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# Influence of Task Predictability on the Activity of Neurons in the Rostral Superior Colliculus During Double-Step Saccades

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**Reyes-Puerta V, Philipp R, Lindner W, Lünenburger L, Hoffmann K-P.** Influence of task predictability on the activity of neurons in the rostral superior colliculus during double-step saccades. *J Neurophysiol* 101: 3199–3211, 2009. First published April 1, 2009; doi:10.1152/jn.90983.2008. Target probability has been shown to modulate motor preparatory activity of neurons in the caudal superior colliculus (SC) of the primate. Here we tested whether top-down processes, such as task predictability, influence the activity of neurons also at the rostral pole of the SC (rSC), classically related to fixation. To investigate this, double-step saccade tasks were embedded in two different paradigms, one containing unpredictable and another containing predictable tasks. During predictable tasks the animals could develop some expectation about the forthcoming second target jump, i.e., anticipate when and where to make the second saccade. Neuronal responses were recorded during both paradigms and compared, revealing the influence of task predictability on the activity of rSC neurons during specific periods of fixation. In particular, neuronal activity stayed significantly lower during the fixation period between two successive saccades in predictable than in unpredictable tasks. In addition there was a learning effect within a session during predictable conditions, i.e., the intersaccadic activity was higher in the early than in the late trials. Further, reaction times for the second saccade were shorter in predictable than in unpredictable tasks. However, we demonstrated that this difference in reaction times cannot be solely accounted for by the reported difference in neural activity, which was mainly influenced by the predictability of the tasks. With these results we show that top-down processes such as predictability are imposed on the activity of neurons in the rostral pole of the primate SC.

## INTRODUCTION

The way in which humans select targets for foveation is context dependent, so that the information extracted from the visual scene is optimized (Yarbus 1967). For instance, changes in target probability are reflected in saccadic reaction times, so that saccades made to more probable targets have shorter latencies (Carpenter and Williams 1995). In addition, target probability has been shown to modulate neural responses in the caudal superior colliculus (SC) (Basso and Wurtz 1997; Dorris and Munoz 1998). In particular, it has been demonstrated that the delay activity of those neurons involved in saccadic motor preparation is modulated by saccadic probability. Thus neurons representing potential target locations tend to fire earlier and with higher frequency when their associated target is more probable in the context of a determined task. Similar delay activity has been found in several cortical structures associated with target selection, such as the frontal eye fields (Sommer and Wurtz 2000) and the lateral intraparietal area (Paré and Wurtz 1997).

In the present experiments we studied whether neurons located at the rostral pole of the SC (rSC) also show a similar modulation in response to task predictability. A group of neurons located at the rSC—called "fixation neurons" by several authors—has been interpreted as showing fixation-related activity, firing tonically during fixation, and pausing during saccades (Munoz and Wurtz 1993a). This activity has been reinterpreted as representing potential target locations close to the fovea, contributing to small saccades and pursuit movements (Krauzlis 2004; Krauzlis et al. 2000). Despite a considerable amount of functional and anatomical data, the potential contribution of the rSC to visual fixation is still under debate (Büttner-Ennever et al. 1999; Guitton et al. 2004; Hafed and Krauzlis 2008; Hafed et al. 2008). A top-down effect was also reported for these neurons by showing that in the context of antisaccades fixation during the instruction period was associated with an increased activity compared with the activity associated with prosaccades (Everling et al. 1999).

To test the influence of task predictability on rSC neurons, tasks containing double-step saccades were used. In these tasks monkeys had to follow the appearance of two successive targets with their gaze. In general, the use of double-step saccades permitted us to control the probability of maintaining fixation during specific periods of time, which can be correlated with neural activity. In particular, our double-step tasks were embedded into two different paradigms. Whereas the mixed paradigm contained only unpredictable tasks, the blocked paradigm presented double-step tasks in an orderly manner so that the animals could develop some expectation about the forthcoming second target jump. Consequently, in these tasks the monkeys could anticipate when and where to make the second saccade. Here we show a critical influence of task predictability on the activity of rSC neurons during the intersaccadic fixation period—that is, the time when the animal fixates between the two successive saccades. Preliminary data can be found in Lünenburger et al. (2003).

Furthermore, we studied the effect of task predictability on saccadic reaction times. Previous studies demonstrated the existence of postsaccadic refractoriness, a term referring to the fact that in visually guided double-step saccades the reaction time of the second saccade is longer than that for the first (Becker and Jürgens 1979; Feinstein and Williams 1972; Lünenburger and Hoffmann 2003; Lünenburger et al. 2000). In general, reaction times for the second saccade were shorter during predictable compared with unpredictable conditions, suggesting that the postsaccadic refractoriness is a modifiable factor. In any case, a

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trial-by-trial correlation between saccadic reaction times and rSC neural data could not be found, which is in accordance with previous results (Dorris et al. 1997). Further, the fact that the predictability influenced both saccadic reaction times and neuronal firing rates could be a confounding factor. A dissociation test between these three variables—neural activity, reaction times, and predictability—was performed to demonstrate that the neural activity is modulated only by predictability.

Taken together, our data provide additional strong evidence for the influence of top-down processes on the activity of rSC neurons. Here we reveal for the first time the influence of target predictability on rSC neurons, which was not found in previous studies (Basso and Wurtz 1997; Dorris and Munoz 1998). Thus the activity of rSC neurons, classically related to fixation, is affected by the cognitive state of the animal and not solely dominated by factors such as saccadic reaction times or gaze position.

## METHODS

The experiments were conducted on two male rhesus monkeys (*Macaca mulatta*, CL and CI, weighing 11 and 9.5 kg, respectively) trained to perform single- and double-step saccades. The animals were seated comfortably in a primate chair and engaged in a setup with the body restrained but the head totally free. The head-unrestrained condition was preferred so that gaze saccades could be studied. Only by means of gaze saccades has it been proven that rSC neurons convey a gaze position error signal (Choi and Guitton 2006), a key factor that could have tremendous consequences on the quality and interpretation of our data. Under these circumstances, the monkeys faced a 60-cm-wide circular translucent screen (at a distance of 27.5 cm). Visual targets (red and blue light-emitting diodes; 1-cm diameter, 1.5 cd/m$^2$) were rear-projected onto this screen via galvanometer-driven mirrors under homemade software control. The animal's behavior was monitored by means of the software, which also recorded the spike events, behavioral events, gaze position, and head position. All procedures were approved by the local ethics committee and followed the European and the German national regulations (European Communities Council Directive, 86/609/ECC; Tierschutzgesetz) as well as the National Institutes of Health *Guidelines for Care and Use of Animals for Experimental Procedures*.

### *Surgery*

After a preoperative training, the monkeys were anesthetized with ketamine hydrochloride (10 mg/kg, administered intramuscularly) followed by pentobarbital sodium (25 mg/kg, administered intravenously). Atropine (1 mg) and supplementary doses of pentobarbital sodium were administered intravenously. Under aseptic conditions, a stainless steel head holder was implanted on the animal's skull and a chamber was placed on the midline over the occipital pole, tilted backward 45° from the vertical and therefore aiming perpendiculary at the SC surface. Search coils were implanted under the conjunctiva around each eye (Judge et al. 1980). A connector for the eye coils was fixed in the acrylic cement that was connected to the head holder. Electrocardiogram, body temperature, blood pressure, and SPCO2 were monitored during the surgery. Analgesics and antibiotics were delivered postoperatively for 2 wk.

### *Recording*

Extracellular recordings of single neurons were made with glass-insulated tungsten microelectrodes (impedance 2–3 MΩ measured at 100 Hz). The electrodes within a guide tube were lowered through the dura by a microdrive mounted on the chamber (Narishige, Tokyo). Activity of single cells was detected in real time by means of a computer-controlled multichannel spike sorter (Plexon, Dallas, TX). Single-unit discharges were separated using an on-line time–amplitude window discriminator and sampled with 1-ms time resolution.

Gaze and head positions were measured with a magnetic search coil system (Remmel, Katy, TX). Separate horizontal and vertical position signals were sampled with a frequency of 500 Hz. The gaze-position signal was also used to monitor stable fixation during the tasks.

All data were fed into a PC-ISA multifunction board (Intelligent Instrumentation PCI-20098C) controlled by homemade software, which monitored the behavior of the animals during the tasks, and stored the recordings.

