

Neuronal Signal Dynamics during Preparation and Execution for Behavioral Shifting in Macaque Posterior Parietal Cortex

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Abstract

■ Cognitive flexibility arises from our ability to shift behaviors depending on demand changes. Behavioral shifting recruits both a preparatory process for an upcoming behavior and an execution process for the actual behavior. Although neuroimaging studies have shown that several brain regions, including posterior parietal cortex (PPC) participated in each component process, it remains unresolved how such processes are implemented at the single-cell level or even whether these processes are distinctively carried out across microstructures in such regions. By recording single-unit activity from PPC of two monkeys performing an analog of the Wisconsin Card Sorting Test, we found that, in the execution process, two types of neurons exhibited activity modulation depending on whether shift was (shift trial) or was not required (nonshift trial): one type showing larger

activity and the other showing smaller activity in the shift trial than in the nonshift trial. In the preparatory process, in contrast, the population activity of both types became larger in the shift trial than in the nonshift trial. The majority of both types exhibited shift-related activity modulation in both processes, whereas the remaining was specialized in the execution process. The former and the latter neurons were spatially intermingled within PPC. Significantly, when the animals performed set shifting spontaneously in prospect of a demand change, the shift-related activity modulation still emerged in both processes. We suggest that both execution and preparation signals are represented within PPC, and that these signals reflect behavioral shifting mechanisms that can be driven by either internal or external triggers. ■

INTRODUCTION

Cognitive flexibility enables us to adapt ourselves to changing environments. This ability requires prompt shifting of our behavior in response to demand changes, a process called behavioral shifting. Human psychological studies propose that two main cognitive processes are recruited in behavioral shifting (Monsell, 2003; Rubinstein, Meyer, & Evans, 2001; Ruthruff, Remington, & Johnston, 2001; Meiran, 1996; Allport, Styles, & Hsieh, 1994). One is the preparatory process through which subjects prepare for an upcoming behavior on receiving a signal for a demand change. The other is the execution process through which subjects actually perform their behavior.

Human neuropsychological studies report that focal cortical damage, including either frontal or nonfrontal damage, causes a severe deficit in behavioral shifts, even though patients were notified of a demand change (Mountain & Snow, 1993; Anderson, Damasio, Jones, & Tranel, 1991; Milner, 1963). Animal lesion studies also support these observations (Fox, Barense, & Baxter, 2003; Rushworth, Hadland, Gaffan, & Passingham, 2003; Dias, Robbins, & Roberts, 1996, 1997; Passingham, 1972). Human fMRI studies have provided more detailed informa-

tion about the functional architecture of the cortices by demonstrating that several brain regions such as posterior parietal cortex (PPC) were recruited in each component process (Chiu & Yantis, 2009; Ruge, Goschke, & Braver, 2009; Badre & Wagner, 2006; Gruber, Karch, Schlueter, Falkai, & Goschke, 2006; Sakai & Passingham, 2003; Monchi, Petrides, Petre, Worsley, & Dagher, 2001; Kimberg, Aguirre, & D'Esposito, 2000; Sohn, Ursu, Anderson, Stenger, & Carter, 2000). However, because of innate limitations in spatial and time resolution in neuropsychological and neuroimaging methodologies, it still remains unclear whether such component processes are distinctively implemented in the identified regions across possible microstructures, which could range from the size of columnar organization to the size of the point-spread function of blood oxygenation level dependent signal.

We addressed this issue in the present study by examining single-unit activity in PPC of two monkeys performing an analog of the Wisconsin Card Sorting Test (WCST). Previously, we examined the preparatory period in which animals started preparation on receiving a signal for a demand change and found a group of PPC neurons, unidimensional shift-related (UDSR) neurons, that were specialized in the preparatory process (Kamigaki, Fukushima, & Miyashita, 2009). Here in the present report, we assessed the execution period in which the monkeys

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started with a blank screen for 3500–4100 msec (preparatory period). Then the screen turned gray and the warning stimulus appeared. The sample stimulus overlaid the warning stimulus with a 500-msec duration (sample period) and three choice stimuli were then presented at the top, right, and left of the sample until the animals selected one of them by a button press (choice period). Every sample and choice stimulus had two attributes, color and shape (called dimension), and the monkeys had to match the sample to the choice in the relevant dimension. After six to eight consecutive correct trials, the relevant dimension changed without informing the monkeys (“dimensional change”), and the response based on the previous dimension was no longer correct. The first trial after the dimensional change was called the “inevitable-error” trial. The first trial with a correct response after the dimensional change was defined as the “shift” trial; other correct trials and inevitable-error trials were defined as “nonshift” trials.

Electrophysiology

The procedure for single-unit recording has been described in detail elsewhere (Kamigaki et al., 2009). The activity of single neurons was recorded extracellularly from the lateral surfaces of PPC, including area 7a and the dorsal prelunate area (DP) (Stoet & Snyder, 2004; Lewis & Van Essen, 2000) (right and left hemispheres in Monkey W and Monkey G, respectively), with a glass-insulated Elgiloy microelectrode (~1 M Ω at 1 kHz). The microelectrode was advanced through the intact dura mater into the cortex using a hydraulic micromanipulator (MO-95C, Narishige, Tokyo, Japan). Recording sites were localized using structural magnetic resonance (MR) images [obtained using a 4.7-T MRI scanner (Biospec 47/40, Bruker) for Monkey W and a 1.5-T MRI scanner (Hitachi Medical, Tokyo, Japan) for Monkey G].

Data Analysis

We analyzed neuronal activity around the choice period, that is, –500 msec to 400 msec from the choice period onset, which we termed the “execution” period. Accordingly, the neuronal activity during this period was called “execution activity.” The start time of the analysis window, that is, –500 msec from the choice period onset, corresponded to the onset of sample stimulus presentation. The end time of the analysis window, that is, 400 msec from the choice period onset, approximately corresponded to the minimal response time of both monkeys, so that the choice stimulus was almost always presented until this time. The execution activity was analyzed using a two-way ANOVA ($p < .05$) with the factor dimension (color or shape), upon which the monkeys’ response was based, and the factor trial type (shift or nonshift trial). Except for the inevitable-error trials, only correct trials were analyzed.

We used only those neurons that were tested in at least three trials for each combination of dimension and trial type factors. A neuron with a significant main effect of trial type was defined as a “shift-related” (SR) neuron because this type of neuron showed the activity modulated depending on whether set shifting was performed (i.e., shift trial) or not (nonshift trial). Of the SR neurons, those neurons that had larger activity in the shift trial than in the nonshift trial were called “increase-type” neurons, whereas those neurons that had smaller activity in the shift trial were called “decrease-type” neurons.

In Figures 3D, 4D, 5B, and 6B, we separately analyzed neuronal activity during the two periods in the execution period, that is, the sample period (500 msec time window preceding the choice period onset) and the choice period (400 msec time window following the choice period onset), which we called “sample activity” and “choice activity,” respectively. We also analyzed the activity of SR neurons during the preparatory period (we used a time window, 1000–2500 msec from the onset of the period), which we called “preparatory activity” (Figures 3–6). There was no overlap between the preparatory and execution periods. For each neuron, spike trains were convoluted with a Gaussian kernel ($\sigma = 50$ msec) and averaged across trials to obtain spike density functions (SDFs). For ensemble SDFs, the individual SDFs of each neuron were normalized using its maximum firing rate during the execution period. We tested whether the population activity was affected by the trial positions relative to the shift trial using one-way ANOVA with a trial position factor (shift trial, or first to third trials after the shift trial) (Figures 5 and 6). If a main effect was significant ($p < .05$), post hoc comparisons were conducted using Fisher’s least-significant difference test.

In order to examine the effect of action selection, we tested whether the behavioral-response direction (top, right or left) modulated the neuronal activity during the choice period (Stoet & Snyder, 2004, 2007). First, we determined a “preferred” direction for each neuron as follows. By using all correct and inevitable-error trials, the mean choice activity (activity during the 400-msec time window following the choice onset) was calculated for each direction (top, right, and left). The direction for which the neuron showed the maximum mean choice activity was defined as a “preferred” direction, and the remaining two directions as “null” directions. We then performed a two-way ANOVA with the factors direction (preferred or null directions) and trial type (shift or nonshift trials) on the choice activity. If a neuron had any significant main effect of direction or interaction ($p < .05$), the neuron was regarded as having “directional selectivity.” To further analyze the effect of behavioral-response direction on the population activity (Figure 5C for increase-type neurons; Figure 6C for decrease-type neurons), we compared the choice activity between the shift and nonshift trials for each of the preferred and null directions separately.

We also analyzed the time course of SR neurons in two different ways. First, we calculated the “peak time” using the activity difference between the shift and nonshift trials. For each cell, the SDFs ($\sigma = 50$ msec) aligned at the sample onset were calculated in the shift and nonshift trials. The SDF in the shift trials was then subtracted by that of the nonshift trials to produce the activity difference. From the sample onset, we searched for the peak time when a neuron showed the maximum absolute activity difference. Second, we calculated the “response onset” using activity in the shift trials. For each cell, the neuronal activity in shift trials was aligned at the sample onset, and its perievent histogram with 10-msec binwidth was created. The mean firing rate (baseline activity) and its standard deviation (*SD*) were calculated in the period of 500–2500 msec after the preparatory period onset in the shift trials. For increase-type neurons, if the activity in the shift trials exceeded $2SD$ from its baseline activity in at least two consecutive bins after the sample onset, the first bin was defined as the response onset. For decrease-type neurons, if the activity in the shift trials fell below $2SD$ from its baseline activity in at least two consecutive bins, the first bin was defined as the response onset. We excluded the neurons in which the response onset could not be detected.

According to a previous study (Katai et al., 2010), we classified each of the SR neurons into two cell types, a putative regular spiking (pRS) neuron or a putative burst spiking (pBS) neuron, on the basis of interspike intervals (ISI). If the ISIs smaller than 5 msec accounted for less than 5% of all the ISIs recorded from the neuron, the neuron was classified as a pRS neuron. Otherwise, the neuron was classified as a pBS neuron.

