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Original article

Saccadic shortening and cerebral hemodynamics during neck flexion and vibrational stimulation on neck extensor muscles

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ABSTRACT

We investigated the effects of voluntary neck flexion (NF) and vibrational stimulation (VS) to the neck extensor muscles on saccadic reaction time (SRT) and the changes in cerebral oxygenated hemoglobin concentration (oxy-Hb). Oxy-Hb was measured from frontal area to observe attentional modulation, sensory-motor area for sensory-motor activity of neck extensor muscles, and occipital area for visual processing. Subjects were 13 young healthy adults, who performed reflexive-visually guided saccades for 2 min in three conditions; sitting in rest neck (RN) position, NF position, and VS to the neck extensor muscles. SRT was shorter in NF and VS than RN. In NF, SRT was the shortest for the first 10 s, and oxy-Hb showed the greatest initial increase in all areas, especially in occipital area by that time. After the initial increase, oxy-Hb decreased and from 1 min again started to gradually increase in all areas. Under VS, oxy-Hb showed a significant decrease in all areas, especially for the first 30 s, without initial increase. By the second minute oxy-Hb returned towards the base-line, with similar changing pattern as in NF, especially in the frontal area. These results could suggest that SRT shortening by NF and VS are regulated by different mechanisms.

Key Words: neck extensor muscles, neck flexion, vibration stimulation, near-infrared spectroscopy, attention, brain activation

INTRODUCTION

Cortical network and oculomotor circuit involved in saccade control and generation is now well characterized, using clinical and functional imaging studies (Pierrot-Deseilligny et al., 1995). In our previous studies, we found that the sensory information from neck extensor muscles can lead to the shortening of saccadic latency and this was regulated in two ways. One was by voluntary neck flexion (NF) (Fujiwara et al., 2000) and another by vibrational stimulation (VS) to the neck extensors (Fujiwara et al., 2001). Our previous studies using visual evoked potential and event-related potentials showed that NF non-specifically activates the brain, related to sensory, discriminative and cognitive processing (Fujiwara et al., 2012; Kunita and Fujiwara, 2004). Meanwhile, in studies with VS, we found greater shortening of the saccadic latency in memory-guided saccades and anti-saccades, which involves higher nervous system than in visually-guided saccades (Fujiwara et al., 2009b). In addition, from pioneer studies, VS induced a visual illusion of displacement of a small visual target viewed in the dark (Biguer et al., 1988) or of head rotation (Karnath et al., 1994), and also many neck reflexes with nucleus of the central nerve in the brainstem were reported (Magnus, 1924). Those studies suggested that these activations are attributed to the extensive level of cortical and subcortical brain activation, with increased afferent information from the neck muscle. However, the activated brain areas and activation time-courses with NF and VS remained unclear.

In the present study we decided to use those methods and observe the attentional and/or brain activation aspects. We used reflexive visually-guided saccades, because they can be performed with no intentional efforts (Mort et al., 2003) but with required activation for a long time, and the observation of saccadic reaction time (SRT) will be important in our study. We hypothesize that SRT shortening by NF and VS would be regulated by different mechanisms, and this could be observed by the different activation of the cerebral cortex, as well as the shortening of SRT.

To investigate the level of cortical activity, it was important to use a method with a high spatial resolution in dynamic condition (such as NF). Studies showed that in the prefrontal cortex, oxygenated hemoglobin

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concentration (oxy-Hb), which is measured by near-infrared spectroscopy (NIRS), is reportedly sensitive to task-related hemodynamic changes (Miyai et al., 2001). We also reported oxy-Hb increase in the prefrontal cortex during anti-saccades in the elderly (Fujiwara et al., 2010) and decrease in the athletes who can perform saccades automatically due to the sport experience (Fujiwara et al., 2009a). In this study, changings in oxy-Hb can be considered as an index of cerebral activation associated with NF and VS. Expecting regional changings in oxy-Hb, we measured frontal area for attentional activation, sensory-motor area of the leg, trunk and neck, and occipital areas for visual processing during saccades. The following working hypotheses were set; 1) SRT would shorten by NF and VS to the neck extensor muscles, 2) changing pattern in cerebral oxy-Hb under NF and VS would be different.

