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VENTROLATERAL PREFRONTAL CORTEX ACTIVITY ASSOCIATED WITH INDIVIDUAL DIFFERENCES IN ARBITRARY DELAYED PAIRED-ASSOCIATION LEARNING PERFORMANCE: A FUNCTIONAL MAGNETIC RESONANCE IMAGING STUDY

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Abstract—To describe the neural substrates of successful episodic long-term memory encoding, we collected functional magnetic-resonance imaging data as participants completed an arbitrary delayed auditory paired-association learning task. During the task, subjects learned predefined but hidden stimulus pairs by trial and error based on visual feedback. Delay period activity represents the retrieval of the relationship between the cue item and its candidate for associates, that is, working memory. Our hypothesis was that the neural substrates of working memory would be related to long-term memory encoding in a performance-dependent manner. Thus, inter-individual variance in performance following a fixed learning set would be associated with differing neural activations during the delay period. The number of learning trials was adjusted such that performance following completion of the learning set varied across subjects. Each trial consisted of the successive presentation of two stimuli (first stimulus and second stimulus [S2]) with a fixed delay interval, allowing extraction of sustained activity during the delay period. Sustained activities during the delay period were found in the bilateral dorsolateral prefrontal cortex, intraparietal sulcus, and left ventrolateral prefrontal cortex, as well as the premotor and pre-supplementary motor areas. The activities did not change in strength across learning, suggesting that these effects represent working memory components. The sustained activity in the ventrolateral prefrontal region was correlated with task performance. Task performance was also positively correlated with the decrement in S2/feedback-related activity during learning in the superior temporal sulcus, a region previously shown to be involved in association learning. These findings are consistent with lesion and neuroimaging studies showing that the ventrolateral prefrontal cortex plays an important role in long-term memory encoding, and raise the possibility that working memory processes interact with long-term memory formation as represented by the covariation of activity in the superior temporal sulcus and the ventrolateral prefrontal cortex.

Key words: controlled selection, fMRI, LTM formation, VLPFC, working memory.

Humans have a remarkable ability to make arbitrary links between events or objects through learning and experience. This dynamic learning process is called long-term memory (LTM) formation (Tulving, 1995). LTM formation supports the encoding and retrieval of memories of events. Recent research in neuropsychology and neuroimaging has underscored the importance of the prefrontal cortex (PFC) in promoting successful LTM formation (for a review see Blumenfeld and Ranganath, 2007). The lateral prefrontal cortex (LPFC) plays a critical role in supporting working memory (WM) (Owen, 1997; D'Esposito et al., 2000), a facet of memory characteristically required in any task that involves the maintenance and manipulation of information over short periods of time (Baddeley, 1992). Hence the role of PFC in LTM formation is probably related to WM processes associated with this region.

Work on the functional segregation of the dorsolateral prefrontal cortex (DLPFC) and the ventrolateral prefrontal cortex (VLPFC) (Owen, 2000; Wager and Smith, 2003; Petrides, 2005; Ranganath, 2006; Blumenfeld and Ranganath, 2007) has suggested that different regions within the PFC might deal with different components of WM processing (Petrides, 1994; Owen et al., 1996; D'Esposito et al., 2000; Ranganath, 2006; Badre and Wagner, 2007). Recently, based on psychophysical and neuroimaging studies, Blumenfeld and Ranganath (2007) proposed that the DLPFC contributes to the organization of multiple pieces of information in WM, enhancing memory for associations between items in LTM. The function of VLPFC is related to

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Abbreviations: BA, Brodmann's area; DLPFC, dorsolateral prefrontal cortex; DMSv, visual delayed-matching-to-sample; EPI, echo-planar imaging; FA, flip angle; fMRI, functional magnetic resonance imaging; FOV, field of view; HRF, hemodynamic response function; IFG, inferior frontal gyrus; IFS, inferior frontal sulcus; IPS, intraparietal sulcus; LPFC, lateral prefrontal cortex; LTM, long-term memory; MNI, Montréal Neurological Institute; MP-RAGE, magnetization-prepared rapid-acquisition gradient echo; MRI, magnetic resonance imaging; PA, paired-association learning; PAa, auditory paired-association learning; PFC, prefrontal cortex; PMd, dorsal premotor area; preCu, precuneus; preSMA, pre-supplementary motor area; rmANOVA, repeated measures of analysis of variance; SPM, statistical parametric map(ping); STS, superior temporal sulcus; S1, first stimulus; S2, second stimulus; TE, echo time; TR, repetition time; VLPFC, ventrolateral prefrontal cortex; WM, working memory.

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the selection of goal-relevant item information that strengthens the representation of these features during LTM encoding (Blumenfeld and Ranganath, 2007). However, few neuroimaging studies have tested both WM and LTM formation simultaneously. Thus, the involvement of WM in LTM formation at the neural level is still largely unknown.

The delayed paired association learning task (Tanabe et al., 2005) provides a means of exploring the relationship between WM and LTM. Tanabe et al. (2005) conducted an experiment using cross-modal and intra-modal delayed paired-association learning (PA) tasks with functional magnetic resonance imaging (fMRI). During the task, following the presentation of the first stimulus (S1), subjects had to select a pairing candidate based on information that was learned in previous trials and retained in memory. When the second stimuli (S2) and feedback (F) were presented, subjects could update information regarding the relationship between S1 and S2. Tanabe et al. (2005) found that the S2/F-related activity in the superior temporal sulcus (STS) peaked early in the learning phase and then decreased, indicating that this area is important for the formation of paired associations. By contrast, fronto-parietal areas including the DLPFC and VLPFC were activated during the delay period, but did not show learning-associated signal changes. Activity in these latter regions might signify a neural substrate for WM processes, which are distinct from, but interact with, LTM formation (Blumenfeld and Ranganath, 2007).

