Electrophysiological Correlates Associated with Involuntary Processing of Single Letters in the Japanese Hiragana Script

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Early processing of print stimuli is reflected by the left-lateralized N170 of event-related potentials (ERPs). However, most previous ERP studies have focused on letter strings, and only a few have investigated single letters in opaque alphabetic writing systems, despite the fact that they are the front-end of visual word recognition and reading development. We examined ERPs for single letters of the highly transparent Japanese Hiragana script, which demonstrated a direct correspondence between graphemes and phonemes. We presented single Hiragana letters along with control stimuli (alphanumeric symbols in Experiment 1; unfamiliar foreign letters in Experiment 2) in rapid succession. The participants were asked to judge the stimulus colors and linguistic properties of letters that were task-irrelevant. In both experiments, Hiragana letters elicited more negative enhancement than control stimuli in the left hemisphere only, which showed a different pattern from the bilateral enhancement associated with single alphabetic letters. These results suggest that the implicit sound activation of Hiragana is automatic, which extends the "phonological mapping hypothesis" on the left-lateralization of N170 onto single letters.

Key words: event-related potential, single letter processing, Japanese Hiragana, transparent writing system

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Introduction

To fluently read a particular text it is essential to be able to rapidly and effortlessly recognize its individual letters. Most models of reading processes assume that individual letters are processed via a front end of visual word recognition modality (e.g., Dehaene et al., 2005; McClelland & Rumelhart, 1981; Pelli et al., 2003). This skill is acquired in initial stages of reading development; furthermore, studies suggest that preschool children’s fluency in naming and understanding of the letters, may predict reading development throughout school ages (Badian, 1998; Neuhaus et al., 2001). Therefore, it is imperative to investigate single letter processing to successfully construct both the theoretical framework and practical assessment of reading development.

The print-tuned and left-lateralized N170 has been previously considered as an electrophysiological manifestation of the initial print processing (Bentin et al., 1999). In expert readers, N170 has been reported as a form of a negative ERP enhancement, specifically correlated with strings of letters, than other visual control stimuli (e.g., symbols or false fonts), which suggests the involvement of orthographic processing in initial perceptual stages of categorization (Simon et al., 2004). Developmental studies suggested that the print-tuned N170 concurrently expressed with the initiation of learning to read (Maurer et al., 2006), and sometimes demonstrated delayed expression in children with developmental dyslexia (Maurer et al., 2006, 2011). These results showed that the print-tuned N170 can reflect the extent of visual expertise for letter stimuli.

Letter-strings specific negative enhancements have also been characterized to have leftward asymmetry (Maurer et al., 2008; Simon et al., 2004). Since N170 lateralization is modulated by orthographic transparency, it indexes the regularity of grapheme-phoneme conversion rules. To illustrate, Maurer and their colleagues reported that, while transparent German pseudowords elicited a left-lateralized N170 (Maurer et al., 2005b), opaque English pseudowords elicited bilateral N170 (Maurer et al., 2005a). Since implicit phonological processing may be involved in a regular orthography (i.e., German) instead of an irregular orthography (i.e., English), it has been suggested that the left-lateralization effect of N170 reflects the grapheme-phoneme mapping processes (phonological mapping hypothesis) (Maurer & McCandliss, 2007). The causal association between phonological processing and the leftward asymmetry of N170 was supported by artificial-script learning; left-lateralized N170 was observed only when participants were required to focus on grapheme-phoneme conversion in learning the new scripts (Yoncheva et al., 2010).

Although there are several ERP studies on letter strings, only a few have focused on single letters. Alphabetic single letters, in comparison with the control stimuli, elicited a negative enhancement with latency and scalp distribution similar to letter-strings specific N170 (Daffner et al., 2014; Stevens et al., 2013). However, it was bilateral, instead of left-lateralized; furthermore, enhancement and the lack of
left lateralization may be due to orthographical opaqueness (Stevens et al., 2013). This finding suggested that the phonological mapping hypothesis can be applied to single letters. Here, we predicted that single letters in the Japanese Hiragana script elicit a left-lateralized N170, since they are highly transparent and can be individually converted into sounds.