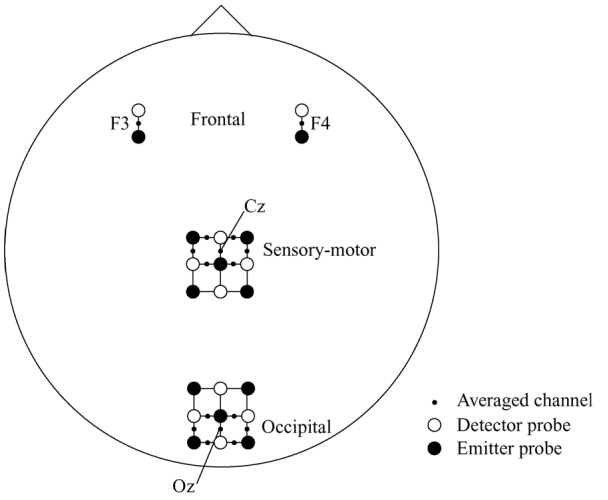

Figure 1. Schematics indicating the arrangement of optical probes for measurement of oxidative-hemoglobin (oxy-Hb) concentration. Small circles indicate analyzing channels for oxy-Hb. Open and filled circles indicate light detector and emitter probes, respectively.

METHODS

Subjects

Subjects were 13 young adults (4 women and 9 men), with a mean age of 21.2 years (standard deviation (SD) = 5.1). No subject reported any history of neurological or orthopedic impairment. In accordance with the Declaration of Helsinki, all subjects provided informed consent by signing a consent form after an explanation of confidentiality ensuring results, protecting privacy right, and experimental protocols, which were approved by our institutional ethics committee.

In preliminary experiment, we measured the SRT during NF and selected subjects who exhibited its shortening. It was expected that vibration to the neck extensors would have a significant effect on SRT in those subjects. We previously reported that the difference in the shortening of the SRT associated with the activation of the neck extensors depended on sport experience, therefore we chose subjects who had a sport experience in a high-speed ball playing, such as table tennis, tennis or baseball (Kunita and Fujiwara, 2009).

Apparatus and data recording

Subjects sat on a steel-frame chair with the back resting against a vertical wall and the trunk secured with a cotton band, to prevent anteroposterior movement. Subjects kept the knees and elbows flexed approximately 90º and their feet and forearms were put on a footrest and armrests stands. A chin stand was used to support the head and to allow the relaxation of the neck extensor muscles as much as possible, because saccadic reaction times shorten when these muscles are activated (Fujiwara et al., 2000). NF angle was defined as the rotational angle of the tragus around the acromion in the sagittal plane, with a quiet sitting posture being as the starting position (0º). Flexion angle was strictly determined using a custom-made angular detector in which the center point was set at the acromion and the distance between the acromion and tragus was regulated. Head inclination angle was determined as the angle between auriculoinfraorbital and the gravitational lines, with an angular detector (Level + Angle Detector, Mitsumoto, Japan) using the principle of pendulum placed on the temple. During measurement, the head inclination angle was kept as that in the quiet sitting posture to maintain constant sensory stimulus from the vestibular organs.

Horizontal electrooculogram (EOG) was recorded from electrodes on the outer canthus of each eye, and vertical EOG from electrodes above and below the right eye. A ground electrode was placed at the center of forehead. To monitor the activity of neck muscles, surface electromyography (EMG) activity from the upper trapezius muscles was recorded from both sides using surface electrodes in bipolar derivation, with a ground electrode on the prominent vertebra. Using a DC amplifier (AN-601G, Nihon Kohden, Japan) for EOG and an AC

bioelectrical amplifier (MA1000, DIGITEX, Japan) for EMG, signals from the electrode were amplified (×2,000), and signals from EMG were bandpass-filtered (5-1000 Hz). Electrode impedance was reduced to less than 10 kΩ.

