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Repetitive Paired-Pulse Transcranial Magnetic Stimulation Over the Visual Cortex Alters Visual Recovery Function

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ABSTRACT

Background: Some repetitive transcranial magnetic stimulation (rTMS) techniques fail to facilitate cortical excitability in the human visual cortex. A more effective and facilitatory method is needed to increase the feasibility of rTMS to explore visual cortex function.
Objective: The present study aimed to develop a novel tool for modulating the visual cortex excitability and examined the influences of repetitive transcranial magnetic paired-pulse stimulation (rPPS) on the visual cortex.
Methods: Optimal interstimulus intervals (ISIs) were determined to assess recovery function of visual evoked potentials (VEPs). Paired stimuli from checkerboard pattern reversals were presented at 11 ISIs from 50–200 ms. Each session consisted of control (S1) and paired (S1 + S2) epochs to extract the S2 response. The recovery function was calculated as the ratio of S2/S1 amplitudes. Subsequently, rPPS was utilized with a 1.5 ms ISI over the visual cortex at the stimulus intensity of the visual masking effect. Amplitudes and suppression ratios of the paired VEPs were compared before and after rPPS. The effect of single pulse TMS was also evaluated.
Results: Paired VEPs resulted in suppressive effects at ISIs up to 200 ms, with an optimal ISI of 90 ms due to small variability and moderate inhibitory effects. There was no significant effect of rPPS on N75-P100 with paired VEPs. Following rPPS, however, P100-N145 inhibition decreased up to 10 min. The single pulse protocol did not result in these effects.
Conclusions: Modulation of VEP recovery by rPPS suggested that rPPS exhibited a disinhibitory effect on the visual cortex.

Introduction

Since Barker et al. [1] first introduced the transcranial magnetic stimulation (TMS) technique, it has been used to explore the human motor system and other areas of neuroscience. Repetitive TMS (rTMS) is able to induce changes in brain activity that last after stimulation [2]. In general, high-frequency (5–20 Hz) rTMS results in a facilitatory effect, whereas low-frequency (≤1 Hz) rTMS exhibits the opposite effect on the cerebral cortex [3–7]. These effects have been attributed to a form of long-term potentiation or depression [8]. Researchers have applied rTMS to the visual cortex, as well as the primary motor cortex (M1), resulting in inhibitory effects—and a smaller number of facilitatory effects—to the visual cortex [9–11]. Amplitude habituation of pattern reversal visual evoked potentials (PR-VEPs) decreases after 1 Hz rTMS; however, 10 Hz rTMS has no significant effect [9,10]. Following 1 Hz rTMS over the visual cortex, visual-induced alpha desynchronization is also reduced [12]. Therefore, a more effective and facilitatory method is needed to increase the feasibility of rTMS to explore visual cortex function.

TMS in combination with paired-pulses has been shown to be more effective than single pulse TMS for stimulating the visual cortex. Gerwing et al. [13] demonstrated that the phosphene threshold (PT) of paired-pulses at an interstimulus interval (ISI) of 50 ms is 70% of the PT associated with single pulse TMS. In addition, using ISIs of 2–20 ms, PT is ~90% of the single pulse PT [14]. Sparing et al. [15] reported that short-interval subthreshold conditioning stimulus at ISIs (2–12 ms) applied to the visual cortex facilitates the perception of phosphene. Recently, Thickbroom et al. [16] developed a repetitive paired-pulse TMS (rPPS) to modulate M1 excitability; this technique consisted of paired TMS stimuli of equal intensity using a 1.5 ms ISI at a rate of 0.2 Hz for 30 min rPPS has a facilitatory effect in the corticospinal system, which lasts up to 10 min after stimulation. This method targets intracortical

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networks generating I-waves [16,17], and increased corticomotor excitability occurs at the supraspinal level [18,19]. This MEP amplitude increment closely resembles spike-timing-dependent synaptic models [17]. Initially, I-wave activities were studied in single motor unit responses *via* needle electrodes [20,21], and later, I-waves were confirmed in epidural activity [22]. In a previous study of a conscious patient with epidural electrodes, a significant MEP amplitude increase was paralleled by a slight increase in epidural volley amplitude [18]. Accordingly, Di Lazzaro et al. [18,23] proposed an additional mechanism other than I-wave networks [18,23]. In contrast, Cash et al. [24] compared I-wave facilitation curves encompassing the first three I-waves before and after rPPS, and concluded that the increased efficacy of synaptic events was associated with the generation of descending I-wave volleys.

The physiological mechanisms involved in the stimulation of non-motor cortical areas remain poorly understood due to difficulties in recording generated output, especially compared with easily obtained recordings from the pyramidal tract following M1 stimulation [25]. For this reason, rPPS has never been applied to visual cortex. Interestingly, periodic firing in cat visual cortex at an interval of 1.4 ms was reported after electrical stimulation of the optic nerve or of the cortical depth [26,27]. Therefore, paired-pulse TMS with an appropriate ISI may be a more effective method for inducing plastic changes in the visual cortex than single pulse TMS. Consistent with this hypothesis, recording of visual evoked single–unit activity during paired-pulse TMS to the visual cortex of anaesthetized cats showed that conditioning stimuli with intensities of 60–130% of the test stimulus caused facilitation of the test response [28]. This effect was strongest with an ISI of 3 ms and declined for longer ISIs [28]. Therefore, the present study hypothesized that the rPPS effect in the visual cortex could provide valuable insights into the mechanisms of rPPS engaged in cortical circuits.

