Learning-set (LS) is a property of insight and hypothesis testing characterized by the ability to solve novel problems based on previous experiences with problem solving. However, the neural organization and mechanisms underlying LS remain unclear. To further characterize this process, positron emission tomography (PET) studies with [(15)O]H₂O were performed to measure regional cerebral blood flow (rCBF) during the learning phase of the two-choice visual discrimination task under the LS paradigm in rhesus monkeys. When comparing studies before and after LS formation, the orbitofrontal and lateral prefrontal cortices were differentially activated, and functional connections between these structures and the striatum, which contributes to habit learning, were altered. We conclude that changes in the lateral prefrontal cortex during problem solving may contribute to the executive function of working memory and also inhibit control of a primitive learning system, thereby promoting LS formation.

Keywords: macaque monkey, PET, prefrontal cortex, visual discrimination

Introduction

Previous studies for the learning-set (LS) performance in the two-choice object discrimination task in monkeys and human infants suggest that they can acquire and use the abstract structure of 'the alternative', which is defined as the relationship between the stimuli and the problem solving strategy beyond a stationary association between a specific stimulus and response (Harlow, 1948; Restle, 1958; Reese, 1989). We recently reported that a rapid learning acquisition with slow response latency was the most prominent behavioral characteristic of performance in monkeys for the LS with the two-choice object discrimination task (Yokoyama et al., 2004). While slow latency in accurate response seems paradoxical, it is actually suggestive of the higher order executive process in which the prefrontal cortex plays a critical role, namely manipulation of information on the basis of the current plan (Fuster, 1997; Petrides, 1998). Other studies have demonstrated difficulty in LS formation following extensive damage of the prefrontal cortex (Warren and Harlow, 1952; Brush et al., 1961).

Functional brain imaging techniques can characterize dynamic changes in neural activities involved in behavioral performance and can be used to investigate neural processes associated with LS (Honda et al., 1998; Sakai et al., 2002). The present study utilizes PET imaging techniques to assess regional cerebral blood flow (rCBF) as an index of neural activity during the learning phase of the task under the LS paradigm in rhesus monkeys. Specifically, changes in the striatum and anterior inferotemporal cortex were assessed, as previous studies have suggested that these neuroanatomical regions may mediate habit learning (Mishkin and Appenzeller, 1987; Teng et al., 2000; Fernandez-Ruiz et al., 2001). Furthermore, interconnectivity of the prefrontal cortex to these areas was analysed to reveal the functional interaction underlying its major function of the higher order aspects of the cognitive behavior.

Materials and Methods

Subjects

Two male juvenile rhesus monkeys (Macaca mulatta) (monkey A, 4 years old; monkey B, 5 years old) were used. Monkeys were maintained and handled in accordance with the recommendations of the US National Institutes of Health, the guidelines of the Central Research Laboratory, Hamamatsu Photonics K.K., and handled in accordance with the recommendations of the US National Institutes of Health and the guidelines of the Central Research Laboratory, Hamamatsu Photonics K.K. After anatomical characterization of the brain by MRI, an acrylic head holder was attached to the top of the monkey's skull. The head holder was used for painless fixation of the monkey's head during the PET scanning and during behavioral testing.