A light-emitting diode (LED) visual stimulator (SLE-5100; Nihon Kohden, Japan) was used to induce saccades. LEDs were alternately illuminated between a central fixation point and four lateral targets (step paradigm) using a function generator (WF1946A; NF, Japan). The central fixation point was illuminated for a random duration of 1-3 s, and one of the lateral targets was subsequently illuminated for 1 s. The four lateral targets were located at 5°and 10° to the right and left from the central fixation point and were randomly presented. LED was placed at the level of the root of the nose, and the distance between the central fixation point and the root of the nose was set at 1 m.

To vibrate the trapezius muscle, a pair of vibrators' heads (EA-02, Electro Design, Japan) driven by hydraulic pressure, was applied to the skin over a central region of upper trapezius muscle at the both side. The vibrators were set perpendicularly against the long axis of the muscle. The vibration frequency and amplitude was set at 60 Hz and 0.5 cm (Fujiwara et al., 2006). Under the vibration stimulation, 70% of maximal pressure was applied to the trapezius muscle, which was detected using the strain-gauge transducer (LU-5KSB34D, Kyowa, Japan) connected to the vibrator. To monitor EMG of the trapezius and the pressure on the trapezius, each signal was sent to the digital oscilloscope (DS-6612, Iwatsu, Japan).

We used 2 NIRS devices to detect the concentration of oxy-Hb. Oxy-Hb in the frontal cortex was recorded by using a NIRO-200 (Hamamatsu Photonics, Japan), with a data sampled at 2 Hz. Two pair of probe holders, with a distance between the light-detector and light-emitter probes of 3 cm was fixed around positions F3 and F4 of the international 10-20 system and on the lines linking F3 to C3 and F4 to C4. Oxy-Hb in the sensory-motor and occipital areas was recorded by using ETG-4000 (Hitachi Medical, Japan) with data sampled at 10 Hz. 2 sets of 4 light-detector and 5 light-emitter probes with the 3-cm distance between each emitter and detector probes (6×6 cm^2 areas) were placed over the sensory-motor and occipital cortex, with reference to Cz and Oz (Figure 1).

Because of uncertainty about differential path-length factor, measured data were not absolute values of oxy-Hb concentration (Hoshi, 2003). Since no specific value of differential path-length factor could be adopted from previous studies (Miyai et al., 2001), the scale unit of the measured values was concentration multiplied by unknown path-length (mmol×mm).

For subsequent analyses, all electrical signals except for those from ETG-4000 were sent online to a computer (Dimension 9150; Dell Japan, Japan) via an A/D converter (AD16-64(LPCI) LA; CONTEC, Japan) with a sampling frequency of 1,000Hz and 16-bit resolution. Text data of signals recorded from ETG-4000 was sent offline to the computer.

Procedure

Saccades were carried out in a step paradigm, in which a fixation point was turned off simultaneously with the appearance of a lateral target, and subjects oriented their gaze as quickly as possible after it appeared. To become familiarized with reflexive-visually guided saccades, subjects performed three trials with 1 min duration. Prior to start the measurements, in order to relax the trapezius muscles, contraction and relaxation of the shoulder girdle elevator muscles were repeated several times, and a deep breath was taken to relax the trapezius muscle. Experimenter verbally instructed the subjects to relax the trapezius muscle, and this relaxation was confirmed with EMG monitoring.

Experimental trials were divided in three major conditions: neck in resting position (RN); NF position; and VS to the neck extensor muscle. In RN and VS, neck position was set at the angle during quiet sitting, and in NF, the angle at 80% of maximum neck flexion for each individual (Fujiwara et al., 2000). In the all conditions, subjects were initially asked to gaze at the central point with resting their chin on the stand and experimenters confirmed stable oxy-Hb in frontal, sensory-motor and occipital areas for 30 s. Then, in RN, saccades were performed with the chin resting. In NF, experimenter lowered the chin stand, subjects voluntarily maintain their NF position, and then saccades were started. In VS, vibrators from the both sides were applied to the trapezius muscle, the experimenters confirmed approximately 70% of maximum pressure, and then vibration stimulation and saccades started. Each condition comprised of 3 trials for 2-min saccades with a 3-min rest between trials. Experiment was finished within 1.5-2 hours. The first experimental condition was RN, and then the order of the

other conditions was randomized for the subject.

