アカゲザルにおける学習中の心拍変動解析による自律神経活動の評価

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The Evaluation of Autonomic Nervous System Activation during Learning in Rhesus Macaques with the Analysis of the Heart Rate Variability

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ABSTRACT. The aims of this study were to measure the activity of the autonomic nervous system using heart rate variability (HRV) during learning tasks and to clarify the relationship between learning to overcome a difficult situation and the autonomic nervous system in monkeys. Two young male monkeys (*Macaca mulatta*) were given simple discrimination learning tasks (DL) and delayed matching to samples tasks (DMTS); Holter-type electrocardiography was done, and HRV was measured. We defined the frequency bands of HRV in rhesus macaques; the low frequency (LF) was 0.01–0.15 Hz, and the high frequency (HF) was 0.15–0.50 Hz. Based on these frequency bands, the LF/HF ratios during learning tasks were analyzed, and a significant increase in the ratio was found during the tasks. The variances in the HF differed between the DL and DMTS tasks; during DMTS tasks, HF variances had a tendency to increase. Our results indicate that increased sympathetic activity accompanied learning and suggest that the parasympathetic nervous system plays a key role during learning, particularly when difficult tasks are being learned.

KEY WORDS: autonomic nervous system, heart rate variability, holter-type electrocardiography, learning tasks, rhesus macaque (*Macaca mulatta*).

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The autonomic nervous system is distributed to involuntary tissues, such as the internal organs, blood vessels, and smooth muscles. It plays an essential role in the maintenance of homeostatic balance by, for example, activating the sympathetic nervous system in response to external stimuli.

Species with developed brains improve their various skill sets through learning; they thereby ensure that they can adapt and live independently, and that they can overcome difficult situations by learning. When an organism is subjected to physical or psychological challenges, excitement and/or attention are also elicited to solve the problem through the activation of the sympathetic nervous system. However, we cannot find a research dealing with how sympathetic nervous activity influences learning, although there are many studies reporting the effects of stress on learning and memory [4, 12]. Such studies have focused on hormones and/or neurotransmitters, such as corticotrophin-releasing factor, adrenocorticotropic hormone, glucocorticoids, vasopressin, oxytocin, norepinephrine (NE), and epinephrine (E), which are released into the brain and the blood following arousing or stressful experiences [4]. The NE concentration in the blood or the urine has been generally used to measure sympathetic nervous system activity. However, the serum half-life of catecholamines is very short (1–3 min) [26], and to collect the blood samples, the animals must be restrained or a surgical procedure is required.

Therefore, heart rate variability (HRV), which indicates changes in autonomic nervous system activity, would be a useful noninvasive method [15]. There are several reports that suggest the usefulness of HRV in studying psychological stresses [5, 16, 31], classical conditioning [10], emotional regulation controlled by the limbic system [29], and prefrontal cognitive function [6, 8].

Electrocardiograms are not readily obtained in animals without using some form of restraint or anesthesia. Nevertheless, monkeys are often used in neurophysiological or psychopharmacological studies dealing with learning, memory, attention, or inhibitory control, because they have higher cognitive function or intelligence [14, 23]. The phylogenetic classification and nervous systems of primates are very similar to humans, and their neurophysiologic characteristics may be the same as in humans [32]. Only a few reports have measured HRV in primates; they dealt only with the influence of anesthesia [2] or ethanol [3] on HRV.

One of the purposes in this study was to use Holter-type electrocardiography in rhesus monkeys to measure the components of HRV without needing to restrain or anesthetize the animals. The second aim of the study was to investigate changes in autonomic nervous activities during learning as reflected by HRV.

MATERIALS AND METHODS

Animals: Two young (40-month-old) male rhesus macaques (*Macaca mulatta*) were studied after the Animal Experiment Ethics Committee of Azabu University approved all of the experiments, including the monkeys' daily care arrangements, although the use of primates that have higher intelligence had been required to reduce its number by the Committee. Their room was maintained at about 21°C with a 12 hr light-and-darkness cycle (lighting time 7:00–19:00). The animals were individually housed in stainless steel cages (700 mm × 700 mm × 700 mm), except when the experimental procedures were carried out.