In Figure 7, to compare neuronal activity between pairs of trial types (shift-success vs. shift-failure trials for Figure 7A, B; shift-success vs. nonshift-following-error trials for Figure 7C, D; spontaneous shift vs. nonshift-following-correct trials for Figure 7E, F), we calculated a difference index for each neuron as follows. First, the mean firing rate during the execution period was calculated for each of two trial types. Then, their difference was divided by their sum, thus giving the difference index for a neuron. To address whether the neuronal activity at the population level was different between the pairs of trial types, we tested the null hypothesis that the difference indices were equal to zero using the Wilcoxon signed rank test.

In order to address the ocular effects on neuronal activity, we conducted ocular analyses in the following two ways: (1) We assessed whether any ocular behaviors specific to the shift trials might have created the shift-related activity modulations of SR neurons. For each SR neuron, we calculated the difference in various ocular parameters during the execution period, including gaze eccentricity and saccadic parameters (saccadic frequency, amplitude, and direction) between the shift and nonshift trials. Then, across all SR neurons, a correlation

analysis was performed between the difference in each ocular parameter and the difference in the neuronal activity (Pearson’s correlation). (2) We examined whether gaze behaviors were differentiated between color and shape dimensions, and such differences might have caused shift-related activity modulations. By using all correct and inevitable-error trials in the neuronal sessions in which all SR neurons were recorded, we compared the difference in gaze eccentricity between dimensions, during the preparatory, sample, and choice periods (*t* test). Then we tested whether these gaze-eccentricity differences between dimensions, if any, might correlate with the shift-related activity modulations. For each neuron, we calculated the absolute activity difference between the shift and nonshift trials, and the absolute gaze-eccentricity difference between dimensions during the preparatory, sample, and choice periods. Correlation analyses were performed between the two differences across all SR neurons during each of the three periods (Pearson’s correlations; Figures 5D and 6D). For each neuron, we also calculated sensitivity for gaze eccentricity as the shift-related activity modulation divided by the absolute gaze-eccentricity difference (Calton, Dickinson, & Snyder, 2002; Boussaoud & Bremner, 1999; Andersen, Essick, & Siegel, 1985).

The switch cost is measured as the performance difference between the first and second trials after the demand change in literature (Kamigaki et al., 2009; Monsell, 2003; Rubinstein et al., 2001; Ruthruff et al., 2001; Meiran, 1996; Allport et al., 1994). We measured the switch cost in our task as the difference in the error rate and the response time between the first and second trials after the inevitable-error trial. We used only correct trials for calculation of the response time.

All statistical analyses were carried out using SAS/STAT (SAS Institute) and MATLAB (MathWorks) software. All of the statistical tests were two-tailed.

RESULTS

Behavioral Performance

We trained two monkeys (Monkey W and Monkey G) to perform an analog of the WCST (Figure 1A; Kamigaki et al., 2009). The monkeys performed this task well and shifted their behavior after an inevitable-error trial. The first trials after the inevitable-error trials were reliably performed based on the new dimension so that the percentages were significantly higher than chance (96.4% and 67.0% for Monkey W and G, respectively; $p < .0001$, binomial test). Performance was even better in subsequent trials: 97.3% and 82.0% in the second trial after the inevitable-error trial for Monkey W and Monkey G, respectively.

Once the monkeys first reached a correct response in a trial after the inevitable-error trial (called shift trial), they faithfully maintained correct responses during the following nonshift trials. Their behaviors around a shift trial are

shown in Figure 1B. The mean percentage of responses based on the new dimension was nearly perfect in the trials immediately after the shift trials (97.1% and 95.0% for Monkey W and Monkey G, respectively) and this was maintained in the subsequent trials. This stable and reliable performance after the shift trials suggests that during the shift trials the monkeys did indeed shift their behavior as well as their internal cognitive set for the new dimension and maintained it during the nonshift trials.

We next calculated the response time, that is, the time required for selecting a choice stimulus after the choice onset. The mean response time averaged across all correct and inevitable-error trials was 762.4 ± 175.3 msec and 662.9 ± 294.6 msec (mean \pm SD) for Monkeys W and G, respectively. This showed that the mean response times for both monkeys came after the end of the execution period, that is, 400 msec after the choice onset, which we used to detect task-related activity (see the next section and Methods).

According to several psychological studies (Monsell, 2003; Rubinstein et al., 2001; Ruthruff et al., 2001; Meiran, 1996; Allport et al., 1994), subjects' performance often declines in the first trials after a demand change as compared with the following trials, which is known as switch cost. We then calculated the switch cost reflected in the error rate and in the response time (see Methods and Kamigaki et al., 2009). The switch cost reflected in the error rate was significantly larger than zero in both monkeys (0.9% and 15.0% in Monkeys W and G, respectively, $p < .01$, t test). The switch cost reflected in the response time was significantly larger than zero in one monkey (76.8 msec in Monkey G, $p < .001$, t test; 0.3 msec in Monkey W, $p = .23$).

Neuronal Database

We recorded single-unit activity from 804 neurons in the lateral surfaces of PPC (Kamigaki et al., 2009; Stoet & Snyder, 2004; Lewis & Van Essen, 2000). We examined the activity around the time of the choice period, "execution period," to detect task-related activity. The activity of each neuron during the execution period was analyzed using a two-way ANOVA [$p < .05$, Dimension (color or shape) \times Trial type (shift trial or nonshift trial)] (Figure 2). Out of the recorded neurons, 234 (29%) showed a significant main effect of dimension, and their activity was modulated depending on the dimension that the animals obeyed. Only 76 neurons (9%) showed a significant interaction between dimension and trial type. In the present study, we focused on those neurons with a significant main effect of trial type (i.e., SR neurons). Among the recorded neurons, 118 (15%) were found to be SR neurons. Of these, neurons with larger activity in the shift trial than in the nonshift trial were called "increase-type" neurons, whereas neurons with the opposite preference

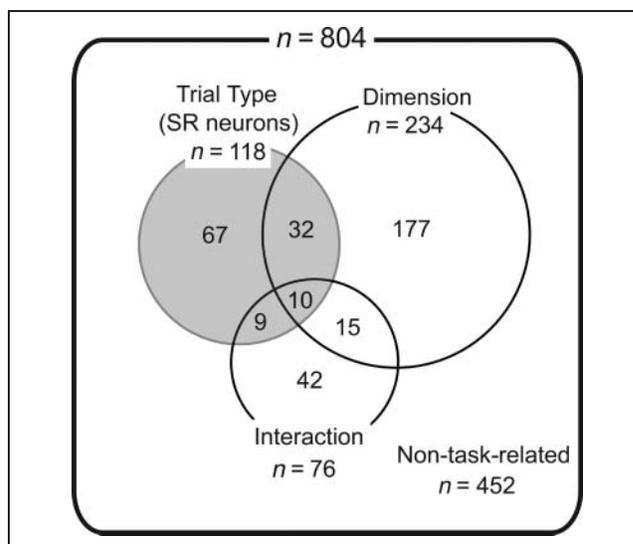


Figure 2. The result of two-way ANOVA during the execution period. A two-way ANOVA with the factors of dimension and trial type was performed for each of the 804 recorded neurons during the execution period. Three circles indicate the main effects of dimension, trial type, and their interaction, respectively. The numbers of neurons with each main effect or their interaction are shown. Lightly shaded area indicates SR neurons.

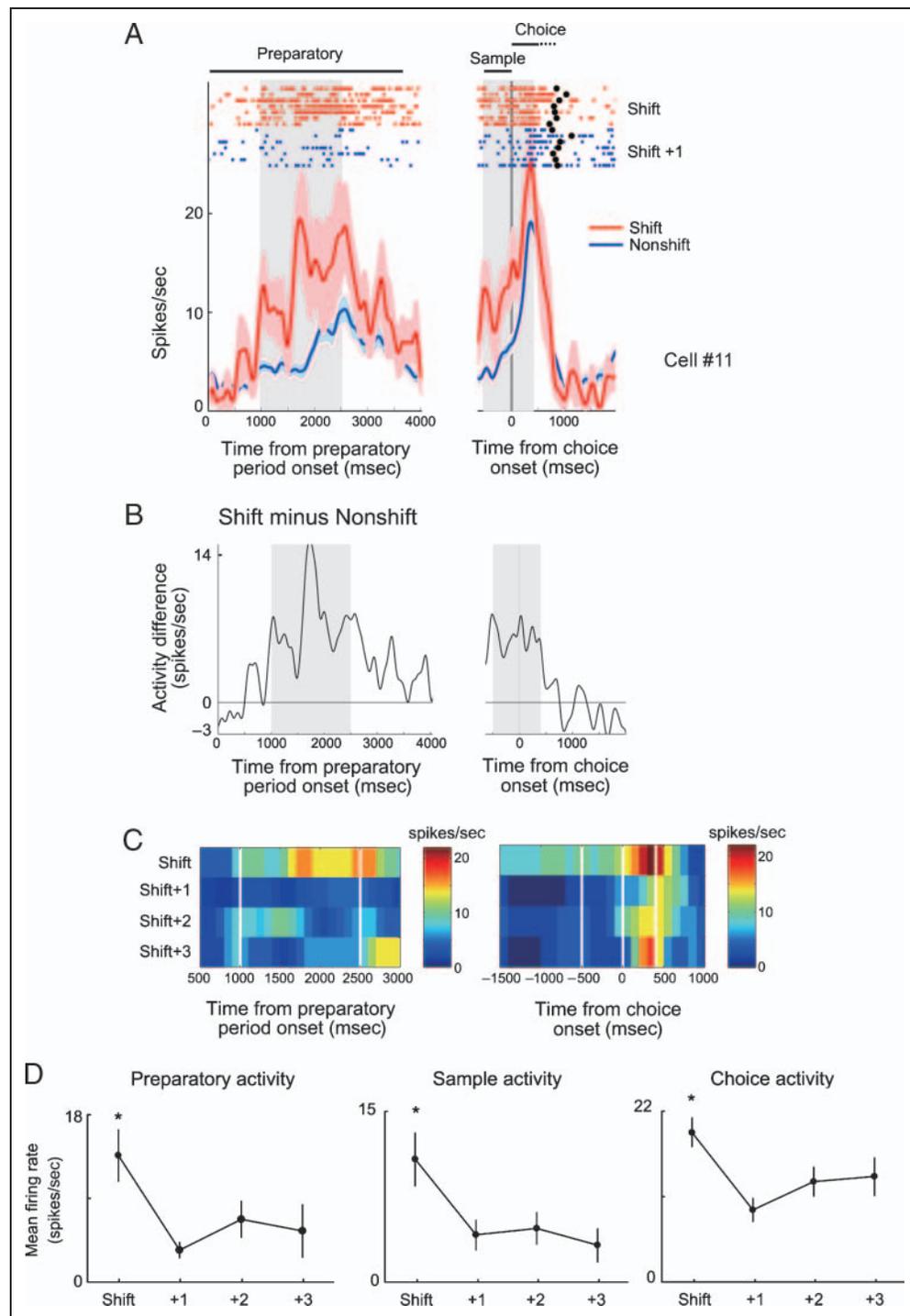
were called "decrease-type" neurons. Out of the 118 SR neurons, 76 (64%) were increase-type neurons, and the remaining 42 (36%) were decrease-type neurons.