### *Paradigms*

Double-step tasks were arranged into two paradigms to determine the effect of task predictability on the activity of rSC neurons: the mixed and the blocked paradigm (Fig. 1). In both paradigms, double-step tasks were designed in a horizontal periphery–center–periphery combination. A visual target was presented at a peripheral position on the screen (target position 1) after the monkey pressed a button near its hip with the hand. First the monkeys had to acquire and maintain fixation for 1,300–1,700 ms in a window of 4° radius around this fixation point, although the first 500 ms after fixation onset were discarded from analysis. Then the fixation point was displaced to the center of the screen (target position 2), where it remained for a defined time before it was moved to another peripheral position (target position 3). In some single-step conditions of the mixed paradigm the target position remained at the center (target position 2). A second fixation period of 800–1,200 ms was introduced to obtain a reward. The window of 4° radius around the fixation point was used to counteract the nonlinearity of the gaze signal error at peripheral target locations, a property that is intrinsic to the eye coil system (Judge et al. 1980). However, note that the gaze position signal was stable and precise at central target positions, remaining in only a small fraction of the valid range (Figs. 2, *A* and *B*, and 3, *A* and *B*, and Supplemental Figs. S1, S2, and S3).[1]

The intersaccadic fixation period is defined as the time starting 50 ms after the first saccade offset and finishing 50 ms before the second saccade onset. The first prolonged fixation period is defined as the time starting 50 ms after the beginning of the fixation at the initial fixation point, finishing 50 ms before the first saccade onset. The second prolonged fixation period is defined as the time starting 50 ms after the second saccade offset, finishing 50 ms before the completion of the trial. The firing rate during prolonged fixation was computed as the mean firing rate of the two prolonged fixation periods. First saccade reaction time is defined as the time between first target jump and first saccade onset. Correspondingly, second saccade reaction time is defined as the time between second target jump and second saccade onset.

### *Unpredictable double-step conditions*

The mixed paradigm presented randomly unpredictable double-step and single-step conditions. Double-step conditions started with a target at the periphery (±15°), continuing with a central fixation (0°), and finishing again at the periphery (±15°, ±7.5°) (Fig. 1*A*). Single-step conditions that started at ±15° and finished at 0° were also presented because they could be confused with possible double-step saccades (see the following text for a complete definition of single-step conditions). In the case of double-step conditions, five different interstimulus interval (ISI) times and four different saccadic combinations yielded a total of 20 conditions. The ISI accounts for the time between the two successive target jumps. We used one fixed ISI of 400 ms (as in the blocked paradigm) and four different variable ISI times, where we used the end of the first saccade to trigger the second target jump. This trigger was set at the time when the monkey's gaze

[1] The online version of this article contains supplemental data.

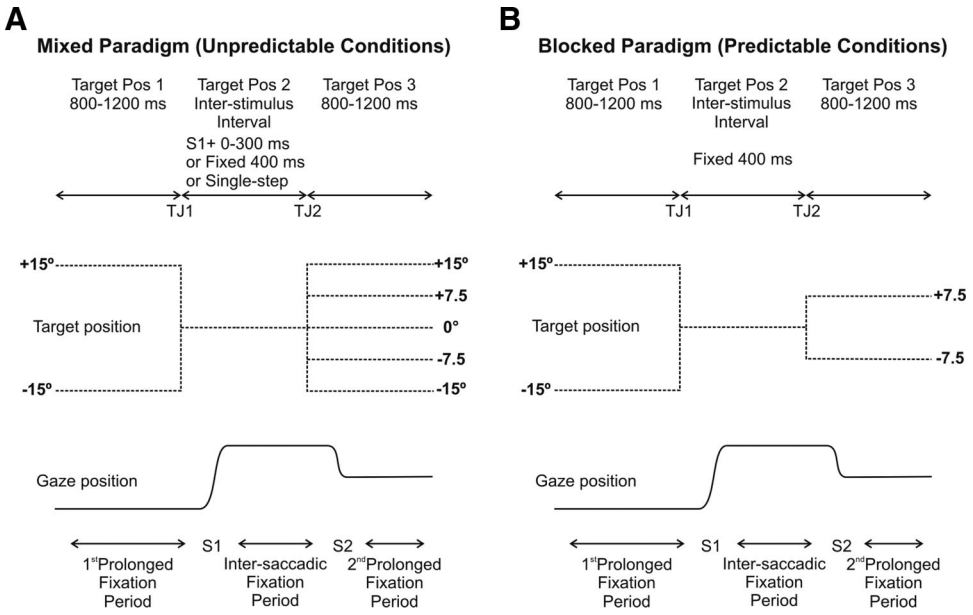


FIG. 1. Spatial and temporal outline of conditions. *A*: mixed paradigm. The description goes from *top* to *bottom*. *Top*: outline showing the periods of constant target location and the times of target jumps (TJ1 and TJ2). *Middle*: target position trace showing the spatial and temporal outline of the 1st and 2nd target jumps. Spatially the tasks are designed in a horizontal 15° periphery-to-center and 15 or 7.5° onward or backward or 0° (no 2nd saccade) combination. The interstimulus interval (ISI) used is variable (triggered by the 1st saccade plus 0 to 300 ms, fixed to 400 ms, or single saccade condition). *Bottom*: gaze position trace matched to the lowest condition. In addition, an outline showing prolonged and intersaccadic fixation periods and saccade onsets (S1 and S2) is presented beneath. *B*: blocked paradigm. Spatially the tasks are designed in a horizontal 15° periphery-to-center and 7.5° onward or backward combination. The ISI used is 400 ms; otherwise, the same conventions are used as in *A*.

entered the control window (4° centered at the second target) and after a predetermined delay of either 0, 100, 200, or 300 ms. Trials using a fixed ISI of 400 ms ended at ±7.5°. Those using variable ISIs ended at ±15°. Target color was always red at all target locations.

### *Predictable double-step conditions*

The blocked paradigm contained four double-step predictable conditions each presented in separate blocks. Double-step tasks started with a target at the periphery (±15°), continuing with a central fixation (0°), and ending again at the periphery (±7.5°) (Fig. 1*B*). The conditions had a fixed ISI of 400-ms duration, which represents the delay between the two target displacements. During this paradigm similar double-step saccade conditions were performed in blocks of typically 15 to 40 trials, allowing the animals to predict the type of task (in this case double-step) and the ISI (400 ms). The sequence of these blocks was also regular over time. Generally, during recording of 20 of the 22 validated neurons in predictable conditions (90.21% of 1,614 predictable trials included in the analysis) onward and backward double-step saccades were presented in separate blocks, so that the direction of the second saccade was also predictable. As a control, during recording of 2 of the 22 validated neurons in predictable conditions (9.78% of 1,614 predictable trials included in the analysis) onward and backward saccades were presented randomly mixed, so that the direction for the second saccade was not predictable. No significant difference was found on the data pertaining to both sets of recordings ($P > 0.05$) and thus the data were pooled and analyzed together. Double-step predictable saccades were cued using a green peripheral target for the first 500 ms under fixation (excluded from analysis); the target color was red otherwise.

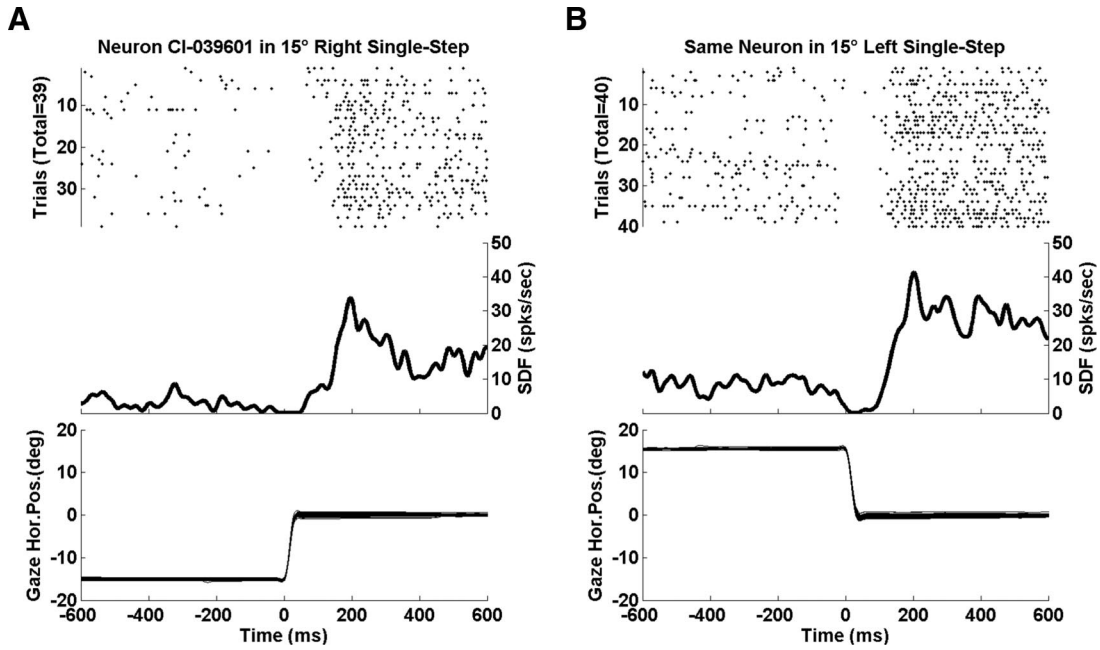


FIG. 2. Neuron CI-039601 responses to conventional single-step tasks. *A*: response to single-step saccade of 15° to the right. *Top*: a raster plot for each trial aligned on saccade onset. *Middle*: the spike density function (SDF) of this neuron, computed as described in METHODS. *Bottom*: gaze horizontal position traces. Positive values correspond to rightward movements. *B*: response to single-step saccade of 15° to the left. Same conventions are used as in *A*.

