PRIMATES, 32(3): 371–378, July 1991

SHORT COMMUNICATION

Effects of Frequency of the Tones on Auditory Event Related Potentials in Monkeys (*Macaca fuscata*) Under Various Hypobaric Hypoxia Conditions of Simulated High Altitudes*

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ABSTRACT. Changes in auditory event related potentials (ERPs) as a function of frequency of tones ranging from 500 Hz to 8,000 Hz were examined under various hypobaric hypoxia conditions of simulated high altitudes up to 6,000 m. A sequence of $P_1-N_1-P_2$ components, corresponding to those of the human subject, was observed with the monkey's ERPs. These component amplitudes changed as a function of frequency of tone. The relationship revealed an inverted U curve with a maximum amplitude at a tone of 2,000 Hz. The following results under hypoxia were noted: marked reduction of amplitude in ERPs induced by hypoxia linked only to a tone's particular frequency, especially for 2,000 Hz. The delay of latency in the respective components of the ERPs was dependent on the degree of hypoxia. These findings seem to suggest the anatomical bases of the neural generators contributing to each component of the ERPs.

Key Words: Auditory event related potentials; Monkey; Hypobaric hypoxia; Simulated high altitude.

INTRODUCTION

The effects of hypobaric hypoxia on cognitive processing in humans have been described by many investigators (e.g. STAMPER et al., 1980). In general, debilitating effects known as acute mountain sickness involving a deterioration of perceptual-motor skills and of performance in memorization or cognitive tasks occur. We as well have investigated and confirmed such changes in physiological and psychological states under simulated high altitude (IMAI et al., 1988; KIDA et al., 1982; MATSUZAWA et al., 1985). Furthermore, such impairment correlated highly with changes not only in the late positive component of event related potentials (ERPs) but also in the earlier components of P_2 and N_2 (KIDA et al., 1988; KIDA & IMAI, 1989). We plan to clarify the neural processes responsible for the ERPs which may relate to the deterioration of cognitive functions caused by hypoxia. Such research would be facilitated by an appropriate animal model.

Monkeys appear to be most appropriate for generating an animal model. Regarding ERPs, a strong similarity in waveform morphology has been found between human and monkey subjects (e.g. ARTHUR & STARR, 1984; NEVILLE & FOOTE, 1984; PALLER et al.,

*This work was supported by the Cooperation Research Program, Primate Research Institute, Kyoto University.

1988). However, as far as we know, the fact that there have been few investigations carried out in monkeys under hypobaric hypoxia due to a simulated high altitude is a problem. The first step of our study, therefore, is to examine the overall endurance and/or adaptation of monkeys in hypobaric hypoxia from a simulated high altitude through measurements of cortical brain activities and autonomic nervous activities, and through behavioral observation.

The focus of the present report is directed to the most fundamental examinations of the effects of the probability of stimulus presentation and of the frequency of the tones under various hypobaric hypoxia conditions.

METHODS AND MATERIALS

All procedures used were in accordance with the current Guidelines for Animal Care of Kyoto University Primate Research Institute. Approval for this research was obtained from the Ethical Committee of RIEM (Research Institute of Environmental Medicine), Nagoya University. Four young Japanese macaques (*Macaca fuscata*) and one human, a male graduate student, served as subjects. Prior to the present experiment, the monkeys were all trained to adapt to the testing situation in a primate chair for more than three weeks in order to minimize the head movements and the body movements during testing. One of the four monkeys served as a pilot subject in testing to determine the experimental protocol at simulated high altitudes. The results for this monkey were discarded from analysis of the present experimental data. For the within-subject comparison, one of the remaining three monkeys was studied twice under identical conditions.

Five experiments were carried out individually using a TABAI decompression chamber (3 m width × 5.4 m depth × 2.5 m height) with a temperature range of 25 ± 1°C at RIEM, Nagoya University. After control data were obtained at sea level, chamber pressure was reduced stepwise to a simulated high altitude of 6,000 m. During this period, the subject was exposed to simulated high altitude of 1,000 m and 2,000 m for approximately 20 min each, followed by exposure to simulated high altitudes of 3,000 m, 4,000 m, 5,000 m, and 6,000 m for 30 min each. The rate of ascent was 100 m per min and of descent was 150 m/min.