Single PPC Activity Related to Behavioral Shifting in Both Preparatory and Execution Periods

An example of an increase-type SR neuron is shown in Figure 3. During both the sample and choice periods (Figure 3A, B, right), this neuron was activated more strongly in the shift trials (red line in Figure 3A) than in the nonshift trials (blue line). In addition to the activity around the choice period, this neuron also exhibited larger activity during the preparatory period (Figure 3A, B, left) in the shift trials (red line in Figure 3A) than in the nonshift trials (blue line). We next examined the effect of the trial positions from the shift trials (Figure 3C, D). The activities during the sample and choice periods were both significantly larger in the shift trials than in the following nonshift trials [$p < .05$, post hoc Fisher's least-significant difference test, following one-way ANOVA (a significant main effect of trial position, $p < .005$); Figure 3D, middle and right]. Moreover, the preparatory activity was also significantly larger in the shift trials than in the following nonshift trials ($p < .01$, post hoc Fisher's least-significant difference test; Figure 3D, left).

The decrease-type neurons showed different patterns of activity modulation. An example of a decrease-type neuron is shown in Figure 4. In contrast to the increase-type neuron shown in Figure 3, during the sample and choice periods (Figure 4A, B, right), the neuron showed smaller

Figure 3. Representative increase-type neuron. (A) Rastergrams and SDFs of an increase-type neuron. Data are aligned to the onset of the preparatory (left) and choice periods (right). Red and blue dots in the rastergrams indicate neuronal discharge in the shift trials and in the first nonshift trials after the shift trials, respectively. For SDFs, red and blue thick lines indicate the mean activity averaged across all shift trials and all nonshift trials, respectively. Envelopes around the SDFs indicate SEMs. Light-gray shading indicates the time window for the analysis during the preparatory period (left) and the sample and choice periods (right) (see Methods). Black points in the rastergrams indicate the times at which the monkey pushed a choice button. (B) Difference in the SDFs between the shift and nonshift trials. (C) Color-coded time courses of the mean firing rates. Data were aligned to the onset of the preparatory period (left) and the choice period (right). From top to bottom, the time courses were sorted according to the trial positions from the shift trial. The two vertical white lines for each panel indicate the analysis window during the preparatory period (left) and the execution period (right). (D) The mean firing rates during the analysis window of the preparatory period (i.e., preparatory activity, left), the sample period (sample activity, middle), and choice period (choice activity, right) as a function of the trial positions from the shift trial. Error bars indicate SEMs. $*p < .02$, Fisher's least-significant difference test, following one-way ANOVA with a significant main effect of trial position ($p < .01$).



activity in the shift trials (red line in Figure 4A) than in the nonshift trials (blue line). However, during the preparatory period (Figure 4A, B, left), the polarity of the activity modulation was reversed: This neuron showed larger activity in the shift trials (red line in Figure 4A) than in the nonshift trials (blue line). We further examined the effect of the trial positions from the shift trials (Figure 4C, D). The sample and choice activities were both significantly smaller in the shift trials than in the following nonshift trials ($p < .05$, post hoc Fisher's least-significant difference

test; Figure 4D, right). In contrast, the preparatory activity was significantly larger in the shift trials than in the following nonshift trials [$p < .001$, post hoc Fisher's least-significant difference test; Figure 4D, left].

Population Analyses of the Shift-related Modulation of PPC Activity

We next performed population analyses for the two types of SR neurons. Figure 5A displays the average time course

of the activity in all the 76 increase-type neurons for each trial position. As expected from the definition of the increase-type neurons, the execution activity was larger in the shift trials than in the following nonshift trials [$p < .001$, post hoc Fisher's least-significant difference test, following one-way ANOVA (a significant main effect of trial position, $p < .001$); Figure 5B, right]. As seen in the example neuron in Figure 3, the same results were obtained irrespective of whether the sample or choice activities were

separately analyzed, which confirmed that shift-related activity modulation was consistently observed for the entire duration of the execution period (Figure 5B, right). During the preparatory period, the population activity was significantly larger in the shift trials than in the following nonshift trials ($p < .001$, post hoc Fisher's least-significant difference test; Figure 5B, left). These similarities in the activity of the execution and preparatory periods, however, might arise from a time-dependent correlation between activity

Figure 4. Representative decrease-type neuron. (A–D) The same format was used as in Figure 3. (A) Rastergrams and SDFs of a decrease-type neuron. (B) Difference in the SDFs between the shift and nonshift trials. (C) Color-coded time courses of the mean firing rates as a function of the trial positions from the shift trial. (D) The mean preparatory activity (left), sample activity (middle), and choice activity (right) as a function of the trial positions from the shift trial. Error bars indicate *SEMs*. * $p < .02$, ** $p < .004$, Fisher's least-significant difference test, following one-way ANOVA with a significant main effect of trial position ($p < .01$).

