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

Vicente F. Reyes-Puerta\textsuperscript{1,2}, Roland Philipp\textsuperscript{1}, Werner Lindner\textsuperscript{1}, Lars Lünenburger\textsuperscript{3}, Klaus-Peter Hoffmann\textsuperscript{1,2}

\textsuperscript{1} Dept. General Zoology and Neurobiology, Ruhr University Bochum, Germany
\textsuperscript{2} International Graduate School of Neuroscience, Ruhr University Bochum, Germany
\textsuperscript{3} Spinal Cord Injury Center, Balgrist University Hospital, Zürich, Switzerland

\textbf{Running Head:} Influence of Predictability on the Rostral SC

\textbf{Corresponding author:}
Prof. Dr. Klaus-Peter Hoffmann
Dept. General Zoology and Neurobiology
Ruhr University Bochum
D-44780 Bochum, Germany
Phone: +49 2343224363
Fax: +49 2343214185
Email: kph@neurobiologie.rub.de

\textbf{Keywords:} superior colliculus; predictability; fixation neurons; saccades; motor preparation

\textbf{Acknowledgments:} This work was supported by the European Commission (MEST-CT-2004-007825 ‘SensoPrim’) and by the German Science Foundation (SFB-509 ‘Neurovision’). We thank Dr. Claudia Distler for surgical procedures, Dr. Dieter F. Kutz for the design of the setup, Hermann Korbmacher for providing the glass-insulated tungsten electrodes, and Margit Bronzel for animal care. We would like also to thank the anonymous reviewers for their helpful comments and suggestions.
ABSTRACT

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 like task predictability influence the activity of neurons also at the rostral pole of the SC (rSC) — classically related to fixation. In order 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 inter-saccadic 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 can not 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). Particularly, 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, e.g. 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 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 et al., 2000; Krauzlis, 2004). 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 et al., 2008; Hafed and Krauzlis, 2008). A top-down effect was reported also for these neurons by showing that in the context of antisaccades fixation during the instruction period was associated with an increased activity as compared to the activity associated with prosaccades (Everling et al., 1999).

In order 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. While 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 inter-saccadic 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 for the first (Feinstein and Williams, 1972; Becker and Jürgens, 1979; Lünenburger et al., 2000; Lünenburger and Hoffmann, 2003). In general, reaction times for the second saccade were shorter during predictable as compared to unpredictable conditions, suggesting that the postsaccadic refractoriness is a modifiable factor. In any case, a 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 in order to demonstrate that the neural activity is only modulated 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 like saccadic reaction times or gaze position.
MATERIALS AND 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 it has been proven that rSC neurons convey a gaze position error signal (Choi and Guitton, 2006), a key factor which 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 LEDs; 1 cm diameter, 1.5 cd/m²) were rear-projected on this screen via galvanometer driven mirrors under home-made software control. The animal’s behaviour was monitored by means of the software which also recorded the spike events, behavioural 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 anaesthetized with ketamine hydrochloride (10 mg/kg, i.m.) followed by pentobarbital sodium (25 mg/kg, i.v.). 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 backwards 45° from the vertical and therefore aiming perpendicularly 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 which 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 weeks.

Recording

Extracellular recordings of single neurons were made with glass-insulated tungsten microelectrodes (impedance 2–3 MΩ measured at 100Hz). The electrodes within a guide tube were lowered through the dura by a microdrive which was mounted on the chamber (Narishige, Tokyo, Japan). Activity of single cells was detected in realtime by means of a computer controlled multi-channel spike sorter (Plexon Inc., Dallas, TX). Single-unit discharges were separated using an online 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 home-made software, which monitored the behaviour of the animals during the tasks and stored the recordings.

Paradigms

Double-step tasks were arranged into two paradigms in order 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-centre-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 1300–1700 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 centre 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 centre (target position 2). A second fixation period of 800–1200 ms was introduced in order 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 which 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. 2A, 2B, 3A, 3B, and Supplemental Figs. S1, S2 and S3).