The aim of the study was to apply rPPS to the visual cortex and to analyze subsequent plastic changes. PT is an index of visual cortex excitability [11,29,30]. However, phosphene perception does not always occur in all subjects, and objective studies are needed. Therefore, the present study adopted recovery function of PR-VEPs as an objective measurement of visual cortex excitability. Visual recovery function was first established by recording paired PR-VEPs while modulating ISIs to evaluate the suppressive effect. Then, visual cortex excitability of PR-VEPs was evaluated using optimal ISI before and after rPPS. Because participating subjects do not always perceive phosphene, special care was taken to determine the rPPS stimulus intensity; a masking effect of TMS was employed over the visual cortex to measure the visual masking threshold [31]. If rPPS over the visual cortex induces significant plastic changes, it may be a useful tool for the study of neurophysiological and psychological aspects of visual processing.

Methods

Subjects

Eighteen healthy individuals (11 men, 7 women; 20–39 years old) participated in the present study. No subjects had neurological, psychiatric, or other medical problems. The right eye and hand were dominant in all subjects. All subjects provided written informed consent, and the study was undertaken with the approval of the Human Ethics Committee of the Faculty of Medicine, Graduate School of Medical Sciences, Kyushu University.

VEP recordings

The subjects were seated in a comfortable chair in a quiet room with dimmed lights. PR-VEPs were recorded from 2.5 cm above the inion with a reference electrode placed on the forehead (FCz). A ground electrode was attached to the vertex (Cz). Ag–AgCl electrodes were applied to the scalp using collodion. Electrode impedance was maintained at <5 kΩ. Signals were amplified (Neuropack 8, Nihon Kohden, Tokyo, Japan) with a 0.5–200 Hz band-pass filter and were digitized at 1 kHz. Visual stimuli consisted of 15′ checkerboard patterns that were presented in the lower visual field. The stimuli subtended 6° of the visual angle from the vertical and horizontal midlines. TMS over the visual cortex induced phosphene and a masking effect in the lower visual field [32,33], so PR-VEPs from lower visual field stimulation were recorded. The stimuli were displayed on a cathode ray-tube monitor with a refresh rate of 100 Hz at a viewing distance of 114 cm. The white and black regions had a luminance of 120 cd/m^2 and 1 cd/m^2, respectively. The contrast was 98%, and the background luminance was set at a mean luminance of 60 cd/m^2. To maintain attention during the recording session, the subjects were requested to fixate on a red point in the center of the screen. The stimuli were generated using the VSG2/5 system (Cambridge Research Systems, Rochester, UK).

Experiment 1: Recovery function of PR-VEPs

Twelve subjects participated in Experiment 1. A single recording session comprised control (S1) and paired (S1 + S2) epochs of pattern reversals to extract S2 responses. Two control (S1) and two paired stimuli (S1 + S2), respectively, were alternated at an ISI of 700 ms (Fig. 1). If one control and one paired stimulus were presented alternatively, each control epoch (S1) always had a paired epoch (S1 + S2) for the previous stimulus, rather than an additional control epoch, and *vice versa*. The ISI of 700 ms allowed the visual cortex to fully recover from the previous effect of S1 or S1 + S2, because PR-VEPs in clinical examination are phase-reversed at a rate of 1 Hz (ISI: 500 ms) [34].

The paired stimuli had 11 different ISIs (50, 60, 70, 80, 90, 100, 120, 140, 160, 180, and 200 ms), and sessions for the various ISIs were recorded in a pseudo-random order. We also recorded paired PR-VEPs with ISIs of 10, 20, 30, and 40 ms from two subjects in a pilot study. A shorter ISI resulted in a stronger inhibitory effect, particularly at 10 and 20 ms ISIs. The purpose of this experiment was to determine the optimal ISI for paired PR-VEPs to estimate stable functional recovery. Therefore, shorter ISIs, which induced strong inhibition, were not utilized in further experiments. A total of 100 epochs were collected for each condition. Each recording session was 140 s, and the combined duration of the recording sessions was ~60 min. The EEG signals were averaged separately for each condition. Responses to the second stimuli (S2) were obtained by subtracting control VEP waveforms (S1) from paired VEPs (S1 + S2). All epochs were utilized after removing those with excessive artifacts by visual inspection. Peak latencies and peak-to-peak amplitudes of N75, P100, and N145 were analyzed. Subtracted VEP latencies were measured from the S2 onset, and VEP amplitude attenuations were normalized to control VEPs.