Data analysis

All analyses were performed using BIMUTAS-II signal analysis software (Kissei Comtec, Japan). SRT was defined as latency from the target appearance to the onset of eye movement. Onset of eye movement was determined by visual inspection of EOG displacement, which was easily discernible from baseline. SRT for each condition was averaged for every 10 s.

In the frontal, sensory-motor, and occipital areas, oxy-Hb concentration which showed similar patterns of waveforms were averaged between F3 and F4, among Cz and the nearest six channels, and among Oz and the nearest six channels, respectively (Figure 1), and the averaged waveforms were used for the following analyses. Oxy-Hb during saccades for each condition was averaged for every 10 s. Baseline was defined as the mean oxy-Hb for 10-s period from 15 s to 5 s before performing saccades for RN and NF and before applying pressure to the trapezius muscles for VS. In NF, upper peak of oxy-Hb during saccades was identified in each area, and in VS, lower peak was identified. Data for oxy-Hb was represented as the difference from the baseline. Peak times were represented as latency to start of saccades.

Statistical analysis

All data were examined using the Shapiro–Wilks tests for normality. A one-way repeated analysis of variance (ANOVA) was used to assess the effects of time course by 10-s period on the SRT for each condition, and to test significant differences among the upper peak of oxy-Hb in NF and mean oxy-Hb in RN and VS in the period corresponding to the peak time for each area and among the lower peak of oxy-Hb and mean oxy-Hbs for each area. A post hoc multiple-comparison analysis using Tukey honestly significant difference test was performed to further examine differences suggested by one-way ANOVA. To examine whether SRT and oxy-Hb for each area in NF and VS significantly differed from those in RN, paired *t*-test was used for each 10 s period. The one-sample *t*-test was used for each 10 s period to assess whether oxy-Hb for each area significantly differed from baseline for each condition. Alpha level was set at $p < 0.05$. All statistical analyses were performed using SPSS 14.0J software (SPSS Japan, Japan).

RESULTS

The averaged SRTs for every 10 s period in each condition are shown in Figure 2. Under RN, the mean SRT was 189.4 ms (SD = 26.3) with no significant differences for 2 min. Under NF, a significant effect of time-course was found on SRT (mean SRT: 177.9 ms (SD = 25.3); $F_{1,12} = 4.37$; $p < 0.01$), with the first 10 s significantly shortened compared to 40, 80 and 110s ($p < 0.05$). SRTs during the first 10, 20 and 30 s were significantly shortened than those in RN (21 ms, $t_{12} = 7.40$, $p < 0.001$; 17 ms, $t_{12} = 6.08$, $p < 0.001$; and 10 ms, $t_{12} = 3.55$, $p < 0.01$, respectively). After 40 s, shortening of SRT fluctuated until the end of the trial with 30 or 20 s cycles, and with a 10 s increase between them. Under VS, mean SRT was 171 ms (SD = 25.2) with no differences between the time periods, but SRTs for all periods were significantly shorter than those in RN ($t_{12} > 11.2$, $p < 0.05$).

The black thick line in Figure 3 shows grand-averaged oxy-Hb waveform for each conditions and brain area.