Behavioral tasks

Two monkeys were trained in the simple vasomotor task (VMT) and the visual discrimination task (VDT) as previously described (Yokoyama et al., 2004). Briefly, the VMT required an immediate lever press below a single visual stimulus that appeared randomly at the right or left side on a display, and the VDT required selection between two different visual stimuli presented simultaneously on the right and left sides on the display. The monkeys were placed on a monkey chair, which was connected to a lever box, facing a CRT display. A Tachisto scope (Iwatsu Iseck) connected to a personal computer (Macintosh PowerBook G3) was used to control the task and to record behavioral data. Each trial was initiated by pressing the center lever on the lever box, resulting in the display of a white fixation spot at the center on a gray background. After 2.5 s, the fixation spot disappeared, and a white square shape appeared randomly at the right or left side on the display. After the appearance of the target, the monkeys were required to release the lever within 3.0 s and to press the right or left lever below the shape within 3.0 s. A drop of water was delivered as a reward immediately after the correct response. Following display of a blank gray background for 150 ms, the fixation spot reappeared, signaling the start of the next trial. Each monkey was allowed to practice the VMT until the task was performed without error and with constant minimal reaction time. The two training problems of the VDT were introduced involving transferable pairs of stimuli. The stimulus of the square shape used in the VMT was also used as a correct stimulus paired with a cross shape in the first training problem, and the cross shape used in the first training problem was transferred to the incorrect stimulus of the second training problem. After practicing with the VDT training problems, PET scans for the pre-LS condition were performed during the acquisition phase of the VDT using a novel pair of visual stimuli and during the VMT without an element of learning as a control. Then, the monkeys underwent several VDT problems using novel pairs as a LS training. After the LS training (monkey A, 17 problems; monkey B, 21 problems), PET scans for the LS condition were performed during the acquisition phase of VDT with additional novel pairs.
PET experiments
The PET study was performed as previously described (Onoe et al., 2001). Thirty-one slices with a center-to-center distance of 3.6 mm were collected simultaneously by PET scanner (SHR7700, Hamamatsu Photonics K.K., Japan). Transaxial resolution of the PET scanner was 2.6 mm at full-width-half-maximum (FWHM) in the enhanced 2-D mode. Scans were performed under unanaesthetized conditions at a sitting position in a scanner that was tilted at 75° from the vertical position. After the delivery of a bolus of [15O]H2O (1.2 GBq in 1.5 ml) via a venous cannula placed into a sural vein, the scan was initiated automatically when the radioactivity in the brain was greater than 30 kcps. The monkey began the behavioral task (40 trials) ~10 s before the start of the PET scan. PET data were collected in 80 s groups (one 40 s period followed by four 10 s periods). Approximately 20 PET scans were performed per day, and the interscan interval was ~15 min. PET experiments were conducted twice weekly, separated by at least 1 day. For the first VDT in the pre-LS condition, 21 scans were conducted in monkey A and 16 scans were conducted in monkey B. VMT was conducted on the same scanning day of VDT (monkey A, 19 scans; monkey B, 14 scans). Successive PET images for each task in the pre-LS condition were obtained from two PET experimental days within one week. In the LS condition after the LS training, PET experiments required an additional 3 weeks. A total of 46 scans for VDT and 21 scans for VMT were collected in monkey A, and a total of 22 scans for VDT and 20 scans for VMT were collected in monkey B. The monkeys solved 3–5 novel problems for VDT during the PET experiment on one day.

Data analysis for PET
PET images summed for the first 60 s epochs were used for statistical analysis. The reconstruction was performed on projection data, after which images were corrected for attenuation using a transmission scan. PET images (voxel size, 1.2 mm3) smoothed with a Gaussian filter of 4 × 4 × 4 mm FWHM were processed using statistical analysis of parametric mapping (SPM96) implemented in MEDx (Sensor Systems PET scanner, SHR7700, Hamamatsu Photonics K.K., Japan). The statistical threshold was set at Z > 3.09. Any region that consisted of less than four-clustered voxels was not considered a significant signal because of the limitation of spatial resolution. To determine the anatomical localization of activated foci, the SPM[Z] PET images were precisely co-registered with the matching MRI image using a three-dimensional alignment program (3D BrainStation, Loats Associates Inc., USA) according to the method described by Onoe et al. (2001). Regions of interest (ROIs) for the caudate nucleus, putamen, lateral prefrontal, orbitofrontal and anterior inferotemporal cortices, which included the activated foci revealed by SPM analysis, were determined on the basis of MRI image of each subject. The mean values of ROIIs from the left and right hemisphere were used for analyzing correlation coefficient between the rCBF values.