*INSERT FIGURE 1 NEAR HERE*

The inter-saccadic 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. 1A). Single-step conditions
which started at ±15° and finished at 0° were also presented, as they could be confused with possible double-step saccades — see below for a complete definition of single-step conditions. In the case of double-step conditions, five different inter-stimulus interval times and four different saccadic combinations yielded a total of 20 conditions. The inter-stimulus interval accounts for the time between the two successive target jumps. We used one fixed inter-stimulus interval of 400 ms (as in blocked paradigm) and four different variable inter-stimulus interval 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 entered the control window (4° centred at the second target) and after a predetermined delay of either 0 ms, 100 ms, 200 ms or 300 ms. Trials using fixed inter-stimulus interval of 400 ms ended at ±7.5°. Those using variable inter-stimulus intervals ended at ±15°. Target colour 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. 1B). The conditions had a fixed inter-stimulus interval of 400ms 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 inter-stimulus interval (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 1614 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 1614 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 therefore the data was 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). Target colour was red otherwise.

**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 centre or at the periphery (±15°, ±7.5°, 0°) and ending at the centre 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 centre of the screen (0°) where the target remained for a defined time before it was moved to another peripheral position (±7.5°, ±15°). Target colour 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 colour (red versus green respectively). However, this distinction was not available during unpredictable conditions.
Blinks

Transient fixation point offsets or blinks were also used in order to characterize the visual properties of the recorded neurons — although they were not included into 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 3582 trials (13.67%) presented a fixed inter-stimulus interval of 400 ms, ending at ±7.5°. Conditions with variable inter-stimulus interval contained 2249 of 3582 trials in total (62.78%) and ended always at ±15°. Finally, we also used 843 of 3582 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 1614 trials (59.90%) containing onward and 647 of 1614 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 Munoz and Wurtz, 1993a and Krauzlis et al., 2000 — 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 centre of the receptive field and the centre of the fovea is less than 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 lower than 1.0 was established in order to ensure stability. 4) *Activity during fixation.* Neurons had to have a minimum firing rate of 5 spks/sec (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 lower than 50% of the firing rate during prolonged fixation. Second saccades in double-step conditions were not used to evaluate the pause in activity, neither were conditions containing a blink. 6) *Fixation activity during blink.* Blink conditions were used in order to
discard neurons showing purely visual activity. Thus, neuronal firing rate had to be higher than 5 spks/sec 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 which 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 side of the central target position (0°), neurons had to show less than 75 % difference in their firing rate between the positions. The inter-saccadic 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 cut-off 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 times the standard deviation of the filtered gaze velocity signal. Only gaze saccades with more than 3° degrees 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 in order 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 equal or higher than 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 in order to ensure proper performance of the D’Agostino-Pearson test (Zar, 1999). For simplicity the mean and standard deviation was used to present neuronal data (firing rates and activity ratios) and the median and interquartile range (IQR) were used to present behavioural data (reaction times and inter-saccadic 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

A total of 88 neurons were recorded in 2 monkeys during 41 penetrations at the rSC. In all of these penetrations, neurons at the superficial layers showed foveal or parafoveal visual receptive fields (less than 2.5º 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 their anatomical as well as functional proximity to rSC neurons, FOPNs could be distinguished easily because of their delayed pause. We only considered 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 Materials and 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 in order 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 a rSC neuron (neuron CI-039601) during single-step saccades is shown in
Fig. 2A and 2B. These single-step saccades were performed intermingled with double-step
saccades during the mixed paradigm (see Materials and Methods). The activity — represented by
the Spike Density Function (SDF) — is tonic during fixation (mean 15.26 spks/sec) 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.

*INSERT FIGURE 2 NEAR HERE*

During the mixed paradigm — made up by unpredictable conditions — this neuron shows a similar pattern of activity (Fig. 3A). This plot shows the condition containing a double-step gaze saccade to the left (forward) with fixed inter-stimulus interval of 400 ms. The activity is tonic during prolonged fixation (mean 10.4 spks/sec before the first saccade and 17.9 spks/sec after the second saccade, respectively) and pauses during saccades (mean firing rate of the two saccade periods is 0 spks/sec). 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 spks/sec, which is higher than the firing rate during the prolonged fixation period (mean of the two periods is 14.2 spks/sec). 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 spks/sec before the first saccade and 17.4 spks/sec 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, as 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. 3B. Again, the activity is tonic during prolonged fixation (mean 10.7 spks/sec before the first saccade and 14.8 spks/sec after the second saccade, respectively) and paused during saccades (mean of the two saccade periods is 0 spks/sec). However, in this case the mean firing rate during the whole inter-saccadic period (4.6 spks/sec) is significantly lower than during the prolonged fixation period.

