indicates that aripiprazole prevents apoptosis in the brain of methamphetamine-treatment rodents. (Abekawa et al., 2011), which supports the significance of our results.

Recent neuroimaging studies have shown significant volume reductions in white matter with abnormal brain connectivity in schizophrenia (Schlosser et al., 2007). The reduced density and compromised morphology of the oligodendrocytes as well as signs of deviant myelination have been evident in schizophrenia (Uranova et al., 2007). Microglial activation in the CNS has been implicated in the pathogenesis of white matter disorders, and microglial cytotoxicity of oligodendrocyte has been reported to mediate through the free radical-related molecules such as NO, $\bullet O_2^-$ and their compound, peroxynitrite ($ONOO^-$) generated by activated microglia (Li et al., 2005; Merrill et al., 1993). On the other hand, one recent imaging report suggests that risperidone, which have anti-inflammatory effects on microglial activation in vitro (Kato et al., 2007), may be specifically impacting later-myelinating intracortical circuitry in patients with schizophrenia (Bartzokis et al., 2009).

Summing up the aforementioned evidence and our results, aripiprazole may thus have therapeutic effects on patients with schizophrenia by reducing the microglial inflammatory/oxidative reactions, which puts forward a novel therapeutic hypothesis beyond dopamine/neuron doctrine in the field of schizophrenia research. Besides schizophrenia, aripiprazole has proved to have therapeutic effects in depression, anxiety and other psychiatric disorders (Mohamed et al., 2009; Weber et al., 2008). In a recent animal study, aripiprazole has proved to have protective effects on the depression-induced oxidative stress in rat brain (Eren et al., 2007). This evidence has accorded with our present result that except for aripiprazole no other antipsychotics have an antioxidative effect via inhibiting superoxide generation from activated microglia. Thus, such wider range of aripiprazole pharmacological effects on various psychiatric diseases may be explained by the present result that aripiprazole inhibits the microglia-induced oxidative stress.

The brain is considered particularly vulnerable to oxidative damage. This intrinsic oxidative vulnerability of the brain suggests that oxidative damage may be a plausible pathogenic candidate of schizophrenia, depression and other psychiatric disorders (Ng et al., 2008). Therefore, our results may imply that aripiprazole acts, at least partially, by a different mechanism of action than other antipsychotics, which may be reflected in its deviating clinical profile. Regarding further studies, the molecular mechanism of the inhibitory effect of aripiprazole on the generation of $\bullet O_2^-$ radicals from PMA-stimulated microglia should be clarified in more detail, and in vivo studies should also be performed in order to confirm the present results.

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**Contributors**

All authors contributed substantially to the scientific process leading up to the writing of the present paper. TAK, the first author, and AM, the principal investigator of the present research made the conception and design of the project and wrote the protocol. The performance of experiments and the data analysis/interpretation were done by TAK, AM, KY, YM, HH, YS, SH, YHH, NS, EH and YM. TAK wrote the first draft of the manuscript. Critical revisions of the manuscript were made by TI, HU and SK. All authors contributed to and have approved the final manuscript.

**Conflict of interest**

All authors declare that they have no financial conflict of interest.

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ORIGINAL PAPER

# Depressive symptoms and apathy are associated with psychomotor slowness and frontal activation

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**Abstract** Affective symptoms, such as depression and apathy, and cognitive dysfunction, such as psychomotor slowness, are known to have negative impacts on the quality of life (QOL) of patients with mental and physical diseases. However, the relationships among depressive symptoms, apathy, psychomotor slowness, and QOL in a non-clinical population are unclear. The aim of the present study was to assess these relationships and examine the underlying cortical mechanisms in a non-clinical population. Fifty-two healthy male volunteers were assessed for depressive symptoms using the Zung Self-rating Depression Scale (SDS), for apathy measured using the Apathy Scale, and QOL using the Short-Form 36 item questionnaire (SF36). The volunteers also performed the Trail Making Test Part A (TMT-A) while undergoing assessment of hemoglobin concentration changes in the frontal cortical surface using 24-channel near-infrared spectroscopy (NIRS). The scores of the SDS and Apathy Scale showed significant negative correlations with the scores of most of subscales of the SF36. In addition, the SDS score had a significant positive correlation with the time to complete the TMT-A. Further, activation of several frontal cortical areas had a significant positive correlation with the scores of the SDS and Apathy Scale. These results suggest that the degree of depressive symptoms and apathy are associated with a lower QOL in a non-clinical population and that cortical hyperactivation during a psychomotor task measured by NIRS may identify objectively individuals with a high degree of depressive symptoms and apathy.

**Keywords** Depressive symptoms · Apathy · Psychomotor slowness · Cortical activation · Quality of life

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## Introduction

Depressive symptoms and apathy have major impacts on the mental and physical health of individuals. Major depressive disorder (MDD), for example, is characterized by depressive symptoms and loss of interest, which is a component of apathy, and is a leading cause of worldwide disability. Worsening of depressive symptoms is associated with a reduced quality of life (QOL) [7, 17, 31]. The presence of subsyndromal depressive symptoms has also been shown to have a negative impact on psychosocial functioning [9]. In addition, there is increasing evidence that depressive symptoms are influential in the onset or progression of various kinds of diseases including Alzheimer's disease [25], coronary disease [11], and diabetes [1]. Furthermore, there is substantial evidence suggesting the negative impacts of depressive symptoms and apathy on QOL in many diseases including HIV [33], Parkinson's disease [21, 29], and brain tumors [14].

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In addition to depressive symptoms and apathy, cognitive decline such as psychomotor slowness has a negative impact on social functioning of individuals. For example, Naismith et al. [20] reported that objectively measured psychomotor slowness is a significant predictor of physical disability in MDD, and Muslimovic et al. [19] reported that psychomotor slowness has a negative effect on QOL in Parkinson's disease. The Trail Making Test (TMT) is a popular neuropsychological instrument and is presumed to be a test of psychomotor skills [12, 27]. Functional neuroimaging studies have reported the involvement of the frontal cortical network in TMT [10, 18, 30, 40].

Meanwhile, neurocircuit abnormalities, an underlying condition in depressive symptoms and apathy in MDD, have been studied using neuroimaging approaches. For example, previous studies reported that anhedonic symptoms and depression severity were associated with reduced caudate volume [26] and decreased activation in the subgenual anterior cingulate cortex [16]. In addition, there is substantial evidence suggesting that psychomotor slowness in MDD is related to the fronto-striatal circuitry. Several studies using positron emission tomography (PET) reported that MDD patients with affective flattening and psychomotor slowness had decreased presynaptic dopamine function in the left caudate [2, 15].

In contrast to overt psychopathology such as MDD, there have been few studies that have examined the relationship among depressive symptoms, apathy and psychomotor slowness in a non-clinical population, and the cortical mechanisms of such symptomatology are unclear. Recently, the development of near-infrared spectroscopy (NIRS) has enabled non-invasive measurement of cortical activation under natural conditions, which enables examination while the subject performs a task related to psychomotor slowness such as the TMT-A. We hypothesized that the degree of depressive symptoms and apathy are associated with psychomotor slowness, as measured by TMT-A, and abnormal cortical activation, as measured by NIRS, as well as low QOL in a non-clinical population. We performed the following study to test this hypothesis directly.

## Methods

### Subjects

Fifty-two healthy male volunteers participated in this study (mean age, 37.4 ± 11.1 years). All subjects were determined to be right-handed using the Edinburgh Handedness Inventory scale [24]. Two experienced psychiatrists together excluded a participant with psychiatric symptoms above the threshold level. No subject had a history of major psychiatric disorder including major depressive disorder and anxiety disorder, neurological disorder, substance abuse, head injury, or major physical illness or was using any psychotropic medications at the time of the study. This study was approved by the Institutional Review Board of Mihara Hospital and the Prefectural University of Hiroshima. Written informed consent was obtained from each subject prior to the study.

### Assessment of depressive symptoms, apathy, and QOL

Each subject was assessed for subjective depressive symptoms, extent of apathy, and QOL.

Subjective depressive symptoms were measured using the Zung Self-rating Depression Scale (SDS), a self-rating scale that consists of 20 questionnaires. The score of the SDS ranges from 20 (best) to 80 (worst), and the average is 35.1 ± 8.0 (mean ± SD) in the Japanese normal control population [5]. A higher score of the SDS is an indicative of a relatively greater degree of depressive symptoms.

Extent of apathy was measured using the Apathy Scale, a self-rating scale for assessing a tendency of apathy that consists of 16 questionnaires. The score of the Apathy Scale ranges from 0 (best) to 42 (worst), and the average is 8.7 ± 6.6 (mean ± SD) in the Japanese normal control population [23]. A higher score of the scale is an indicative of a relatively greater degree of apathy.

