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Correlation between prefrontal cortex activity during working memory tasks and natural mood independent of personality effects: An optical topography study

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ABSTRACT

Interactions between mood and cognition have drawn much attention in the fields of psychology and neuroscience. Recent neuroimaging studies have examined a neural basis of the mood–cognition interaction that which emphasize the role of the prefrontal cortex (PFC). Although these studies have shown that natural mood variations among participants are correlated with PFC activity during cognitive tasks, they did not control for personality differences. Our aim in this study was to clarify the relationship between natural mood and PFC activity by partialling out the effects of personality. Forty healthy adults completed self-report questionnaires assessing natural mood (the Profile of Mood States) and personality (the NEO Five-Factor Inventory and the Behavioral Inhibition/Activation Systems scales). They performed verbal and spatial working memory (WM) tasks while their PFC activity was measured using optical topography, a non-invasive, low-constraint neuroimaging tool. Correlation analysis showed that the level of negative mood was inversely associated with PFC activity during the verbal WM task, which replicated our previous findings. Furthermore, the negative correlation between negative mood and PFC activity remained significant after controlling for participants' personality traits, suggesting that natural mood is an independent contributing factor of PFC activity during verbal WM tasks.

1. Introduction

Over the past decades, psychologists have shown that many cognitive processes, such as working memory (WM), are closely related to a person's subjective mood (Mitchell and Phillips, 2007). Neuroimaging studies have begun to elucidate the underlying brain mechanisms, which have implicated the prefrontal cortex (PFC) as a key region responsible for the mood–cognitive interaction (Pessoa, 2008). For instance, a functional magnetic resonance imaging (fMRI) study showed that dorsolateral PFC activation during an N-back working memory (WM) task was decreased when negative mood was induced by the viewing of aversive film clips (Qin et al., 2009). Another fMRI study reported that PFC activity during WM tasks (using words and faces as stimuli) was modulated by positive and negative mood inductions in a stimulus-specific (words vs. faces) manner (Gray et al., 2002). Although some studies have demonstrated how brain activations related to cognitive processes are affected by emotions using cognitive tasks involving emotional stimuli (e.g., emotional Stroop tasks), they did not directly focus on the interaction between mood and WM (Canli et al., 2004; Herrington et al., 2005). Thus, the relationship between mood and PFC activity during WM tasks is still worth investigating.

While most neuroimaging studies examined the mood–cognition interaction using laboratory-based mood-induction procedures, it should be noted that experimentally induced and natural moods in our daily lives are not necessarily identical. Experimentally induced moods are qualitatively different from natural mood in many aspects such as intensity, stability, and persistency (Rusting, 1998; Sison and Mather, 2007). It has been argued that experimentally induced moods tend to be more intense than natural mood (Sison and Mather, 2007). Induced moods are also susceptible to demand characteristics (Sison and Mather, 2007; Westermann et al., 1996), which may involve an emotion-regulation process (Ochsner and Gross, 2005); therefore, they would have different influences on cognition than natural mood. Moreover, experimentally induced moods are typically elicited by viewing or listening to ~10 min of emotional stimuli (e.g., film clips and music) and rapidly disappear after the experiment (Qin et al., 2009). Thus, they usually have acute

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effects on cognition. On the other hand, natural mood is formed in daily life and often lasts much longer (hours to days or weeks). Indeed, experimentally induced moods and natural moods sometimes exert completely opposite effects on cognitive performance (Parrot and Sabini, 1990). In recent neuroimaging research using optical topography (OT) (Maki et al., 1995), which can measure brain activity under natural circumstances (e.g., sitting on a chair in an office or living room), researchers have focused on the relationships between natural mood and PFC activity during cognitive tasks. For example, Suda et al. (2009) showed that individuals who reported higher levels of psychological fatigue in daily circumstances exhibited lower levels of PFC activation during a verbal fluency task (VFT). A study from our group also showed that natural variations in negative mood among healthy adults are inversely correlated with PFC activity during a verbal WM task (but not during a spatial WM task) (Aoki et al., 2011). Here, we define the WM as "the temporary retention of information that was just experienced but no longer exists in the external environment" (D'Esposito, 2007), and use simple delayed response tasks. These studies have demonstrated that the PFC plays an important role in the interaction of natural mood and cognition, extending the findings of fMRI studies that relied on experimentally induced moods.

One important limitation of these OT studies, however, is that none controlled for personality differences among participants. Personality traits of individuals are known to affect natural mood in daily lives (Gable et al., 2000) and to be associated with PFC activity during cognitive tasks including WM (Gray and Braver, 2002; Kumari et al., 2004). Thus, observed correlations between natural mood and PFC activity can be "spurious relationships" that just indirectly reflect the associations between personalities and PFC activity. To show that natural moods may be an independent contributing factor of PFC activity during cognitive tasks, one should separate the relationship of natural mood with PFC activity from that of personality.

In this study, we investigated whether the relationship between natural mood and PFC activity during cognitive tasks is explained by personality differences. First, we intended to replicate our previous findings: natural variations in the negative mood levels of healthy adults (as indicated by the scores of the Profiles of Mood States [POMS]) were inversely correlated with PFC activity during a verbal WM task, but did not correlate with PFC activity during a spatial WM task (Aoki et al., 2011). The POMS is a self-report questionnaire that is suitable for evaluating individuals' natural moods in their current life situations (Aoki et al., 2011; Canli et al., 2004; McNair and Heuchert, 2003). Next, we examined whether and how the correlation between natural mood and PFC activity would change after the effects of personality were partialled out. We focused on two sets of personality variables: Neuroticism and Extraversion measured using the NEO Five-Factor Inventory (NEO-FFI) (Costa and McCrae, 1992), and the Behavioral Inhibition System (BIS) and Behavioral Activation System (BAS) measured using the BIS/BAS scales (Carver and White, 1994). These measures have often been used to investigate the relationships between personality and brain activity (Canli, 2004; Canli et al., 2004; DeYoung et al., 2009; Gray and Braver, 2002).

2. Methods

2.1. Participants

Forty healthy adults (10 females, 30 males; mean age=38.4 yr, S.D.=7.4 yr, and range=25–52 yr) participated in this study. Three males were left-handed as determined by the Edinburgh Handedness Inventory (Oldfield, 1971), while the other 37 participants were right-handed. Inclusion or exclusion of the left-handed participants did not affect the main findings reported in this article. Of note, none had participated in our previous study (Aoki et al., 2011); thus, the sample was completely independent. The study was approved by the Ethics Committee of Hitachi, Ltd. All participants provided written informed consent prior to participation.

2.2. Mood and personality measures

Natural moods of the participants were assessed using a short form of the Japanese version of the POMS (Yokoyama et al., 1990). The participants rated 30 mood-related adjectives on a 5-point scale ranging from 0 ("not at all") to 4 ("extremely") on the basis of how they had been feeling during the past week (McNair et al., 1971; Yokoyama et al., 1990). While the POMS has six mood subscales (tension, depression, anger, vigor, fatigue, and confusion), the positive mood score (Pos: the score for the vigor subscale) and the negative mood score (Neg: the sum of the scores for the other five subscales) were used in the analysis, as in previous studies (Aoki et al., 2011; Canli et al., 2004).

Personality was assessed using the Japanese version of the NEO-FFI (Shimonaka et al., 1999) and the BIS/BAS scales (Takahashi et al., 2007). The NEO-FFI is a 60-item self-report questionnaire that assesses the "Big Five" personality traits (Neuroticism, Extraversion, Openness, Agreeableness, and Conscientiousness) (Costa and McCrae, 1992). We particularly focused on Neuroticism and Extraversion, because these two personality variables have been extensively studied for their neurobiological substrates (Canli, 2004; Canli et al., 2004). The BIS/BAS scales consist of 20 statements describing how a person reacts to potentially punishing or rewarding events. The participants rated each item on the basis of how much it held true for them using a 4-point scale (1=does not hold true; 4=holds true) (Carver and White, 1994; Takahashi et al., 2007).

