

## Day-to-Day Variation in the Hoffmann Reflex in Females

Megumi MURATA\*, Hidetaka YAMAGUCHI\*\*, Kazutoshi SEKI\*\*\*,  
Terumasa TAKAHARA\*\*\*\*, Tatsuya SAITO\*  
and Sho ONODERA\*\*\*\*\*

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### Abstract

The present study aimed to investigate changes in the Hoffmann reflex (H-reflex) at the same time on different days and to compare the findings between genders. Four males and five females in healthy subjects agreed to participate in the study and provided informed consent. All subjects were nonsmokers, and none of the females were consuming any oral contraceptives. The H-reflex was elicited in the right soleus muscle by constant current stimulation of the tibial nerve in the popliteal fossa with the subjects in the prone position. As a reference, the maximal amplitude of the H-wave relative to that of the M-wave ( $H_{\max}/M_{\max}$ ) was measured to determine changes in  $\alpha$ -motoneuron excitability.  $H_{\max}/M_{\max}$  of 6 to 9 trials and was represented by the coefficient of variation (CV). The CV of  $H_{\max}/M_{\max}$  in females was significantly higher than that in males. The level of  $H_{\max}/M_{\max}$  for males was highly reproducible, suggesting that fewer factors alter  $\alpha$ -motoneuron excitability in males compared with those in females. Several female subjects experienced symptoms of depression and suffered from body and menstrual pain. Variations in  $H_{\max}/M_{\max}$  values were considerably greater for females than for males, suggesting that the  $\alpha$ -motoneuron might be influenced by the menstrual cycle phase as well as by individual differences.

### 1. Introduction

The Hoffmann reflex (H-reflex) is a neurophysiological parameter that can be used to evaluate motor output in humans [1]. The H-reflex is measured using a noninvasive test that can be applied in unanesthetized human subjects, regardless of sex and age [2, 3]. The H-reflex is the response evoked by the electrical stimulation of peripheral nerves. During percutaneous stimulation of a peripheral nerve, because

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\* Doctoral Program in Health Science, Graduate School of Health Science and Technology, Kawasaki University of Medical Welfare, Kurashiki, Okayama 701-0193, Japan

E-Mail: w6312001@kwmw.jp

\*\* Department of Sports Social Management, Sports Health Course School of Social Science, KIBI International University, Takahashi, Okayama 716-8508, Japan

\*\*\* Department of Service Management, Faculty of Service Industries, University of Marketing and Distribution Sciences, Kobe, Hyogo 651-2188, Japan

\*\*\*\* Department of Human Arts and Sciences, Faculty of Human Sciences, University of Human Arts and Sciences, Saitama, Saitama 339-8539, Japan

\*\*\*\*\* Department of Health and Sports Science, Faculty of Health Science and Technology, Kawasaki University of Medical Welfare, Kurashiki, Okayama 701-0193, Japan

of its large diameter, Ia afferents that innervate muscle spindle sensory receptors will be recruited before motor axons with a smaller diameter. The amplitude of the H-reflex is considered to be an index of activity in the spinal cord as a final common neural pathway, and it is used to evaluate the activity of the upper spinal organs and the input from sensory systems [4-8]. The action potentials evoked by the H-reflex are known as the H-wave and M-wave. The H-wave amplitude can differ among individuals, and there are few reports that have examined day-to-day variations in H-wave amplitude [9]. In particular, little is known about day-to-day variations in the H-reflex in females.

In women who menstruate, menstruation occurs at intervals of approximately 4 weeks. Bleeding occurs as the uterine mucous membrane is shed, and the bleeding is usually preceded by ovulation and predecidual changes in the endometrium. Physical symptoms, including headaches and cramps, and physical signs, including breast changes, occur in most menstruating females. The H-reflex is inhibited by pain [10] and increased with increased activity of the parasympathetic nerve system [11]. Therefore, it is likely that the H-reflex will change more in females than in males. The present study investigated changes in the H-reflex at the same time on different days in males and females and compared the findings between genders. The primary aim of this study was to investigate day-to-day variations in the maximal amplitude of the H-wave relative to that of the M-wave ( $H_{\max}/M_{\max}$ ) in females.