QOL was measured using the Medical Outcomes Study Short-Form 36-item questionnaire (SF36) [39]. SF36 is used widely to assess physical and mental well-being in social and individual contexts. Eight subscales are derived, referring to 8 health concepts: physical functioning (SF36-PF), role functioning-physical (SF36-RP), bodily pain (SF36-BP), general health (SF36-GH), vitality (SF36-VT), social functioning (SF36-SF), role functioning-emotional (SF36-RE), and mental health (SF36-MH). Each subscale ranges from 0 (worst health) to 100 (best health), and a score of 50 represents the mean score for the population.

### Activation task

The activation task consisted of a 30-s pre-task baseline, a TMT-A, and a 70-s post-task baseline. Each subject sat on a comfortable chair in a quiet room, and the subject was ordered to keep their head immobile as much as possible and to not speak. During the test, the subjects were required to draw a line as rapidly as possible joining consecutive numbers (1–25), which were pseudorandomly arranged on each page. We used series of 4 TMT-A sheets, which had different circle position patterns. The time required for completing the test (TMT time) was determined as a measure of task performance. During the pre-task and

post-task periods, the subjects were instructed to draw lines repeatedly between two spots on a paper.

### NIRS measurement

In this study, changes in [oxy-Hb] and [deoxy-Hb] were measured using a 24-channel NIRS machine (Hitachi ETG-100) at two wavelengths of near-infrared light (i.e., 780 and 830 nm). Absorption was measured, and [oxy-Hb] and [deoxy-Hb] were calculated. The distance between the pair of emission and detector probes was 3.0 cm, and it was considered that the machine could measure points at a depth of 2–3 cm from the scalp, that is, the surface of the cerebral cortex [8, 35]. As shown in Fig. 1, the probes of the NIRS machine were placed on the subject's bilateral frontal region. The frontal probes measured hemoglobin concentration changes at 24 measurement points in a 6 ± 15 cm area, with the lowest probes positioned along the Fp1–Fp2 line according to the international 10/20 system used in electroencephalography. The absorption of near-infrared light was measured with a time resolution of 0.1 s. The obtained data were analyzed using the ''integral mode''. The pre-task baseline was determined as the mean across the last 10 s of the 30-s pre-task period, and the post-task baseline was determined as the mean across the last 5 s of the 70-s post-task period. Linear fitting was applied to the data between these two baselines. The moving average method was used to exclude short-term motion artifacts in the analyzed data (moving average window: 5 s).

### Data analyses

The analysis focused on [oxy-Hb] changes. Changes in [oxy-Hb] were assumed to more directly reflect cognitive activation than [deoxy-Hb] changes, as shown by a stronger correlation with blood-oxygenation level-dependent (BOLD) signals measured by fMRI [32].

NIRS data that clearly contained motion artifacts determined by a close observation of the subjects were excluded from analyses.

To examine the relationship among affective symptoms (SDS, Apathy Scale) and QOL (SF36), task performances (TMT time) and [oxyHb] changes during TMT, Pearson correlation analyses were conducted.

Statistical analysis was performed using PASW 18.0 software (Tokyo, Japan).

## Results

### Correlation between affective symptoms and QOL

Averaged scores of the SDS, Apathy Scale, and SF-36 are shown in Table 1. As shown in Table 2, the SDS negatively correlated with the SF36-RP ($r = -0.285$, $p = 0.041$), SF36-BP ($r = -0.279$, $p = 0.045$), SF36-GH ($r = -0.574$, $p < 0.001$), SF36-VT ($r = -0.635$, $p < 0.001$), SF36-RE ($r = -0.434$, $p = 0.002$), and SF36-MH ($r = -0.640$, $p < 0.001$). The Apathy Scale negatively correlated with the SF36-PF ($r = -0.367$, $p = 0.007$), SF36-GH ($r = -0.316$, $p = 0.023$), SF36-VT ($r = -0.459$, $p = 0.001$), SF36-RE ($r = -0.413$, $p = 0.002$), and SF36-MH ($r = -0.433$, $p = 0.001$). These results suggest that depressive symptoms and apathy are closely related to a lower QOL.

### Correlation between affective symptoms and task performance

The average TMT time was 75.4 ± 18.3 (mean ± SD) seconds. The score of the SDS was positively correlated

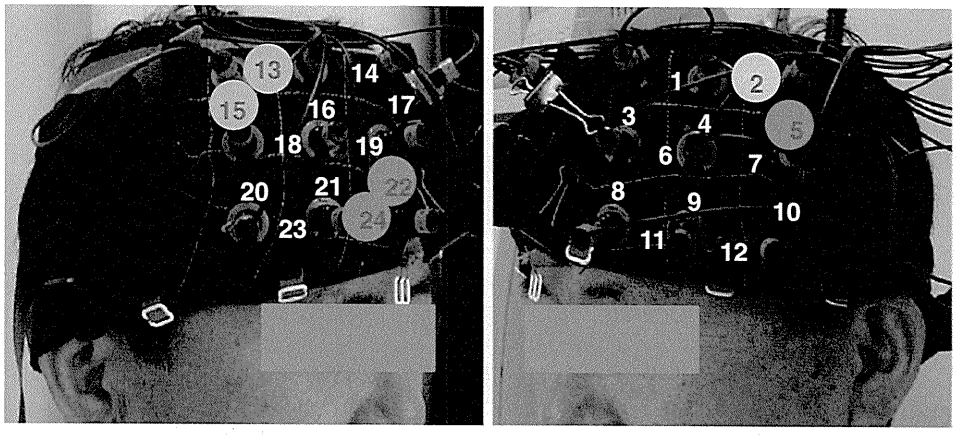


**Fig. 1** Probe setting and channels showing significant correlations with the SDS and Apathy Scale. *Yellow area* indicates a channel showing significant correlations with the SDS. *Blue areas* indicate channels showing significant correlations with the Apathy Scale. *Green areas* indicate channels showing significant correlations with both the SDS and Apathy Scale

**Table 1** Affective symptoms and QOL

| | Mean | SD |
|---|---|---|
| SDS | 36.8 | 7.7 |
| Apathy Scale | 9.8 | 6.0 |
| SF36-PF | 54.9 | 4.4 |
| SF36-RP | 50.5 | 7.0 |
| SF36-BP | 50.6 | 9.9 |
| SF36-GH | 50.9 | 10.9 |
| SF36-VT | 49.0 | 10.0 |
| SF36-SF | 50.7 | 8.5 |
| SF36-RE | 50.3 | 8.0 |
| SF36-MH | 49.4 | 9.2 |

*SD* standard deviation, *SDS* Zung Self-rating Depression Scale, *SF36* Medical Outcomes Study Short-Form 36-item questionnaire, *PF* physical functioning, *RP* role functioning, *BP* bodily pain, *GH* general health, *VT* vitality, *SF* social functioning, *RE* role emotional, *MH* mental health

**Table 2** Correlation coefficients between affective symptoms and QOL

| | SDS | Apathy Scale |
|---|---|---|
| SF36-PF | −0.261 | −0.367** |
| SF36-RP | −0.285* | −0.273 |
| SF36-BP | −0.279* | −0.207 |
| SF36-GH | −0.574** | −0.316* |
| SF36-VT | −0.635** | −0.459** |
| SF36-SF | −0.189 | −0.218 |
| SF36-RE | −0.434** | −0.413** |
| SF36-MH | −0.640** | −0.433** |

*SDS* Zung Self-rating Depression Scale, *SF36* Medical Outcomes Study Short-Form 36-item questionnaire, *PF* physical functioning, *RP* role functioning, *BP* bodily pain, *GH* general health, *VT* vitality, *SF* social functioning, *RE* role emotional, *MH* mental health

* $p < 0.05$; ** $p < 0.01$

with TMT time ($r = 0.357$, $p = 0.009$), suggesting that participants with depression took a longer time to complete the task. In contrast, there was no significant correlation between the score of the Apathy Scale and TMT time ($r = 0.261$, $p = 0.062$).

Correlation between affective symptoms and [oxy-Hb] changes during task

As shown in Table 3 and Fig. 1, [oxy-Hb] changes during the TMT-A was positively correlated with SDS in CH2 ($r = 0.442$, $p = 0.021$), CH13 ($r = 0.400$, $p = 0.013$), and CH15 ($r = 0.528$, $p = 0.006$) and with Apathy Scale in CH5 ($r = 0.451$, $p = 0.046$), CH13 ($r = 0.372$, $p = 0.021$), CH15 ($r = 0.0.711$, $p < 0.001$), CH22($r = 0.339$, $p = 0.017$), and CH24($r = 0.361$, $p = 0.009$). No channel showed [oxy-Hb] changes during the TMT-A that were negatively correlated with the SDS or the Apathy Scale. These results suggest that participants with depression and apathy required greater levels of functional activation in several brain areas to complete the task.

