Neurodynamics of Cognitive Set Shifting in Monkey Frontal Cortex and Its Causal Impact on Behavioral Flexibility

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Abstract

■ Flexible behavior depends on the ability to shift an internal cognitive set as soon as external demand changes. According to neuropsychological studies in human and nonhuman primates, selective lesion to the PFC impairs flexible behavioral shifting. Our previous fMRI study demonstrated that the prefrontal regions showed transient activation related to set shifting in humans and monkeys. To investigate the underlying neural processing, we recorded single-unit activities while monkeys performed a cognitive-set-shifting task, which required shifting between shape-matching and color-matching behaviors. We identified a group of neurons in the inferior arcuate region that exhibited selective activity when the monkeys were required to shift their cognitive set. These shift-related neurons were localized in the focal area along the posterior bank of the inferior arcuate sulcus. Reversible inactivation of this area ipsilateral to the response hand with a small volume of muscimol (even with 0.5 μl) selectively impaired the performance of behavioral shifting. Moreover, this selective behavioral impairment strongly correlated with the dose of muscimol. These results demonstrated localized neural processing for cognitive set shifting and its causal role for behavioral flexibility in primates. ■

INTRODUCTION

Cognitive flexibility requires rapid shifting of the internal cognitive set upon a change in external demands. Individuals with selective damage to the PFC often show difficulty in flexible set shifting and persist with a response that is no longer appropriate, a phenomenon known as "perseveration" (Buckley et al., 2009; Dias, Robbins, & Roberts, 1996; Passingham, 1972; Milner, 1963). Our previous fMRI study identified the arcuate sulcus region in the PFC as being activated during set shifting in humans and monkeys (Nakahara, Hayashi, Konishi, & Miyashita, 2002). Other human fMRI studies further demonstrated that the PFC is critically involved in cognitive flexibility through dynamic interactions with other cortical regions in the brain-wide networks (Leber, Turk-Browne, & Chun, 2008; Badre & Wagner, 2006; Dosenbach et al., 2006; Konishi, Chikazoe, Jimura, Asari, & Miyashita, 2005; Rushworth, Walton, Kennerley, & Bannerman, 2004; Braver, Reynolds, & Donaldson, 2003; Sakai & Passingham, 2003; Yantis & Serences, 2003; Monchi, Petrides, Petre, Worsley, & Dagher, 2001; Kimberg, Aguirre, & D'Esposito, 2000; Sohn, Ursu, Anderson, Stenger, & Carter, 2000). However, because of the limited spatial and time resolution of fMRI data, the underlying neural mechanisms at the single-cell level remain unclear. Although electrophysiological studies performed in monkeys indicate that neural activities in several brain regions, including prefrontal and parietal cortices, can represent a maintained cognitive set or "rule" (Stoet & Snyder, 2009; Johnston, Levin, Koval, & Everling, 2007; Mansouri, Matsumoto, & Tanaka, 2006; Genovesio, Brasted, Mitz, & Wise, 2005; Wallis, Anderson, & Miller, 2001), there is little direct information at the single-cell resolution about how shifting of cognitive set is implemented in the cortical regions. Recently, we successfully trained monkeys to promptly perform set shifting, mostly within a single trial. This enabled us to examine the dynamics of shift-related neural processing, and we identified a group of cells in the posterior parietal cortex that showed increased activity only when the monkeys were required to shift their cognitive set (Kamigaki, Fukushima, & Miyashita, 2009, 2011).

On the basis of our fMRI study described above (Nakahara et al., 2002), this study investigated neural processing in the frontal cortex while the monkeys performed set shifting. Furthermore, we tested the causal relationship between the neural processing and flexible behavior. Similar to the group of posterior parietal cells identified in our previous study (Kamigaki et al., 2009), we found a group of frontal cells showing selective activity only when the monkeys prepared to shift their behavior. However, unlike

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the posterior parietal cells, these frontal cells were also activated when the next requirement of set shifting approached. Moreover, these shift-related cells were localized in the focal area along the inferior arcuate regions of the frontal cortex. Reversible inactivation of this focal area using muscimol selectively impaired the performance of set shifting. These results provide the first evidence for the localized dynamic neural processing of set shifting and its causal impact on the flexible behavior in the primate brain.

METHODS

General Procedures

Three male monkeys (*Macaca fuscata*; 8.5–9.4 kg) served as subjects in this study. The care and use of these animals conformed to the NIH Guide for the Care and Use of Laboratory Animals and to the regulations of The University of Tokyo School of Medicine. Head-holding devices and recording chambers (Crist Instruments, Hagerstown, MD) were attached to the monkeys' skulls under aseptic conditions, following general anesthesia with sodium pentobarbital (2.5 mg/kg body weight/hr, IV). For the behavioral task, the stimuli were presented using a 17-in. LCD monitor. A response device with one center button and three response buttons (top, right, and left of the center button) was attached to the primate chair in front of the monkeys' hands, and the monkeys responded by pushing one of the three response buttons. Eye movement was monitored using a PC-based CCD camera system (Kamigaki et al., 2009). We also monitored EMG activity from oral and hand muscles (see Recording Procedures for details). Neuronal data, EMG data, and eye positions were acquired and stored in a computer running a custom-made program in LabVIEW (National Instruments, Austin, TX).

Behavioral Task

We trained the three monkeys to perform a cognitive-set-shifting task (Figure 1A, B). In this task, the monkeys were required to match the sample to the choice stimulus based on the relevant dimension, color, or shape (see below for details). The trial sequence appeared at one of four quadrants on the screen (called "target quadrant"), and its upper/lower position instructed the relevant dimension (Monsell, 2003; Meiran, 1996): The upper and lower position indicated that the color and shape were relevant, respectively, for Monkey I. For Monkeys M and Y, we used the opposite relation in which the upper/lower indicated the shape/color was relevant.

The trials started with a blank screen ("preparatory" period, 600–1000 msec). Four quadrants then appeared with one target quadrant (light gray), and the remaining three (dark gray; "ready" period). After the monkeys pressed the center button, a warning stimulus (0.1° × 0.1° in size) was presented in the target quadrant ("warning" period, 400 msec for Monkeys I and M and 500 msec for Monkey Y), and then a sample stimulus overlaid the warning stimulus ("sample" period, 400 msec for Monkeys I and M and 500 msec for Monkey Y). Three choice stimuli then appeared on the top, left, and right of the sample ("choice" period). Each sample and choice stimulus consisted of a color patch (red, blue, or green) superimposed on a white shape (circle, triangle, or cross). Consequently, each stimulus had two dimensions: color and shape. Among the three choice stimuli presented, one matched the sample color, another matched its shape, and the third matched neither dimension (Figure 1A). The sample stimulus was randomly chosen from 3 × 3 combinations of the color and shape dimensions. The same color and shape attributes were not chosen more than three consecutive times. The color patch was 0.7° × 0.7° in size in the sample stimuli and 0.5° × 0.5° in size in the choice stimuli. The size of the shape was 2.1° × 2.1° in the sample stimuli and 1.5° × 1.5° in the choice stimuli. The choice period lasted until the monkeys responded by pressing a response button (up to 3000 msec). Upon the monkeys' response, a visual feedback was presented for 500 msec ("feedback" period). If the response was correct, only the matched shape or color attribute remained on the screen, and a liquid reward (a drop of fruit juice) was simultaneously delivered. If the response was incorrect, a reward was omitted and a blank screen appeared.

For every trial, the target quadrant changed its position between left and right. After six to eight consecutive correct trials in one dimension, the relevant dimension changed ("dimension change"; Figure 1B), and the target quadrant changed its position between upper and lower without changing left/right positions.

Recording Procedures

The activity of single neurons was recorded extracellularly from the posterior bank of the inferior arcuate region (Petrides, Cadoret, & Mackey, 2005; Romo, Hernández, & Zainos, 2004; Kurata & Hoffman, 1994; Kurata & Tanji, 1986; Figure 2; ipsilateral [left] hemispheres in Monkeys I and Y and both contralateral and ipsilateral hemispheres [left and right] in Monkey M) using a glass-coated tungsten or elgiloy microelectrode (Kamigaki et al., 2009, 2011; ~1 MΩ at 1 kHz). The microelectrode was advanced through the intact dura mater into the cortex along a stainless steel guide tube using a hydraulic micromanipulator (MO-95C, Narishige, Tokyo). We took two different approaches for targeting the cortex. One approach was used for Monkey M by inserting the microelectrode with 20° angles tilted from the vertical line in the coronal plane, without tilting in the sagittal plane (Figure 2A). Another approach was used for Monkeys I and Y by inserting the microelectrode with 45° angles tilted from the orbitomeatal line in the sagittal plane without tilting in the coronal plane (Figure 2C). Recording sites were localized using structural MR images obtained by a 4.7-T MRI scanner (Biospec 47/40, Bruker, Ettlingen, Germany).