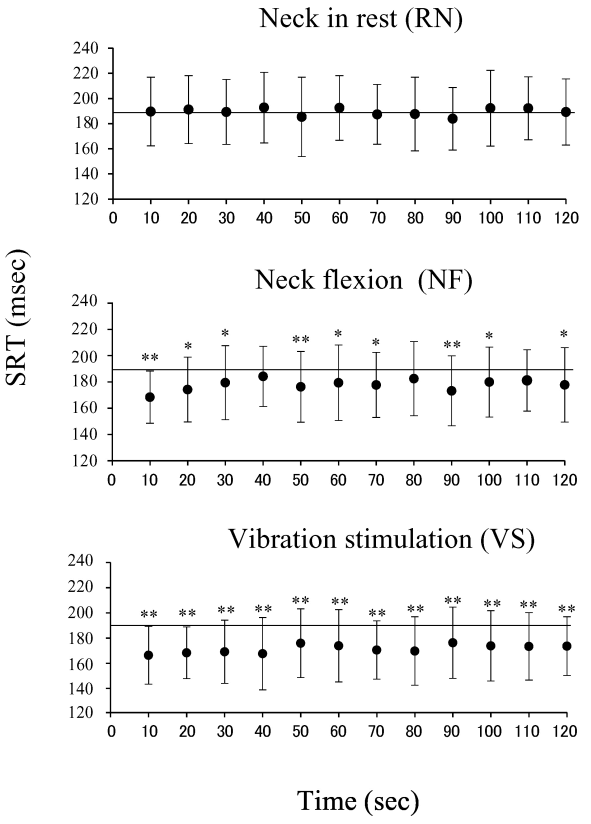

Figure 2. Means and standard deviations of saccadic reaction time. Asterisks indicate differences with respect to rest neck position. *$p < 0.05$, **$p < 0.01$.

White circles show averaged values for every 10 s for 2 min. Under RN, oxy-Hb was significantly increased than the baseline only in 80 s of frontal area (t_{12} = 2.43, p < 0.05), and significantly decreased in 20 s of sensory-motor (t_{12} = 2.52, p < 0.05), and in 20 and 30 s of occipital areas (t_{12} > 3.14, p < 0.05).

Under NF, for first about 10 s, oxy-Hb showed remarkable increase in all areas, with the upper peaks in frontal area at 9.7 s (SD = 6.5), in sensory-motor area at 7 s (SD = 4.1) and in occipital area at 9.1 s (SD = 3.0). These peak times showed no significant differences among the areas. In all areas, these initial peak values of oxy-Hb in NF were significantly larger than the mean oxy-Hbs for the corresponding 10 s in RN and VS (in frontal area, $F_{1,11}$ = 17.8, NF and RN: p < 0.01; NF and VS: p < 0.001; in sensory-motor area, $F_{1,11}$= 9.04, NF and RN: p < 0.05; NF and VS: p < 0.01; in occipital area, $F_{1,11}$= 36.7, NF and RN: p < 0.001; NF and VS: p < 0.001). During these periods, mean oxy-Hb was significantly increased than the baseline in sensory-motor (at 10s, t_{12} = 2.62, p < 0.05) and occipital areas (at 10, 20 s, t_{12} < 3.92, p < 0.05). After that, in frontal area, mean oxy-Hb significantly decreased than the baseline at 30, 40 s (t_{12} > 2.53, p < 0.05). In sensory-motor and occipital areas, mean oxy-Hbs were significantly increased than the baseline at last 30 (t_{12} = 2.68, p < 0.05) and 40s (t_{12} = 3.04, p < 0.05), respectively.

In contrast, under VS, oxy-Hb gradually decreased with the lower peaks in frontal area at 37.2 s (SD = 10.4), in sensory-motor area at 34.4 s (SD = 18.0) and in occipital area at 25.9 s (SD = 9.3). These peak times showed no significant differences among the areas. In all areas, there were significant differences among the lower peak values and the mean oxy-Hbs for every 10-s period (in frontal area, $F_{1,12}$ = 8.00, in sensory-motor area, $F_{1,12}$ = 6.70, in occipital area, $F_{1,12}$ = 4.85, ps < 0.05). Mean oxy-Hbs for the first 10 s showed significant differences compared with the lower peaks (ps < 0.05), but those for 20 s and 30 s were not significantly different in all areas. Additionally, oxy-Hb in frontal area decreased from the baseline for the first 90 s (t_{12} > 5.73, p < 0.05), from 10 to 30 s in sensory-motor (t_{12} > 3.10, p < 0.05) and in occipital area it decreased in the same pattern as in frontal area (t_{12} > 4.77, p < 0.05).