Experiment 2: Effect of rPPS on VEP recovery function

Fourteen subjects participated in Experiment 2. The eight subjects were identical to those from Experiment 1. rPPS was delivered to the visual cortex for 30 min, and control and paired PR-VEPs, with a 90 ms ISI, were recorded before and after rPPS every 5 min for 30 min. Peak latencies and peak-to-peak amplitudes of N75, P100, and N145 were measured for control and subtracted VEPs. The ratios of VEPs (S2/S1) for each peak were calculated as visual cortex excitability. For the paired VEPs, the time required for recording of all the recovery cycles was about 60 min. Therefore, a 90 ms ISI was chosen for the small intersubject variability and moderate inhibition of the amplitude ratio, as determined in Experiment 1.

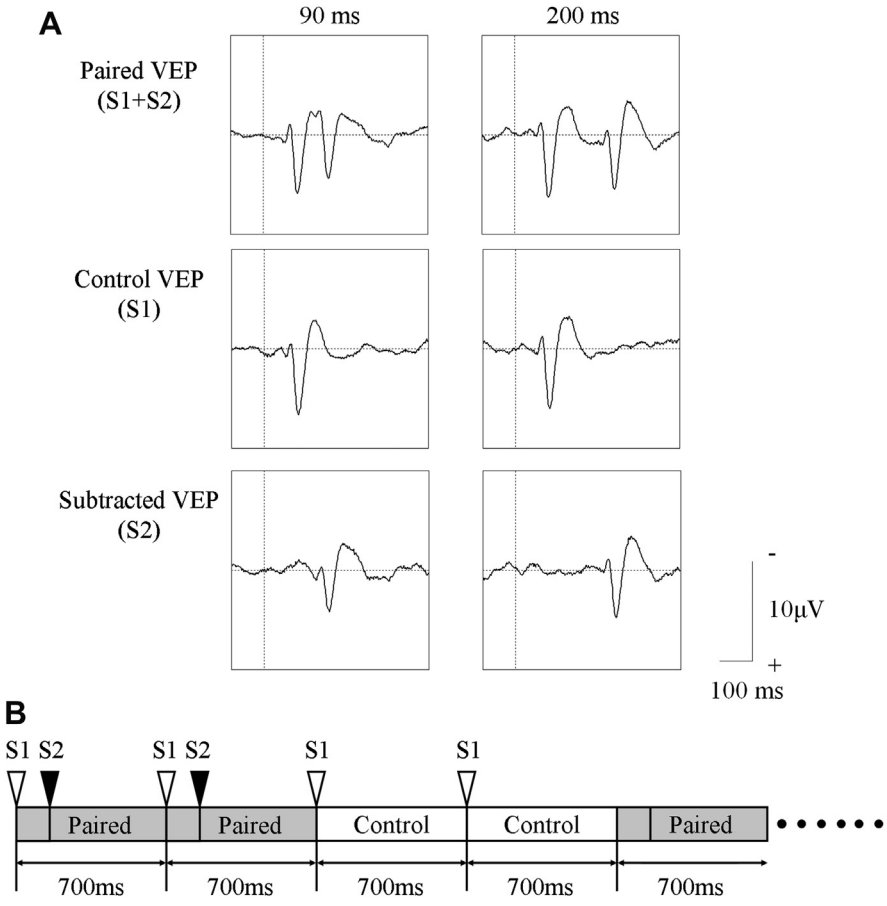

Figure 1. Grand averaged waveforms of paired pattern reversal VEPs at two ISIs (90 ms and 200 ms) (A) and the protocol for presenting paired stimuli (B). (A) Paired VEPs are obtained through two successive pattern reversals (S1 + S2), whereas control VEPs are elicited from a single pattern reversal (S1). Subtracted VEPs (S2) are calculated by subtracting control VEPs from paired VEPs. (B) S1 and S2 indicate time of reversals. Therefore, paired stimuli consist of two reversals (S1 and S2), and control stimuli have one reversal (S1). Subtracted VEPs represent responses to the second stimuli (S2).

TMS and rPPS

Two Magstim 200 stimulators (Magstim, Whitland, UK) were connected using a Bistim module (Magstim), and a round 90 mm in diameter coil was placed over the occipital cortex. The position of the lower coil edge was approximately 2 cm above the inion. The direction of the induced current in the brain was anti-clockwise, and rPPS was delivered at an ISI of 1.5 ms. Paired stimuli intensity was to the threshold of the visual masking effect (66.4 ± 4.0%; mean ± SD), as described below. rPPS was delivered every 5 s for 30 min (360 stimuli).

Visual masking effect for determining rPPS intensity

TMS over the visual cortex has been shown to suppress visual perception [31], which results from visual masking between visual information and TMS responses. PT is known to result in high test-retest reliability [35]. However, the success rate for inducing phosphene with single pulse TMS is highly variable. Therefore, obtaining a stable PT measurement requires a large number of TMS experiences [33]. Previously, our group encountered difficulty in obtaining phosphene in optic flow perception [36]. Therefore, the masking effect threshold was utilized to assess the rPPS effect.