The monkey was comfortably restrained in a primate chair with Velcro straps placed around the arms and legs. The head of the monkey was not physically restricted. Ag-AgCl electrodes were affixed with electrode paste and collodion along the midline of the scalp and referred to linked earlobes with a neck ground and impedance at 10 KΩ or less. All reported latency and amplitude measurements for the ERP waves were from the Cz electrodes (in the 10/20 International system). Eye movements were monitored in recording from a electrode positioned at the Fpz. Electrical activities were amplified (band pass 0.15 – 100 Hz) and were stored on FM tape (SONY KS-616) together with tone signals for the purpose of off-line analysis using an ATAC 450 (NIHON KOHDEN).

The five kinds of pip tones, 500 Hz, 1,000 Hz, 2000 Hz, 4,000 Hz, and 8,000 Hz, were generated by a SANEI Model 7S11 signal processor and delivered through a full-field speaker positioned 100 cm from the subject. The intensity of all tones was 80 dB SPL (for the human subject), and the rise-fall and plateau times were 4 msec and 2 msec, respectively. The tone stimulus presentation was controlled by a TKK Time Programmer.

A stimulus sequence included two different tones, one being 500 Hz and the other any one of the remaining four tones. There were thus four combinations of two tones paired each other. For two monkeys (*M1*, *M2*) and the human subject, the tone of 500 Hz was the

frequent tone, and for the other monkey (*M3*) and the monkey in the second experiment (*M1*), the 500 Hz tone was the rare tone. The frequent/rare stimulus ratio was 80/20, and tones were randomly presented at a rate of 1.0 Hz during a stimulus sequence of approximately 6 min. Under each simulated altitude condition, subjects were tested individually, using at least two stimulus sequences with different combinations of tones. The sequences were separated by a stimulus-free interval of more than 5 min. The combinations of two tones were counterbalanced throughout the various high altitude conditions, so that the ERP data for all the combinations of two tones could be obtained at a low altitude (0 m and 1,000 m) at an intermediate altitude (3,000 m and 4,000 m), and a severe high altitude (5,000 m and 6,000 m), respectively.

ERPs were also recorded from a human subject, under conditions analogous to those described for monkeys. The same stimulus sequences were presented to the subject, who was not given any explicit instructions regarding the stimulus sequences. Eye movements were monitored using an electrode located below the right eye.

EEG signals were averaged for each of the tone stimuli for each of three conditions of hypobaric hypoxia. Trials containing excessive eye movement or muscle artifacts were rejected (approximately 35% of the trials during a stimulus sequence of 6 min). Following artifact rejection, the number of trials averaged, which differed among monkeys, altitude conditions, and tone stimuli, was approximately 40 (32 to 48) for each of the rare stimuli and 160 (120−180) for each of the frequent stimuli.

RESULTS

The ERPs recorded from all the monkeys showed a strong similarity to those recorded from the human subject. They consisted of a positive wave (P_1) followed by a negative wave (N_1), and a late positive wave (P_2). Figure 1 shows the ERPs to the rare and frequent tone stimuli in the human and two monkey subjects under two different conditions of simulated high altitude. There was no difference in the basic waveform morphology of ERPs between species, although the latencies of the major components in the monkeys were earlier than those in the human.

Visual inspection of Figure 1 shows that the characteristics of the ERPs in the monkeys were as follows: (1) The amplitude of ERPs obtained at low altitude of 0 m to 1,000 m changed as a function of tone frequency, with the greatest amplitude being produced by the tone of 1,000 Hz for *M3* and of 2,000 Hz for *M1*. (2) No effect of stimulus probability on the ERPs was found. (3) The ERPs recorded under hypobaric hypoxia of 5,000 m to 6,000 m showed a decrement in amplitude and an increment in latency. However, the ERPs elicited by the tone of 500 Hz were relatively unchanged, whether rare or frequent. Result (2) was confirmed by the second experiment, performed with monkey *M1*. That is to say, the relationship between ERP magnitude and stimulus tone frequency was an inverted U curve with a peak point at 2,000 Hz, regardless of whether the 500 Hz tone was the frequent tone (first experiment) or the rare tone (second experiment). On the other hand, in the case of the ERPs in the human, an N_2 wave with a peak latency of about 180 msec appeared for the rare tone stimuli when they were presented at the low altitude. In addition, the ERPs for the human were less influenced by hypobaric hypoxia and by the frequency of tone stimuli studied here than those in the monkeys.