### *Single-step conditions*

Single-step conditions were used during both paradigms to characterize the recorded neurons. In the mixed paradigm they were randomly intermingled with double-step conditions, starting at the center or at the periphery (±15°, ±7.5°, 0°) and ending at the center or at the periphery (±15°, ±7.5°, 0°). In the blocked paradigm they were presented sequentially in blocks, typically after the double-step blocks were finished. They always started at the center of the screen (0°), where the target remained for a defined time before it was moved to another peripheral position (±7.5°, ±15°). Target color was red at all target locations for every condition. During predictable conditions, single- and double-step saccades were distinguishable at the beginning of the trial by target color (red vs. green. respectively). However, this distinction was not available during unpredictable conditions.

### *Blinks*

Transient fixation point offsets or blinks were also used to characterize the visual properties of the recorded neurons, although they were not included in the analysis. This characterization allowed us to perform a validation process on our neurons (see *Data analysis* for further details). During the mixed paradigm, blinks were generally presented during the intersaccadic fixation period of double-step saccades with a variable duration of 100, 150, 200, or 300 ms. These blinks were randomly introduced within unpredictable double-step conditions. During the blocked paradigm, blinks were presented within the second period of prolonged fixation of double-step and single-step saccades and before finishing every trial. Therefore they were presented in a regular manner and with fixed blink duration of 300 ms.

### *Proportion of trials*

The proportion of performed trials in each paradigm is a critical aspect for the analysis and interpretation of the collected data. In the mixed paradigm, the main factor is the proportion of trials finishing at different target locations. In this paradigm, 490 of 3,582 trials (13.67%) presented a fixed ISI of 400 ms, ending at ±7.5°. Conditions with variable ISI contained 2,249 of 3,582 trials in total (62.78%) and always ended at ±15°. Finally, we also used 843 of 3,582 trials (23.53%) presenting single-step saccades starting at ±15° and finishing at 0°. In the blocked paradigm, the proportion of performed trials was approximately equal for each condition. We used 967 of 1,614 trials (59.90%) containing onward and 647 of 1,614 trials (40.08%) containing backward conditions. The median number of trials per block was 20.

### *Data analysis*

All off-line analyses were performed in Matlab (The MathWorks). Neurons fulfilling the following criteria [adapted from Krauzlis et al. (2000) and Munoz and Wurtz (1993a)] were validated and further analyzed. *1*) *Visual receptive field.* Only neurons recorded during penetrations containing foveal or parafoveal visual receptive fields were included—i.e., when the distance between the center of the receptive field and the center of the fovea is <2.5°. Receptive fields were registered at superficial layers at the beginning of each penetration. *2*) *Depth.* Neurons had to be recorded between 0.8 and 3.5 mm below the dorsal surface of the SC. *3*) *Firing rate stability.* Neurons had to show stability in the firing rate during recordings. Recording stability was ensured by computing the coefficient of variation in mean firing rate of trials for every block. A coefficient of variation <1.0 was established to ensure stability. *4*) *Activity during fixation.* Neurons had to have a minimum firing rate of 5 spikes/s (average) during prolonged fixation. *5*) *Pause in activity during saccades.* Neurons had to show a pause in activity during saccades. Pause is considered as having a firing rate during the saccade period <50% of the firing rate during prolonged fixation. Second saccades in double-step conditions were not used to evaluate the pause in activity, nor were conditions containing a blink. *6*) *Fixation activity during blink.* Blink conditions were used to discard neurons showing purely visual activity. Thus neuronal firing rate had to be >5 spikes/s during the blink period of blink conditions, which is also the minimum accepted firing rate during prolonged fixation. *7*) *Activity in different horizontal orbital positions.* As reported in Munoz and Wurtz (1993a) and Krauzlis et al. (2000), rSC neurons showed generally symmetrical activity at different static horizontal gaze positions. However, a variable degree of asymmetry could be observed in our data, a property that was not reported previously (see Supplemental Fig. S2). As a rule, during conditions in which gaze position was at both the left and the right sides of the central target position (0°), neurons had to show <75% difference in their firing rate between the positions. The intersaccadic interval was not used to perform this calculation. Consequently, we discarded those neurons showing a stronger degree of asymmetry in their activity.

Gaze and head position signals were filtered with a second-order Butterworth filter (28-Hz cutoff frequency). Eye position relative to the head was computed as the difference between the gaze and head signals in space. Velocity and amplitude criteria were used to detect the onset and offset of gaze movements. The onset and offset velocity thresholds were calculated as 2.5-fold the SD of the filtered gaze velocity signal. Only gaze saccades with >3° amplitude were detected. Trials recorded during double-step conditions containing a different quantity than two detected saccades were discarded. In the same way, trials recorded during single-step conditions containing a different quantity than one detected saccade were discarded.

Spike density functions (SDFs) were computed using a Gaussian kernel of 10-ms SD and a time resolution of 1 ms. SDFs were used for the presentation and analysis of individual neurons.

Statistical tests were used to test the hypothesis that two independent samples containing reaction times or firing rates represent similar distributions. Generally the following approach was used. First, each distribution of data was tested for normality (D'Agostino–Pearson test) within each group. If both samples were normally distributed and the sample size in both was ≥20, parametric tests (independent sample *t*-test) were applied at the 5% significance level for between-group comparisons. If at least one sample deviated significantly from normality or had a small size (<20), nonparametric tests (Mann–Whitney *U* test) were performed—again at the 5% significance level. A minimum sample size of 20 was set to ensure proper performance of the D'Agostino–Pearson test (Zar 1999). For simplicity the mean and SD was used to present neuronal data (firing rates and activity ratios) and the median and interquartile range (IQR) were used to present behavioral data (reaction times and intersaccadic interval durations).

Regressions were computed using exponential [$y = a \cdot \exp(b \cdot x)$] and linear functions $y = x\beta$ to model our data. We used the least-square-fit method in these regressions. The goodness of fit was indicated by the coefficient of determination $R^2$. Pearson's correlation coefficients were also used when computing correlations between neuronal firing rates and saccadic reaction times.

## RESULTS

In all, 88 neurons were recorded in two monkeys during 41 penetrations at the rSC. In all of these penetrations, neurons at the superficial layers showed foveal or parafoveal visual receptive fields (<2.5° eccentricity). Five neurons in three of these penetrations were following omnipause neurons (FOPNs), as described by Mustari et al. (1997). As previously reported by these authors, we occasionally encountered first FOPNs dorsally and rSC neurons more ventrally in the same penetrations. Despite both their

anatomical and their functional proximity to rSC neurons, FOPNs could be distinguished easily because of their delayed pause. We considered only the remaining 83 neurons for further analysis.

Of the 83 rSC neurons recorded, 41 fulfilled all the criteria to be considered as fixation-related neurons (criteria listed in METHODS following Munoz and Wurtz 1993a). In brief, the validated neurons lie in the intermediate layers of the rostral SC, show tonic activity during fixation, and pause before and during saccades. When the fixation spot is extinguished they maintain some tonic activity, disclosing that they are not just purely visual neurons. A careful validation process was applied to warrant a homogeneous group of cells similar to those previously described. The remaining 42 neurons violated one or several of the applied criteria and were discarded from the main analysis.

However, a closer inspection of the discarded neurons reveals the richness and variability of neuronal responses located at the rSC. Twelve of these neurons (28.57%) showed directional sensitivity in their activity during saccades; generally they showed a pause of activity during ipsiversive saccades and tonic activity during (small and medium) contraversive saccades. Some of them showed interesting patterns of activity during predictable double-step saccades, as can be observed in one example described in Supplemental Fig. S1. Nine neurons (21.42%) showed a marked gaze position effect in their activity; typically they showed very low tonic activity during fixation at peripheral positions of the ipsilateral visual hemifield, and higher tonic activity during fixation at central and contralateral positions of the visual hemifield (Supplemental Fig. S2). Three neurons (7.14%) showed activity during fixation and a late pause during saccades; the pause started after the saccade onset but before the saccade offset, contrary to FOPNs, in which the pause starts after the saccade offset. Four neurons (9.52%) showed low or no activity while the foveated target was briefly extinguished; therefore they were considered as purely visual neurons.

Of the 41 validated neurons, 19 neurons were recorded during the mixed paradigm, 16 during the blocked paradigm and 6 during both paradigms.

### *Neuronal responses*

A typical response of the rSC neuron (neuron CI-039601) during single-step saccades is shown in Fig. 2, *A* and *B*. These single-step saccades were performed intermingled with double-step saccades during the mixed paradigm (see METHODS). The activity, represented by the spike density function (SDF), is tonic during fixation (mean 15.26 spikes/s) and paused during both saccades: one 15° to the right and one 15° to the left. The activity level measured during fixation falls within the range of previously published values (Munoz and Wurtz 1993a). Note that in this case the activity is lower before the saccade in both conditions, independently of the position of the gaze. Correspondingly, the activity increases quickly in both conditions after the saccade.