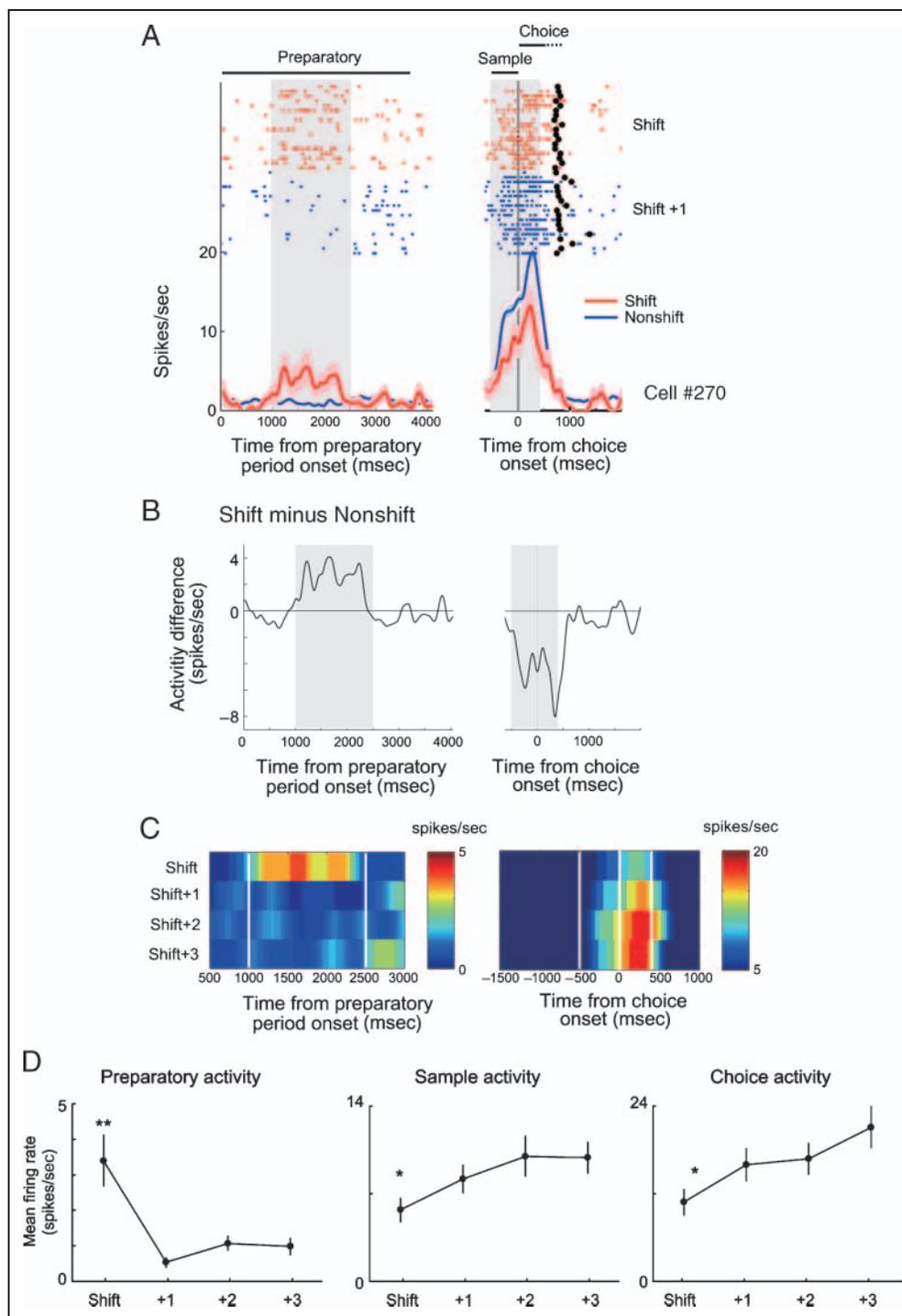
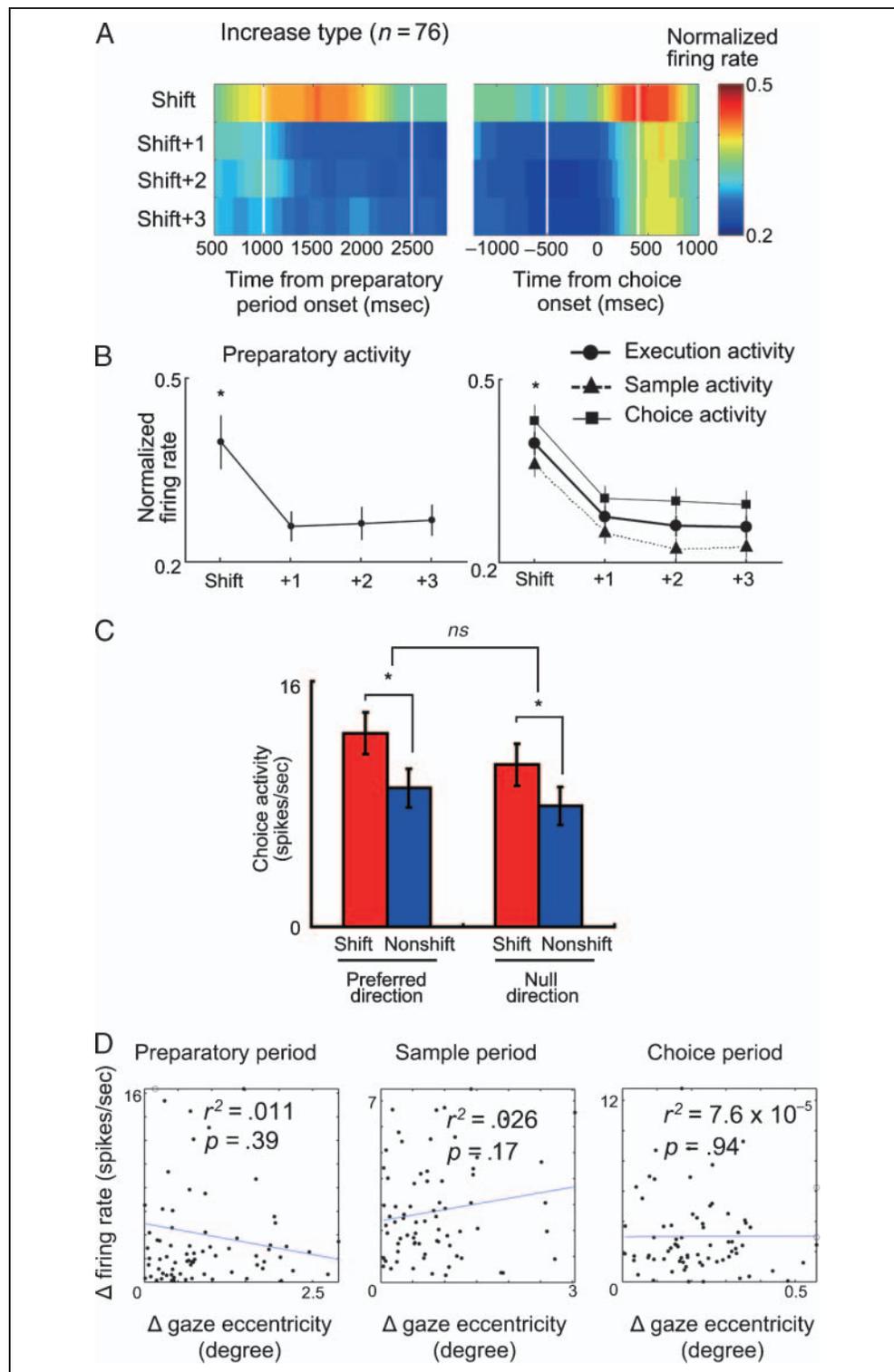


Figure 5. Population activity of increase-type neurons ($n = 76$). (A) Color-coded time courses of the normalized activity averaged across all the neurons. Data are aligned to the onset of the preparatory period (left) and the choice period (right). From top to bottom, the time courses were sorted according to the trial positions from the shift trial. The two vertical white lines for each panel indicate the analysis window during the preparatory period (left) and the execution period (right) (including the sample and choice periods). (B) The mean normalized preparatory activity (left) and execution activity (right) as a function of the trial positions from the shift trial. The execution activity was also shown in the same right panel. Error bars indicate SEMs. $*p < .001$, Fisher's least-significant difference test, following one-way ANOVA with a significant main effect of trial position ($p < .01$). (C) The choice activity sorted by direction (preferred or null directions) and trial type (shift or nonshift trials) (see Methods). Red and blue bars denote the shift and nonshift trials, respectively. Error bars indicate SEMs. $*p < .001$. The activity differences between the shift and nonshift trials were not significantly different for preferred and null directions ($p = .072$). (D) Scatterplot of the absolute gaze-eccentricity difference between dimensions (x -axis) versus the absolute activity difference between shift and nonshift trials (y -axis) during each trial period (from left to right, preparatory, sample, and choice periods, respectively). Unfilled circles represent outliers with values over the range in the x - or y -axis.



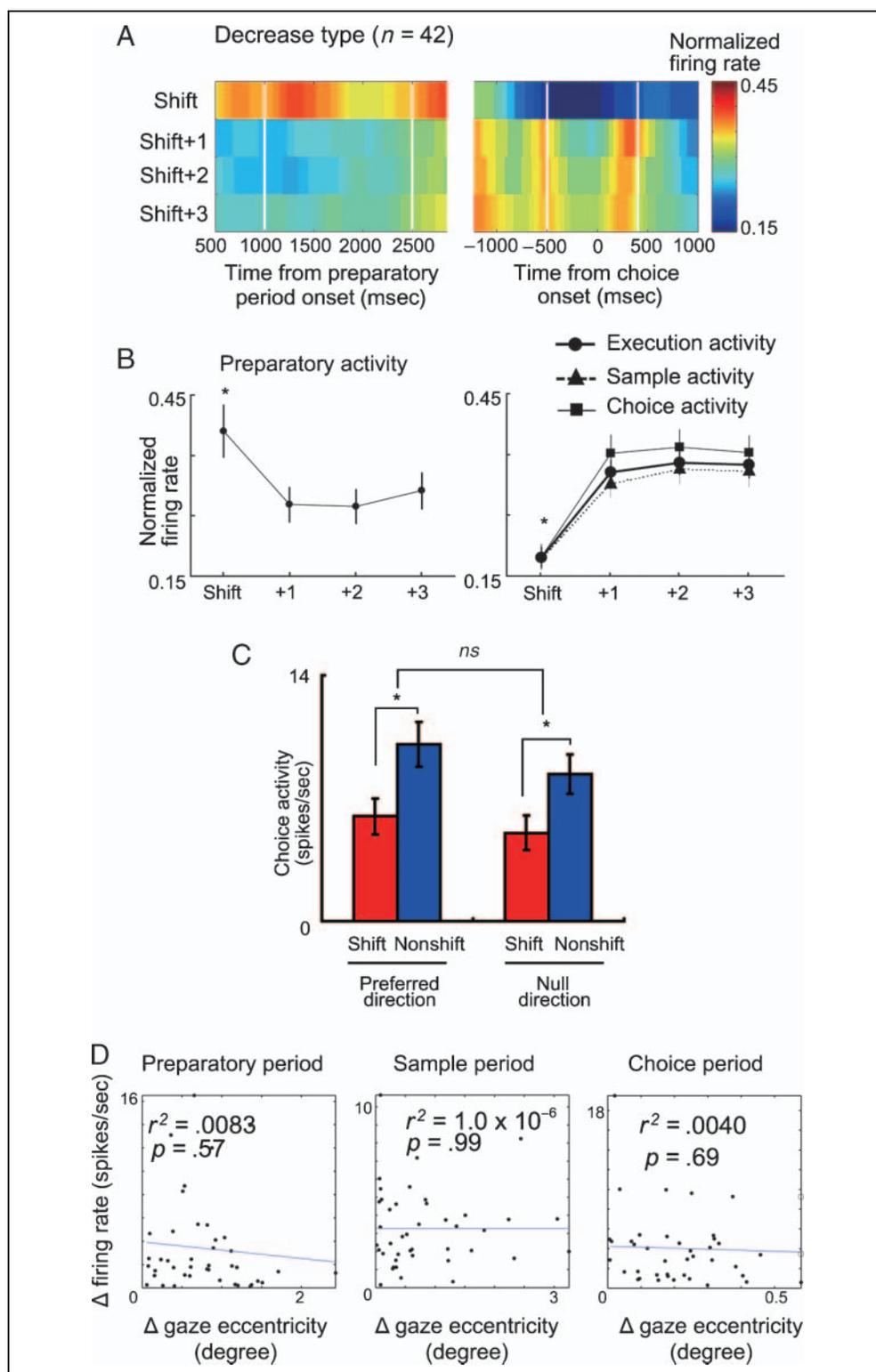
in these two consecutive trial periods. For each neuron, we then tested whether the preparatory and execution activities showed a trial-by-trial correlation in the shift trials (Pearson's correlation, $p < .05$), however, only a small minority of increase-type neurons (8 of 76; 11%) exhibited a significant correlation. Thus, it is unlikely that similarities in the activity of the two consecutive periods arose

from a simple time-dependent correlation. Out of the 76 increase-type neurons, 41 (54%) exhibited the preparatory activity with a significant main effect of trial type ($p < .05$, two-way ANOVA with Dimension \times Trial type). This indicated that these 41 neurons had a significant shift-related activity modulation both during the execution and preparatory periods, whereas the remaining 35 had a significant

shift-related activity modulation only during the execution period. There was no clear spatial segregation between these 41 neurons and the remaining 35. We next examined whether, in the shift trials, cell-by-cell fluctuation of the execution activity was coupled with that of the preparatory activity. Across these 41 neurons, the execution activity was

positively, but not significantly, correlated with the preparatory activity (Pearson's correlation, $r = .10$, $p = .52$). Previously, we found a group of PPC neurons that discriminated between the shape-to-color and color-to-shape shifts during the preparatory period (UDSR neurons; Kamigaki et al., 2009). To address whether the present increase-type