Figure 2 indicates mean changes in peak latency of the ERPs in monkeys as a function of tone frequency under three conditions of simulated high altitude. The mean values indicated in this and subsequent figures were calculated based on the combined data of ERPs

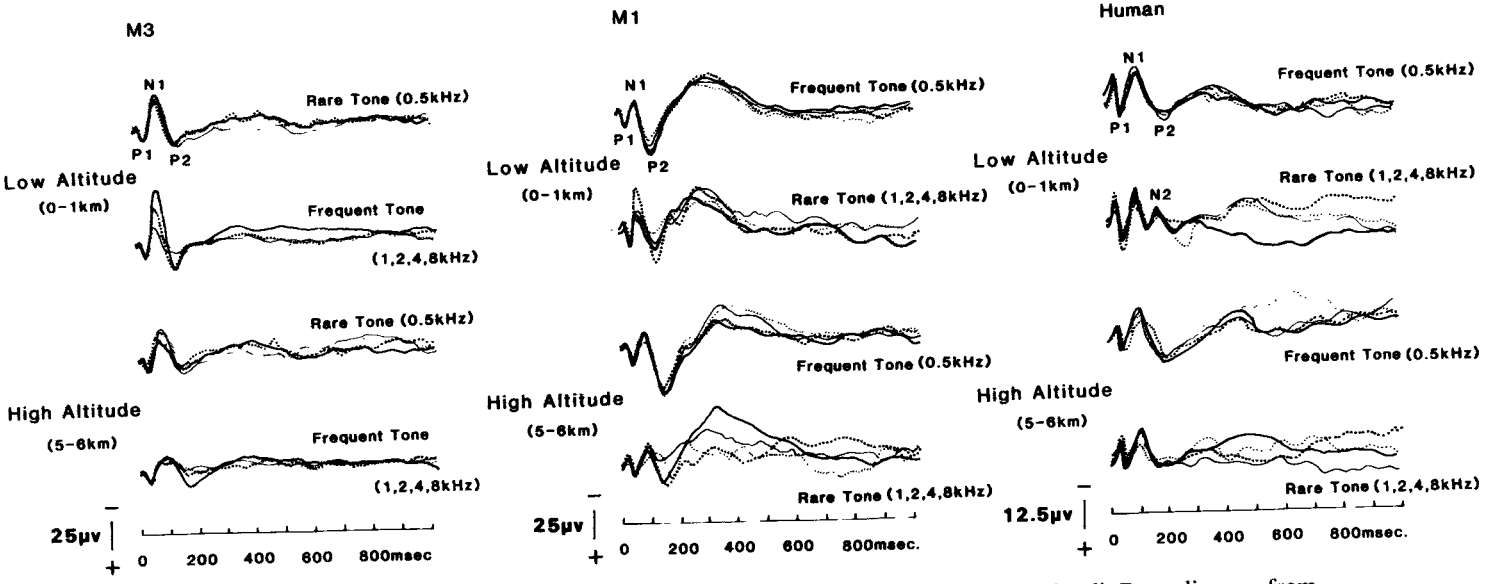

Fig. 1. ERPs in two monkeys and a human to the rare and frequent tone stimuli. Recording are from Cz, and were obtained at sea level and low altitude of 1,000 m and at high altitudes of 5,000 m and higher. A stimulus sequence included the two different tones, one of which was 500 Hz, and the other 1,000 Hz, 2,000 Hz, 4,000 Hz, or 8,000 Hz. There were thus four combinations of paired tones. The ERPs for 500 Hz and 1,000 Hz tones are indicated by thick solid traces, for 500 Hz and 2,000 Hz tones by thick dotted traces, for 500 Hz and 4,000 Hz tones by thin solid traces, and for 500 Hz and 8,000 Hz tones by thin dotted traces. For monkey *M3*, the tone of 500 Hz was the rare stimulus in all combinations. For monkey *M1* and the human, the tone of 500 Hz was the frequent stimulus. Stimulus occurrence at time zero.

