Title: Neural activity in the human brain signals logical rule identification

Authors: Kaori Tachibana¹, Kyoko Suzuki¹, Etsuro Mori¹, Naoki Miura², Ryuta Kawashima³, Kaoru Horie⁵, Shigeru Sato⁵, Jun Tanji⁷, and Hajime Mushiake⁸

Addresses:

¹ Department of Behavioral Neurology and Cognitive Neuroscience, Tohoku University Graduate School of Medicine, Sendai, Japan
² Department of Intelligent Mechanical Systems Engineering, Kochi University of Technology, Kochi, Japan
³ Department of Functional Brain Imaging, IDAC, Tohoku University, Sendai, Japan
⁴ RISTEX, Japan Science and Technology Agency, Kawaguchi, Japan
⁵ Graduate School of International Cultural Studies, Tohoku University, Sendai, Japan
⁶ LBC Research Center, Tohoku University, Sendai, Japan
⁷ Brain Science Institute, Tamagawa University, Tamagawa Gakuen, Machida, Japan
⁸ Department of Physiology, Tohoku University Graduate School of Medicine, Sendai, Japan

Corresponding author: Hajime Mushiake

Department of Physiology, Tohoku University Graduate School of Medicine
2-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan
Email: hmushiak@mail.tains.tohoku.ac.jp; Tel: +81 22 717 8072; Fax: +81 22 717 8077

Running head: Neural activity during logical rule identification
Abstract

To select an appropriate action, we conform to a behavioral rule determined uniquely in each behavioral context. If the rule is not predetermined and must be discovered, we often test hypotheses concerning rules by applying one candidate rule after another. The neural mechanisms underlying such rule identification are still unknown. To explore which brain areas are involved in the process of logical rule identification, and to determine whether such areas differ from those taking part in implementing the rule to find a suitable action, we measured brain activation using functional magnetic resonance imaging (fMRI) while subjects performed a rule-identification task. The subjects were required to select a red or blue square on a screen based on either a “sequence rule” or a “probability rule”. Positive or negative feedback to the subject’s choice led the subject to identify the correct rule. We found that the posterior medial frontal cortex (pMFC), caudate nucleus, fusiform gyrus, and middle temporal cortex exhibited significant activation during the period when subjects underwent the hypothesis testing. Among these brain areas, the pMFC and caudate nucleus were also activated in response to the critical feedback signals selectively during the trials when the subjects identified a rule. Furthermore, we found a significant enhancement in effective connectivity between the active regions in the pMFC and caudate regions.
43  **Keywords:** posterior medial prefrontal cortex, caudate nucleus, fMRI, rule identification
We routinely perform actions under the guidance of a behavioral rule uniquely
determined in each behavioral context (Bunge 2004). To determine the most suitable
behavioral rule under conditions that require rule discovery, we typically test hypotheses
by applying one plausible rule, followed by the application of the next rule until a correct
rule is logically identified. Psychological studies have dealt with the behavioral
properties characterizing some aspects of rule discovery (Gorman and Gorman 1984;
utilizing Wason’s 2-4-6 task (Wason 1960) have clarified the behavioral processes
involved in rule identification. Wason’s task requires subjects to generate a triplet of
numbers to elucidate a rule that governs the generation of sequences in triplets of
numbers. It was reported that subjects identified an appropriate rule by first determining a
hypothetical rule, and then generating a triplet sequence according to that rule.
Subsequently, the subjects evaluated the hypothetical rule serially, based on positive or
negative feedback to the triplet sequence (yes or no) until a correct rule was identified
logically. It was found that subjects identified the currently correct rule by testing either to
confirm or to reject their hypothesis. Once a correct rule was identified, the subjects
generated the triplets readily by implementing said rule. Subsequent studies generated the
view that the rule identification procedure involves at least two behavioral processes: hypothesis testing and rule decision. According to this viewpoint, hypothesis testing is considered as a serial process of accumulating evidence for alternative hypotheses before a decision to finalize the rule finding (Gold and Shadlen 2002; 2007).

It is of great interest to know which brain region is involved in the process of behavioral rule finding. Recent brain imaging studies in humans and single-cell studies in non-human primates have suggested the involvement of the lateral prefrontal cortex in implementing behavioral rules to guide actions (Bunge et al. 2003; Hoshi et al. 2000; Parker and Gaffan 1998; Sakai and Passingham 2000; Wallis et al. 2001). The basal ganglia also play a role in learning a behavioral rule (Pasupathy and Miller 2005; Samejima et al. 2005; Seger and Cincotta 2006). Furthermore, the roles of cortical and subcortical areas in attentional set shifting and behavioral monitoring have been studied using the Wisconsin Card Sorting task (WCST(Milner 1963; Nelson 1976) in humans (Konishi et al. 1998; Monchi et al. 2001; Nagahama et al. 1996). However, it is not known which brain areas are involved in the process of rule identification, and to what extent such areas differ from those implementing the rule for finding a suitable action.

Notably, the WCST is excellent for examining the process of set shifting, but the behavioral condition used in this test is not appropriate for sorting out the process of rule
identification and rule implementation. To answer these questions we designed a behavioral task that dissociated the process of logical rule identification from the process of rule implementation. Furthermore, our behavioral task required several steps of hypothesis testing before rule identification was accomplished. Thus, the current study is focused on the process of logical rule identification and not on the reward-based rule learning. We will show that two brain regions, one in the cerebral cortex (the posterior medial frontal cortex) and the other in the striatum (the caudate), were active foci of particular interest when subjects identified a behavioral rule based on the process of hypothesis testing.
Materials and Methods

Subjects

Twenty-six healthy right-handed subjects (22 males and 4 females, mean age 22.2 years; age range 18–33 years) volunteered for this study. All the subjects had normal vision and none had a history of neurological or psychiatric illness. Before participating in the study, written informed consent was obtained from each subject. All procedures were carried out in accordance with the guidelines approved by the Tohoku University 21st Century Center of Excellence Program in the Humanities and the Helsinki Declaration of Human Rights. The subjects wore a head-mounted mirror to see objects projected by a computer and operated push buttons in a small box held in the right hand. They were required to push the right (or left) button to select the right (or left) square appearing on the screen.