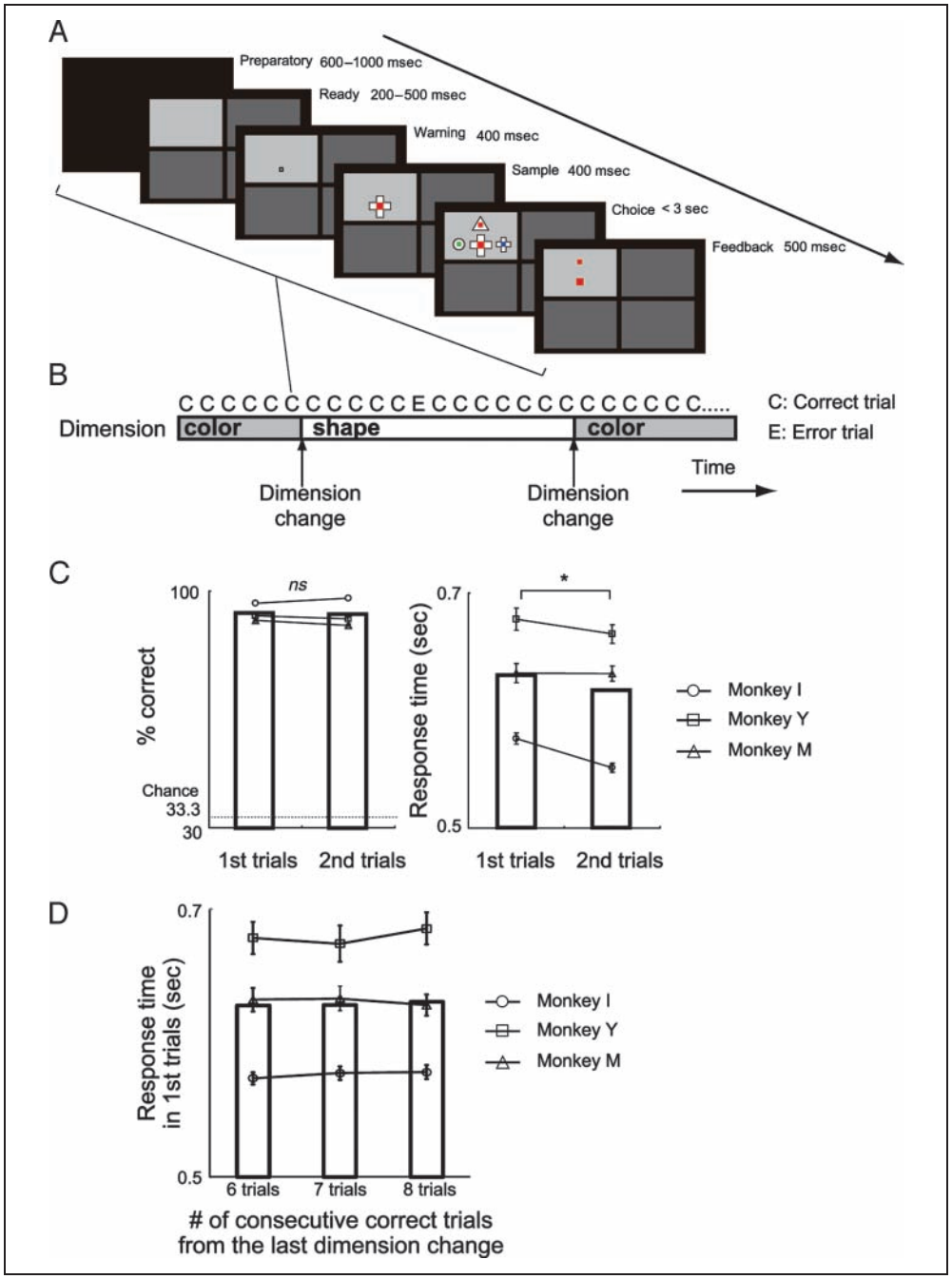

Figure 1. A cognitive-set-shifting task and behavioral performance. (A) Trial periods. The stimulus sequence was presented at one of four quadrants on the screen ("target quadrant"). The task required monkeys to match a sample stimulus to one of three choice stimuli based on one relevant dimension, color, or shape. The relevant dimension was instructed by the upper/lower position of the target quadrant (e.g., the upper for color and the lower for shape in Monkey I, counterbalanced among monkeys). A trial started with a blank screen ("preparatory" period). Four quadrants then appeared with one "target" quadrant (light gray) and the remaining three quadrants (dark gray; "ready" period). Warning and sample stimuli were sequentially presented within the target quadrant ("warning" and "sample" periods, respectively). Three choice stimuli were then presented and the animals selected one of them by pressing one of the three choice buttons (top, right, and left; "choice" period). Upon the monkeys' response, a visual feedback was presented ("feedback" period). (B) Task sequence. Animals had to respond successively based on one relevant dimension. After six to eight consecutive correct trials, the relevant dimension changed ("dimension change"), and the monkeys had to shift their behavior to respond based on the other dimension. (C) Mean percentage of correct trials and RTs in the first and second trials from dimension change for each monkey. The dotted line in the left indicates the chance level, 33.3%. *$p < .04$. (D) RT in the first trials as a function of the number of consecutive correct trials from the last dimension change (six to eight trials) for each monkey. Error bars indicate *SEM*.

While the monkeys performed the task, we monitored neuronal activity using a sound monitor and on-line rastergrams and searched for neurons that showed any change in activity during any trial period. EMG activity was monitored bilaterally with surface and wire electrodes (Kurata, 2007; Kurata & Hoshi, 2002) from the following muscles of Monkey M: biceps and triceps brachii, anterior deltoid, trapezius upper, and masseter muscles. EMG data were amplified (×1000–10,000), band-pass filtered between 20 and 5 kHz, sampled at 1 kHz through an A/D converter, and stored in a computer running a LabVIEW program. EMG was full-wave rectified to build perievent histograms with 50-msec bins (Figure 5A).

Drug Injection

After completion of the neuronal recording experiments, we injected a $GABA_A$ receptor agonist, muscimol, into the identified inferior arcuate region of Monkey M to reversibly inactivate the region (Figures 6 and 7). In each muscimol

injection experiment, we injected 0.5–2.0 μl muscimol (Sigma, concentration of 5 μg/μl) dissolved in 0.1 M phosphate-buffered saline. The target hemisphere was ipsilateral to the hand used for the task (Figure 6A) to minimize the potential effects on the response hand (Kurata & Hoffman, 1994). For each injection session, we conducted muscimol injection at a different coordinate, without any duplication of the same coordinates (see Figure 7C for the spatial distribution of the injection sites). Before injection in each daily session, the behavioral performance was tested for 1000 trials in the cognitive-set-shifting task (served as a "preinjection" condition). As a "postinjection" condition, the test of behavioral performance started immediately after the completion of injection (3030–5200 trials per daily session). After the completion of muscimol injection experiments, we conducted saline injection experiments in separate day sessions as a control. In each saline injection experiment, we injected 0.1 M phosphate-buffered saline for 0.5–1.0 μl and tested the behavioral performance in the same way as in the muscimol injection experiments. Using a microinjector (IM-9B, Narishige, Tokyo), we injected muscimol or saline at a rate of 0.05–0.1 μl/min through a glass micropipette that was inserted into the cortex by the same hydraulic microdrive used for the microelectrodes. During insertion of the micropipette, we monitored the insertion depth with a micrometer scale