We computed the relation of inter-saccadic 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 inter-saccadic firing rate is significantly lower than the prolonged firing rate during the predictable condition. The results are shown in Fig. 3C for the unpredictable and in Fig. 3D 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 grey 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 inter-saccadic fixation firing rate as compared to the prolonged fixation firing rate. Thereby, the inter-saccadic to prolonged firing rate ratio is higher in the
unpredictable (median=1.20, IQR=1.33) than 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 Material and Methods — comparing the inter-saccadic to prolonged firing rate ratio of the trials recorded during unpredictable to the trials recorded during predictable conditions. In order to represent predictable conditions we gathered trials recorded using a fixed inter-stimulus interval of 400 ms. To represent unpredictable conditions we gathered trials recorded using fixed inter-stimulus interval of 400 ms, and trials recorded using variable inter-stimulus interval triggered by the first saccade reaction time plus a predetermined delay of 200 ms. The median inter-stimulus interval in this second condition was 438 ms. Half of these neurons (three of six) showed a significantly lower inter-saccadic to prolonged firing rate ratio in predictable as compared to 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 above. We computed the relation of inter-saccadic 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 used again the same tasks as in the previous analysis to represent
predictable and unpredictable conditions. In total we used 20 neurons and 1112 trials to represent unpredictable and 22 neurons and 1614 trials to represent predictable conditions.

The results are shown in Fig. 4A for unpredictable and in Fig. 4B for predictable conditions, respectively. In these figures, each symbol represents the mean inter-saccadic and prolonged fixation activity of one neuron. Triangles represent neurons recorded during both predictable and unpredictable conditions (six 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 fixed inter-stimulus interval of 400 ms or using variable inter-stimulus with a delay of 200 ms in order to be included into this analysis.

*INSERT FIGURE 4 NEAR HERE*

We tested whether the difference between inter-saccadic and prolonged firing rate is statistically significant — as described in Material and Methods. For each neuron we compared the resulting distribution of inter-saccadic 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 inter-saccadic than prolonged firing rate and one (16.66%) higher. During unpredictable conditions, two neurons (33.33%) showed significantly lower inter-saccadic 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 inter-saccadic fixation firing rate as compared to prolonged fixation firing rate during predictable conditions. However, this general effect disappears during unpredictable conditions. Consequently and as we previously stated, the ratio of inter-saccadic to prolonged fixation firing rate is generally lower in predictable than 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 sixteen neurons recorded only during predictable conditions, fifteen (93.75%) showed significantly lower inter-saccadic than prolonged firing rate, and one (6.25%) higher. Within the pool of fourteen neurons recorded only during unpredictable conditions, seven (50%) showed significantly lower inter-saccadic than prolonged firing rate, three (21.42%) higher, and four (28.57%) no significant difference.

Fig. 4C shows the difference in inter-saccadic 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 during predictable conditions (median=0.45, IQR=0.72).

Learning effects were tested using the data collected during unpredictable and predictable conditions. In order to see the temporal evolution of the decrease in inter-saccadic firing rate during the predictable blocks, we calculated the ratio between inter-saccadic and prolonged
firing rate over the progression of the trials in the blocks. For each trial number within a block, we computed the mean inter-saccadic to prolonged firing rate ratio using all trials from all neurons recorded in that position in both paradigms. The result is shown in Fig. 4D. Regressions were computed using exponential functions \( y = a \cdot \exp(b \cdot x) \) for both groups and represented by black (unpredictable) and grey (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) did not reach significance as well — note also the variability of the responses. In any case, there is a visible learning effect in the predictable conditions, as the inter-saccadic activity is higher in early than 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 1112 trials in unpredictable and 22 neurons and 1614 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. 5A. In unpredictable conditions a median of 187 ms and an interquartile range of 27 ms were obtained, while 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).