Results
Learning performance in the LS condition showed a more rapid increase in percent correct than that in the pre-LS condition, indicating that the monkeys could learn more efficiently based on their experiences. Response latency was also greater in the LS condition when compared with the pre-LS condition (Fig. 1), which is consistent with our previous report (Yokoyama et al., 2004). Behavioral data within the time window used for PET analysis are presented in Table 1. Two-way ANOVA revealed that percent correct was significantly different between the two tasks (VMT versus VDT) [monkey A, F(1,18) = 114.8, P < 0.0001; monkey B, F(1,13) = 22.2, P < 0.0001] but there was no effect of significant conditions or presence of a tasks × conditions interaction. In contrast, release latency showed a significant tasks × conditions interaction. There was no significant difference in trial number between the two tasks.

Areas of increased rCBF when comparing the learning phase of the VDT and the VMT in the pre-LS and LS conditions are illustrated in Figure 2. As expected, activations within the striatum (the putamen in monkey A; the caudate nucleus and putamen in monkey B) were observed in the pre-LS condition. However, these activations were not observed in the LS condition. Significant activations in the anterior inferotemporal cortex were observed in the LS condition in monkey A and under both conditions in monkey B. The orbitofrontal cortex was significantly activated in the pre-LS condition but not in the LS condition in both monkeys. In contrast, a significant activation of the lateral prefrontal cortex was observed in both monkeys in the LS condition but not in the pre-LS condition.

The functional relationship between the activated cortical areas and the striatum (caudate nucleus or putamen) was

Figure 1. Learning performances of individual problems in the pre-LS (a, c) and LS (b, d) conditions during the PET study in monkey A (a, b) and in monkey B (c, d). Closed circles and solid lines show mean values of percent correct, and open circles and dotted lines show mean values of response latency. The values in the pre-LS condition were from one problem, while those in the LS condition were means and SDs (error bars) of multiple problems (n = 20 in monkey A and n = 13 in monkey B).
evaluated by comparing the rCBF values between the two brain regions under the pre-LS and LS conditions (Table 2). Scatter plot diagrams for the correlations of signal intensity showed significant positive correlations between the orbitofrontal cortex and caudate nucleus in the pre-LS and LS conditions (Fig. 3). In contrast, there was a significant negative correlation between the lateral prefrontal cortex and the putamen in the LS condition but not in the pre-LS condition. A significant positive correlation was also observed between the anterior inferotemporal cortex and the caudate in the pre-LS condition but not in the LS condition.

**Discussion**

Activation of the lateral prefrontal cortex during the learning phase was specifically observed in the LS condition. This contrasts with previous reports that demonstrated that ablation of the lateral prefrontal area in non-human primates had no effect on learning performance of the VDT (Passingham, 1972; Mishkin and Appenzeller, 1987). However, the number of solved problems that the subjects experienced before the ablation in that study was smaller than in the present study, which may indicate a more immature state of LS formation and could account for the difference in results. Another study

### Table 1

Behavioral data in the PET study

<table>
<thead>
<tr>
<th>Tasks</th>
<th>Percent correct (%)</th>
<th>Release latency (ms)</th>
<th>Trial number</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monkey A</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Pre-LS condition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VDT</td>
<td>77.8 ± 20.2</td>
<td>418.3 ± 33.8</td>
<td>10.9 ± 0.4</td>
</tr>
<tr>
<td>VMT</td>
<td>100.0 ± 0.0</td>
<td>400.0 ± 44.9</td>
<td>10.8 ± 0.8</td>
</tr>
<tr>
<td>LS condition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VDT</td>
<td>76.4 ± 24.3</td>
<td>518.3 ± 79.4</td>
<td>9.8 ± 1.0</td>
</tr>
<tr>
<td>VMT</td>
<td>100.0 ± 0.0</td>
<td>426.6 ± 56.4</td>
<td>11.2 ± 0.4</td>
</tr>
<tr>
<td><strong>Monkey B</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-LS condition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VDT</td>
<td>80.8 ± 24.5</td>
<td>323.3 ± 10.2</td>
<td>11.8 ± 0.9</td>
</tr>
<tr>
<td>VMT</td>
<td>97.6 ± 3.9</td>
<td>314.5 ± 9.6</td>
<td>11.7 ± 0.5</td>
</tr>
<tr>
<td>LS condition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VDT</td>
<td>79.2 ± 24.2</td>
<td>400.4 ± 62.0</td>
<td>11.4 ± 0.8</td>
</tr>
<tr>
<td>VMT</td>
<td>99.6 ± 1.9</td>
<td>301.4 ± 7.9</td>
<td>12.2 ± 0.4</td>
</tr>
</tbody>
</table>