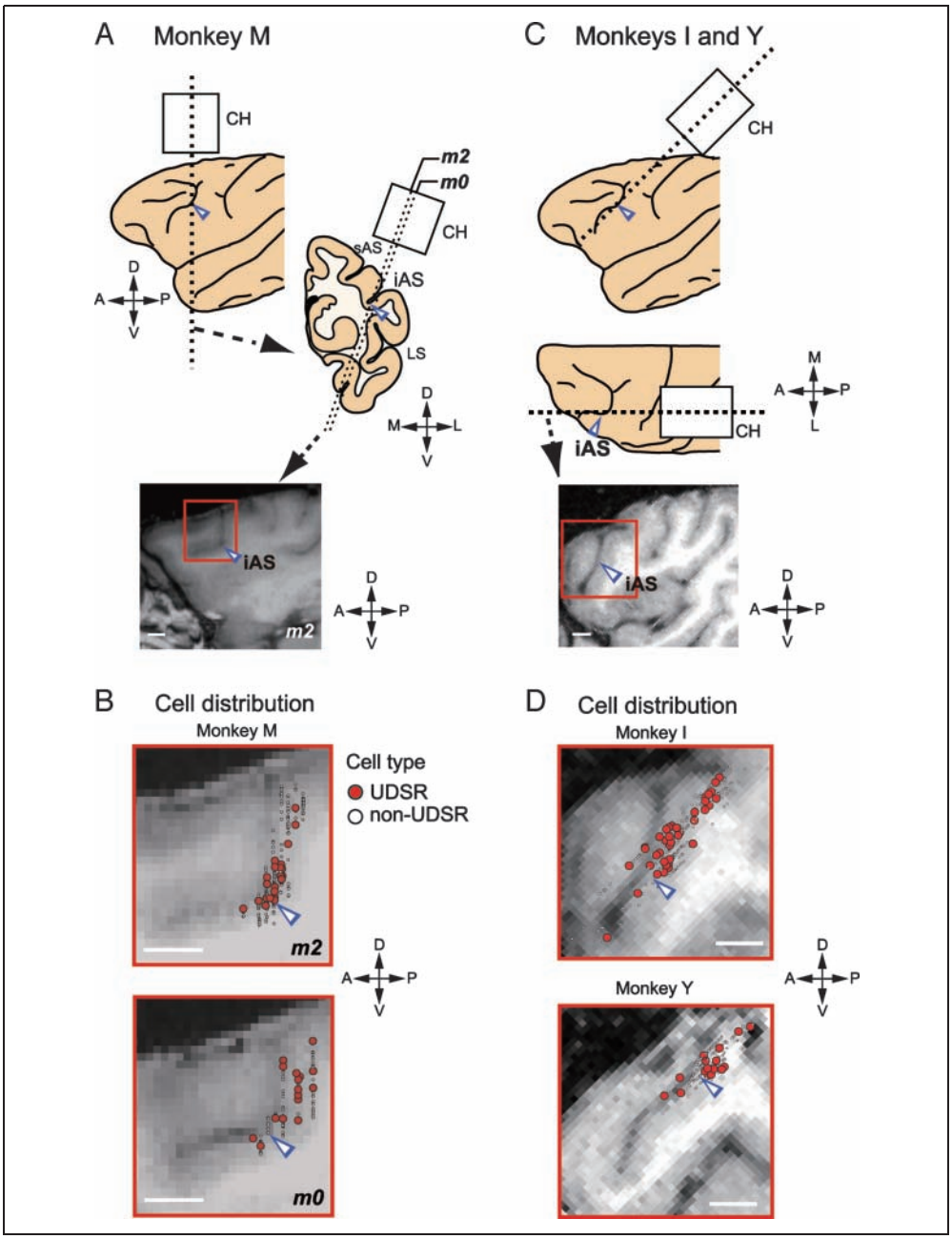

Figure 2. Recording sites. (A) Schematic illustration of the recording approach used for Monkey M. We inserted the microelectrode with 20° angles tilted from the vertical line in the coronal plane, without tilting in the sagittal plane. The structural MR image shows a parasagittal plane (*m2* plane). The part surrounded by the red rectangle is enlarged in (B). "CH" indicates recording chamber. Arrowheads denote the inferior arcuate sulcus (iAS). LS = lateral sulcus; sAS = superior arcuate sulcus. (B) The spatial distribution of UDSR cells (red) and other cells (white) in the posterior bank of iAS in Monkey M. Cells were superimposed on two different planes (*m2* and *m0*, separated by 2 mm from each other) of the MR images. Cells in the planes with 1 mm medial and 1 mm lateral from the *m2* plane were also superimposed on the *m2* plane, and those in the planes with 1 mm lateral from the *m0* plane were also plotted on the *m0* plane. (C) The recording approach used for Monkeys I and Y. We inserted the microelectrode with 45° angles tilted from the orbitomeatal line in the sagittal plane without tilting in the coronal plane. The MR image shows a sagittal plane. (D) Spatial distribution of UDSR cells in Monkeys I and Y (top and bottom, respectively). Cells in 2 mm medial to 1 mm lateral from the plane of the MR image were superimposed on the same plane for Monkey I, and cells were 2 mm medial to 2 mm lateral for Monkey Y. All scale bars indicate 4 mm.

on the microdrive manipulator as well as a scale on the micropipette. This system enabled the injection at known coordinates within the recording chamber.

To further confirm the injection sites, we conducted two approaches after the completion of all the injection experiments. First, we injected an MRI contrast agent, gadolinium (1.0 μl, 50 mM, Magnescope, Guerbet, Tokyo, Japan), at a rate of 0.05 μl/min, through the same microdrive manipulator at the same coordinate as in the muscimol injection, and obtained MR images (fast spin-echo, in-plane resolution = 0.39 × 0.39 mm^2, slice thickness = 1.5 mm, repetition time = 1259 msec, echo time = 12 msec). Second, metal deposits were placed at the electrode tip position by passing an anodic direct current (5 μA for 5 min) through the microelectrode (Koyano et al., 2011; Fujimichi et al., 2010). To detect the metal deposits by MRI, a fast spin-echo scan was performed (in-plane resolution = 0.25 × 0.25 mm^2, slice thickness = 1.0 mm, repetition time = 3000 msec, echo time = 60 msec).

Data Analysis

Neuronal activity was analyzed during the last 300 msec in the preparatory period, in which monkeys would prepare for the upcoming stimulus. To probe the task-related activity of each cell during this time window, we performed a two-way ANOVA with the factors Dimension (color or shape), upon which the monkeys' response was based, and Trial Number (first trial from dimension change or other trials). Only correct trials were included in the analysis. We used only those neurons that were tested in at least the six first trials. Cells showing a significant interaction between the two factors by ANOVA ($p < .05$) were called unidimensional shift-related (UDSR) cells (Kamigaki et al., 2009), because they were typically selectively activated when the monkeys were required to shift their behavior into one dimension, but not into the opposite dimension (Figure 3A–D). To further examine the other task-related activity in our database, we also performed the same two-way ANOVA ($p < .05$) on each trial period (Figure 3F).

For each UDSR cell, perievent histograms with a 50-msec bin width of spike trains were aligned at the end of the preparatory period. For ensemble population activity histogram, firing rates of each cell were averaged across trials and then normalized using their peak value during any periods of the trial. The preferred dimension of each cell was then determined as the dimension in which a cell showed greater activity in the first trial from dimension change. To analyze the population activity, normalized mean firing rates of each cell were first calculated, and a two-way ANOVA with Dimension (preferred or nonpreferred) and Trial Number (first to eighth trial from dimension change) was then performed on the normalized activity (Figure 3C, D). If the interaction between the two factors was significant ($p < .05$), post hoc comparisons were conducted using Tukey's honestly significant difference test.

For behavioral analyses during the unit-recording sessions (Figure 1C), we calculated the mean error rate and RT in the first and second trials from dimension change, across all daily recording sessions using all correct and error trials. The switch cost for the error rate and for the RT were then calculated as the difference between the two trials (Monsell, 2003; Meiran, 1996; Allport, Styles, & Hsieh, 1994). For behavioral examinations in the muscimol/saline injection experiments (Figures 6 and 7), we similarly analyzed the mean error rate and RT across all daily injection sessions. To directly compare the switch cost between pre- and postinjection conditions, the switch cost was normalized, that is, the difference in the RT between the first and second trials was divided by their sum (termed $RTD_{1st–2nd}$ in Figure 6B).

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

RESULTS

Behavioral Performance

We successfully trained three monkeys to promptly shift their behavior in a cognitive-set-shifting task. This task required the monkeys to match a sample stimulus to one of three choice stimuli based on one relevant dimension, color, or shape, as instructed with the presented stimulus (see Methods and Figure 1A, B). After six to eight consecutive correct trials in one dimension, the relevant dimension changed and the monkeys had to shift their cognitive set to respond based on the new dimension.

All the monkeys shifted their behavior as soon as the relevant dimension changed. Already in the first trials from dimension change, the percentage correct was over 90% for all monkeys, and this performance was maintained in the subsequent second trials. The switch cost (Monsell, 2003; Meiran, 1996; Allport et al., 1994), a performance decline in the first trial compared with the second trial, was not significant for the error rate, $F(1, 254) = 1.29$, $p > .2$ (no main effect of Trial Number by two-way ANOVA with Trial Number [first, second] × Monkey factors), but was significant for the RT, $F(1, 254) = 4.28$, $p < .04$ (Figure 1C).