*INSERT FIGURE 5 NEAR HERE*

*INSERT TABLE 1 NEAR HERE*

The histograms for the second saccade reaction times are shown in Fig. 5B. In unpredictable conditions, a median of 210 ms and interquartile range of 64 ms was obtained, while 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 as compared to 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 as compared to 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 1614 (13.32%) trials showed a second reaction time shorter than 120 ms. These saccades showing unusually short latencies (express saccades) are neither present within the first reaction time distribution nor during unpredictable second saccades. Control tests were performed in order to find out whether express saccades were preceded by lower inter-saccadic firing rates on a trial-by-trial basis. The distribution of inter-saccadic firing rates was computed on a trial-by-trial basis for two subpopulations of neurons — one presenting express and other presenting regular saccades in the predictable condition. The result is shown in Fig. 5C, 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 versus 8.1 spks/sec respectively) or means (17.8 versus 11.7 spks/sec 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) — neither during predictable (-0.15 Pearson’s correlation coefficient) nor 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 inter-saccadic firing rates, the link between the two variables was manifest at the level of the population. Thus, the obtained difference in inter-saccadic 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 or not, we used conditions with variable inter-stimulus intervals recorded in the mixed paradigm. This paradigm contained only unpredictable conditions. Four tasks with variable inter-stimulus interval were analyzed, in which we used the first saccade reaction time in order to trigger the second target jump (as described in Materials and Methods). Each task contained two directions (onward and backward relative to the first saccade) and two different starting points (+15º and -15º horizontally from the centre of the screen), such that each task actually contained four different movement patterns. The four tasks are here referred as S1+0, S1+100, S1+200 and
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, 622 trials (14 neurons) in S1+200 and 384 trials (10 neurons) in S1+300. A total of 2249 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. 6A 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 300ms 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 for the first (Feinstein and Williams, 1972; Lünenburger and Hoffmann, 2003).

Further we tested the relationship between the second target jump delay and the mean firing rate in the inter-saccadic period. In this case the firing rate was computed using the mean firing rate
of individual neurons. The results are shown in Fig. 6B. 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 inter-saccadic fixation period increased as the second target jump delay increased. In any event, the difference between the prolonged and inter-saccadic fixation firing rates was only significant during the two first tasks (p<0.05), demonstrating that the activity of rSC neurons needed at least 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 Supplementary Fig. S5.

Thus, there was no direct relationship between rSC neurons firing rates and saccadic reaction times. As observed in Fig. 6A and 6B, a direct relationship between inter-saccadic 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 which could be related to the inter-saccadic firing rates is the duration of the inter-saccadic intervals. As expected, the duration of the inter-saccadic interval increased as the delay between the first saccade offset and the second target jump increased (Table 1). The increase in inter-saccadic 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 inter-
saccadic firing rates and the duration of the inter-saccadic intervals. As the inter-saccadic intervals duration increased, the inter-saccadic firing rates increased as well.

We wanted to test whether there was an influence of task predictability on inter-saccadic interval durations in the data used in the previous sections. In order to do that, we computed the distribution of inter-saccadic intervals in predictable and unpredictable conditions (Fig 7A). As previously stated, to represent predictable conditions we gathered trials recorded in the blocked paradigm using a fixed inter-stimulus interval of 400 ms. To represent unpredictable conditions we gathered trials recorded in the mixed paradigm using fixed inter-stimulus interval of 400 ms, and trials recorded using variable inter-stimulus interval triggered by the first saccade reaction time plus a predetermined delay of 200 ms. In sum we used 1112 trials from 20 neurons to represent unpredictable and 1614 trials from 22 neurons to represent predictable conditions.

In general, trials showed a significantly shorter inter-saccadic 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 inter-saccadic 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 inter-saccadic interval durations (which was in turn consequent of the difference in task predictability).

*INSERT FIGURE 7 NEAR HERE*
To dissociate between the influence of inter-saccadic interval duration and task predictability on neuronal firing rates, we selected a subsample of trials collected in predictable and unpredictable conditions for which the inter-saccadic 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 grey background (Fig 7A).

