Analysis of Sleep EEGs by the Interval Histogram Method —Validity of the Baseline Night as a Control and the Effect of Ethyl Loflazepate (CM6912)—

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Summary: The effects of an anxiolytic drug, ethyl loflazepate (CM6912) on sleep EEGs were investigated by the interval histogram method originally developed. EEGs were classified into each of the δ_2 - β_2 and σ_1 wave-form parameters, and the individual frequencies were determined on the third baseline, second drug and the first recovery nights in each sleep stage. In comparison with the second baseline night, the σ_1 waves in stage 2 sleep and the δ_1 waves in REM sleep were decreased and the α and σ_1 waves in stage 3 sleep were increased on the third baseline night. In consideration of the 5% significance level and the remarkable influence of drug administration, described below, no major problems were encountered in the use of the night as a control in this experimental schedule. The main characteristics of administration of CM6912 manifested as decreases in the slow wave, increases in the fast wave, and an increase in the σ_1 wave in sleep stages other than stage 1. These tendencies were remarkable on the second drug night and were still evident even on the recovery night. Increases in σ_1 wave were related to increases in sleep spindles. The increases in the β_2 wave were particularly remarkable in REM sleep.

Key words interval histogram method, analysis, EEG, sleep, stage, benzodiazepine, ethyl loflazepate, CM6912

Introduction

The main roles of an automatic sleep analysis system are to reduce the work of investigators' reading record paper and to provide the materials for objective evaluation based on automatic mea-

surements of various sleep and wave-form parameters. Therefore, such an analysis should be highly accurate, and the results would depend on the analytical procedure utilized for EEGs, i.e., the most reliable index of sleep. The authors have adopted the interval

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histogram method originally developed for the analysis of EEGs (Kuwahara et al. 1988, 1996) and applied it to sleep experiments using various drugs (Mizuki et al. 1988; Tanaka et al. 1988; Suetsugi et al. 1992, 1995). In this report, investigation of the effects of a benzodiazepine anxiolytic drug, ethyl loflazepate (CM6912), on EEGs, as assessed by the analytical procedure, is described.

Materials and Methods

The analysis included polysomnograms (stored in a data recorder) recorded from 6 healthy male students (19-28 years of age, mean 21.5 years) given 4mg of CM6912 (Mizuki et al. 1988). According to the experimental schedule, the period from the first to third nights was regarded as the baseline night, the fourth and fifth nights as the drug night, and the sixth night as the recovery night (a placebo was administered on the baseline and recovery nights). The polysomnograms stored in the data recorder were reproduced at a speed of 10 times and analyzed with an automatic sleep analysis system (Kuwahara et al. 1996). EEGs (C4-A1) were classified by the interval histogram method into each of the following wave-form parameters and the individual frequencies were determined: δ_2 (0.5-2 Hz), δ_1 (2-4 Hz), θ (4-7.5 Hz), α (7.5-13.5 Hz), β_1 (13.5-20 Hz), β_2 (20-30 Hz) and σ_1 (11.1-14.7 Hz). The σ_1 wave indicates the proportion against 100 for the quantity of δ_2 - β_2 waves observed.

The sleep stage diagrams shown in the upper columns of Figs 1 and 2 were automatically evaluated, according to

flow chart, on the basis of the wave-form parameters determined by the interval histogram method (shown below the columns), and such wave-form parameters as the number of sleep spindles (Spindle), slow waves of 75 μV or more at 0.5-2 Hz (H-V δ), mean EMG amplitude (EMG-1, EMG-2) and the number of reverse phases between EOG channels (EOG). The final check was done visually by investigators. The standardized sleep manual of Rechtschaffen and Kales (1968) was used for evaluation of sleep stages. Changes in wave-form parameters of δ_2 - β_2 and σ_1 waves were investigated on the baseline, drug and recovery nights in each sleep stage in this study. The first baseline night was excluded from the analysis in consideration of the first night effect (Agnew et al. 1966). The second and third baseline nights were also assessed for validity for their use as the control. The paired *t*-test was used for statistical evaluation. A super mini-computer (ECLIPSE MV/20000, Data General) was used for the data analysis.