2.3. WM tasks

The tasks were presented through the Platform of Stimuli and Tasks (software developed by Hitachi Ltd., Central Research Lab.). The participants performed two types of WM task (verbal and spatial), each of which had two load (two- and four-item) conditions. Thus, four task conditions were included in total: Verbal/2 items, Verbal/4 items, Spatial/2 items, and Spatial/4 items.

The WM tasks had a delayed-response paradigm (Fig. 1A). Each trial started with a 1500-ms presentation of the target stimuli (S1), which was followed by a delay of 7000 ms. A probe stimulus (S2) was then presented for 2000 ms or until the participant made a response. The inter-trial interval was determined such that the duration between the S2 onset of a trial and the S1 onset of the next trial was randomized between16 and 24 s. We expect that this randomization of the inter-trial intervals reduces the influence of participants' anticipations toward the task onset and spontaneous low-frequency oscillations. Only a fixation cross was presented during the interval and the delay period. A change in the brightness of the fixation cross (500 ms prior to S1 onset) was used as a visual cue for trial onset. Auditory cues, 1000- and 800-Hz pure tones of 100-ms duration, were presented at the onsets of the visual cue and S2, respectively.

In the verbal WM task, two (in the two-item condition) or four (in the four-item condition) Japanese characters in *Hiragana* were presented as S1, and a Japanese character in *Katakana* was presented as S2. *Hiragana* and *Katakana*, syllabaries in the Japanese writing system, represent the same set of syllables with different symbols. The participants indicated by pressing a button whether the character presented as S2 corresponded to any of the characters presented as S1. Because the characters presented as S1 and S2 were presented in different Japanese morphograms (i.e., *Hiragana* and *Katakana*), the participants were prompted to make their judgments on the basis of the phonetic information conveyed by the characters, not on the basis of their form. In the spatial WM task, S1 was the location of two (in the two-item condition) or four (in the four-item condition) white squares out of eight locations, and S2 was the location of a white square. The participant's task was to judge whether the location of the white square presented as S2 was identical to any of the locations of the white squares presented as S1.

2.4. Procedure

Participants completed the POMS questionnaire at the beginning of the experiment. Next, they received computer-automated instructions that were followed by a brief practice session to familiarize them with the tasks. OT measurements were then conducted while the participants performed the WM tasks. The tasks were organized into two sessions, one for the verbal WM task and the other for the spatial WM task, with the order counterbalanced across participants. Each session consisted of 16 trials (eight for each WM-load condition), and sessions were separated by a short break (approximately 1 min). The duration of the OT measurements was approximately 15 min, and the whole experiment took about 45 min. The participants' personalities were assessed after the OT measurement.

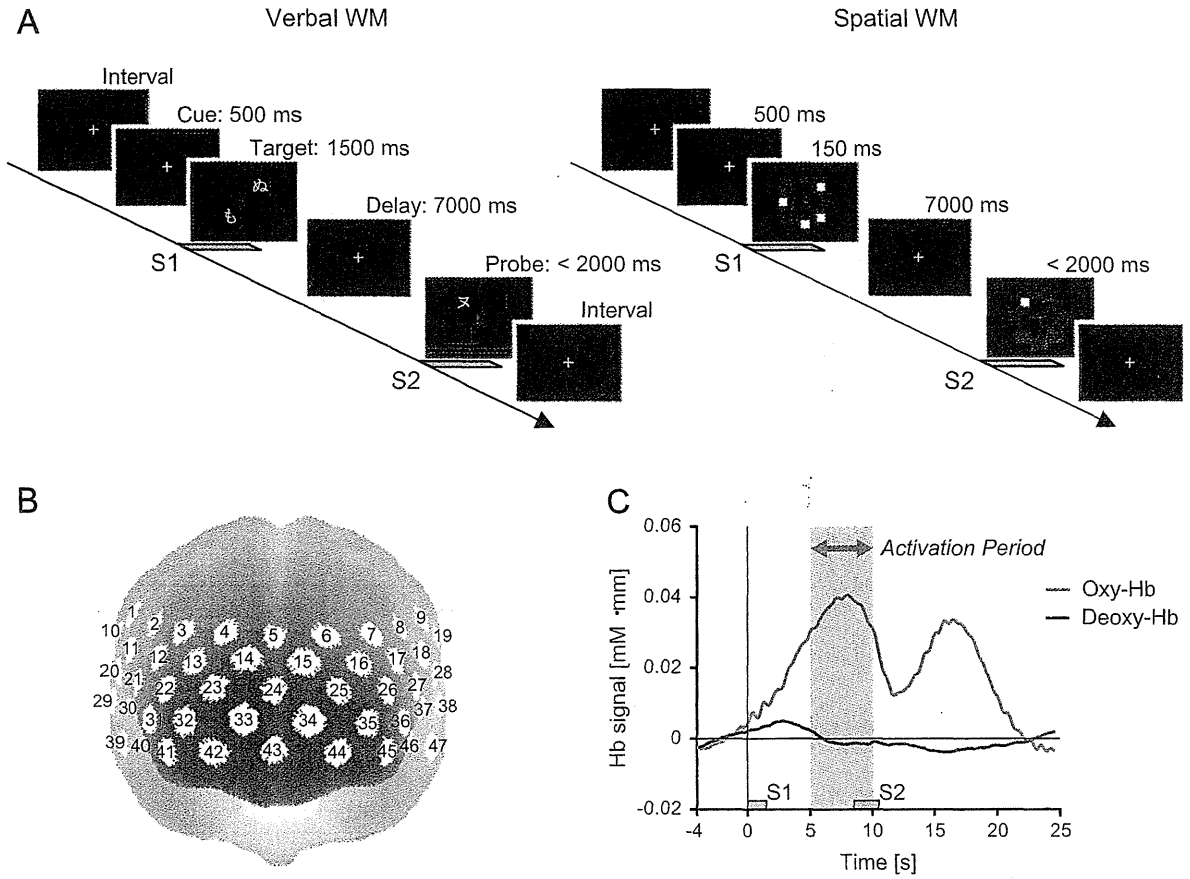

Fig. 1. Task and settings. (A) Schematic diagrams of verbal and spatial WM tasks. Participants were instructed to remember a "target" stimulus (the stimulus to be memorized, S1) and report whether the character (verbal WM task) or location of a white square (spatial WM task) presented in a subsequent "probe" stimulus (the stimulus to be judged, S2) was identical to one of the items in S1. (B) Locations of OT channels (47 measurement points) registered in MNI space based on spatial registration (Singh et al., 2005). (C) Definition of activation period. Graph shows oxy- and deoxy-Hb signal changes during WM tasks for a representative channel (Ch 22), averaged across all blocks, task conditions, and participants. The 5-s activation period (starting 5 s after S1 onset) is indicated by the green rectangle. Gray rectangles indicate time periods for S1 and S2 presentation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.5. OT measurement

We used a 47-channel OT system (ETG-7100, Hitachi Medical Corporation, Japan) to measure hemodynamic activity in the PFC. The system uses two wavelengths of near-infrared light (695 and 830 nm), and the relative changes in the oxy- and deoxy-Hb concentrations were calculated on the basis of the modified Beer–Lambert law (Maki et al., 1995). A 3 × 10 probe set consisting of 30 optodes (15 near-infrared light sources and 15 detectors), with an inter-optode distance of 30 mm, was placed on the participant's forehead. This configuration formed 47 measurement points (i.e., channels [Chs]), each one corresponding to a source–detector pair (Fig. 1B). The average power of each light source was 2 mW (for both wavelengths), and the sampling rate was set to 10 Hz.

To estimate the locations of the OT channels in the Montreal Neurological Institute (MNI) space, we used a spatial registration method (Okamoto and Dan, 2005; Singh et al., 2005). The three-dimensional coordinates of the 30 optode locations and scalp landmarks (based on the international 10–20 system: Fp1, Fp2, Fz, T3, T4, C3, and C4) were recorded for 11 volunteers using a 3D-magnetic space digitizer (3D probe positioning unit for OT system, EZT-DM101, Hitachi Medical Corporation, Japan). These data were input to an algorithm (downloaded from http://www.jichi.ac.jp/brainlab/tools.html) and used to estimate the MNI coordinates of the 47 OT channels. Probabilistic anatomical labeling based on the Talairach Daemon database (Lancaster et al., 2000, 2007) was also performed for each OT channel (Okamoto et al., 2009). The results of the spatial registration were used to create activation/correlation maps.

