and Guitton 2000a). In this particular behavior, the visual axis becomes relatively immobile (plateaus) at a number of locations prior to reaching the salient target. Some cats frequently use multiple-step gaze shifts to reorient their visual axis. We showed in Bergeron and Guitton (2000a) that most cat SCFNs paused during the gaze saccades as well as during the plateaus at the beginning of horizontal contralaterally directed multiple-step sequences. SCFN activity resumed only when the visual axis arrived, in the dark, at a particular angular distance from the target position. The subsequent tonic activity was not modulated in relation to saccades in the gaze trajectory. By comparison, Paré and Guitton (1998) showed, for a single example of a multiple-step gaze shift, that an OPN paused for every saccadic gaze step and fired tonically during every fixation period; a pattern quite different from that of SCFNs.

Here we compare SCFN and OPN discharges during horizontal multiple-step gaze shifts to the left and right. We confirm that during gaze saccades the firing pattern of OPNs is “motor-like” and reliably indicates, by its alternating periods of tonic activity and pauses, whether the gaze trajectory is in the fixation or saccade modes. By comparison, SCFNs do not carry motor-like signals. We show further, for both cell-types, that the tonic activity during gaze plateaus increases as the angular distance between the visual axis and the target [gaze position error (GPE)] goes to zero. Thus information on how close the visual axis is to a salient target is available in a very low-level brain stem region, notably OPNs, which are just two synapses away from ocular motoneurons.

Brief reports of some of these results have been presented previously (Bergeron and Guitton 2000b, 2001).

METHODS

Animal preparation

All surgical and experimental protocols were approved by the Animal Care Committee of the Montreal Neurological Institute and complied with the Canadian Council on Animal Care policy on the use of laboratory animals. Cats underwent initial training and were selected because they frequently oriented using multiple-step rather than single-step gaze shifts. Note however that the relative use of
multiple-steps varied from day to day. Seven cats were prepared for chronic single-unit recordings in the brain stem: five (cats N, M, P, H, and T) for the study of SCFNs in the rostral SC and two different animals (cats G and L) for the study of OPNs in the nucleus raphe interpositus.

Anesthesia was induced with an intramuscular injection of ketamine hydrochloride (10 mg/kg). The cats were intubated and maintained on anesthesia using halothane. During surgery, heart rate, respiratory rate, and body temperature were monitored. A wire coil consisting of three turns of teflon-coated multistrand stainless steel wire (California Fine Wire) was sutured to the sclera of one eye for recording eye movements (Robinson 1963). The wire leads passed subcutaneously to an acrylic skull implant that was anchored to the skull with T-shaped stainless steel bolts. A stainless steel cylinder, constructed to hold a small micropositioner (Narashige), was positioned vertically on the midline of the cranial surface at the anteroposterior stereotaxic coordinate A-P: 0 (Berman 1968) to permit access to the SC for single-unit recording. For access to the OPN region, the cylinder was positioned just rostral to the lambdoid crest, centered on the midline, and tilted 25° back from the frontal plane.

We determined, using antidromic stimulation in two cats (N and T), whether a collicular cell was a tecto-reticular neuron. We implanted into the predorsal bundle and fixed to the explant a bipolar concentric stimulating electrode (SNEX-100; Kopf). This electrode was lowered into the brain stem at an angle 20°–30° posterior to the frontal plane, to a site just rostral to the abducens nucleus (stereotaxic coordinates P 5.5, H –5.0, ML 0; Berman 1968) within the predorsal bundle. The final stimulating electrode position was determined by trial and error during surgery: the electrode position was adjusted while continuously stimulating such that a recording microelectrode in the SC could record antidromically activated SC cells and/or strong evoked potentials.

The connector for the eye-coil, the recording cylinder, and the stimulating electrode were embedded in the explant. A screw, attached to the explant, held a second search coil used to measure head movements. A thin stainless steel U-shaped crown was also embedded in the posterior perimeter of the explant for the purpose of attaching the cat’s head to a universal joint; it attached to a freely rotating vertical shaft (see Behavioral procedures).

After the surgery, an intramuscular injection of gentamicin (15 mg/kg) or cefazolin (35 mg/kg) was administered as a prophylactic measure against infection. This treatment was continued on a daily basis for 10 postoperative days. At the end of the surgery, an analgesic medication (buprenorphine hydrochloride, 0.01 mg/kg) was given and continued for 2 days. Cats recovered for ≥10 days before experimental procedures commenced.

Behavioral procedures

During the experiments, the alert cat was enveloped in a loosely fitting cloth bag and placed in an open-top box that gently restrained its body and limb movements. The animal box allowed full ranges in horizontal and vertical-up head motion except for downward head motion, which was restricted to about 35° below normal head posture. Only horizontal gaze shifts were analyzed in this study. Two cats—cat M for SCFNs and cat L for OPNs (see Tables 1 and 2, respectively)—were studied with the head completely unrestrained. For the others, the U-shaped crown in the explant was attached, via two universal joints, to a vertical shaft rotating in low-friction bearings. The universal joints minimized constraint on the animal’s orienting behavior. The results, summarized in Tables 1 and 2, suggest cell discharges were not affected by whether or not the head was attached to the shaft. Information about calibration procedures for our search coil in magnetic field technique (Robinson 1963) have been reported elsewhere (Guitton et al. 1984).

To obtain gaze shifts we used a “barrier paradigm” (Munoz and Guitton 1991). A cat faced an opaque black barrier of variable width (10°–60°) directly in front at a distance of about 35 cm. The target was pured cat food placed in a small (3°) receptacle attached to a stem held by the experimenter. Initially in a trial, the food target was hidden behind the barrier and consequently there was no visible target of significance to the cat. To obtain an orienting gaze shift, the receptacle (not stem) was suddenly protruded from the barrier at the edge of one of the two vertical sides. In this condition, the hungry cat redirected its visual axis from its initial position on the blank screen to the target and was fed. To obtain the time of target appearance, the rapidly moving food target intercepted an infrared detector located at the very edge of the barrier, which generated a marker pulse fed to the computer. The target’s trajectory also was measured using a search coil attached to the food receptacle. The calibration of this coil signal was obtained by referring it to the actual physically measured target position. Using these two methods, our estimate of the time of appearance of the target’s edge was within ±3 ms.

The barrier paradigm permitted a wide range of gaze shift amplitudes, not only because of the variable barrier width, but also because the overall gaze shift required to fixate the target depended on the initial gaze position that varied from trial to trial. Ambient lighting was provided by a fluorescent light with very fast decay time (~100 μs). Visually guided gaze shifts were performed in the light. These were randomly interleaved with gaze shifts made in complete darkness to the remembered location of the target. To obtain such memory-guided gaze shifts, ambient lighting was extinguished for 1 s, beginning 120 ms after an infrared detector was triggered by the passing food target. When the light was turned on, the cat frequently generated a “corrective” gaze saccade, but these were small, on average 2.1 ± 1.7° (SD) for our seven cats. We took final intended gaze position as that within the last 200 ms before ambient lighting was reestablished.

Single-unit recording

Munoz and Wurtz (1993a) proposed five criteria for a cell to be classified as a monkey SCFN: 1) SCFNs are located in the rostral SC; 2) their motor activity increases when the animal actively fixates a visible target; 3) this activity persists when the target momentarily disappears but the animal maintains fixation in the dark; 4) for saccades >15°, the tonic activity of most cells pauses at the onset of ipsilateral and contralateral saccades and the pause lasts for the duration of the saccades; and 5) at the end of primarily contraversive saccades to a target, the tonic activity of most cells exceeds that at the start, even if the target has disappeared and the saccade is made in the dark. In prior work we have shown that SCFNs in the head-unrestrained cat—the condition studied here—have very similar properties, save for criterion 4: cat SCFNs pause at the onset of primarily contralateral gaze shifts (Bergeron and Guitton 2000a; Munoz and Guitton 1989, 1991; Munoz et al. 1991). We also showed that many of these SCFNs are tecto-reticular neurons. By comparison, for the head-fixed cat, Peck and Barot (1997) describe omnidirectional pauses for large saccades.