We employed a task similar to that by Okumura et al. (2015), which required identification of random targets that could be differentiated by using different colors among frequent standards in rapid stimulus presentation (Figure 1). This experimental design may have a number of advantages to elucidate the early implicit processing of letters/letter strings. The task to detect non-linguistic, simple visual feature imposes low load and may elucidate the mechanism behind involuntary aspects of processing or the extent of learning. Despite the non-linguistic nature of the task, Hiragana letter strings elicited a typical left-lateralized enhancement than the alphanumeric symbol strings as control stimuli in expert readers, suggesting an involuntary activation of phonological representations (Okumura et al., 2015).

Here, we compared the ERPs in response to single letters in Japanese Hiragana with alphanumeric symbols. Previous studies have frequently used this form of comparison between a well-learned print stimuli and less-learned symbols (Bentin et al., 1999; Maurer et al., 2005a, 2005b). However, the alphanumeric symbols used in here may involve either semantic or numerical operational associations, which may elicit neural responses different from that of letters. Therefore, in Experiment 2, we used unfamiliar foreign letters (i.e., Thai letters) as control stimuli presenting neither phonological nor semantic association for Japanese readers. We also controlled the number of letters utilized in this study.

**Experiment 1**

We explored ERP manifestations associated with the implicit processing of single letters in the transparent Japanese Hiragana script, with various alphanumeric symbols used as controls.

**Method**

Thirteen native graduate and undergraduate students (9 women and 4 men, aged 20–34 years) participated. All participants were right-handed and had normal or corrected-to-normal vision, without any reading difficulties. They were provided written, informed consent conforming to the Declaration of Helsinki and received financial remuneration for participating. This experiment was approved by the ethics committee at Hokkaido University.

Stimuli consisted of 14 Hiragana letters (い, う, え, あ, か, こ, し, た, と, に, ま, み, り) and alphanumeric symbols (@, &, #, %, ∀, +, =, ʃ, ꞏ, Ꞑ, ₿, $, ¥, §). We used letters identical to those used by Okumura et al. (2015), which were familiar to Japanese readers, and presented them individually. As shown in Figure 1, all stimuli (0.9° by 0.9°) were presented against a gray background at the center of a computer monitor. A frame with four curbs aligned in a rectangle (2.7° by 7.1°) was always displayed during blocks to indicate the location of stimulus presentation and fixation. The viewing distance was maintained at 70 cm. Each stimulus was presented for 100 ms with a random inter-stimulus
interval of 300–600 ms (50 ms/step). Hiragana letters and symbols were repeated seven times within each block; there were two blocks in total. We controlled the stimulus presentation and response collection via E-prime software (version 2.0; Psychology Software Tools, Sharpsburg, Pennsylvania, USA). Initially, the participants received a short practice block. The participants fixated on a central rectangle during the experimental blocks and were required to press a button with their right thumb as quickly as possible when they identified the navy letters/symbols (i.e., target), which were occasionally presented alongside stimuli in black color (i.e., non-target stimuli). Each block contained 175 non-target and 21 target stimuli.

Behavioral performance, including the percentage of correct target identifications (hits) and reaction times (RTs) for the hits, was measured. Responses that occurred within 150–800 ms after presenting the target were scored as correct, and responses to non-target stimuli were classified as false alarms (FAs). The behavioral results were subjected to t-tests.