Three random alphabet letters were presented for 30 ms in a triangular arrangement. Each letter subtended 0.8°, and the fixation point was positioned at the center of the three letters. A single TMS over the occipital cortex was delivered with a stimulus onset asynchrony of 105 ms after letter presentation. The optimal position of the magnetic coil for letter suppression in the right lower quadrant was determined over the scalp. The experiment started with an intensity of 50% of maximum stimulator output. The intensity was increased in 5% steps, and then decreased by 2% steps, to determine the threshold, which was defined as minimum intensity to induce suppression in $\geq$ 5 out of 10 stimuli. Paired stimuli at this threshold intensity, which was produced by a round coil, suppressed right and left lower quadrant letters. These results demonstrated that rPPS stimulated both hemispheres of the visual cortex.

Experiment 3: Effect of repetitive single pulse TMS on the VEP recovery function

Seven subjects were recruited from Experiment 2 to participate in Experiment 3. VEP amplitudes and amplitude ratios were compared with estimate visual cortex excitability before and after 30 min of repetitive single pulse TMS (rSPS) using the same ISI (5 s) and intensity as in Experiment 2. The VEP recording method was also identical.

Statistical analysis

The effects of the ISI on latencies and amplitude ratios in Experiment 1 were evaluated using a linear regression model with the subjects as the random effect. The rPPS effects on VEP amplitudes and the amplitude inhibition ratio in Experiment 2 were evaluated using a contrast method [37]. First, the baseline value was

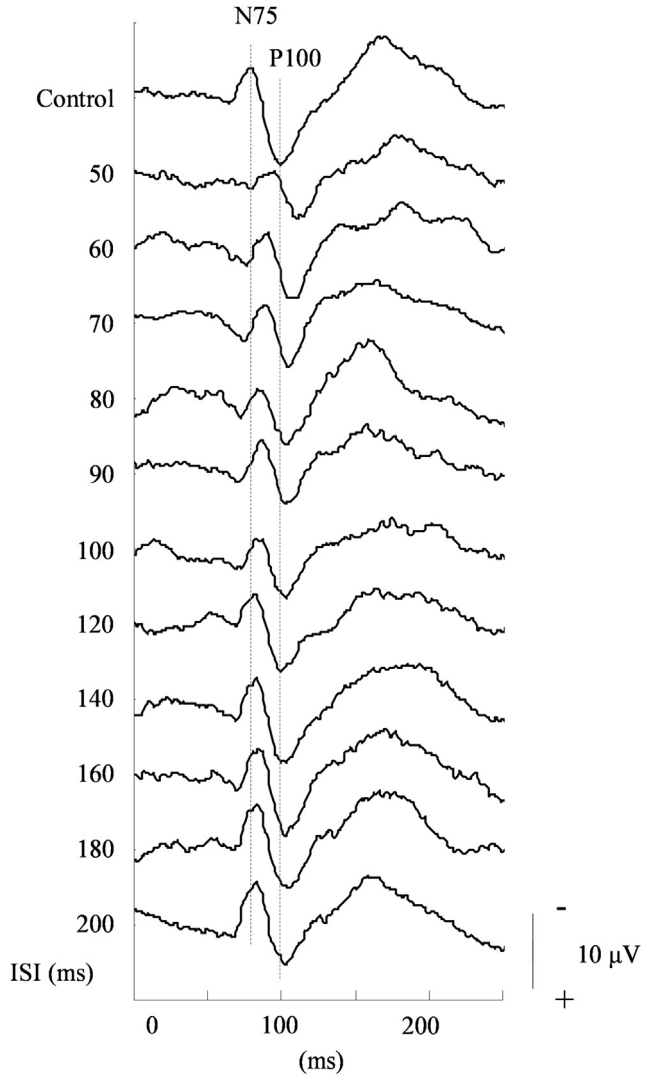

Figure 2. Control and subtracted VEPs with various ISIs in a representative subject. The top waveform shows control VEPs obtained from single pattern reversal stimulation. The lower waveforms represent subtracted VEPs for various ISIs (50–200 ms) and aligned with the stimulus onset of S2. Latencies of N75 and P100 components are more delayed with decreasing ISIs, and N75-P100 and P100-N145 amplitudes are more attenuated with decreasing ISIs, respectively. The dotted vertical lines represent N75 and P100 peak latencies in control VEPs.

compared with combined data after rPPS (baseline vs. 0–30 min after rPPS or rSPS). A post-hoc contrast method was then employed for further analysis (baseline *vs.* each time point after rPPS or rSPS). Data are presented as mean ± standard error mean. $P < 0.05$ was considered statistically significant throughout the study.