Figure 6. Population activity of decrease-type neurons ($n = 42$). (A–D) The same format was used as in Figure 5. (A) Color-coded time courses of the normalized activity averaged across all the neurons. (B) The mean normalized preparatory activity (left) and execution activity (right) as a function of the trial positions from the shift trial. Error bars indicate SEMs. $*p < .001$, Fisher's least-significant difference test, following one-way ANOVA with a significant main effect of trial position ($p < .01$). (C) The choice activity sorted by direction (preferred or null directions) and trial type (shift or nonshift trials). $*p < .001$. The activity differences between the shift and nonshift trials were not significantly different for preferred and null directions ($p = .12$). (D) Scatterplot of the absolute activity difference between shift and nonshift trials versus the absolute gaze-eccentricity difference between dimensions during each trial period (from left to right, preparatory, sample, and choice periods, respectively). Unfilled circles represent outliers with values over the range in the x - or y -axis.



neurons had the same characteristics, we tested ($p < .05$, t test) the activity difference between the two shift directions for each neuron. Among the 76 increase-type neurons, only 10 (13%) and 3 (4%) neurons showed the execution and the preparatory activity with a significant difference between the shift directions, respectively, which

implies that discharge patterns of increase-type neurons were different from those of UDSR neurons. Because parietal neurons are known to be affected by the direction of action selection (Stoet & Snyder, 2004, 2007; Goldberg, Bisley, Powell, & Gottlieb, 2006; Colby & Goldberg, 1999), we tested the effect of the behavioral-response direction on the shift-related activity modulation (see Methods). Even when we focused on the choice activity that can be most sensitive to the behavioral-response direction, the activity of increase-type neurons was still significantly different between the shift and nonshift trials, irrespective of whether we focused on the trials with a preferred direction or those with a null direction ($p < .001$ for both directions; Figure 5C). Moreover, when the choice activity was analyzed using a two-way ANOVA with the factors trial type (shift or nonshift trial) and direction (preferred or null direction), there was no significant interaction between these two factors ($p > .07$; Figure 5C). This indicates that directional preference had little effects on the shift-related activity modulation of increase-type neurons.

Figure 6A shows the average time course of the activity for all the 42 decrease-type neurons. As expected from the definition of the decrease-type neurons, the execution activity was significantly smaller in the shift trials than in the following nonshift trials ($p < .001$, post hoc Fisher's least-significant difference test; Figure 6B). We obtained the same results by separately analyzing the sample and choice activity (Figure 6B, right). In contrast, during the preparatory period, the population activity was significantly larger in the shift trials than in the following nonshift trials ($p < .001$, post hoc Fisher's least-significant difference test; Figure 6B). Out of the 42 decrease-type neurons, 22 (52%) exhibited the preparatory activity with a significant main effect of trial type ($p < .05$, two-way ANOVA). Thus, these 22 neurons had a significant shift-related activity modulation both during the execution and preparatory periods, whereas the remaining 20 had a significant shift-related activity modulation only during the execution period. These 22 neurons and the remaining 20 were spatially intermingled in the recording sites. Across these 22 neurons, the execution and preparatory activities in the shift

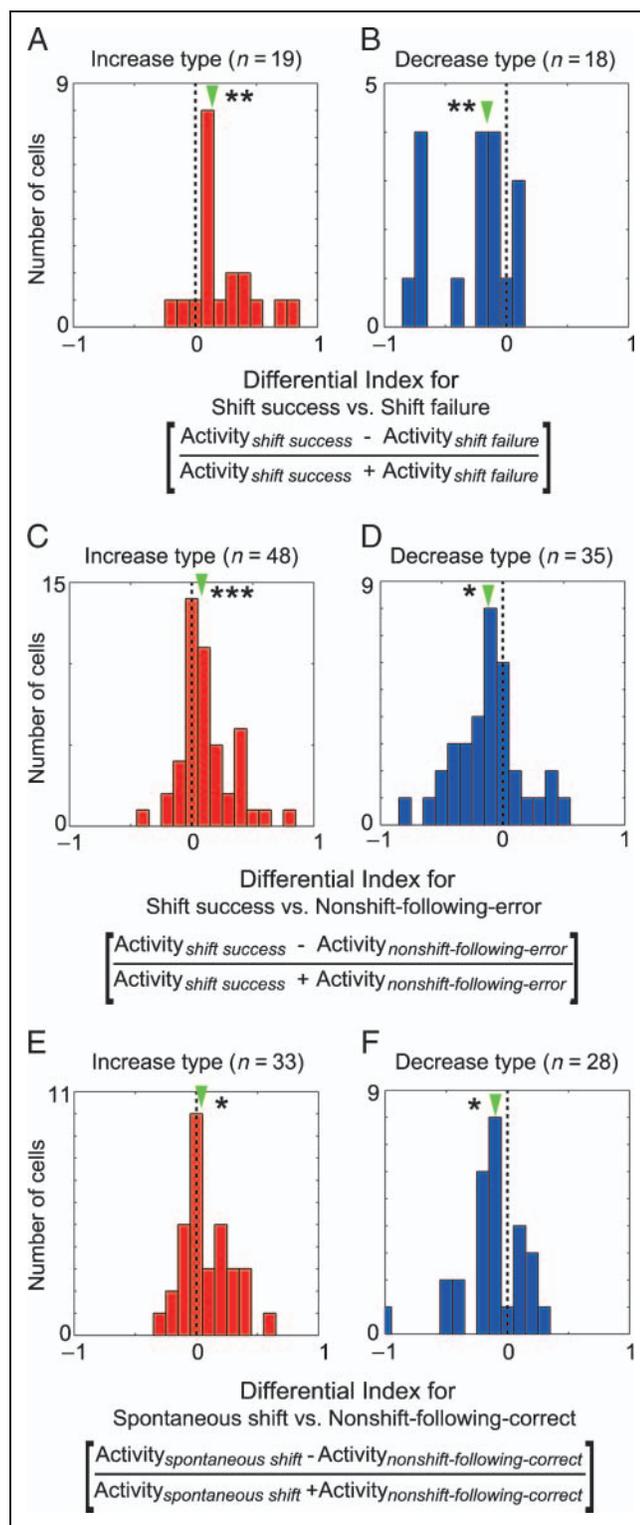


Figure 7. SR activity and its relationships to behavioral outcomes. (A, B) The distribution of the difference indices for the execution activity in the shift-success versus shift-failure trials. For A and B, 19 increase-type and 18 decrease-type neurons with two or more shift-failure trials were analyzed, respectively. The green arrowhead for each panel indicates the median of the difference indices. (C, D) The distribution of the difference indices for the execution activity in the shift-success versus nonshift-following-error trials. For C and D, 48 increase-type and 35 decrease-type neurons with three or more nonshift-following-error trials were analyzed, respectively. (E, F) The distribution of the difference indices for the execution activity in the spontaneous shift versus nonshift-following-correct trials. For E and F, 33 increase-type and 28 decrease-type neurons with two or more spontaneous shift trials were analyzed, respectively. * $p < .04$, ** $p < .003$, *** $p < .0004$, Wilcoxon signed rank test.

trials tended to, but not significantly, show a negative correlation (Pearson's correlation, $r = -.38$, $p = .083$). For each neuron, we further examined whether the activity was different ($p < .05$, t test) between the two shift directions. Among the 42 decrease-type neurons, no (0%) and only four neurons (10%) exhibited the execution and the preparatory activity with a significant difference between the shift directions, respectively. This suggests that the firing patterns of decrease-type neurons were distinct from those of UDSR neurons. Next, we tested the effect of behavioral-response direction in decrease-type neurons, as already tested in increase-type neurons. Their choice activity was significantly different between the shift and nonshift trials, for both preferred and null directions ($p < .001$; Figure 6C). Furthermore, the choice activity had no significant interaction between trial type and direction factors ($p > .1$, a two-way ANOVA; Figure 6C), which confirmed that directional preference had little effects on the shift-related activity modulation of decrease-type neurons.

In each monkey, we performed the same population analyses and confirmed that statistically similar results were obtained from the two monkeys: For increase-type neurons, the execution activity was significantly larger in the shift trials than in the following nonshift trials ($p < .001$ and $p < .01$ for Monkey W and Monkey G, respectively), and the preparatory activity was also significantly larger in the shift trials ($p < .001$ and $p < .01$ for Monkey W and Monkey G, respectively). For decrease-type neurons, the execution activity was significantly smaller in the shift trials ($p < .001$ and $p < .01$ for Monkey W and Monkey G, respectively), whereas the preparatory activity was significantly larger in the shift trials ($p < .05$ for both monkeys).

Because a shift trial followed an absence of reward in our task, we examined the possibility that the absence of reward might have caused a possible context modulation on neuronal activity. We conducted three series of analyses, that is, analyses of "shift-failure" trials, "nonshift-following-error" trials, and "spontaneous shift" trials (for details, see the next section), and all these demonstrated, in both types of SR neurons, that both the execution and preparatory activity modulations were not due to a possible context modulation caused by the reward contingencies.