Next we tested whether this increase in the RT at the first trial might have been derived from overt attention shift. We analyzed the relationship between eye position and RT in the first trial. We included only correct trials for this analysis. For each daily session, we calculated the mean horizontal and vertical eye position and the mean RT in the first trials. Then, in each monkey, we calculated the correlation between the mean horizontal/vertical eye position and the mean RT across all daily sessions. However, we found no significant correlations between eye position and RT in any of the monkeys (Pearson's correlation coefficient $r = .020$, $p = .9$ and $r = .075$, $p = .63$ for horizontal and vertical eye positions, respectively, in Monkey I;

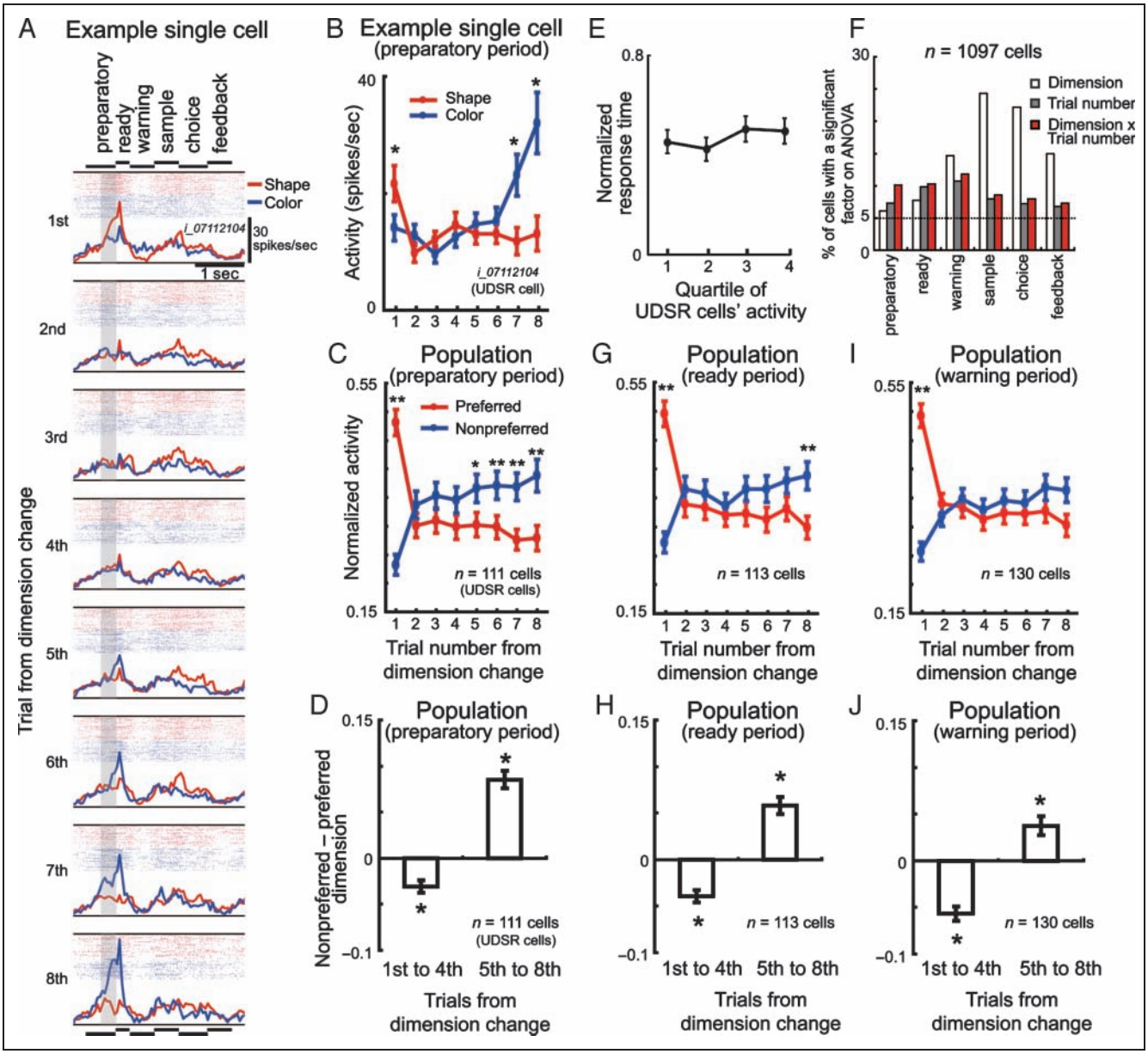

Figure 3. Neuronal activity of cells in the inferior arcuate sulcus. (A) Representative UDSR cell that exhibited activity modulated by the trial number from dimension change. The data were aligned at the end of the preparatory period. The hatched region denotes the last 300 msec of the preparatory period used for the analysis. Note that the first trial in each panel is the trial in which the monkey was notified of the dimension change and successfully performed set shifting between dimensions. The preparatory period in the first trial preceded the ready period in which the dimension change was signaled to the monkey. (B) Mean activity of the same cell during the preparatory period as a function of the trial number from dimension change. Error bars indicate *SEM*. *$p < .05$. (C) Population activity of all UDSR cells ($n = 111$) during the preparatory period as a function of the trial number from dimension change. Error bars indicate *SEM*. *$p < .01$, **$p < .005$. In (B) and (C), the activity in the first trial was recorded during the preparatory period preceding the ready period in which the dimension change was signaled to the animals. (D) Activity difference of UDSR cells between dimensions (nonpreferred − preferred dimension) during the preparatory period in first to fourth and fifth to eighth trials. Error bars indicate *SEM*. *$p < .001$. (E) Normalized RT as a function of the quartile of UDSR cells' activity. Error bars indicate *SEM*. (F) Percentage of cells among all recorded cells ($n = 1097$) with a significant factor by two-way ANOVA (Dimension [color, shape] × Trial Number [first, other trials], $p < .05$) during each trial period. The dotted line denotes 5%, a chance level expected from the p value for the ANOVA. (G, I) The same format as in (C), but the population activity of cells with a significant interaction during the ready period (G) ($n = 113$), and during the warning period (I) ($n = 130$). *$p < .01$, **$p < .005$. (H, J) The same format as in (D), but the data for the cells in (G) and (I), respectively. *$p < .001$.

$r = -.30$, $p = .12$ and $r = .15$, $p = .26$ for horizontal and vertical eye positions, respectively, in Monkey Y; $r = .23$, $p = .098$ and $r = .043$, $p = .75$ for horizontal and vertical eye positions, respectively, in Monkey M). These results demonstrated that the RT in the first trial was not significantly correlated with eye position. Thus, it is unlikely that the increase in the RT in the first trial was derived from overt attention shift reflected in eye position.

In our task, the dimension change occurred after six to eight consecutive correct trials, so it is likely that the

monkeys expected the next dimension change and became more flexible as the number of consecutive correct trials in one dimension increased. We examined whether the RT in the first trial became smaller as the number of consecutive correct trials increased since the last dimension change. For each daily session, we calculated the mean RT in the first trial as a function of the number of consecutive correct trials (Figure 1D). In each monkey, we performed a correlation analysis between the mean RT and the number of consecutive trials across the daily sessions. However, we did not find a significant correlation in any of the monkeys (Pearson's $r = .059$, $p = .50$ for Monkey I; $r = .044$, $p = .69$ for Monkey Y; $r = -.026$, $p = .74$ for Monkey M). We also calculated the switch cost as the difference in the RT between the first and second trial from the dimension change and analyzed the correlation between the switch cost and the number of consecutive trials. However, we found no significant correlation in any of the monkeys (Pearson's $r = .08$, $p = .33$ for Monkey I; $r = .11$, $p = .34$ for Monkey Y; $r = .042$, $p = .58$ for Monkey M). We suggest that, although the monkeys might have expected the next dimension change, the behavioral RT was not so sensitive as to reflect the monkey's readiness and flexibility to perform set shifting.

Neuronal Activity of Cells in the Inferior Arcuate Sulcus

We isolated 1097 single units from the posterior bank of the inferior arcuate sulcus (Romo et al., 2004; Petrides & Pandya, 2002) in the three monkeys (Figure 2; see below for the detailed description about the recording sites). As in our previous study of posterior parietal cells (Kamigaki et al., 2009), we first focused on the preparatory period, in which the animals would prepare for the upcoming trial before the explicit instruction of the relevant dimension (see Methods and Figure 1A). We found that 111 cells (10%) showed a significant interaction between Dimension (color, shape) and Trial Number (first, other trials) on two-way ANOVA (UDSR cells; see Methods for the definition). A typical UDSR cell (Figure 3A, B) showed increased activity in the first trial during the preparatory period, just before the ready period in which the dimension change was signaled to the monkey. This increased activity was selective only for the shape dimension, but not for the color dimension. In contrast, in the seventh and eighth trials in which the next dimension change approached, the cell showed greater activity in the color dimension than in the shape dimension ($p < .05$, Tukey's least significant difference test; Figure 3A, B).

Population analysis showed that, in the first trial, the activity of UDSR cells was larger in the preferred dimension, but in the fifth to eighth trials, activity in the nonpreferred dimension became significantly higher than in the preferred dimension ($p < .05$, Tukey's honestly significant difference test; Figure 3C). Compared with the preferred dimension, the mean activity in the nonpreferred dimension was significantly smaller in the first half of the trials (first to fourth trials) but was larger in the latter half (fifth to eighth trials; $p < .001$, t test; Figure 3D). We also confirmed that, in each of the three monkeys, the activity in the preferred dimension was significantly smaller in the latter half of the trials, compared with the nonpreferred dimension ($p < .02$ for all monkeys). We suggest that this activity modulation in the latter half of the trials was related to the monkey's expectation or flexibility to perform set shifting. During the latter half of the trials of the nonpreferred dimension, the monkey's readiness or expectation to shift to the preferred dimension should increase. This increased flexibility to perform set shifting might be associated with the increase in activity of UDSR cells. Conversely, UDSR cells did not exhibit increased activity during the latter half of the trials of the preferred dimension probably because the monkey was in a flexible state for shifting to the nonpreferred dimension.