Results

Recovery curve for PR-VEPs

Grand averaged waveforms for paired stimulation (S1 + S2), control (S1), and subtraction (S2) are shown in Fig. 1. Amplitude of subtracted VEPs with a 90 ms ISI was less than in the control, although a 200 ms ISI resulted in no apparent reduction. In Fig. 2, the subtracted waveforms for each ISI demonstrated that latency delay and attenuation of the amplitudes were most prominent with shorter ISIs and became less apparent with longer ISIs. The amplitude ratio of S2 and S1 was smallest at 50 ms ISI for N75-P100 and at 60 ms ISI for P100-N145 (0.59 ± 0.07 and 0.70 ± 0.05, respectively;

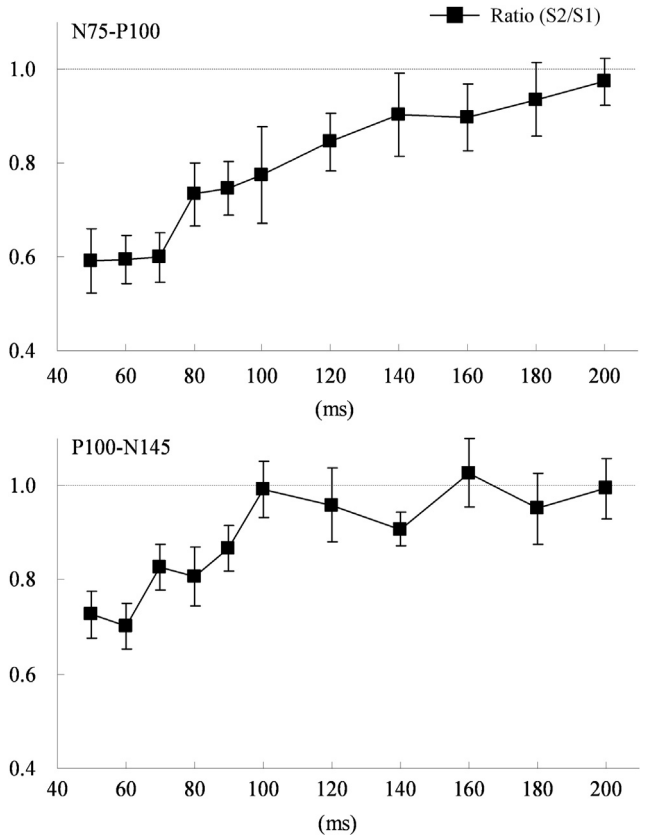

Figure 3. Recovery curve for amplitude ratios of VEP components ($n = 12$). Amplitude ratios (S2/S1) are shown for N75-P100 and P100-N145. Both N75-P100 and P100-N145 are attenuated at shorter ISIs and gradually recover with longer ISIs. The dotted horizontal lines represent a ratio of 1.0, indicating complete recovery. Values are expressed as mean ± SEM.

Fig. 3). The amplitude ratios exhibited linear recovery at 200 ms (N75-P100: $P < 0.001$; P100-N145: $P < 0.001$; Fig. 3). Amplitude suppression at 90 ms ISI was most stable and had a moderate value for both peaks (N75-P100, 0.75 ± 0.06; P100-N145, 0.87 ± 0.05). In addition, latency changes were substantial for N75 ($P < 0.001$) and P100 ($P < 0.001$), but were not evident for N145. The latency delay compared with control VEP (S1) was greatest at 50 ms ISI for N75 and P100, respectively (10.3 ± 1.7 and 6.3 ± 1.3 ms).

rPPS effects on the VEP recovery function

No subjects reported adverse side effects from the rPPS protocols, although all subjects experienced tolerable cutaneous sensations and/or neck muscle contractions as a result of occipital TMS. Based on results from Experiment 1, 90 ms ISI was adopted to evaluate visual cortex excitability.

Amplitudes from control VEPs (S1) and N75-P100 and P100-N145 were not significantly modulated by rPPS ($P = 0.54$; Fig. 4A). Similarly, amplitudes from subtracted VEPs (S2) for N75-P100 and P100-N145 were not significantly affected by rPPS ($P = 0.102$; Fig. 4A). Subsequently, amplitude ratios (S2/S1) for N75-P100 and P100-N145 were calculated (Fig. 4B). Amplitude ratios at 90-ms ISI before rPPS were 0.66 ± 0.03 for N75-P100 and 0.76 ± 0.04 for P100-N145, respectively. However, the N75-P100 amplitude ratio was not significantly different after rPPS (Fig. 4B). In contrast, the P100-N145 amplitude suppression ratio significantly increased after rPPS ($P = 0.006$; contrast method, baseline *vs.* 0–30 min). An increase in the P100-N145 amplitude ratio was also

