Flurazepam Effects on Slow-Wave Sleep: Stage 4 Suppressed but Number of Delta Waves Constant

Abstract. *Repeated administration of flurazepam reduced stage 4 sleep (high delta-wave concentration) but produced a greater increase in stage 2 duration so that total sleep time was increased. Computer analysis revealed that the increased amount of stage 2 (low delta-wave concentration) sleep provided a number and duration of delta waves sufficient to offset the loss of delta activity in stage 4. However, the amplitude of the average delta wave was reduced. These results demonstrate the value of direct quantification of delta-wave activity, the variable that underlies visual classification of slow-wave sleep into stages 2 to 4. They also give rise to new hypotheses regarding the relative absence of side effects in spite of profound stage 4 suppression by flurazepam and the mechanisms by which total sleep time is increased by this drug.*

Flurazepam, a widely prescribed hypnotic (sleeping pill), has long been known to suppress the stage 4 component of slow-wave (NREM) sleep (*1*, *2*). It shares this property with other benzodiazepines (*3*). Flurazepam also alters other sleep variables, reducing rapid eye movement (REM) activity and increasing sleep spindles and beta activity (*4*).

The suppression of stage 4 sleep by benzodiazepines becomes manifest after several nights of administration. In our study, stage 4 sleep was reduced to less than 20 percent of the baseline level by the end of 1 week. No tolerance to stage 4 suppression has been observed (*1*).

The classification of an epoch of sleep as stage 4 depends upon the occurrence of electroencephalogram (EEG) waves falling within a specific frequency range (usually 0 to 3 hertz), which meet amplitude (usually 50 to 75 μv) and density (number of waves or time occupied per epoch) criteria (*5*). Benzodiazepine suppression of visually scored stage 4 could result from alteration of any of these three variables. We evaluated these possibilities with a computer program that measures directly the delta EEG activity upon which visual classification of NREM sleep into stages 2, 3, and 4 is based (*6*, *7*).

Our subjects were four medical students. They received 15 mg of flurazepam orally before sleep on the first drug night and then 30 mg per night for the next seven nights. Sleep recordings (*8*) from three consecutive nights from an earlier study constituted the baseline. The first two and the last three nights under drug and the first three withdrawal nights were recorded. Although no placebo was used in baseline and withdrawal conditions, the ink-written records were coded and scored for sleep stages without knowledge of drug condition (single blind).

The left frontal EEG lead was recorded on a tape recorder (Vetter model A) at 15/16 of an inch per second with flutter compensation. A time code, written every 10 seconds on both magnetic tape and ink records, permitted us to identify segments of tape corresponding to visually scored NREM sleep. These NREM segments were analyzed off-line at four times the recorded speed on a PDP-12 computer with a program (PANV35) that measures wave periods and amplitudes in specifiable frequency bands (*6*, *7*). The output of this program was transferred to nine-track tape and analyzed on an IBM 370 system (*6*).

Both the computer-derived and the visually scored measures were analyzed with a multiple regression/correlation procedure for repeated-measures analysis of variance (*9*). This method enabled us to control for Type I errors that might arise with multiple statistical comparisons.

Table 1 shows the mean values for the baseline, the last three nights under drug, and the first three nights of withdrawal for visually scored sleep stages. The expected effects of flurazepam were observed: the duration of stage 4 sleep was markedly decreased, that of stage REM was moderately decreased, and REM's were substantially reduced. The increase in the duration of stage 2 sleep was greater than the combined reduction in stages 4 and REM; thus, total sleep was significantly increased. During the first three withdrawal nights, stage 4 remained suppressed. The duration of REM sleep approached baseline levels and, although total NREM sleep fell, it remained significantly above baseline so that total sleep time continued above baseline.

Table 2 presents the computer-derived measures of delta activity (0 to 3 hertz) during NREM sleep. The total integrated amplitude per night declined substantially under the drug as a result of a decrease in the size of the average delta wave. However, the number of waves in the 0- to 3-hertz band and the time they occupied showed no significant change.

Although flurazepam did not reduce the total number or duration of delta

Table 1. Mean values for visually scored measures of sleep in baseline, drug, and withdrawal conditions. Means are based on four subjects and three nights in each condition. Statistical comparisons with the baseline were made with *t*-tests after a significant *F* was obtained across conditions (*9*).