Our first hypothesis was that WM interacts with LTM formation in a performance-dependent manner. To test this hypothesis, we conducted an fMRI experiment with an intra-modal audio-auditory paired-association learning task (PAa). In order to introduce inter-individual differences in learning about stimulus pairs, we halved the number of training trials (which constitute the learning period) used in a previous study (Tanabe et al., 2005) in which all of the participants successfully learned the paired-association task by the end of the final session. We also recruited a larger number of subjects (n=28). This experimental design ensured a larger variance in task performance at the end of the (shortened) learning period in the present study. In addition, we introduced a visual delayed matching-to-sample (DMSv) task that was interleaved with the PAa task. To minimize contamination between the learning and the control tasks, we used a different stimulus modality in the two tasks. The DMSv task entailed active maintenance of item (S1) without learning, whereas the PAa task required additional processing such as retrieval of the relationship between the cue item (S1) and its candidate of associates during the delay period. Therefore performance in the DMSv task should have been high throughout the experiment, allowing us to exclude the possibility that individual differences in vigilance and/or attentiveness contributed to performance in the learning task. We expected that individuals who were faster at learning the paired associations would show more prominent activation of the WM-related fronto-parietal areas during the delay period.

Our second hypothesis was that the learning-related decrement in the S2/F-related activity in the STS is correlated with performance across subjects. Because of the induced variability in performance in the present study, the decrement-specific regions could not be defined. Thus, we determined the location of LTM formation-related STS by referring to a previous fMRI study (Tanabe et al., 2005).

EXPERIMENTAL PROCEDURES

Subjects

In total, 28 healthy volunteers participated in the experiment (13 females and 15 males; mean age=24.8 years; age range=20–39 years). All of the subjects had normal or corrected-to-normal visual acuity and normal hearing, and were right-handed according to the Edinburgh Handedness Inventory (Oldfield, 1971). The protocol was approved by the Ethical Committee of the National Institute for Physiological Sciences, Japan. The experiments were undertaken in compliance with national legislation and the Code of Ethical Principles for Medical Research Involving Human Subjects of the World Medical Association (Declaration of Helsinki). All of the subjects gave their written informed consent for participation.

Experimental design and task procedure

A PAa task was interleaved with a DMSv task. We employed a different stimulus modality to minimize contamination between the learning and the control tasks. Although the experimental design of the PAa and the DMSv tasks was similar, the DMSv task had fewer WM demands and was therefore easier rather than the PAa task. For this reason we could use the DMSv task as a control for vigilance and/or general attentiveness. In the PAa task, the subjects had to identify three predefined audio-auditory pairs out of 15 possible pairs in a trial and error manner. The order of presentation of each pair of stimuli (i.e. S1 and S2) was varied, and 30 possible different patterns of S1–S2 presentation were produced from the six sounds. In the DMSv task, subjects judged whether the second visual stimulus was the same as the first. The sound stimuli were generated by temporally modulating 500 ms white noise (sampling rate=44.1 kHz; stereo sound) using MATLAB 6.5 (MathWorks, Natick, MA, USA) and GoldWave 4.26 (GoldWave, Inc., St. John's, NL, Canada). The sound waves are shown on the left side of Fig. 1a. We used the same visual stimuli for the DMSv task as in our previous study (Tanabe et al., 2005); these comprised two-dimensional amorphous texture patterns that were downloaded free of charge from the Internet (http://page.freett.com/amorphis). The size and contrast of the stimuli were modified to 4×4° and subtended a visual angle of 19×14° (Fig. 1b).

Stimulus presentation and response collection were performed using Presentation 0.90 (Neurobehavioral Systems, Albany, CA. USA) software implemented on a personal computer (Dimension 8200; Dell Computer, Co., Round Rock, TX, USA). A liquid crystal display (LCD) projector (DLA-M200L; Victor, Yokohama, Japan) located outside and behind the scanner projected the stimuli through another waveguide onto a translucent screen, which the subjects viewed via a mirror attached to the head coil of the magnetic resonance imaging (MRI) scanner. The auditory stimuli were presented via MRI-compatible headphones (Hitachi Advanced Systems, Yokohama, Japan). For each subject, the volume of the sound was adjusted to an appropriate level for task execution in the context of the MR scanner noise. Responses were collected via an optical button-box (Current Designs, Inc., Philadelphia, PA, USA).

The task was explained to the subjects in detail, and they were trained to recognize all of the auditory and visual stimuli prior to the scanning session. The subjects were instructed not to use verbalization or labeling strategies to memorize the presented