2.6. Data analysis

Analysis was performed using the plug-in-based software Platform for Optical Topography Analysis Tools (developed by Hitachi Ltd., Central Research Lab.) run on MATLAB (The MathWorks, Inc., USA). We mainly analyzed the oxy-Hb signal because previous studies indicated that the contrast-to-noise ratio for the oxy-Hb signal is higher than that for the deoxy-Hb signal (Sato et al., 2011b; Strangman et al., 2002). Indeed, a number of previous OT studies investigating PFC activity during cognitive tasks have focused exclusively on the oxy-Hb signal (Kopf et al., 2011; Ruocco et al., 2010). The time-continuous data of Hb (both the oxy- and deoxy-Hb) signals recorded during a session were divided into 28.5-s task blocks, such that each block consisted of a 4-s pre-task period (starting 4 s before the S1 onset), an 8.5-s task period (during the 1.5-s S1 presentation and the 7-s delay period), a 12-s recovery period (starting immediately after S2 onset), and a 4-s post-task period. Blocks containing an oxy-Hb signal change larger than 0.4 mM • mm over two successive samples (200-ms period) were discarded on a channel-by-channel basis, which provided an objective criterion for rejecting data contaminated by motion artifacts (Aoki et al., 2011; Sato et al., 2011a). The Hb signals of the remaining blocks were smoothed with a 5-s moving average and baseline-corrected by linear regression based on the least squares method using the data from the pre-task and post-task periods of each block.

To evaluate an individual's PFC activation in response to the WM tasks, we calculated 'activation values' for each task condition in a channel-wise manner with the same method used in our previous study (Sato et al., 2011a). First, a 5-s 'activation period' (starting 5 s after S1 onset, taking into consideration the delay of hemodynamic responses to neuronal activity) was determined as the time window of interest. The window included the peak of the oxy-Hb signal increase during the task block (Fig. 1C). The mean oxy-Hb signal values (expressed in mM • mm) during the activation and pre-task periods (defined as above) were calculated for each block. The differences between these values (i.e., the oxy-Hb signal values during the activation period minus those during the pre-task period) were averaged across blocks and divided by the standard deviations (across blocks) to take into account the trial-to-trial variability (Sato et al., 2011a).

In the subsequent group analysis, we performed channel-wise statistical tests on the activation values. Significant PFC activation at the group level for each task condition was assessed by using one-sample t tests of the activation values (vs. zero). The differences in PFC activation across task conditions were evaluated using two-way repeated-measures analysis of variance (ANOVA) of the activation values with the WM types (verbal, spatial) and the WM loads (two items, four items) as within-subject factors. To analyze the relationships between mood, personality, and PFC activity (and also task performance), we calculated correlation coefficients between the questionnaire scores, behavioral measures (accuracy and reaction time [RT]), and activation

values for each task condition. We used the Spearman rank correlation because the relationships between questionnaire scores and behavioral or neural measures are not necessarily regarded as linear (Schroeter et al., 2004). To examine whether the correlations were influenced by confounding factors such as age, gender, handedness, and task performance (both accuracy and RT), we also performed partial correlation analysis. Performance measures were included as control variables because task performance could be related to PFC activity (higher task performance tended to be associated with greater PFC activation, particularly for the spatial WM task: see Supplementary Table S1 and Fig. S1). In these channel-wise analyses, we used the false discovery rate (FDR) method to correct for multiple comparisons among the 47 channels (Singh and Dan, 2006). The false discovery rate (FDR) threshold was set to 0.05 for each topographical map in order not to report more than 5% false positives (on average). We also used permutation tests to calculate "map-wise" *P*-values (Groppe et al., 2011), which indicate the probabilities associated with the following null hypothesis (H_0): "there is no significant correlation between the POMS scores and PFC activity during the WM task in any of the 47 channels consisting of an OT map." We set the "test statistics" as the number of channels, in a map consisting of 47 channels, whose uncorrected *P*-values (determined by the channel-wise Spearman correlation analysis) were lower than 0.05. Under the null hypothesis, the value of the test statistics is expected to be 2.35 (=5% of 47 channels) on average and follow a Poisson distribution due to false positives. For each map-wise test, we performed 10,000 permutations of the POMS scores of participants and computed the test statistics for each permuted data set to generate a distribution of the test statistics (see Supplementary Fig. S2). By comparing the value of the test statistics for the actual (non-permuted) data with this distribution, we obtained a map-wise *P*-value. Permutation tests can successfully control the familywise error rate and automatically adjust to the degree of correlation between multiple tests (Groppe et al., 2011). Thus, they are particularly suitable for the analysis of multi-channel OT data (Singh and Dan, 2006).

We performed several additional analyses to examine the correlations between the POMS scores and other measures of PFC activation. First, we checked if the differences in the oxy-Hb signal changes between the two-item and four-item conditions (4 items−2 items), as well as the sums of the oxy-Hb signal changes between the two conditions (2 items+4 items), were correlated with the POMS scores. Second, we tested whether the second peak of the oxy-Hb signals during the WM tasks (Fig. 1C) correlated with the POMS scores. Third, we tested whether the correlations between the POMS scores and the deoxy-Hb signal during the first and second peaks were significant. These results are reported in Supplementary Tables S2 and S3.

3. Results

3.1. Mood and personality scores

The mood and personality scores of participants are shown in Table 1. The distributions of these scores matched those obtained from the Japanese normative samples (Shimonaka et al., 1999; Takahashi et al., 2007; Yokoyama et al., 1990).

Correlations between mood and personality scores are shown in Table 2. Significant correlations were observed between the Pos and Extraversion scores ($rho=0.61$, $P<0.001$) as well as the Pos and BAS scores ($rho=0.55$, $P<0.001$). Similarly, significant correlations were observed between the Neg and Neuroticism scores ($rho=0.50$, $P=0.001$) as well as between the Neg and BIS scores ($rho=0.44$, $P=0.004$).

3.2. Task performance

The accuracy and RT data are shown in Table 3. Two-way repeated-measures ANOVA was used to examine the effects of WM type (verbal/spatial) and WM load (2 items/4 items) on task performance. A significant main effect of WM load on RT ($F=77.9$, $P<0.001$) was observed, which indicates slower responses under the higher WM-load conditions (i.e., 4 items). A significant main effect of WM type ($F=7.15$, $P=0.011$) and a significant WM-type × WM-load interaction ($F=4.42$, $P=0.042$) were also observed, suggesting that the participants took longer to perform the verbal WM task than the spatial one. The WM-load effects on RT (RTs in the 4-item conditions minus those in the 2-item conditions) were larger for the spatial WM task (99.9 ms for the verbal WM task and 174.6 ms for the spatial WM task; $t=2.10$, $P=0.042$; paired *t*-test). This was due to the RT difference in the 2-item conditions between the verbal and spatial WM task ($t=3.86$, $P<0.001$; paired *t*-test). RTs in the four-item conditions were not different between the tasks ($t=1.13$, $P=0.266$; paired *t*-test). The main effects and the interaction were not significant for accuracy ($F<2.47$, $P>0.124$), which was possibly due to the ceiling effect (especially for the WM-type main effect).

Table 1
Basic statistics for mood and personality scores.

Factors	Mean	S.D.	Range
Mood			
Pos	7.3	3.7	0–14
Neg	29.2	17.0	2–87
Personality			
Neuroticism	23.2	6.6	7–37
Extraversion	24.4	6.3	14–37
BIS	18.5	3.6	10–25
BAS	37.7	5.1	28–52

Note: Pos: POMS positive mood scores; Neg: POMS negative mood scores.

Table 2
Correlations between mood and personality scores.

Factors	Pos	Neg	Neuroticism	Extraversion	BIS	BAS
Mood						
Pos	–					
Neg	−0.32*	–				
Personality						
Neuroticism	−0.11	0.50**	–			
Extraversion	0.61**	−0.39*	−0.27	–		
BIS	−0.27	0.44**	0.58**	−0.36*	–	
BAS	0.55**	−0.36*	−0.16	0.53**	−0.22	–

Note: Pos: POMS positive mood scores; Neg: POMS negative mood scores. All values are Spearman's rank correlation coefficients.