We next assessed whether the activity of UDSR cells reflected the monkey's readiness to perform set shifting. We speculated that the monkeys might have shorter RTs as UDSR cells exhibited larger activity in the first trial of the preferred dimension. To address this issue, we analyzed the relationship between the RT and the firing rates of UDSR cells in two different ways. First, for each UDSR cell, we focused on the first trials of the preferred dimension. We then binned these trials into the quartile based on the firing rate of each cell during the preparatory period. The RT was averaged in trials of each quartile and normalized to its minimum and maximum. Figure 3E shows the normalized RT as a function of the quartile of neural activity (first to fourth quartiles). We included only those cells that had at least one trial for each of all the quartiles (70 of the 111 UDSR cells). Across these UDSR cells, we analyzed the correlation between the normalized RT and the quartile of neural activity. However, we found no significant correlation (Pearson's $r = .059$, $p = .32$). Second, we normalized the firing rate of each UDSR cell to its minimum and maximum during the preparatory period in the first trials of the preferred dimension. The RT was also normalized to its minimum and maximum in these trials of each cell. We then calculated the correlation between the normalized RT and the normalized firing rate across all the 111 UDSR cells. However, no significant correlation was found (Pearson's $r = .012$, $p = .61$). These results could not demonstrate that the monkey's RT in the first trial significantly correlated with the activity of UDSR neurons. As already shown above (see Behavioral Performance and Figure 1D), the behavioral RT was not sensitive enough to reflect the monkey's readiness and flexibility for set shifting. Therefore, although UDSR cells could be related to the monkey's readiness and flexibility, we were not able to find a significant relationship between the RT and the activity of UDSR cells.

These UDSR cells were localized in the focal area, especially near the bending sector of the inferior arcuate

sulcus, consistently across the three monkeys (Figure 2A, B, for Monkey M, and Figure 2C, D, for Monkeys I and Y). This suggests neural processing for set shifting was localized in the inferior arcuate region, at least within our recording sites.

In addition to the preparatory period in which UDSR cells were probed, we also analyzed other trial periods and found task-related cells for each period (see Methods; Figure 3F). Among them, 113 and 130 cells exhibited a significant interaction on the two-way ANOVA ($p < .05$) during the ready and warning periods, respectively. During these periods, the monkeys should know the relevant dimension by the instruction (Figure 1A) and prepare for the upcoming stimuli to respond. Figure 3G and I show the population activity of the 113 and 130 cells, respectively, as a function of the trial number. Similar to UDSR cells in Figure 3C, in the first trial, the activity of the two populations was larger in the preferred dimension than in the nonpreferred dimension. However, in contrast to UDSR cells, the activity in the nonpreferred dimension did not become significantly higher than in the preferred dimension, even when the trial number increased (i.e., the fifth to eighth trials; $p > .1$, Tukey's honestly significant difference test), except for the eighth trial of the 113 cells ($p < .005$). Figure 3H and J shows that, in these two populations, the activity in the nonpreferred dimension was significantly smaller in the first half of the trials (first to fourth trials) but was larger in the latter half (fifth to eighth trials; $p < .001$, t test), which was similar to the results of UDSR cells in Figure 3D. However, compared with UDSR cells, the activity difference between dimensions in the latter half of the trials was significantly smaller in the two populations ($p < .05$ for the 113 cells in the ready period and $p < .005$ for the 130 cells in the warning period; t test). These results suggest that, although these cell populations defined by the ready and warning periods shared activity properties with UDSR cells, the activity of UDSR cells was more predictive of the upcoming dimension, which may make a unique contribution to flexible behavior.

The Effect of Spatial Attention on the Activity of UDSR Cells during the Cognitive-set-shifting Task

Although our recording sites in the posterior bank of the inferior arcuate region did not include areas related to spatial attention according to the literature (Rizzolatti & Luppino, 2001; Kurata & Tanji, 1986), we tested any possible effects of spatial attention on the activity of UDSR cells. To address this issue, we analyzed gaze position that is assumed to directly reflect spatial attention, because we did not require the monkeys to fixate during the task.

First, we examined the relationship between eye position and the activity of UDSR cells at the population level. The mean horizontal and vertical eye positions were calculated during the last 300 msec of the preparatory period, the same time window used to probe UDSR cells. For each session in which each UDSR cell was recorded, we compared the difference in eye positions between the color and shape dimensions of the first trial. These eye position differences might have systematically affected the differential activity of UDSR cells in the first trial. However, we found no significant correlations between the difference in eye positions and the difference in the neuronal activity across all 111 UDSR cells (Pearson's $r = -0.1$, $p = .32$; $r = -0.12$, $p = .22$ for horizontal and vertical eye positions, respectively; Figure 4A).

Second, to assess the effects of eye position on each UDSR cell, we performed, cell-by-cell, an ANCOVA including the mean eye positions during the last 300 msec of the preparatory period in addition to the task factors (Eye Position [mean horizontal, vertical eye position] × Dimension [color or shape] × Trial Number [first, other trials], $p < .05$). Even after regressing out the eye-position-related factors on neural activity using this ANCOVA, 79 of the 111 UDSR cells (72%) still showed a significant interaction between Dimension and Trial Number factors. Moreover, population analysis of these 79 cells showed that, in the first trial, the activity of UDSR cells was greater in the preferred dimension, but when the next dimension change approached (sixth to eighth trials), the cells showed significantly greater activity in the nonpreferred dimension ($p < .05$, Tukey's honestly significant difference test; Figure 4B, left). Consistent with the activity of the original 111 UDSR cells, the mean activity in the nonpreferred dimension, compared with the preferred dimension, was significantly smaller in the first half of the trials (first to fourth trials) but larger in the latter half (fifth to eighth trials; $p < .01$, t test; Figure 4B, right).

These two series of analyses suggest that it is unlikely that spatial attention, as reflected by gaze position, produced the differential activity of UDSR cells, depending on the trial number from dimension change.

EMG Activity during Task Performance and Its Relation to Neural Activity of UDSR Cells

To examine whether the activity modulation of UDSR cells was influenced by any differences in hand and oral movements during task performance, we analyzed EMG activity in hand and oral muscles (see Methods for details). Figure 5A shows EMG activity in representative oral and hand muscles (right masseter, right and left anterior deltoid, and right triceps; note that the right hand was used for the task) in a neural session in which a UDSR cell was simultaneously recorded. We were able to analyze EMG activity in these muscles for a total of 26 neural sessions with UDSR cells (Figure 5B, top). Population analysis of EMG activity for these 26 sessions showed that the right masseter muscle was active in the feedback period, and the right anterior deltoid and right triceps muscles were active in the choice and feedback periods, whereas the left anterior deltoid muscle was inactive in either period (Figure 5B, top). Even when we focused on the active

period for each muscle, the muscles showed no significant interaction by two-way ANOVA (Dimension [color, shape] × Trial Number [first to eighth]; $p > .8$, for right masseter, right anterior deltoid, and right triceps; Figure 5B, bottom; see Figure 3C for comparison). Therefore, the differential activity between dimensions of UDSR cells depending on the trial number could not be explained by EMG activity in hand and oral muscles.

To test the effect of hand/oral movements on each of the 26 UDSR cells, we next performed, for each cell, an ANCOVA including the mean EMG activity during the last 300 msec of the preparatory period, in addition to the two task factors and eye position factors (EMG [eight muscles: right and left anterior deltoid, right and left triceps, right and left trapezius upper, right biceps, and right masseter] × Dimension [color or shape] × Trial Number [first, other trials] × Eye Position [horizontal, vertical eye position], $p < .05$). Of the 26 UDSR cells, 21 (81%) still showed a significant interaction between Dimension and Trial Number factors by this ANCOVA ($p < .05$). Moreover, consistent with the activity of the original 111 UDSR cells, the mean activity of the 21 cells in the nonpreferred dimension, compared with the preferred dimension, was significantly smaller in the first half of the trials (first to fourth trials) but larger in the latter half (fifth to eighth trials; $p < .05$, t test).

These results demonstrated that the activity of UDSR cells could not be derived from any systematic differences in hand and oral movements during the task (see Discussion for further descriptions about the relation between neural activity and motor commands).