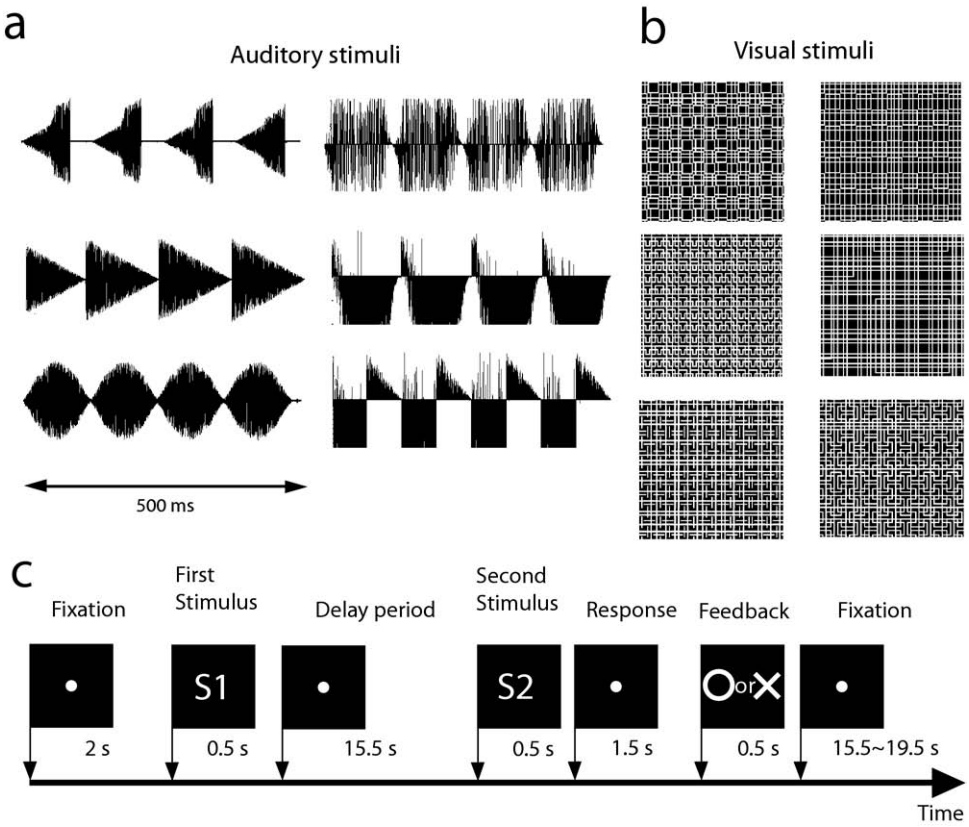


Fig. 1. Stimuli and schematic diagram of a trial. (a) Auditory stimuli for the PAa learning task are shown in waveform. (b) Visual stimuli for the DMSv task. These stimuli were the same as those used in the visual paired-association learning (PAv) learning task in our previous study. (c) Schematic diagram of a trial. Subjects were instructed to perform the PAa task if the S1 was auditory. They were required to execute the DMSv task if the S1 was visual. Subjects remembered the S1 (and/or the stimulus with which it is paired in the case of the PAa task) during a 15.5 s delay period within which they viewed a blue fixation stimulus. Then, a S2 (auditory in PAa, visual in DMSv) was shown. The subjects responded with a button press with the pre-assigned finger (to indicate "a pair" or "not a pair" in the PAa task, and "the same" or "not the same" in the DMSv task) when the fixation stimulus turned red. Then, visual feedback was presented, allowing the subjects to learn the auditory pairs via trial and error in the PAa task. In the DMSv task, subjects could confirm whether their response was correct using the visual feedback. The two tasks were interleaved and presented pseudo-randomly.

stimuli and the relationships between them throughout the experiment. During the sessions, the subjects' eyes were required to fixate a point. Each trial consisted of the successive presentation of a pair of stimuli (S1 and S2) with a fixed S1–S2 interval (15.5 s); the duration of each stimulus was 500 ms (Fig. 1c). If the S1 was presented auditorily, the subject was required to perform the PAa task; if the S1 was presented visually, the subject performed the DMSv task. The S2 stimulus subsequently disappeared and the fixation point turned red, cuing the subject to respond using a pre-assigned button with either the right index or middle finger. The subjects reported whether S1 and S2 were "a pair" or "not a pair" in the PAa task, or "the same" or "different" in the DMSv task. The subjects were asked to respond as quickly and accurately as possible. Pictorial positive (o) or negative (x) feedback (F) was presented 1500 ms after the disappearance of S2. The subjects were asked to correctly pair the stimuli using this feedback information in the PAa task. As feedback was also given in the DMSv task, the subjects could verify whether each response was correct. If the subject missed the stimuli (i.e. did not hear or see S1 and/or S2), he/she was asked to make no response. These missed trials were excluded to calculate accuracy rates (correct response rate). The two tasks were randomly interleaved, and the inter-trial intervals were pseudo-randomized to be 15.5, 17.5, or 19.5 s in length; 10 s was added every six trials allow for scans of baseline neural activity. Each session contained 18 trials (nine trials of the PAa task and nine trials of the DMSv task), and a total of six sessions were run. The number of the trials in the PAa task containing paired stimuli which had been associated, and trials containing non-associated paired stimuli was four and five trials per session, respectively. This experimental structure was the same as that used in our previous study (Tanabe et al., 2005). However, to introduce inter-individual differences in stimulus pair learning, we halved the number of training trials (which constitute the learning period) used in the previous study. Together with recruitment of a larger number of subjects, this experimental design ensured a larger variance in task performance following the (shortened) learning period.

MRI data acquisition

All images were acquired using a 3 T MR scanner (Allegra, Siemens, Erlangen, Germany). For functional imaging during the sessions, an interleaved T2*-weighted gradient-echo echo-planar imaging (EPI) procedure was used to produce 34 continuous 4-mm-thick transaxial slices covering the entire cerebrum and cerebellum (repetition time [TR]=2000 ms; echo time [TE]=30 ms; flip angle [FA]=75°; field of view [FOV]=192 mm; 64×64 matrix; voxel dimensions=3.0×3.0×4.0 mm). Oblique scanning was used to exclude the eyeballs from the images. For anatomical imaging, T1-weighted magnetization-prepared rapid-acquisition gradient echo (MP-RAGE) images scanned at the same locations as those used for the EPI were obtained from each subject

(TR=1460 ms; TE=4.38 ms; FA=8°; FOV=192 mm; number of slabs=1; number of slices per slab=36; voxel dimensions=0.9×0.8×4.0 mm). To acquire a finer structural whole-head image, MP-RAGE images were also obtained (TR=2500 ms; TE=4.38 ms; FA=8°; FOV=230 mm; number of slabs=1; number of slices per slab=192; voxel dimensions=0.9×0.9×1.0 mm).

Each session consisted of a continuous series of 365 volume acquisitions with a total duration of 12 min 14 s. To avoid subject fatigue, several breaks (approximately 10 min per break) were inserted within the six sessions (in a typical case, the order of presentation was three sessions/break/three sessions). The total duration of the experiment was approximately 180 min, including the instruction period, the practice time, and the acquisition of the structural MR and fMR images.

Image preprocessing

The first seven volumes of each session were eliminated to allow for stabilization of the magnetization, and the remaining 358 volumes per session (a total of 2148 volumes per subject for six sessions) were used for the analysis. To enable the results to be compared with those of our previous study (Tanabe et al., 2005), the same software and procedure were adopted for the analysis. Statistical Parametric Mapping software (SPM99, Wellcome Department of Imaging Neuroscience, London, UK) was used for preprocessing. After correcting for differences in slice timing within each image volume, all of the volumes were realigned for motion correction. Each structural image volume from the same slice position was co-registered with the image volume of the eighth scan, and the whole-head MP-RAGE image volume was then co-registered with this structural image volume. The whole-head image volume was normalized to the Montréal Neurological Institute (MNI) T1 image template using a nonlinear basis function. The same parameters were applied to all of the EPI volumes, which were spatially smoothed in three dimensions using a 10-mm full-width at half-maximum gaussian kernel.