## 2. Methods

### 2.1. Subjects

Four healthy males (Age:  $20.8 \pm 0.5$  years, Height:  $169.9 \pm 2.5$  cm, Weight:  $67.5 \pm 2.8$  kg; mean  $\pm$  standard deviation) and five healthy females (Age:  $22.0 \pm 1.7$  years, Height:  $159.8 \pm 1.3$  cm, Weight:  $53.9 \pm 1.9$  kg, Menstrual cycle length:  $28.9 \pm 4.5$  days) volunteered to participate in this study. All subjects were nonsmokers and did not use prescribed or recreational drugs. None of the females were consuming any oral contraceptives. Three menstrual cycle phases were defined on the basis of changes in basal body temperature (oral digital thermometers, MC-672L, OMRON, Japan) over a month: the menstrual phase (2-3 days after the beginning of menstruation), the follicular phase (the phase between the days of measurement in the menstrual phase and the luteal phase), and the luteal phase (2 weeks before the beginning of menstruation). All subjects provided written informed consent prior to participation. All procedures were approved by the Ethics Committee of the Kawasaki University of Medical Welfare and conformed with the Declaration of Helsinki.

### 2.2. Measurements

The H-reflex was measured on the basis of the amplitude of the H-wave and that of the M-wave on an evoked electromyogram. The H-reflex in each subject was measured at the same time on different days (about 1 week). The subjects were in the prone position during measurements, and the H-reflex was elicited in the right soleus muscle (SOL) by electrical stimulation of the tibial nerve in the popliteal fossa with a single square pulse of 1-ms duration (Stimulator: SEN-3301 and Isolator: SS104-J, NIHON KOHDEN, Japan). The maximal M-wave ( $M_{\max}$ ) was defined as the peak amplitude of the M-wave in response to increases in stimulus intensity. Surface electromyography (EMG) was conducted by placing disposable bipolar electrodes on the center of the SOL (10 mm diameter, interelectrode distance 20 mm). The interelectrode resistance was less than 5 k $\Omega$  both before and after the experiment. EMG signals were amplified using a bioamplifier system (JB-210J, NIHON KOHDEN, Japan) and were band-pass filtered at 15-10,000 Hz. All signals were digitized using an AD converter (Power Lab 16/30, AD instruments, Japan) with a 10-kHz sampling frequency and stored on a personal computer. As a reference, the maximal H-wave ( $M_{\max}/M_{\max}$ ) was measured to determine changes in  $\alpha$ -motoneuron excitability, and H-wave and M-wave recruitment curves were constructed from recorded data to identify the amplitudes of  $H_{\max}$  and  $M_{\max}$ . The Coefficient of Variation (CV) in  $H_{\max}/M_{\max}$  was calculated from the data of 6-9 trials.

### 2.3. Statistical analysis

Values are expressed as means  $\pm$  standard deviation and coefficient value (CV). Statistical calculations were carried out with the Stat View 5.0 software program for Macintosh. The unpaired *t*-test was used to compare the CV of  $H_{\max}/M_{\max}$  between male and female. The statistical significance was set to  $P < 0.05$ .

### 3. Results

Figure 1 showed all the raw data plotted to recruitment curves of both the H-wave and M-wave obtained from 6-9 trials conducted in ones typical for each gender. Figure 1 calibrated with relative units such as the ratio of the threshold of H-wave to that of M-wave on the abscissa, and the ratio of the amplitude of H-wave to the mean maximal amplitude of M-wave on the ordinate. In females, the recruitment curves were changeable when compared to males.