An electroencephalogram (EEG) was recorded using an electrode cap (EasyCap GmbH, Herrsching, Germany) with 25 Ag/AgCl electrodes aligned according to the extended International 10–20 System (Fp1/2, F7/8, F3/4, Fz, T7/8, C3/4, Cz, P7/8, P3/4, Pz, PO7/8, PO3/4, POz, O1/2, and Oz). All channels were referenced using the tip of the nose. We recorded bipolar electrooculogram (EOG) via electrodes placed at Fp1 and below the left eye (vertical) and at the outer canthi of both eyes (horizontal). The EEGs and EOGs were amplified using a SynAmps amplifier (NeuroScan, Sterling, VA, USA), filtered with a band-pass of 0.1–30 Hz, and sampled at 500 Hz. Electrode impedance was maintained below 10 kΩ. The continuous EEG signals were divided into epochs from 200 ms before to 800 ms after the onset of the stimulus and were baseline-corrected relative to the 200 ms pre-stimulus interval. Epochs with artifacts above 75 µV at any EEG or EOG channel and those immediately after the target were excluded before averaging. The averaged epoch numbers were 109 (SE = 8) for letters and 110 (SE = 8) for symbols.

The intervals of mean amplitude for analyses were chosen considering the visual inspection of grand average ERPs and peaks of the difference waves (Figure 2). The intervals were 140–180 ms and 200–300 ms. The differences between letters and symbols were maximal at PO7/PO8. The mean amplitudes were subjected to a two-way repeated-measures ANOVA that included hemisphere (left/right) and stimulus type (letter/symbol). Partial eta squared ($\eta^2_p$) was used as a measure of effect size.

Results

The mean RTs of detecting the target were 446.72 ms (SE = 9.23) for letters and 441.47 ms (SE = 10.60) for symbols. Although the hit rates were slightly low (for letters, $M = 75.91\%$, $SE = 5.52$; for symbols, $M = 73.38\%$, $SE = 6.41$), we observed zero false alarms for all participants. The pattern of results may suggest that the participants had carefully executed the designated task. The mean RTs and hit rates did not differ between letters and symbols, $p > .1$.

The left column of Figure 2 shows ERP results in Experiment 1. Subtraction ERPs between letters and symbols showed a posterior negative effect with a peak at approximately 160 ms, and showed significant expression at the left occipito-temporal sites (PO7/PO8). This effect was tested for the mean amplitude during 140–180 ms post stimulus, and there was a two-way interaction between the hemisphere and the stimulus, $F(1, 12) = 6.98, p < .03$, $\eta^2_p = .37$. In post-hoc tests, we noted a significant simple main effect of stimulus type at the left electrode site, $F(1, 12) = 6.94, p < .03$, $\eta^2_p = .37$, but not at the right electrode site, $F(1, 12) = 1.99, p > .1$, $\eta^2_p = .14$, which indicated that the negativity for Hiragana letters was greater than that for symbols only at the left electrode site. We also tested simple main effects of hemisphere, but they were not significant either for letters, $F(1, 12) < 0.01$, $p > .9$, $\eta^2_p < .01$, nor symbols, $F(1, 12) = 0.73, p > .4$, $\eta^2_p = .05$.

Moreover, we also tested this print sensitivity at P7/P8 to compare our data to previous studies regarding
Hiragana letter strings, which showed prominent print-tuning effects at relatively anterior electrode sites (Okumura et al., 2015; Uno et al., 2017). There was only a significant main effect of stimulus, $F(1, 12) = 5.58, p < .04, \eta_p^2 = .32$, and the interaction between hemisphere and stimulus did not reach a significant level, $F(1, 12) = 3.23, p = .1, \eta_p^2 = .21$.

In addition to the negative enhancement for letters in the left hemisphere, we identified a difference between letters and symbols in a posterior P2 latency range. A main effect of stimulus was significant, $F(1, 12) = 8.13, p < .05, \eta_p^2 = .40$, between 200–300 ms, indicating that the ERPs for letters were more negative than those for symbols. This effect of stimulus type did not interact with either of the hemispheres, $F(1, 12) = 2.70, p > .1, \eta_p^2 = .18$.