**Table 3** Correlation coefficients between affective symptoms and [oxy-Hb] changes during TMT

| Channels | SDS | Apathy Scale |
|---|---|---|
| 1 | 0.38 | 0.28 |
| 2 | 0.442* | 0.36 |
| 3 | 0.28 | −0.05 |
| 4 | 0.09 | 0.09 |
| 5 | 0.18 | 0.451* |
| 6 | −0.09 | 0.29 |
| 7 | −0.06 | −0.02 |
| 8 | 0.21 | 0.19 |
| 9 | −0.01 | 0.06 |
| 10 | −0.30 | 0.17 |
| 11 | 0.11 | 0.22 |
| 12 | −0.06 | 0.21 |
| 13 | 0.400* | 0.372* |
| 14 | −0.04 | −0.12 |
| 15 | 0.528** | 0.711** |
| 16 | −0.02 | 0.23 |
| 17 | 0.12 | 0.22 |
| 18 | −0.09 | −0.01 |
| 19 | 0.06 | 0.15 |
| 20 | −0.10 | 0.08 |
| 21 | −0.14 | 0.07 |
| 22 | 0.14 | 0.339* |
| 23 | −0.07 | 0.16 |
| 24 | 0.06 | 0.361** |

*SDS* Zung Self-rating Depression Scale

* $p < 0.05$; ** $p < 0.01$

## Discussion

In this study, we demonstrated that depressive symptoms and apathy negatively affect brain function and QOL in a non-clinical population. An unexpected, but interesting result was that depressive symptoms had a greater negative impact on task performance than apathy. In this study, we showed that the score of the SDS was positively correlated with the TMT time, but the degree of apathy was not correlated with the TMT time. We also showed that participants with a high degree of depressive symptoms and apathy had a greater [oxy-Hb] increase in many frontal cortical regions.

The degree of depressive symptoms and apathy were associated with most of indices of the SF-36. Our results are consistent with those reported by McCall et al. [17], who showed that an increasing severity of depression was associated consistently with worse QOL in MDD. Our results are also consistent with those of Oguru et al. [21], who reported that both the Apathy Scale and Beck Depression Inventory scores were negatively correlated with QOL in Parkinson's disease. Together, our results suggest that the presence of depressive symptoms and apathy has a negative impact on individual QOL.

The degree of depressive symptoms was associated significantly with psychomotor slowness, but the degree of apathy was not related to psychomotor slowness. The relationship between psychomotor slowness and age has been shown in previous studies [3, 34]. In our study, age was positively correlated with the TMT time, but there was no correlation between age and affective symptoms (data not shown). Psychomotor slowness in MDD has been shown in previous studies. For example, slower response times in MDD were observed on the TMT, Rule Shift Cards, and Stroop test [6]. Our results are consistent with those reported by Rosenberg et al. [28], who showed that the Geriatric Depression Scale was associated with incident impairment on all cognitive tests including the TMT-A in healthy older women. However, our results are inconsistent with those reported by Feli et al. [4], who showed that apathy correlated with a measure of information processing speed (Stroop test B) in older MDD patients. The reason for this inconsistency is unclear, but one possible reason is a difference between the tasks for psychomotor slowness. The TMT-A may not be sufficiently sensitive to detect the effects of apathy on brain function.

We also showed that participants with high degree of depressive symptoms and apathy had a greater [oxy-Hb] increase in many frontal cortical regions. Previous neuroimaging studies on cognitive impairment in MDD have demonstrated brain activation patterns with hypo-(e.g., Okada et al. [22]) and hyper-(e.g., Walter et al. [38]) activation of frontal cortical regions [13, 37]. In such studies, performance must be taken into account before attempting interpretation, and hyperactivation in context of equal or poorer performance is usually interpreted as 'inefficiency'. In this study, we found hyperactivation in the context of equal or poorer performance with a high degree of depressive symptoms and apathy, that is, inefficiency. Our results are consistent with those of Wagner et al. [36], who reported prefrontal hyperactivation with equal performance of the Stroop test in MDD using fMRI, and with results of Walter et al. [38], who reported that prefrontal hyperactivation with poor performance of Working Memory task in MDD using fMRI. Our results suggest that participants with a high degree of depressive symptoms and apathy require greater cortical resources to perform the same task. Furthermore, lower QOL and psychomotor slowness caused by depressive symptoms and apathy may be related to such inefficient frontal activation.

Our results are inconsistent with our hypothesis. We found that apathy was associated with low QOL and frontal cortical inefficiency, but was not correlated with psychomotor slowness. One potential explanation is that the effects of apathy may be more sensitively measured by cortical [oxy-Hb] changes detected by NIRS than by behavioral output. Thus, our present methods combining behavioral and NIRS measurement enabled us to detect the effects of apathy on brain function that would be difficult to detect by behavioral output alone.

There are several limitations in this study that should be taken into consideration. First, the participants were all male because women can have potentially influential factor such as mood fluctuations across the menstrual cycle, and our findings may not be generalizable to a female population. Second, assessments of depressive symptoms and apathy are based on self-rating scales without a structured diagnostic interview (e.g., SCID). Third, age and IQ were not controlled. They are potential factors capable of affecting not only psychomotor slowness, but also brain function and QOL. Fourth, depressive symptom was measured using the SDS. Although the SDS was developed specifically for patients with a diagnosis of major depression, the SDS is commonly used even in healthy subject study, since the scale is simple and less burdensome for subjects. Fifth, power analysis and multiple comparisons were not conducted in our study as in most previous NIRS studies. Further studies should take these factors into account. With these limitations in mind, this study provides evidence to support the hypothesis that depressive symptoms and apathy were associated with psychomotor slowness and abnormal cortical activation, as well as low QOL in a non-clinical population.

In conclusion, the degree of depressive symptoms and apathy were associated with lower QOL, and participants with high degree of depressive symptoms and apathy have inefficient cortical activations. On the basis of the findings, we assume that cortical hyperactivation during a psychomotor task measured by NIRS may be used to identify objectively individuals with a high degree of depressive symptoms and apathy. Further functional neuroimaging studies focusing on depressive symptoms and apathy at a non-clinical level may elucidate the brain mechanisms underlying depressive symptoms and apathy. These studies may be beneficial for promoting the QOL of healthy subjects and patients suffering from depressive symptoms and apathy.

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**Conflict of interest** None.



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# The Effect of Negative and Positive Emotionality on Associative Memory: An fMRI Study

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## Abstract

In general, emotion is known to enhance memory processes. However, the effect of emotion on associative memory and the underling neural mechanisms remains largely unexplored. In this study, we explored brain activation during an associative memory task that involved the encoding and retrieval of word and face pairs. The word and face pairs consisted of either negative or positive words with neutral faces. Significant hippocampal activation was observed during both encoding and retrieval, regardless of whether the word was negative or positive. Negative and positive emotionality differentially affected the hemodynamic responses to encoding and retrieval in the amygdala, with increased responses during encoding negative word and face pairs. Furthermore, activation of the amygdala during encoding of negative word and neutral face pairs was inversely correlated with subsequent memory retrieval. These findings suggest that activation of the amygdala induced by negative emotion during encoding may disrupt associative memory performance.

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## Introduction

The ability to learn and remember new associations between previously unrelated information is an important aspect of declarative memory. Declarative memory is associative, linking together component parts, such as words and objects, either directly or via spatial, temporal or other relationships. Previous neuroimaging studies have provided crucial information concerning the neural correlates that underlie this process. A variety of associative encoding tasks results in robust hippocampal activation, including the encoding of word pairs [1,2,3,4] and triplets [5,6], object pairs [7], and name–face pairs [8,9,10].

On the other hand, the relationship between memory and emotion is of paramount importance, given that people experience various affective states over the course of daily life. Although a recent review on memory and emotion has demonstrated that emotion may enhance memory processes that occur at all stages, including encoding, storage, and retrieval [11], we previously reported that negative emotionality does not necessarily promote good memory performance and associated hippocampal activation [12]. This discrepancy may be due to procedural differences. The most likely explanation is that the encoding of an association between items may have played a key role. The possible effects of emotionality associated with memory for paired items is unclear, even though the medial temporal lobe (including the hippocampus) showed greater activation for emotional items than for neutral items during both encoding [13] and retrieval [14] in studies of memory for single items. Furthermore, although much functional neuroimaging evidence has linked the memory-enhancing effect of emotion to amygdalic modulation during encoding [13,15,16,17,18,19,20] and retrieval [14], whether similar emotionality effects can be observed on associative memory remains unclear. Indeed, emotion does not necessarily enhance memory. When faced with negative events, people tend to pay attention to central features of such events while ignoring peripheral details [21,22]. As a result, memory of the negative event itself is enhanced, whereas memory of peripheral events is impaired. Furthermore, the difference between memory for gist and memory for detail can be more pronounced for negative than for positive events [23].