Task sequence during fMRI scanning (Fig. 1A)

The subjects were asked to find a correct behavioral rule to select a target color and then to perform a two-choice task based on the rule discovered. In each trial, the subjects were required to gaze at a central fixation spot (yellow dot) on the screen for 1500–2500 msec, and then select a red or blue square, which appeared simultaneously on
either side of the fixation spot, in an attempt to predict which color was correct in that trial (target color). The right-left placement of the red and blue squares was counterbalanced.

Subjects were informed that two rules were involved in determining a correct choice: a “sequence rule” and a “probability rule”. In the sequence rule, the computer selected three target colors that appeared in a fixed temporal sequence (such as red-blue-red) that remained invariant in a block of trials. In the probability rule, the computer determined a target color in each trial stochastically with a fixed ratio of 2:1 in a block (e.g., red in 8 trials and blue in 4 trials, appearing unpredictably in a block). To avoid confusion, under the probability rule condition, the computer was programmed to ensure that the sequence of the three target colors in the initial three trials in a block did not appear in the next three trials. The repetition of either a red or blue target three times in a row was also avoided.

The subjects were required to respond within 1500 msec (choice period). The subjects were told that the probability condition was programmed in that manner. When the subject selected a color, the unselected color was turned off. At the end of the choice period, visual feedback was presented at the center of the screen for 1500 msec to indicate whether the choice was correct (white circle) or incorrect (white cross). If the subjects failed to respond within the choice period, an exclamation mark was displayed on the screen. After the feedback signal was turned off, a yellow fixation point was displayed on
the screen for 1500–2500 msec as a variable inter-trial interval. Each block consisted of
12 to 15 trials in order to prevent the subjects from anticipating the end of individual
blocks of trials. At the end of each block, a long inter-trial interval was established before
initiating the subsequent block so that the subjects performed 12 blocks of trials in about
15 min. The subjects completed a total of 160 trials in one session. All of the subjects
participated in practice sessions that included 24 trials before entering the sessions for
functional magnetic resonance imaging (fMRI) scanning. The practice session was
performed in a separate room.

Self-judgment task (Fig. 1B)

To examine when and how the study subjects identified the rules to select the
correct target colors, we introduced a modified version of the behavioral task in which we
required the subjects to report when they identified a current rule. All of the subjects
performed this task before they entered the scanner. For this task, we added a push button
to the aforementioned right-left choice buttons (Fig. 1B) to report when the subjects
identified the rule. After the feedback sign consisting of either the circle or cross was
turned off, a white triangle appearing at the top of the fixation point inquired whether the
subject judged that a current rule was found. During presentation of the triangle (1500
msec), the subjects pressed the middle button only when they judged that they had identified the strategic rule for the first time in a trial block. Otherwise, the middle button was not pressed. This judgment-reporting task indicated when the subjects first identified the rule in a block, and whether they identified the rule by positive or negative feedback.

The next trial commenced with a variable inter-trial interval of 1500-2500 msec. This modified version of the task included 108 trials.

Logical principles used in identifying rules

Before the practice sessions, the subjects were given a detailed explanation of the principles of the rule-finding task required by this study. The subjects were told explicitly that they had to find a correct target color in accordance with one of two strategic rules: either sequential or probabilistic. The subjects had to determine which behavioral rule governed the target choice during the current block of trials at an initial stage in each block of choice trials. Initially, for the first three trials, the subjects chose red or blue by trial and error (C₁–C₃). Based on feedback signals, they learned a set of three correct answers (A₁–A₃). We defined these three initial trials in each block as the “triplet detection period.” The subjects were informed that if the current rule was “sequential”, then the three target colors (A₁–A₃) should appear again in the three
subsequent choice trials. Therefore, the subjects adopted the strategy of selecting the first target color coinciding with $A_1$ as the subsequent choice ($C_4$). If $C_4$ was incorrect (i.e., resulted in negative feedback), then the current rule was deemed “probabilistic” and the subjects should subsequently conform to the probabilistic rule. If $C_4$ was correct, then the subjects chose $C_5$ as the target coinciding with the target for $A_2$. If $C_5$ was incorrect, then the current rule was deemed probabilistic; otherwise, the rule remained indeterminate, forcing the subjects to select $C_6$ as coinciding with $A_3$. If this choice was incorrect, again the current rule was probabilistic. If $C_6 = A_3$, then the current rule was “sequential”. To sum up, the choices $C_4$–$C_6$ were critical in determining the strategic rule used to select the target color in the hypothesis-testing procedure (see Fig.2). In other words, the behavioral task was designed in a manner in which positive feedback to $C_6$ led to the identification of the sequential rule, whereas negative feedback to any one of $C_4$ to $C_6$ led the subjects to identify the probabilistic rule. Therefore, we defined the trial whereby the choice of $C_6$, for identifying the sequential rule, or any one of $C_4$–$C_6$, for identifying the probabilistic rule to be a Rule Decision trial. According to the results of the self-judgment task, a small number of subjects tended to report the rule identification at a trial that was one trial later (Rule Decision +1) than expected. In these cases, we redefined the Rule Decision trial for these subjects and used the Rule Decision trial as a reference for analyzing the image data.
Furthermore, to clarify the effect of rule identification on behavior and brain activation, we realigned the trials based on the Rule Decision trial and defined them as the realigned choice number \((R_{C_n})\) (Fig. 2). After the subjects identified the rule as being either sequential or probabilistic, they implemented that rule in the subsequent trials to select a correct color. We defined these trials \((R_{C1} \text{ and thereafter})\) as rule-implementation trials.