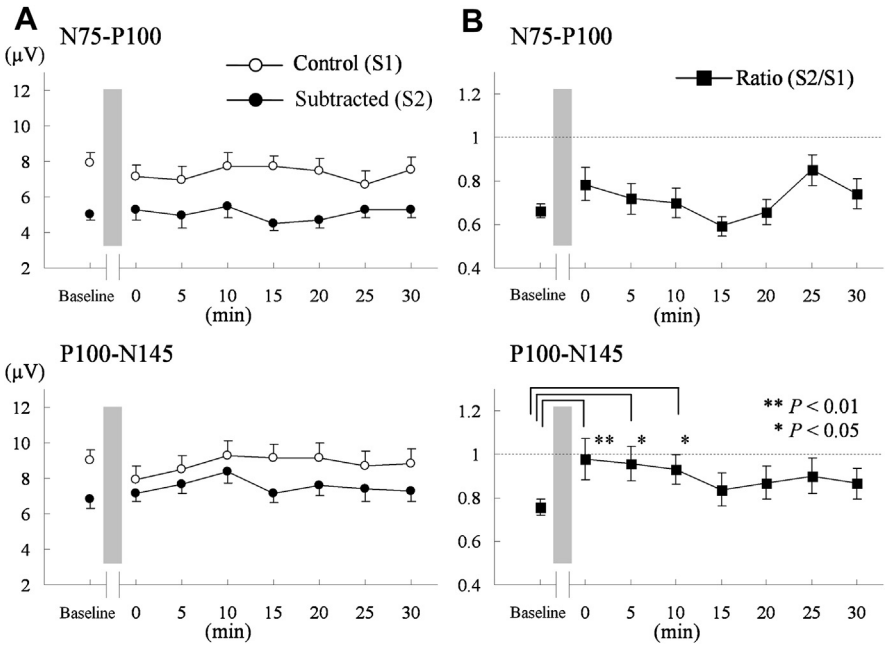

Figure 4. Changes in control (S1) and subtracted VEP (S2) amplitudes (A) and the amplitude ratios (S2/S1) (B) in major components before and after rPPS ($n = 14$). Amplitudes from control and subtracted VEPs are not significantly modulated by rPPS (A); however, the P100-N145 ratio significantly increases after 10 min rPPS, indicating disinhibition of paired VEPs (B). The N75-P100 ratio is not significantly modulated by rPPS. Values are expressed as mean ± SEM.

significant during the limited early period of 0, 5, and 10 min after rPPS ($P = 0.007$, $P = 0.015$, $P = 0.034$, respectively; Fig. 4B).

In Fig. 4A (lower panel) it appears that the main effect after stimulation (0 min) is due to a reduction of the control VEP amplitude (S1), and not to an increase in amplitude of the subtracted VEP (S2). To investigate this possibility, paired comparisons between the baseline value and each of the values at 0, 5, and 10 min were performed. The S1 amplitude at 0 min showed a trend towards a decrease ($P = 0.06$; Fig. 4A), but the amplitudes at 5 and 10 min did not show any significant changes ($P = 0.34$ and $P = 0.60$, respectively; Fig. 4A).

In contrast to the above results, there were no significant changes in VEP amplitudes (Fig. 5A) or amplitude ratios after rSPS, respectively (Fig. 5B).

Discussion

The present study utilized visual masking thresholds with a round coil to consistently stimulate the visual cortex. Paired PR-VEPs with various ISIs (up to 200 ms) induced only a suppressive effect. Following rPPS with an optimal ISI of 90 ms, P100-N145 inhibition decreased for 10 min, which represented disinhibition of the paired PR-VEPs. Modulation of the VEP recovery function, which was induced by rPPS with the visual masking threshold, provides new insight into plastic changes in the visual cortex.

PT is the gold standard for determining TMS stimulus intensity over the visual cortex [11,30]. However, phosphene is not always perceived by every healthy control. Therefore, it is difficult to consistently study visual cortex physiology across subjects. The effects of interventional TMS on the visual cortex have been estimated by PT changes [11]. However, phosphene perception has some limitations as a detailed assessment method for excitability changes in the visual cortex. For example, inhibitory continuous theta burst stimulation has been shown to increase PT, whereas facilitatory intermittent theta burst stimulation does not significantly alter PT [11]; these results suggested that PT determination is not sufficiently sensitive to reveal small changes. In addition, TMS at the PT intensity, as well as timing of the masking effect, delivered to early visual areas did not significantly affect VEPs in the visual suppression task, but the values were significantly altered by TMS at the visual masking threshold [38]. The present study utilized a stimulus intensity that induced a visual masking effect [31]. Initially, it was predicted that a figure-of-eight coil would be suitable for stimulating a single hemisphere. However, PR-VEPs from a lower quadrant of the visual field resulted in much smaller amplitudes, because of the small visual stimulus field in the pilot study. Therefore, a lower-quadrant PR-VEP was not sufficient to evaluate precise VEP recovery function. Altogether, results demonstrated that the visual masking technique was complicated, but provided a more stable method for activating the visual cortex in the general population.