We used this subgroup to compute again the difference in inter-saccadic to prolonged firing rate ratio between predictable and unpredictable conditions (Fig 7B). Again, the ratio was significantly higher (p<0.01) during unpredictable (median 1.04, IQR=0.80) than 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 inter-saccadic 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 the one 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 inter-saccadic to prolonged fixation activity ratio was inversely proportional to the level of task predictability (Fig. 4C). Moreover, second saccade reaction times were shorter during predictable conditions (showing express saccades) as compared to unpredictable conditions (Fig. 5B). Our second and important finding demonstrated a learning effect during predictable conditions (Fig. 4D). Thus, rSC neurons fired at similar frequency during the inter-saccadic and prolonged fixation periods at the beginning of each block; however, in predictable conditions the inter-saccadic 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 mainly influenced by the neuronal activity at the level 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 inter-saccadic firing rates and second saccade reaction times in tasks with variable inter-stimulus interval (Fig. 6A and 6B). Further, we did not observe significant changes in first saccade reaction time (Fig. 6A) or prolonged firing rate before the first saccade (Fig. 6B) 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 inter-saccadic interval was shown to be related to the rSC inter-saccadic activity. In order to dissociate between neural activity, inter-saccadic interval and
predictability, we performed an analysis in a subsample of trials for which the inter-saccadic interval was held approximately constant (Fig. 7). Again, a clear influence of predictability was shown in our data, as the neural activity was significantly different despite the similarity in inter-saccadic 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 (FEF) 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 which are involved in the planning of saccades and in the formation of an attention map of the salient environment (Paré and Wurtz, 1997; Bisley and Goldberg, 2003). 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 behaviours 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 V1. 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 vs. 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 (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 — as compared to 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 (Figure 6 in Hafed and Krauzlis, 2008), actually
both effects — increase and decrease — were found in individual neurons. Even more intriguing is that those neurons which showed a decrease in their response during smooth pursuit had a preferred eccentricity closer to the fovea (Supplemental Figure 3 in Hafed and Krauzlis, 2008). Thus, it is probable that those neurons — which represent targets closer to the fovea and which show a decrease of activity during smooth pursuit — are the ones called fixation neurons by several other authors. Probably they are the neurons which reflect more closely the behavioral state of fixation, and the ones 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 a saccade or a smooth eye movement is produced (Hafed and Krauzlis, 2008). This last point is supported by our data, as the activity of rSC neurons did not show lower but higher activity before express as compared to 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 compute dynamically the GPE signal. Further, the difference in activity during inter-saccadic 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 therefore 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 to 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 or not. 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 feed-back loop or as a feed-forward top-down process remains open. A feed-back loop could be implemented via the central mesencephalic reticular formation (cMRF) or via the cerebellum. As discussed above the top-down influence could come from cortical areas like 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 inter-saccadic 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 higher than 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. A total of 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 (Quaia et al., 1999; Choi and Guitton, 2006; Wilimzig et al., 2006; Hafed and Krauzlis, 2008), 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 show clearly that task predictability influences the activity of rSC neurons. This result confirms that the activity of rSC neurons reflects not only the behavioural state of visual fixation but also motor preparatory signals, as it was shown previously 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.
REFERENCES


FIGURE LEGENDS

Figure 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 first and second target jumps. Spatially the tasks are designed in a horizontal 15° periphery-to-centre and 15° or 7.5° onward or backward or 0° (no second saccade) combination. The inter-stimulus interval used is variable (triggered by the first 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 inter-saccadic 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-centre and 7.5° onward or backward combination. The inter-stimulus interval used is 400 ms. Otherwise same conventions are used as in panel A.

Figure 2: Neuron CI-039601 responses to conventional single-step tasks. A, Response to single-step saccade of 15° to the right. Upper panel contains a raster plot for each trial aligned on saccade onset. Middle panel contains the Spike Density Function (SDF) of this neuron computed as described in Materials and Methods. Lower panel represents 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 panel A.
Figure 3: Neuron CI-039601 responses to unpredictable and predictable double-step tasks. **A,** Response to unpredictable condition. Upper panel contains a raster plot for each trial aligned on second target jump. Middle panel contains the Spike Density Function (SDF). Lower panel represents target (dashed) and gaze (solid) horizontal position traces. **B,** Same neuron response to predictable condition. Same conventions are used as in panel A. **C,** Comparison of Firing Rates (FRs) during inter-saccadic (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 grey line represents linear regression using collected data. **D,** Comparison of firing rates during inter-saccadic (y-axis) vs. prolonged fixation (x-axis) periods for same neuron in the presented predictable condition. Same conventions are used as in panel C.