**MRI data acquisition**

Functional and anatomical images were acquired using a 1.5-T Siemens Symphony (Siemens, Erlangen, Germany). Gradient-echo planar (GE-EPI) MRI sensitive to a blood oxygenation level-dependent (BOLD) contrast was acquired using the following settings: repetition time 3000 ms, echo time 50 ms, FOV 192×192 mm², matrix size 64×64, flip angle 90°, slice thickness 3 mm, gap 1 mm, and number of slices 32. A total of 347 volumes (about 17 min) were collected during the experiment. The first three dummy scans in each run were discarded to allow for T1 equilibration.

Image processing and statistical analysis of the fMRI data were performed using Statistical Parametric Mapping (SPM2; Wellcome Department of Imaging Neuroscience, London, UK, http://www.fil.ion.ucl.ac.uk) and MATLAB (MathWorks, Natick, MA, USA). The effect of head motion across the scans was corrected by realigning all of the
scans to the first scan. A mean image created from the realigned EPI images was coregistered with the structural T1 image and the structural images were normalized spatially to a standard template of $2 \times 2 \times 2$ mm voxels in the Montreal Neurological Institute (MNI) space. The derived spatial transformation was applied to the realigned EPI images. Subsequently, the normalized EPI images were smoothed spatially with a Gaussian filter of 8 mm full-width half-maximum to reduce noise and minimize the effects of normalization errors.

The data for the individual subjects were analyzed statistically using the general linear model in SPM2. The fMRI time series data were modeled by a series of events convolved with a canonical hemodynamic response function (HRF). Global changes were adjusted by proportional scaling, and the low-frequency confounding effects were removed using an appropriate high-pass filter.

Event-related analysis for detecting task–phase specific activation

To examine the activity changes related to each behavioral event during the task phase, we conducted an event-related analysis for each subject and a random-effect analysis for multi-subject data. For the single-subject-level analysis, we estimated the activity changes in response to the feedback signal for the following four contrasts obtained from
each subject: (1) the response to behavioral events at the Rule Decision trial minus the
response at the peri-Rule Decision trials (the mean of responses to behavioral events for
the two trials immediately preceding [PRE] and following [POST] the Rule Decision
period); (2) the sequence rule (from RC1 to RC6) minus the probability rule (from RC1 to
RC6); (3) the probability rule (from RC1 to RC6) minus the sequence rule (from RC1 to
RC6), and (4) the trials C1 to C3 minus all the trials following C4 for both rule conditions.

If the subjects made an unexpected choice on the Rule Decision trial, i.e., when the
subjects were given positive feedback on the Rule Decision trial in the probability rule
condition block, or when they were given negative feedback on the Rule Decision trial in
the sequence rule condition block, the data in that block were not included for further
analysis. As for the direct comparison between the sequence rule and the probability rule
(i.e. contrasts (2) and (3)), only the trials during which the subjects were given positive
feedback were included in the analysis.

For the random-effects analysis, contrast images for each subject were computed
using a one-sample t-test (in a between-subject random-effect analysis). The t values
were transformed to a unit normal Z distribution to create a statistical parametric map for
each contrast. The statistical threshold was set at P < 0.001 uncorrected for multiple
comparisons at the voxel level. Only activations involving contiguous clusters of at least
100 voxels are reported here. Finally, the resulting activation maps were constructed and superimposed on stereotactically standardized T1-weighted MRI images. All coordinates are reported in MNI space.

**Regression analysis for detecting activation involved in hypothesis testing**

To investigate brain activity during a process of hypothesis testing, we conducted a regression analysis separately on responses to feedback signals. As a measure of neural activity reflecting the hypothesis testing process, we estimated the activity changes during the course of evidence accumulation, according to the sequential analysis framework proposed by Gold and Shadlen (2007). To do this, we used a regressor defined as Decision Variable that represents accumulated information:

\[
\text{Decision Variable} = -\sum \log P(e)
\]

where \(P(e)\) was calculated as the likelihood function of an evidence using Bayesian updating procedure. We used a binominal distribution as the likelihood function, which was a conditional probability of seeing the evidence such as positive or negative feedback during the hypothesis testing. The conjugate prior of the binominal distribution is the beta distribution \(B(x, y)\). As a prior probability distribution at the beginning of hypothesis testing, we tentatively used \(B(2, 2)\). Subsequently, a posterior subjective probability
distribution for each rule was updated based on the observation of positive or negative feedback at trials for C4 to C6. We estimated the value of the likelihood function from the posterior probability distribution of the beta function as follows.

\[ P = \frac{x+k}{x+y+n} \]

where \( n \) is the number of trial and \( k \) is the number of either positive or negative feedback.

This sequence of evidence accumulation was performed until the rule-decision was attained. According to the sequential analysis framework (Gold and Shadlen 2007), the decision variable is updated until the decision variable reaches a certain criterion. We estimated the decision criterion based on subject’s performance during the pre-scan, self-judgment task. The trial at which the rule decision occurred was in principle the Rule Decision trial, defined above (see the section, Logical principles used in identifying rules).

We found that this, indeed, was the case for a majority of subjects. However, for two subjects, the trial of rule decision was one trial later than the logically-defined trial in the pre-scan, self-judgment task. For these two subjects, therefore, we adopted a higher decision criterion so that their trial of rule decision was adjusted by one trial later than logically estimated trial. Using the decision variable obtained through the procedures explained so far, we performed the regression analysis of fMRI data for individual
subjects. Subsequently, we performed the random-effect analysis using a one-sample student’s $t$-test.