Learning Task: In this study, two learning tasks, discrimination learning (DL) and delayed matching to sample

(DMTS), were given to the monkeys. These tasks have often been used in cognitive studies [9, 18, 25, 28]. The DL task appears to be easy for monkeys, while the DMTS task appears to be relatively difficult because of the inherent use of working memory to complete each trial. The relationship between learning and peripheral autonomic nervous system activity was explored by comparing nervous system activity during DL and DMTS.

During the DL task, the monkey was seated in front of a monitor and had to closely watch the screen. One color stimulus — red (R), blue (B) or green (G) — was presented randomly on the screen. The monkey was required to push the same colored key that was positioned under the screen. For a correct response, the subject received a reward and a particular sound. If the monkey pushed an incorrect key, it did not receive a reward and heard a sound different from the sound associated with a correct response. Subsequently, the task was given again. The interval between trials was set at 3 sec.

The R and G color stimuli were also used for the DMTS task. All stimuli were 200 × 200 pixels (6.25 cm × 6.25 cm); each colored square was presented randomly. At the beginning of the trial, one R or G color stimulus was initially shown in the center of the screen (cue stimulation) for 3 sec. After cue stimulation, there was a 3 sec delay with no stimulation on the screen. After the delay, the R and G color stimuli were presented in random order at the bottom of the screen (select stimulation). These stimuli were given until the monkey responded by pushing one of the two keys that was installed under the monitor. A correct response was defined as choosing the key that corresponded to the cue stimulation color. No reward was given for an erroneous response, whereas a food reward was given for a correct choice. The interval between trials was set at 4 sec.

We recorded ECG and HRV for the learning activity including the operant conditioning. Both tasks were terminated after about 20 min to allow their measurements to be retrieved and to avoid burdening the subjects. We also examined both the correct response rate (CR) and the reaction time (RT), which was the time until the subject pressed any key after the select stimulations were shown.

Holter-type Electrocardiography and Heart Rate Variability: We used a Holter-type electrocardiograph machine (106 mm × 29 mm × 81 mm, 240 g, QR-1300; Fukuda M-E Co., Ltd., Tokyo, Japan), which allowed the animal full freedom of action during each learning task.

We used standard, disposable electrodes. Body hair on the sites to which the electrodes were attached was removed to prevent artifacts. Although 5 electrodes were required to use the 2 channels of the machine, we used only 1 channel, since only 3 electrodes were attached to the subject: a positive electrode was placed on the xiphoid process, a negative electrode was placed on the rib, and a ground electrode was placed on a thoracic rib on the right side.

The subject wore a jacket made of stretchable fabric that was placed over the electrodes. A hook and loop fastener was attached to the jacket to allow it to be easily fitted and removed. Extra leads or an ECG recorder were placed in the jacket to provide camouflage. We recorded ECGs for the subjects without having to restrain them or anesthetize them. The stored R-R interval data were analyzed by fast Fourier transformation (FFT). The representative power spectra of the R-R interval fluctuations are shown in Fig. 1. The low frequency (LF) and the high frequency (HF) were calculated by integrating the spectrum.

Experimental Procedure: Three electrodes were attached to each subject, and the jacket was placed on each subject. ECGs were recorded continuously for 6 hr, and the recordings were repeated 3 times to determine the frequency bands in the rhesus macaque.

In the learning task phase, the subject was first kept at rest in a cage, and the ECG was recorded for 5 min (Pre); then, each learning task was given. After 20 min of the learning task, the subject was returned to the cage, and an ECG was recorded for 5 min (Post). Thus, approximately 30 min of ECG data were collected during each session, and the session were repeated 12 times over 24 days. The heart rate, LF, HF, and LF/HF were analyzed every 5 min using a software program (HS-1000; Fukuda M-E Co., Ltd., Tokyo, Japan).

Statistical Analysis: Results are expressed as means ± SE. One-way factorial ANOVA, multiple comparison tests, and Tukey tests were used as post hoc tests to compare the data obtained for every 5 min with the correct response rate (CR) and the reaction time (RT). HF and LF/HF changes that occurred during the 2 learning tasks were compared using two-way repeated-measures ANOVA. The relationships among the variables were examined using Spearman's rank correlation test.