Figure 4: Population responses. **A,** Comparison of Firing Rates (FRs) during inter-saccadic (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 inter-saccadic than prolonged firing rate. Grey-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 inter-saccadic (y-axis) vs. prolonged fixation (x-axis) periods
for the population of neurons recorded in predictable conditions. Same conventions are used as in panel B. **C**, Comparison of inter-saccadic to prolonged firing rate ratio between neurons recorded during predictable and unpredictable conditions. The y-axis represents ratio of inter-saccadic to prolonged firing rate. Solid bars represent mean ratio. Error bars represent standard deviation. *p<0.05** **D**, Evolution of inter-saccadic to prolonged firing rate 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 inter-saccadic to prolonged firing rate. 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 (grey) conditions using exponential functions.

Figure 5: Reaction times in unpredictable and predictable conditions. **A**, Distribution of first saccade reaction times in unpredictable (black) and predictable (grey) 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 second saccade reaction times in unpredictable (black) and predictable (grey) conditions. Same conventions are used as in panel A. **C**, Distribution of inter-saccadic firing rates (FRs) during trials presenting regular and express saccades on a trial-by-trial basis. The x-axis represents the mean inter-saccadic firing rates in spks/sec. The y-axis represents the percentage of trials showing a specific firing rate. The bin size used to compute the histogram is 5 spks/sec.
Figure 6: Saccadic reaction times and neuronal firing rates (FRs) in unpredictable tasks with variable inter-stimulus interval. **A**, Relation between second target jump delay and saccadic reaction times. The x-axis contains conditions with increasing delay between first saccade onset and second target jump onset. The y-axis represents saccadic reaction time. Empty grey and solid black circles represent median reaction times for first and second saccades, respectively. The error bars represent upper and lower quartiles. ***p<0.001 **B**, Relation between second target jump delay and neuronal firing rates. The y-axis represents firing rate. Empty grey and solid black squares represent mean firing rates for inter-saccadic and prolonged fixation, respectively. Otherwise same conventions as in panel A. *p<0.05

Figure 7: Dissociation between inter-saccadic interval durations, firing rates and predictability. **A**, Distribution of inter-saccadic interval durations in unpredictable (black) and predictable (grey) 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. Grey area represents subsample of trials used to compute the ratio comparison in panel B. **B**, Comparison of inter-saccadic to prolonged firing rate ratio between neurons recorded during predictable and unpredictable conditions using the subsample of trials marked in panel A. This subsample contains trials for which the inter-saccadic interval was held approximately constant. The y-axis represents ratio of inter-saccadic to prolonged firing rate. Solid bars represent mean ratio. Error bars represent standard deviation. **p<0.01
SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1: Neuron CI-036604 during predictable double-step saccades. This neuron did not pass our validation process (see Materials and Methods) but showed interesting patterns of activity during predictable conditions. In these plots same conventions are used as in Figures 2 and 3. Two different double-step tasks are plotted (left-left and left-right) aligned on three different events (second target jump, first saccade onset and second saccade onset). The two tasks present a fixed inter-stimulus interval of 400 ms. Panels A, B and C show the three alignments (second target jump, first saccade onset and second saccade onset) for the left-left task. Panels D, E and F show the same for the left-right task. This neuron shows a burst of activity when performing a gaze saccade to the contralateral visual hemifield (7.5° to the right, panel C) and a pause when performing a saccade to the ipsilateral visual hemifield (7.5° to the left, panel F). Note that the activity associated to the first saccade (15° to the left) is different depending on the direction of the following second saccade — to the left (panel B) or to the right (panel E). The activity at the onset of the saccade is 35.5 spks/sec in the first condition and 4.8 spks/sec in the second. This difference could be only due to the effect of predictability, since the performed first saccade is the same in both conditions (15° to the left). Note also that the activity before the first saccade and after the second one is similar in both conditions.