In this study, we hypothesized that negative emotion does not necessarily promote good associative memory performance, and that the amygdala has disparate influences on associative memory for positive and negative information. We used fMRI to investigate the effect of emotional (negative or positive) item valence on brain activation during an associative memory task, and examined the relationship between memory performance and brain activation affected by emotion during encoding and retrieval.

## Results

### Behavioral results

During the fMRI protocol, 15 healthy volunteers performed a novel face-emotional word paired associate task consisted of 'encoding', in which subjects were asked to remember pairs of neutral face and emotional (positive or negative) words, and 'control' and 'retrieval', in which subjects were asked to indicate

the word that was previously paired with that face (Fig. 1; see Methods for details). The mean correct response rates (mean ± SD) during retrieval were 48.9±14.7% for negative word and neutral face pairs and 58.1±13.3% for positive word and neutral face pairs. Accuracy rates across the two emotional conditions differed significantly (paired t-test, $t=-2.208$, $p=0.044$).

### Group analysis on each contrast

We performed fMRI group analysis on the four contrasts, subtracting the control condition from each experimental condition, according to a random effect model. All activations satisfying our criteria for significance are shown in Tables 1 and 2. We observed significant activation of the hippocampus during all of the 4 conditions, and significant activation of the left amygdala only during the negative encoding condition.

### Correlation between activations in regions detected by one sample t-tests and associative memory performances

We conducted a secondary correlation analysis to examine the relationship between brain activation in regions detected by one sample t-tests and associative memory performance. There wasn't positive correlation between brain activation and associative memory performance in any areas. In contrast, this analysis revealed that left amygdala and hippocampus activation during encoding of negative word and neutral face pairs (contrast estimate of 'negative encoding – control') was inversely correlated ($r=-0.527$, $p=0.043$, and $r=-0.519$, $p=0.047$, respectively) with successful retrieval (Table 3).

### Differential effects of negative and positive emotion on encoding and retrieval

A 2×2 ANOVA was performed to examine the differential effects of negative and positive emotion on encoding and retrieval. All activations satisfying our criteria for significance are shown in Table 4. This analysis revealed significant emotion×task interactions in the left amygdala (Fig. 2A). Post hoc analysis (corrected by Bonferroni) of the averages of contrast estimates of voxels in this cluster revealed that the 'Encoding of negative word and neutral face pairs' showed a greater BOLD response compared with the 'Encoding of positive word and neutral face pairs' ($F=7.761$, $p=0.012$) and the 'Retrieval of negative word and neutral face pairs' ($F=8.335$, $p=0.015$) in this area (Fig. 2B).

### Correlation analysis between amygdala activation and associative memory performance

We conducted a secondary correlation analysis to examine the relationship between amygdala activation (mentioned above and shown in Fig. 2A) and the corresponding behavioral performance. This analysis revealed that amygdala activation during encoding of negative word and neutral face pairs (contrast estimate of 'negative encoding – control') was inversely correlated ($r=-0.850$, $p=0.00006$), and that during encoding of positive word and neutral face pairs (contrast estimate of positive encoding – control) was positively correlated ($r=0.599$, $p=0.018$) (Fig. 3A) with successful retrieval (Fig. 3B). Amygdala activation during retrieval was not significantly correlated with a correct response rate regardless of whether the words were positive ($r=0.274$, $p=0.322$) or negative ($r=-0.165$, $p=0.557$).

## Discussion

In this study, we explored the effect of emotion on associative memory performance and its underling neural mechanisms. The hippocampus showed activations during the encoding and retrieval of word and face pairs regardless of whether the words were negative or positive. However, there wasn't positive correlation between activations in these regions and associative

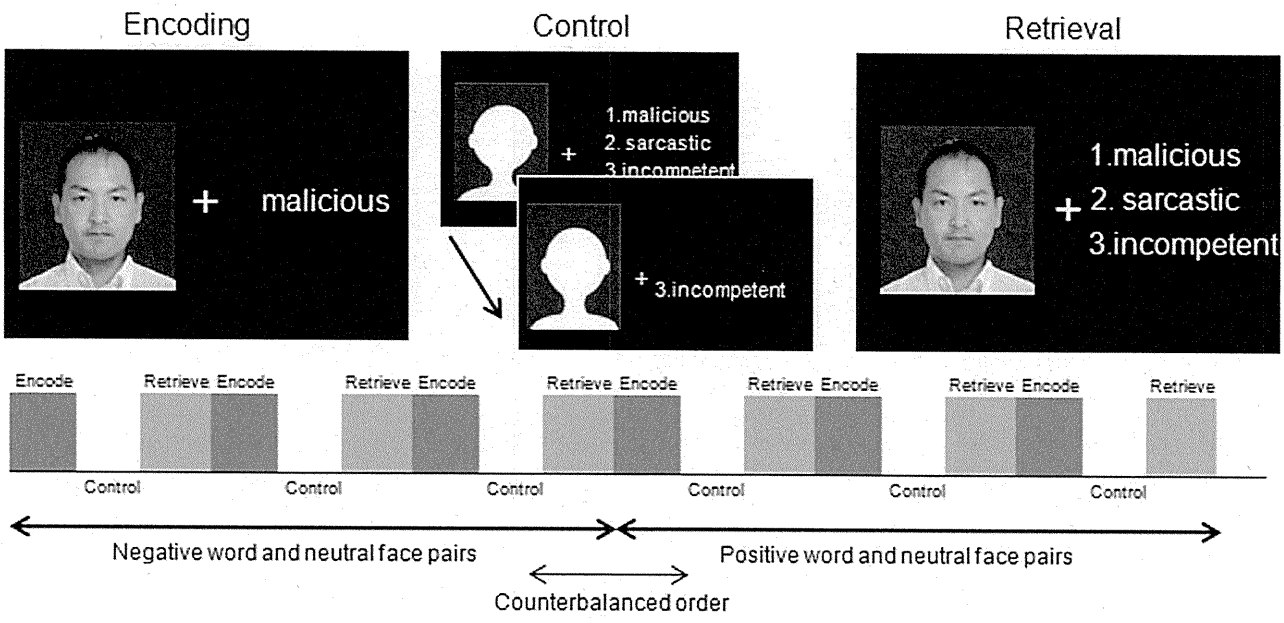


**Figure 1. Face-Word Association Paradigm.** Subjects were asked to learn pairs of neutral face and emotional (positive or negative) words related to personality-trait by pressing a button. After the control task in which subjects were asked to press one of the target button, each face was shown with 3 words and subjects were asked to indicate, via button press, which word was previously paired with that face. (The picture of one of the authors was used in the Figure instead of that from the database of SOFTPIA JAPAN to protect the privacy of subjects participated in the database).
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**Table 1.** Results of one sample t-test for negative word and neutral face pairs.