* $P<0.05$; uncorrected.
** $P<0.005$; uncorrected.

Table 3
Task performance.

Task conditions		Accuracy (%)		RT (ms)	
		Mean	S.D.	Mean	S.D.
Verbal WM	2 items	96.9	6.8	1235	200
	4 items	95.6	9.2	1335	211
Spatial WM	2 items	95.3	10.5	1116	186
	4 items	93.1	8.9	1291	255

3.3. Group average of PFC activity during WM tasks

The PFC was reliably activated in response to the WM tasks, as shown in the group-level activation maps (Fig. 2A) and the time courses (Fig. 2B). One-sample *t* tests of the activation values (calculated as described in Section 2 (Methods)) showed significant increases in the oxy-Hb signal during the activation period of the WM tasks for all conditions ($t>2.29$, FDR <0.05). Two-way repeated-measures ANOVA revealed a main effect of WM-load on the activation values for eight channels (Chs 4, 5, 6, 7, 22, 24, 41, 43; $F>8.07$, FDR <0.05), confirming greater PFC activation under higher WM-load conditions. Neither a significant WM-type main effect ($F<9.40$, FDR >0.05) nor a WM-type × WM-load interaction ($F<8.96$, FDR >0.05) was observed.

The overall activation patterns and the locations of activation foci (channels for which the activation values were greatest) were highly similar across task conditions, as shown in Fig. 2A.

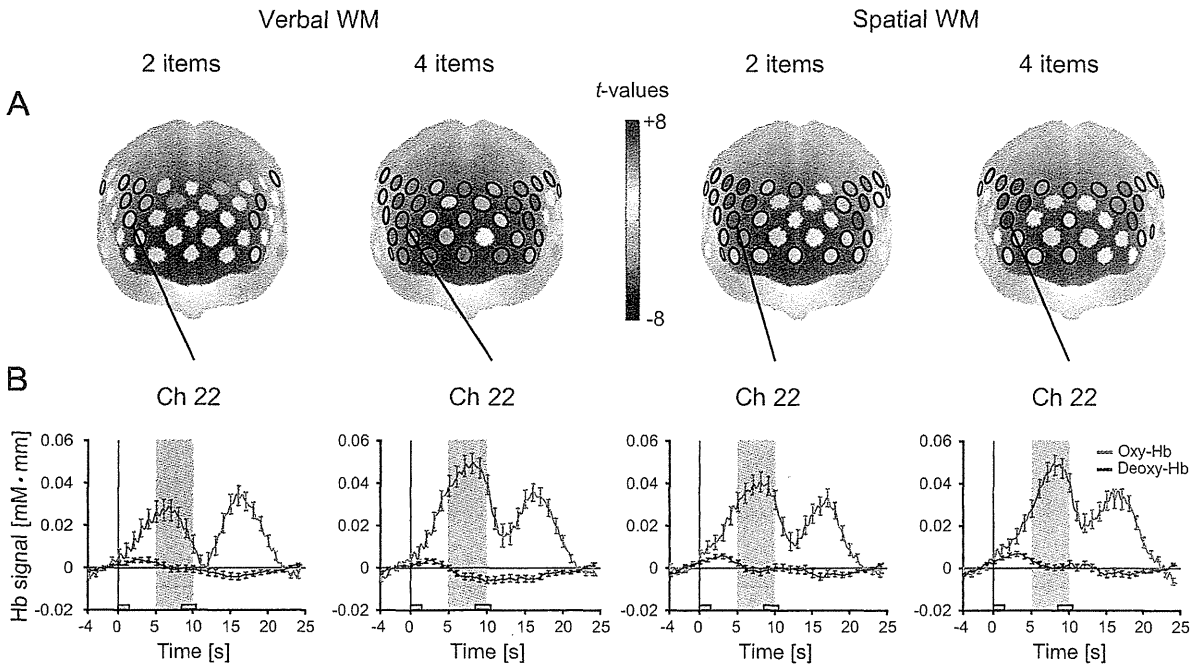

Fig. 2. Hb-signal changes during verbal and spatial WM tasks. (A) Activation maps showing oxy-Hb signal changes in response to the WM task for each task condition. Color scale indicates Student's *t* values (one-sample *t* tests of activation values vs. zero) for each channel. Channels with significant *t* values (FDR < 0.05) are circled with a bold black line. (B) Time courses for oxy- and deoxy-Hb signal changes for a representative channel (Ch 22), representing grand average (with standard error bars) across all participants. Green rectangles indicate activation periods.

To examine the similarity of PFC activation patterns across task conditions, we calculated the correlations between the activation values for any two task conditions on a channel-by-channel basis (see Supplementary Table S4). The activation values between different task conditions were highly correlated (median $rho > 0.42$, $P < 0.007$), especially between the conditions within each WM type (median $rho > 0.54$, $P < 0.001$).

3.4. Relationships between mood scores and PFC activity

The relationships between the POMS scores (i.e., Pos and Neg) and PFC activity for the WM tasks were examined using correlation analysis performed on a channel-by-channel basis (Fig. 3; see also Supplementary Table S1).

The Pos scores were not significantly correlated with activation values for any task condition (map-wise $P > 0.304$, permutation test). However, the correlation between the Neg scores and the activation values for the Verbal/4-item condition was significant (map-wise $P=0.018$, permutation test). The strongest correlation was observed in Ch 34 ($rho = -0.48$, FDR = 0.07; see Fig. 4), which was located in the anterior PFC (BA 10) as estimated using the spatial registration method (Lancaster et al., 2007; Okamoto et al., 2009; Tzourio-Mazoyer et al., 2002). When we used *P*-values derived from one-tailed tests on the ground of our *a priori* hypothesis that the Neg scores would be negatively correlated with the PFC activity during the verbal WM task, nine channels survived the FDR-corrected threshold (Chs 3, 13, 22, 23, 24, 32, 33, 34, and 43; $rho = -0.48$ to -0.39, FDR < 0.05). Moreover, when we controlled for several confounding variables (age, gender, handedness, accuracy, and RT), significant correlations were found for five channels (Chs 24, 33, 34, 35, and 43; $rho = -0.62$ to -0.47, FDR < 0.05). These channels comprised a "cluster" located in the anterior PFC. In contrast, no significant correlation was found between the Neg scores and the activation values for the other task conditions (map-wise $P > 0.115$, permutation test). Critically, the correlations between the Neg scores and activation values for the Verbal/4-item condition were still significant for 18 channels after the Neuroticism and Extraversion scores were included as additional controlling variables (Chs 3, 4, 9, 13, 15, 23, 24, 25, 26, 33, 34, 35, 40, 41, 42, 43, 44, 45; $rho = -0.65$ to -0.43, FDR < 0.05). Significant correlations also survived in four channels when we controlled for the BAS and BIS scores in addition to age, gender, handedness, accuracy, and RT (Chs 24, 33, 34, and 35; $rho = -0.55$ to -0.50, FDR < 0.05). Larger range and standard deviation of the Neg score (Table 1) did not account for these results, because similar results (i.e., significant correlations after controlling for personality effects) were obtained for the POMS fatigue subscale, whose range and standard deviation were comparable with other mood and personality variables (see Supplementary Fig. S3).

We also examined whether activation differences between the two-item and four-item conditions for the WM tasks were associated with the POMS scores. For this purpose, we calculated correlations between the differences in the activation values (the 4-item conditions minus 2-item conditions) and the POMS scores. However, there was no significant correlation in any condition, either between Pos or Neg scores (map-wise $P > 0.164$, permutation test; see Supplementary Table S2).

3.5. Relationships between personality scores and PFC activity

We also examined the correlations between personality scores and PFC activity. Among the four personality variables, no significant correlation was observed in any task condition (map-wise $P > 0.054$, permutation test), with an exception of the correlation between the BAS scores and PFC activity for the Verbal/2-item condition (map-wise $P=0.006$, permutation test; Ch 25; $rho = 0.55$, FDR < 0.05). Moreover, this correlation was no longer significant when mood scores (the Pos and Neg) and the other confounding variables (age, gender, handedness, accuracy, and RT) were controlled for (map-wise $P=0.085$, permutation test). Thus, we found no evidence for a direct relationship between personality and PFC activity independent of participants' moods.