Our group has developed a revised method that utilized paired PR-VEPs to obtain a detailed VEP recovery curve. Almost 30 years ago, Mitchell et al. [39] reported a recovery cycle of PR-VEPs in experiments using ISIs with 10, 15, 20, 30, 40, and 100 ms. In the Mitchel et al. [39] study, there was no clear pattern of recovery with respect to amplitude. The discrepancy between these two sets of results is likely due to methodological differences in PR stimulation. The luminance and check size of their visual stimuli were 12 times higher and 3 times larger than that of our stimuli, respectively (black and white checkered luminance were 17 cd/m^2 and 1440 cd/m^2, respectively, and the check size was 47′). Therefore, their PR stimulation may have stimulated not only the spatial frequency channel, but also the luminance channel. Recent studies using pattern onset (PO) [40] and flash stimuli [41] have demonstrated the suppressive effects of paired stimuli. Despite this, PO-VEPs are limited for ISIs less than 80 ms, because visual stimuli have a finite pulse length (13.3 ms) [40]. Cantello et al. [41] have reported successful implementation of a protocol for paired-pulse flash-VEPs. However, the flash VEP response ordinarily has a highly complex shape with high intersubject variability [34]. In addition, visual subsystems process visual information differently, including luminance, spatial frequency, color, and motion. Flash VEPs primarily evaluate the luminance channel, while PR-VEPs assess the spatial frequency (or contrast) channel [34]. Therefore, the

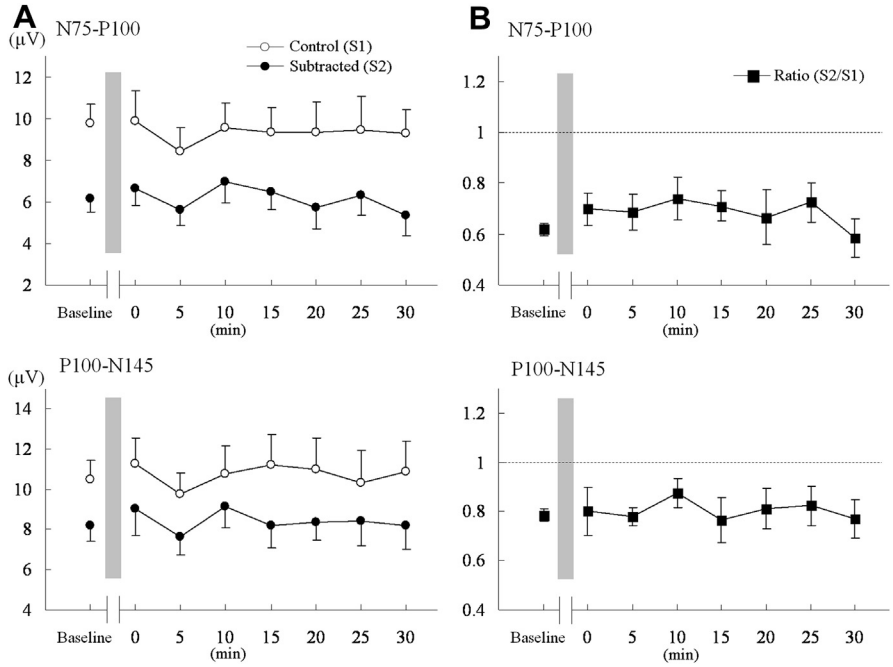

Figure 5. Changes in S1 and S2 amplitudes (A) and the amplitude ratios (S2/S1) (B) in major components before and after rSPS ($n = 7$). S1 and S2 amplitudes are not significantly modulated by rSPS. Similarly, the N75-P100 and P100-N145 ratios are not significantly modulated by rSPS. Values are expressed as mean ± SEM.

suppressive effects of flash- and PR-VEPs may reflect different aspects of visual channels. Overall, the present method enabled recordings of stable PR-VEPs for short ISIs (down to 50 ms). Although the P100 amplitude has not been utilized for clinical purposes due to the large intersubject variability, results from the present study suggested the use of an amplitude ratio between S2 and S1 to reduce amplitude variability and increase sensitivity for evaluating cortical excitability. The origin of paired VEP (PR-VEP, PO-VEP, and F-VEP) suppression has not yet been conclusively identified [39–41]. Our study supports a cortical origin of the suppression, but detailed animal experiments will be needed to elucidate the underlying mechanism.

The present results confirmed amplitude suppression and latency delay with shorter ISIs, but did not demonstrate amplitude facilitation with longer ISIs, as observed in a previous PO-VEP study [40]. The Höffken et al. [40] study measured amplitudes of C1 (positive peak about 100 ms) and C2 (negative peak following C1), and the amplitude ratio was measured as the peak-to-peak amplitude of C1–C2. Results from the present study suggested that the difference in facilitatory effects between these two studies may be due to difference in neuronal generators of VEP components between the two stimulus methods. PR-VEPs comprise N75, P100, and N145 peaks, whereas PO-VEPs comprise C1, C2, and C3 peaks [42]. The sensitivity of each VEP component may differ in response to check size, contrast, and stimulus duration [43–45], which likely results in differing behaviors in response to PR-VEPs and PO-VEPs.