Before recording SCFNs, we first confirmed that our electrodes were in the rostral SC by exploring the SC’s motor map using electrical stimulation (train duration, 300 ms; frequency, 300 Hz; current, 5–30 μA). The map’s organization was deduced based on the amplitude and direction of gaze shifts evoked at different electrode positions (Paré and Guitton 1994). With this method it was possible to predict the stereotaxic location of the rostral SC’s fixation zone. We then confirmed this location by applying electrical stimulation and verifying that we could interrupt a gaze shift in mid-flight. We recorded SCFNs in the intermediate and deep layers—about 1.2–3 mm below the dorsal surface—of the rostral zone of the SC. When we encountered a cell in the rostral SC, we first verified qualitatively that its discharge properties were compatible with criteria 2–5 above, as applied to the cat and described in our prior studies. We then focused
TABLE 1. Parameters describing SCFN activity

<table>
<thead>
<tr>
<th>SCFNs</th>
<th>$f_i$ (Hz)</th>
<th>$R_2$ (ms)</th>
<th>$P$ (ms)</th>
<th>$f_{e}$ (Hz)</th>
<th>$f_{i}$ (Hz)</th>
<th>$\Delta f$ (Hz)</th>
<th>GPE at 1.64s $\Delta f$ (c.l.)</th>
<th>First spike GPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>16</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>0.73</td>
<td>0.36</td>
</tr>
<tr>
<td>M5♣</td>
<td>7</td>
<td>0.59</td>
<td>0.38</td>
<td>5</td>
<td>49</td>
<td>48</td>
<td>23 ± 5</td>
<td>NP</td>
</tr>
<tr>
<td>M5♣*</td>
<td>7</td>
<td>0.45</td>
<td>0.34</td>
<td>0</td>
<td>38</td>
<td>39</td>
<td>16 ± 3</td>
<td>NP</td>
</tr>
<tr>
<td>M5♣*</td>
<td>28</td>
<td>0.67</td>
<td>0.16</td>
<td>0</td>
<td>55</td>
<td>54</td>
<td>16 ± 1</td>
<td>NP</td>
</tr>
<tr>
<td>N12</td>
<td>25</td>
<td>0.57</td>
<td>0.71</td>
<td>0</td>
<td>112</td>
<td>113</td>
<td>17 ± 3</td>
<td>22</td>
</tr>
<tr>
<td>N13</td>
<td>6</td>
<td>0.56</td>
<td>0.69</td>
<td>0</td>
<td>48</td>
<td>45</td>
<td>18 ± 6</td>
<td>13</td>
</tr>
<tr>
<td>N23c+</td>
<td>1</td>
<td>0.85</td>
<td>0.79</td>
<td>0</td>
<td>34</td>
<td>33</td>
<td>10 ± 2</td>
<td>7</td>
</tr>
<tr>
<td>N26+</td>
<td>38</td>
<td>0.45</td>
<td>0.49</td>
<td>0</td>
<td>63</td>
<td>64</td>
<td>13 ± 2</td>
<td>10</td>
</tr>
<tr>
<td>N27a+</td>
<td>12</td>
<td>0.38</td>
<td>0.65</td>
<td>0</td>
<td>46</td>
<td>45</td>
<td>11 ± 2</td>
<td>12</td>
</tr>
<tr>
<td>N29c+</td>
<td>11</td>
<td>0.54</td>
<td>0.45</td>
<td>0</td>
<td>41</td>
<td>45</td>
<td>11 ± 6</td>
<td>6</td>
</tr>
<tr>
<td>N37a+</td>
<td>0</td>
<td>0.70</td>
<td>0.70</td>
<td>0</td>
<td>18</td>
<td>18</td>
<td>6 ± 2</td>
<td>4</td>
</tr>
<tr>
<td>N38</td>
<td>12</td>
<td>0.54</td>
<td>0.35</td>
<td>0</td>
<td>57</td>
<td>57</td>
<td>8 ± 1</td>
<td>7</td>
</tr>
<tr>
<td>N40</td>
<td>16</td>
<td>0.65</td>
<td>0.10</td>
<td>0</td>
<td>31</td>
<td>31</td>
<td>9 ± 1</td>
<td>9</td>
</tr>
<tr>
<td>N42a</td>
<td>15</td>
<td>0.85</td>
<td>0.25</td>
<td>0</td>
<td>39</td>
<td>40</td>
<td>18 ± 2</td>
<td>18</td>
</tr>
<tr>
<td>P47a</td>
<td>37</td>
<td>0.74</td>
<td>0.46</td>
<td>16</td>
<td>83</td>
<td>65</td>
<td>21 ± 3</td>
<td>NP</td>
</tr>
<tr>
<td>P48</td>
<td>14</td>
<td>0.77</td>
<td>0.79</td>
<td>0</td>
<td>30</td>
<td>50</td>
<td>6 ± 1</td>
<td>5</td>
</tr>
<tr>
<td>P48b</td>
<td>7</td>
<td>0.57</td>
<td>0.50</td>
<td>0</td>
<td>63</td>
<td>66</td>
<td>18 ± 2</td>
<td>19</td>
</tr>
<tr>
<td>T5+</td>
<td>21</td>
<td>0.57</td>
<td>0.76</td>
<td>0</td>
<td>40</td>
<td>43</td>
<td>30 ± 6</td>
<td>25</td>
</tr>
<tr>
<td>Mean</td>
<td>15 ± 1</td>
<td>0.59 ± 0.5</td>
<td>0.50 ± 0.2</td>
<td>1 ± 4</td>
<td>0.50 ± 0.2</td>
<td>0.50 ± 0.2</td>
<td>15 ± 6</td>
<td>13 ± 6</td>
</tr>
</tbody>
</table>

For each cat, cells are listed according to the chronological order in which they were recorded. $f_i$, firing frequency before target onset when cat fixes opaque barrier in light; $f_e$, asymptotic firing frequency at large GPE; $f_{i}$, firing frequency at end of multiple-step gaze shift in dark; $\Delta f$, calculated value of $f_i$ – $f_{e}$, c.l., 95% confidence limit.

* Not sufficient data.

♣ Head completely unrestrained.

* Tecto-reticular neuron.

NP, no pause in discharge; could not calculate the preferred GPE from first spike.

NM, not significantly modulated.

on obtaining as much data as possible on the discharge properties during multiple-step gaze shifts.

By stimulating antidromically, in cats N and T, the main descending axons at a site in the predorsal bundle just rostral to the abducens nucleus, it was possible to verify in these cats whether the recorded SCFNs were tecto-reticular neurons. Our techniques have been determined according to the criteria described by Lipski (1981). For the two cats studied with this technique, cells identified as tecto-reticular neurons are indicated in Table 1.

The region explored to find OPNs extends from the rostral margin of the abducens nucleus ±1.2 mm anterior, 1.0–3.0 mm below the margin of the IVth ventricle, and ±0.6 mm lateral from the midline (Evinger et al. 1982; Ohgaki et al. 1987; Paré and Guittion 1998; Strassman et al. 1987). To record single-unit activity we used tungsten microelectrodes (1–2 MΩ; Frederick Haer). The electrode was advanced manually through a cannula to 5 mm over the region investigated, and the hydraulic microdrive lowered the electrode while searching for cells. Action potentials were amplified, filtered (bandpass 300 Hz to 10 kHz), and displayed on an oscilloscope. The identification of OPNs was based on their discharge properties during head-fixed eye-saccades and single-head-unrestrained gaze shifts, which have been well described in numerous studies (head-fixed, reviewed in Moschovakis et al. 1996; head-unrestrained, Paré and Guittion 1998; Phillips et al.1999). During the experiments, target, eye and gaze positions, cell activity, light offset, and duration of dark period were stored on DAT tape (TEAC RD-200T) for off-line analysis.

**Data analysis**

The gaze and head coils gave signals proportional to eye and head positions in space, respectively. Gaze (G) = eye-position-in-space = eye-in-head (E) + head-in-space (H). In off-line analysis, using the data stored on DAT tape, we calculated E from G and H. Movement traces were filtered at 1 kHz and digitized at 2 kHz with data acquisition software and subsequently analyzed with MatLab. The start and end of saccadic eye and gaze trajectories were determined by a velocity threshold corresponding to 25°/s. For saccadic head movements the threshold was 15°/s, respectively. Action potentials were converted to logic pulses via a time-amplitude window discriminator (BAK Electronics). Spike density histograms were generated by substituting for each spike a Gaussian function with a width of 10 ms (MacPherson and Aldridge 1979; Richmond et al. 1987) and then summing all the Gaussians together to generate a continuous function in time. Mean firing frequency was calculated by counting the number of spikes in an interval and dividing by the duration of that interval. The relevant interval durations are given in RESULTS.

During multiple-step gaze shifts, we will show in RESULTS that the firing frequency during plateaus increased smoothly as GPE decreased to zero (e.g., Figs. 1B and 3A). To describe the change in firing frequency with gaze position error, we fitted the data to the following Gaussian relationship

$$f_f = f_{i} + \sigma \cdot e^{\frac{-(GPE - \mu)^2}{2\sigma^2}}$$

where $f_f$ is the firing frequency at large GPE, $f_{i}$ is the firing frequency at the end of a multiple-step sequence, $\Delta f = f_{i} - f_{e}$, and $\sigma$ is a constant providing a measure of the width of the bell-shaped curve and equivalent to 1 SD in statistical analyses.