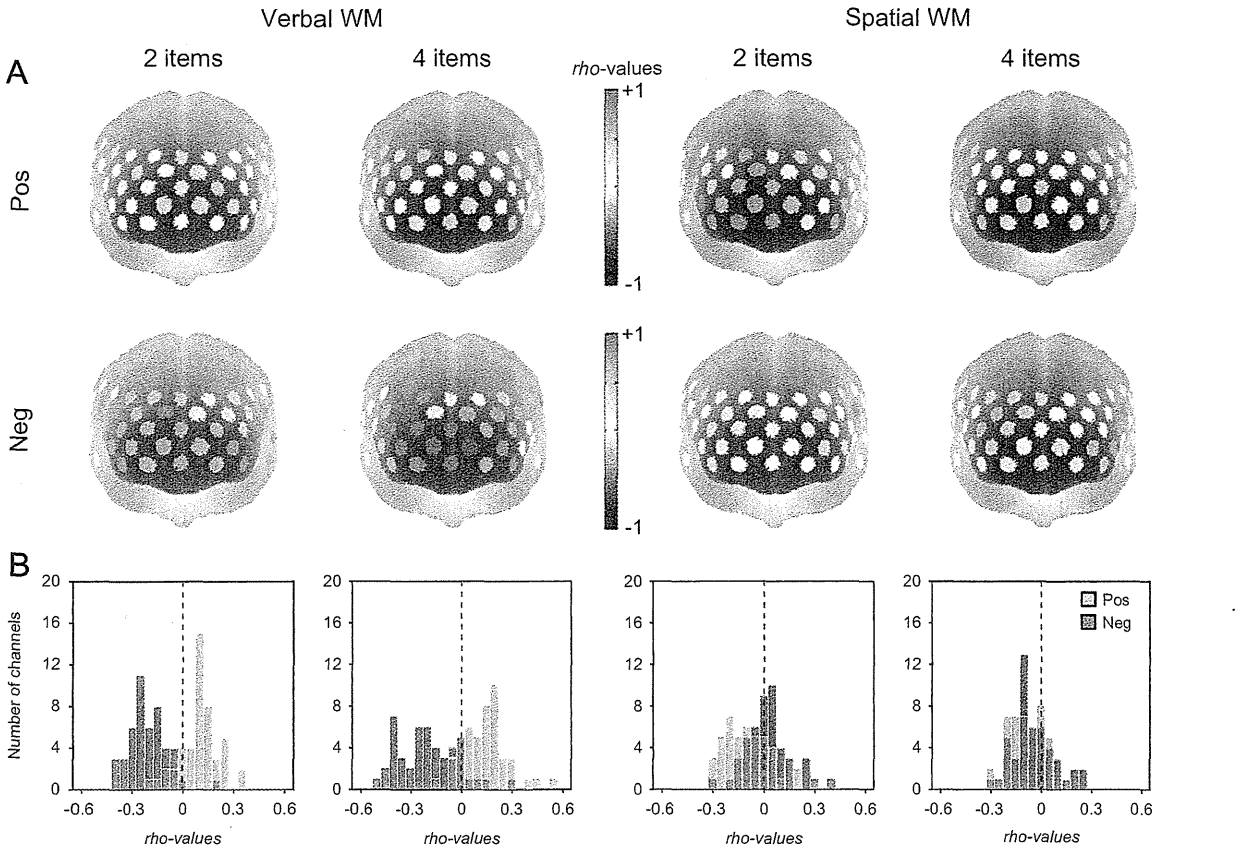

Fig. 3. Relationships between mood and PFC activity. (A) Correlation maps showing relationships between POMS positive or negative mood (Pos and Neg) scores and activation values for each task condition. Color scale indicates Spearman's *rho* values for each channel. (B) Histograms showing distributions of *rho* values for each correlation map, indicating overall tendencies of correlation between mood measures and PFC activity.

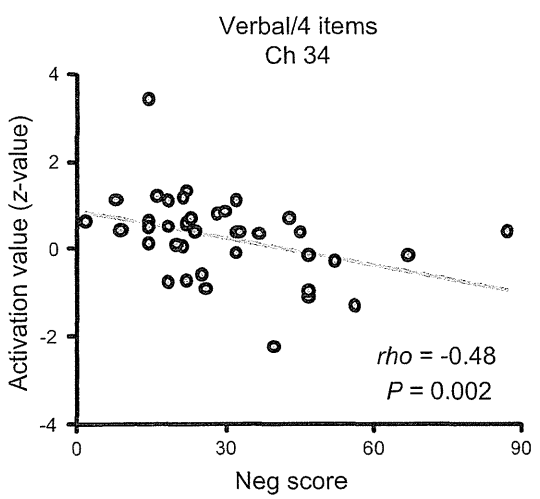

Fig. 4. Correlation plot of mood scores with PFC activity. Negative correlation between individuals' Neg scores and activation values for the Verbal/4-item condition. Plot shows the relationship for the channel with the lowest *rho* value (Ch 34).

4. Discussion

Our aim of this study was to clarify the relationship between natural mood and PFC activity while controlling for personality differences among individuals. We found that the level of negative mood (as indicated by the POMS negative mood scores) was inversely correlated with PFC activity during a verbal WM task under a high WM-load condition, which was consistent with the results of our previous study (Aoki et al., 2011). Moreover, this relationship remained significant even after controlling for individual differences in personality traits (Neuroticism–Extraversion or BIS–BAS) in addition to other confounding factors (i.e., age, gender, handedness, and task performance). These results suggest that natural negative mood has a unique relationship with PFC activity during verbal WM tasks, which is not attributed to the effects of personality.

4.1. PFC activity during verbal and spatial WM tasks

We observed significant PFC activations during the WM tasks for all task conditions. The activation pattern was highly comparable to those reported in our previous studies using essentially the same tasks (Aoki et al., 2011; Sato et al., 2011a) and to those reported in other OT studies using WM tasks (Ehlis et al., 2008; Schecklmann et al., 2010; Schreppel et al., 2008; Tsujimoto et al., 2004). Moreover, it was consistent with those found in many studies of WM tasks using other neuroimaging modalities such as fMRI and positron emission tomography (Smith and Jonides, 1999; Smith et al., 1996; Wager and Smith, 2003). We also observed a WM-load dependency of PFC activity (i.e., higher activation under a high WM-load condition compared to that under a low WM-load condition) for several channels, as predicted. Taking these findings together, we conclude that OT is suitable for measuring WM-related activity in the PFC.

We found no statistical difference in PFC activity between the verbal and spatial WM tasks. These tasks are thought to induce different PFC activation patterns: verbal WM tasks activate the left PFC relatively more, whereas spatial WM tasks activate the right PFC relatively more (Smith et al., 1996). However, such

laterality-based distinction between verbal and spatial WM tasks remains elusive (Ray et al., 2008). Drawing conclusions from our data concerning the activity difference in the PFC between verbal and spatial WM tasks is difficult because the visual stimuli and task performance were not perfectly matched across the WM types in the present study. Further studies are needed to clarify whether and how verbal and spatial WM functions are associated with differential PFC activation.

Time courses of the hemodynamic responses also replicated the results of our previous studies (Aoki et al., 2011; Sato et al., 2011a). As expected, the oxy-Hb signals increased in response to the presentation of the target stimulus (S1), and they reached maximum during the pre-defined activation period. The oxy-Hb signals increased again after the presentation of the probe stimulus (S2). Such two-peaked temporal activation patterns were similar across all four conditions (Fig. 2B). The two peaks may be associated with the WM processes of encoding/maintenance and retrieval, respectively.