The Effect of Muscimol Injection on Behavioral Performance

Next, we reversibly inactivated this area in the inferior arcuate region and examined its causal impact on behavioral shifting (Figure 6A and Methods). We injected a small volume of muscimol (0.5–2.0 μl, 5 μg/μl, cf. Fogassi et al., 2001; Shima & Tanji, 1998) into a hemisphere ipsilateral to the hand used for the task to minimize any potential effects on the response hand. As a control, we also conducted saline injection experiments after muscimol injection experiments (see Methods). Although muscimol injection

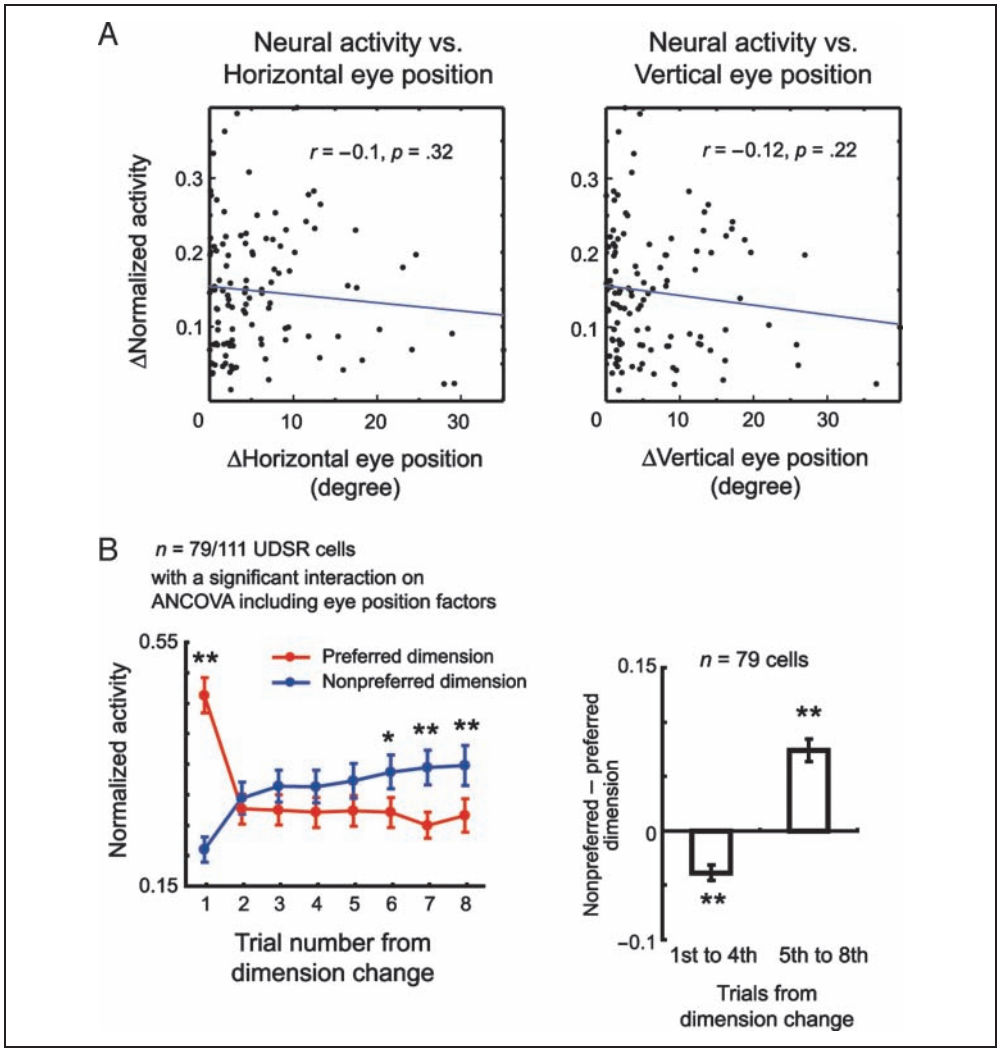

Figure 4. Relationship between eye position and neural activity. (A) Scatter plots show the relation between activity of UDSR cells and eye position during the preparatory period across all recording sessions for UDSR cells ($n = 111$). For each recorded session of UDSR cells, the mean horizontal and vertical eye positions during the preparatory period were calculated for the first trial from dimension change. The absolute differences in the normalized activity and in eye position between dimensions were then calculated. The activity difference was plotted against the difference in eye position. (B) Population activity of UDSR cells ($n = 79$ of 111) that showed a significant interaction by ANCOVA including eye position factors ($p < .05$). The left shows the normalized activity as a function of the trial number from dimension change in the 79 UDSR cells. The right shows the activity difference (nonpreferred − preferred dimension) averaged across the first to fourth and across the fifth to eighth trials from dimension change. *$p < .05$, **$p < .001$.

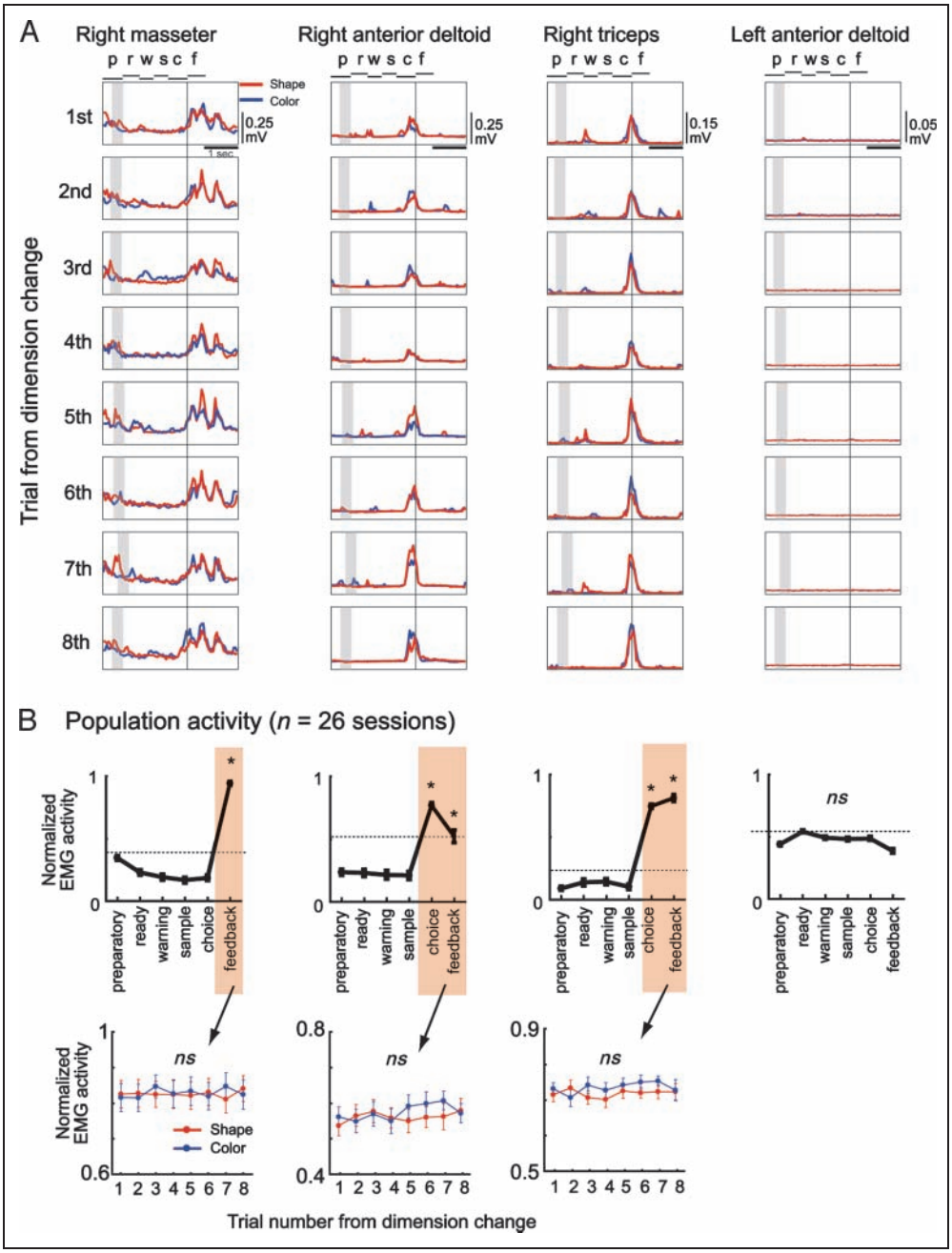

Figure 5. EMG activity during the task. (A) Example of EMG activity in a neural session in which a UDSR cell was recorded. EMGs of representative muscles (right masseter, right and left anterior deltoid, and right triceps) are shown as a function of the trial number from dimension change. Note that the monkey used the right hand for the task. The red and blue traces denote the shape and color dimensions, respectively. At the top, "p, r, w, s, c, and f" indicate preparatory, ready, warning, sample, choice, and feedback periods, respectively. The data were aligned at the end of the choice period. The hatched region indicates the last 300 msec of the preparatory period. (B) Population EMG activity in each muscle for 26 sessions in which UDSR cells were recorded. For each session, the EMG activity was normalized to its minimum and peak activity in each trial and averaged across all correct trials. The graph shows the mean EMG activity averaged across all the 26 sessions. Note that error bars for *SEM* are smaller than the data points. The hatched period denotes the "active" period, in which the muscle showed greater activity than baseline activity + 2 *SD* (indicated by a broken line). The bottom shows the population EMG activity during the active period plotted as a function of the trial number from dimension change. No significant interaction was found in either of the muscles ($p > .8$, two-way ANOVA with Dimension [color, shape] × Trial Number [first to eighth]).