During the mixed paradigm—made up by unpredictable conditions—this neuron shows a similar pattern of activity (Fig. 3*A*). This plot shows the condition containing a double-step gaze saccade to the left (forward) with fixed ISI of 400 ms. The activity is tonic during prolonged fixation (mean 10.4 spikes/s before the first saccade and 17.9 spikes/s after the second saccade, respectively) and pauses during saccades (mean firing rate of the two saccade periods is 0 spike/s). The pause in activity is present during both saccades, which have different amplitudes (15 and 7.5°, respectively), and remains obvious when aligning the plots at saccade onset, as the SDF goes to 0 (Supplemental Fig. S3). The mean firing rate during the intersaccadic period is 22.9 spikes/s, which is higher than the firing rate during the prolonged fixation period (mean of the two periods is 14.2 spikes/s). There is no gaze position effect for this neuron, since the firing rates in the opposite unpredictable condition—double-step saccade to the right (forward)—are 8.8 spikes/s before the first saccade and 17.4 spikes/s after the second saccade, respectively (not shown). In other words, the activity of this neuron is not modulated by gaze position but by the history in the task because the activity at the left gaze position is low when the eye starts from left and high when the eye comes from the right gaze position.

The activity of the same neuron recorded during the blocked paradigm, made up by predictable conditions, is presented in Fig. 3*B*. Again, the activity is tonic during prolonged fixation (mean 10.7 spikes/s before the first saccade and 14.8 spikes/s after the second saccade, respectively) and paused during saccades (mean of the two saccade periods is 0 spike/s). However, in this case the mean firing rate during the whole intersaccadic period (4.6 spikes/s) is significantly lower than that during the prolonged fixation period.

We computed the relation of intersaccadic to prolonged firing rates on a trial-by-trial basis for the two presented blocks of this neuron: one recorded during an unpredictable and the other during a predictable condition. With this comparison we check whether the intersaccadic firing rate is significantly lower than the prolonged firing rate during the predictable condition. The results are shown in Fig. 3*C* for the unpredictable and in Fig. 3*D* for the predictable condition, respectively. In the unpredictable condition 7 of 18 trials (38.88%) fell under the dashed line with slope equal to 1. In contrast, in the predictable condition 31 of 37 trials (83.78%) did so. Regressions using the linear model $y = x\beta$ were computed for both groups (solid gray line). The slope of the regression is 1.41 in the unpredictable condition and 0.31 in the predictable one. A higher slope indicates higher intersaccadic fixation firing rate compared with the prolonged fixation firing rate; thus the intersaccadic to prolonged firing rate ratio is higher in the unpredictable (median = 1.20, IQR = 1.33) than that in the predictable condition (median = 0.26, IQR = 0.66). This difference is highly significant ($P < 0.001$).

In total, six neurons—including the neuron CI-039601 presented in Figs. 2 and 3—were recorded during both predictable and unpredictable tasks. All these neurons were recorded in different experiments. We performed statistical analyses for each neuron (as described in METHODS), comparing the intersaccadic to prolonged firing rate ratio of the trials recorded during unpredictable to the trials recorded during predictable conditions. To represent predictable conditions we gathered trials recorded using a fixed ISI of 400 ms. To represent unpredictable conditions we gathered trials recorded using a fixed ISI of 400 ms and trials recorded using variable ISI triggered by the first saccade reaction time plus a predetermined delay of 200 ms. The median ISI in this second condition was 438 ms. Half of these neurons (three of six) showed a significantly lower intersaccadic to prolonged firing rate ratio in predictable com-

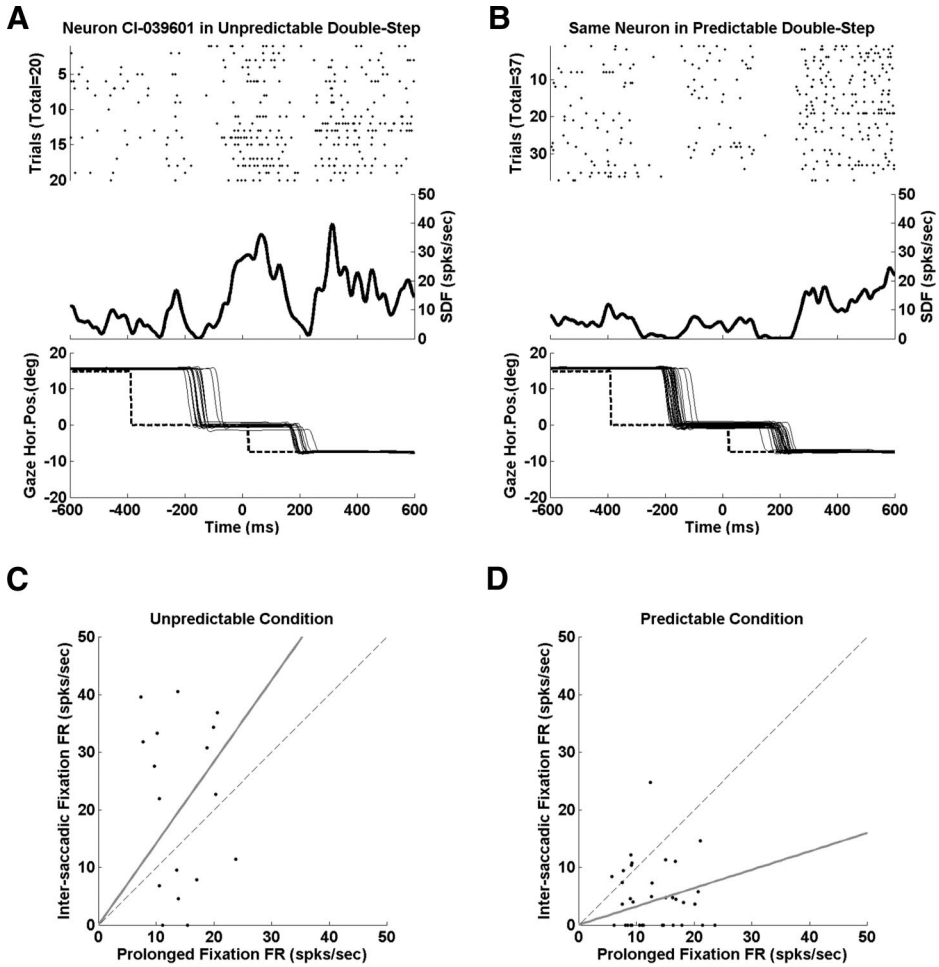


FIG. 3. Neuron CI-039601 responses to unpredictable and predictable double-step tasks. *A*: response to unpredictable condition. *Top*: a raster plot for each trial aligned on 2nd target jump. *Middle*: the SDF. *Bottom*: target (dashed) and gaze (solid) horizontal position traces. *B*: same neuron response to predictable condition. Same conventions are used as in *A*. *C*: comparison of firing rates (FRs) during intersaccadic (*y*-axis) vs. prolonged fixation (*x*-axis) periods on a trial-by-trial basis for the same neuron in the presented unpredictable condition. Dashed line represents linear relationship with slope equal to 1. Solid gray line represents linear regression using collected data. *D*: comparison of FRs during intersaccadic (*y*-axis) vs. prolonged fixation (*x*-axis) periods for same neuron in the presented predictable condition. Same conventions are used as in *C*.

pared with unpredictable conditions ($P < 0.05$). The other three neurons did not show significant differences.

For our analysis of neurons that were not recorded in both the predictable and unpredictable conditions, we used the same approach as for neuron CI-039601 described earlier. We computed the relation of intersaccadic to prolonged firing rate for each neuron grouping the data into two sets: one containing recordings performed during unpredictable and the other during predictable conditions. Here we again used the same tasks as in the previous analysis to represent predictable and unpredictable conditions. In all we used 20 neurons and 1,112 trials to represent unpredictable and 22 neurons and 1,614 trials to represent predictable conditions.

The results are shown in Fig. 4*A* for unpredictable and in Fig. 4*B* for predictable conditions, respectively. In these figures, each symbol represents the mean intersaccadic and prolonged fixation activity of one neuron. Triangles represent neurons recorded during both predictable and unpredictable conditions (6 neurons). Circles represent neurons recorded only during predictable (16 neurons) or only during unpredictable conditions (14 neurons)—note that neurons recorded during the mixed paradigm must have trials recorded using a fixed ISI of 400 ms or using variable ISI with a delay of 200 ms to be included into this analysis.