4.2. Negative mood and PFC activity during verbal WM task

Our first aim was to replicate our previous work (Aoki et al., 2011). We found significant negative correlations between the level of negative mood and PFC activity only in the verbal WM task but not in the spatial WM task, despite the fact that group averages of PFC activity were statistically equivalent for these tasks. This result is highly consistent with our previous finding based on a completely independent sample ($n=29$), which also revealed a specific correlation between negative mood and PFC activity during a verbal WM task (Aoki et al., 2011). Such reproducibility of the results across the studies suggests a reliable relationship between negative mood measured with the POMS and PFC activity during the verbal WM task. Furthermore, our results are in agreement with previous OT and fMRI studies, which showed that PFC activity during cognitive tasks is attenuated when the participants have higher levels of negative mood (Qin et al., 2009; Suda et al., 2009).

Note that we did not restrict the correlation analysis to the channels that showed significant activation for the WM tasks. We included all 47 channels to control the sensitivities of the correlation maps with the same multiple comparison adjustments among the 47 channels. This approach can be a useful method to identify individual differences in brain activity as a previous study that focused on individual differences demonstrated correlations in brain regions where significant activations were not found at the group level (Canli et al., 2002).

The difference in the relationships of mood with PFC activity between the verbal and spatial WM tasks is also consistent with previous studies. Behavioral experiments have shown that verbal and spatial cognitive functions are selectively modulated by mood states (Bartolic et al., 1999; Gray, 2001), although underlying brain mechanisms remain to be elucidated. The selective effects of neurotransmitters (e.g., dopamine and serotonin) on mood and cognitive functions possibly mediate such specific interactions between mood and cognition (Ashby et al., 1999; Robbins and Roberts, 2007), which should be clarified in future studies.

The difference between verbal and spatial WM in relation to negative mood has another implication to the present results. While the effect of extracerebral hemodynamic changes (e.g., skin blood flow) should be taken into consideration in interpreting OT data, it is unlikely that the present observation concerning the correlation between negative mood and PFC activity is due to these changes because they cannot account for the task-specific correlation between negative mood and verbal WM tasks.

4.3. Relationship between mood and PFC activity independent of personality effects

Our second aim was to separate the contributing factors of natural mood and personality to inter-individual variations in PFC activity during WM tasks. We assessed both the natural mood and personality of participants and evaluated the effect of each factor using partial correlation analysis. This approach has been used to separate the effects of mood states and personality traits in previous behavioral psychological and neuroimaging studies (Bishop, 2009; Canli et al., 2004; Suhr and Tsanadis, 2007). The correlation between negative mood and PFC activity during the verbal WM task was not attenuated after personality scores were included as control variables, suggesting that the observed relationship between negative mood and PFC activity during the verbal WM task is not an indirect association intervened by personality effects. This extends our previous findings by showing the unique contribution of natural mood on PFC activity, which is not simply attributed to personality differences among individuals.

Another approach for separating the effects of mood and personality is to adopt within-subject design and measure mood and brain activity of the same individuals for multiple occasions (Liston et al., 2009). Using this approach, we recently showed that changes in the levels of depressed mood *within* individuals are negatively correlated with PFC activity during the same verbal WM task (Sato et al., 2011a). This study also demonstrated that the relationship between natural mood and PFC activity is independent of some trait factors, which is in line with the present results.

Although natural mood and personality are closely related, they are psychologically distinctive constructs. While recent growth in "personality neuroscience" has begun to elucidate neurobiological substrates for personality (DeYoung et al., 2009), they might not fully cover the neurobiological substrates for natural mood. For example, the relationships of personality with brain activity may reflect genetic variations among individuals, whereas those of mood may reflect the levels of cortical neurotransmitter concentrations that vary within individuals from time to time. The present results showing the unique relationship between natural mood and PFC activity independent of personality are important for considering the distinction of the neural substrates of mood and personality.

4.4. Limitations

There are some limitations to be discussed in this study. First, the gender ratio in the participants was imbalanced (female:male=1:3). However, we speculate that our finding is not dependent on a gender difference because the partial correlation analysis controlled for gender replicated the results. Moreover, Canli et al. (2004) did not mention any evidence of a fundamental difference between male and female participants in how brain activation is associated with either personality trait or mood state. It might be interesting in the future study to examine gender differences in terms of the relationship between brain activity and mood states with larger and more gender-balanced samples.

Second, we need to consider limitations of our WM tasks. Although we found greater activation values in a high-load (4-item) condition than in a low-load (2-item) condition in eight channels, we did not find significant correlations of the negative mood with the PFC activity related to the contrast between the two load conditions. This might be because the WM-load difference between the two conditions was relatively small. Indeed, the accuracies were not significantly different between the two-item

and 4-item conditions in the verbal WM task. Alternatively, the observed negative correlation between participants' negative moods and the PFC activity might not be attributed to the pure WM-related activity, but rather might be derived from other verbal-related cognitive process shared across both low and high WM-load conditions of the verbal WM task. In addition, as we found the mood states were correlated with activation values for the first peak (a 5-s period starting 5 s after S1 onset) not for the second peak (a 5-s period starting 5 s after S2 onset; see Supplementary Table S3), the activity of our main finding might reflect rehearsal process in the maintenance period and not the central executive. Thus, it is not clear if the WM alone reflects the PFC activity, and future studies are necessary to clarify the cognitive components responsible for the relationship between the PFC activity and mood states.

In addition, it might be pointed out that the changes in brightness of the fixation cross and/or the auditory cues in the WM tasks could influence the PFC activity. Although we did not test the influence of the slight changes in the visual and auditory stimuli on the brain signals, one characteristic of our study was a selective correlation with negative mood in the verbal WM task, which was not found in the spatial WM task. As these two WM tasks share the changes in brightness of the fixation cross and the auditory cues, it is clear that these visual/auditory stimuli themselves did not intervene in our main finding.

4.5. Conclusion

In spite of some limitations described above, we demonstrated that individuals experiencing higher levels of negative moods during the past week (as assessed with the POMS) showed lower levels of PFC activity during a verbal WM task, which replicated the results of our previous study based on an independent sample (Aoki et al., 2011). Moreover, this relationship was not explained by individual differences in personality traits or by age, gender, handedness, or task performance. The results extend our previous work by controlling for personality differences among individuals and provide valuable insight into the neurobiological substrates of natural mood, which should be distinguished from those of personality.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.pscychresns.2012.10.009.

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Transdermal delivery of adriamycin to transplanted Ehrlich ascites tumor in mice

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Abstract:

The transdermal delivery of anti-cancer drugs is considered to be unfeasible due to the low permeability of drugs through the dermis. However, we previously showed that a thioglycolate-based depilatory agent increases the drug permeability of mouse skin. In the present report, we investigated the skin permeability and efficacy of the anti-cancer drug adriamycin increased when administered transdermally to mice in combination with a thioglycolate-based depilatory agent. Adriamycin in combination with depilatory treatment significantly reduced Ehrlich tumor growth in hairless mice as compared to that of non-depilatory-treated hairless mice. In addition, our transdermal delivery method for adriamycin increased the therapeutic effectiveness of this agent by decreasing toxicity. Moreover, measurement of adriamycin autofluorescence revealed that transdermally applied adriamycin penetrate the dermis after depilatory agent treatment. These results indicate that the transdermal delivery of anti-cancer drugs is feasible by pretreating skin with a thioglycolate-based depilatory agent.

Keywords: Trandermal drug delivery; Thioglycolate; Adriamycin

1. Introduction

Oral and intravenous administration are the two main drug delivery routes for anti-cancer drugs. Transdermal delivery is considered to be unfeasible for cancer treatment due to the low permeability of drugs through the dermis. However, the skin is the largest organ in the body and an obvious route for both local and systemic drug delivery. Thus, the transdermal delivery of anti-cancer drugs may be useful in the clinical settings if the skin permeability of drugs can be increased.

We previously showed that the skin permeability of gentamicin increased when combined with a thioglycolate-based depilatory agent [1]. Ultrastructural studies revealed that alteration and expansion of intracellular spaces in the epidermis and dermis were responsible for the increase of drug permeability of depilatory agent-treated skin. Transdermal drug delivery possesses several advantages over oral and intravenous drug administration [2], in that it: 1) bypasses gastrointestinal incompatibility and the hepatic 'first-pass' effect; 2) reduces side-effects through optimization of blood concentration-time profiles; 3) involves patient-activated/patient-modulated delivery, which enhances patient compliance; 4) enhances target specificity; and 5) reduces medical treatment costs.