PPC has been reported to show neuronal activity related to gaze position and saccade (Goldberg et al., 2006; Andersen & Buneo, 2002; Colby & Goldberg, 1999; Bremmer, Distler, & Hoffmann, 1997). Therefore, we evaluated the effect of ocular parameters on neuronal activity in two ways (see Methods). First, we tested whether the activity of SR neurons was correlated with various ocular parameters, including gaze eccentricity and saccadic parameters (saccadic frequency, amplitude, and direction). However, there were no significant correlations for either type of SR neurons (Pearson's correlations; $r^2 < .03$, $p > .1$, for all parameters in increase-type neurons; $r^2 < .08$, $p > .07$, for all parameters in decrease-type neurons). Second, we examined the possibility whether the monkeys directed their gaze to different

positions depending on the color and shape dimensions, and such gaze differences between dimensions produced the shift-related activity modulation. During the preparatory period, there was no significant difference in gaze eccentricity between dimensions (mean difference = 0.01° ; $p = .94$; t test). During the sample and choice periods, gaze eccentricity was slightly, but significantly, larger in the shape dimension than in the color dimension (the mean difference was 0.5° and 0.4° during sample and choice periods, respectively; $p < .001$). This result implies the possibility that the monkeys might have shifted their gaze or spatial attention between the color and shape dimensions. Then we tested whether these small amounts of gaze-eccentricity difference could have systematically caused the shift-related activity modulation. However, we found no significant correlations between gaze-eccentricity difference and the shift-related activity modulation during any periods in both types of SR neurons [Pearson's correlations; $r^2 < .03$, $p > .1$, for all periods in increase-type neurons (Figure 5D); $r^2 < .01$, $p > .5$, for all periods in decrease-type neurons (Figure 6D)]. Moreover, if we assume that such small gaze-eccentricity differences caused the shift-related activity modulation, the mean sensitivity for gaze eccentricity of SR neurons would be extremely large (for increase-type neurons, 31, 9.3 and 30 spikes/sec per degrees of gaze eccentricity, during the preparatory, sample, and choice periods, respectively; for decrease-type neurons, 7.9, 18 and 85 spikes/sec per degrees of gaze eccentricity, during the three periods, respectively). However, such high sensitivities are unlikely for the PPC neurons according to literature (Calton et al., 2002; Boussaoud & Bremmer, 1999; Andersen et al., 1985). Thus, although we cannot exclude the alternative possibility that the monkeys shifted their gaze or spatial attention between dimensions, it is at least implausible that the shift-related activity modulation exclusively arose from the gaze parameters (see Discussion).

Two Types of Shift-related Neurons and Their Characterizations

We classified SR neurons into increase/decrease-type neurons by whether their execution activity was larger/smaller in the shift trials than in the nonshift trials. We next inspected whether these two types further differed in other aspects of discharge patterns.

From the time courses plotted in Figures 5A and 6A, we speculated that there might be a temporal difference between increase- and decrease-type neurons during the execution period. Then we analyzed the time course of their activities in two ways (see Methods). First, we calculated the peak time as the time when a neuron showed the maximum absolute difference in activity between the shift and nonshift trials. The peak time of decrease-type neurons, 106 ± 89 msec (mean \pm SE from the sample onset), showed a tendency to precede the peak time of increase-type neurons (204 ± 74 msec), but this difference was not significant ($p = .41$, t test). Second, we analyzed

the response onset of activity in the shift trials. For this analysis, we calculated the mean firing rate (baseline activity) and its standard deviation (*SD*) in the period of 500–2500 msec from the preparatory period onset. The response onset was defined as the time when the activity in the shift trials exceeded or fell below 2 *SD* from its baseline activity. The response onset of increase-type neurons was 441 ± 65 msec (mean \pm *SE*, $n = 67$) from the sample onset, whereas the response onset of decrease-type neurons was 59 ± 25 msec (mean \pm *SE*, $n = 24$). We excluded neurons in which the response onset was not detected from this analysis. The response onset of decrease-type neurons significantly preceded the response onset of increase-type neurons ($p < .001$, *t* test).

As shown in Figure 2, a large proportion of SR neurons (43%; $n = 51/118$) had a significant main effect of dimension (color/shape) or an interaction on the two-way ANOVA during the execution period, which indicated that these cells were dimension-sensitive. We then examined whether the relevant dimension might differentially affect the neuronal activity of increase- and decrease-type neurons. To test this possibility, we performed the following two analyses. First, we analyzed whether the preference for the dimension was congruent between the shift trials and other trials. Using all shift and nonshift trials, we determined the “preferred dimension” for each cell in which the cell showed larger execution activity compared with another dimension. The activity in the shift trial was then compared between preferred and nonpreferred dimensions. Some neurons exhibited “congruent” dimension sensitivity, as indicated by the greater level of activity in the shift trials of the preferred dimension than that of the nonpreferred dimension. The other neurons exhibited “incongruent” dimension sensitivity, showing the opposite preference on shift trials. Of the 118 SR neurons, 42, 9, and 67 (35.6%, 7.6%, and 56.8%) exhibited congruent, incongruent, and no dimension sensitivity, respectively. We then examined the distributions for the increase- and decrease-type neurons separately [for increase-type neurons, 32, 8, and 36 (42%, 11%, and 47%) exhibited congruent, incongruent, and no dimension sensitivity, respectively; for decrease-type, 10, 1, and 31 (24%, 2%, and 74%) exhibited congruent, incongruent, and no dimension sensitivity, respectively]. The distributions were significantly different between increase- and decrease-type neurons ($p < .02$, χ^2 test). The proportions of dimension-sensitive cells, either congruent or incongruent, were larger for increase-type than decrease-type neurons. Second, we examined the distributions of cells exhibiting dimension sensitivity for the execution and preparatory periods separately. We found that the distributions were significantly different between increase- and decrease-type neurons ($p < .02$, χ^2 test; 41% of increase-type neurons showed dimension sensitivity only during the execution period, 12% did both during the execution and preparatory periods, 5% did only during the preparatory period, and 42% did during neither periods;

for decrease-type neurons, the corresponding distribution was 21%, 5%, 19%, and 55%, respectively). The total proportions of dimension-sensitive cells during the execution and preparatory periods were higher in increase-type than in decrease-type neurons. These two analyses imply that the subset of SR neurons had an additional function to maintain the relevant dimension, and that increase-type neurons are more extensively dedicated to this function than decrease-type neurons.

Next, we tested the possibility whether increase- and decrease-type neurons consisted of different proportions of cell types. According to the interspike intervals (ISI) during the task, we classified each neuron into a putative regular spiking neuron (pRS) or a putative burst spiking neuron (pBS) (see Methods and Katai et al., 2010). We found that 67% and 65% of increase- and decrease-type neurons, respectively, were pRS neurons. Moreover, the median, mean, and standard deviation of the ISI were not significantly different between increase- and decrease-type neurons ($p > .07$, *t* test). This result suggests that increase- and decrease-type neurons did not consist of different proportions of pRS and pBS cells.

In summary, the two types of SR neurons showed a temporal difference in the response onset and exhibited different sensitivities to the relevant dimension. Despite these differences in firing properties, they did not consist of different proportions of cell types.

PPC Activity and Its Relationships to Behavioral Outcomes

We next addressed whether failure of set shifting affected the shift-related activity modulation during the execution period. After a dimensional change, but before successful set shifting, the monkeys occasionally made some errors other than an inevitable error, suggesting a failure to shift their cognitive set in those error trials. We therefore examined the execution activity during these error trials that intervened between the inevitable-error trial and the first correct trial after the dimensional change. We will call these intervening error trials “shift-failure” trials and call the first correct trial after a dimensional change a “shift-success” trial instead of a “shift” trial. We had a prediction that the shift-related modulation of the execution activity would be weaker in the shift-failure trials than in the shift-success trials, so that the increase-type and decrease-type neurons would show larger and smaller activity in the shift-success trials than in the shift-failure trials, respectively. The results actually confirmed our prediction. Of the 76 increase-type neurons, 19 had two or more shift-failure trials. For each of these 19 neurons, we calculated a difference index that denotes the activity difference between the shift-success and shift-failure trials (see Methods). The distribution of the difference indices of the 19 neurons was significantly shifted to a positive value ($p < .003$, Wilcoxon signed rank test; Figure 7A). Of the 42 decrease-type neurons, 18 had two or more

shift-failure trials. The distribution of the difference indices was significantly shifted toward a negative value ($p < .002$, Wilcoxon signed rank test; Figure 7B). We also examined the preparatory activity in both types of SR neurons that had a significant shift-related preparatory activity. In contrast to the execution activity, we predicted that, for both types of SR neurons, the preparatory activity would be larger in the shift-success trials than in the shift-failure trials. The results actually confirmed our prediction. Among the 41 increase-type neurons with a significant shift-related preparatory activity (see the previous section), we analyzed 10 that had two or more shift-failure trials. The distribution of the difference indices was significantly shifted toward a positive value ($p < .04$, Wilcoxon signed rank test). Out of the 22 decrease-type neurons with a significant shift-related preparatory activity, 12 had two or more shift-failure trials. The distribution of the difference indices of these 12 decrease-type neurons was also significantly shifted toward a positive value ($p < .03$, Wilcoxon signed rank test). These results in the two types of SR neurons indicate that both the execution and preparatory activities significantly depended on the success or failure of set shifting. They also had an important implication in the reward-related issues. Because both shift-success and shift-failure trials followed a lack of reward but they differed only in the success/failure of set shifting, we can conclude that successful set shifting, but not a possible context modulation produced by the lack of reward, was the critical factor influencing the shift-related activity modulation.

