Mental rotation of alphabet characters affects the face-sensitive N170 component
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Face-sensitive N170 component of event-related potential is sensitive to face inversion, which has been defined as the face-inversion effect. Moreover, a previous study suggested that similar to the face-inversion effect of the face-sensitive N170 affected by mental rotation of the face, object-related N170 of three-dimensional objects was also affected by the mental rotation of two-dimensional objects. The purpose of the present study was to compare the relationship between face-sensitive N170 during face perception (upright and inverted faces) and object-related N170 during character perception (normal and mirror version for alphabet characters). The results indicated that the latency of N170 for mirror version for alphabet characters was significantly longer than that for normal version for alphabet characters, and the latency of N170 for inverted faces was significantly longer than that for upright faces. Therefore, the results of the present study clearly indicate that face-sensitive N170 components are related to the mental rotation of normal and mirror version for alphabet characters. These results suggest the novel possibility that face-sensitive N170 latency can be used as a biomarker for mental rotation and that mental rotation is related to the fusiform area as a neural generator of N170 in the human brain. NeuroReport 31: 897–901 Copyright © 2020 The Author(s). Published by Wolters Kluwer Health, Inc.

Keywords: biomarkers, event-related potential, face-inversion effect, face-sensitive N170, mental rotation, object-related N170

Introduction
N170 has been reported as a face-sensitive event-related potential (ERP) component [1,2]. N170, a negative component of the ERP that emerges on bilateral posterior temporal electrodes, appears nearly 170 ms after presentation of human face. In addition, N170 amplitude was greater for human faces than for nonface stimuli and scrambled faces [3–10]. Moreover, because the face-inversion effect (FIE) affects the N170 component, the N170 component has been reported to be delayed further in latency and larger in amplitude when the faces were inverted [1,3,11–14]. A previous study compared N170 amplitude and latency for five different rotations of the face (0, 45, 90, 135, 180 degrees) [14]. This result indicated the highest N170 amplitude for faces rotated by 90 degrees, medium N170 amplitude for inverted faces (faces rotated by 180 degrees), and smallest N170 amplitude for upright faces (faces rotated by 0 degrees) [14]. Therefore, it may be presumed that the FIE of face-sensitive N170 is affected by the mental rotation of upright and inverted faces.

Furthermore, the object-related N170 component has been reported to reflect the perception of two-dimensional (2D) and three-dimensional (3D) objects [15,16]. Tanaka [16] directly compared the relationship between face-sensitive N170 during face perception (upright and inverted faces) and object-related N170 during object perception (2D and 3D objects, for example, triangles and squares). Results of this study showed that the latency of N170 for 3D objects was significantly more delayed than that for 2D objects and a similar delayed latency of N170 for inverted faces than upright faces [16]. A previous study suggested that 3D objects (e.g., solid triangles) may be recognized by mental rotation of 2D objects (e.g., plane triangles) which must be complicatedly, mentally rotated at various angles and directions [15]. Therefore, Tanaka [16] suggested that similar to the FIE of face-sensitive N170 that is affected by mental rotation of the face, the object-related N170 of 3D objects was affected by mental rotation of 2D objects.

The first study of mental rotation had used 3D objects as the stimulus [17]. Subsequently, Cooper and Shepard [18] had used alphabet characters as the stimulus. Cooper and Shepard [18] presented asymmetrical alphabet characters (e.g., R) as its normal or mirror image version (horizontally reversed alphabet character) in the mental rotation task. If FIE of N170 is affected by the mental rotation, it may be presumed that the FIE of N170 is not only affected by the mental rotation of 2D and 3D objects, but also by mental rotation of normal and mirror version of alphabet characters. Therefore, the purpose of the present study is to directly compare the relationship...
between face-sensitive N170 during face perception (upright and inverted faces) and object-related N170 during character perception (normal and mirror version for alphabet characters). More specifically, the purpose of the present study was to clarify whether face-sensitive N170 components are related to the mental rotation of normal and mirror version for alphabet characters. Based on this hypothesis, results of this study will demonstrate that the object-related N170 latency of mirror version for alphabet characters will be longer than the normal version for alphabet characters, similar to the influence of mental rotation of faces on the FIE of face-sensitive N170. Moreover, P1 is another face-sensitive, positive ERP component, that appears on medial occipital electrodes, nearly 100ms after human face presentation [2]. Because Tanaka [16] found that the latency of P1 for 3D objects was significantly longer than that for 2D objects, this study also analyses P1.

**Methods**

**Participants**

Twenty-two healthy, right-handed, East Asian participants [10 women; age 18–29 years (mean age 21.1 years)] participated in this study. All participants, with normal or corrected-to-normal vision, were recruited among students of Otemon Gakuin University and were paid for their participation. Written informed consent was obtained from each participant before the study and the study was approved by the Ethics Committee of Otemon Gakuin University.