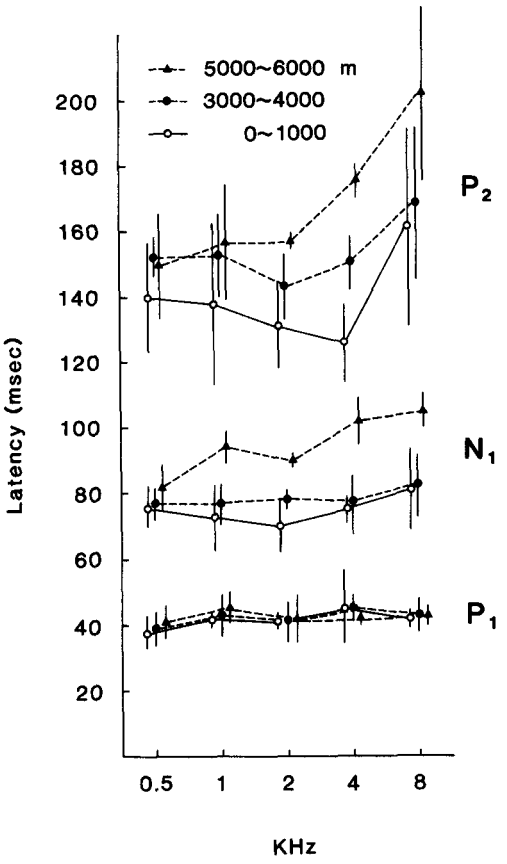

Fig. 2. Mean peak latency and ±S.D. in each component of the ERPs in monkeys as a function of frequency of tone under three conditions of hypobaric hypoxia.

both to rare tones and to frequent tones since there was no significant difference between them.

The latency of the P_1 wave was almost unchanged. No significant difference in the latency anywhere in the frequency range and in the simulated altitudes was found. The latency of the N_1 wave was also relatively unchanged under simulated altitudes below 4,000 m, but the N_1 latency delayed with increasing frequency of tone stimuli under altitudes of 5,000 m to 6,000 m. A two-way analysis of variance (Altitude × Frequency of Tone) showed a significant main effect of altitude, $F(2, 30)=10.2$, $p<0.01$, but there was neither a significant effect of frequency of tone ($F>0.1$) nor a significant interaction between the two factors ($F>0.1$). The latency of the P_2 wave was strongly influenced both by the altitude condition and by tone frequency. On the whole, the higher the altitude conditions, the more delayed the latency, and the higher the frequency of the tone stimuli, the slower the latency. A two-way analysis of variance (Altitude × Frequency of Tone) showed significant effects of altitude and frequency of tone, $F(2, 30)=14.0$, $p<0.01$, and $F(4, 30)=7.1$, $p<0.01$, respectively. There was no significant interaction between them.

Figure 3 shows mean changes in amplitude of the ERPs as a function of tone frequency