To determine how well this nonlinear equation fits the data, we used...
TABLE 2. Parameters describing OPN activity

<table>
<thead>
<tr>
<th>OPNs</th>
<th>( f_1 )</th>
<th>( R_2 )</th>
<th>( P )</th>
<th>( f_1 )</th>
<th>( f_1 )</th>
<th>( \Delta f )</th>
<th>GPE at 1.64( \sigma ) ( \pm ) c.l.</th>
<th>( R_2 )</th>
<th>( P )</th>
<th>( f_1 )</th>
<th>( f_1 )</th>
<th>( \Delta f )</th>
<th>GPE at 1.64( \sigma ) ( \pm ) c.l.</th>
</tr>
</thead>
<tbody>
<tr>
<td>G2</td>
<td>89</td>
<td>0.64</td>
<td>0.06</td>
<td>75</td>
<td>195</td>
<td>109</td>
<td>13 ( \pm ) 4</td>
<td>0.16</td>
<td>0.80</td>
<td>116</td>
<td>167</td>
<td>47</td>
<td>8 ( \pm ) 5</td>
</tr>
<tr>
<td>G2a</td>
<td>115</td>
<td>0.62</td>
<td>0.88</td>
<td>93</td>
<td>195</td>
<td>99</td>
<td>8 ( \pm ) 1</td>
<td>0.70</td>
<td>0.16</td>
<td>75</td>
<td>192</td>
<td>111</td>
<td>7 ( \pm ) 1</td>
</tr>
<tr>
<td>G3</td>
<td>76</td>
<td>0.56</td>
<td>0.10</td>
<td>35</td>
<td>78</td>
<td>43</td>
<td>9 ( \pm ) 1</td>
<td>0.28</td>
<td>0.36</td>
<td>50</td>
<td>70</td>
<td>20</td>
<td>6 ( \pm ) 1</td>
</tr>
<tr>
<td>G4a</td>
<td>157</td>
<td>0.44</td>
<td>0.07</td>
<td>116</td>
<td>200</td>
<td>97</td>
<td>11 ( \pm ) 3</td>
<td>0.44</td>
<td>0.12</td>
<td>134</td>
<td>246</td>
<td>97</td>
<td>11 ( \pm ) 3</td>
</tr>
<tr>
<td>G4b</td>
<td>28</td>
<td>0.55</td>
<td>0.87</td>
<td>16</td>
<td>53</td>
<td>35</td>
<td>9 ( \pm ) 1</td>
<td>0.80</td>
<td>0.70</td>
<td>19</td>
<td>58</td>
<td>38</td>
<td>5 ( \pm ) 1</td>
</tr>
<tr>
<td>G4d</td>
<td>110</td>
<td>0.68</td>
<td>0.42</td>
<td>64</td>
<td>146</td>
<td>80</td>
<td>9 ( \pm ) 1</td>
<td>0.77</td>
<td>0.10</td>
<td>50</td>
<td>152</td>
<td>98</td>
<td>7 ( \pm ) 1</td>
</tr>
<tr>
<td>G5</td>
<td>100</td>
<td>0.55</td>
<td>0.36</td>
<td>59</td>
<td>139</td>
<td>77</td>
<td>13 ( \pm ) 4</td>
<td>0.42</td>
<td>0.67</td>
<td>61</td>
<td>126</td>
<td>62</td>
<td>7 ( \pm ) 2</td>
</tr>
<tr>
<td>G6</td>
<td>89</td>
<td>0.67</td>
<td>0.49</td>
<td>78</td>
<td>156</td>
<td>78</td>
<td>13 ( \pm ) 1</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>G6a</td>
<td>120</td>
<td>0.67</td>
<td>0.31</td>
<td>46</td>
<td>143</td>
<td>91</td>
<td>14 ( \pm ) 2</td>
<td>0.57</td>
<td>0.44</td>
<td>68</td>
<td>125</td>
<td>56</td>
<td>7 ( \pm ) 1</td>
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<tr>
<td>G6c</td>
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<td>0.77</td>
<td>0.77</td>
<td>31</td>
<td>126</td>
<td>92</td>
<td>8 ( \pm ) 1</td>
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<tr>
<td>G6e</td>
<td>140</td>
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<td>0.66</td>
<td>30</td>
<td>145</td>
<td>115</td>
<td>8 ( \pm ) 1</td>
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<tr>
<td>L7</td>
<td>98</td>
<td>0.26</td>
<td>0.58</td>
<td>50</td>
<td>79</td>
<td>35</td>
<td>3 ( \pm ) 2</td>
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<tr>
<td>L7a</td>
<td>85</td>
<td>0.53</td>
<td>0.43</td>
<td>41</td>
<td>121</td>
<td>81</td>
<td>16 ( \pm ) 5</td>
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<td>114</td>
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<tr>
<td>L7b</td>
<td>55</td>
<td>*</td>
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<td>*</td>
<td>*</td>
<td>*</td>
<td>0.53</td>
<td>0.85</td>
<td>13</td>
<td>44</td>
<td>29</td>
<td>3 ( \pm ) 1</td>
</tr>
</tbody>
</table>

Mean 93 \( \pm \) 34 0.56 \( \pm \) 0.14 0.53 \( \pm \) 0.3 56 \( \pm \) 28 135 \( \pm \) 45 79 \( \pm \) 26 10 \( \pm \) 4 0.55 \( \pm \) 0.22 0.46 \( \pm \) 0.3 60 \( \pm \) 41 129 \( \pm \) 63 66 \( \pm \) 33 6 \( \pm \) 3

For each cat, cells are listed according to the chronological order in which they were recorded. \( f_1 \), firing frequency before target onset when cat fixes opaque barrier in light; \( R_2 \), goodness of fit of Eq. 1 = variance accounted for (VAF); \( P \), runs test; \( P > 0.05 \) indicates data do not deviate significantly from Gaussian distribution about line; \( f_1 \), asymptotic firing frequency at large GPE; \( f_1 \), firing frequency at end of multiple-step gaze shift in dark; \( \Delta f \), calculated value of \( f_1 - f_1 \) \( \pm \) c.l., 95% confidence limit.

* Not sufficient data.

GraphPad Prism version 3.0 for Windows (GraphPad Software Inc.) to perform the nonlinear regression analyses. We specified parameter \( f_1 \) and the software adjusted \( \sigma \) and \( f_1 \) to provide the optimal fit to the data points. In the two tables summarizing the data analysis, we provide three quantitative criteria that evaluate the goodness of fit: the nonlinear regression coefficient \( (R_2) \), the “Runs test,” and the 95% confidence limits on the fitted parameter \( \sigma \).

The nondimensional value \( R_2 \) quantifies the goodness of fit and is a fraction between 0 and 1 given by the equation

\[
R_2 = 1.0 - \frac{SS_{reg}}{SS_{tot}} \tag{2}
\]

where \( SS_{reg} \) is the sum of squares of the distance of the experimental points from the nonlinear curve and \( SS_{tot} \) is the sum of squares of the distance of the points from a horizontal straight line through the mean of all \( y \) axis values. This is the same equation used for linear regression analysis, and \( R_2 \) gives the variance-accounted-for (VAF). When \( R_2 = 1 \), all points lie on the curve. Note that \( R_2 \) cannot be used as the only criterion for goodness of fit. A high \( R_2 \) indicates that the points are close to the curve and accounts for much of the variance, but in a nonlinear fit, the curve can deviate systematically from the data points being, say, above a certain sequence of points and then below the next sequence, etc. Hence the “Runs test” is used, which gives the probability \( (P, \text{one-tail}) \) that the points are randomly distributed about the curve. In statistical analysis, a probability of 5% or less is commonly used as the criterion for stating that two populations are statistically different; i.e., for rejecting the null hypothesis. Accordingly, we used the criterion that \( P \) should be \( >5\% \) for the fit to be statistically acceptable; i.e., that there is greater than a 1 in 20 chance that the observed distribution of points about the fitted line could have been obtained following a pick of data from a random distribution. For example, a low \( P \) value such as 0.01 indicates that in a large population of points, randomly distributed about the curve, there is less than a 1% chance of selecting a subgroup with the observed distribution of points about the curve; i.e., the fit is not good and another nonlinear curve might be tried. The value \( P = 0.05 \) is the least expected; as we shall see, 85% of our fits had a \( P > 0.20 \).

RESULTS

To obtain many trials with multiple steps, it was generally necessary to record from a cell for at least about 1 h; a difficult problem in the head-unrestrained condition, because head movements cause small displacements of the brain stem relative to the microelectrode tip, and contact with a cell is easily lost. Thus difficulties imposed by recording stability and multiple-step behavior limited the number of cells that could be studied in each cat. Recording stability was more of a problem with SCFNs than with OPNs. For each SCFN and OPN that we studied in each cat. Recording stability was more of a problem with SCFNs than with OPNs. For each SCFN and OPN that we retained for analysis, we had between 8 and 60 (average about 20) of each of single- and multiple-step gaze shifts from which we could quantitatively assess a cell’s discharge pattern.