**Experiment 2**

In Experiment 1, we observed an early print-tuning effect in the left hemisphere, and subsequent bilateral negative ERPs for single letters relative to familiar alphanumeric symbols. The early leftward response supported our prediction described in the Introduction, but it distributed over more posterior electrode sites. Experiment 2 used unfamiliar controls to confirm that these negative ERPs for single letters were not confounding of neural activities associated with the meaning of the alphanumeric symbols. Moreover, the accuracy of color discrimination in Experiment 1 was
somewhat low, so that in Experiment 2 more detectable, blue colored targets were used instead of the navy-blue color. This change could allow us to test the phonological mapping hypothesis of the left-lateralization of N170 for single letters in a task with a lower demand.

Method
The methods were identical to those in Experiment 1, unless noted otherwise. Twelve undergraduate students, who were native Japanese speakers and did not declare any reading difficulties, participated in this experiment (3 women and 9 men, aged 19–22 years). Thai letters (e.g., ฤ, ฦ, ฅ, ฅ, ฅ, ฅ, ฅ) were used as the control stimuli, which were unfamiliar for most Japanese university students, and all participants in this experiment reported that they could not read them. Hiragana and Thai letters consisted of 42 letters. Color of target stimuli was blue, which was more detectable than that in Experiment 1. Each stimulus was repeated twice within a block, and there were three blocks in total. Each block contained 156 non-target and 12 target stimuli. The averaged epoch numbers were 146 (SE = 10) for Hiragana and 145 (SE = 10) for Thai letters.

Results
The mean RTs of target detection were 400.83 ms (SE = 12.68) for letters and 399.04 ms (SE = 10.97) for Tai letters. The hit rates were higher than those in Experiment 1 (for Hiragana letters, M = 98.06%, SE = 1.02; for Thai letters, M = 95.34%, SE = 1.83) and there were no false alarms for all participants. The mean RTs and hit rates did not differ between Hiragana and Tai letters, p > .08.

The right column of Figure 2 shows ERP results in Experiment 2. Hiragana letters elicited a larger negative enhancement at occipito-temporal sites with a peak at approximately 160 ms than the Thai letters. Thus, the effect was tested in the same time window of 140–180 ms as Experiment 1 and its left-lateralization was presented by the two-way interaction between hemisphere and stimulus, $F(1,11) = 5.36, p < .05, \eta^2_p = .33$. In post-hoc tests, we noted a significant simple main effect of the stimulus type at the left electrode site, $F(1,11) = 5.65, p < .05, \eta^2_p = .34$, but not at the right electrode site, $F(1,11) = 0.56, p > .4, \eta^2_p = .05$. The differences between hemispheres were not significant for Hiragana, $F(1,11) = 0.05, p > .8, \eta^2_p = .00$, and for Thai letters, $F(1,11) = 0.49, p > .4, \eta^2_p = .04$. At P7/P8 electrode sites, any statistical effects were not significant, ps > .1.

At a later time window (200–300 ms), we observed a difference between Hiragana and Thai letters. This effect was supported by a main effect of stimulus, $F(1,11) = 9.06, p < .02, \eta^2_p = .45$. Although there was a significant interaction between the stimuli and hemispheres, $F(1,11) = 6.73, p < .03, \eta^2_p = .38$, the post-hoc tests demonstrated that the negative deflection for letters was observed in both left, $F(1,11) = 6.09, p < .04, \eta^2_p = .36$, and right hemisphere, $F(1,11) = 10.21, p < .01, \eta^2_p = .48$.

Therefore, we found similar results across Experiment 1 and Experiment 2.