Results from the present study demonstrated that the amplitude ratio of paired PR-VEP P100-N145—not N75-P100—increased after rPPS. Immediately after rPPS, the P100-N145 amplitude of S1 appeared to be decreased (Fig. 4A). However, a paired comparison did not show any significant alteration of S1. Thus, the observed effect was not simply derived from a decrease of the control amplitude S1. A contrast method revealed that S1 and S2 of P100-N145 were not significantly modulated, but rather that the S2/S1 ratio significantly increased after rPPS in the limited early period (up to 10 min). These results indicated that rPPS exhibited a disinhibitory effect on the late VEP component, but not the early one. Although the mechanisms of disinhibition remain poorly understood, several possibilities for the site of modulation, including visual cortices and other visual pathways, exist. N75 and P100 might originate from V1, whereas N145 is generated in V1 or the extrastriate cortex [34]. The late component of monkey PR-VEPs, which is comparable to the human N145, has been reported to originate from the visual areas, including V1–V3 [46]. rPPS might modulate extrastriate cortical function, resulting in disinhibition of P100-N145. rPPS over the visual cortex increased subtracted P100-N145 amplitude. However, the control P100-N145 amplitude did not significantly change. Because a high-contrast checkerboard pattern was utilized for PR-VEP recording, the control VEP amplitude was saturated at this contrast level such that a ceiling effect occurred. In contrast, moderate suppression from paired PR-VEPs with 90 ms ISI was sufficient for rPPS modulation.

Previous studies of rTMS at 1-Hz over the visual cortex have demonstrated an inhibitory effect on electrophysiological estimation, but rTMS at 10-Hz has often failed to result in an effect. Results from rTMS studies of the visual cortex showed that PR-VEP amplitudes and VEP habituation are reduced after inhibitory rTMS, but facilitatory rTMS has no effect on either factor [9,10]. Because 30 min of rSPS did not lead to disinhibition or any other effect, rPPS appeared to have modulatory effects over the visual cortex. In fact, paired-pulse TMS has been reported to reduce PT compared with single pulse TMS, irrespective of ISIs [13,14]. rPPS is expected to be safer than facilitatory rTMS with high-frequency stimuli, because rPPS stimulates at a low frequency (0.2 Hz). Therefore, rPPS is a promising method for inducing plastic changes in the visual cortex, as well as in the motor cortex.

rPPS over the visual cortex resulted in a facilitatory effect for up to 10 min, as previously observed for M1 [16]. However, rPPS on the visual cortex was less effective than rPPS on M1. There are several possible explanations for these differences. First, Di Lazzaro et al. [18] reported that rPPS over M1 induces increased MEP amplitude, with a slight increase in I-waves. They suggested that rPPS over M1 with I-wave periodicity might involve certain circuits, in addition to those involved in I-wave generation [18,23]. In contrast, Cash et al. [24]

have confirmed that repeated I-wave facilitation by rPPS at I-wave intervals leads to a sustained increase in corticospinal excitability. There is no direct evidence of I-wave discharges in the output of V1 neurons in humans. However, periodic firing in the cat visual cortex at an interval of 1.4 ms was demonstrated after electrical stimulation of the optic nerve or of the cortical depth [26,27]. In addition, paired-pulse TMS to the cat visual cortex caused facilitation of the test stimulus, suggesting the presence of interactions of poly-synaptic, repetitive inputs [28]. This repetitive synaptic event may be analogous to the rPPS mechanism of I-wave generation in M1 [17,24]. Therefore, the mechanisms of rPPS with 1.5-ms ISI over the visual cortex could be similar to the mechanism of I-waves in M1. More specifically, they might be related to a TMS-induced summation effect as a result of repetitive monophasic pulses [19] and/or plastic changes in neuronal circuits [18,23]. Future studies are needed, with other ISIs, to better elucidate the specificity of 1.5 ms ISI. Second, the effects of interventional rTMS might depend on cortical excitability, because voluntary muscle contraction during rTMS over M1 alters this effect [47]. The visual cortex is continuously active during rPPS due to the processing of visual information, whereas the motor cortex is more easily maintained in a resting state during rPPS. Therefore, rPPS over the visual cortex could be less effective than over the motor cortex. Third, differences in neural generators between VEPs and MEPs might contribute to differences in the rPPS effect. The current induced by TMS flows parallel to the surface of the scalp, thereby trans-synaptically activating pyramidal neurons in M1 [48]. rPPS-activated neurons are primarily activated by MEP measurements. In contrast, PR-VEPs are complex responses, and each VEP component involves different cortical layers or areas [34,46]. Therefore, neurons activated for VEPs might have limited overlap with rPPS-activated neurons. Finally, it is well known that the motor threshold of M1 and PT do not correlate, and that the motor threshold is typically less than the PT [49,50]. In addition, Sparing et al. [15] demonstrated that phosphene induction is not inhibited in any condition of short-interval subthreshold conditioning stimuli. These findings suggest that TMS efficacy could differ over motor and visual cortices and is likely related to varying excitability of cortical areas. TMS efficacy could also be related to individual variability in the amount of exposed surface in the striate cortex [51].

In summary, the effects of rPPS on the visual cortex were analyzed using a recovery curve for paired PR-VEPs. Results demonstrated that P100-N145 inhibition decreased after rPPS with 90 ms ISI. Therefore, rPPS with a visual masking threshold provides a promising tool for exploring plastic changes in the visual cortex.

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