We tested whether the difference between intersaccadic and prolonged firing rate is statistically significant (as described in METHODS). For each neuron we compared the resulting distribution of intersaccadic firing rates to the distribution of prolonged firing rates. First, we present the results for the six neurons recorded during both predictable and unpredictable tasks (data plotted using triangles). During predictable conditions, five neurons (83.33%) showed significantly lower intersaccadic than prolonged firing rate and one (16.66%) higher. During unpredictable conditions, two neurons (33.33%) showed significantly lower intersaccadic than prolonged firing rate, two (33.33%) higher, and two (33.33%) no significant difference. In other words, this last result shows that rSC neurons have generally lower intersaccadic fixation firing rate compared with prolonged fixation firing rate during predictable conditions. However, this general effect disappears during unpredictable conditions. Consequently, and as we previously stated, the ratio of intersaccadic to prolonged fixation firing rate is generally lower in predictable than that in unpredictable conditions.

Similar proportions were obtained when computing the results for neurons recorded only during predictable or only during unpredictable tasks, showing that neurons recorded during both paradigms follow a general pattern. Of the 16 neurons recorded only during predictable conditions, 15 (93.75%) showed significantly lower intersaccadic than prolonged firing rate, and one (6.25%) higher. Within the pool of 14 neurons recorded only during unpredictable conditions, 7 (50%) showed significantly lower intersaccadic than prolonged

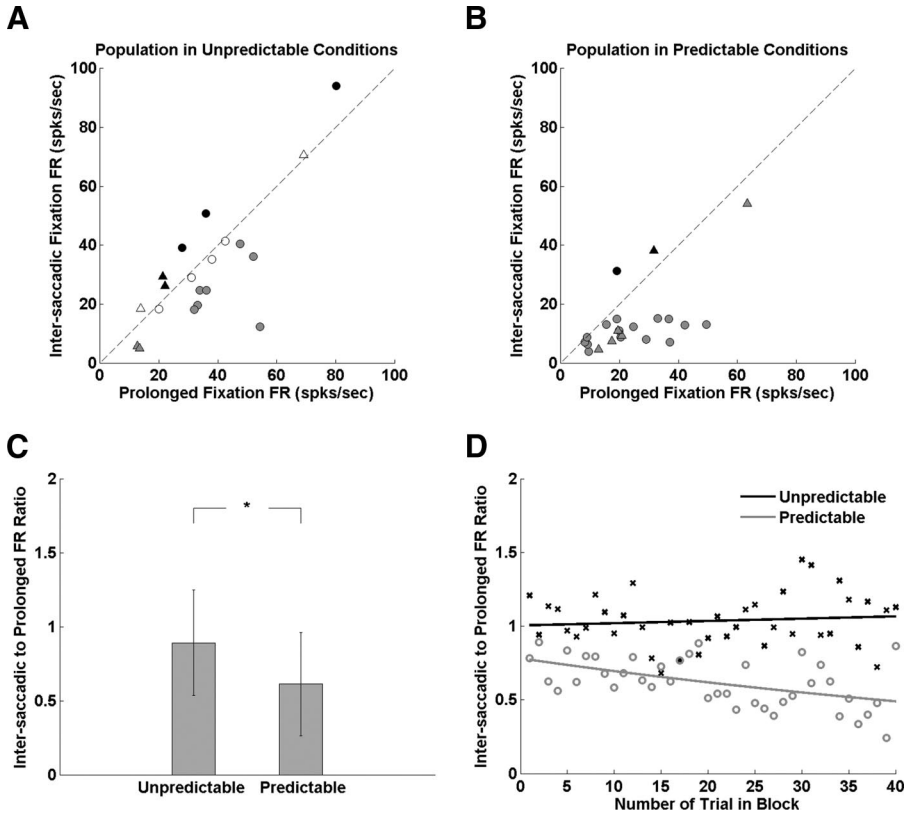


FIG. 4. Population responses. *A*: comparison of FRs during intersaccadic (*y*-axis) vs. prolonged fixation (*x*-axis) periods for the population of neurons recorded in unpredictable conditions. Each element in this plot represents the mean values of one individual neuron. Triangles represent neurons recorded during both predictable and unpredictable conditions. Circles represent neurons recorded only during predictable or only during unpredictable conditions. Black-filled elements represent neurons showing significantly higher intersaccadic than prolonged firing rate. Gray-filled elements represent neurons showing significantly higher prolonged firing rate. Empty elements represent neurons showing no significant difference. Dashed line represents linear relationship with slope equal to 1. *B*: comparison of firing rates during intersaccadic (*y*-axis) vs. prolonged fixation (*x*-axis) periods for the population of neurons recorded in predictable conditions. Same conventions are used as in *B*. *C*: comparison of intersaccadic to prolonged firing rate ratio between neurons recorded during predictable and unpredictable conditions. The *y*-axis represents ratio of intersaccadic to prolonged firing rate. Solid bars represent mean ratio. Error bars represent SD. $*P < 0.05$. *D*: evolution of intersaccadic to prolonged FR ratio during the progression of trials in the blocks. The *x*-axis represents the consecutive number of a trial within a block. The *y*-axis represents mean ratio of intersaccadic to prolonged FR. Each data point is computed using all data from different neurons recorded for a certain trial number within a block. Regressions are computed for unpredictable (black) and predictable (gray) conditions using exponential functions.

firing rate, 3 (21.42%) higher, and 4 (28.57%) no significant difference.

Figure 4*C* shows the difference in intersaccadic to prolonged firing rate ratio between predictable and unpredictable conditions. The mean ratio of individual neurons in both groups was used to compute this test. The ratio was significantly higher ($P < 0.001$) during unpredictable (median = 0.93, IQR = 0.85) than that during predictable conditions (median = 0.45, IQR = 0.72).

Learning effects were tested using the data collected during unpredictable and predictable conditions. To see the temporal evolution of the decrease in intersaccadic firing rate during the predictable blocks, we calculated the ratio between intersaccadic and prolonged firing rate over the progression of the trials in the blocks. For each trial number within a block, we computed the mean intersaccadic to prolonged firing rate ratio using all trials from all neurons recorded in that position in both paradigms. The result is shown in Fig. 4*D*. Regressions were computed using exponential functions [$y = a \cdot \exp(b \cdot x)$] for both groups and represented by black (unpredictable) and gray (predictable) thick lines. The parameters obtained were $a = 1.004$, $b = 0.001$ for unpredictable conditions and $a = 0.78$, $b = -0.01$ for predictable conditions. The coefficient of determination was $R^2 = 0.01$ for unpredictable and $R^2 = 0.26$ for predictable conditions. The ratio decays during the progression of the trial in predictable conditions, while maintaining the initial level in unpredictable conditions.

Further we tested whether the difference between the two groups (predictable and unpredictable conditions) was statistically significant right from the beginning or developed during the progression of the block. The third trial was the first showing statistical significance of the difference. However, and due to the limited number of trials, some other trial numbers (later in the block) also did not reach significance; note too the variability of the responses. In any case, there is a visible learning effect in the predictable conditions because the intersaccadic activity is higher in early than that in late trials. A control was performed using only trials from neurons recorded in both predictable and unpredictable conditions. The result obtained was qualitatively similar (Supplemental Fig. S4).

### *Saccadic reaction times*

In previous studies dealing with visually guided double-step saccades, the reaction time of the second saccade was always found to be longer than for the first one (Feinstein and Williams 1972; Lünenburger et al. 2003). We wanted to test whether this effect is influenced by task predictability as well. For this purpose, saccadic reaction times were estimated for unpredictable and predictable conditions. The neurons and trials used here are the same as in the population analyses of the previous section (20 neurons and 1,112 trials in unpredictable and 22 neurons and 1,614 trials in predictable conditions). Histograms were computed using percentage of trials, so that the integral of each histogram is equal to 100%. The histograms for the first gaze saccade reaction time are shown in Fig. 5*A*. In unpredictable conditions a median of 187 ms and an interquartile range of 27 ms were obtained, whereas in predictable conditions the median was 178 ms and interquartile range 38 ms. Medians of individual conditions are presented in Table 1. The 9-ms difference between both groups is highly significant ($P < 0.001$, Mann–Whitney *U* test).