Supplemental Figure 2: Neuron CI-028204 during predictable double-step and single-step tasks. This neuron did not pass our validation process (see Materials and Methods) due to a strong effect of the gaze position on its activity. In these plots same conventions are used as in Figures 2
and 3. Four different double-step tasks are shown: right-right in panel $A$, left-left in panel $B$, right-left in panel $C$ and left-right in panel $D$, in which trials are aligned on second target jump. Single-step tasks are shown on panel $E$ (7.5° Right) and panel $F$ (7.5° Left), in which trials are aligned on saccade onset. Note that the activity presents a strong degree of asymmetry, showing a lower level at peripheral positions of the ipsilateral visual hemifield (15° Right), and a higher tonic level during fixation at more central positions (7.5° Right, 0° and 7.5° Left) and peripheral positions of the contralateral visual hemifield (15° Left). Therefore, the gaze position showing lowest activity is 15° Right (mean 8.5 spks/sec) and the one showing highest activity is 7.5° Left (mean 39.2 spks/sec). Note also that task predictability modulates the activity of this neuron, since the activity during the inter-saccadic fixation period in double-step tasks is considerably lower than during the first prolonged fixation period in single-steps — even though the activity is related to the same gaze position.

Supplemental Figure 3: Activity of Neuron CI-039601 aligned on saccades. The activity related to the unpredictable double-step left-left task is shown aligned on first saccade onset (panel $A$) and on second saccade onset (panel $B$). The activity related to the predictable double-step left-left task is shown as well aligned on first saccade onset (panel $C$) and on second saccade onset (panel $D$). The main difference between the two tasks — predictable and unpredictable — is the activity level during the inter-saccadic fixation period (as discussed in Results). Note the monotonic increase of activity after the end of the first and second saccades in the unpredictable task. Note also that the activity drops earlier before the first and second saccades during the predictable task. The well known modulation of presaccadic activity in caudal neurons by task
predictability (Basso and Wurtz, 1997; Dorris and Munoz, 1998) and the presence of lateral inhibitory interactions between rostral and caudal neurons (Munoz and Istvan, 1998) could account for this difference in activity level before the saccades.

Supplemental Figure 4: Evolution of inter-saccadic to prolonged firing rate ratio during the progression of trials in the blocks (control). In contrast to Fig. 4D, here we use only data related to the six neurons recorded during both predictable and unpredictable conditions. Again, the x-axis represents the consecutive number of a trial within a block, and the y-axis represents mean ratio of inter-saccadic to prolonged firing rate. Regressions are computed anew for unpredictable (black) and predictable (grey) conditions using exponential functions. The same tendency as in Fig 4D can be observed, i.e., the inter-saccadic activity is higher in early than in late trials during predictable conditions, while it remains stable during unpredictable ones.

Supplemental Figure 5: Mean Spike Density Function (SDF) of neurons in unpredictable tasks with variable inter-stimulus interval. A, Mean SDF aligned on first saccade onset. The x-axis represents time relative to the saccade onset. The y-axis represents mean firing rate. SDFs of individual neurons were computed and pooled in order to calculate the mean SDF. Different colours represent conditions with increasing second target jump delay. Continuous lines represent mean SDF until second target jump. Dashed lines represent mean SDF after second target jump. Note that the activity increases until a transient response around 100 ms after the saccade offset (in conditions where the target stays in the fovea). After this transient response the activity increases monotonically — even after the second target jump — until the second saccade
(see panel B). **B**, Mean SDF aligned on second saccade onset. Dashed lines represent mean SDF until median second target jump. Continuous lines represent mean SDF after median second target jump. Otherwise same conventions as in panel A. The activity starts dropping at about 150 ms before the second saccade onset, independently of the condition. However, the level from which the activity starts to drop is different in each condition. As a consequence, there is a gradual growth of inter-saccadic firing rate as the second target jump delay increases.
Table 1: Summary of behavioural and neuronal data during double-step tasks. Median first saccade reaction times (RT1), second saccade reaction times (RT2), inter-saccadic interval durations (IID), and mean inter-saccadic firing rates (IFR), prolonged firing rates (PFR) and inter-saccadic 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 standard deviation. The tasks presented are: predictable with 400 ms inter-stimulus interval (400 ms Predictable), unpredictable with 400 ms inter-stimulus interval (400 ms Unpredictable), and unpredictable with variable inter-stimulus 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).