SCFN discharges during contralateral and ipsilateral multiple-step gaze shifts

We report here on 18 cells recorded in the rostral SC of five cats and classified as SCFNs based on prior descriptions of SCFN discharges during single-step gaze shifts in the head-unrestrained cat and head-fixed monkey (see Single-unit recording). For these same neurons, we reported previously that following the initial pause in activity at gaze shift onset, the appearance of the first spike after the pause was determined by GPE (Bergeron and Guittion 2000a). Two cats were studied for antidromic responses. In cat T, we recorded only one SCFN, an identified tecto-reticular neuron. In cat N we identified 5/10 SCFNs as tecto-reticular neurons (labeled in Table 1). Note that this observation does not imply that 50% of SCFNs in cat N were output cells. Indeed, in cat N the antidromic stimulating electrode ceased to function while we recorded cell N37a. (In Table 1, the cells are listed from top to bottom in the chronological order that they were tested.) The mean conduction time of our tecto-reticular neurons was 0.56 \( \pm \) 0.23 ms.
We begin with an illustrative example (Fig. 1) of the discharge of a typical SCFN during a single-step gaze shift (Fig. 1A, see also Bergeron and Guitton 2000a; Munoz and Wurtz 1993a). This will serve as an introduction to the discharges associated with multiple steps, the behavior of interest in this paper. Note that this saccadic gaze shift is composed of an eye saccade summed with a head movement. When the gaze saccade ended, the visual axis was stabilized in space by an ocular counter-rotation that compensated for head rotation. In our paradigm, just before target presentation, the cat’s gaze was immobile on a blank opaque barrier devoid of salient targets. In this condition, SCFN discharge frequency was low. SCFN discharges in monkey are also low when there is no salient target (Munoz and Wurtz 1993a). After target presentation, this low tonic “background” discharge paused for large (for this cell, about >10°) single-step gaze saccades directed contralaterally (Fig. 1A, Table 1), but not ipsilaterally (data not shown). In this example, the discharge resumed halfway through the gaze saccade, and firing frequency reached a maximum at the end. Note the small burst evoked by the onset of ambient lighting in this and other discharges illustrated in this figure.

During single steps, gaze moved without interruption, in a saccade-like trajectory, until it arrived on target. In multiple steps, the progression of gaze toward the target was interrupted by periods of relative gaze immobility. Figure 1B shows the discharge of the same SCFN, cell N26, during a multiple-step gaze shift and its associated eye and head components. The cat moved its visual axis through a total angle of about 30° using three gaze saccades separated by two gaze position plateaus of different duration. During the plateau phases, the eye moved opposite to and compensated for head motion and gaze velocity was usually very low, frequently zero, as in the present example.

For this SCFN, there was a pause before the onset of the contralateral gaze shift, similar to that for the single step (Fig. 1A). The activity resumed at a certain point during the multiple-step sequence. We showed in Bergeron and Guitton (2000a), for those single-step and multiple-step gaze shifts in which there was a pause in activity at gaze shift onset, that the first spike in the resumption of SCFN discharge after the pause occurred when the visual axis arrived, not at overall gaze shift end but, at a specific angular distance from the target; i.e., a specific GPE. This discharge property was independent of the number and amplitude of steps and the duration of inter-step plateaus. Every cell had its “preferred” GPE at which activity resumed after the pause, irrespective of whether gaze was in a plateau position or moving during a saccade. In Bergeron and Guitton (2000a), we did not provide a quantitative analysis of the tonic discharge pattern of SCFNs during the plateau phases of movements in either the ipsilateral or contralateral directions. This information must be known to better understand the encoding of movement commands by the collicular motor map and also to evaluate the possible influence of SCFNs on OPNs. We provide this information here.

Figure 2 shows the activity of SCFN, cell M56, during a number of multiple-step gaze shifts in the contralateral (A–D) and ipsilateral (E–H) directions, respectively. The multiple-step gaze shifts vary in overall amplitude from about 25° (bottom traces) to 60° (top traces). In Fig. 2, A–D, the same contralateral gaze shifts are shown, but they are aligned on different criteria; the beginning of the first step (Fig. 2A); the end of the first step (Fig. 2B); the end of the second step (Fig. 2C); and the end of the multiple-step sequence (Fig. 2D). In Fig. 2, E–H, the same ipsilateral gaze shifts are shown, as in A–D, on different features of a gaze shift. The different alignments on the end of sequential gaze steps permit an analysis of the change in tonic activity during plateaus as gaze approaches the target. Furthermore, these different alignments permit important comparisons with the OPN discharge illustrated in Fig. 5.

For these overall large gaze shifts, Fig. 2, A and B, shows that the activity of SCFN M56 ceased just after target presentation, before the first gaze saccade in all contralateral trials, thereby yielding a clear cessation of activity (pause), as seen in the histogram at gaze onset and during the first contralateral gaze saccade. By comparison, Fig. 2, E and F, shows that near the start and during the first ipsilateral gaze saccades, there was a slight reduction, not a pause, in overall activity.

Comparing each panel in Fig. 2 shows that the firing frequency at the end of the second step (C and G) was lower than that at gaze-end (D and H) and higher than at gaze-start (A and E). Indeed all SCFNs, of which cell M56 is an example, displayed a gradual increase in activity to reach a maximum firing frequency at the end of both contralateral and ipsilateral multiple-step gaze shifts in the dark. To analyze this property quantitatively, we calculated (see Methods) the mean firing frequency in all plateaus having a duration >75 ms and velocity <25°/s. The mean firing frequency at the end of a multiple-step sequence (ffo) was calculated in the 200-ms period following the last gaze saccade.

Figure 3, A and B, shows quantitatively, as a function of GPE, the pattern of increase in mean firing frequency of cell M56 during plateaus between the start and end of contralateral and ipsilateral gaze shifts, respectively (see Data analysis). Recall that GPE = 0° is synonymous with gaze-end. Note, in Fig. 3, A and B, that the firing frequency, ffo, during plateaus at large GPEs—i.e., the early phases of the gaze displacement—was lower than the background firing frequency, fbo, measured just before target presentation when the cat faced the opaque barrier. Furthermore, for this cell, ffo for contralateral movements (Fig. 3A) was 0, but not for ipsilateral movements (Fig. 3B). Figure 3, C and D, shows analogous results for a SCFN (cell P47a) whose direction-dependent activity pattern was opposite to that of cell M56: its tonic activity during the initial plateaus, at large GPE, being zero for contralateral gaze shifts but not for ipsilateral gaze shifts. Note that cell M56 (Fig. 3, A and B), as all cells in cat M, was recorded in an animal whose head was completely unrestrained. By comparison, cell P47a was recorded in a cat whose head was attached to the universal joint-vertical shaft arrangement (see Methods). Figure 3 and Table 1 suggest that the SCFNs recorded in cat M had characteristics similar to those in the other cats.

For contralateral movements (Table 1), 15 of the 17 SCFNs for which we had sufficient data had discharge characteristics similar to those of cell M56: there was a pause in the histograms at the start of the gaze shifts (ffo = 0), and subsequently, firing frequency increased inversely with GPE. For one other cell (P47a), there was a reduction, not a pause, in activity relative to ffo at the start, but like all cells the activity increased as the gaze shift progressed. [In the remaining cell (H1), we had insufficient data.] By comparison, for ipsilateral gaze
shifts (Table 1), the modulation of firing frequency was more variable: \( ff_i \) was either statistically less, equal to, or greater than \( ff_o \), and on average for the population, \( ff_i \) was not different from \( ff_o \) (\( P > 0.05 \)). In three cells, the discharge was not modulated (NM in Table 1) with GPE. Note that we found no cells with histograms showing \( ff_i = 0 \) for gaze shifts in both directions.

The data in Fig. 3 suggest that when modulated, the \( ff \) rises smoothly as GPE decreases and makes a smooth transition to peak firing frequency at GPE = 0°. Examples of mathematical relations that could be used to fit this behavior are Gaussian and sigmoid functions. We analyzed all cells using GraphPad Prism (see METHODS) to determine whether either of these functions had a better fit to the data points. For all cells, we found that a sigmoid function did not provide a better fit (\( F \) test, \( P > 0.05 \)). Furthermore, there were no significant differences (\( P > 0.05 \)) in the ability of these functions either to maximize the variance-accounted-for (\( R^2 \)) or to improve the results of the Runs test (see METHODS). We chose the Gaussian function (Eq.1) because it has been widely used in describing other properties of collicular neural activity, e.g., population activity profiles on the motor map (Ottes et al. 1986) or movement fields of saccade-related cells (e.g., Edelman and Goldberg 2001).