The histograms for the second saccade reaction times are shown in Fig. 5*B*. In unpredictable conditions, a median of 210

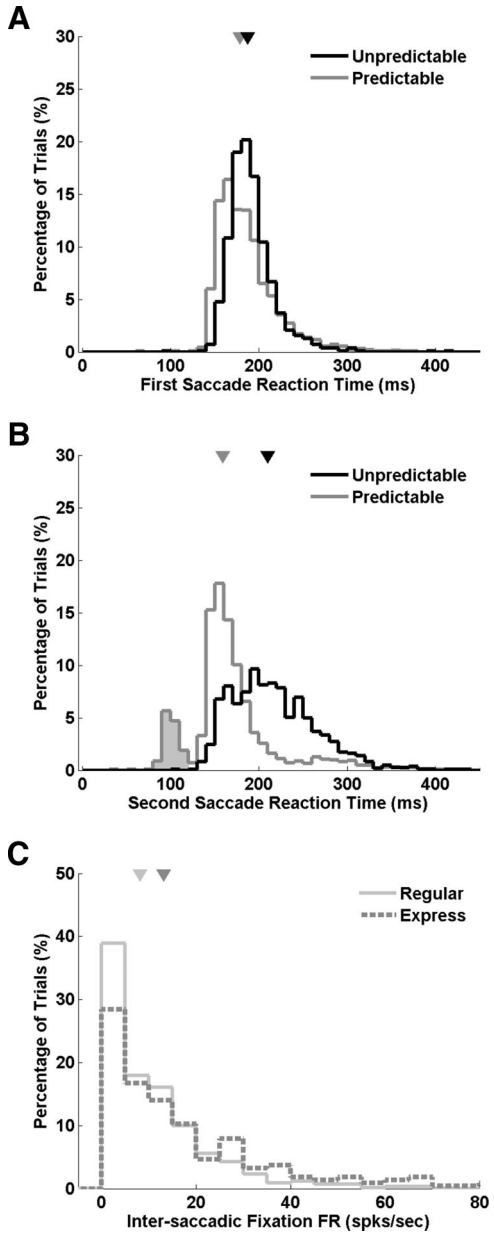


FIG. 5. Reaction times in unpredictable and predictable conditions. *A*: distribution of 1st saccade reaction times in unpredictable (black) and predictable (gray) conditions. The *y*-axis represents the percentage of trials showing a certain reaction time plotted on the *x*-axis. The bin size used to compute the histogram is 5 ms. Filled triangles represent medians for both groups. *B*: distribution of 2nd saccade reaction times in unpredictable (black) and predictable (gray) conditions. Same conventions are used as in *A*. *C*: distribution of intersaccadic FRs during trials presenting regular and express saccades on a trial-by-trial basis. The *x*-axis represents the mean intersaccadic firing rates in spikes/s. The *y*-axis represents the percentage of trials showing a specific FR. The bin size used to compute the histogram is 5 spikes/s.

ms and interquartile range of 64 ms were obtained, whereas in predictable conditions the median was 159 ms and the interquartile range 35 ms. A Mann–Whitney *U* test yielded a *P* value $<0.001$, providing strong evidence that both groups have different medians. During both unpredictable and predictable conditions, backward double-step saccades showed a shorter reaction time compared with onward double-step saccades (in accordance with Dorris et al. 1999) (Table 1). No other significant difference was observed between backward and onward conditions.

Thus saccadic reaction times were faster during predictable compared with unpredictable conditions. This was particularly true for the second saccade and suggests that the postsaccadic refractoriness is not an unmodifiable factor, but can be modulated by top-down processes as predictability.

During predictable conditions, 215 of 1,614 (13.32%) trials showed a second reaction time $<120$ ms. These saccades showing unusually short latencies (express saccades) are not present, either within the first reaction time distribution or during unpredictable second saccades. Control tests were performed to find out whether express saccades were preceded by lower intersaccadic firing rates on a trial-by-trial basis. The distribution of intersaccadic firing rates was computed on a trial-by-trial basis for two subpopulations of neurons: one presenting express and the other presenting regular saccades in the predictable condition. The result is shown in Fig. 5*C*, in which two similar distributions can be observed. A higher firing rate was found before express saccades than before regular saccades, when comparing medians (13.0 vs. 8.1 spikes/s, respectively) or means (17.8 vs. 11.7 spikes/s, respectively). The difference was significant but not high enough to arrive at definitive conclusions.

We also tried to detect correlations using all the trials recorded during predictable and unpredictable conditions separately. In concordance with Dorris et al. (1997), no clear trial-by-trial correlations were obtained between rSC neurons' firing rates and saccadic reaction times (for saccades of 7.5 and 15° amplitude), either during predictable ($-0.15$ Pearson's correlation coefficient) or during unpredictable conditions ($-0.06$ Pearson's correlation coefficient).

### *Dissociation between reaction times, firing rates, and predictability*

Although the second saccade reaction times were not correlated on a trial-by-trial basis with the intersaccadic firing rates, the link between the two variables was manifest at the level of the population. Thus the obtained difference in intersaccadic firing rates could be due to the observed difference in second saccade reaction times and not to the existence or lack of predictability. In other words, reaction times could be a confounding factor for testing the link between predictability and firing rates.

For the purpose of checking whether the reaction times are directly linked to rSC neurons' firing rates, we used conditions with variable ISIs recorded in the mixed paradigm. This paradigm contained only unpredictable conditions. Four tasks with variable ISIs were analyzed, in which we used the first saccade reaction time to trigger the second target jump (as described in METHODS). Each task contained two directions (onward and backward relative to the first saccade) and two different starting points ($+15°$ and $-15°$ horizontally from the center of the screen), such that each task actually contained four different movement patterns. The four tasks are here referred to as S1 + 0, S1 + 100, S1 + 200, and S1 + 300. Second target jump delay is defined as the time between the first saccade onset and the second target jump. The number of trials (and neurons) in the four tasks was as follows: 638 trials (14 neurons) in S1 + 0; 605 trials (13 neurons) in S1 + 100;

TABLE 1. *Summary of behavior and neuronal data during double-step tasks*

| Task | Number of Neurons | Number of Trials | RT1, ms | RT2, ms | | | IID, ms | IFR, spikes/s | PFR, spikes/s | Ratio IFR/PFR |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Onward | Backward | Both | | | | |
| 400 ms, Predictable | 22 | 1,614 | 178 (38) | 163 (32) | 152 (36) | 159 (35) | 352 (40) | 14.2 ± 11.9 | 24.8 ± 14.2 | 0.61 ± 0.34 |
| 400 ms, Unpredictable | 12 | 490 | 187 (36) | 187 (42) | 170.5 (36) | 179 (40) | 364 (46) | 33.8 ± 25.6 | 36.7 ± 21.4 | 0.92 ± 0.41 |
| S1 + 0 ms | 14 | 638 | 189 (27) | 319 (68.5) | 259 (42.25) | 289 (70) | 288 (70) | 17.5 ± 10.6 | 37.5 ± 10.6 | 0.48 ± 0.27 |
| S1 + 100 ms | 13 | 605 | 187 (28) | 262 (53) | 219 (34.75) | 239 (59) | 346 (58.5) | 28.9 ± 20.9 | 41.4 ± 15.5 | 0.69 ± 0.32 |
| S1 + 200 ms | 14 | 622 | 187 (24) | 251 (56.75) | 215 (40.75) | 233.5 (52) | 438 (52) | 37.6 ± 23.0 | 42.7 ± 17.4 | 0.87 ± 0.32 |
| S1 + 300 ms | 10 | 384 | 183 (22) | 250 (42) | 215 (40) | 233.5 (49) | 539 (50) | 42.9 ± 25.3 | 42.4 ± 19.9 | 0.98 ± 0.24 |

Median first saccade reaction times (RT1), second saccade reaction times (RT2), intersaccadic interval durations (IID), and mean intersaccadic firing rates (IFR), prolonged firing rates (PFR), and intersaccadic to prolonged firing rate ratios (Ratio IFR/PFR) are presented. Values are divided into onward and backward conditions for the second saccade reaction times. Reaction times are presented using median and interquartile range. Firing rates are presented using mean and SD. The tasks presented are: predictable with 400-ms interstimulus interval (400 ms Predictable), unpredictable with 400-ms interstimulus interval (400 ms Unpredictable), and unpredictable with variable interstimulus interval triggered by the first saccade reaction time plus a determined delay of 0, 100, 200, or 300 ms (S1 + 0 ms, S1 + 100 ms, S1 + 200 ms, and S1 + 300 ms, respectively).

622 trials (14 neurons) in S1 + 200; and 384 trials (10 neurons) in S1 + 300. In all, 2,249 trials were recorded in these tasks.

First, the relationship between the second target jump delay and saccadic reaction times was analyzed. The results are shown in Fig. 6*A* for the four different tasks. Medians are presented in Table 1. In general, there was no noticeable difference in first saccade reaction time for the four tasks, demonstrating the lack of predictability in these tasks. The second saccade reaction time was clearly higher in the S1 + 0 task, probably because in this task the second target jump was made during the first saccade—a condition in which one would expect a longer reaction time. Second saccade reaction times were fairly stable in the other three conditions (Table 1). In any case, the difference between the first and second saccade reaction times was significant throughout the four tasks ($P < 0.001$), demonstrating that postsaccadic refractoriness was still present 300 ms after the saccade. This result was expected, as generally in visually guided double-step saccades the reaction time of the second saccade is longer than that for the first (Feinstein and Williams 1972; Lünenburger and Hoffmann 2003).