RESULTS

Frequency Band in Rhesus Macaques: The analyses of the ECG data recorded for 18 hr revealed that the frequency bands in rhesus macaques consisted of LF band of 0.01–0.15 Hz and HF band of 0.15–0.50 Hz (Fig. 1). Using these frequency bands, LF, HF, and LF/HF data were calculated every 5 min during the experiments. The relationships between heart rate (HR) and HF or LF/HF are shown in Fig. 2. There was a negative correlation between HF and HR ($rs = -0.683$, $P < 0.01$, Spearman's rank-correlation coefficient), and a positive correlation between LF/HF and HR ($rs = 0.643$, $P < 0.01$, Spearman's rank-correlation coefficient).

Correct Response Ratio (CR) and Reaction Time (RT) during DL and DMTS: The correct response ratio (CR) and the reaction time (RT) for DL and DMTS are shown in Fig. 3. The CR was significantly lower during DMTS than during DL ($P < 0.01$, paired *t*-test), while the RTs were significantly longer during DMTS than during DL ($P < 0.01$, paired *t*-test).

Sympathetic Nerve Activities by Heart Rate Variability Analysis: The means of LF/HF during Pre-task, Task, and Post-task periods are shown in Fig. 4. The results of one-way factorial ANOVA and Tukey tests showed significant

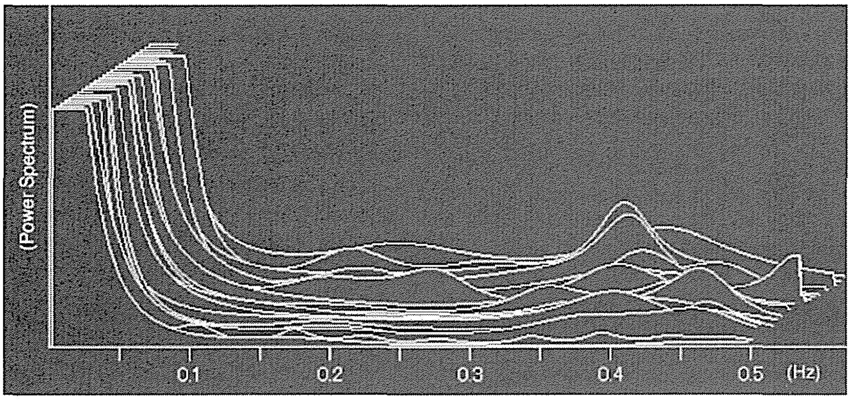

Fig. 1. Spectrum pattern of heart rate variability. This spectrum pattern was obtained from ECG data recorded for 6 hr on three separate occasions. There were two major frequency components of heart rate fluctuations, which were defined as the low frequency component (0.01–0.15 Hz, LF) and the high frequency component (0.15–0.50 Hz, HF). We analyzed the low and high frequency components by integrating the spectrum for the respective bandwidth.

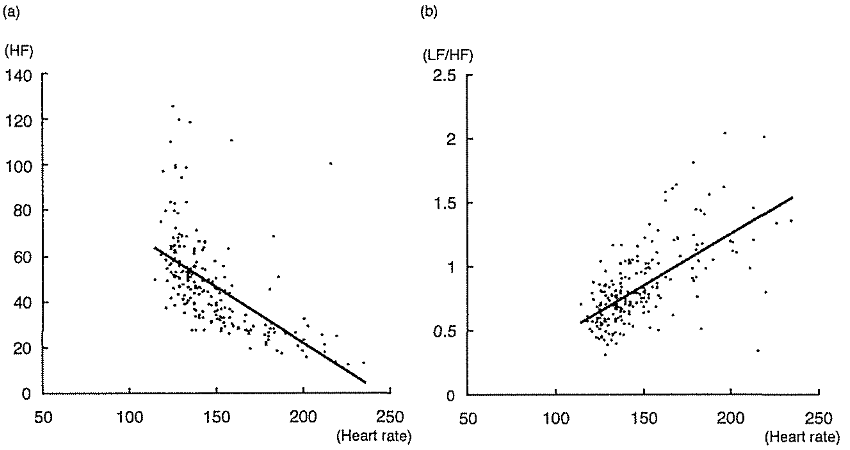

Fig. 2. The correlations between heart rate (HR) and: (a) LF/HF (a: rs = 0.64, $P < 0.01$, n = 219, Spearman's rank-correlation coefficient); (b) HF (b: rs = −0.68, $P < 0.01$, n = 219, Spearman's rank-correlation coefficient).