ROI analysis for detecting activation commonly found during hypothesis testing and at the occurrence of rule decision

To better characterize the brain activity accompanying the rule-identifying process, we looked for brain regions that commonly exhibited activation during the period of hypothesis testing and at the time of rule decision. For this purpose, we conducted conjoint regions of interest (ROI) analysis based on the statistical parametric map obtained by the regression analysis (for detecting activation involved in hypothesis testing) and on the parametric map obtained by the event-related analysis (for detecting rule decision-specific activation). We defined the ROIs as spheres centered on the peaks of clusters that appeared when the statistical threshold was established at $P < 0.001$ with a radius of 8 mm. The mean percentage signal change (relative to a fixation period inserted between blocks) within each ROI was calculated for each subject and task using Mars Bar (http://marsbar.sourceforge.net/).

Dynamical causal modeling analysis among ROIs in multiple brain regions
Finally, to investigate a functional linkage among the ROIs, we applied dynamic causal modeling (DCM) to estimate the effective connectivity among ROIs in multiple brain regions (Friston et al. 2003; Marreiros et al. 2008). For each subject, the time series of all ROIs were extracted in a sphere region (radius = 8 mm) from the effects of interest f-contrast ($P < 0.05$, uncorrected). To detect changes in the effective connectivity concerning rule identification behavior, we established four behavioral periods as the modulation factors, including triplet encoding, hypothesis testing, and rule implementation periods. We then calculated the intrinsic and effective connectivities for the four behavioral periods using the two-state model (Marreiros et al. 2008).
Results

Behavioral data for the pre-scanning task and fMRI scanning task

Table 1 shows the behavioral data collected during the pre-scanning and fMRI scanning tasks. Before fMRI scanning, all of the participants were asked to perform the rule-identification exercise as a pre-scanning task. A two-way repeated-measures ANOVA on reaction time for the three behavioral periods (triplet detection, hypothesis testing, and rule implementation) and the two rule conditions (sequence and probability rule) showed a statistically significant main effect for the periods ($F[2,50] = 11.663, P < 0.0001$) but not for the rules ($F[1,25] = 1.851, P>0.1$). We performed a two-way repeated-measures ANOVA on the reaction time for the three periods and two rules for the fMRI scanning task, which also showed a statistically significant main effect for the periods [$F(2,50) = 19.578, P < 0.0001$] but not for the rules ($F[1,25] = 0.423, P>0.5$).

To examine when the subjects identified the current rule in each trial block, we plotted the cumulative frequency of choices for which subjects reported that they had identified the rule for the first time in the individual blocks (Fig. 2B). The subjects identified the sequence rule predominantly at the sixth choice, while the probability rule was identified mostly during the fourth, fifth, and sixth choices. These results demonstrated that the subjects performed the task predominantly in accordance with the
instructed principles of determining the behavioral rule, as illustrated in Figure 2. The success rate in finding a correct target color was 46.9 ± 8.9% during the triplet detection period, 95.5 ± 7.3% during the sequence-rule implementation period, and 62.0 ± 9.6% during the probability-rule implementation period (mean ± SD for all subjects).

Neural activity during hypothesis testing

To study neural activity during the period of hypothesis testing, we performed a regression analysis to investigate the brain areas involved in the process of accumulating evidence for the identification of alternative rules (see Methods). We found that the activity in the following brain areas increased significantly ($P < 0.001$) during the period of hypothesis testing: the pMFC (BA6/8/32), caudate nucleus, putamen, fusiform gyrus, middle temporal cortex, and occipital lobe (BA17; Fig. 3 and Table 2).

Neural activity at trials when the behavioral-rule decision is made

We compared brain activity at the Rule Decision trial with that measured at the PRE and POST trials based on the event-related analysis explained in the Methods section. From these comparisons, we determined a significant increase in activity in response to behavioral events during the Rule Decision trial in four brain regions: the
pMFC (area 6/8/32), caudate nucleus, ventrolateral prefrontal cortex (VLPFC; area 44), and the Insula ($P < 0.0001$; area 48; Fig. 4). This activation pattern was totally different from that of the rule implementation period as shown below. Table 3 summarizes the results of the random-effect analysis of this contrast.

We looked for brain regions commonly exhibiting activation during the period of hypothesis testing and at the time of rule decision, based on the conjoint ROI analysis (see Methods). We found that the caudate nucleus and pMFC were active not only as a result of the regression analysis but also based on the direct comparison analysis (Fig. 5). To examine the activation in these two regions quantitatively, we performed a ROI-based analysis for these areas by extracting data on the mean percent signal changes at each ROI (right panels in Fig. 5). In both regions, the percent signal changes increased gradually as the trials approached the Rule-Decision trial.
Effective connectivity of RULE-Decision selective activity between cortical and sub-cortical areas

To examine the functional connectivity between cortical and sub-cortical areas, we performed the DCM analysis on the activity in the pMFC and caudate nucleus for each behavioral epoch (see Methods). Although the intrinsic connectivity between the pMFC and caudate nucleus remained unaltered through the four behavioral epochs, the effective connectivity in the hypothesis-testing phase was significantly larger than in other phases ($P < 0.001$; Figs. 6A, B). This finding demonstrated that the functional connectivity between the caudate and pMFC areas was enhanced selectively during the period of rule identification.

Neural activity during the triplet detection period

We examined brain activations during the triplet detection period (Fig. 7A). A significant activity increase was found in the left anterolateral prefrontal cortex (area 10) and its posterior-adjacent area (area 46), left inferior parietal cortex (area 7), left precuneus (area 7), right pre-supplementary motor area (pre-SMA), right occipital cortex (area 19), left lingual gyrus (area 18), and bilateral cerebellum. Table 4 summarizes the results of the random-effect analysis of this contrast. Apparently, the spatial distribution
of active foci in this behavioral period differed markedly from the distribution observed during rule identification.