| Region | BA | Side | peak level | | cluster level | | x | y | z |
|---|---|---|---|---|---|---|---|---|---|
| | | | Z | p | $k_E$ | p | | | |
| Encoding | | | | | | | | | |
| Cerebellum | | R | 5.50 | 0.002 | 25 | 0.002 | 42 | −52 | −30 |
| Inferior Frontal Gyrus | 47 | L | 5.21 | 0.007 | 30 | 0.001 | −42 | −18 | −10 |
| Hippocampus | | R | 3.97 | 0.008[a] | 71[a] | 0.005[a] | 28 | −38 | 0 |
| Hippocampus | | L | 3.37 | 0.050[a] | 1[a] | 0.044[a] | −30 | −22 | −16 |
| Amygdala | | L | 2.91 | 0.050[b] | 1[b] | 0.046[b] | −30 | 0 | −22 |
| Retrieval | | | | | | | | | |
| Inferior Frontal Gyrus | 47 | L | 5.70 | 0.001 | 132 | 0.000 | −34 | 18 | −6 |
| Cerebellum | | R | 5.44 | 0.003 | 89 | 0.000 | 44 | −54 | −28 |
| Precentral Gyrus | 9 | L | 5.42 | 0.003 | 57 | 0.000 | −38 | 4 | 38 |
| Lingual Gyrus | 18 | L | 5.34 | 0.004 | 451 | 0.000 | −10 | −82 | −14 |
| Cerebellum | | L | 5.29 | 0.005 | 92 | 0.000 | 0 | −60 | −44 |
| Medial Frontal Gyrus | 32 | L | 5.14 | 0.009 | 65 | 0.000 | −8 | 12 | 50 |
| Angular Gyrus | 39 | L | 5.13 | 0.009 | 76 | 0.000 | −32 | −62 | 38 |
| Cuneus | 17 | L | 4.92 | 0.021 | 8 | 0.010 | −24 | −80 | 12 |
| Middle Occipital Gyrus | 19 | L | 4.83 | 0.030 | 19 | 0.003 | −26 | −92 | 4 |
| Cerebellum | | R | 4.82 | 0.031 | 11 | 0.006 | 8 | −74 | −50 |
| Superior Temporal Gyrus | 38 | L | 4.76 | 0.036 | 4 | 0.018 | −46 | 16 | −16 |
| Inferior Occipital Gyrus | 18 | L | 4.74 | 0.042 | 5 | 0.015 | −34 | −88 | −10 |
| Cuneus | 17 | R | 4.73 | 0.044 | 2 | 0.026 | 20 | −96 | −10 |
| Hippocampus | | L | 3.65 | 0.023[a] | 14[a] | 0.023[a] | −24 | −30 | −6 |

BA, Brodmann area; L, Left; R, Right; Z, Z value of the peak activation within the cluster; Coordinates for the peak voxel are listed as MNI coordinates. p, corrected p value for whole brain or region of interest ([a] bilateral hippocampus which include 1667 voxels or [b] bilateral amygdala which include 306 voxels); $k_E$, cluster size (voxels) difined by the same peak-level FWE thresholds and used for the cluster level testing.

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**Table 2.** Results of one sample t-test for positive word and neutral face pairs.

| Region | BA | Side | peak level | | cluster level | | x | y | z |
|---|---|---|---|---|---|---|---|---|---|
| | | | Z | p | $k_E$ | p | | | |
| Encoding | | | | | | | | | |
| Cerebellum | | R | 4.85 | 0.031 | 5 | 0.013 | 42 | −52 | −30 |
| Cerebellum | | L | 4.72 | 0.048 | 1 | 0.032 | −6 | −50 | −40 |
| Hippocampus | | R | 4.45 | 0.002[a] | 103[a] | 0.002[a] | 36 | −36 | −6 |
| Hippocampus | | R | 4.04 | 0.007[a] | 4[a] | 0.035[a] | 40 | −16 | −22 |
| Hippocampus | | L | 3.54 | 0.033[a] | 13[a] | 0.022[a] | −32 | −22 | −18 |
| Hippocampus | | L | 3.49 | 0.038[a] | 1[a] | 0.043[a] | −26 | −40 | 4 |
| Retrieval | | | | | | | | | |
| Cerebellum | | R | 5.91 | 0.000 | 174 | 0.000 | 42 | −54 | −30 |
| Middle Occipital Gyrus | 18 | L | 5.89 | 0.000 | 877 | 0.000 | −30 | −34 | −14 |
| Insula | 13 | R | 5.73 | 0.001 | 121 | 0.000 | 34 | 24 | 6 |
| Precuneus | 7 | L | 5.61 | 0.002 | 130 | 0.000 | −28 | −72 | 38 |
| Medial Frontal Gyrus | 6 | L | 5.46 | 0.003 | 234 | 0.000 | −6 | 14 | 50 |
| Inferior Frontal Gyrus | 47 | L | 5.22 | 0.009 | 45 | 0.000 | −34 | 18 | −4 |
| Inferior Occipital Gyrus | 18 | R | 5.19 | 0.010 | 71 | 0.000 | 22 | −92 | −12 |
| Cerebellum | | R | 5.10 | 0.014 | 8 | 0.006 | 10 | −74 | −34 |
| Inferior Frontal Gyrus | 9 | L | 5.05 | 0.017 | 128 | 0.000 | −38 | 4 | 34 |
| Fusiform Gyrus | 19 | R | 4.98 | 0.023 | 13 | 0.002 | 32 | −80 | −20 |
| Cerebellum | | R | 4.97 | 0.023 | 13 | 0.002 | 2 | −62 | −42 |
| Cerebellum | | R | 4.94 | 0.026 | 11 | 0.003 | 36 | −72 | −28 |
| Cuneus | 18 | L | 4.88 | 0.033 | 5 | 0.010 | −24 | −82 | 12 |
| Caudate | | L | 4.86 | 0.035 | 11 | 0.003 | −12 | −8 | 18 |
| Inferior Frontal Gyrus | | L | 4.84 | 0.038 | 5 | 0.010 | −46 | 16 | 2 |
| Hippocampus | | L | 4.40 | 0.002[a] | 114[a] | 0.001[a] | −24 | −30 | −4 |
| Hippocampus | | R | 4.12 | 0.006[a] | 151[a] | 0.000[a] | 24 | −26 | −8 |

BA, Brodmann area; L, Left; R, Right; Z, Z value of the peak activation within the cluster; Coordinates for the peak voxel are listed as MNI coordinates. p, corrected p value for whole brain or region of interest ([a] bilateral hippocampus which include 1667 voxels or [b] bilateral amygdala which include 306 voxels); $k_E$, cluster size (voxels) difined by the same peak-level FWE thresholds and used for cluster level testing.

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memory performances, and on the contrary, there was significant negative correlations between left hippocampus activation during negative encoding and the rate of successful retrieval. In contrast, left amygdala activation was observed only during encoding with negative emotionality, and there was also significant negative correlations between this amygdala activation and successful retrieval. In addition, a 2×2 ANOVA and subsequent Post-hoc analysis detected the region activated specifically during encoding with negative emotionality in the left amygdala. Furthermore, this amygdala activation was inversely correlated with subsequent memory retrieval with high significance. These results suggest that amygdala activation induced by negative emotionality may disrupt associative memory encoding.

Although research on memory and emotion has demonstrated that emotional (both positive and negative) events are often better remembered than neutral events [13,14], we reported previously that negative emotionality does not enhance memory for associated word pairs [12]. In this study, we demonstrated that paired items with negative emotionality are more poorly remembered than those with positive emotionality. In this discrepancy, the encoding of an association between items may have played a role, as compared to encoding single items. This hypothesis is supported by the results of previous studies [21,22,24] demonstrating that negative emotion enhances memory for gist, but reduces memory for detail. Although the face and emotional word associative memory assessment in the present study are quite different from the gist and event detail assessments used by previous studies, it is possible that the negative word meanings operate as gist, while the relationships between word and face pairs operate as more peripheral, less salient aspect of the encoding task. This emotion-related effect of memory may be

**Table 3.** Correlation between activations in regions detected by one sample t-tests and associative memory performances.

| | Region (peak coordinate) | Correct respons rate (negative) | Correct respons rate (positive) |
|---|---|---|---|
| Encoding (negative) | Hippocampus (28 −38 0) | $r=-0.446$, $p=0.096$ | |
| | Hippocampus (−30 −22 −16) | $r=-0.527$, $p=0.043$* | |
| | Amygdala (−30 −0 −22) | $r=-0.519$, $p=0.047$* | |
| Retrieval (negative) | Hippocampus (−24 −30 −6) | $r=0.037$, $p=0.895$ | |
| Encoding (positive) | Hippocampus (36 −36 −6) | | $r=0.121$, $p=0.668$ |
| | Hippocampus (40 −16 −22) | | $r=0.130$, $p=0.643$ |
| | Hippocampus (−32 −22 −18) | | $r=-0.077$, $p=0.786$ |
| | Hippocampus (−26 −40 4) | | $r=-0.000$, $p=0.999$ |
| Retrieval (positive) | Hippocampus (−24 −30 −4) | | $r=-0.140$, $p=0.618$ |
| | Hippocampus (24 −26 −8) | | $r=0.079$, $p=0.780$ |

*r*, correlation coefficiency; *p*, p-value;
*, $p<0.05$.
doi:10.1371/journal.pone.0024862.t003

mediated by the amygdala, as suggested by the absence of the effect in individuals with amygdala damage [25]. However, the biological mechanism of such phenomenon has not been examined in detail in human functional neuroimaging studies. The pronounced inverse correlation between amygdala activation induced by negative emotionality and the correct response rate shown in this study provide direct evidence that amygdala activation during encoding is a mediator of this phenomenon.