We compared mean oxy-Hbs for every 10s between RN and NF or VS (asterisks in Figure 3). Under NF, oxy-Hb were significantly smaller than RN in frontal area at 40 s (t_{12} = 2.00, p < 0.05), with no changings in sensory-motor, and was significantly larger in occipital area for the first 30 s (t_{12} > 4.49, p < 0.05) and from 90 until 110 s (t_{12} > 4.28, p < 0.05). Under VS, oxy-Hb was significantly smaller in frontal from 30 s to 100 s (t_{12} > 4.26, p < 0.05), with no changings in sensory-motor, and significantly

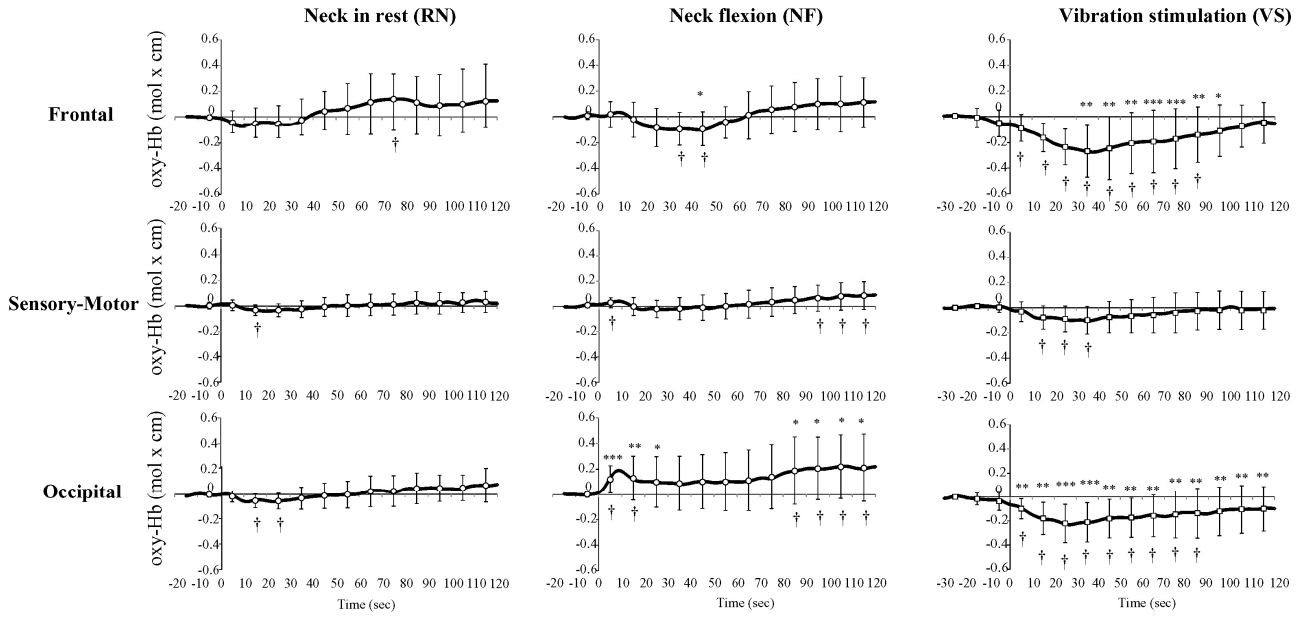

Figure 3. Grand-average waveform for oxy-Hb and means for each 10 s, in the neck in rest, neck flexion and vibration stimulation conditions in the frontal, sensory-motor and occipital areas. † indicates a significant difference from baseline. Asterisks indicate differences with respect to rest neck position. *p < 0.05, **p < 0.01, ***p < 0.001.

smaller in occipital areas for all 2 min ($t_{12} > 4.13$, $p < 0.05$).