We next examined the execution activity in the nonshift trial that follows an erroneous response. We named these trials the “nonshift-following-error” trials. The nonshift-following-error trials were the same as the shift-success trials in that both trials followed a lack of reward, but were critically different in the following point. Before the shift-success trial, the monkeys made their responses based on one dimension for at least six consecutive trials by reliably maintaining their cognitive set, but this was not the case before the nonshift-following-error trial. Thus, we conjectured that set-shifting processes were more strongly required in the shift-success trials than in the nonshift-following-error trials. Figure 7C and D exactly confirmed our conjecture. We could analyze 48 increase-type neurons with three or more nonshift-following-error trials. The distribution of the difference indices for the shift-success versus nonshift-following-error trials was significantly shifted toward a positive value ($p < .0004$; Wilcoxon signed rank test; Figure 7C). We then analyzed 35 decrease-type neurons with three or more nonshift-following-error trials. The distribution of the difference indices was significantly shifted toward a negative value ($p < .02$; Wilcoxon signed rank test; Figure 7D). The preparatory activity was also analyzed for both types of SR neurons that had a significant shift-related preparatory activity. Among the 41 increase-type neurons with a significant shift-related preparatory activity, 17 had three or more nonshift-following-error trials. The distribution of the difference indices was signifi-

cantly shifted toward a positive value ($p < .04$, Wilcoxon signed rank test). Out of the 22 decrease-type neurons with a significant shift-related preparatory activity, 14 had three or more nonshift-following-error trials. The distribution of the difference indices was significantly shifted toward a positive value ($p < .03$, Wilcoxon signed rank test). Because both the shift-success and nonshift-following-error trials came after a lack of reward, but differed in their demand of set shifting, the results above indicate that the activity difference between these trials was ascribable to set shifting per se, but not a possible context modulation produced by the lack of reward.

Finally, we examined whether the shift-related modulation of the execution activity could be produced without any explicit signals for a dimensional change. We focused on the trial in which the monkeys spontaneously shifted their cognitive sets. We sometimes observed a case in which the monkeys maintained a correct response based on one dimension in four or more consecutive trials, but suddenly changed their response into that based on the other dimension even though they received reward in the previous trial and got no explicit events signaling a dimensional change. We defined these trials “spontaneous shift” trials. Then, we compared these trials with the nonshift trials that followed a correct response, which we named “nonshift-following-correct” trials. We could analyze 33 increase-type neurons with two or more spontaneous shift trials. The distribution of the difference indices for the spontaneous shift versus nonshift-following-correct trials was significantly shifted toward a positive value ($p < .04$; Wilcoxon signed rank test; Figure 7E). We next analyzed 28 decrease-type neurons with two or more spontaneous shift trials, and found that the distribution of the difference indices was significantly shifted toward a negative value ($p < .03$; Wilcoxon signed rank test; Figure 7F). We next examined the preparatory activity in both types of SR neurons that had a significant shift-related preparatory activity. Among the 41 increase-type neurons with a significant shift-related preparatory activity, 12 had two or more spontaneous shift trials. The distribution of the difference indices was significantly shifted toward a positive value ($p < .02$; Wilcoxon signed rank test). Out of the 22 decrease-type neurons with a significant shift-related preparatory activity, 16 had two or more spontaneous shift trials. The distribution of the difference indices was significantly shifted toward a positive value ($p < .005$, Wilcoxon signed rank test). Thus, when the monkeys shifted their cognitive set spontaneously without any explicit signals, SR neurons also exhibited shift-related activity modulation both during the execution and preparatory periods, as they did in the shift-success trials. Moreover, because both the spontaneous shift and nonshift-following-correct trials equally followed a reward delivery but differed in the occurrence of set shifting, the results suggest that the activity difference between these trials was ascribable to set shifting per se, and not to a possible context modulation caused by reward.

However, these differences between the spontaneous shift and nonshift-following-correct trials might merely reflect a difference between error and correct trials. Then we focused on the other error trials, “simple error” trials, in contrast to spontaneous shift trials. We reasoned that the internal drive to make a dimension shift should be relatively weaker in the simple error trials than in the spontaneous shift trials, because the monkeys did not reliably maintain correct responses in the simple error trials. The difference indices for the spontaneous shift versus the simple error trials during the execution period were significantly shifted toward positive in increase-type neurons ($p = .026$; Wilcoxon signed rank test; $n = 23$) and negative in decrease-type neurons ($p = .040$; $n = 18$). In this analysis, we included those neurons with two or more spontaneous shift and simple error trials. The difference indices during the preparatory period were also significantly shifted toward positive both in increase-type ($p = .043$; $n = 8$) and decrease-type neurons ($p = .043$; $n = 9$), when we analyzed those neurons with a significant shift-related preparatory activity. The results suggest that the activity in the spontaneous shift trials indeed represented the internal drive to make a dimension shift rather than a mere erroneous response.

In summary, these series of analyses demonstrated that the shift-related modulations of both the execution and preparatory activities could be critically attributable to successful set shifting per se, but not to a possible context modulation caused by the reward contingencies. Moreover, the analysis using spontaneous shift trials demonstrated that the shift-related modulations of both the execution and preparatory activities could be produced just internally without any explicit signals for a dimensional change.

Cognitive Processes in the Execution Period

We have shown that the shift-related activity modulation was consistently observed both during the sample and choice parts in the execution period. However, the sample and choice periods included different task events and different cognitive processes, which might differentially affect the activity during these two periods. Then we analyzed the effect of various potential cognitive processes on neuronal activity for each sample and choice part of the execution period.

First, to examine the effect of behavioral outcome, we analyzed the activity contrast between shift-success versus shift-failure trials, as shown in Figure 7A, but for each sample and choice periods separately. Then we calculated the difference index for shift-success versus shift-failure trials in each neuron. The difference indices were significantly shifted to positive in each period for increase-type neurons ($p = .00016$ and $.040$ in the sample and choice periods, respectively; Wilcoxon signed rank test; $n = 19$), and for decrease-type neurons ($p = .018$ and $.038$ in the sample and choice periods, respectively; $n = 18$). We then tested

whether the difference index varied between the sample and choice periods, and found that the difference index was significantly larger in the sample period than in the choice period for increase-type neurons ($p = .040$; t test), but not for decrease-type neurons ($p = .69$). This suggests that increase-type neurons amplified the signal of successful shift in the sample period compared with the choice period, whereas decrease-type neurons maintained the corresponding signal for the entire duration of the execution period.

Second, to test whether the relevant dimension affected the activity of SR neurons during the sample and choice periods, we performed a two-way ANOVA with dimension and trial type as factors for each SR neuron ($p < .05$). If one neuron had a significant main effect of dimension or interaction, the neuron was regarded as being affected by dimension. Out of the 76 increase-type neurons, 59% ($n = 45$) and 51% ($n = 39$) were affected by dimension in the sample and choice periods, respectively. Among the 42 decrease-type neurons, 40% ($n = 17$) and 40% ($n = 17$) were affected by dimension in the sample and choice periods, respectively. The results imply that the effect of dimension did not differ between the sample and choice periods. We also emphasize that approximately half of the SR neurons were affected by dimension. This suggests that the subsets of SR neurons were also involved in the representation of the relevant dimension.

Third, we examined whether SR neurons showed selectivity for the presented sample stimulus during each period. To this end, we tested whether neuronal activity was modulated depending on the type of sample stimulus using a two-way ANOVA with color (red, blue, green) and shape (circle, triangle, cross) of the sample stimulus as factors. If the neuronal activity exhibited any significant main effects or interaction ($p < .05$), the neuron was regarded as exhibiting stimulus selectivity. For increase-type neurons, 9% ($n = 7/76$) and 16% ($n = 12/76$) showed stimulus selectivity during the sample and choice periods, respectively. For decrease-type neurons, 10% ($n = 4/42$) and 17% ($n = 7/42$) showed stimulus selectivity during the sample and choice periods, respectively. As would be expected from the properties of parietal neurons, only a small proportion of neurons showed stimulus selectivity. However, this proportion may have been underestimated because the stimulus was not necessarily presented in the receptive field of the recorded neurons, but in the foveal position for most of the cases.

Fourth, in a similar line of analysis, we also examined whether SR neurons exhibited selectivity for the target choice stimulus that the monkeys selected in the choice period. We did not analyze the activity in the sample period because the target stimulus did not yet appear until this period. We tested whether neuronal activity was modulated depending on the type of the target stimulus using a two-way ANOVA with color (red, blue, green) and shape (circle, triangle, cross) of the target stimulus as factors. If neuronal activity exhibited any significant main

effects or a significant interaction ($p < .05$), the neuron was regarded as showing target-stimulus selectivity. Of the increase-type neurons, 20% ($n = 15/76$) showed target stimulus selectivity during the choice period. Of the decrease-type neurons, 12% ($n = 5/42$) showed target stimulus selectivity. The proportion of neurons showing stimulus selectivity was again small. For the same reasons mentioned above, the proportions may have been underestimated in our analysis.

Finally, we analyzed the effect of action selection by testing whether the direction of behavioral responses modulated activity in the choice period. We did not analyze sample period activity, for the same reason described above. Using a two-way ANOVA with direction (preferred or null directions) and trial type (shift or nonshift trials) as factors, we examined whether SR neurons showed directional selectivity during the choice period (see Methods). We considered a neuron to show directional selectivity if it exhibited any significant “direction” effect, that is, a significant main effect of direction or a significant interaction between direction and trial type ($p < .05$). We found that approximately one third of SR neurons showed directional selectivity: 34% ($n = 26/76$) of increase-type neurons and 36% ($n = 15/42$) of decrease-type neurons were directionally selective. We note that the activity was still significantly different between the shift and nonshift trials, irrespective of whether we focused on the trials with a preferred response direction or those with a null response direction (Figure 5C for increase-type neurons and Figure 6C for decrease-type neurons). We suggest that although some SR neurons were affected by action selection, SR neurons were still significantly involved in behavioral shift itself.

These lines of evidence suggest that although various cognitive processes in each of the sample and choice periods could have modulated the activity of some SR neurons, SR neurons were consistently recruited for the shift-related cognitive process during both sample and choice parts of the execution period.