Neural activity during the rule-implementation period

Finally, to investigate whether any of the examined brain areas showed differential activation related to the two behavioral strategic rules used during the rule implementation period, we compared the brain activities associated with the sequence-rule and probability-rule trials directly (Figs. 7B and C). We found significant increases in activity selective for the sequence rule in the bilateral anterior cingulate cortex (area 11/25), right middle cingulate cortex (area 23), right inferior frontal cortex (area 48), right precentral gyrus (area 6), left insula (area 48), left lingual gyrus (area 37), left cuneus (area 18), and left precentral gyrus (area 4). In contrast, we found significant increases in activity selective for the probability rule in the right angular gyrus (area 7/40), left middle frontal gyrus (area 10), right middle frontal gyrus (area 46), left inferior parietal lobule (area 40), bilateral superior frontal gyrus (area 8), left middle frontal gyrus (area 6), bilateral insula (area 47), and right middle temporal gyrus (area 20). These selective patterns of activity were manifest after identifying current rules. Table 5 summarizes the results of the random-effect analysis of this contrast.
Discussion

In this study, we analyzed brain activity while the subjects performed a behavioral task designed to differentiate the behavioral processes required for identification and implementation concerning behavioral rules in separate temporal epochs. We found that multiple cortical and subcortical areas were involved differently in each of the three behavioral processes. Of particular interest was the activity related to rule identification. First, we located brain areas that were active during the process of hypothesis testing as determined by regression analysis. Second, with an event-related analysis, we detected brain areas that were activated specifically during the sequence rule decision (following positive feedback to the subject’s choice) and during the probability rule decision (following negative feedback). From our conjoint ROI analysis, we found that two brain regions emerged as active sites specifically related to rule identification. The pMFC and caudate nucleus exhibited a progressive increase in activity during the hypothesis-testing period and were also active in response to feedback signals leading to the decision of both the sequence and probability rules. Furthermore, we found that the effective connectivity between the pMFC and caudate regions was enhanced selectively during the period of hypothesis testing. These findings suggest the importance of these two areas in logically identifying behavioral rules.
pMFC as a focus for rule identification

The posterior aspect of the medial frontal cortex (pMFC), which was found to be the activity focus during rule identification in this study, is located in a transitional area that includes the posterior part of the anterior cingulate cortex (areas 24 and 32) ventrally and the medial frontal association areas corresponding to areas 8 and 6 (or the pre-SMA) dorsally. The ventral part of the focal area corresponds approximately to the posterior part of the rostral cingulate zone (RCZp) in the cingulate cortex (Paus et al. 1993; Picard and Strick 1996). The RCZp is thought to be situated between the caudal cingulate zone (CCZ) and the adjacent rostral cingulate zone (RCZa). The RCZa has been implicated in conflict monitoring (Botvinick et al. 1999; Carter et al. 2000; Casey et al. 2000) and attentional control (Davis et al. 1997; Kwan et al. 2000), and is viewed as a human homologue of CMAr in monkeys (Picard and Strick 2001; Shima and Tanji 1998). The functional role played by the RCZp remains unclear, but its potential role in action selection has been proposed (Botvinick et al. 1999; Crosson et al. 1999; Rubia et al. 2001). The posterior aspect of the anterior cingulate cortex, including the RCZp, has also been proposed to participate in conflict monitoring (Botvinick et al. 1999; Kerns 2006; van Veen and Carter 2005) and in performance monitoring (Ridderinkhof et al. 2004; Taylor...
et al. 2006; Ullsperger and von Cramon 2004; Walton et al. 2004). Our study revealed an aspect of the role of the RCZp that is different from action selection, conflict monitoring, or performance monitoring: logical rule identification. Notably, the behavioral factor of incompatible response tendency is not much of a critical factor in rule identification as in conflict monitoring. The behavioral factor of free choice (Walton et al. 2004) or error prevention (Carter et al. 2000) in performance monitoring is not necessarily crucial for rule identification. Rather, the process of hypothesis testing involving evidence accumulation characterizes the logical rule identification.

The pre-SMA has been studied extensively, and is thought to participate in a range of action controls requiring subjects to inhibit voluntary actions (Nachev et al. 2005; Nachev et al. 2007; Sumner et al. 2007) or to generate actions regulating motion sequences (Isoda and Tanji 2004; Nakamura et al. 1999; Sakai et al. 1999; Shima and Tanji 2000), updating motor plans (Shima et al. 1996), changing from a pre-existing motor plan to another (Matsuzaka and Tanji 1996; Rushworth et al. 2002), learning new condition-action associations (Hernandez et al. 2002), or switching from automatic to controlled action (Isoda and Hikosaka 2007). Our findings suggest that the pre-SMA, in conjunction with adjacent areas, is also involved in the identification of behavioral rules.
It remains to study whether different sets of pre-SMA neurons are involved in each aspect of behavioral control, such as in task switching and rule finding.

Involvement of the caudate nucleus in behavioral-rule identification

In this study, we found increased activity in the region of the striatum largely corresponding to the caudate nucleus during the behavioral period of rule identification, regardless of whether the rule was sequential or probabilistic. Recent studies demonstrating the involvement of the striatum in classification learning (Seger and Cincotta 2005), category learning (Little et al. 2006), reinforcement learning (Haruno et al. 2004; Samejima et al. 2005), and conceptual reasoning (Rao et al. 1997) are relevant to our findings. All of these studies revealed the importance of the caudate in evaluating feedback signals reflecting the outcome of the subject’s selection of action for behavioral learning. In our study, in contrast, the subjects were informed of two potential rules that govern the target-selection task. The behavioral requirement, therefore, was the rule selection by hypothesis testing and rule decision, but not rule learning. Despite these differences in behavioral requirements, it is interesting that the activation of the caudate was found in common. There is a possibility that different portions of the caudate, despite their overlap, are activated depending on whether the task requirement is rule
learning or selection/decision of behavioral rule. Alternatively, a common factor of evaluating feedback signals for behavioral revisions may give rise to caudate activation. A contribution of the striatum to the evaluation of action results has also been implicated in studies using reversal learning (Cools et al. 2002; Cools et al. 2004; Monchi et al. 2001; Rogers et al. 2000). However, it is noteworthy that in our study, the caudate activity increase occurred only during the rule identification period, and not during the triplet detection period. This finding of context-dependent encoding of feedback signals in the caudate is consistent with a context encoder theory for the striatal function proposed by Houk and Wise (Houk and Wise 1995).