impaired the overall performance for error rates and RTs (see the next paragraph and Figure 7), the RT in the first trial was greatly increased compared with the other trials ($p < .01$, Tukey's honestly significant difference test). As a result, the switch cost for the RT became significantly larger after muscimol injection, $F(1, 1679) = 15.65$, $p < .0001$ (a main effect of Injection by two-way ANOVA with Injection [pre-, postinjection] × Session [1- to 5-day sessions] factors), but not after saline injection, $F(1, 1169) = .12$, $p > .7$ (Figure 6B). This behavioral effect observed only in the muscimol condition, but not in the saline condition, could not be produced by an accumulation of general tissue damage, because all the muscimol injection experiments were conducted before saline injection experiments.

Moreover, the increased switch cost after muscimol injection was significantly correlated with the dose of muscimol (Pearson's $r = .97$, $p < .001$; Spearman's $r = .95$, $p < .002$; Figure 6C). These effects were not observed between the third and fourth trials, $F(1, 1679) = 1.01$, $p > .3$ for muscimol and $F(1, 1169) = .03$, $p > .8$ for saline (no main effect of Injection by two-way ANOVA; $p > .05$, no significant correlation with muscimol dose; Figure 6D, E). The results

demonstrated that muscimol injection selectively increased the switch cost in a dose-dependent manner.

To further assess the behavioral effect caused by muscimol injection in more detail, we calculated the mean RT and the mean error rate across all daily injection sessions, as a function of the trial number from dimension change (Figure 7). For the error rate and RT, we performed a three-way ANOVA (Injection [pre-, post-injection] × Trial Number [first to fifth] × Session [day sessions]). In the muscimol experiments (Figure 7A), for the error rate, we found a significant main effect of Injection, $F(1, 20) = 414.6$, $p < .001$, and main effect of Trial Number, $F(4, 20) = 3.27$, $p = .032$, but no significant interaction between Injection and Trial Number, $F(4, 20) = 2.43$, $p = .081$. Post hoc comparison showed that there was no significant difference between the first and second trials ($p > .9$, Tukey's honestly significant difference test). For the RT, there was a significant main effect of Injection, $F(1, 7920) = 763.1$, $p < .001$, main effect of Trial Number, $F(4, 7920) = 77.29$, $p = .001$, and an interaction between Injection and Trial Number, $F(4, 7920) = 3.06$, $p = .016$. Furthermore, post hoc comparison showed that the response was significantly longer in first trial than in the second trial ($p < .001$, Tukey's honestly significant difference test). In the saline injection condition (Figure 7B), there were no significant main effects or interactions, for the error rate and RT ($p > .1$). These results showed that the overall behavioral performance, reflected in the error rate and RT, was impaired after muscimol injection into the ipsilateral inferior arcuate sulcus region (Figure 7C). However, the RT in the first trial was more enlarged relative to that in the other trials, so that the

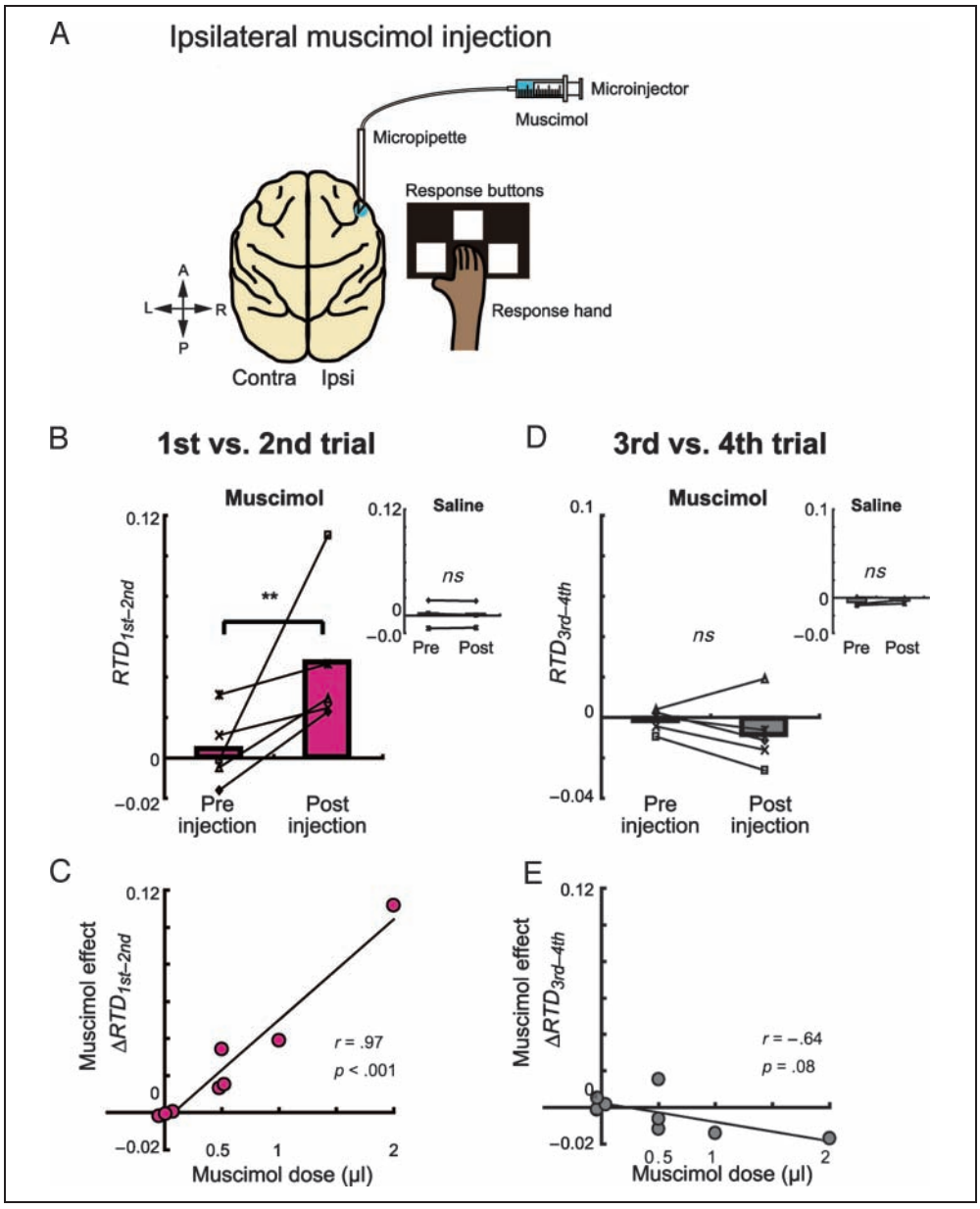

Figure 6. Behavioral effect caused by ipsilateral muscimol injection. (A) Experimental set up for muscimol injection. (B) RT difference between the first and second trials from dimension change divided by their sum ($RTD_{1st–2nd}$) before and after the muscimol injection. The inset shows data for saline control. (C) Muscimol effect on *RTD* between the first and second trials ($\Delta RTD_{1st–2nd}$: postinjection $RTD_{1st–2nd}$ minus preinjection $RTD_{1st–2nd}$) was plotted against the muscimol dose (0.5–2 μl). Data for "0" (μl) in the abscissa represent values during saline injections as convention of muscimol dose. (D, E) Same as in (B, C), but the difference between the third and fourth trials.