For cells M56 and P47a, the best-fit curves using Eq.1 are shown in Fig. 3 with parameters, \( \Delta ff \) and \( \sigma \), given for this and other cells in Table 1. Note that it was not possible to analyze the relationship between firing frequency during plateaus and GPE for the SCFNs marked by asterisks in Table 1 because the multiple-step gaze trajectories in certain directions had gaze-plateau positions that did not cover a sufficiently large range of GPEs. For our sample, both the VAF (\( R^2 \)) and the Runs test
DISCHARGES OF SCFNs AND OPNs DURING MULTIPLE-STEP GAZE SHIFTS

The open triangle on each panel in Fig. 3 marks the point on the GPE axis where the Gaussian function reaches 1.64σ where the Gaussian function is fit to the data points. A Gaussian function (see METHODS, Eq. 1) is fit to the data points. A threshold GPE is defined as occurring at GPE = 1.64σ. fi0 firing frequency before target presentation when cat faced opaque barrier. fi, asymptotic value of firing frequency during plateaus at large GPEs. Parameters in Eq. 1 are A: Δff = 54, fi0 = 0, σ = 9.8; B: Δff = 46, fi0 = 8, σ = 12.8; C: Δff = 65, fi0 = 16, σ = 12.8; D: Δff = 72, fi0 = 0, σ = 8.5. Points within the 5° range in GPE, delimited by the vertical dotted lines in A and B, are from plateaus whose firing frequency was further analyzed in Fig. 4.

FIG. 3. Mean firing frequency of SCFNs during gaze position plateaus (●) increases as gaze-position error (GPE) of plateau decreases. Data from 2 SCFNs are shown. Cell M56 paused (i.e., ff0 = 0) at the start of all contralateral gaze shifts about 28° (A), but not before ipsilateral gaze shifts (B). Cell P47a paused before ipsilateral gaze shifts about 30° (D) but not before contralateral gaze shifts (C). A Gaussian function (see METHODS, Eq. 1) is fit to the data points. A threshold GPE is defined as occurring at GPE = 1.64σ. fi0 firing frequency before target presentation when cat faced opaque barrier. fi, asymptotic value of firing frequency during plateaus at large GPEs. Parameters in Eq. 1 are A: Δff = 54, fi0 = 0, σ = 9.8; B: Δff = 46, fi0 = 8, σ = 12.8; C: Δff = 65, fi0 = 16, σ = 12.8; D: Δff = 72, fi0 = 0, σ = 8.5. Points within the 5° range in GPE, delimited by the vertical dotted lines in A and B, are from plateaus whose firing frequency was further analyzed in Fig. 4.

indicate that Eq. 1 is a plausible model: P was always >0.05, and P > 0.2 in 85% of the sample. The mean R2 values show that, on average, the VAF was 0.59 ± 0.5 and 0.53 ± 0.2 for contralateral and ipsilateral movements, respectively. Note also that the experimentally derived mean Δff = fi - fi0 = 49 and 36 spikes/s for contralateral and ipsilateral movements, respectively, is similar (t-test, P > 0.05) to the value calculated by Eq. 1, 50 and 45 spikes/s, respectively. The mean firing frequency during plateaus at large GPEs (ff0, the horizontal asymptote in Fig. 3) was significantly lower for contralateral (1 spike/s) than for ipsilateral (17 spikes/s) movements (t-test, P < 0.05). This is because of the much greater likelihood of a pause in the former. Note also in Table 1 that the mean firing frequency (ff0) at the end of ipsilateral and contralateral movements was statistically similar (t-test, P > 0.05). Note that ipsilaterally and contralaterally directed gaze shifts end at opposite positions relative to the body midline. Therefore there is no modulation of gaze-end firing frequency with either head, gaze and target positions relative to the body.

Noisy firing rates are characteristic of movement-related discharges in SC (e.g., Edelman and Goldberg 2001; Goossens and Van Opstal 2000). In Fig. 3, the considerable variability in the value of ff0 is a specific example of the important inter-trial variability in firing frequency. Noisy firing rates can lead to large relative variations in the estimate of mean firing frequency, particularly for the combination of low firing frequency and short plateau duration. For example, a mean firing frequency of 20 spikes/s implies that in a 100-ms plateau there will be 2 spikes. Clearly, a variability of ±1 spike in the count will lead to a large range, 10–30 spikes/s, in the estimate of mean frequency.

Apart from the dependence of firing frequency on GPE and the presence of unaccountable-for noise, there is the possibility that part of the variability in firing frequency at a given GPE might be reduced if a dependence were found on some characteristics of the eye, head, or gaze trajectories. Although no clear rationale for this can be offered, we nevertheless attempted for each cell to find a link between mean firing frequency during gaze plateaus at a fixed GPE and some feature of the concurrent eye position, head, or gaze velocity. For our example cell M56, we analyzed the discharge during plateaus that occurred within the 5° range of GPEs spanned by the vertical dotted lines in Fig. 3, A and B. This range was selected because it contained a large number of points. We found no significant modulation of SCFN M56 activity with either head velocity (Fig. 4, A and B), eye position (Fig. 4, C and D), or gaze velocity (data not shown) during plateaus. We analyzed each cell in our population using the same range of GPEs but with a mean of the range that varied from cell to cell, selected to ensure sufficient points for analysis. In no cell was there a significant modulation of firing frequency; the mean linear regression correlation coefficients were r = 0.22 ± 0.12 (for eye position), r = 0.22 ± 0.13 (for head velocity), and r = 0.23 ± 0.14 (for gaze velocity).

The open triangle on each panel in Fig. 3 marks the point on the GPE axis where the Gaussian function reaches 1.64σ from its peak. This is the point where 5% of the area under the curve lies on one side and 95% on the other. This point conveniently identifies the GPE at which the change in firing frequency begins to increase significantly above its asymptotic low value (ff0) at large GPE. We call the value of GPE marked by the triangle, the cell’s “threshold GPE.” As indicated in Fig. 3, A and B, for SCFN M56, the threshold GPE = 16° and 21° for contralateral and ipsilateral movements, respectively. Note in Table 1 that the threshold GPE varied across cells. Note also that the identified tecto-reticular SCFNs did not have discharges that differed from those of the other cells. Therefore we did not treat them as a separate population. The range of threshold GPEs encoded by SCFNs for contralateral gaze shifts...
was 6–30°; the mean ± 95% confidence limits = 15° ± 6°. For ipsilateral gaze shifts, the threshold GPE of some cells could not be determined because their activity remained constant after target presentation and was not modulated (marked NM in Table 1) with GPE. If we exclude these points from our population characteristics, we obtain for ipsilateral gaze shifts the range 6–21° (mean = 17° ± 5°).

For cells that paused at the beginning of multiple-step gaze shifts, indicated by $f_0 = 0$ in Table 1, we calculated in Bergeron and Guitton (2000a) the equivalent of the present threshold GPE as the value of GPE at the time of the first spike that follows the pause in activity at gaze onset. These values are given in Table 1 for the same trials used to calculate the Gaussian fits. The two calculation methods give similar results. For example, for contralateral movements, the “time-of-first-spike” method gives a mean GPE ± SD = 13° ± 6° whereas, for the same cells and trials, the present analysis using Eq. 1 gives a mean threshold GPE ± 95% confidence limits = 14° ± 6°. There is no significant difference between the two calculation methods ($t$-test, $P > 0.05$). The advantage of the present approach is that it permits a quantitative evaluation of the full discharge pattern and a convenient analysis of those firing frequency profiles that show no pause in activity at gaze shift onset.

OPN discharges during leftward and rightward multiple-step gaze shifts

OPNs are located close to the midline, and we could not determine with certainty whether a neuron was on the right or left sides of this axis. Therefore when describing OPN discharge characteristics, we refer to gaze shifts as being toward the left or right, not ipsilateral or contralateral. For comparison with SCFNs, 14 OPNs were recorded in two different cats and classified as OPNs according to their discharge pattern during single-step gaze shifts (Paré and Guitton 1998). We have not reported on the present OPNs in a previous paper. Figure 1C illustrates the characteristic pause in OPN tonic activity that is known to begin just before the onset of single-step gaze saccades of all amplitudes and directions and end just before the end of these gaze saccades (Paré and Guitton 1998). Thus the tonic discharge of OPNs ceases (pauses) for a time period equal to the duration of the gaze, not saccadic eye or head movement, trajectory. Note also that, unlike SCFNs (e.g., Fig. 1A), OPNs discharged briskly up to the onset of a gaze saccade even when the animal’s visual axis was fixed on the opaque barrier on which there was no target of interest. This difference between the discharges of SCFNs and OPNs, before gaze shift onset, is reminiscent of that shown by Everling et al. (1998) during the gap task in monkey.