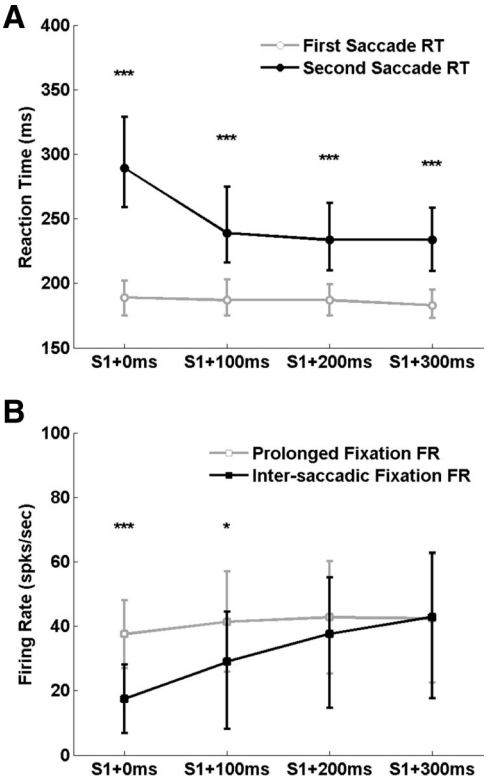


FIG. 6. Saccadic reaction times and neuronal FRs in unpredictable tasks with variable ISI. *A*: relation between 2nd target jump delay and saccadic reaction times. The *x*-axis contains conditions with increasing delay between 1st saccade onset and 2nd target jump onset. The *y*-axis represents saccadic reaction time. Empty gray and solid black circles represent median reaction times for 1st and 2nd saccades, respectively. The error bars represent upper and lower quartiles. ***$P < 0.001$. *B*: relation between 2nd target jump delay and neuronal firing rates. The *y*-axis represents FR. Empty gray and solid black squares represent mean FRs for intersaccadic and prolonged fixation, respectively; otherwise, the same conventions as in *A*. *$P < 0.05$.

Further we tested the relationship between the second target jump delay and the mean firing rate in the intersaccadic period. In this case the firing rate was computed using the mean firing rate of individual neurons. The results are shown in Fig. 6*B*. We found no important differences along these four tasks in the firing rate during the prolonged fixation period. However, and contrary as expected, firing rates during the intersaccadic fixation period increased as the second target jump delay increased. In any event, the difference between the prolonged and intersaccadic fixation firing rates was significant only during the two first tasks ($P < 0.05$), demonstrating that the activity of rSC neurons needed ≥100 ms to recover totally after the saccade. The only possibility to explain this result is that firing rate increased monotonically after the end of the first saccade. This property of the discharge is further analyzed in Supplemental Fig. S5.

Thus there was no direct relationship between rSC neurons firing rates and saccadic reaction times. As observed in Fig. 6, *A* and *B*, a direct relationship between intersaccadic firing rates and second saccade reaction times was not found after the saccade. Therefore the hypothesis that the difference in firing rates obtained in the previous section is due to the difference in reaction times is very unlikely. This is also supported by the lack of correlation obtained on a trial-by-trial basis.

However, another variable that could be related to the intersaccadic firing rates is the duration of the intersaccadic intervals. As expected, the duration of the intersaccadic interval increased as the delay between the first saccade offset and

the second target jump increased (Table 1). The increase in intersaccadic interval duration was not linear because of the higher second saccade reaction times in condition S1 + 0. In any case, a relationship was observed between intersaccadic firing rates and the duration of the intersaccadic intervals. As the intersaccadic intervals duration increased, the intersaccadic firing rates increased as well.

We wanted to test whether there was an influence of task predictability on intersaccadic interval durations in the data used in the previous sections. To do that, we computed the distribution of intersaccadic intervals in both predictable and unpredictable conditions (Fig. 7*A*). As previously stated, to represent predictable conditions we gathered trials recorded in the blocked paradigm using a fixed ISI of 400 ms. To represent unpredictable conditions we gathered trials recorded in the mixed paradigm using a fixed ISI of 400 ms and trials recorded using variable ISI triggered by the first saccade reaction time plus a predetermined delay of 200 ms. In sum we used 1,112 trials from 20 neurons to represent unpredictable and 1,614 trials from 22 neurons to represent predictable conditions.

In general, trials showed a significantly shorter intersaccadic interval ($P < 0.001$) in predictable (median 352 ms, IQR = 40) than in unpredictable conditions (median 401 ms, IQR = 72). Thus task predictability influenced not only neuronal firing rates, but also intersaccadic interval durations. As a result, it could be possible that the measured difference in neuronal firing rates was only a consequence of the difference in intersaccadic interval durations (which was in turn consequent on the difference in task predictability).

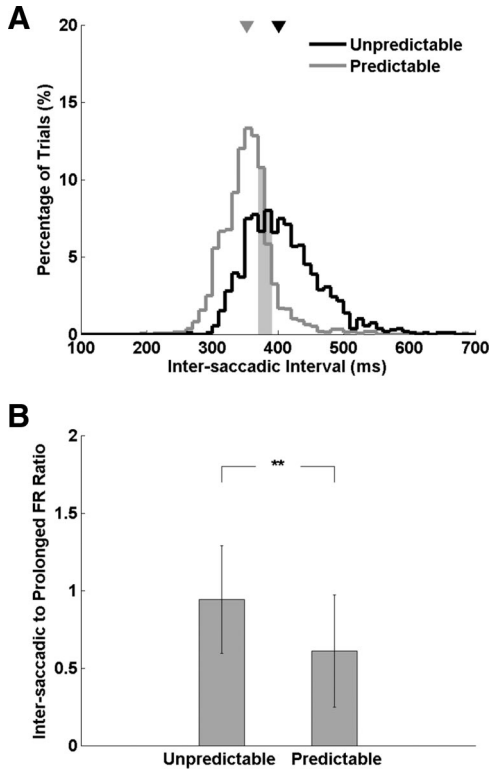


FIG. 7. Dissociation between intersaccadic interval durations, firing rates, and predictability. *A*: distribution of intersaccadic interval durations in unpredictable (black) and predictable (gray) conditions. The *y*-axis represents the percentage of trials showing a certain reaction time plotted on the *x*-axis. The bin size used to compute the histogram is 5 ms. Filled triangles represent medians for both groups. Gray area represents subsample of trials used to compute the ratio comparison in *B*. *B*: comparison of intersaccadic to prolonged FR ratio between neurons recorded during predictable and unpredictable conditions using the subsample of trials marked in *A*. This subsample contains trials for which the intersaccadic interval was held approximately constant. The *y*-axis represents ratio of intersaccadic to prolonged FR. Solid bars represent mean ratio. Error bars represent SD. $^{**}P < 0.01$.

To dissociate between the influence of intersaccadic interval duration and task predictability on neuronal firing rates, we selected a subsample of trials collected in predictable and unpredictable conditions for which the intersaccadic interval was held approximately constant (between 370 and 390 ms). The only variable that we had in this subsample of trials was the difference in task predictability. The subsample is marked on the plotted distribution using a gray background (Fig. 7*A*).

We used this subgroup to compute again the difference in intersaccadic to prolonged firing rate ratio between predictable and unpredictable conditions (Fig. 7*B*). Again, the ratio was significantly higher ($P < 0.01$) during unpredictable (median 1.04, IQR = 0.80) than that during predictable conditions (median 0.47, IQR = 0.66). This difference was significant ($P < 0.01$). With this result we show that the difference in rSC neurons' firing rates was modulated only by the predictability of the tasks, discarding any confounding factor such as reaction times or intersaccadic interval duration.

## DISCUSSION

### *Influence of predictability on the rSC*

Our results revealed a critical influence of task predictability on the activity of rSC neurons, similar to that demonstrated for the caudal SC (Basso and Wurtz 1997; Dorris and Munoz 1998). Two main findings support this notion. First, the activity of rSC neurons changed significantly depending on the level of predictability of the task. The intersaccadic to prolonged fixation activity ratio was inversely proportional to the level of task predictability (Fig. 4*C*). Moreover, second saccade reaction times were shorter during predictable conditions (showing express saccades) compared with those during unpredictable conditions (Fig. 5*B*). Our second and important finding demonstrated a learning effect during predictable conditions (Fig. 4*D*). Thus rSC neurons fired at similar frequency during the intersaccadic and prolonged fixation periods at the beginning of each block; however, in predictable conditions the intersaccadic fixation firing rate became lower than the prolonged fixation firing rate during the progression of the block.

A link between rSC neural activity and saccadic reaction times (for saccades of 7.5° and 15° amplitude) seems to be manifest at the level of the population (in accordance with Dorris and Munoz 1995). However, we showed that the difference in neuronal activity is not influenced by reaction times, but by the presence or lack of predictability in our tasks. Two arguments support this statement. First, the link between reaction times and neuronal activity was not obvious when computing trial-by-trial correlations (related results were obtained by Dorris et al. 1997). Neither our analysis performed on express saccades, nor the analysis performed using all trials in predictable or unpredictable conditions, supported the existence of trial-by-trial correlations between neural activity and reaction times. As previously suggested (Dorris et al. 1997), it is probable that the saccadic reaction times for medium and large saccades are influenced mainly by the neuronal activity at the level of the caudal SC.