Although we do not know the neural mechanisms responsible for the disruption of associative memory encoding with negative emotionality by amygdala activation, one possible mechanism is that amygdala activation enhances the attention to the negative word itself and reduces the attention to the association of the items required for task performance. This interpretation is consistent with the idea that the amygdala focuses processing resources on the most salient information, as Easterbrook originally proposed

**Table 4.** Results of 2×2 ANOVA.

| Region | BA | Side | peak level | | cluster level | x | y | z |
|---|---|---|---|---|---|---|---|---|
| | | | Z | p | $k_E$ | | | |
| Main effect of task | | | | | | | | |
| Encoding>Retrieval | | | | | | | | |
| Angular Gyrus | 39 | L | 6.46 | 0.000 | 312 | −56 | −62 | 38 |
| Parahippocampal Gyrus | 37 | R | 4.94 | 0.007 | 29 | 40 | −38 | −20 |
| Superior Frontal Gyrus | 9 | L | 4.71 | 0.020 | 7 | −20 | 44 | 44 |
| Superior Frontal Gyrus | 6 | L | 4.56 | 0.038 | 2 | −16 | 14 | 60 |
| Middle Temporal Gyrus | 39 | L | 4.53 | 0.042 | 2 | −40 | −66 | 16 |
| Hippocampus | | R | 4.86 | 0.000[a] | 112[a] | 38 | −18 | −20 |
| Hippocampus | | L | 3.56 | 0.024[a] | 6[a] | −32 | −26 | −16 |
| Retrieval>Encoding | | | | | | | | |
| Inferior Occipital Gyrus | 17 | L | >8 | 0.000 | 13337 | −10 | −92 | −12 |
| Insula | 13 | R | 5.72 | 0.000 | 217 | 32 | 26 | 2 |
| Midbrain | | L | 5.63 | 0.000 | 500 | −4 | −20 | −6 |
| Inferior Frontal Gyrus | 47 | L | 5.41 | 0.001 | 164 | −30 | 22 | −4 |
| Inferior Frontal Gyrus | 9 | L | 5.31 | 0.001 | 53 | −58 | 4 | 30 |
| Hippocampus | | L | 4.12 | 0.003[a] | 8[a] | −20 | −30 | −4 |
| Main effect of emotion | | | | | | | | |
| No area | | | | | | | | |
| Interaction Task×Emotion | | | | | | | | |
| Amygdala | | L | 3.02 | 0.036[b] | 6[b] | −24 | −8 | −16 |

BA, Brodmann area; L, Left; R, Right; Z, Z value of the peak activation within the cluster; Coordinates for the peak voxel are listed as MNI coordinates. p, corrected p value for whole brain or region of interest ([a] bilateral hippocampus which include 1667 voxels or [b] bilateral amygdala which include 306 voxels); $k_E$, cluster size (voxels) difined by the same peak-level FWE thresholds.
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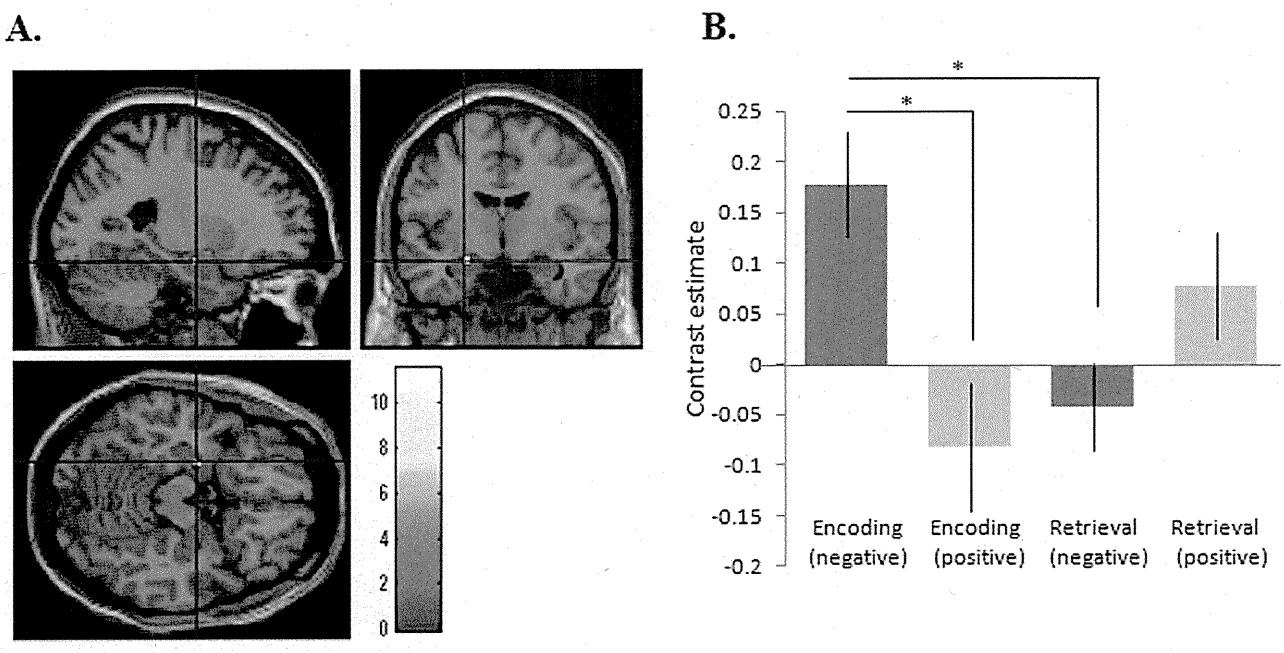


**Figure 2. Differential effects of negative and positive emotion on encoding and retrieval.** A. shows the brain region in which task×emotion interaction was detected (MNI coordinate: x = −24, y = −8, z = −16). B. shows the graph displaying the contrast estimates (mean ± SE) for the region of interest shown in Figure 2A for the 4 contrasts (negative encoding, positive encoding, negative retrieval, and positive retrieval compared to the relevant control) *: $p<0.05$.
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[26]. In fact, our regression analyses also revealed a significant inverse correlation between the correct response rate and the magnitude of brain activation in the left hippocampus. This means that activation of this region also disrupted rather than contributed to the associative memory processing. Given the fact that amygdala activity has been reported to correlate with subsequent memory for emotional material [15,16,27] and the influence of the amygdala on the efficacy of encoding is believed to be expressed through its effect on the hippocampus, it is plausible that the amygdala may focus processing recourses automatically on the negative words and not the association of paired items required for task performance.

In addition to the results mentioned above, amygdala activation during the positive encoding was positively correlated with the task performance of associative memory in this study. Although the mechanism of this inverse effect of amygdala activation on

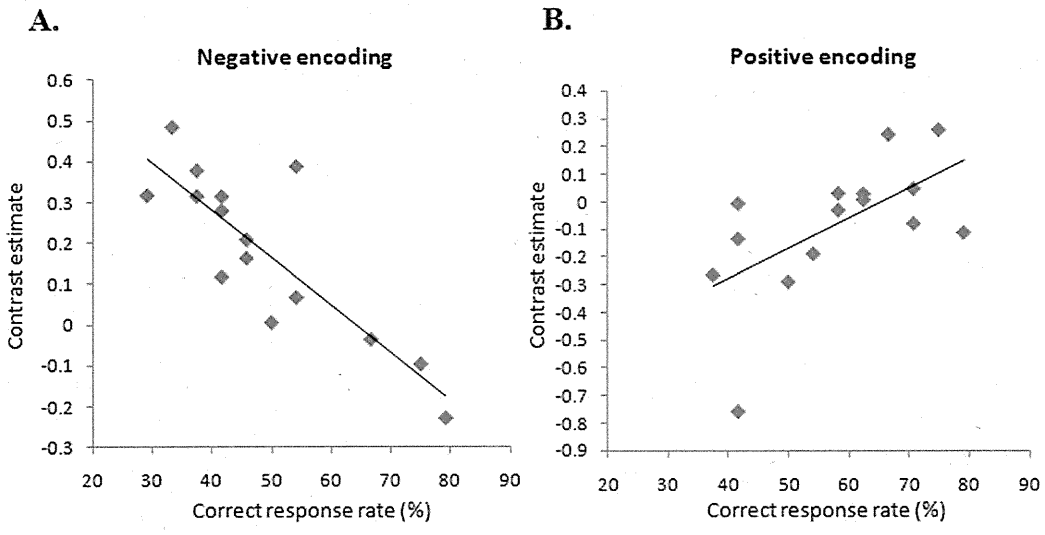


**Figure 3. Correlation between amygdala activation and associative memory performance.** A. shows the graph illustrates the inverse correlation between correct response rate of negative word - face pairs and the contrast estimates during encoding of negative word - face pairs in the region of interest shown in Figure 2A. B. shows the graph illustrates the positive correlation between correct response rate of positive word - face pairs and the contrast estimates during encoding of positive word - face pairs in the region of interest shown in Fig. 2A.
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associative memory is unclear, results of previous studies of patients with amygdalar damage suggest that the amygdala can both potentiate and reduce gist memory, depending on the encoding context [25]. Although additional research is required to better understand the circumstances (positive or negative, single item or paired item) in which amygdala activity can disrupt or facilitate memory encoding, the number of cues to which an organism attends may be modulated by emotional valence and not by arousal mediated by the amygdala.