DISCUSSION

Although neuroimaging studies have suggested that several brain regions such as PPC were involved in set shifting (Chiu & Yantis, 2009; Ruge et al., 2009; Badre & Wagner, 2006; Gruber et al., 2006; Sakai & Passingham, 2003; Nakahara et al., 2002; Monchi et al., 2001; Rushworth, Paus, & Sipila, 2001; Kimberg et al., 2000; Sohn et al., 2000; Konishi et al., 1998), it remains obscure how the two possible component processes in set shifting, namely, preparatory and execution processes, were implemented within the identified regions. In the present study, we examined single-unit activity in PPC of the monkeys performing set shifting and found that, during the execution period, a group of neurons showed shift-related activity modulation, which we termed “SR” neurons. The SR neurons were further divided into two types, one type exhibiting larger activity and the other showing smaller activity in the shift trial than

in the nonshift trial. Even though both types of SR neurons were defined by the activity during execution period, the shift-related modulation was also observed during the preparatory period. The population activity of both types was significantly biased toward larger activity in the shift trial than in the nonshift trial. The majority of both neuron types exhibited shift-related activity modulation also during the preparatory period, whereas the remaining did only during the execution period. In our previous study, another group of PPC neurons was found to show shift-related activity selectively during the preparatory period (UDSR neurons; see Kamigaki et al., 2009, and the next paragraph). The SR neurons that were involved also in the preparatory processes and those specialized only in the execution process, as well as UDSR neurons, were all spatially intermingled within PPC. These observations suggest that each of the subprocesses of behavioral shifting, preparation and execution, dynamically recruited functional cell groups within PPC. Furthermore, in the spontaneous shift, the shift-related activity modulation also emerged during both the execution and preparatory periods. This finding of the spontaneous shift, however, may be ascribed to the activity difference between correct and erroneous responses, as reported in previous studies (Johnston, Levin, Koval, & Everling, 2007; Mansouri, Matsumoto, & Tanaka, 2006; Everling & DeSouza, 2005). These studies showed that neuronal activity in the prefrontal and anterior cingulate regions discriminated between correct and erroneous trials. These previous observations differ from the present finding in the following two ways. First, because the previous studies did not discriminate between spontaneous shift and simple error trials, their activity differences could reflect mere differences between error and correct trials. In contrast, the present SR neurons exhibited greater shift-related activity modulation in spontaneous shift trials than in the simple error trials. This suggests that the activity of SR neurons reflected an internal drive to make a dimension shift. Second, in the previous reports, neuronal activity was modulated depending on whether the monkeys obeyed one behavioral rule or another. Unlike SR neurons in the present study, the activity of such rule-dependent neurons does not directly relate to behavioral shifting itself.

We previously identified another shift-related PPC neurons, UDSR neurons, by focusing the activity during the preparatory period (Kamigaki et al., 2009). Among the present SR neurons that were defined by the activity during the execution period, only a small subset was included in the previous UDSR neurons (11% of increase-type neurons and 14% of decrease-type neurons). The discharge patterns of the two populations, SR and UDSR neurons, mainly differed in two points. First, the majority of SR neurons showed shift-related activity during both the preparatory and execution periods, whereas almost all the UDSR neurons showed shift-related activity only during the preparatory period (see Supplemental Figure 6C in Kamigaki et al., 2009). This suggests that UDSR neurons were mainly specialized in the preparatory process, whereas SR neurons

were involved in both processes. Second, only a few SR neurons showed execution and preparatory activities with a significant difference between the shape-to-color and color-to-shape shifts (4% and 13% of increase-type neurons and 10% and 0% of decrease-type neurons during the execution and preparatory periods, respectively; see Results), whereas a larger percentage of UDSR neurons (51%) discriminated the two shift directions during the preparatory period. We found no clear spatial segregation between SR and UDSR neurons in PPC. Because these two populations shared few neurons and exhibited distinct discharge patterns, they may contribute to neurally separable processes in behavioral shifting, although they functioned in close proximity to each other within PPC.

We next discuss three possibilities that the activity of SR neurons might be related to factors other than behavioral shifting *per se*. (1) Because a shift trial followed an absence of reward in our task, the absence of reward might have caused a possible context modulation on the neuronal activity (Schultz, 2007; Schultz & Dickinson, 2000). For this issue, we have provided three lines of evidence against this possibility. First, the modulations of the execution and preparatory activities were significantly stronger in the shift-success trials than in the shift-failure trials, even though both trials followed a lack of reward. Second, the activity modulations were significantly larger in the shift-success trials than in the nonshift-following-error trials, despite the fact that both trials followed an absence of reward. Third, the activity modulations were significantly larger in the spontaneous shift trials than in the nonshift-following-correct trials, even though both trials followed a reward delivery. These three comparisons with the same reward conditions demonstrated that the modulations of both the execution and preparatory activities could be due to behavioral shifting, but not to a possible context modulation caused by the reward contingencies. (2) Because the neuronal activity in PPC is also known to be related to ocular parameters (Goldberg et al., 2006; Andersen & Buneo, 2002; Colby & Goldberg, 1999; Bremmer et al., 1997), ocular behaviors might have affected the activity of SR neurons. Moreover, our analysis indicated that gaze eccentricity was significantly different between the shape and color dimensions during the sample and choice periods, therefore, we cannot rule out the alternative possibility that the monkeys might have used a strategy for solving our task to shift their gaze or spatial attention between the small color object and the large shape object. Even in this case, however, monkeys should have known which object to attend to, so that they promptly shifted their behavior in accordance with the task demands. These behaviors can still be regarded as a type of behavioral shift on demand, and therefore can serve as an experimental model of cognitive flexibility. Furthermore, according to our correlation and gaze-sensitivity analyses, it is unlikely that gaze behavior exclusively caused the shift-related activity modulation. Thus, we suggest that, although spatial attention and gaze behavior might have modulated the neu-

ronal activity, the activity of SR neurons still reflects the processing of behavioral shift *per se*, rather than mere gaze-related factors.

Because the execution period included different task events, namely, the sample and choice periods, we further scrutinized the activity of SR neurons for each of the two periods separately in relation to potential cognitive processes. First, to assess the effect of behavioral outcome, we analyzed the activity contrast between the shift-success versus shift-failure trials for each period. The activity contrast was significant in each period both for increase- and decrease-type neurons. We further found that the activity contrast was significantly larger in the sample period than in the choice period for increase-type neurons, but not for decrease-type neurons. This suggests that increase-type neurons amplified the signal of successful shifts in the sample period compared with the choice period, whereas decrease-type neurons maintained the corresponding signal for the entire duration of the execution period. Second, we showed that approximately half of SR neurons were affected by the relevant dimension during both the sample and choice periods. The relevant dimension in our task corresponds to behavioral rule/strategy, which is more generally called behavioral set (Johnston et al., 2007; Johnston & Everling, 2006; Mansouri et al., 2006; Stoet & Snyder, 2003, 2004). Thus, the result implies that the subsets of SR neurons were also involved in maintaining behavioral set. Third, we examined the stimulus selectivity for the sample stimulus and also for the target stimulus. We found that relatively small percentages of SR neurons (20% or less) showed stimulus selectivity. These percentages were lower than those reported in the previous reports (Janssen, Srivastava, Ombelet, & Orban, 2008; Sereno & Maunsell, 1998), and we may have underestimated these proportions because the stimulus was not necessarily presented in the receptive field of the recorded neurons, but in the foveal position for most of the cases. Finally, to test the effect of action selection during the choice period, we analyzed whether the direction of behavioral responses modulated the neuronal activity. Consistent with the previous reports in PPC (Stoet & Snyder, 2004, 2007; Goldberg et al., 2006; Colby & Goldberg, 1999), we found that approximately one third of SR neurons exhibited sensitivity to the behavioral-response direction. However, the activity of SR neurons was still significantly different between the shift and nonshift trials, irrespective of either a preferred or null direction. In summary, we suggest that although the activity of some SR neurons might reflect dynamic interplays of various cognitive processes and a subset of them might be involved in these processes to some extent, SR neurons were consistently dedicated to the shift-related cognitive process.

In the present study, we found two types of SR neurons, increase- and decrease-type neurons. During the preparatory period, both types showed positive shift-related activity modulation, whereas during the execution period, they exhibited distinct shift-related activity

modulation: Increase-type neurons showed positive modulation and decrease-type neurons showed negative modulation. The firing patterns of the two types cannot be explained by a simple feedforward model in which decrease-type neurons monosynaptically inhibit increase-type neurons. Although the mean response onset of decrease-type (59 msec from the sample onset) preceded that of increase-type (441 msec) neurons, the temporal difference was large (382 msec) (see Results). Thus, it is unlikely that decrease-type neurons send inhibitory monosynaptic projections to increase-type neurons. Moreover, this model predicts that decrease-type neurons consist of larger proportions of inhibitory/burst spiking (BS) neurons compared with increase-type neurons, but the ISI analysis indicates that this is not the case. Therefore, we suggest that increase- and decrease-type neurons may form more complicated circuitries for behavioral shift processing.

According to many fMRI studies, PPC may function cooperatively with other brain regions, including prefrontal cortex (PFC), in each component process of set shifting (Chiu & Yantis, 2009; Ruge et al., 2009; Badre & Wagner, 2006; Sakai & Passingham, 2003; Yantis & Serences, 2003; Corbetta & Shulman, 2002; Nakahara et al., 2002; Monchi et al., 2001; Rushworth et al., 2001; Sohn et al., 2000). At present, because single-unit recording studies in PFC have exclusively focused on the preparatory process (Quilodran, Rothé, & Procyk, 2008; Isoda & Hikosaka, 2007; Johnston et al., 2007; Johnston & Everling, 2006; Mansouri et al., 2006; Genovesio, Brasted, Mitz, & Wise, 2005; Wallis, Anderson, & Miller, 2001; Whiete & Wise, 1999; Shima & Tanji, 1998), they have not yet clarified the neuronal mechanisms that link execution and preparatory processes in the lateral and medial PFC regions. Further researches will be needed to examine how the two component processes of set shifting were implemented at the single-cell level in several brain regions outside PPC.

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