Adriamycin (doxorubicin hydrochloride) is an anthracycline antibiotic that is commonly used in the treatment of a wide range of cancers, carcinomas and soft tissue sarcomas. Here, we investigated whether thioglycolate-based depilatory agent-treatment increases the skin permeability of adriamycin and its anti-tumor activity for cancer cells grown underneath the skin. To determine the efficacy of our depilatory method, the anti-tumor effect and distribution of adriamycin applied as a cream to Ehrlich solid tumor-bearing hairless mice were examined by measuring tumor size and adriamycin autofluorescence, respectively.

2. Experimental Section

Adriamycin cream was prepared by mixing Adriacin (Kyowa Hakko Kirin Co., Ltd., Tokyo, Japan) with White Ointment (Nikko Pharmaceutical Co., Ltd., Gifu, Japan) using a planetary centrifugal mixer, AR-100 (Thinky INC., Tokyo, Japan). The final concentration of adriamycin was 0.2 or 0.6 mg/g of cream. Five-week-old female hairless mice (HR1; body weight, approximately 20 g) were obtained from Japan SLC, Inc. (Shizuoka, Japan) and were housed individually under controlled temperature and humidity conditions, and had free access to water and food. The present study was approved by Animal Ethics Committee of the University of Tokyo.

Ehrlich carcinoma cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum at 37 °C in a humidified 5% CO_2 atmosphere. To prepare cells for transplantation into mice, exponentially growing cells were harvested, washed, and then resuspended in RPMI 1640. Ten million of Ehrlich carcinoma cells were transplanted subcutaneously into the backs of hairless mice. Three days after the transplantation, mice were randomly divided into 4 groups containing 6 animals per group.

Depilatory cream was obtained from Reckitt Benckiser Co., Ltd. (Tokyo, Japan) and was applied once every 3 days for 1 min to the mouse skin, which was then rinsed with warm water to remove the cream. Adriamycin cream (0.5 g) was then gently applied by rubbing onto the skin of the transplantation site daily for 21 days (a total of 7 treatments with depilatory cream were performed during this time). Treatment started 3 days after tumor cell transplantation when tumors reached a diameter of approximately 1 cm. At the end of the treatment, the mice were euthanized with an overdose of ether. Euthanasia and carcass disposal were performed in accordance with the institutional guidelines for animal care of the University of Tokyo. Solid tumors were washed with saline after being excised, and then weighed.

To examine the distribution of adriamycin, 10-μm cryosections of the tumor specimens were prepared and then examined under a fluorescence microscope (Axioplan, Carl Zeiss GmbH, Oberkochen, Germany) to visualize adriamycin, which has excitation and emission peaks of 488 nm and 556/582 nm, respectively. The obtained images were optimized for contrast and brightness using Photoshop CS5 software (Adobe Inc., San Jose, CA, US).

3. Results and Discussion

Adriamycin is a potent anti-cancer agent that is clinically useful for the treatment of acute leukemias, malignant lymphomas, and carcinomas [3]. In the present study, we have shown that the use of depilatory treatment allows for the potential transdermal delivery of this anti-cancer drug. We examined the anti-tumor effect of adriamycin administered transdermally to hairless mice bearing solid tumors that were induced by transplantation of Ehrlich's carcinoma cells, which are widely used to form xenografts. Ehrlich carcinoma is a transplantable, poorly differentiated malignancy and grows in both solid and ascitic forms [4]. The anti-cancer effects of adriamycin with the depilatory treatment were evaluated by the inhibition of tumor growth, which was determined by comparing the weight and size of harvested tumors from untreated control and treated mice. As shown in Fig. 1, transdermal treatment with adriamycin cream led to a reduction in tumor size compared to the untreated control group. The effect of adriamycin in solution was also examined by applying the solution (0.1 mg/0.2 mL saline/ day) directly onto skin, but no reduction in tumor size was observed (data not shown). Moreover, subcutaneous injection of the adriamycin solution (0.1 mg/0.2 mL saline/day) into the transplantation site resulted in the death of all mice by the 15th consecutive day of treatment, whereas all mice administered adriamycin transdermally lived until the end of experiment.

We next compared the anti-tumor effects of adriamycin of two different doses and intervals during a 14-day treatment period. The daily transdermal administration (0.05 mg/0.5 g) and intermittent (once every 3 days) subcutaneous injection of adriamycin (0.05 mg/0.2 mL) had equivalent anti-cancer effects on tumor weight (Fig. 2). In contrast, the transdermal administration of adriamycin resulted in statistically higher increase in the body weight gain rate ($p<0.05$), which was used as a measure of toxicity, compared to subcutaneous injection, and was statistically similar to the control. These data suggest that the transdermal delivery of adriamycin can increase its therapeutic effectiveness by diminishing toxicity without affecting its anti-tumor activity.

We also examined the distribution of adriamycin following its application in cream form to the back area of hairless mice pre-treated with and without a depilatory agent. Adriamycin was detected in thin sections of the treatment area by its autofluorescence [5]. As shown in Fig. 3, only transdermally administered adriamycin with the depilatory agent was observed in the epidermis to dermis and reached the underlying muscle layer.

We previously reported that liquid chromatography-tandem mass spectrometry (LC-MS/MS) can be used to validate the effect of a depilatory agent on the *in-vivo* permeation of gentamicin [1]. LC-MS/MS analysis of the sera and muscle tissue extracts of hairless mice confirmed that the treatment drug was not detectable in the non-depilatory-treated group, but was present in the depilatory-treated-group. Using electron microscopy, we previously observed a large expansion of the intercellular gaps and extraordinary spaces in the basal and prickle-cell layers in depilatory agent-treated mice [1]. These results indicate that alteration and expansion of the intracellular spaces in the basal and prickle-cell layers of dermis may be due to the shrinkage of cells in those layers, which in turn leads to reduced resistance. Lee *et al.* [6] have shown that depilatory agents enhance transepidermal drug delivery by reducing the resistance of both the transcellular and intercellular

routes of the stratum corneum. These findings, together with our present results for adriamycin, suggest that the combination of a skin impermeable drug with a depilatory agent increases drug penetration into the epidermis, where it produces a loco-regional of systemic effect through the vascular network.

Transdermal drug delivery systems offer many advantages over conventional administration routes [7]. However, given the low permeability of external molecules through the skin, it remains a minor portal of entry for drugs in the clinical setting [8]. Therefore, various approaches aimed at decreasing the resistance of skin to drug penetration have been investigated [9]. For example, Herai *et al.* [10] found that the penetration enhancer monoolein significantly increased the *in-vitro* skin permeation and retention of adriamycin in the stratum corneum. In addition, Han *et al.* [11] reported that although transdermal adriamycin delivery is enhanced by liposomal formulations, topical applications have a few limitations with regard to delivery capacity and speed. The liposome-mediated delivery of adriamycin proceeds through follicular routes and has a significant synergistic effect in combination with iontophoresis.