Results and Discussion

Validity of the baseline night when used as the control

There were no significant changes in stage 1 or 4 sleep on the third baseline night in comparison with the second baseline night, whereas the σ_1 waves in stage 2 sleep and the δ_1 waves in REM sleep tended to be decreased and the α and σ_1 waves in stage 3 sleep tended to be increased (Table 1).

Some investigators have described the first night effect as follows: the effect is decreased on and after the

TABLE 1.
Changes in EEGs on the second and third baseline nights in each sleep stage

Stage	Stage 1		Stage 2		Stage 3		Stage 4		Stage REM	
	Baseline Night	Second Third	Baseline Night	Second Third	Baseline Night	Second Third	Baseline Night	Second Third	Baseline Night	Second Third
δ_2	12.3±0.9	12.6±0.8	16.7±1.2	17.1±0.9	34.7±0.9	33.7±0.9	45.5±1.9	45.3±3.6	13.6±0.5	14.2±0.7
δ_1	15.3±1.7	13.9±1.4	18.2±1.6	18.1±1.4	19.7±1.2	19.5±0.7	18.1±1.1	18.0±1.4	19.1±1.1	18.4±0.7*
θ	21.6±1.7	20.7±2.1	23.7±0.9	23.7±1.1	19.5±1.9	19.5±1.6	15.3±1.3	15.5±1.6	25.7±0.6	25.5±1.0
α	22.3±3.5	23.0±2.6	24.1±3.5	23.6±3.4	15.4±1.9	16.4±2.2*	12.2±2.3	12.4±2.8	20.8±1.3	21.2±1.7
β_1	13.7±1.4	14.2±1.9	11.4±1.6	11.3±1.5	6.6±1.0	6.8±0.9	5.5±0.6	5.5±0.7	10.2±0.6	10.2±0.6
β_2	14.8±1.5	15.6±3.3	5.9±0.8	6.1±1.2	4.0±1.0	4.1±1.3	3.4±1.0	3.2±0.9	10.6±1.1	10.5±1.2
σ_1	11.7±1.0	12.3±1.1	14.7±2.0	14.3±1.7*	7.7±0.8	8.1±1.0*	6.3±1.1	6.4±1.5	9.6±0.7	9.7±0.6

*: $P < 0.05$

second night according to Agnew et al. (1966); according to Touyz et al. (1978), there was no significant difference between the baseline night and the drug night (when a placebo was administered) or between the baseline night and the recovery night in the experimental schedule including two adaptation nights, 2 baseline nights, 3 drug nights (when a placebo was administered) and 2 recovery nights. From these findings, the first night effect may have been present on the second baseline night, but it was estimated to have already been absent or to have been minimal on the third baseline night. The reasons for the previously described tendencies of some parameters in stage 2, stage 3 and REM sleep on the third baseline night remain obscure. However, in consideration of the 5% significance level and the remarkable influence of drug administration, described below, no major problems were encountered in the use of the night as a control in this short-term experimental schedule. Thus, the mean value of the night was used as the wave-form parameter for the baseline night.

The observation of time-course changes

Figures 1 and 2 show sleep stage diagrams and time-course changes in the wave-form parameters measured on the third baseline night and the second drug night in one subject. The third baseline night following the sleep-onset was characterized by increases in β_2 and γ waves and decreases in δ_2 to θ waves in stage 1 sleep, increases in σ_1 waves and β_1 waves, which were synchronous with increases in σ_1 waves, in stage 2 sleep, increases in δ_2 waves in stage 3 and stage 4 sleep, and increases in β_2 waves in REM sleep (Fig. 1). The second drug night was characterized by a tendency for the waves in stage 1 sleep similar to those observed on the third baseline night. In stage 2 sleep, the σ_1 wave was markedly increased, while α and β_1 waves were increased in a pattern synchronous with the increase in σ_1 waves. These increases may have been attributable to the frequency band which overlapped that of the σ_1 waves. In stage 3 and stage 4 sleep, δ_2 waves were decreased and β_1 and σ_1 waves were increased. In REM sleep, β_2 waves were markedly increased

Fig. 1. Sleep stage diagrams and time-course changes in wave-form parameters measured on the third baseline night when a placebo was administered. Zero on the time axis corresponds to the time 22:30 when the experiment started.