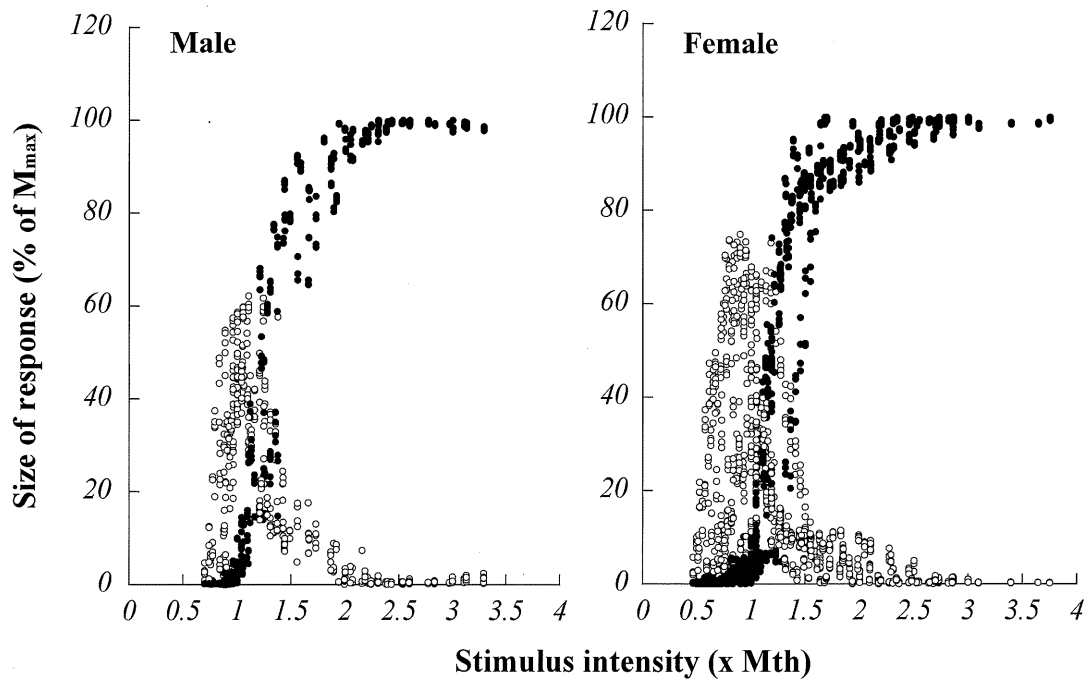


Fig. 1 Superimposed recruitment curves of the H-wave and M-wave constructed from all plots in 6-9 trials on a single subject for each gender.

The filled circle indicates the relationship between stimulus intensity and H-wave amplitude. The open circle indicates the relationship between the stimulus intensity and M-wave amplitude. Both axes were calibrated by the relative scales, i.e. the ratio of the amplitude of H-reflex relative to the mean maximal amplitude of M-wave on the ordinate, and the ratio of the threshold of the H-wave relative to that of the M-wave on the abscissa.

The CV of  $H_{\max}/M_{\max}$  was 3.5-6.4% in males and 5.9-31.8% in females, thus being higher females than males (Table 1). To evaluate the relationship between the CV of gender presented in Table 2, the CV of females was significantly higher than that of the males ( $P < 0.05$ ).

Table 1  $H_{\max}/M_{\max}$  for males (left) and females (right). For each female, measurements were made during the menstrual, follicular, and luteal phases of the menstrual phase

Subject	Number of measurements (time)	$H_{\max}/M_{\max}$ (%)	Subject	Number of measurements (time)	$H_{\max}/M_{\max}$ (%)
A	6	45.0 ± 2.1 (4.6)	E	9	51.9 ± 6.8 (13.2)
B	6	54.1 ± 3.0 (5.5)	F	9	25.7 ± 5.7 (22.3)
C	6	53.5 ± 3.4 (6.4)	G	9	56.2 ± 5.8 (10.3)
D	6	89.3 ± 3.2 (3.5)	H	9	52.4 ± 16.8 (31.8)
			I	9	66.4 ± 3.9 (5.9)

The numbers in parentheses are the coefficient of variation (%).

Table 2 The CV of  $H_{\max}/M_{\max}$  when compared between genders

	Males	Females
CV (%)	5.0 ± 1.2	16.7 ± 10.4 *

CV: Coefficient of Variation (%)

Data are means ± standard deviation (Males; n=4, Females; n=5)

\* P < 0.05: Males vs Females

As illustrated in Table 3, the five female subjects were nulliparous, with an average menstrual cycle length of 28.9 ± 4.5 days. The  $H_{\max}/M_{\max}$  differed among females at each stage of the menstrual cycle phase.