Figure 1. Effect of transdermally administered adriamycin on tumor growth in hairless mice.
In mice treated with adriamycin cream (0.1 or 0.3 mg/day), the tumor size and weight (middle and bottom sections) were significantly smaller than those in the untreated control group (upper sections). A higher dose of adriamycin (bottom section) slightly improved the inhibition of tumor growth. Scale bar = 1 cm

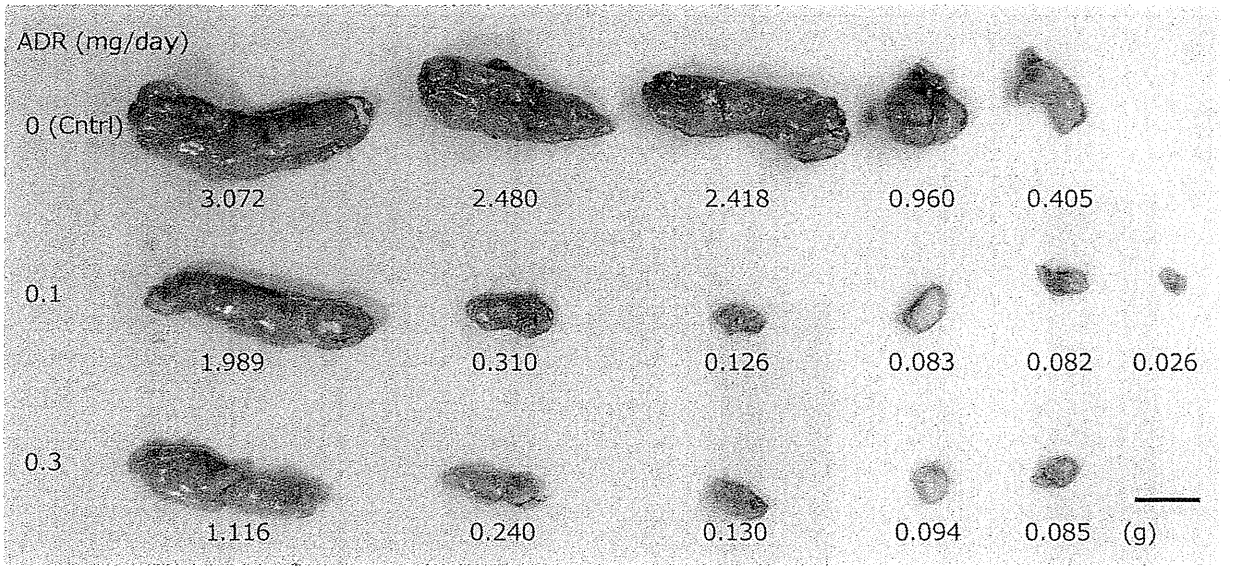


Figure 2. Comparison of transdermal and subcutaneous administration of adriamycin by weight gain rate and tumor weight.

The body weight gain rate (black bars, left axis) and tumor weight (striped bars, right axis) of tumor-bearing mice transdermally administered (TD) and subcutaneously injected (SC) with adriamycin were compared. The daily transdermal administration and the once every three days subcutaneous injectiond of adriamycin resulted in similar anti-cancer effects, as estimated by the tumor weight. However, transdermal adriamycin administration led to a higher weight gain rate in mice than subcutaneous injection ($p<0.05$, Welch's t-test), and was statistically similar to the level in the untreated control. Data are shown as the mean ± S.D.

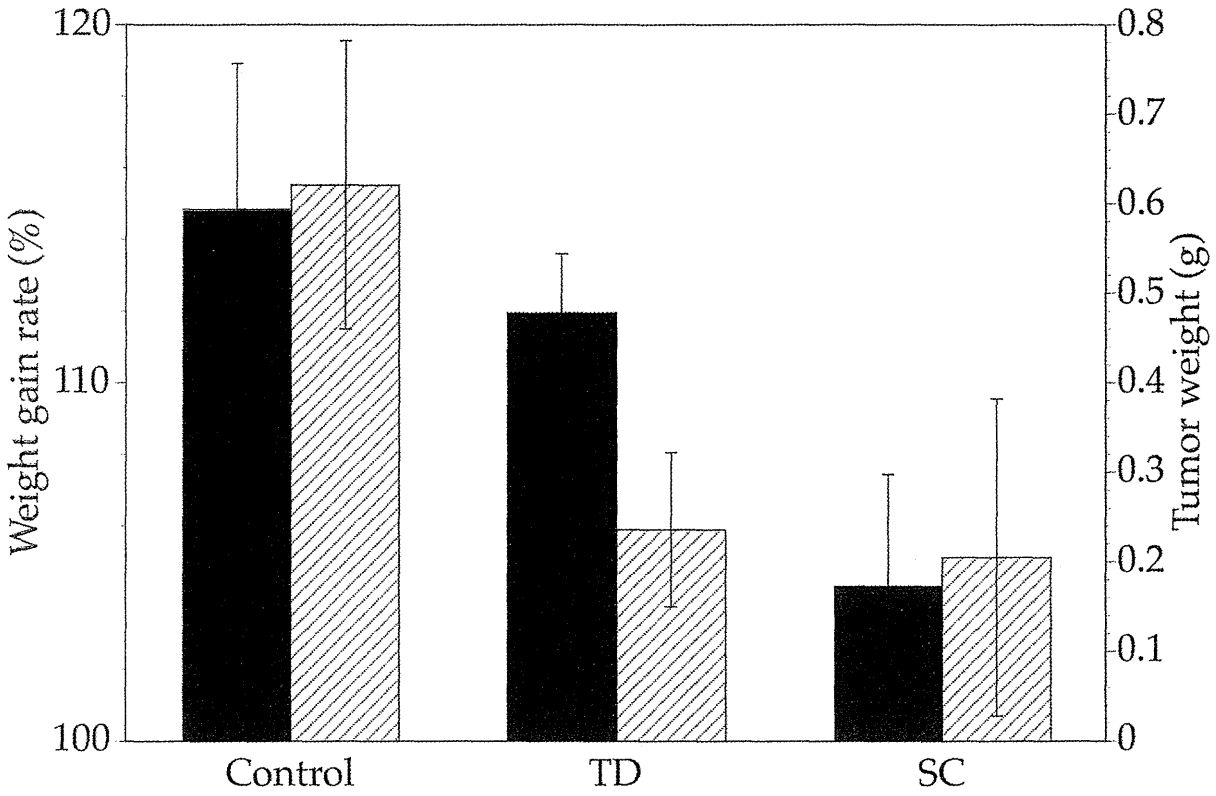
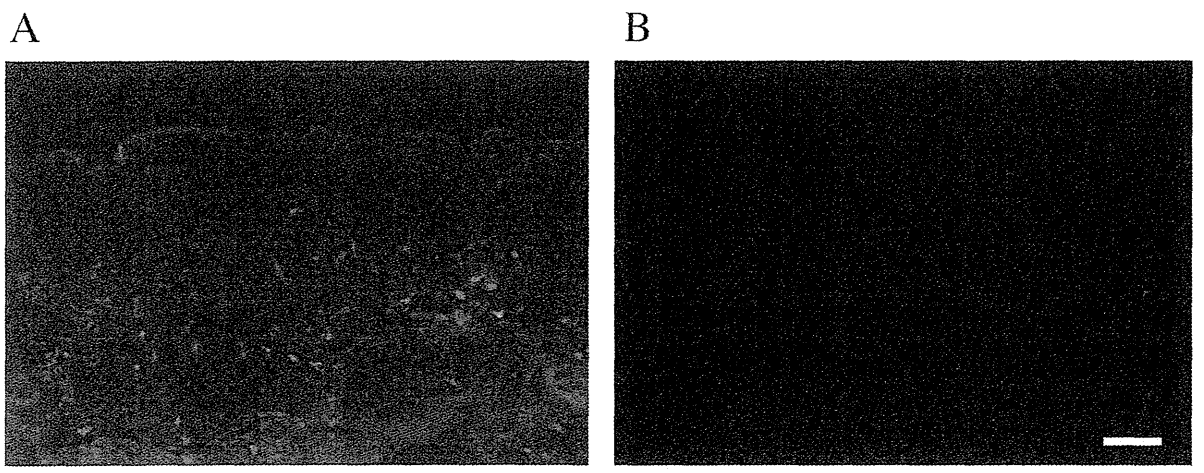


Figure 3. Distribution of transdermally administered adriamycin.

Adriamycin cream was transdermally applied to the skin of hairless mice for three consecutive days with (A) or without (B) pretreatment with a depilatory agent. The distribution of adriamycin was then detected based on its autofluorescence. Adriamycin was observed in the dermis/fascia only after pretreatment with the depilatory agent. Scale bar = 10 μm.



4. Conclusions

In conclusion, our results suggest that the pretreatment of skin with a depilatory agent increases the anti-tumor effect against Ehrlich solid tumor. Our present cancer treatment method involves the use of a thioglycolate-based depilatory agent to increase the permeability of the dermal surface and has proven to be more convenient and effective than injection. Thus, depilatory agent-treatment may be useful for the local application and systemic delivery of anti-cancer drugs.

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Conflict of Interest

The authors declare no conflict of interest.

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