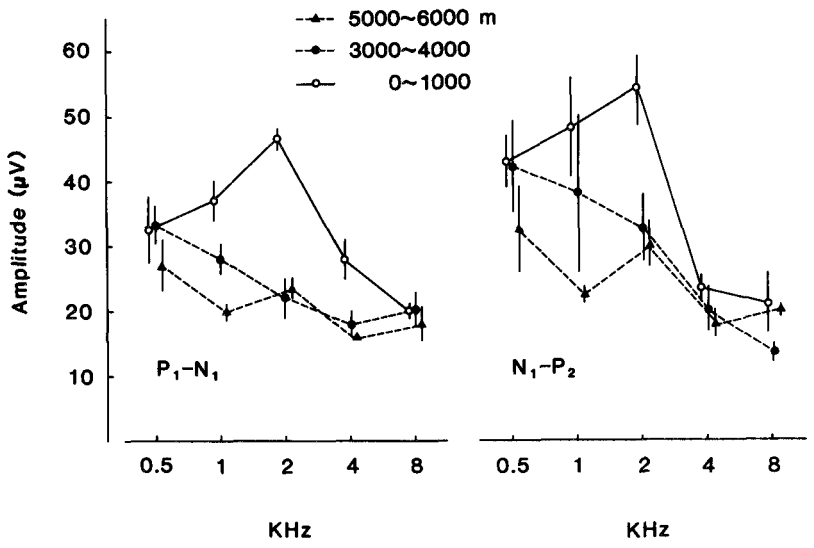

Fig. 3. Mean peak-to-peak amplitude and ±S.D. in each component of the ERPs in monkeys as a function of frequency of tone under three conditions of hypobaric hypoxia.

under three conditions of simulated altitude. At sea level and an altitude of 1,000 m, the peak-to-peak amplitude between P_1 and N_1 revealed an inverted U shape as a function of tone frequency. The greatest amplitude in P_1-N_1 waves was observed at 2,000 Hz. However, under the conditions of simulated high altitudes over 3,000 m, the inverted U shape pattern disappeared, and the amplitude of the P_1-N_1 waves decreased as stimulus frequency increased. A two-way analysis of variance of P_1-N_1 amplitude values showed significant main effects of altitude and frequency of tone, $F(2, 30)=23.9$, $p<0.01$ and $F(4, 30)=11.3$, $p<0.01$, respectively. There was also significant interaction between them, $F(8, 30)=3.9$, $p<0.01$. For the peak-to-peak amplitude between N_1 and P_2, a similar tendency was also found. A two-way analysis of variance of N_1-P_2 amplitude values showed significant main effects of altitude and frequency of tone, $F(2, 30)=17.0$, $p<0.01$ and $F(4, 30)=24.2$, $p<0.01$, respectively. A significant interaction was also found between them, $F(8, 30)=3.2$, $p<0.01$.

DISCUSSION

The changes in latency of the ERPs during hypobaric hypoxia differed among the ERP components P_1, N_1, and P_2. The delay of latency in the P_2 component occurred at an earlier stage of hypoxia, when the delay of latency in N_1 component was not present. The N_1 latency delayed under altitudes of 5,000 m to 6,000 m. However, the early positive component, the P_1 wave, remained unchanged in its latency even under severe hypoxia of up to 6,000 m. According to GASTAUT and FISHER-WILLIAMS (1959), the depression of electrical brain activity during acute hypoxia extends progressively from the telencephalon to the diencephalon, and then to the mesencephalon, during which time the metencephalon continues to show normal electrical activity. The results mentioned above, which were obtained using procedures fundamentally different from those of the present study, seem to suggest

exploration of the anatomical bases of the neural generators contributing to each ERP wave.

With respect to the changes in amplitude of the ERPs (P_1-N_1, N_1-P_2) in the monkeys, the following results were noted. First, a relationship exhibiting an inverted U shape was observed between ERP amplitude and tone stimulus frequency when the tones were presented under the low altitude conditions (below 1,000 m). This relationship differed basically from the relationship between auditory sensitivity and tone frequency found in monkeys (FOBES & KING, 1982; KOJIMA, 1988). Second, the inverted-U relationship disappeared altitudes of 3,000 m or higher. The amplitudes of P_1-N_1 waves and N_1-P_2 waves demonstrated a marked decrement only when the tones of 2,000 Hz were presented. In contrast, the amplitudes of ERPs to the tones of 500 Hz, 4,000 Hz, and 8,000 Hz remained unchanged even under severe hypobaric hypoxia. Eventually the effects of hypoxia on ERP amplitudes were limited to those elicited by a particular tone stimulus frequency. Such a change was not found with the human subject.

There was another difference between the human and the monkey. For the human subject, an N_2 component was evidently evoked by the rare tone, although the N_2 disappeared under hypobaric hypoxia. The ERPs in the monkeys did not vary with stimulus probability. We found no evidence of N_2 component in the monkeys. The difference appears to reflect the difference in attentiveness and/or awareness to the probability of a particular stimulus within a sequence of stimuli between human and monkey.