In a multiple-step sequence (Fig. 1D), the pattern of activity is similar to that for single-steps; OPNs are active during every gaze position plateau and pause for every gaze saccade. This figure also suggests that the activity is not related to the characteristics of the head trajectory and that the tonic firing frequency during plateaus increase as GPE goes to zero. We will consider these points below. Figure 5 shows the discharge of a typical OPN, cell G4d, during a series of multiple-step gaze shifts. Different trials during right and left gaze shifts are aligned in the same manner as for the SCFN in Fig. 2. The activity pauses just before the beginning of each gaze saccade in both the left and right directions and then increases abruptly at the end of each step. This activity pattern is clearly very different from that seen in SCFNs (Fig. 2). In particular, the high tonic OPN activity that precedes and follows the first gaze step is strikingly different from that in SCFNs.

Note however, a striking similarity between the discharges of the two cell types. For right (Fig. 5, A–D) and left (Fig. 5, E–H) gaze shifts, the activity of this typical OPN during each plateau increases as each step brings gaze closer to the final destination. Thus activity in OPN G4d at the end of the multiple-step sequence was higher than at the start, just as for SCFNs. The increase in activity of OPN G4d as GPE went to zero is shown quantitatively in Fig. 6. A and B, for left and right movements, respectively. Note that the firing frequency during plateaus at large GPE ($f_0$), is lower than that before target presentation ($f_0$). This property is similar to that of SCFNs.

Figure 6 and Table 2 show that Eq. 1 provides a realistic description of the dependence of this OPN’s firing frequency on GPE. The VAF was 68% and 77% for right and left movements, respectively, and the Runs test indicated that the distribution of points about the line did not differ significantly from Gaussian. For cell G4d, the threshold GPE ± 95% confidence limits was 9° ± 1° and 7° ± 1° for right and left movements, respectively. These values are not significantly different from each other ($t$-test, $P > 0.05$). The quantity $A_f$, calculated from the best-fit procedure, was 82 and 98 for right and left movements, respectively. These values are very similar to the experimental values, $A_f = f_0 - f_0$ = 82 and 102, respectively, calculated from the data. However for this cell, the asymptotic value of mean firing frequency ($f_0$) at high GPE for rightward movements, 64 spikes/s, was significantly different ($t$-test, $P < 0.05$) than the value, 50 spikes/s, for leftward movements.

J Neurophysiol • VOL. 88 • OCTOBER 2002 • www.jn.org
Table 2 lists the parameters for all OPNs. Complete data for left and right movements were obtained in 10/14 OPNs. The remaining four OPNs provided data for one direction of movement only (asterisks in Table 2). Across the population, there was a significant difference (t-test, \( P < 0.05 \)) between the mean threshold GPEs for right (10° ± 4°) and left (6° ± 3°) movements. This difference was not due to an over-representation of cells for which we had only movements to the right. Indeed, when we consider only those 10 cells for which we had data for movements to both sides, the right-left difference was still significant. Given that we could not assign OPNs to a particular side of the brain, we believe this difference is due to a sampling bias. Indeed, there was no significant difference (t-test, \( P > 0.05 \)) across the OPN population when averages are compared for \( f_f \) and \( f_c \) for left and right movements, respectively. Note also from Table 2 that the cells recorded in cat L, whose head was completely unrestrained, have characteristics similar to those in the other cats.

As shown in Fig. 6, there was, for all cells, considerable scatter about the best-fit line. Can we account for it? We have proposed that multiple-steps gaze shifts are created by a nystagmic eye movement pattern (Fig. 1, B and D) generated by alternating vestibular-driven quick and slow phases (Bergeron and Guitton 2000a; Guitton et al. 1984). Brain stem inputs onto OPNs, important for the generation of quick-phases, are eye position and head velocity (e.g., Galiana 1991; see DISCUSSION).

To verify whether firing frequency was modulated by these inputs, we analyzed the discharge during plateaus that occurred at a nearly fixed GPE. This procedure is similar to that used in our analysis of SCFNs (Fig. 4). For the OPN population we...
found no significant modulation, during plateaus, of OPN firing frequency with either head velocity ($r = 0.21 \pm 0.1$), eye position ($r = 0.24 \pm 0.15$), or gaze velocity ($r = 0.25 \pm 0.14$).

Combining the results of the present analysis of SCFNs with those in our initial study (Bergeron and Guitton 2000a), we conclude that it is GPE, not time, that is critical for the development of the firing frequency pattern in these cells. It is also important to verify this property in OPNs. We saw, in Fig. 6, A and B, that the GPE of a plateau is important for determining the OPN firing frequency profile. However, in any one multiple-step gaze shift, time and GPE are correlated, and one might argue that the OPN discharge is not dependent on GPE but rather on the time relative to gaze-end. To distinguish between the influence of each quantity, for OPN G4d, we selected regions along the abscissa in Fig. 6, A and B, indicated by the vertical lines, for which the time of occurrence of a plateau was not correlated to the firing frequency during the plateau (Fig. 6, C and D; $r = 0.1$ and 0.03 for left and right movements, respectively; not significant; $P > 0.05$). For these same data, we found that firing frequency was significantly correlated to the GPE of the plateau (Fig. 6, E and F; $r = 0.47$ and 0.46, respectively; $P < 0.005$), but not to the time of the plateau (Fig. 6, G and H; $r = 0.25$ and 0.13, respectively; $P > 0.05$). We performed this analysis for all our OPNs. In six cells, time and GPE were always correlated, and thus we could not distinguish between these parameters. In the remaining eight cells, the mean ± SD correlation coefficient between firing frequency and GPE averaged across left and right movements was $r = 0.40 \pm 0.17$, whereas for time $r = 0.16 \pm 11$. We conclude that OPN firing frequency, like that of SCFNs, is influenced by GPE and not by the time relative to the end of the gaze shift.

Population activity of SCFNs and OPNs during multiple-step gaze shifts

Figure 7, A and B, shows, for all our SCFNs, the best-fit curves, using Eq. 1, through each cell’s discharge for contralateral and ipsilateral multiple-step gaze shifts. (Recall that the parameters applicable to each curve are given in Table 1.) In each panel, the thick line represents an estimate of the population discharge and was obtained by calculating the average line through the ensemble of Gaussian fits. Figure 7, D and E, and Table 2 give the same information for OPNs.

The thick curves in Fig. 7, A, B, D, and E, provide insight into how the hypothetical global discharge in a population of SCFNs and OPNs encodes GPE. Figure 7C shows the population average, ±2 SD, for combined ipsilateral and contralateral SCFN discharges, obtained as the mean of the thick lines in Fig. 7, A and B. We show this because OPNs may receive combined projections from both SCs, a point that will be considered in the DISCUSSION. For comparison, Fig. 7F shows the population average ±2 SD for the mean of the left and right population OPN discharges. The heavy dashed lines in Fig. 7, C and F, show the population average of only those cells in Tables 1 and 2 for which $R^2 \geq 0.65$; this is very similar to the population averages.

Relationship between firing frequencies at start and end of trials

For most OPNs and all SCFNs, the firing frequency ($f_f$), before target presentation, when the cat faced the opaque barrier in the light but with no salient target, was lower than the firing frequency in the dark at GPE = 0 ($f_f$) (see Tables 1 and
2). Figure 8 compares $f_{e}$ to $f_{o}$ for OPNs and SCFNs. Note that the two frequencies are linked: the higher the $f_{o}$, the higher the $f_{e}$. Furthermore, the points seem to fall about a line parallel to, and offset from, the dotted unity diagonal line. This suggests that the effect of landing on target is to add a constant frequency increment to $f_{o}$. The average $f_{o}$ for all SCFNs recorded was 15 ± 11 spikes/s (Table 1) and for OPNs was 93 ± 34 spikes/s (Table 2). By comparison, SCFNs had a mean (pooling both contralateral and ipsilateral) of $f_{e} = 51 ± 20$ spikes/s and OPNs a mean (left plus right) of 132 ± 54 spikes/s. The average increase in frequency ($f_{e} - f_{o}$) for SCFNs and OPNs was very similar, 36 and 39 spikes/s, respectively. Perhaps SCFNs provide this increment to OPNs.

Response of SCFNs and OPNs to light onset during fixation

It is well known that many cells in the SC’s deep layers, including tecto-reticular neurons, show a transient increase in discharge after the appearance of a visual stimulus in their receptive field (Berthoz et al. 1986; Guitton and Munoz 1991; reviewed in: Guitton 1991; Sparks 1986). In particular, SCFNs have visual responses with receptive fields that include the area centralis (Munoz and Guitton 1989, 1991; Peck 1989; Peck and Baro 1997). OPN also have visual responses with receptive fields near central vision (Everling et al. 1998; Evinger et al. 1982).

In our paradigm, a burst discharge could be evoked in 12/14 OPNs and all SCFNs when ambient lighting was turned on after the cat’s gaze had reached, in the dark, the location of the remembered target. We calculated the onset of the burst as the point where the neural activity reached 2 SD above background firing rate whose average was $f_{e}$. Figure 9, A and B, shows histograms giving the mean onset latency of the visual response for each cell. The population averages were as follows: SCFNs, 54 ± 20 ms; OPNs, 58 ± 22 ms. This difference was not significantly different and thus yielded no insight into whether SCFNs drove OPN visual responses.