Evaluation of sustained delay activity

The sustained activity during the S1–S2 delay period was analyzed using a conventional statistical parametric mapping (SPM) approach. To depict the neural substrates of the task, the neural responses to S1 and S2/feedback (S2/F), as well as the delay activity between S1 and S2, were modeled with a hemodynamic response function (HRF) which combines two gamma functions (as described by Friston et al., 1998a,b, 2007) without a temporal derivative for each subject. Contrast images of the sustained activity of each subject were used for the group analysis with a random-effects model, in order to make inferences at the population level (Friston et al., 1999, 2007). The resulting set of voxel values for each contrast constituted an SPM of the t statistic (SPM{t}), which was transformed into normal distribution units (SPM{Z}). The threshold for the SPM{Z} was set at $Z>3.09$ ($P<0.001$) at the voxel level and $P<0.05$ (minimum cluster volume was 353 voxels) with a correction for multiple comparisons at the cluster level for the entire brain (Friston et al., 1996, 2007), unless otherwise indicated.

Correlation between sustained activity and task performance

To depict correlations between neural activities during the delay period and performance across the subjects, we conducted a correlation analysis between the sustained activity during the delay period for each subject and the individual's task performance of the final session. Task performance was measured as response accuracy, the number of correct response divided by total number of PA trials responded to in the session. As the subject was asked to make no response when he/she missed the stimuli (i.e. did not hear or see S1 and/or S2), these missed trials were excluded from the analysis. Parameter estimate (β) of the regressor of delay period was adopted as the measure of the sustained activity. The parameter estimates of the regressor of interest in each voxel constitute contrast images, representing the percent signal change relative to the mean global MR signal, which was scaled to 100 (Friston et al., 2007). Contrast images of the sustained activity of each subject were used for the group analysis in a random-effects model, incorporating task performance in the final session as a covariate (regressor).

At the local maximum within each region showing an effect in the correlation analysis (i.e. anterior IFS), we calculated the β estimates of the sustained activity during each session in each subject, and plotted this against session for each individual.

Evaluation of the learning effect in the STS in response to S2/F

The component of brain activity in the STS showing a learning effect was observed based on the peak activity in response to S2/F. The method used to obtain the time course of each trial from the MR signal time series data was the same as that in our previous study (Tanabe et al., 2005). The MR signal data were first filtered with high-pass (cutoff frequency at 120 s) filters within a session. PAa(1, 1) is the scan volume acquired during the initial presentation of the auditory S1, and PAa(i, 1) is the ith presentation of S1 (i=1∼54). The ith PAa-condition trial consists of PAa(i,1), PAa(i, 2), PAa(i, j) . . . . PAa(i, 20), which represents the consecutive scan volumes acquired with a time interval of 2 s (j=1∼20). Within each trial, percentage normalization was then performed. Thus PAa(i, j) is the percentage signal change of the jth scan volume in the ith PAa trial compared with baseline (average of the first two-volume scan points, j=1, 2) of the same trial. A typical linear detrending method, which computes the least squares fit of a straight-line to the data and subtracts the resulting function from the data, was also applied within each trial. These MR signal data were averaged in each session, so that six time series data were obtained in the present study.

To depict the individual differences in learning, we analyzed the correlations between accuracy in the final PAa session and the MR signal decrease from the first to the final session in the STS at the thirteenth scan. The STS locus (x=−60, y=−22, z=−4) and scan point (thirteenth scan) in the present study were the same as those used by Tanabe et al. (2005) in which a learning-related decrement of the S2/F related activity was found only in the STS.

RESULTS

Performance

To evaluate performance between different tasks and sessions, a two-way repeated measures of analysis of variance (rmANOVA) incorporating the effects of both task and session was conducted. This revealed that there were significant main effects of session ($F_{(5,135)}=9.08$; $P<0.001$), task ($F_{(1,27)}=175.15$; $P<0.001$) and a significant interaction between task and session ($F_{(5,135)}=5.85$; $P<0.001$). Fig. 2a shows that the learning effect (measured as improvement in performance) in the PAa task is more prominent in than that in the DMSv task, the latter of which showed a saturation in accuracy as learning progressed. In contrast, a two-way rmANOVA of RT showed significant main effects of session ($F_{(5,135)}=5.685$; $P<0.001$) and task ($F_{(1,27)}=101.519$; $P<0.001$), but no significant interaction ($F_{(5,135)}=1.54$; $P=0.18$). Fig. 2b shows the reduction in RT for both tasks, suggesting the