Data were means±S.D. evaluated within the time window of 30 s from the start of PET scanning.

**Figure 2.** The regions showing significant increase of rCBF (when comparing VDT to VMT) overlaid on MRI images that were aligned with the orbitomeatal (OM) line (a, monkey A; c, monkey B). Graphs indicate percent increases of rCBF over the VMT level of regions indicated by arrows on the corresponding slices (b, monkey A; d, monkey B) in the pre-LS (yellow columns) and LS (red columns) conditions. Z-scores of each region are as follows: OFC (4.42), LPFC (3.48), Pu (4.60) and AIT (3.43) in monkey A, and OFC (4.03), LPFC (3.46), Pu (4.13) and AIT (4.93) in monkey B. LPFC, the lateral prefrontal cortex; OFC, the orbitofrontal cortex; Cd, the caudate nucleus; Pu, the putamen; and AIT, the anterior inferotemporal cortex.
reported more rapid forgetting of a specific stimulus-response association in subjects following LS formation (Bessemer, 1971) and supports the idea that a newly introduced working memory process plays a critical role in LS condition. This working memory process of monitoring the consequence of their selection on the preceding trial (Petrides, 1998), which is required for the strategic response in the LS performance, may contribute to the development of an abstractive plan as 'the alternative'. The lateral prefrontal cortex is also involved in the ability to shift attention between different parts of a stimulus (Dias et al., 1996, 1997), while orbital regions are more concerned with learning whether or not a particular stimulus is associated with reward (Pears et al., 2003). The ability to focus on parts of the stimuli that are relevant for discrimination may be an aspect of LS formation.

The lateral prefrontal cortex in the LS condition showed a negative correlation with the putamen that was significantly activated in the pre-LS condition but not in the LS condition. This result indicates that a functional inhibitory connection between the lateral prefrontal cortex and putamen was plastically acquired during the LS formation. Functional organization of inhibitory processes is an intrinsic property of the prefrontal cortex; although the original hypothesis claimed that inhibitory control was a function of the ventral prefrontal cortex (Mishkin, 1964), recent studies have revealed that the ability to suppress inappropriate response tendencies is present throughout the prefrontal cortex (Diamond and Goldman-Rakic, 1989; Funahashi et al., 1993; Fuster, 1997). These results strongly suggest that the inhibitory connection of the lateral prefrontal cortex to the putamen acquired during the LS formation is required for the suppression of a primitive, habit-like learning system that is dependent on the connections between the striatum and other portions of the cortex.