DISCUSSION

Research in oculomotor control has led to the definition of a neural fixation system (Goldberg 2000; Leigh and Zee 1999). Apart from SCFNs and OPNs, neurons implicated in fixation behavior have been found in the substantia nigra pars reticulata (Hikosaka and Wurtz 1983), thalamus (Schlag and Schlag-Rey 1984), subthalamus nucleus (Matsumura et al. 1992), zona incerta (Ma 1996), frontal eye field (Bruce et al. 1985; Burman and Bruce 1997; Hanes et al. 1998), supplementary eye field (Bon and Luchetti 1992, 1995; Lee and Tehovnik 1995; Schall 1991; Schlag et al. 1992), prefrontal cortex (Suzuki and Azuma 1977), and posterior parietal cortex (Lynch et al. 1997; Sakata...
et al. 1980). The role of each of these nodes and how they interact both anatomically and functionally is not understood.

Controversy as to whether SCFNs gate saccades

In this paper we are concerned with the collicular fixation zone and its link to OPNs. In the classic visually triggered saccade task—a target comes on when the fixation point goes off—SCFNs discharge tonically during the fixation period, are silent for the duration of the saccade to the target, and discharge again when gaze is on target. This discharge pattern, being similar to that of OPNs, has suggested that SCFNs gate and suppress saccades (Munoz and Guitton 1989, 1991; Munoz and Wurtz 1993a; Munoz et al. 1991; Quaia et al. 1999). They can do this via two known routes: 1) by inhibiting the SC’s motor map, via collicular inhibitory interneurons (Munoz and Guitton 1991; Munoz and Istvan 1998; Paré et al. 1994) and 2) by activating OPNs (Büttner-Ennever et al. 1999; Everling et al. 1998; Gandhi and Keller 1997; Munoz and Guitton 1989, 1991; Munoz and Wurtz 1993a,b; Munoz et al. 1991; Paré and Guitton 1994), which, in turn, inhibit the brain stem saccadic burst generator (reviewed in Scudder et al. 2002).

The role of SCFNs in providing the “go” and “stop” signals for saccades has been questioned. Regarding the latter, lesions of the SC’s rostral pole do not produce hypermetric saccades (Munoz and Wurtz 1993b) as would be predicted if the stop signal were removed. Evidence against the go signal has been obtained by investigating what triggers short latency (about 100 ms) “express” saccades. These saccades are generated in the “gap-task” in which a fixation point is extinguished a short time (the gap) before target presentation (for discussion, see Dorris et al. 1997; Everling et al. 1998). The short latency of express saccades compared with longer-latency regular saccades, which occur when there is no gap, has been attributed to disengagement of ocular fixation wherein the elimination of inhibitory fixation activity before target presentation reduces saccade reaction time (Dorris et al. 1997; Reuter-Lorenz et al. 1991; reviewed in Findlay and Walker 1999). Recent studies cast doubt on whether SCFNs have this control ability. Although SCFNs show reduced activity in the gap period, their change in discharge is not correlated to saccade reaction time (Dorris et al. 1997; Everling et al. 1998). Rather, the shortened reaction time is correlated to increased collicular motor activity in “buildup neurons” that drive the movement (Dorris et al. 1997).

The following observations have also been used to support the argument that SCFN discharges do not control fixation, but rather lie on a functional continuum with more caudal cells on the motor map. First, some SCFNs have a burst discharge preceding very small (as little as 0.6°) saccades in the contralateral direction (Krauzlis et al. 2000; Munoz and Wurtz 1993a). Such cells have motor-related discharges with movement fields, just like neurons that encode larger saccades in the SC’s classical motor map (Munoz and Wurtz 1995a; Munoz et al. 1991). Indeed saccades can be made to target displacements as small as 3.5 arc min (Wyman and Steinman 1973), but we do not know whether the rostral SC is involved in their generation. Second, the tonic activity of monkey SCFNs encodes small eye position-errors between a target and the current location of the visual axis (Krauzlis et al. 1997, 2000). This tonic activity in both buildup neurons and SCFNs is not necessarily a motor-related discharge, obligatorily linked to saccade generation (Bergeron and Guitton 2000a; Berthoz et al. 1986; Grantyn and Berthoz 1985; Krauzlis et al. 2000; Mays and Sparks 1980; Munoz and Guitton 1991; Munoz and Wurtz 1993a, 1995a). The encoding of eye (gaze) position-error is a property common to both SCFNs and neurons with buildup activity in the collicular motor map (Munoz and Guitton 1991; Munoz and Wurtz 1995a,b; Peck and Baro 1997). Finally, saccades perturbed by stimulation of the SC’s fixation zone show deviations from their normal trajectory that are characteristic of deviations produced by stimulating the motor map but not the OPN area (Gandhi and Keller 1999a). In sum, the points in the preceding paragraph have emphasized a motor role for the SC’s fixation zone, arguing that it is the rostral extension, for encoding very small saccades, of the classic collicular motor map.

Despite these experiments that question the direct role of the rostral SC in assuring fixation and in gating saccades, it is important to recall that there is much evidence showing that the level of activity in the SC’s fixation zone influences saccade generation and latency. Indeed, activation of the SC’s fixation zone either pharmacologically, by low frequency electrical stimulation, or naturally via attentive fixation, either suppresses or increases the latency of saccadic eye and gaze movements (Munoz and Wurtz 1993b; Paré and Guitton 1994; Paré et al. 1994). Furthermore, it is important to account for projection of the SC’s rostral pole, probably of SCFNs, to OPNs (Büttner-Ennever et al. 1999).

SCFNs encode GPE

An important point regarding the activity of SCFNs during multiple-step gaze shifts is that their firing frequency pattern is different from what might be predicted on the basis of their discharge pattern during single-step gaze shifts. A given cat SCFN was either silent or discharged at low frequency for the initial small gaze saccades and plateaus at the beginning of multiple-step gaze shifts to either side. By comparison, toward the end of a multiple-step sequence, the same SCFN was active at a much higher frequency during both comparable gaze saccades and plateaus. Thus the pattern of SCFN activity was not correlated to gaze-saccade occurrence, for either ipsilateral or contralateral movements. Indeed, we never observed burst discharges for the small contraversive saccades that occurred at the end of multiple-step sequences.

Table 1 shows that most SCFNs had different threshold GPEs in the ipsilateral and contralateral directions. However, on average across cells, the mean threshold GPE was the
same—about 16°—in both the contralateral and ipsilateral directions. Krauzlis et al. (2000) also showed that the firing frequency of SCFNs encodes GPE. Their results complement ours, but cannot be directly compared. In our studies (see also Bergeron and Guitton 2000a), we have defined a cell’s threshold GPE as occurring when gaze approaches the target, at the gaze position relative to the target where the activity of the cell just begins to increase significantly above background. This occurred at the “tail” of the Gaussian, e.g., to the left of the curve in Fig. 3A. By comparison, in the study of Krauzlis et al. (2000), the preferred GPE occurred at the value of GPE where cell activity peaked, which in different SCFNs encoded a continuum of GPEs between 0° and about 5°. Due to noisy peak discharges (e.g., Fig. 3), we could not distinguish in our data whether the peak in activity occurred at small nonzero values of GPE, as found by Krauzlis et al. (2000). Within the accuracy of our measurements, we can only assert that the firing frequency of all our SCFNs peaked when the visual axis arrived on or was very close (GPE approximately ±3°) to the target, but we cannot exclude the possibility that some SCFNs had peak discharges at small, nonzero, GPEs.

Cat tecto-reticular neurons located on the SC’s motor-map have a preamble discharge—analogous to the buildup activity in buildup neurons of the monkey’s SC (Munoz and Wurtz 1995a)—that precedes the motor burst (Grantyn and Berthoz 1985, 1987; Munoz and Guitton 1991; Munoz et al. 1991; Olivier et al. 1993). The preamble encodes a specific range of GPEs—a GPE field—determined by the cell’s position on the topographically coded motor-map. Each tecto-reticular neuron has a preferred GPE, which defines a GPE map coextensive with the visual and gaze motor maps (Munoz and Guitton 1991). GPE fields are large (Munoz and Guitton 1991) and are the outcome of a large ensemble of neurons on the SC map that is active when the visual axis is at a specific distance from the target. Thus tecto-reticular neurons at the 15° location on the map have a firing frequency in their preamble discharge that peaks at GPE = 15°, but these cells also discharge tonically for a large range of GPEs, notably at GPE = 0° when the animal is fixating the target (Munoz and Guitton 1991). Our SCFNs—some of which were identified tecto-reticular neurons (Table 1)—had GPE fields that centered on, or near, 0°. Thus from caudal to rostral SC, there is a continuous representation of GPE.