In earlier neuroimaging studies, the caudate nucleus was considered as being in an active state in association with a mental shift to a new response set (set shifting (Cools et al. 2002; Cools et al. 2004; Monchi et al. 2001; Rogers et al. 2000). In a more recent report, however, activation of the caudate nucleus was interpreted as representing the planning of a self-generated novel action, rather than a set-shifting task (Monchi et al. 2006). Considering our findings, one possible interpretation of the results reported by Monchi et al. could be that the subjects found an appropriate rule (for color or number matching) under their behavioral condition defined as “with shift” trials.
Destrebecqz et al. (2005) reported observing a functional connectivity between the caudate nucleus and pMFC when the study subjects were engaged in the explicit learning of a sequence-generation task. Based on our observation of correlated activity between the caudate and pMFC, we interpret the caudate-pMFC interaction as being enhanced when subjects become consciously aware of a behavioral rule. This interpretation is also consistent with a recent discovery that behavioral changes follow the development of a functional link between the caudate and prefrontal cortex (Pasupathy and Miller 2005).

In theory, there is a possibility that the activity during the rule-identification period could vary depending on whether the subjects succeeded in identifying a hypothesis or failed to do so. However, in practice, the subjects rarely (in less than 5%) failed to identify a rule and the data during the unsatisfactory trials were too few to perform this comparison.

Activity during the triplet detection period

During the behavioral period corresponding to triplet detection, we observed active foci in areas that differed entirely from those observed during the rule-identification period. The activity focused in the lateral prefrontal cortex and parietal
cortex during this period is in accord with current understandings of the function of these
frontoparietal areas in relation to the short-term storage of working memory and
behavioral-rule learning (Goldman-Rakic 1987; Miller and Cohen 2001; Tanji and Hoshi
2008; Wallis et al. 2001). However, other behavioral factors, such as attention, that vary
selectively during the triplet detection period could contribute to the activation.

Activity during behavioral-rule implementation

The spatial pattern of brain activation during this behavioral stage following the
behavioral-rule identification differed depending on whether the behavioral rule was the
sequence rule or probability rule. Under the sequence-rule condition, the subjects’
selection of the target color was deterministic, following the sequence of three target
colors presented during the triplet detection period, and was remembered thereafter.
Prominent activation foci that we found for sequence-rule implementation were in the
ventromedial prefrontal cortex, medial temporal lobe, and precuneus. These active foci
are consistent with those reported in previous studies with a common behavioral context;
the ventromedial prefrontal cortex was found to be activated in a sequence-learning task
(Werheid et al. 2003), a decision-making task (Daw et al. 2006), and during
decision-making when conforming to the prior probability of the current choice.
These studies imply that the medial prefrontal cortex plays an important role in predictive action selection that is based on previously acquired knowledge. As for the medial temporal cortex, a previous study suggested that the anterior medial temporal lobe is involved in explicit learning of a rule-based categorizing task (Nomura et al. 2007). In our study, once the subjects identified the behavioral rule, they were likely to predict the next target color before the stimulus was presented.

In contrast, under the probability rule condition, significant activations were observed in the bilateral anterior lateral prefrontal cortex, right parietal cortex, and anterior cingulate cortex. These regions are consistent with previous studies reporting brain activation in an uncertain decision-making paradigm (Blair et al. 2006; Critchley et al. 2001; Elliott et al. 1999; Huettel 2006; Ito et al. 2003; Krain et al. 2006; Paulus et al. 2003; Simmons et al. 2006; Volz et al. 2003; Walton et al. 2004). It should be noted that under the probabilistic condition, the selection of target colors was always made with uncertainty. Activities in the dorsolateral prefrontal and parietal cortices and in the insula reportedly reflected the extent of uncertainty in a probabilistic decision-making task (Huettel et al. 2005). Recent reports are accumulating evidence that points to the role of the insula in detecting and representing uncertainty (e.g., Preuschoff et al. 2008). Other neuroimaging studies found that the DLPFC and parietal cortex were active when the
subject was planning the forthcoming behavioral selection in the context of problem solving (Brass et al. 2005; Mushiake et al. 2002; Newman et al. 2003). In interpreting the present data during the rule-implementation stage, however, the presence of negative feedback under the probabilistic (but not sequential) condition should also be considered.