**Stimuli**

Ten faces were downloaded from various websites as stimuli, with five East Asian young women and five men and were unfamiliar to the study participants. A total of 20 stimuli (stimuli faces were of two types – either upright or inverted) were developed using Photoshop 12 (Adobe Systems Inc, San Diego, California, USA). Normal and mirror version of alphabet characters were 10 asymmetrical alphabet characters (B, C, D, F, G, K, P, R, S and Z). Twenty alphabet stimuli (alphabet characters for stimuli were of two types – either normal or mirror version) were developed using the Paint function of Windows 10 (Microsoft Corporation, Redmond, Washington, USA).

All stimuli with faces and alphabet characters were airbrushed using Photoshop 12 to eliminate any outstanding features or blemishes and were presented on a white background. All face stimuli were presented with a front-on view and in grayscale, and all alphabet characters stimuli were drawn by lines of black color; their mean luminance was adjusted so as to equate this across all stimuli (luminance values = 13.5 cd/m²) using Photoshop 12 (see Fig. 1, for examples). Each stimulus of this study was subtended at visual angles of 6.9° × 7.2° and presented at the center of a 22-inch cathode ray tube (CRT) monitor (MITSUBISHI, Diamondtron M2, RDF223G, Chiyoda, Tokyo, Japan). The screen resolution was 1280×1024 with a refresh rate of 100 Hz.

**Procedure**

All participants were seated comfortably 1.0m in front of a 22-inch CRT monitor. Multi Trigger System (Medical Try System, Kodaira, Tokyo, Japan) was used for stimuli presentation and reaction time recording. Each trial was composed of: (I) a fixation mark (+) presented for 500 ms, (II) a stimulus presented for 500 ms, and (III) a judgment screen presented for 1000 ms. The intertrial interval varied between 1000 and 1500 ms. The four types of stimuli (upright or inverted faces, normal and mirror version characters) were presented in random order and with equal probability. On the judgment screen, the participant was instructed to identify the type of stimuli as quickly and accurately as possible and to respond by pressing one of the four buttons that were assigned to button number 1, 2, 3, or 4 with their right index finger. However, the four types of stimuli were randomly assigned a button number (1, 2, 3, or 4) for each participant. The participants performed 10 practice trials, followed by 300 experimental trials separated into three blocks.

**Recording and analysis**

Electroencephalography and electrooculography data were recorded by a 128-channel Sensor Net (Electrical Geodesics, Inc., Eugene, USA) using Ag/AgCl electrodes using the 10-5 system [19]. The electroencephalography and electrooculography electrode signals were recorded continuously (500Hz sampling rate, band-pass filter at 0.01–30Hz) using a common vertex (Cz) reference, and re-referenced to the common average with off-line. The impedance was maintained below 5 kΩ for all electrodes. Periods containing artefacts exceeding 140μV in both vertical and horizontal electrooculography voltages were excluded from the averages. Electroencephalography and electrooculography data were analyzed using the standard EGI Net Station 5.2.01 package.

Stimulus-locked ERPs were separately extracted for upright and inverted faces, as well as for normal and mirror version characters, from 200 ms before stimulus presentation to 300 ms after it, and were baseline corrected using the 200 ms pre-stimulus window. For analyses of the P1 latency and amplitude of the positive peak, electrode sites O1 and O2 were selected, and the electroencephalography signal in a window of 60 ms before presentation to 110 ms afterwards were selected. For analyses of the N170 latency and amplitude of the negative peak, the electrode sites P7, PO7, PO8, and P8 were selected, and the electroencephalography signal window 110 ms before presentation to 180 ms post-presentation of the stimulus were selected. The mean reaction time and ERP latency and amplitude (peak to peak) were analyzed for each participant for each stimulus type.
Statistical analysis
For the reaction time analyses, a one-way repeated-measures analysis of variance (ANOVA) of all stimulus types (normal and mirror version characters as well as upright and inverted faces) was used. For the P1 latency and amplitude analyses, a two-way (4 × 2) repeated-measures ANOVA with each of the stimulus types and electrodes (O1 and O2) was used; N170 latency and amplitude were analyzed using a three-way (4 × 2 × 2) repeated-measures ANOVA for all stimulus type, hemispheres (left and right), and electrodes (P7 vs. PO7 and PO8 vs. P8), with post hoc comparisons performed using Bonferroni's correction. Greenhouse–Geisser corrections were applied to P values associated with repeated-measures comparisons with multiple degrees of freedom.

Results
Behavioral results
There was no main effect of all stimulus types on the reaction time (mean ± SD): normal version characters, 357.75 ± 78.97 ms; mirror version characters, 364.62 ± 94.24 ms; upright faces, 344.75 ± 83.02 ms; and inverted faces, 339.79 ± 84.02 ms [F (3, 63) = 2.40; P = 0.080; η² = 0.10].