DISCUSSION

In the present study, we compared NF and VS with RN to investigate their effects on saccadic shortening, and attentional and/or brain activation level by measuring the activity in cerebral cortex. With NF, SRT shortened for the first 30 s, and then the shortening was repeated with 30 or 20 s cycles. These cycles may indicate the process of attentional activation and its fatigue (Bahill and Stark, 1975; Corbetta and Shulman, 2002). The results for saccadic shortening with NF is consisting with our previous studies (Fujiwara et al., 2000), however, the shortening in SRT during the first 30 s is a new important finding and may suggest about the early phase of attentional activation accompanied with NF. It is known that attention can be induced under voluntarily control, which is top-down mechanism requiring subjects' efforts, and by involuntarily control, which is typically bottom-up process, controlled by external stimuli. Importantly, these top-down and bottom up attentional processes in many cases interacts between each other to optimize attentional processing (Coull, 1998; Egeth and Yantis, 1997). From our results we may suggest that NF would activate both top-down and bottom-up processing. These were shown with faster SRT and oxy-Hb increase simultaneously for the first 10 s in all areas, suggesting about the intentional state to voluntarily activate the cortex for saccade performance (top-down). Especially, occipital area showed the largest initial oxy-Hb increase. Nevertheless, such kind of initial increase rapidly decreased and remained stable for 1 min. Especially in frontal area, NF was significantly smaller than RN for 40s, suggesting that presumably neck afferent muscles information increased the attention (bottom-up) to support reflexive saccades in brainstem (Robinson and Kertzman, 1995). However, from the second minute oxy-Hb started to gradually increase in all areas, which may indicate the process of attentional activation and its fatigue (top-down) (Coull, 1998). Another important finding was that only in occipital area, significant increase from the baseline was found during NF. It is known that visual information reaches visual cortex via the lateral geniculate body in the thalamus (Tobimatsu and Celesia, 2006). Even though in this study we did not provided any data for subcortical regions, we may speculate about the role of thalamus during the NF. Thalamus which is one of the main centers of brain activation plays an important role as a relay portion for sensory information. The sensory information track to the cortex in the thalamus may be affected by the ascending brain activation originating from the brainstem reticular formation and/or descending brain activation originating from the frontal lobe, which includes attention (Berthoz, 1996). As we found the greatest initial and secondary increased oxy-Hb in occipital areas, and considering aforementioned, our results may suggest that NF could influence both neural pathways related to saccadic shortening. We may also suggest about the role of the frontal cortex, from the reports that the attentional modulation in the visual associated cortex would greatly depend on top-down signals from prefrontal cortex (Gazzaley et al., 2007).

In the sensory-motor area, NF showed a little increase also by the end of second minute. Such kind of increases could be a result of activation in sensory-motor area related to maintenance of NF position and recruited a large number of motor units to eliminate the effect of fatigue (Enoka and Stuart, 1992).

Under VS, SRT was shortened, however, this shortening did not fluctuate as in the NF and oxy-Hb was significantly decreased between first 10 and 30 s in all areas, and unlike NF, they did not over-crossed the baseline. These may suggest that vibration probably had a greater effect to the ascending brain activation in subcortical brainstem regions (Burke et al, 1976; Roll et al., 1991) and may directly influenced SRT. However, by the end of the saccades oxy-Hb amplitude starts to increase towards the baseline, which means about the involvement of the cortical activity. This was especially well observed in frontal area, as by the last 20 s vibration stimulation was not significant from the rest and may suggest about the attentional activation. Perhaps, the change of oxy-Hb with VS might be considerably influenced by the change of blood flow in the all cerebral cortex. However, the above-described phenomenon of oxy-Hb would be important as the relative activation change of the cerebral cortex overlapping with the whole blood flow. We will investigate these phenomena in details by the near future.

CONCLUSION

SRT for the NF shortened with fluctuating pattern, whereas for the VS this SRT shortening was relatively

stable. However, oxy-Hb under NF for the first 10 s, showed the greatest initial increase in all areas, especially in occipital area. After the initial increase, oxy-Hb decreased and from 1 min again started to gradually increase in all areas. Under VS, oxy-Hb showed a significant decrease in all areas, especially for the first 30 s, without initial increase. By the second minute oxy-Hb returned towards the base-line, with similar changing pattern as in NF, especially in the frontal area. These results could suggest that SRT shortening by NF and VS are regulated by different mechanisms.

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