differences among the 3 phases for DL (one-way factorial ANOVA, $P < 0.01$): between Pre-task and Task (Tukey, $P < 0.01$); between Task and Post-task (Tukey, $P < 0.01$). There were also significant differences between Pre-task and Task for DMTS (Tukey, $P < 0.01$) and between Task and Post-task for DMTS (Tukey, $P < 0.01$). LF/HF increased during the learning tasks and decreased after the tasks. Although there was no significant difference in LF/HF between DL and DMTS (DL, 1.72 ± 0.04 *vs.* DMTS, 1.65 ± 0.04) (Fig. 4), there was a significant difference over time in the changes in HF between DL and DMTS (DL, 8.63 ± 0.98 *vs.* DMTS, 12.0 ± 0.77 at 20 min of Tasks, two-way repeated-measures ANOVA, $P < 0.01$, Fig. 5).

DISCUSSION

The present study demonstrated the usefulness of HRV obtained by a Holter-type electrocardiograph for analyzing changes in autonomic nervous system activity under normal conditions. It also highlighted the importance and necessity of moderate peripheral autonomic nervous activation during learning. We had the high reproductivity in the autonomic nervous system activation during the experiments, although we used two animals.

HR is under the control of the autonomic nervous system. The HF corresponds to parasympathetic nervous system activity (cardiac vagus nerve activities). The LF/HF ratio corresponds to sympathetic nervous system activity in rhesus macaques due to the correlations among HR, HF, and LF/HF [22, 24].

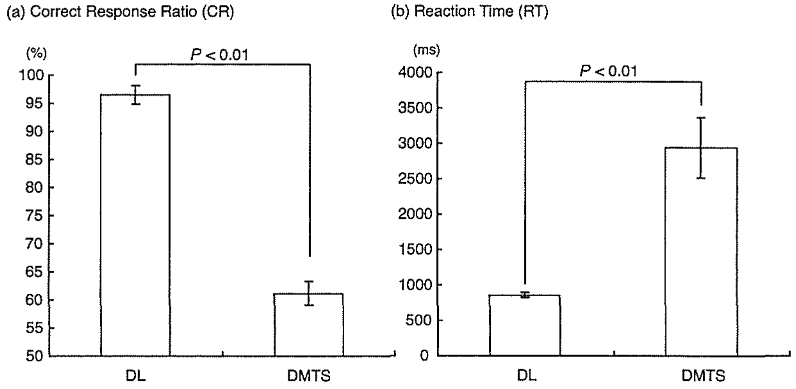

Fig. 3. The correct ratio (CR: a) and reaction time (RT: b) for the DL and DMTS tasks. Values are means ± SE. There were significant ($P < 0.01$) differences between DL and DMTS.

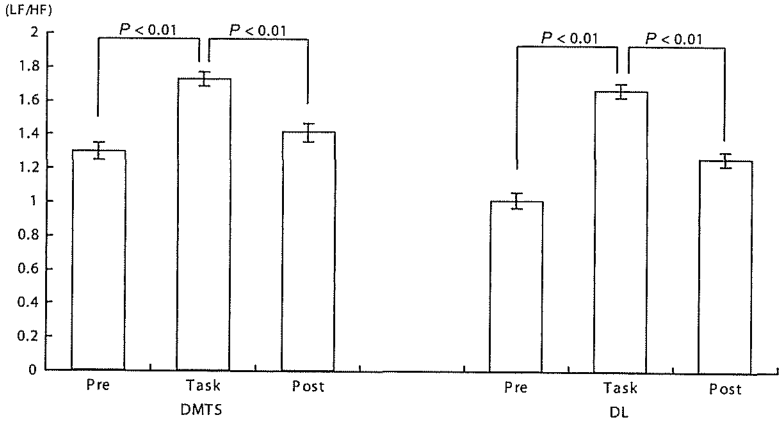

Fig. 4. Changes in the LF/HF ratio. DMTS pre: before performing the DMTS task. DMTS: during the DMTS task. DMTS post: after performing the DMTS task. DL pre: before performing the DL task. DL: during the DL task. DL post: after performing the DL task. Values are means ± SE. There were significant ($P < 0.01$) differences among pre-task, task, and post-task for both DL and DMTS tasks.