Discussion
We aimed to clarify whether single letters of the transparent Japanese Hiragana elicit an early negative enhancement that resembles the N170 with leftward asymmetry, which has been observed for transparent letter strings. In the present study, single letters elicited a greater negative enhancement than single symbols during 140–180 ms over left occipito-temporal sites in Experiment 1. This print-tuning effect was also observed when control stimuli was Thai letters in an easier task in Experiment 2. The leftward asymmetry and latency range of the print-tuning effect corresponded with the typical letter-string specific N170, which had been previously reported in several studies (Bentin et al., 1999; Maurer et al., 2008; Okumura et al., 2015). Although the absolute N1 amplitudes for letters did not differ between left and right hemispheres in both experiments, such comparisons may rely on an oversimplified assumption.
that the two hemispheres are anatomically symmetrical (Zhao et al., 2012, 2014). Thus, we focused on differences between letters and symbols in each hemisphere and found the left-lateralized N170 print-tuning effect for single letters regardless of control stimuli and task demands.

Previous studies showed bilateral N170 for single letters in opaque alphabetic script (Daffner et al., 2014; Stevens et al., 2013). In contrast, Hiragana single letters are significantly transparent and it seems reasonable to assume that participants implicitly converted letters into sounds during the color detection tasks. Therefore, our finding on the N170 with leftward asymmetry supports the phonological mapping hypothesis of the left-lateralization of N170 (Maurer & McCandliss, 2007) for single letters. Furthermore, the similarity in latency across the N170s for single letters and that for words/non-words suggests that phonological mapping of single letters could concurrently exist in the cortical stage of processing as that for letter strings. However, N170 for single letters was maximal at the occipital sites (PO7/PO8) in our study, while that for the Hiragana words and nonwords was distributed slightly more anteriorly (P7/P8) (Okumura et al., 2014, 2015; Uno et al., 2017). This topographical difference was consistent with the cortical hierarchy of visual word recognition: the posterior parts of left occipito-temporal cortex represent individual letters, while the anterior parts are involved in processing the letter strings (Dehaene et al., 2005; Vinckier et al., 2007).

Importantly, while the print-tuned N170 in left hemisphere have often been observed with the uses of a one-back repetition detection (Maurer et al., 2005a, 2005b) or lexical decision task (e.g., Simon et al., 2004), we observed that such a response for single letters, as well as in our studies for letter strings (Okumura et al., 2015; Uno et al., 2017), in tasks that did not explicitly require to "read". This may be because attention directed toward letters during color discrimination facilitated the task-irrelevant phonological processing, through the mechanism of object-based attention-spreading across features (Duncan, 1984). Therefore, our task should be useful to examine the extent of automaticity of activation of letter/letter-string representations with their properties.

In the present study, we observed that posterior P2 amplitudes (200-300 ms) were greater in Experiment 1 than those in Experiment 2, which may be due to different task demands across the experiments. More importantly, the P2s were decreased in amplitude for letters compared to control stimuli, and the effects had a bilateral rather than left-lateralized distribution, in both experiments. Mere overlapping of prolonged N170 for letters does not account for the P2 effects because of the difference in scalp distributions. The smaller P2 for letters may be associated with transparency and/or saliency of Hiragana. Previous studies showed decreased P2 for pronounceable letter strings (Appelbaum et al., 2009; Sereno et al., 1998), which implies an interaction between orthographic and phonological processing. P2 can also be decreased for salient stimuli regardless of physical stimulus properties, reflecting less requirements of processing resources for salient stimuli (Straube & Fahle, 2010; Straube et al., 2010). Further investigations are required for elucidating the functional significance of P2 in print processing.

To the best of our knowledge, this is the first study to report the ERPs for single letters in transparent writing systems. The finding of N170 may provide fundamental data for future developmental studies to examine the processing of single letters of Japanese Hiragana. Our task does not require to discriminate linguistic, but simple visual properties, so that it would be less demanding even for children with or without reading difficulties. Thus, the task used here should be useful to investigate the development of early reading processes from preschool children throughout school age, and the impact of intervention for reading. Since processing single letters may be "front-end" for visual word recognition, and single letters should be acquired at first in reading development, investigations of electrophysiological responses to single letters as well as those to letter strings are important for revealing
the developmental changes of reading processes.

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