Table 3  $H_{\max}/M_{\max}$  during each menstrual cycle phase in females

Subject	Number of measurements (time)	Menstrual phase (%)			Follicular phase (%)			Luteal phase (%)		
E	9	53.9	47.1 (8.6)	45.3	53.1	47.7 (5.4)	42.2	61.6	61.3 (6.8)	54.8
F	9	30.7	29.2 (8.2)	22.6	26.9	23.0 (8.1)	18.8	36.8	22.1 (15.8)	21.0
G	9	62.6	51.1 (12.1)	50.5	64.3	58.7 (13.9)	50.4	59.9	58.7 (10.3)	49.6
H	9	73.3	49.5 (39.6)	33.7	73.1	45.8 (35.3)	37.8	74.9	45.1 (36.1)	38.8
I	9	70.1	63.8 (6.3)	62.6	73.8	68.2 (10.3)	63.0	68.6	64.8 (5.6)	63.0

The numbers in parentheses are the difference between maximum and minimum  $H_{\max}/M_{\max}$  values.

#### 4. Discussion

In our study, the CV of  $H_{\max}/M_{\max}$  was significantly higher in females when compared to males. Funase et al. [9] reported that the pattern of recruitment curves and  $H_{\max}/M_{\max}$  did not change in the same subject. However, they did change among different subjects. Previous studies have also reported the effects of

exercise [12, 13], environment [14, 15], disease [16-18], and body position [19, 20] on the basis of changes in the H-reflex amplitude. The present study was conducted 2 to 3 times per week, and the experimental environment and body position were the same for all subjects. The small variations in  $H_{\max}/M_{\max}$  values observed for the male subjects in this study may be a result of endogenous factors. Furthermore,  $H_{\max}/M_{\max}$  for the male subjects was highly reproducible, suggesting that factors that alter the excitability of spinal  $\alpha$ -motoneurons are fewer in males than in females.

Symptoms such as headache and cramps and physical signs such as breast changes are expected among females in the age group included in the present study. Some subjects had also complained of depression and body and menstrual pain. Urbscheit et al. [21] reported that the excitability of spinal  $\alpha$ -motoneurons measured using the ankle reflex increased during cooling. In addition Onodera et al. [11] found that spinal  $\alpha$ -motoneuron excitability increased with an increase in the activity of the parasympathetic nervous system. Matsumoto et al. [22] reported that the autonomic nervous system partially controlled muscular activity. Additionally, Stachenfeld et al. [23] found that the actions of progesterone and estradiol worked in opposite directions in the regulation of core body temperature. Therefore, it is possible that the interaction between estradiol and progesterone had an effect on cardiac parasympathetic activity. Furthermore, Mazzocchio et al. [10] found that the H-wave amplitude was decreased by pain. Thus, it is possible that symptoms such as pain during the menstrual cycle as well as ovarian hormone levels increase cardiac sympathetic nervous activity and change the excitability of spinal  $\alpha$ -motoneurons.

Lagerquist et al. [24] investigated diurnal variations in the H-wave amplitude and reported that the SOL H-wave amplitude was higher during the evening than during the early morning. Conversely, there was no difference in  $H_{\max}/M_{\max}$  during the same time period [25]. Yamaguchi et al. [26] observed that, in individual subjects, the H-wave amplitude increased throughout the day, suggesting that the H-wave amplitude was affected by various factors, including sleep time and physical activity. The greater variations in  $H_{\max}/M_{\max}$  in females compared with those in males are possibly related to changes during the menstrual cycle phase, in addition to individual differences.

The present study reports new findings on the activity of the nervous system in females, as assessed using the H-reflex, and confirms the supposition that the nervous system in females responds differently than that in males. Previous studies have reported that variations in the motor unit [27] over time relate to temperature [28] changes caused by fluctuating estradiol levels during the menstrual cycle phase in females. Consequently, hormonal activity is the key to the present study, but future studies may identify new explanations for the changes in motor activity in females.

## 5. Conclusion

The CV of  $H_{\max}/M_{\max}$  was significantly higher in females when compared to males. Furthermore,  $H_{\max}/M_{\max}$  was highly reproducible among males, suggesting that factors that alter the excitability of  $\alpha$ -motoneurons are fewer in males than in females.  $H_{\max}/M_{\max}$  was highly variable in females, suggesting that both the menstrual cycle and individual differences are important factors contributing to changes in  $\alpha$ -motoneurons excitability.

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