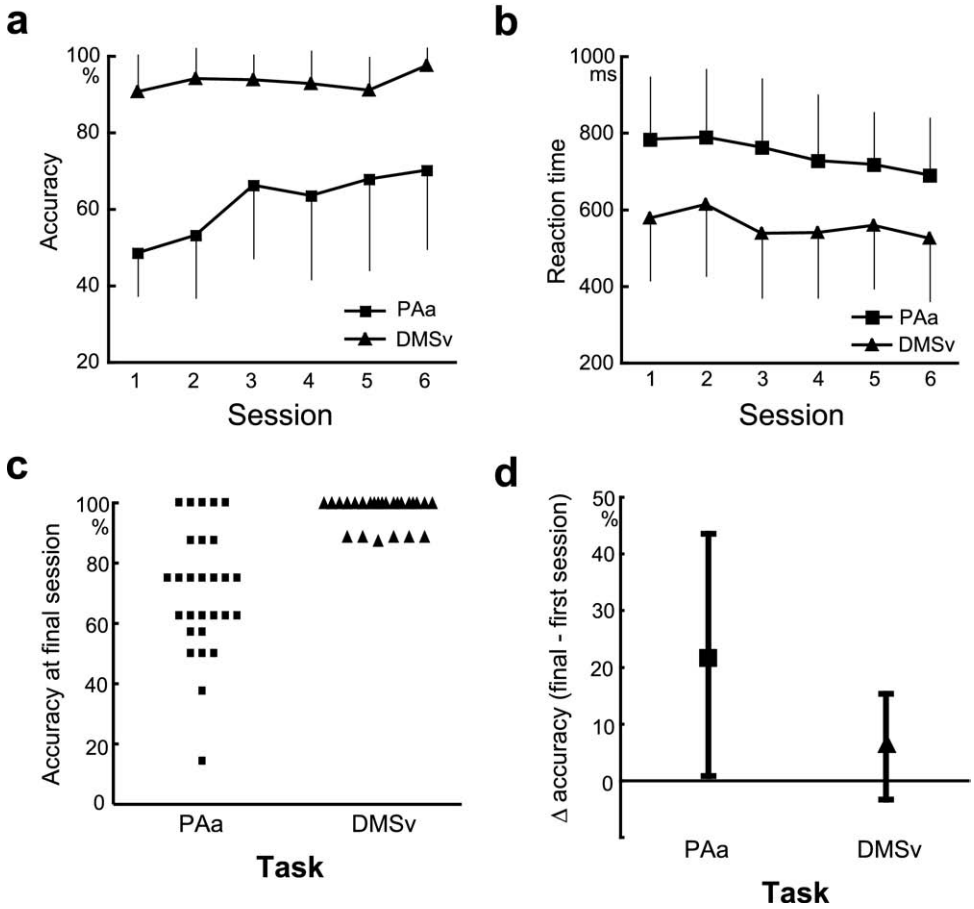


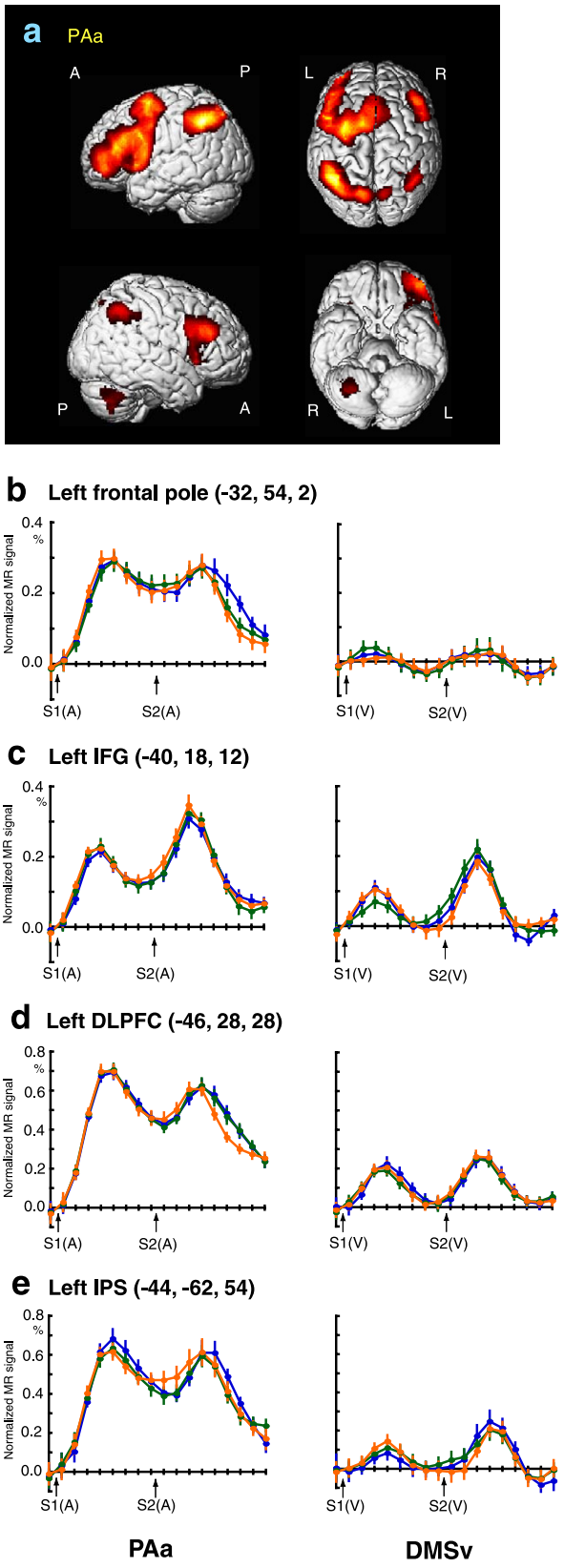
Fig. 2. Plots of the behavioral data. (a) Time course of response accuracy in the PAa and DMSv tasks. Filled square, PAa task; filled triangle, DMSv task. Error bars indicate ±1 SD. (b) Time course of reaction time in the PAa and DMSv tasks. Filled square, PAa task; filled triangle, DMSv task. Error bars indicate ±1 SD. (c) Accuracy rates of all subjects during the final session in the PAa and DMSv tasks. Each dot indicates an individual subject. Filled square, PAa task; filled triangle, DMSv task. (d) Difference in accuracy during the first and final sessions. The filled square shows the average performance on the PAa task whereas the filled triangle shows the average performance on the DMSv task. Error bars indicate ±1 SD.

presence of a non-specific learning effect such as familiarity with the task procedure. Fig. 2c shows the accuracy rates (correct response rates) during the final session in both the PAa and the DMSv tasks. The results showed a large variance in the accuracy rate during the PAa task (filled square; mean=70.7%, standard deviation (SD)= 20.7%), whereas the subjects' performance was highly accurate with only a small variance during the DMSv task (filled triangle; mean=97.6%, SD=4.75%). To confirm this, the difference in the accuracy rate between the first and final sessions (Δ accuracy) was calculated for each individual (Fig. 2d). These results also showed a large variance in PAa task performance (mean=22.1%, SD= 20.7%) compared to the DMSv task (mean=5.88%, SD=9.33%; Fig. 2b). The results of the DMSv showed that vigilance and/or attentiveness was maintained throughout the experiment for all subjects. During the debriefing session following the experiment, many of the subjects stated that they had not learned all of the pairs in the PAa task; this is likely to be due to the small number of trials allowed for learning the pair associations. The subjects who performed at <100% accuracy during the final session reported that the some of the pairings were ambiguous, and that they required feedback to deduce the correct pairs during the final session.