Second, the link between neural activity and reaction times was abolished when analyzing these two variables after saccades. The postsaccadic activity of rSC neurons after the first saccade was not found to be responsible for the increase of the second saccade reaction time in double-step saccades. No direct relationship was found between intersaccadic firing rates and second saccade reaction times in tasks with variable ISI (Fig. 6, *A* and *B*). Further, we did not observe significant changes in first saccade reaction time (Fig. 6*A*) or prolonged firing rate before the first saccade (Fig. 6*B*) in these tasks, proving the stability of our recordings. Up to now there is no clear candidate for a neural correlate of the observed postsaccadic refractoriness in visually guided double-step saccades. Importantly, a similar refractoriness is observed in the microsaccades of fixation (Nachmias 1959) and also in the quick phase of nystagmus after voluntary saccades (Judge 1973), thus raising a considerable possibility that refractoriness is the result of some kind of general gating process. In any case, our data are inconsistent with the notion that rSC activity is responsible for the increased second saccade reaction time.

However, the duration of the intersaccadic interval was shown to be related to the rSC intersaccadic activity. To dissociate between neural activity, intersaccadic interval, and predictability, we performed an analysis in a subsample of trials for which the intersaccadic interval was held approximately constant (Fig. 7). Again, a clear influence of predictability was shown in our data because the neural activity was significantly different despite the similarity in intersaccadic interval duration.

### *Anatomical projections to the rSC*

Several cortical and subcortical areas could be responsible for the influence of task predictability onto the activity of the rSC. The frontal eye fields (FEFs) send diverse delay activity signals related to movement, memory, and vision to the SC (Sommer and Wurtz 2000). Neurons that projected from the FEF to the rSC had foveal visual responses and paused during the gap period in gap tasks. Many of the latter neurons also had presaccadic pauses and postsaccadic increases in activity. These rostral-projecting neurons may influence the activity of rSC neurons with respect to task predictability. Also the lateral intraparietal area (LIP) contains SC-projecting neurons that are involved in the planning of saccades and in the formation of an attention map of the salient environment (Bisley and Goldberg 2003; Paré and Wurtz 1997). In general, SC-projecting LIP neurons were qualitatively similar to SC-projecting FEF neurons. The dorsolateral prefrontal cortex (DLPFC) has been also found to send signals to the rSC, conveying information related to complex behaviors requiring the implementation of cognitive control (Johnston and Everling 2006). Further, the basal ganglia play a crucial role on the SC activity through the caudate nucleus (CD) and the substantia nigra pars reticulata (SNpr) (Hikosaka and Wurtz 1983). The SNpr exerts a sustained inhibition on the SC to control the potential chaos induced by all the excitatory inputs coming from cortical areas as the FEF, LIP, and primary visual cortex. The CD contributes to the initiation of movement by removing the sustained inhibition coming from SNpr. Because the information carried by the basal ganglia is often related to memory and expectation, their contribution in task predictability could be crucial.

### *Gaze position error versus target location*

The two most prominent theories that try to model the activity of rSC neurons with respect to its oculomotor function are the *Gaze Position Error* hypothesis (Choi and Guitton 2006; Guitton et al. 2004) and the *Target Location* hypothesis (Hafed and Krauzlis 2008; Krauzlis et al. 2000). The first hypothesis argues that rSC neurons convey a signal representing a gaze position error (GPE), so that their activity is inversely proportional to the distance to the target. The second one argues that the whole SC forms a continuous map of target locations, so that rSC neurons represent foveal and parafoveal targets. These are not identical explanations because the first involves a monotonic and general relationship between firing rate and position error—not related to specific targets—and the second one involves tuning for specific target locations. Further, the GPE hypothesis would predict a decrease on the activity of rSC neurons during smooth pursuit, compared with the activity during fixation, due to the higher distance between target and gaze positions. However, the second hypothesis would predict a general increase of activity because the increase in spatial uncertainty of the target location would activate a larger population of SC neurons to represent the goal. Although previous studies found a general increase on rSC activity during smooth pursuit (Fig. 6 in Hafed and Krauzlis 2008), actually both effects—increase and decrease—were found in individual neurons. Even more intriguing is that those neurons that showed a decrease in their response during smooth pursuit had a preferred eccentricity closer to the fovea (Supplemental Fig. S3 in Hafed and Krauzlis 2008). Thus it is probable that those neurons that represent targets closer to the fovea and that show a decrease of activity during smooth pursuit are the ones called "fixation neurons" by several other authors. Probably they are the neurons that more closely reflect the behavioral state of fixation and those being able to convey GPE signals. However, it is very unlikely that visual fixation depends only on the activity of these neurons. On the contrary, it is very reasonable that visual fixation is ensured as long as the activity in both colliculi is balanced and that only after an imbalance between the two colliculi is a saccade or a smooth eye movement produced (Hafed and Krauzlis 2008). This last point is supported by our data because the activity of rSC neurons did not show lower but higher activity before express compared with regular saccades. Although still speculative, a higher activity in both rostral and caudal neurons would produce a higher and faster imbalance between the two colliculi, resulting in an express saccade.

In any case, two main arguments bring our results in agreement with the GPE hypothesis. First, the diversity of the collected data makes it likely that some of the rSC neurons contain the necessary information to compute a GPE signal. In particular, gaze-position–dependent neurons and directional neurons could provide adequate inputs to dynamically compute the GPE signal. Further, the difference in activity during intersaccadic fixation can be accounted for by the GPE hypothesis. We can hypothesize that during predictable conditions the monkey could anticipate that the desired displacement is to the second and not to the first target. Under this assumption the GPE was not 0 at the first saccade target and thus the activity of these neurons remained low until the end of the second saccade that completed the sequence. Thus the pause in

rSC neurons' activity was prolonged in accordance with the longer overall double gaze shift duration. However, during unpredictable conditions the monkey could not anticipate whether the first target would be the final target position. As the probability that the first target is the final one increases, the activity of rSC neurons increases as well. The answer to the question whether the modulation of the activity between the two saccades would come via a feedback loop or as a feedforward top-down process remains open. A feedback loop could be implemented via the central mesencephalic reticular formation (cMRF) or via the cerebellum. As discussed earlier the top-down influence could come from cortical areas such as FEF, DLPFC, or LIP.

On the other hand, one could also use the Target Location hypothesis to account for the difference observed in our data. Thereby in predictable conditions the desired target location would shift during the intersaccadic fixation period and, as a consequence, the entire distribution of active neurons would shift as well. Thus the firing rate modulations of rSC neurons with gaze error would be a manifestation of the spatial tuning characteristics of these neurons (Hafed and Krauzlis 2008).

There is no clue provided by our data to distinguish between the two presented hypotheses. The saccades embedded in our tasks were always $>7.5°$ in amplitude, so that no effects of predictability in saccades executed to parafoveal locations were studied. Further, Krauzlis (2003) reported modest but significant correlations between rSC neuron activity and the latency of small saccades, another factor that could not be tested with our data. In any case, there was no relation between the preferred eccentricity of our neurons (foveal or parafoveal) and the measured influence of predictability on their activity. Overall, we suggest that these two hypotheses are actually not contradictory, taking into account the variability of responses recorded in this area, suggesting that there might be at least two different types of neurons intermingled in the rostral SC.

### *Diversity of rSC neural responses*

In general, the richness and variety of rSC neuronal responses was higher than expected. In all, 42 of the 83 recorded rSC neurons were discarded for further analysis on the basis of unusual attributes in their responses, which distinguished them from the previously described rSC neurons. This fact could have implications in the characterization and functional interpretation of the rSC neuronal activity. Our experiments were made in head-free monkeys, a factor that could influence the variability of responses observed.

For instance, a group of neurons presented directionality in their response, showing saccadic activity for contraversive and pause during ipsiversive saccades. These neurons are clearly involved in saccade preparation, possibly responsible for the small saccades necessary to maintain steady fixation. Another substantial proportion of neurons showed a strong gaze-position effect in their activity. This finding would be in contradiction to the results of Munoz and Wurtz (1993a). The gaze-position–dependent neurons could be involved in monitoring target position in extraretinal coordinates; note that similar results have been obtained for the caudal SC (Campos et al. 2005). Further, some rSC neurons showed an effect in the time course of the activity. Thus the mean time from pause onset to saccade onset varied between different neurons. This property could reflect that neurons at the rSC are involved in the processing of visuomotor information at different stages. Finally, purely visual neurons are probably involved in retinal feedback mechanisms necessary to maintain steady fixation.

In sum, although many models consider the rSC as having only one functional role (Choi and Guitton 2006; Hafed and Krauzlis 2008; Quaia et al. 1999; Wilimzig et al. 2006), the relatively large size of this area and the diversity of its neuronal responses may reflect the simultaneous participation of this area in various processes. Under this assumption, the involvement of the rSC in active fixation, small saccades and pursuit movements would not be mutually exclusive but synergistic.

### *Conclusions*

Taken together, our data clearly show that task predictability influences the activity of rSC neurons. This result confirms that the activity of rSC neurons reflects not only the behavioral state of visual fixation but also motor preparatory signals, as previously shown for caudal SC neurons. Thus the activity of the rSC, which is potentially part of the fixation system of the primate, is influenced by cognitive processes.

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