There are certain limitations in this study that should be taken into consideration. First, we did not include a neutral word condition, so there is the possibility that the significant difference of associative memory performance between negative and positive condition was due to the enhancing effect of the positive word rather than the disrupting effect of the negative word. Although our previous study demonstrated that the correct response rate of negative word pairs was significantly lower than that of neutral word pairs [12], it remains unclear whether the effect of a negative word on face-word associative memory performance would be significant relative to neutral stimuli. However, it is plausible that amygdala activation during negative encoding disrupt the associative memory performance from the pronounced inverse correlation between amygdala activation and associative memory performance. Second, we did not evaluate unpleasantness during the task, so the direct relationship between amygdala activation and negative emotionality is not necessarily clear in this study, although it is reasonable to consider that amygdala activation was induced by negative emotionality of words. Third, as in previous studies [10,28], our control (baseline) task did not include real faces, so our results of one sample t-test included the regions which were related to the face perception as well as memory and emotion. However, face perception was equally included in each task (negative encoding, positive encoding, negative retrieval, or positive retrieval), and our results of a 2×2 ANOVA was never confounded by face perception. Fourth, we could not examine whether amygdala activation was positively or negatively correlated with the memory for the words themselves, so we could not directly compare the role of amygdala activation between single item memory and associative memory encoding. Further study is needed to address this issue. Finally, we did not use an event-related subsequent memory design which would be appropriate to directory justify the role of brain activation on associative memory performance, but the block-design in which we can raise the BOLD signal to measurable level in shorter scanning run. The major reason of this selection of design was that we were interested in examining the neural activity of associative memory performance in each individual subject, and applying such paradigm to psychiatric disorders like depression that cannot be forced into longer scanning run.

In conclusion, we observed that associative memory encoding is differentially modulated by amygdala activation according to the valence of emotionality. In particular, robust inverse correlation between the amygdala activation during encoding with negative emotionality and associative memory performance was observed. These findings suggest that amygdala activation induced by negative emotion may automatically focus processing resources on the most salient information, and disrupt associative memory encoding directed by instruction.

## Materials and Methods

### Ethics Statement

The study was conducted under a protocol that was approved by the Ethics Committee of Hiroshima University. All subjects submitted informed written consent of their participation.

### Subjects

Fifteen healthy volunteers (6 men and 9 women), aged 21–27 years (mean age ± SD = 23.6±1.9 years), with no history of neurological or psychiatric illness, participated in the study. All subjects showed a similar level of intelligence as assessed by the Japanese Adult Reading Test (JART) (112.5±5.6).

### Experimental task

During the fMRI protocol, subjects performed a novel block-designed face-emotional word paired associate task. We developed this task from a face-name paired associate task [10,28] that included 3 distinct conditions: encoding, distracter (active baseline), and recognition. The task consisted of 18 blocks, each of which was preceded by an instruction slide informing the subject whether the block was encoding, control, or retrieval condition. Of these 18 blocks, 6 were encoding conditions, 6 were control conditions, and 6 were retrieval conditions. Conditions were interleaved and repeated 6 times (Fig. 1). The duration of each condition was 24 seconds and the preceding instruction slide was shown for 4 seconds. This resulted in a total task period of 9 minutes.

During encoding, pairs of a face and an emotional word were presented serially every 3s, and subjects were asked to remember each face-emotional word pair by pressing a button. The active baseline (control) required subjects to press a button when 2 of 3 words disappeared (randomly within a 3s interval). We used the same emotional words during the corresponding control condition, so as to focus on how emotions modulate the associative memory processing and not on emotional responses themselves. During the retrieval condition, each face was shown with 3 emotional words every 3s, and the subjects were asked to indicate, via button press, which word was previously paired with that face. Forty-eight neutral faces paired with 6 emotional words (3 positive and 3 negative) were used during the experiment, because of the difficulty to select 48 appropriate emotional personality trait words. Although this means that the same words were repeatedly presented with different faces, a different face was presented every time and this task did not require the ability to overcome interference. That is, 48 pairs were presented within encoding condition, and no face-word pair was repeated during the experiment. Each retrieval block tested memory for only the pairs that were in the preceding encoding block, but the presentation of faces was not in the same order in the retrieval block as they were presented in the encoding block. Neutral faces were selected from the database of SOFTPIA JAPAN (The database is not available on line to protect the privacy of subjects participates in the database). Three positive words and 3 negative wards were selected from Anderson's list of personality-trait words translated into Japanese, and were rated on emotional valence and familiarity by a different group of participants [29]. Positive words were from the top 20 positive words and negative words were from the bottom 20 negative words of this list. Positive and negative words were matched in familiarity and word length. Each face and word pair was presented only once during the encoding tasks. For the retrieval tasks performed after the encoding tasks, the remaining 2 of the 3 words were used as distracters. The negative and positive conditions were counterbalanced across the subjects. Stimuli were generated using a personal computer with Presentation software (Neurobehavioral Systems, Inc.; San Francisco, CA). Using an angled mirror, participants viewed the stimuli on a back projection screen mounted outside the scanner bore.

### Acquisition of MRI data

Imaging data were acquired using a GE 3.0 T scanner (General Electric, Milwaukee, Wisconsin). A time course series of 190

volumes per participant (including pre- and post-task period) was acquired with echo planar imaging sequences (TR = 3000 ms, TE = 27 ms, FA = 90deg, Matrix size = 64×64, FOV = 256 mm, 4 mm slice thickness, 32 slice, no gap). Functional scans lasted 9 minutes 30 seconds. After functional scanning, structural scans were acquired using T1-weighted gradient echo pulse sequences (TR = 7.2 ms, TE = 2.1 ms, FA = 20deg, Matrix size = 256×256, FOV = 256 mm, 1 mm slice thickness, 184 slice).

### Analysis

Data were analyzed using the statistical parametric mapping software package, SPM8 (Wellcome Department of Cognitive Neurology, London, UK). The first 5 volumes of the fMRI run (pre-task period) were discarded to ensure a steady-state MR signal, and the remaining 185 volumes were used for the statistical analysis. Each set of functional volumes was realigned to the first volume, spatially normalized to a standard template based upon the Montreal Neurological Institute (MNI) reference brain, and spatially smoothed using an 8-mm FWHM Gaussian kernel.

We modeled four contrasts for each individual, using a general linear model that included each condition (negative encoding, positive encoding, negative retrieval, and positive retrieval) compared to the relevant control conditions. Then, second level analyses were performed according to a random effect model. First, one sample *t*-tests were performed for each contrast. The statistical threshold of $p<0.05$, corrected for whole-brain family wise error (FWE) at a peak level was used, except for *a priori* hypothesized regions, which were thresholded at $p<0.05$, and corrected for small volume (search volume is *a priori* region of interest mask) FWE at a peak level. These *a priori* regions of interest included the hippocampus and amygdala, a region implicated in the processing of memory and emotion. The hippocampal and amygdalic region of the interest mask was created in Montreal Neurological Institute (MNI) space using the WFU Pick Atlas [30]. We used WFU Pick Atlas only for creating the hippocampal and amygdalic region of interest mask, and all other analyses were conducted by using SPM 8.

Second, Pearson's correlation analyses were performed using the averages of contrast estimates (negative encoding-control, positive encoding-control, negative retrieval-control, and positive retrieval-control) of voxels within the clusters detected by the one sample t-test, in order to examine whether activations of these regions during each condition were correlated with corresponding memory performances. Third, a 2×2ANOVA with factors of task (encoding or retrieval) and emotion (negative or positive) was performed. The statistical threshold for this analysis was also set at $p<0.05$, corrected for FWE at a peak level, and small volume correction (SVC) were applied for the hippocampus and amygdala. Finally, Pearson's correlation analyses were performed using the averages of contrast estimates (negative encoding-control, positive encoding-control, negative retrieval-control, and positive retrieval-control) of voxels within the same amygdala cluster detected in the interaction task×emotion interaction shown in Fig. 2, in order to examine whether activations of this region during each condition was correlated with corresponding memory performances.