Event-related potential results
Figure 2 shows the grand-averaged electroencephalography waveforms (P1 component) for each stimulus type. P1 latency for normal version of the character stimuli was 86.82 ± 11.64 ms at O1 while it was 85.00 ± 13.94 ms at O2. P1 latency for mirror version of the character stimuli was 91.45 ± 13.46 ms at O1 and 88.91 ± 13.44 ms at O2. Moreover, P1 latency for the upright face stimuli was 91.09 ± 9.47 ms at O1 as well as 91.45 ± 11.46 ms at O2. P1 latency for the inverted face stimuli was 94.00 ± 13.17 ms at O1 together with 94.45 ± 12.34 ms at O2. There was a significant main effect of all stimulus types on the P1 latency [F (3, 63) = 4.94, P = 0.010, η² = 0.19]. Moreover, P1 latency for the inverted face stimuli was longer than that for the normal version character stimuli (P < 0.01), and N170 latency for the mirror version of the character stimuli was longer than that for the normal version of the character stimuli (P < 0.01), and N170 latency for the inverted face stimuli was longer than that for the upright face stimuli (P < 0.01). Table 1 also reports the mean N170 peak amplitude for all types of stimuli at the four-electrode sites (P7/P8 and PO7/PO8). There was a significant main effect of all stimulus types [F (3, 63) = 4.64, P = 0.01, η² = 0.18]. However, for the multiple comparisons, there was no significant difference in all stimulus types.

Discussion
N170 latency for the mirror version of the alphabet character stimuli was more delayed than the normal version of the alphabet character stimuli, similarly, N170 latency for the inverted face stimuli was more delayed than the upright face stimuli. There was no significant difference in P1 latency for between the normal and mirror version of the alphabet characters. Therefore, the results of the present study clearly indicate that face-sensitive N170 components are related to the mental rotation of normal and mirror version of the alphabet characters. These results suggest that analogous to the FIE of face-sensitive N170 that is...
influenced by the mental rotation of faces, object-related N170 of the mirror version of alphabet characters was influenced by the mental rotation of normal version of alphabet characters. This suggests a novel possibility that the face-sensitive N170 latency can be used as a biomarker for mental rotation.

Table 1  Mean N170 latency (ms) and amplitude (peak to peak, μV) for all stimulus types at four-electrode sites

| Electrode | Normal version characters | Mirror version characters | Upright faces | Inverted faces |
|-----------|--------------------------|--------------------------|--------------|---------------|
| P7        | 139.55 ± 9.82            | 145.18 ± 14.20           | 140.27 ± 8.51| 147.91 ± 10.30|
| PO7       | 139.45 ± 10.52           | 142.36 ± 12.47           | 140.09 ± 8.83| 146.55 ± 11.62|
| PO8       | 136.82 ± 11.10           | 140.27 ± 12.58           | 140.45 ± 12.15| 145.82 ± 10.92|
| P8        | 137.36 ± 10.74           | 141.73 ± 13.01           | 140.27 ± 13.17| 148.73 ± 16.14|

Previous studies that conducted a meta-analysis of the brain regions in neuroimaging studies associated with mental rotation indicated that mental rotation was accompanied by increased activity in the intraparietal sulcus and adjacent regions [20,21]. As suggested in the above study, N170 reflects mental rotation. In addition,
the neural generators of N170 are estimated to lie in the fusiform area of the brain by functional MRI studies [22]. Combining results of the present study with previous studies [20,21] suggests that mental rotation is not only related to the intraparietal sulcus and adjacent regions but is also related to the fusiform area of the brain. Furthermore, previous study measured regional cerebral blood flow during the performance of mental rotation tasks that required cognitive spatial transformations of alphanumeric stimuli by PET and found that there were significant increases in the right posterosuperior parietal cortex and the left inferior parietal cortex [23]. In working memory tasks, the posterior parietal cortex is centrally involved in manipulation processes, whereas activation of the middorsolateral prefrontal cortex is related to the monitoring of the information that is being manipulated [24]. Combining results of the present study with previous studies [20–24], it may be presumed that mental rotation is related to various regions of the human brain.

A previous study compared the FIE of N170 using six upright and inverted visual categories (human faces, cars, chairs, shoes, houses, and grebbles), and found that only the inverted human faces delayed and enhanced N170 [12]. However, another study compared the FIE of N170 in upright and inverted human faces along with upright and inverted Chinese characters and reported that Chinese characters also elicit the FIE of N170 [25]. Cognition of both faces and Chinese characters is believed to rely on configural information [25]. Because the present study used alphabet characters as a stimulus, it may be presumed that delayed N170 latency of the mirror version in the present study reflected configural information of alphabet characters.

In conclusion, the findings of the present study clearly indicate that the face-sensitive N170 component is related to the mental rotation of normal and mirror version for alphabet characters. These findings suggest a novel possibility that the face-sensitive N170 latency can be used as a biomarker for mental rotation and that mental rotation is associated with the fusiform area as a neural generator of N170 in the human brain.

Acknowledgements

Conflicts of interest
There are no conflicts of interest.

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