Sustained activity during the delay period

To detect WM-related activity, sustained activity elicited by the PAa task during the delay period was analyzed. Activation of the LPFC, the inferior frontal gyrus (IFG), the pre-supplementary motor area (preSMA), the dorsal part of the premotor area (PMd), the intraparietal sulcus (IPS), the precuneus (preCu), and the right cerebellum was observed (Fig. 3a, Table 1). To verify that these activated regions did not change during learning, we applied regression analysis to the delay-period data, similar to that used in our previous study (Tanabe et al., 2005). The results showed that no significant changes were observed in these brain regions (data not shown). Time course plots of the averaged signals of those regions in the first, fourth, and sixth sessions revealed sustained activation during the delay period throughout the sessions (i.e. there was no increase or decrease as the sessions proceeded) in the PAa task (Fig.

3b–e, left panels). The DMSv task did not activate these areas during the delay period (Fig. 3b–e, right panels).



Prefrontal activation correlated with task performance

Correlation analysis was performed to depict the areas where activation was correlated with task performance during the delay period. The results showed that only the left VLPFC activity was highly correlated with task performance in the final session (Fig. 4a). Overlap between this region and those that were associated with WM during the delay period was explored by applying an inclusive mask of PAa. This revealed two clusters in the left VLPFC (Fig. 4b): one in the anterior portion of the LPFC/inferior frontal sulcus (IFS; Brodmann's area (BA) 10; Fig. 4c, left panel), and one in the VLPFC/IFG (BA 45/47; Fig. 4c, right panel). Fig. 4d shows the correlation between task performance during the final session and the local signal change (parameter estimate) maximum in the anterior IFS ($x=-32$, $y=36$, $z=6$) and the IFG ($x=-46$, $y=18$, $z=0$) (Fig. 4d; left IFS, $r=0.70$, $P<0.01$; left IFG, $r=0.60$, $P<0.01$). To confirm the left-lateralization of this activity, we also examined the coordinates of this local signal change on the opposite side of the brain (Fig. 4d; right IFS, $r=-0.19$, $P=0.34$; right IFG, $r=0.36$, $P=0.06$). In addition, the correlation between the activity in the bilateral DLPFC (left, $x=-44$, $y=28$, $z=30$; right, $x=50$, $y=30$, $z=34$) and task performance confirmed that only the left VLPFC activity was correlated with task performance (Fig. 4d; left DLPFC, $r=0.002$, $P=0.99$; right DLPFC, $r=0.07$, $P=0.72$).

In the anterior IFS, no subject showed a statistically significant correlation between the estimated delay period activity and performance accuracy in each session, with averaged correlation coefficient (r) of -0.07 (± 0.37). The averaged delay period activity did not change across session (Fig. 4e black; repeated measures ANOVA, $F_{(5,135)}=0.418$; $P=0.84$) but varied across subjects: delay period activity was higher throughout the sessions in the subjects who performed well (Fig. 4e red and orange), and low in those whose performance was lower (Fig. 4e sky blue and blue).

The learning effect and S2/F-related activity in the STS

We then focused on the learning-related signal change following presentation of S2/F. To examine the precise learning-related activity changes, we calculated the correlation between the MR signal change in the STS and task performance across subjects. An individual's MR signal change was calculated as follows: MR signal change= (mean MR signal at the thirteenth scan period during the first session of the PAa task)–(mean MR signal at the

Fig. 3. Sustained neural activities during the delay period. (a) Activation map of the PAa task. (b–e) Time courses of activity. (b) Left frontal pole ($x=-32$, $y=54$, $z=2$). (c) Left IFG ($x=-40$, $y=18$, $z=12$). (d) Left DLPFC ($x=-46$, $y=28$, $z=28$). (e) Left IPS ($x=-44$, $y=-62$, $z=54$). The time course was collapsed across trials in sessions # 1 (blue), # 4 (green), and # 6 (orange) in the PAa (left) and DMSv (right) tasks. Error bars indicate ± 1 standard error of the mean (SEM). For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

Table 1. Fields showing sustained activity during the delay period in the PAa task

Condition	Cluster level *P*-value	Cluster size	MNI coordinates			*Z*-value	Voxel level *P*-value	Side	Location	BA
			x	*y*	*z*					
PAa	<0.001	4366	−44	−54	48	7.57	<0.001	L	IPS	7/40
	−10	−72	46	5.97	<0.001	L	preCu	7		
	10	−68	52	4.64	<0.001	R	preCu	7		
	<0.001	12,001	−30	2	62	7.23	<0.001	L	PMd	6
	−44	30	28	6.62	<0.001	L	DLPFC	46		
	−32	28	10	6.62	<0.001	L	IFG	45		
	−12	6	60	6.13	<0.001	L	preSMA	6		
	−36	52	8	6.04	<0.001	L	FP	10		
	<0.001	2335	40	30	26	5.81	<0.001	R	DLPFC	9/46
	40	18	14	4.39	<0.001	R	IFG	45		
	<0.001	1605	40	−50	52	5.42	<0.001	R	IPS	7/40
	0.004	707	32	−62	−38	5.25	<0.001	R	Cerebellum	

The result of the random-effects analysis for the sustained activity during the delay period in the PAa task is listed. The extent threshold was set at $P<0.05$ (minimum cluster volume 353 voxels) with a correction for multiple comparisons at the cluster level for the entire brain. The height threshold was set at $Z>3.09$ ($P<0.001$, uncorrected) at voxel level.

thirteenth scan period during the sixth session of the PAa task). The results showed a statistically significant correlation between PAa task performance and STS signal change during the PAa task (Fig. 5, $r=0.57$, $P<0.01$). Across participants, larger decreases in the STS signal from the early to late learning phases of the PAa task occurred in the subjects who learned the pairing associations well; no such decrease in STS activity was observed across DMSv sessions.