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## Author Contributions

Conceived and designed the experiments: GO YO YK S. Yoshimura KO ST HY S. Yamawaki. Performed the experiments: GO YK SA YN. Analyzed the data: GO YK. Wrote the paper: GO YO YK KO S. Yoshimura S. Yamawaki.

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# Altered γ-secretase activity in mild cognitive impairment and Alzheimer's disease

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We investigated why the cerebrospinal fluid (CSF) concentrations of Aβ42 are lower in mild cognitive impairment (MCI) and Alzheimer's disease (AD) patients. Because Aβ38/42 and Aβ40/43 are distinct product/precursor pairs, these four species in the CSF together should faithfully reflect the status of brain γ-secretase activity, and were quantified by specific enzyme-linked immunosorbent assays in the CSF from controls and MCI/AD patients. Decreases in the levels of the precursors, Aβ42 and 43, in MCI/AD CSF tended to accompany increases in the levels of the products, Aβ38 and 40, respectively. The ratios Aβ40/43 *versus* Aβ38/42 in CSF (each representing cleavage efficiency of Aβ43 or Aβ42) were largely proportional to each other but generally higher in MCI/AD patients compared to control subjects. These data suggest that γ-secretase activity in MCI/AD patients is enhanced at the conversion of Aβ43 and 42 to Aβ40 and 38, respectively. Consequently, we measured the *in vitro* activity of raft-associated γ-secretase isolated from control as well as MCI/AD brains and found the same, significant alterations in the γ-secretase activity in MCI/AD brains.

## INTRODUCTION

Senile plaques, the neuropathological hallmark of Alzheimer's disease (AD), are composed of amyloid β-protein (Aβ). Aβ is derived from β-amyloid precursor protein (APP) through sequential cleavage by β- and γ-secretases. β-Secretase cleaves at the luminal portion (β-site) of APP to generate a β-carboxyl terminal fragment of APP (βCTF), an immediate substrate of γ-secretase, to produce different Aβ species (for a review see Selkoe, 2001). The most abundant secreted Aβ species is Aβ40,

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whereas the species that has two extra residues (Aβ42) is a minor one (<10%); however, the latter is the one that deposits first and predominates in senile plaques (Iwatsubo et al, 1994).

Presenilin 1/2 make up the catalytic site of γ-secretase. The enzymatic properties of γ-secretase that cleave the transmembrane domain of βCTF have been an enigma, although recent studies provided partial elucidation of this mechanism (Qi-Takahara et al, 2005; Takami et al, 2009). γ-Secretase has two product lines, which successively convert the Aβ49 and Aβ48 that are generated by ε-cleavage, to shorter Aβs by releasing tri- or tetrapeptides in a stepwise fashion. Aβ49 is successively cleaved mostly into Aβ40 via Aβ46 and Aβ43, while Aβ48 is similarly cleaved into Aβ38 via Aβ45 and Aβ42 (see Fig 1). Importantly, the differences between the amounts of released tri- and tetrapeptides determine the levels of the different Aβ species produced (Takami et al, 2009). Thus, the true activity of γ-secretase is defined by the amounts of tri- and tetrapeptides released, but not by the amounts of Aβ species produced. Of note, the most abundant species Aβ40 is derived not from Aβ42, but from Aβ43. Also Aβ38 is derived mainly from Aβ42 (Fig 1). The longer Aβs in cerebrospinal fluid (CSF) including Aβ49 and 46 as well as Aβ48 and 45 must be generated at negligible levels, but may neither be secreted to the interstitial fluid (ISF) nor recruited to CSF. This suggests that the status of brain, and possibly neuronal, γ-secretase could be accurately assessed by measuring all four Aβ species generated by the two product lines of γ-secretase.

Using enzyme-linked immunosorbent assays (ELISAs), we quantified Aβ40 and 43 and Aβ38 and 42 in CSF samples from control subjects and mild cognitive impairment (MCI)/AD patients. The CSF concentrations of Aβ43 and Aβ42 were found to be significantly lower in MCI/AD compared with controls. The ratio of Aβ38/42, which represents the ratio of product/precursor and thus the cleavage efficiency of Aβ42, was plotted against the ratio of Aβ40/43, which represents the ratio of product/precursor in the other product line and thus the cleavage efficiency of Aβ43. The ratio of Aβ38/42 was largely proportional to that of Aβ40/43, indicating that the two cleavage processes are tightly coupled, but both were generally higher in MCI/AD patients compared to control subjects These results

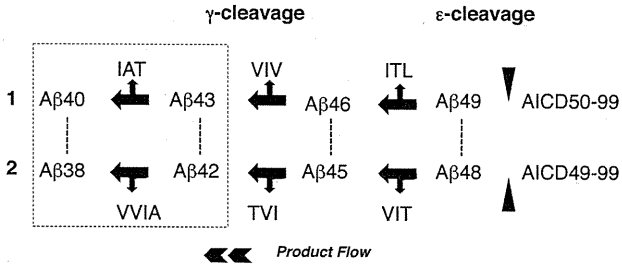


**Figure 1. Generation of Aβs through stepwise processing of βCTF.** At the first step, βCTF is cleaved at the membrane-cytoplasmic boundary (ε-cleavage), producing AICD (APP intracellular domain) 50–99 and 49–99. Counterparts Aβ49 and 48 in turn are cleaved in a stepwise fashion, releasing tri- and tetrapeptides. One product line converts Aβ49 mostly to Aβ40 via Aβ46 and Aβ43. The other product line converts Aβ48 to Aβ38 via Aβ45 and Aβ42. It should be noted that the differences between the amounts of released tri- or tetrapeptide determine the amounts of Aβs produced. Broken lines indicate corresponding Aβs on the two product lines.

suggest that the activity of brain γ-secretase in MCI/AD is enhanced at the conversion of Aβ43 to Aβ40 and Aβ42 to Aβ38, which would result in significantly lower CSF concentrations of Aβ42 and 43. In support of this hypothesis, the activities of raft-associated γ-secretase from control and MCI/AD brains were found to be significantly different: although the total Aβ production was similar, the γ-secretase in MCI/AD brains produced significantly larger ratios of Aβ40/43 and Aβ38/42 than the enzyme in control brains. This raises the possibility that lower CSF levels of Aβ42 and 43 simply reflect the altered γ-secretase activity in the MCI/AD-affected brains.

## RESULTS

The CSF concentrations of Aβs were in the following order: Aβ40 > Aβ38 > Aβ42 ≫ Aβ43 in all CSF samples examined (Table 1 and Supporting Information Fig S2A). The relative amounts of Aβs were constant across the samples: Aβ38:40 ratio in CSF was ~1:3, and Aβ42:43 ratio was ~10:1. The CSF

**Table 1. Subject characteristics and CSF concentrations of Aβs**

| | Control | MCI | AD | ANOVA ***p-value |
|---|---|---|---|---|
| Age (years) | 74.9 ± 7.5 | 72.5 ± 6.6 | 72.3 ± 8.2 | |
| *N* (male/female) | 21 (10/11) | 19 (7/12) | 24 (7/17) | |
| MMSE score | 28.7 ± 1.9 | 25.7 ± 2.6 | 19.6 ± 3.3 | |
| ApoE ε4 | 3 (14.3%) | 10 (52.6%) [a] | 14 (58.6%) [a] | |
| Aβ38 (pM) | 594.5 ± 286.3 | 669.4 ± 247.6 | 760.57 ± 269.4 | |
| Ln(Aβ38) | 6.28 ± 0.46 | 6.44 ± 0.38 | 6.56 ± 0.41 | NS |
| Aβ40 (pM) | 1607.9 ± 712.9 | 1939.5 ± 698.0 | 2292.6 ± 799.6 | |
| Ln(Aβ40) | 7.28 ± 0.47 | 7.51 ± 0.38 | 7.68 ± 0.35 | 0.007 |
| Aβ42 (pM) | 133.1 ± 53.4 | 83.2 ± 49.4** | 90.3 ± 40.1 [a] | |
| Ln(Aβ42) | 4.80 ± 0.47 | 4.25 ± 0.60 | 4.40 ± 0.47 | 0.004 |
| Aβ43 (pM) | 11.8 ± 5.7 | 6.8 ± 5.6** | 7.0 ± 4.6** | |
| Ln(Aβ43) | 2.32 ± 0.60 | 1.59 ± 0.86 | 1.76 ± 0.62 | 0.004 |

[a]2 MCI subjects were homozygous for ε4, while 4 AD subjects were homozygous for the allele.
**$p < 0.05$; Dunnett's *t*-test after log-transformation for comparing between control and MCI or AD.
***$p$-value of analysis of variance after log-transformation.