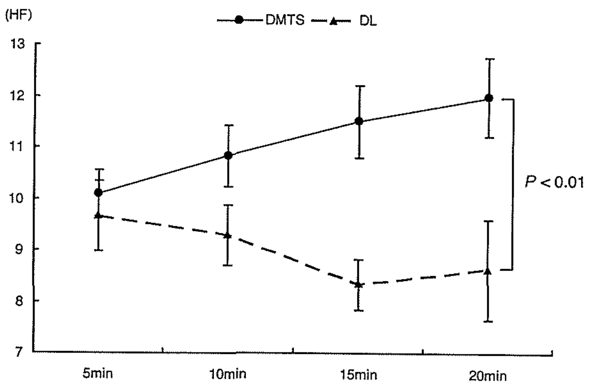

Fig. 5. The changes in HF over time every 5 min. Values are means ± SE. There were significant differences in the changes in HF over time for each learning task (two-way repeated-measures ANOVA, $P < 0.01$).

Frequency Bands in Rhesus Macaques: In this study, we defined the LF band as extending from 0.01–0.15 Hz and the HF band as extending from 0.15–0.50 Hz in rhesus macaques.

The frequency domain (power spectrum) changes at various times of day based on routine activities, such as sleep and waking, according to the balance between the sympathetic and parasympathetic nervous systems [19]. LF is increased and HF is decreased by an increased peripheral sympathetic tone under load conditions. These changes may vary with the type of stress, such as psychosocial, social, or exercise stress. Although ECG studies in primates have been reported, anesthesia or some form of restraint of the animal was needed to obtain the ECGs [11, 13, 30]. ECG measurements under such restrictive conditions do not reflect the actual situation in active animals [1, 17, 21, 27]. The present study succeeded in using electrocardiography equipment attached to the rhesus macaques during task performance. The frequency bands in this study are useful for doing HRV research into the autonomic nervous system activity of rhesus macaques; further investigation using more subjects may be necessary.

Relevance between Learning and the Peripheral Autonomic Nervous System Activity: Regarding task achievement, DL was easy and simple to solve for subjects, whereas DMTS, which required a working memory to solve the task that included a delay time, was more difficult (Fig. 3). We recorded ECGs while the subjects performed the learning tasks and analyzed HF and LF/HF based on the set of frequency bands (LF and HF power). In our study, doing the DL and DMTS tasks increased LF/HF. This suggests that learning behavior is accompanied by peripheral sympathetic nervous activation whether the task is simple or difficult. Some studies have shown that LF/HF and HF are increased as a result of physical training in both horses [20] and humans [8].

The changes in HF differed between DL and DMTS; HF tended to be low for DL, while HF tended to increase over time for DMTS. Gianaros *et al.* have suggested that, in humans, activation of the ventral and medial regions of the prefrontal cortex during a working memory task is accompanied by changes in HF; both a decrease and an increase in HF can occur [6]. Other researches have indicated that brain regions, including the amygdala-hippocampal complex, integrate cardiac autonomic activity with cognitive and emotional behavioral processes [7, 29]. These findings suggest the possibility that parasympathetic nervous activity, which is regulated by the ventral and medial regions of the prefrontal and anterior cingulated cortex, the insula, the amygdala-hippocampal complex, and the cerebellum [6], may be necessary for performing learning tasks. Alternatively, a difficult task like DMTS may be accompanied by an increase in parasympathetic nervous activity.

In this study, we were able to assess autonomic nervous system activity in rhesus macaques without any restraints by analyzing HRV obtained by Holter-type electrocardiography. We found that the parasympathetic nervous system may play a key role in the learning activity of humans and animals.

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