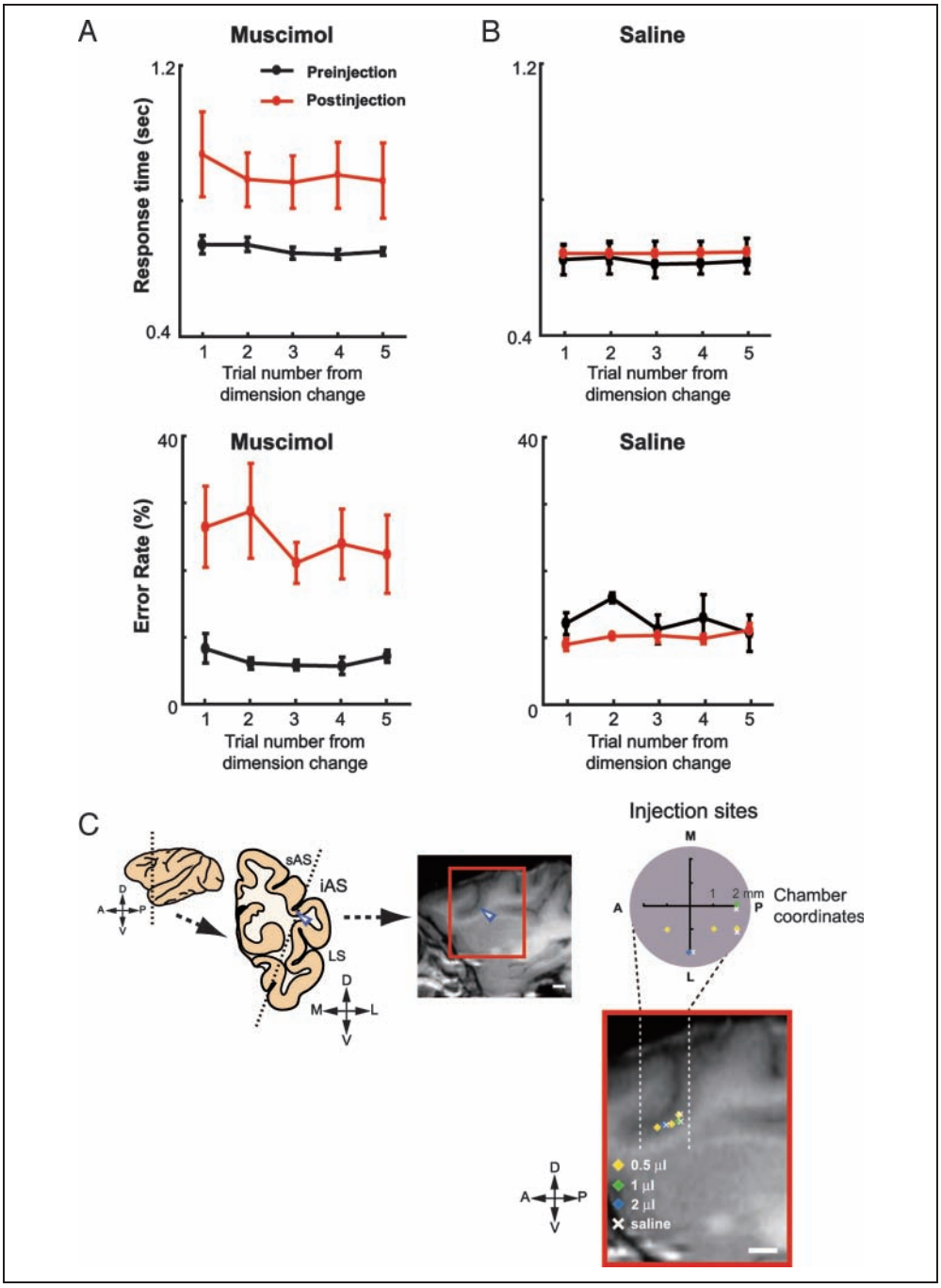

Figure 7. RTs and error rates caused by muscimol injection. (A) The mean RT (top) and error rate (bottom) were plotted as a function of the trial number from dimension change before and after muscimol injection (black and red, respectively). (B) The same as in (A), but in the saline injection experiments. (C) Injection sites were shown on the chamber coordinates and also on the structural MR image of the parasagittal plane. Arrowheads indicate iAS. Scale bar = 4 mm.

switch cost, as quantified by the difference between the first and second trials, became larger after muscimol injection. This behavioral effect specific to the switch cost could not be attributed to any potential motor deficits. These findings suggest that the process required for set shifting, as quantified by the switch cost, was more severely disrupted by the inactivation of the identified area in the inferior arcuate region.

DISCUSSION

This study found that a group of neurons (termed UDSR neurons) was activated in one dimension in the first trial after a dimension change, where set shifting is most strongly required. The same neurons were also activated in the opposite dimension as the next dimension change approached. Their shift-related activity was observed during the preparatory period before explicit instruction of the relevant dimension. This provides a novel insight into the neuronal mechanism for shifting cognitive set in the frontal cortex.

Recently, we also identified a group of neurons in the posterior parietal cortex that selectively increased activity when monkeys prepared to shift their behavior into a specific dimension (Kamigaki et al., 2009). However, unlike the current UDSR neurons, the previous cell groups

in the posterior parietal cortex tended not to be activated in the opposite dimension even when the next dimension approached. We suggest that the present UDSR cells in the inferior arcuate sulcus regions play a critical role in flexible set shifting by their activity predictive of the forthcoming relevant dimension. Moreover, the current group of UDSR neurons was localized in the focal area in the inferior arcuate sulcus. Consistent with this localization, our previous fMRI study using monkeys found that the inferior arcuate region exhibited transient activation in response to set shifting (Nakahara et al., 2002). This study identified dynamic neuronal processing selectively recruited for cognitive set shifting in this focal cortical area. Further inactivation experiments demonstrated that the identified region actually contributes to behavioral shifting.

Here, we discuss the possibility that the shift-related activity of UDSR cells could be derived from other confounding factors, rather than set shifting per se. First, there was a possible effect of spatial attention on neural activity. However, as we did not require the animals to fixate during the task, it can be assumed that the gaze position directly reflects spatial attention. We conducted two series of gaze analyses and demonstrated that the shift-related activity of UDSR cells could not be derived from gaze position. As such, it is unlikely that spatial attention affected neural activity. Second, our recording sites included areas associated with hand and oral movement (Kurata, 2007; Petrides et al., 2005; Dum & Strick, 2002; Kurata & Hoshi, 2002; Rizzolatti & Luppino, 2001); therefore, any systematic differences in these effector movements might have induced activity in UDSR cells. However, our analyses of EMG activity in hand and oral muscles indicated that the activity of UDSR cells could not be explained by the activity of these muscles during the task performance. Although we can exclude the effects of explicit motor commands on neural activity, it is still possible that UDSR cells are associated with implicit motor functions. Previous work suggests that frontal areas, including our recording sites, are involved in implicit motor commands such as transformation of extrinsic, visuospatial coordinates into motor output (Muhammad, Wallis, & Miller, 2006; Schwartz, Moran, & Reina, 2004; Kakei, Hoffman, & Strick, 2001). In this study, we cannot exclude the possibility that the activity of UDSR cells might, at least in part, reflect the transformation of the signal for set shifting into specific motor responses in addition to set-shifting processing.

Previous single-unit recording studies also examined neural activity in the prefrontal regions using set-shifting/task-switching paradigms (Stoet & Snyder, 2009; Mansouri et al., 2006; Genovesio et al., 2005; Wallis et al., 2001). In these paradigms, subjects have to maintain an appropriate configuration of mental resources, termed a "cognitive set," "task set," or "rule" (Monsell, 2003). In addition to this maintenance process, subjects are required to intermittently shift the maintained cognitive set/rule upon instruction. Most studies focused primarily on the former process and found "rule-selective" neural activity (Stoet & Snyder, 2009; Mansouri et al., 2006; Genovesio et al., 2005; Wallis et al., 2001); the latter processes remain largely unknown. Several studies reported neural activity related to switching of action, including arm movements or saccades (Zhou & Thompson, 2009; Isoda & Hikosaka, 2007; Johnston et al., 2007; Bichot & Schall, 2002; Shima & Tanji, 1998; Chen & Wise, 1995), but neural activity related to shifting of a more abstract cognitive set has not previously been investigated. This study provides the first evidence of dynamic neural processing for shifting an abstract cognitive set. Our reversible inactivation experiments further revealed a causal relationship between neural processing in the focal frontal region and the behavioral performance of set shifting. A previous lesion study on monkeys showed that distinct prefrontal areas were causally related to dissociable components of rule-guided behavior (Buckley et al., 2009), but the behavioral assessments were limited because the animals gradually switched behavioral rules after many trials and errors. In the current study, we overcame this limitation by successfully training the monkeys to promptly shift their behavior. As a result, we were able to demonstrate that the focal frontal region selectively contributes to flexible shifting of behaviors.

In addition to our recording sites, it is likely that other prefrontal regions, such as dorsolateral-prefrontal, orbitofrontal, and anterior cingulate cortices, play distinct roles in set shifting in area-specific manners, as suggested by human neuroimaging studies (Leber et al., 2008; Badre & Wagner, 2006; Dosenbach et al., 2006; Konishi et al., 2005; Rushworth et al., 2004; Braver et al., 2003; Sakai & Passingham, 2003; Yantis & Serences, 2003; Monchi et al., 2001; Kimberg et al., 2000; Sohn et al., 2000). Further studies are needed to address the unique functional contributions of distinct prefrontal regions to cognitive flexibility as well as the neural interaction between the regions.

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