Concerning the differences in ERPs between human and monkey, further examinations and considerations in terms of parameters of the stimulus conditions or in terms of biologically significant sounds of monkeys are needed. A decrease in probability of the presentation of a rare tone may have evoked N_2 and P_3 components, like NEVILLE and FOOTE's experimental results (frequent/rare stimulus ratio was 0.92/0.08). Also, the particular increase in ERPs for the tone of 2,000 Hz presented here may be related to any meaningful call stimuli. At this time, these are unknown. However, we could record the ERPs from the monkeys under various simulated high altitudes of up to 6,000 m. Furthermore, from observation of the behavior of monkeys throughout the entire experiment, monkeys are certainly viable for experiments under severe hypobaric hypoxia. Such a study possibly promotes a better understanding of the ERPs in humans.

Acknowledgements. We wish to thank Ms. R. AKAMATSU, Laboratory Primate Center, Primate Research Institute, Kyoto University for her animal care during the present experiment and Dr. M. TANAKA and Mr. A. IMAI, Research Institute of Environmental Medicine, Nagoya University for their assistance in operating the decompression chamber. This study is a part of the project called Kyoto University Medical Research Expedition to Himalaya. In May 1990, two monkeys reached the altitude of 5,640 m of Mt. Xixabangma in Tibet Himalaya. We thank all the monkeys who participated the project.

REFERENCES

ARTHUR, D. L. & A. STARR, 1984. Task-relevant late positive component of the auditory event-related potential in monkeys resembles P_{300} in humans. *Science*, 223: 186–188.

FOBES, J. & J. KING, 1982. Auditory and chemoreceptive sensitivity in Primates. In: *Primate Behavior*, J. FOBES & J. KING (eds.), Academic Press, New York, pp. 245–257.

GASTAUT, H. & M. FISCHER-WILLIAMS, 1959. The physiopathology of epileptic seijures. In: *Handbook of Physiology, Section 1: Neurophysiology Vol. 1*, J. FIELD, H. W. MAGOUN, & V. E. HALL (eds.), American Physiological Society, Washington, D. C., pp: 338–339.

IMAI, A., M. KIDA, T. MANO, & K. KOGA, 1988. Effects of simulated high altitude on the human orienting responses. *Annal. Res. Inst. Environ. Med.*, 39: 136–141. (in Japanese)

KIDA, M. & A. IMAI, 1989. Changes in the short term memory processes and the event related potentials under the simulated high altitude. *Annal. Res. Inst. Environ. Med.*, 40: 135–139. (in Japanese)

————, ————, S. MORI, A. TAKABAYASHI, T. MANO, & K. KOGA, 1988. Changes in the signal detection processes and the event related potentials under the simulated high altitude. *Annal. Res. Inst. Environ. Med.*, 39: 129–135. (in Japanese)

————, K. KOGA, K. INOMATA, R. OSAKA, & S. SUGIMOTO, 1982. Changes in phychological processes under high-altitude conditions. *Environ. Med.*, 26: 67–74.

KOJIMA, S., 1988. Auditory characteristics of Japanese macaque, chimpanzee and humans. *52nd. Ann. Convent. Japan. Psychol. Assoc.* (Abstract)

MATSUZAWA, T., M. KIDA, & K. KOGA, 1985. A vigilance performance task using handheld computers at a high altitude of 8,000 meters in the Himalayas. *Annal. Res. Inst. Environ. Med.*, 36: 238–248. (in Japanese)

NEVILLE, H. J. & S. L. FOOTE, 1984. Auditory event-related potentials in the Squirrel monkeys: Parallels to human late waves responses. *Brain Res.*, 298: 107–116.

PALLER, K. A., S. ZOLA-MORGAN, L. R. SQUIRE, & S. A. HILLYARD, 1988. P_3-like brain waves in normal monkeys and in monkeys with medial temporal lesions. *Behav. Neurosci.*, 102: 714–725.

STAMPER, D. A., R. T. STERNER, & S. M. ROBINSON, 1980. Evaluation of an acute mountain sickness questionnaire: Effects of intermediate-altitude staging upon subjective symptomatology. *Aviation, Space Environmen. Med.*, 51: 379–387.

—— Received *October 22, 1990*; Accepted *April 27, 1991*

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