Identification of a rule about the world or about the best behavioral strategy

In this study the subjects were instructed to identify whether the target-selection rule was sequential or probabilistic. When the probabilistic rule was identified and at the behavioral stage of target-color selection, the subjects were instructed to guess whether the target was red or blue, taking the probability into consideration. Most of the subjects did this task of rule implementation by matching probabilities. However, three subjects adopted the strategy of picking the more probable color because it has a 2/3 chance of being correct. For these subjects, we attempted to perform a separate image analysis on brain activation during the task period of rule implementation. We found activation foci in the bilateral orbitofrontal cortex and inferior parietal cortex, in addition to the middle frontal cortex. However, during the behavioral stage of rule identification, detectable active sites in the three subjects were indistinguishable with those found in the rest of 23 subjects.
Acknowledgments

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References


Daw ND, O'Doherty JP, Dayan P, Seymour B, and Dolan RJ. Cortical substrates for


Hampton AN, Bossaerts P, and O'Doherty JP. The role of the ventromedial prefrontal


Huettel SA, Song AW, and McCarthy G. Decisions under uncertainty: probabilistic context influences activation of prefrontal and parietal cortices. *J Neurosci* 25: 3304-3311,


Monchi O, Petrides M, Petre V, Worsley K, and Dagher A. Wisconsin Card Sorting revisited: distinct neural circuits participating in different stages of the task identified by


**Nelson HE.** A modified card sorting test sensitive to frontal lobe defects. *Cortex* 12:


Shima K, and Tanji J. Neuronal activity in the supplementary and presupplementary motor areas for temporal organization of multiple movements. *J Neurophysiol* 84:


Figure legends

Figure 1

Diagrams illustrating the temporal sequence of the behavioral tasks used in this study. (A) The task used during fMRI scanning. Red and blue squares appeared on the screen on opposite sides of a fixation point (yellow dot) as targets. The subjects were required to select the correct color (target color) within 1500 msec by pressing the left or right button with either the index or ring finger. The selected color remained on the screen for 1500 msec. Subsequently, a feedback signal appeared at the screen center notifying that the choice was correct (with a circle) or incorrect (with a cross). (B) The pre-scanning task in which the subjects reported when they judged that they had identified the behavioral rule. After presentation of the feedback signal, a triangle was displayed at the top of the screen for 1500 msec. The subjects pressed the middle button only when they judged that they had identified the rule for the first time in a trial block.

Figure 2

(A) A diagram schematically illustrating the principle that governed how the behavioral task was performed during the three essential periods: triplet detection, hypothesis testing, and rule implementation. During triplet detection, the subjects made three choices (C₁ –
by guessing a target color. Based on feedback signals, the subjects knew the correct answers (A₁–A₃) and remembered them. During this task period, they built a hypothesis that A₁–A₃ might be a sequence that could appear as the target colors repeatedly in the following trials. Subsequently, in the hypothesis-testing period, the subjects selected A₁, A₂, and A₃ as their choices C₄, C₅, and C₆ respectively. If the subjects received negative feedback for any of C₄–C₆, they identified the rule as “probability”. If the subjects received positive feedback for the choice of C₆, they identified the rule as “sequence”. The first choice subsequent to the discovery of the current rule was redefined as RC₁, which was the beginning of the rule-implementation period. (B) Cumulative frequency curves indicating the occurrence of rule identification at the ordinal number of choices during the pre-scanning, self-judgment task. When performing under the sequence rule (thick line), the subjects identified the rule predominantly at the sixth choice. For the probability-rule judgment (thin line), the subjects reported that they identified the rule during the fourth to sixth choices.

Figure 3

Areas of significant activation associated with rule identification, as identified through regression analysis. This scan identified those brain areas involved in the process of
accumulating evidence for the identification of alternative rules. (A) The pMFC is located in a transitional area between the cingulate cortex and medial association areas (areas 6, 8, and 32). The main focus of activation found in other brain areas includes the caudate nucleus (B), fusiform gyrus (C), and middle temporal cortex (D).

Figure 4

Brain areas that were active during trials at which the behavioral-rule decision was made. This figure shows regions with a significant increase in activity in response to behavioral events during the rule decision trial.

Figure 5

Brain regions commonly activated during hypothesis testing and at trials of behavioral-rule decision. The left panels show two foci of activation indicating the result of conjoint ROI analysis (regression analysis and direct comparison analysis). The right panels show the percent signal changes in the two regions corresponding to the pMFC (A) and in the caudate (B). In both regions, the percent signal changes increase progressively as the trials approached the Rule Decision trial (RC0), irrespective of the behavioral rule. The error bars indicate the standard error of the mean.
Results of DCM analysis to detect functional connectivity between the pMFC and the caudate. (A) Intrinsic connectivity calculated for each epoch appeared similar. (B) The effective connectivity between the pMFC and Caudate nucleus was enhanced selectively during the hypothesis-testing phase ($P < 0.001$). The error bars indicate the standard deviation.

(A) Brain activation during the task period of triplet detection. (B) Cortical areas in which the activation was greater during sequence-rule than during probability-rule implementation. (C) Cortical areas where the activation was greater during probability-rule implementation. The statistical significance level of activation is $P < 0.001$. To perform the comparison to obtain the contrast (B) and (C) for the two sets of rule-implementation, we performed the analysis on only trials in which positive feedback was obtained during rule identification.
Table1. Reaction time in each period for both tasks.

<table>
<thead>
<tr>
<th></th>
<th>Triplet detection period</th>
<th>Hypothesis testing period</th>
<th>Rule implementation period</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-scanning task</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sequence rule</td>
<td>608.1 ± 112.7</td>
<td>564.5 ± 107.2</td>
<td>551.7 ± 100.4</td>
</tr>
<tr>
<td>Probability rule</td>
<td>618.4 ± 150.9</td>
<td>534.4 ± 98.7</td>
<td>542.3 ± 94.1</td>
</tr>
<tr>
<td><strong>fMRI scanning task</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sequence rule</td>
<td>645.9 ± 98.4</td>
<td>590.7 ± 81.7</td>
<td>594.2 ± 98.0</td>
</tr>
<tr>
<td>Probability rule</td>
<td>634.7 ± 111.1</td>
<td>592.7 ± 90.2</td>
<td>584.6 ± 87.9</td>
</tr>
</tbody>
</table>

Values are means ±SD; msec.
Table 2. Brain regions showing significant signal increases as data accumulation.