(Fig. 2). Thus, the evaluation of time-course changes allows visual observation of the relationship between sleep stage and EEG changes.

Changes in EEG patterns due to administration of CM6912

Tables 2 through 6 show changes in wave-form parameters in each sleep stage on the baseline night (average for the second and third baseline nights), first drug night, second drug night, and the recovery night. Individual stages showed the following characteristics.

Stage 1: On the first drug night, δ_1 to α waves were decreased and β_1 and β_2 waves were increased. On the second drug night, δ_2 waves were also decreased in addition to the above tendencies. On the recovery night, the tendencies of δ_1 and θ waves to be decreased and of β_1 and β_2 waves to be increased persisted. There was no significant change in the σ_1 wave (Table 2).

Stage 2: On the first and second drug nights, δ_2 to θ waves were decreased, α to β_2 waves were increased, and the σ_1 wave was markedly increased. These

Fig. 2. Sleep stage diagrams and time-course changes in wave-form parameters measured on the second drug night when ethyl loflazepate (CM6912) was administered. Zero on the time axis corresponds to the time 22:30 when the experiment started.

tendencies were more marked on the second drug night, and were still remarkable even on the recovery night (Table 3).

Stage 3: On the first and second drug nights, δ_1 and θ waves were decreased and α to β_2 waves and the σ_1 wave were increased. These tendencies were somewhat more marked on the second drug night and persisted even on the recovery night (Table 4).

Stage 4: On the first drug night, δ_2 to θ waves were decreased. On the second drug night, δ_2 and δ_1 waves were

decreased and this tendency persisted even on the recovery night. On the first and second drug nights, α to β_2 waves, as well as the σ_1 wave, were increased, and this tendency was remarkable on the second drug night. On the recovery night, β_1 , β_2 and σ_1 waves were still increased (Table 5).

Stage REM: On the first drug night, θ and α waves were decreased. On the second drug night, δ_2 to α waves were decreased, while on the recovery night δ_2 to θ waves were decreased. On the first and second drug nights, β_1 , β_2 and

TABLE 2.
*Changes in EEGs due to drug
administration in stage 1 sleep*

(%)	Baseline Night	Drug Night (CM6912)		Recovery Night
		First	Second	
δ_2	12.4±0.6	11.3±1.3	10.6±0.5**	10.6±2.0
δ_1	14.6±1.2	12.2±1.3*	11.9±0.4**	11.8±1.5*
θ	21.2±1.7	17.4±1.8*	17.8±0.8*	17.9±1.5*
α	22.7±3.1	20.5±2.0*	20.8±1.6*	21.5±1.9
β_1	14.0±1.5	17.8±1.4**	18.3±1.5**	17.9±2.2**
β_2	15.2±2.3	20.9±3.7**	20.6±1.9**	20.4±3.1*
σ_1	12.0±0.9	12.7±0.5	13.1±0.7	13.2±1.2

TABLE 3.
*Changes in EEGs due to drug
administration in stage 2 sleep*

(%)	Baseline Night	Drug Night (CM6912)		Recovery Night
		First	Second	
δ_2	16.9±1.0	14.3±1.0**	12.9±0.5***	13.0±0.5***
δ_1	18.2±1.5	15.3±0.8**	14.0±0.6***	14.2±0.6***
θ	23.7±1.0	20.8±0.8***	19.7±0.8***	20.0±0.9***
α	23.9±3.5	28.1±3.7***	29.9±3.4***	29.1±4.0***
β_1	11.3±1.6	14.7±1.8***	16.2±1.4***	16.2±1.6***
β_2	6.0±1.0	6.9±1.3*	7.3±1.4**	7.5±1.3**
σ_1	14.5±1.9	20.2±1.5***	23.0±2.1***	22.4±2.1***