OPN discharges

Recently, OPNs in the head-fixed cat have been subdivided into two classes, complex and saccade-related (Petit et al. 1999). OPNs in the former class have a lower mean discharge frequency and pause during multiple-step eye saccades and are not reactivated during each inter-saccadic plateau. The latter are the classic OPNs that pause during each saccade and discharge tonically during plateaus. Their pauses in activity are more tightly coupled to saccade start and end than are those of the complex OPNs. Also, the time course of recovery after the pause is faster for saccade OPNs than complex OPNs. We could not, in the head-unrestrained cat, subdivide our OPNs into subtypes using the criteria proposed by Petit et al. (1999).

The prototypical OPN response during a multiple-step gaze shift was a steady firing (f_{pr}) until first gaze saccade onset, a pause in activity during every gaze saccade, a decrease in firing frequency to a fixed lower level (f_{fl}) during plateaus until GPE ~8° was reached, and a gradually rising discharge during each subsequent plateau until gaze was on target (Figs. 5 and 6). At overall gaze shift end, GPE = 0° and OPN firing frequency (f_{pr}) was greater than f_{fl}.

Signals responsible for OPN tonic activity

The many different levels of OPN tonic firing frequency during multiple-step gaze shifts clearly indicate that OPN membrane properties do not simply maintain these cells in a bistable state (see also discussion in Everling et al. 1998). The source of the signal responsible for the tonic activity in OPNs is still unclear. In a thorough review, Moschovakis et al. (1996) postulated that brain stem burst neurons as well as the SC project onto local interneurons (“latch neurons”) in the OPN region that inhibit OPNs. More recently, this problem has been considered in detail (Yoshida et al. 2001). There are also cerebellar projections to the OPN region that arise from the fastigial nucleus (anatomy: Gonzalez-Ruiz et al. 1988; Langer and Kaneko 1984; Noda et al. 1990; physiology: Fuchs et al. 1993; Helmchen et al. 1994; Ohtsuka and Noda 1991) and the basal interstitial nucleus (Gonzalo-Ruiz et al. 1988; Kawagoe et al. 1998). Other projections to OPNs arise from the vestibular (VN) ↔ prepositus hypoglossi (PH) nuclei complex (VN: Ito et al. 1984, 1986; Langer and Kaneko 1990; PH: Ito et al. 1984; Langer and Kaneko 1984, 1990), which provide eye position and head velocity signals that neural network models implicate in the generation of vestibular quick phases (Chun and Robinson 1978; Galiana 1991). Everling et al. (1998) reviewed other inputs to OPNs that arise in the brain stem, some whose functions are totally mysterious, others which may modulate OPN activity during sleep.

The question naturally arises as to what is the influence of the burst generator on OPNs during plateau phases of multiple-step gaze shifts. OPN firing frequency should vary inversely with burst generator firing frequency. The firing frequency of burst neurons is proportional to gaze velocity (Cullen and Guitton 1997; Cullen et al. 1993; Sylvestre et al. 2001); however, gaze velocity was generally very low during plateaus, suggesting little modulation of OPNs by burst neurons. This is compatible with our experimental observation that OPN firing frequency is not related to plateau gaze velocity.

An increase in head velocity combined with a counter-rotation of the eye during a plateau phase increase the probability of triggering quick phases (Galiana 1991). Such signals could decrease OPN tonic activity as the probability of triggering a quick phase increases. However, neither our observations nor those of others have shown that OPN firing frequency is modulated by head velocity. Other studies have reported that the firing frequency of a minority of OPNs is modulated by eye position (Cohen and Henn 1972; Paré 1995; reviewed in Moschovakis et al. 1996). However in none of the OPNs in our sample was the firing frequency modulated by eye position during the plateaus of multiple-step gaze shifts.

SCFN influence on OPN tonic activity

The activity pattern of OPNs and SCFNs was clearly dissociated during the period between target presentation and the onset of the first gaze saccade (as in the “gap” task studied by
Everling et al. 1998), as well as during saccades themselves. However, as the multiple-step sequence evolved, both SCFNs and OPNs encoded in their tonic discharge how close gaze was to the final goal, irrespective of the details—in terms of number of gaze saccades, their amplitude, and intersaccade plateau durations—of the gaze trajectory utilized to reach this goal. The firing frequency (ff) at the end of the multiple-step sequence was higher in both cell types compared with frequency (ff) before the target was presented when the cat faced the blank barrier (Tables 1 and 2; Fig. 7). Indeed, the frequency difference, ff − ff, was the same for both SCFNs and OPNs, supporting an SCFN-OPN drive. Remarkably, although they are only two synapses away from oculo-motorneurons, OPNs received information about gaze position relative to the salient target during a complex multi-step gaze trajectory. Recall that a functional link between the two cell types is possible because OPNs are monosynaptically activated by electrical stimulation of the SC’s rostral pole (Paré and Guitton 1994; Yoshida et al. 2001).

It is often assumed that the collicular projection to OPNs arises predominantly, if not exclusively, from the rostral contralateral SC. The situation is more complex. Büttner-Ennever et al. (1999) have studied, anatomically in monkey, the SC-OPN projection. OPNs on one side of the midline do indeed receive projections from the rostral pole of the contralateral SC via cells with thick axons projecting in the crossed predorsal bundle. However, OPNs also receive crossed projections from cells with thin axons emanating from the small saccade zone (≤15°), just caudal to the rostral pole. Furthermore, the rostral pole of the monkey SC projects to OPNs on both sides via the uncrossed lateral descending pathway that descends ipsilateral to its collicular source.

Munoz et al. (1991) suggested that SCFNs are tecto-reticular neurons. We studied this property in two of our five cats (see METHODS). Our sample is limited, but the six SCFNs that we identified antidromically had a mean conduction time to the rostral pole of the monkey SC of 0.56 ± 0.23 ms, which is compatible with their being part of the fast-conducting thick axon projection in the crossed pathway (Guitton and Munoz 1991). Furthermore, among these six collicular output neurons, we did not find different classes of responses (Table 1). All had properties similar to those of the other SCFNs, suggesting that we were recording from a homogeneous population with SCFN-like properties. Thus at least some SCFNs were output neurons, but we cannot comment on their relative importance.

There is a problem in studying quantitatively the influence of SCFNs on OPNs. Consider a simple scenario: if an OPN on the right side received dominant projections from SCFNs on the left, we would predict that this OPN’s discharge should be characterized by a higher ff before left (ipsilateral to this SCFN, see Table 1) than right gaze shifts. We could not do this analysis because OPNs could not be categorized according to the side of the brain they are on. Rather, those listed in the “left” and “right” response columns of Table 2 are probably a mixture of cells on the left and right sides of the brain stem. To circumvent this problem, we consider here the simplified hypothesis that the population response of a mixed group of left and right OPNs resembles a mixed contralateral and ipsilateral SCFN input. The average threshold GPE of OPNs (9° ± 3.8°; for left and right movements pooled) was significantly less (P < 0.001) than that of SCFNs 16° ± 5.5°; average of contralateral and ipsilateral), indicating that SCFN activity began rising above background before that of OPNs. If SCFNs contribute to OPN tonic activity, then this result suggests that OPNs do not respond to SCFN inputs until they rise above a threshold.

CONCLUSIONS

Our experiments investigated the role of the rostral SC in gating saccades via control of OPN discharges. They revealed even greater decoupling, between OPN and SCFN activity during gaze saccades than that proposed following studies of the head-fixed monkey (Everling et al. 1998; Gandhi and Keller 1999a,b). SCFN activity determined neither the onset nor offset of saccades in multiple-step gaze shifts. In a very recent model of the saccadic system (Quaia et al. 1999), SCFNs provide a go signal for saccades. The structure of this model is based on recordings made in the head-fixed animal. However, in the head-unrestrained condition, other mechanisms must be considered such as the quick phase generator. We argue that this brain stem reflex is not turned off during orienting gaze shifts and is responsible for the multiple eye saccades in the gaze shifts studied here. Thus if accurate gaze shifts are to be made, the contribution of quick phases to gaze displacement must be “known” at higher levels such that an accurate evaluation can be made of current GPE. Accurate control of when to terminate the gaze trajectory can be made via feedback to the SC, which is reflected in the discharge of SCFNs. Put another way, the gradual increase in both SCFN and OPN tonic activity as GPE approaches 0° implies feedback to these cells regarding the progress of gaze toward the target. Given that some evidence supports feedback to the SC (Bergeron and Guitton 2000a; Choi et al. 2001; Matsumoto et al. 2001; Munoz and Wurtz 1995b; Munoz et al. 1991; Port et al. 2000; Witzman et al. 1988, 1991), we speculate that it is SCFNs that provide OPNs with the high activity at GPE = 0°. The induced increase in OPN activity at GPE = 0° reduces the probability of short latency intrusive saccades when gaze arrives at this important location.

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