<table>
<thead>
<tr>
<th>Region of activation</th>
<th>L/R</th>
<th>Area</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Z value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pMFC</td>
<td>L</td>
<td>6/8/32</td>
<td>-4</td>
<td>18</td>
<td>48</td>
<td>4.75</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>R</td>
<td></td>
<td>10</td>
<td>14</td>
<td>4</td>
<td>4.93</td>
</tr>
<tr>
<td>Putamen</td>
<td>L</td>
<td></td>
<td>-16</td>
<td>14</td>
<td>-2</td>
<td>4.52</td>
</tr>
<tr>
<td>Fusiform gyrus</td>
<td>R</td>
<td>19</td>
<td>28</td>
<td>-80</td>
<td>-8</td>
<td>4.34</td>
</tr>
<tr>
<td>Middle temporale cortex</td>
<td>R</td>
<td>22</td>
<td>58</td>
<td>-46</td>
<td>10</td>
<td>4.17</td>
</tr>
</tbody>
</table>
Table 3. Brain regions showing significant signal increases at the RULE-ID trial.

<table>
<thead>
<tr>
<th>Region of activation</th>
<th>L/R</th>
<th>Area</th>
<th>coordinates of peak activation</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Z value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pMFC</td>
<td>L</td>
<td>6/8/32</td>
<td></td>
<td>-4</td>
<td>18</td>
<td>50</td>
<td>4.79</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>R</td>
<td></td>
<td></td>
<td>10</td>
<td>10</td>
<td>4</td>
<td>5.04</td>
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<tr>
<td>VLPFC</td>
<td>L</td>
<td>44</td>
<td></td>
<td>-44</td>
<td>10</td>
<td>28</td>
<td>4.29</td>
</tr>
<tr>
<td>Insula</td>
<td>L</td>
<td>48</td>
<td></td>
<td>-36</td>
<td>22</td>
<td>6</td>
<td>4.21</td>
</tr>
</tbody>
</table>
Table 4. Brain regions showing significant signal increases at the Triplet detection period.

<table>
<thead>
<tr>
<th>Region of activation</th>
<th>L/R</th>
<th>Area</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Z value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inferior parietal lobule</td>
<td>L</td>
<td>7</td>
<td>-32</td>
<td>-74</td>
<td>42</td>
<td>5.67</td>
</tr>
<tr>
<td>Precuneus</td>
<td>L</td>
<td>7</td>
<td>-8</td>
<td>-72</td>
<td>50</td>
<td>5.64</td>
</tr>
<tr>
<td>Middle frontal gyrus</td>
<td>L</td>
<td>10</td>
<td>-34</td>
<td>54</td>
<td>8</td>
<td>5.29</td>
</tr>
<tr>
<td>pMFC</td>
<td>R</td>
<td>6/8/32</td>
<td>4</td>
<td>16</td>
<td>50</td>
<td>4.87</td>
</tr>
<tr>
<td>Occipital cortex</td>
<td>R</td>
<td>19</td>
<td>34</td>
<td>-70</td>
<td>32</td>
<td>4.83</td>
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<tr>
<td>Lingual gyrus</td>
<td>L</td>
<td>18</td>
<td>-26</td>
<td>-94</td>
<td>-12</td>
<td>4.78</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>L</td>
<td></td>
<td>-34</td>
<td>-62</td>
<td>-24</td>
<td>6.44</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>R</td>
<td></td>
<td>40</td>
<td>-58</td>
<td>-30</td>
<td>6.08</td>
</tr>
</tbody>
</table>
Table 5. Brain regions revealed by contrasts between sequence rule trials and probability rule trials.

<table>
<thead>
<tr>
<th>Region of activation</th>
<th>L/R</th>
<th>Area</th>
<th>coordinates of peak activation</th>
<th>Z value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>x  y  z</td>
<td></td>
</tr>
</tbody>
</table>

**SEQUENCE RULE > PROBABILITY RULE**

- Anterior cingulate cortex: L/R 11/25, 0 32 -2 6.25
- Middle cingulate cortex: R 23 4 -16 46 6.04
- Precuneus: L 23 -8 -58 24 5.69
- Rolandic opeculum: R 48 64 2 10 5.63
- Precentral gyrus: R 4/6 56 -8 48 5.53
- Insula: L 48 -42 6 -10 5.50
- Lingual gyrus: L 37 -26 -48 -6 5.03
- Cuneus: L 18 -8 -88 22 4.69
- Precentral gyrus: L 4 -36 -22 60 4.66
- Hippocampus: L 20 -26 -16 -20 4.53

**PROBABILITY RULE > SEQUENCE RULE**

- Angular gyrus: R 7/40 36 -58 42 6.54
<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Middle frontal cortex</td>
<td>L</td>
<td>10</td>
<td>-36</td>
<td>60</td>
<td>12</td>
</tr>
<tr>
<td>Middle frontal cortex</td>
<td>R</td>
<td>46</td>
<td>44</td>
<td>56</td>
<td>2</td>
</tr>
<tr>
<td>Inferior parietal lobule</td>
<td>L</td>
<td>40</td>
<td>-44</td>
<td>-54</td>
<td>50</td>
</tr>
<tr>
<td>Superior frontal cortex</td>
<td>L/R</td>
<td>8</td>
<td>0</td>
<td>30</td>
<td>44</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>L</td>
<td>-10</td>
<td>-78</td>
<td>-28</td>
<td>5.23</td>
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<tr>
<td>Middle frontal cortex</td>
<td>L</td>
<td>6</td>
<td>-36</td>
<td>6</td>
<td>60</td>
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<tr>
<td>Insula</td>
<td>R</td>
<td>47</td>
<td>32</td>
<td>24</td>
<td>-4</td>
</tr>
<tr>
<td>Insula</td>
<td>L</td>
<td>47</td>
<td>-32</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>Middle temporal cortex</td>
<td>R</td>
<td>20</td>
<td>56</td>
<td>-30</td>
<td>-10</td>
</tr>
</tbody>
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