TABLE 4.
*Changes in EEGs due to drug
administration in stage 3 sleep*

(%)	Baseline Night	Drug Night (CM6912)		Recovery Night
		First	Second	
δ_2	34.2±0.5	33.9±2.2	32.9±2.0	32.1±2.1*
δ_1	19.6±0.9	17.3±0.7***	16.5±0.7***	16.3±0.9***
θ	19.5±1.7	17.2±1.3***	16.5±1.1***	16.8±0.8**
α	15.9±2.0	18.1±3.5*	18.8±3.2**	19.0±2.9***
β_1	6.7±1.0	9.1±1.4**	10.5±1.3***	10.7±1.5***
β_2	4.1±1.1	4.4±1.1**	4.8±1.2**	5.1±1.3***
σ_1	7.9±0.9	11.1±2.3**	12.8±2.3***	13.1±2.3***

TABLE 5.
*Changes in EEGs due to drug
administration in stage 4 sleep*

(%)	Baseline Night	Drug Night (CM6912)		Recovery Night
		First	Second	
δ_2	45.4±2.7	43.0±3.4*	39.9±3.3**	41.0±3.0*
δ_1	18.1±1.2	16.2±0.6**	15.0±0.4**	15.6±1.5**
θ	15.4±1.4	14.0±1.1*	14.4±0.7	14.2±1.1
α	12.3±2.5	14.7±3.8*	16.5±3.4***	15.1±2.1*
β_1	5.5±0.6	8.0±1.3**	9.5±1.5***	9.4±1.6***
β_2	3.3±0.9	4.1±1.0***	4.7±1.1***	4.7±0.9***
σ_1	6.4±1.3	9.3±2.5**	11.4±2.8***	10.4±1.7***

TABLE 6.
*Changes in EEGs due to drug
administration in REM sleep*

(%)	Baseline Night	Drug Night (CM6912)		Recovery Night
		First	Second	
δ_2	13.9±0.5	12.6±1.4	11.6±1.5*	11.9±1.3*
δ_1	18.8±0.8	17.2±1.6	15.8±1.8*	15.7±1.1**
θ	25.6±0.8	22.9±1.4**	21.9±1.7**	22.4±1.4**
α	21.0±1.5	19.5±1.0**	19.6±0.9**	20.3±1.0
β_1	10.2±0.6	13.1±1.2**	14.3±1.9**	13.8±1.1**
β_2	10.6±1.1	14.7±3.2*	16.9±3.8***	16.0±2.8***
σ_1	9.6±0.6	10.6±0.5**	11.0±0.7**	11.0±0.5**

*: P<0.05, **: P<0.01, ***: P<0.001

σ_1 waves were increased, and this tendency was still evident on the recovery night. The increases in the β_2 wave on the second drug night and the recovery night were particularly remarkable (Table 6).

As described above, the main effects of CM6912 on EEGs manifested as decreases in the slow wave, increases in the fast wave, and an increase in the σ_1 wave in sleep stages other than stage 1. These tendencies were remarkable on the second drug night and were still evident even on the recovery night. However,

these characteristics were not consistent among all sleep stages. Specifically, the following characteristics, which differed slightly from each other, were observed: 1) slow waves including the α wave were decreased in stage 1 and REM sleep; 2) fast waves including the α wave were increased in stage 2, 3 and 4 sleep; 3) the slow wave components of the δ_1 and θ waves were decreased in stage 3 sleep and the slow wave components of the δ_2 and δ_1 waves were decreased in stage 4 sleep. The persistent tendencies on the recovery night were regarded as being attributable to benzodiazepine drugs, which have a long biological half-life, continuing to exert their effects even after cessation of administration (Oswald et al. 1982).

Benzodiazepine drugs block the stimuli interrupting sleep by suppressing the hypothalamus and the cerebral limbic system, which are involved in emotion. In general, these drugs are also characterized by effects which increase stage 2, while decreasing other sleep stages and increasing sleep spindles and fast waves. CM6912 has been described as having these characteristics (Mizuki et al. 1988). The increase in sleep spindles in stage 2 is closely related to the increase in σ_1 waves. CM6912 is also considered to increase the amplitude of sleep spindles and to prolong their duration. These tendencies were recently suggested to be present in stages 3 and 4 (Kuwahara et al. 1996). The details of these observed tendencies, including the relationships in REM sleep, should be further analyzed.

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