The striatum mediates learning of the VDT in the pre-LS condition by allowing gradual learning of habits for simple stimulus-reward associations (Teng et al., 2000; Fernandez-Ruiz et al., 2001). Further, the striatum may play a role in transition of visual information from the extrastriate cortex to the premotor

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**Table 2**
Pearson's correlation coefficients between brain regions showing differential activity in the pre-LS and LS conditions

<table>
<thead>
<tr>
<th></th>
<th>LPFC</th>
<th>OFC</th>
<th>AIT</th>
<th>Cd</th>
<th>Pu</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPFC</td>
<td>-</td>
<td>-0.49</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>OFC</td>
<td>-0.21/0.24</td>
<td>-</td>
<td>0.26/0.38</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AIT</td>
<td>0.34/0.61</td>
<td>0.50/0.38</td>
<td>-</td>
<td>-</td>
<td>0.25/0.55</td>
</tr>
<tr>
<td>Cd</td>
<td>-0.49/0.35</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Pu</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

The left lower diagonal matrix is based on data from the pre-LS condition and the right upper diagonal matrix on data from the LS conditions. Values at the left side of a slash are of monkey A and those at the right side are of monkey B. The level of the coefficients to be shown is set at $P < 0.1$ in consideration of small number of data. The bold coefficients are at significant level of $P < 0.05$, and blanks are no correlations. The underlines are in common between both subjects.

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**Figure 3.** Scatter plots diagrams of rCBF between the striatum (caudate nucleus or putamen) and the cortical areas in which significant activation was observed during learning either in the pre-LS or LS condition (a, monkey A; b, monkey B). Pearson's correlation coefficients are shown at right-upper of each graph. The level of the coefficients was set at $P < 0.1$ in consideration of small number of data. The bold coefficients are at a significant level of $P < 0.05$, and NC indicates no significant correlations.
cortex, thereby reinforcing the stimulus-response linkage. The positive correlation between the anterior inferotemporal cortex and the striatum observed in the pre-LS condition suggests that the anterior inferotemporal cortex could serve as a direct input origin of visual information. In the LS condition, this functional connection was reduced, and the anterior inferotemporal cortex activity showed a significant correlation with that of the lateral prefrontal cortex. This result is consistent with reports that demonstrate that while interaction of the temporal cortex with basal forebrain is critical for associative learning and not for strategy implementation, interaction of the temporal cortex with the prefrontal cortex is not required for simple associative learning (Gaffan et al., 2002). This reflects the cognitive process of visual information in the LS condition.

The orbitofrontal cortex that was activated in the pre-LS condition mediates reward-related processing associated with motivational control in learning behaviors (Jones and Mishkin, 1972; Tremblay and Schultz, 1999, 2000). Thus, the lack of significant activation of the orbitofrontal cortex in the LS condition may indicate that reward-prediction values are not critical for learning in the LS condition. However, the positive correlations between the orbitofrontal cortex and caudate nucleus in the pre-LS and LS conditions suggest that the orbitofrontal cortex supports the reward-related aspects of the learning process.

Changes in neural networks reflect the distinctive connections of the cortical areas with the striatum involved in learning before and after the LS formation (Fig. 4). The negative correlation between the lateral prefrontal and striatum is especially unique after the LS formation. In fact, the lateral prefrontal cortex may mediate the higher-order representa- tional process for selections as well as suppress the functional network between the striatum and anterior inferotemporal cortex that regulates the non-cognitive, habit learning with short latency of response in the VDT. Indeed, this suppression could provide a mechanistic explanation for the long latency of response observed as one of the distinctive behavioral features in the LS performance. Since change in effective connectivity indicates plasticity of inter-region functional networks (Buchel et al., 1999), experiences dependent on behavioral transfiguration observed in the LS formation could result from plastic changes in effective connectivity between the striatum and lateral prefrontal cortex. Finally, the maturation of neural circuits for the inhibitory control of the lateral prefrontal cortex during the LS formation with its executive function of the working memory could contribute to the flexibility of learning beyond a stationary association.

### Notes

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### References


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**Figure 4.** Hypothetical neural networks for learning of VDT before and after the LS formation. Enclosed ROI names indicate significantly activated areas compared with the VMT, and shaded ones indicate no activation in each condition. Solid and broken lines between brain regions are adopted on positive and negative correlations commonly observed in both subjects, respectively. The thickness of these lines indicates intensity of value in correlation coefficients. Abbreviations are identical to those indicated in Figure 2.