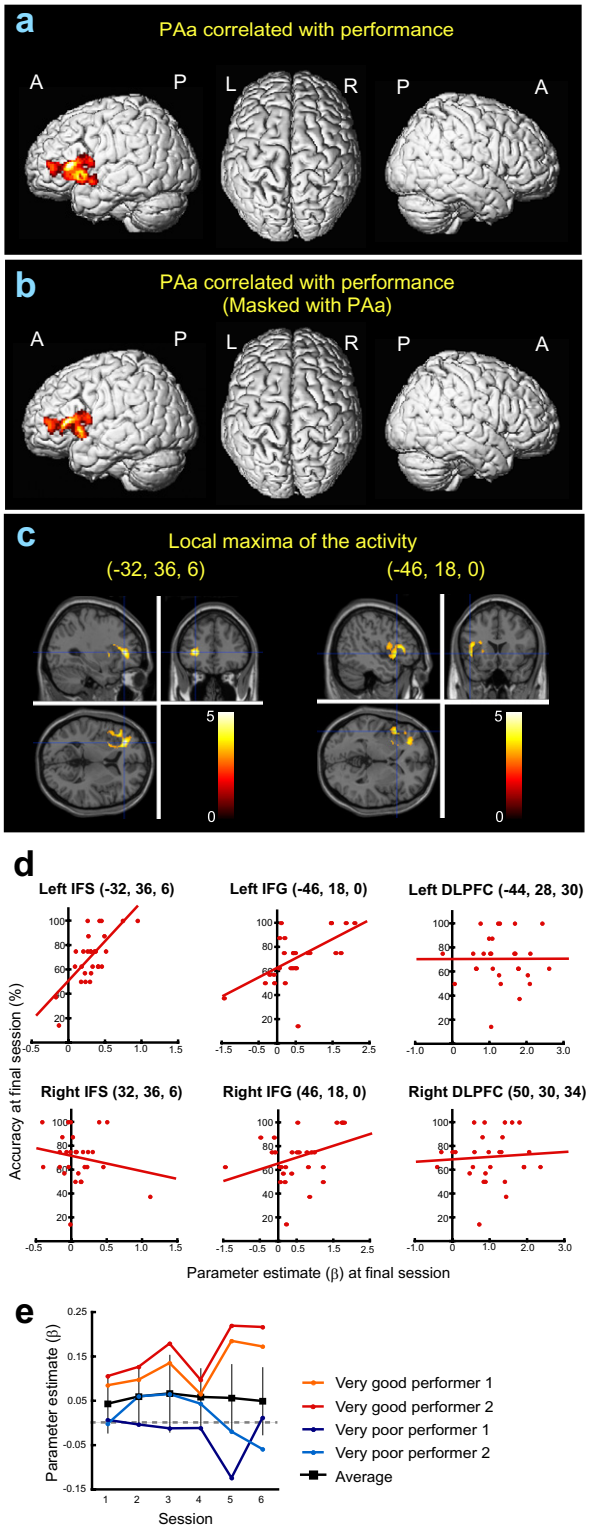
DISCUSSION

As expected, accuracy in the PAa task varied greatly between subjects, even during the final testing session (Fig. 2a, b). Response accuracy in the DMSv task was high across testing blocks for all subjects, indicating that vigilance and attentiveness were maintained throughout the experiment. This suggests that the variable performance observed in the PAa task is not related to individual differences in general attentiveness. Subjects reported no difficulty in switching between the tasks, and cognitive or perceptual interference between the tasks is unlikely given that performance on the DMSv task remained high throughout the experiment.

In the present study, we focused on the WM function, which refers to the processes that enable the maintenance and manipulation of information just experienced, but no longer existing in the external environment, or retrieved from LTM, during paired association learning. In the PA task, fronto-parietal areas (e.g. the DLPFC, VLPFC, preSMA, PMd, IFG and IPS) were active throughout the delay period. The stimulus-related effect shows a pattern with two peaks, likely reflecting a non-specific attentional effect, as this was also observed in the DMSv task. As no auditory area showed delay period activity (see supplementary figure), the delay period activity of the fronto-parietal areas is unlikely to be directly related to the prior presentation of acoustic stimuli. The activated areas shown in Fig. 3 coincide well with those that were identified by Tanabe et al. (2005) as being activated during the delay period of a delayed PA paradigm, irrespective of the modalities (auditory, visual) of the paired stimuli. The present and the previous studies (Tanabe et al., 2005) adopted a paired-association task that enables investigation of the neural substrates of memory formation and organization (Eacott and Gaffan, 1992; Gutnikov et al., 1997; Hasegawa et al., 1998; Sakai and Miyashita, 1991; Tomita et al., 1999). The delay inserted between presentation of stimuli requires subjects to hold a stimulus “on line” before responding to a choice of S2, and hence recruits WM (Baddeley, 1992; Smith and Jonides, 1998). In the present and the previous studies (Tanabe et al., 2005), we added to the experimental design a learning component, that is, the selection of the correct paired association item, as well as the retention of this information during the delay period.

Here, selection is considered as a top-down process, because selection of the item paired to S1 was based on information about the association acquired by previous trial and error learning. Recent evidence from lesion and electrophysiological studies suggests that association memory retrieval requires top-down modulation (Eacott and Gaffan, 1992; Gutnikov et al., 1997; Hasegawa et al., 1998; Tomita et al., 1999). This notion is further supported by human functional neuroimaging studies (Bunge et al., 2004; Ranganath et al., 2004). One of the functions of the LPFC is to make selections from multiple conceptual representations during task performance (Kan and Thompson-Schill, 2004). According to the biased-competition model (Desimone and Duncan, 1995), selection is mediated by an attentional template that encodes stimulus properties relevant to the goal of a task (Kan and Thompson-Schill, 2004). In the present study, this template can be considered to be the learned relationship between the items. When the probe signal (S1) was presented, selection of the paired stimulus was biased by this attentional template. Thus, sustained activity in the fronto-parietal cortices during paired association learning might represent the active

and transient relationship between the items (attentional templates), the selected items, and the selection process itself (Fuster et al., 2000). Recent studies implicate the parietal cortex in WM storage (Vogel et al., 2005), whereas frontal areas are responsible for executive control of functions (Bor and Owen, 2006).



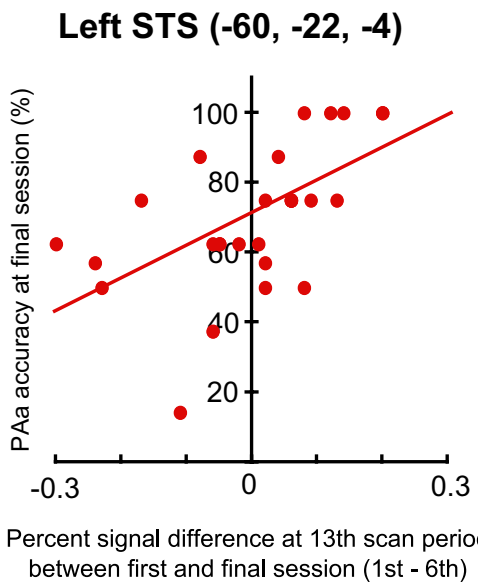


Fig. 5. Correlation between the decrement of brain activity in the STS and task performance. Correlation between the PAa performance in the final session and the difference in MR signal between the first and sixth session in the STS. The STS locus (x=−60, y=−22, z=−4) and scan point (thirteenth scan) were the same as those used by Tanabe et al. (2005).

Sustained activity during the delay period was unchanged as learning progressed (Fig. 3b–e). This finding suggests that the workload related to WM was unchanged during learning in each individual, on the other hand, the delay period activity varies across subjects. We hypothesized that better performance in the paired association learning task would be associated with more prominent activation of the WM-related fronto-parietal areas during the delay period. The results demonstrate a statistically significant correlation between association learning task performance, as observed in the final testing session, and the strength of the sustained activity during the delay period in the left VLPFC (Fig. 4a). This region overlaps with fronto-parietal areas that might support WM processes

Fig. 4. Correlation between brain activation during the PAa task and task performance in the final session. (a) Regions showing brain activity that correlated with task performance (across the whole brain). (b) Common regions showing brain activity that correlated with task performance and learning-related sustained activity during the delay period (masked inclusively with a PAa activation map). (c) Local maxima of the activity superimposed on sections of a high-resolution MR image. The panel on the right shows the IFS region (x=−32, y=36, z=6). The panel on the left shows the IFG region (x=−46, y=18, z=0). (d) Correlation between the accuracy at the final session in the PAa task and the parameter estimate (β) at the final session in the bilateral IFS (x=±32, y=36, z=6), IFG (x=±46, y=18, z=0), and DLPFC (left, x=−44, y=36, z=6; right, x=50, y=30, z=34), respectively. (e) Effect sizes of activation (parameter estimates) in the IFS in each session averaged across subjects (black, filled square), for two subjects with good performance (red and orange filled circles, respectively) and two subjects with poor performance (sky blue and blue filled circles, respectively). Error bars indicate ±1 SD. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

(Fig. 4b). As there was no such correlation with the DLPFC or parietal activation, the VLPFC might represent specific subcomponents of WM function in associative memory learning (Petrides, 1994; Owen et al., 1996; D'Esposito et al., 2000; Ranganath, 2006). In the present study, the learning performance of each subject was predicted by the magnitude of the VLPFC activity during the delay period; thus subjects who learned the association faster showed higher delay-related VLPFC activity. Previous psychological studies have reported that inter-individual performance differences in WM capacity are partly determined by the efficiency with which irrelevant items are excluded (Vogel et al., 2005; Bor and Owen, 2006), and therefore related to the selection process. Hence, correlation between task performance requiring LTM encoding and the sustained activity in the left VLPFC during the delay period might suggest that a subcomponent of WM responsible for stimulus selection is involved in LTM formation.

According to previous studies, the VLPFC is involved in the active retrieval and selection of relevant items (Petrides, 1994; Ranganath and D'Esposito, 2005; Badre and Wagner, 2007; Murray and Ranganath, 2007). One possible explanation for the correlation between the VLPFC activity and task performance is that the learning process requires controlled (top-down) selection to identify the object to be retrieved (Badre and Wagner, 2007; Blumenfeld and Ranganath, 2007). "Proactive interference" demonstrates how learning a previous association (A–B) makes it more difficult to learn a new and overlapping association (A–C) (Barnes and Underwood, 1959; Badre and Wagner, 2005). The PA tasks used in the present study required the subjects to form paired associations that were compared with feedback information in each trial. Thus, in a subsequent trial, it was essential to direct attention towards the goal-relevant information (i.e. correct association) or to inhibit the influence of irrelevant information (i.e. incorrect association). Overcoming proactive interference requires the engagement of controlled selection processes to resolve this competition (Badre and Wagner, 2005, 2007; Blumenfeld and Ranganath, 2007); presumably, the better the resolution of the proactive interference, the easier the retrieval of the associated stimuli. Using a recent probe task with positron-emission topography, Jonides et al. (1998) showed that left IFG activity was related to the resolution of proactive interferences. D'Esposito et al. (1999) demonstrated similar results with fMRI (for a review, see Jonides and Nee, 2006). Thus, the VLPFC might contribute to PA through the resolution of proactive interference for the active retrieval and selection of relevant items.

In the present study, the decrement of the S2/F-related activity in the STS was correlated strongly with performance on the PAa task across subjects (Fig. 5). Although both STS and VLPFC are related to LTM formation in a performance-dependent manner, the former showed a learning-related temporal change in activity, whereas the latter showed time-invariant activation. This implies that the VLPFC and the STS contribute to different stages of LTM formation. Previously Tanabe et al. (2005) found that the S2/F-related STS activity gradually decreases as learning proceeds. S2/F stimuli prompt the comparison of the items held in WM with S2, leading to the possible updating of learned associations. The arbitrary relationship between two paired stimuli gradually becomes strengthened as learning proceeds and feedback becomes increasingly congruent with expectations, and progressively less work is required to build this link. Thus, the STS is part of a neural system that supports LTM formation, functioning as a "comparator" (Tanabe et al., 2005), the function of which is to evaluate goal-relevant similarities and differences between items in the perceived item and those held in WM. Without selection of the relevant items in the WM, a "comparator" could not function. Thus it is conceivable that WM processes might be the primary determinant of performance in tasks that rely on LTM formation.

CONCLUSION

In the present study, we demonstrated that the activity of the left VLPFC during the delay period is highly correlated with final task performance in a delayed PA task. This provides direct evidence for a relationship between WM-related activity and learning of paired associations over time, and supports the hypothesis that WM processes, such as active selection and retrieval, are of central importance to the VLPFC's contribution to successful LTM formation.

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APPENDIX

Supplementary data

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.neuroscience.2009.02.078.

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