Condition	Total sleep (min)	NREM sleep			REM sleep			
		Stage 2 (min)	Stage 3 (min)	Stage 4 (min)	Latency (min)	Duration (min)	Eye movements	
							Total*	Density†
Baseline	410.1	180.1	59.1	35.2	67.3	135.7	549.7	0.28
Flurazepam (30 mg)	431.8§	268.5‖	50.2	6.4‖	100.2‡	106.7§	290.3‖	0.18‖
Withdrawal	429.3‡	243.7‖	53.4	4.8‖	97.9‡	127.3	457.0	0.24

*Number of 4-second epochs of REM sleep with eye movement activity. †Proportion of 4-second epochs of REM sleep with eye movements. ‡$P < .05$. §$P < .01$. ‖$P < .001$.

Table 2. Mean values for computer measures of 0- to 3-hertz (delta) activity during NREM sleep. Statistical treatment, number of subjects, and nights in condition are the same as in Table 1. Because of an anomalous response of the Krohn-Hite filter to a square-wave calibration pulse, the values for amplitude in this table are too low. The true absolute values may be obtained by increasing the results for amplitude by 19 percent. For further details, see (*6*).

Condition	Total activity per night			Concentration of activity per 20-second epoch		
	Integrated amplitude (μv·sec)	Baseline crossings* (No.)	Time in band† (sec)	Integrated amplitude (μv·sec)	Baseline crossings* (No.)	Time in band† (sec)
Baseline	211,890	30,904	9680	260.1	38.1	11.9
Flurazepam (30 mg)	152,090§	29,424	9350	156.3§	30.2§	9.6§
Withdrawal	146,430§	27,378‡	8410	164.2§	30.5§	9.4§

*Number of half-waves within the 0- to 3-hertz band. †Total time occupied by waves in the 0- to 3-hertz band. ‡$P < .01$. §$P < .001$.

waves, their density fell. This change was manifested by a reduction in the number of and time occupied by delta waves in the average 20-second epoch of NREM sleep (Table 2). Integrated amplitude, which showed an absolute decline under the drug, manifested, as a consequence, the largest reduction in values per epoch of NREM sleep.

The results indicate that stage 4 reduction under flurazepam was associated with reduced delta density in NREM sleep and smaller delta waves. However, total NREM sleep increased as a result of an absolute increase in stage 2 duration. Since stage 2 contains an appreciable amount of delta activity, this increase permitted the total number of delta waves and the total time occupied by the 0- to 3-hertz band to remain at baseline levels.

Under drug withdrawal, REM duration increased. The duration of NREM fell below drug levels but remained significantly above the baseline (Table 1). Because NREM duration declined, the total number of and time occupied by delta waves decreased significantly, even though the density of delta activity during withdrawal was the same as that under the drug. These reductions were modest (about 11 percent below the baseline) compared to the persisting reduction of visually scored stage 4 to 86 percent below baseline values (Table 1).

Several investigators (*10, 11*) have emphasized findings which suggest that the stage 4 component of NREM sleep is of considerable biological importance. This evidence includes the high negative correlation of stage 4 sleep with age (*12*) and its strong positive correlation with the duration of preceding wakefulness (*13*). Moreover, when total sleep time is experimentally curtailed, the amount of stage 4 sleep is preserved or increased (*14*), although there is a net loss of other sleep stages (especially REM).

In spite of this indirect evidence of its importance, experimental deprivation of stage 4 sleep has not produced substantial behavior deficit (*15*). Nor is the marked stage 4 suppression by flurazepam, which is far greater than that produced by barbiturates (*1*), accompanied by more severe "hangover" or other side effects (*16*).

Our method of analysis may help explain these inconsistencies. The apparent decrease in number of delta waves with stage 4 suppression by flurazepam is compensated by delta activity in increased stage 2 sleep. [It is of interest that this increase occurred in the first NREM period of the night, which normally contains about half (*10*) of the total stage 4 sleep (*17*).] We hypothesize that similar compensatory changes may have occurred in experimental stage 4 deprivation; these other studies did not directly measure delta waves. Although increased stage 2 sleep compensated for the loss in number of delta waves in stage 4, there was a net decline in the integrated amplitude of the 0- to 3-hertz band as a result of decreased amplitude of the average wave under flurazepam. The functional significance of this last change and of the altered distribution of delta waves remain to be established.

A second hypothesis raised by these findings is that some of the increase in total sleep time produced by benzodiazepine hypnotics results from a "slowing" of the metabolic activity of sleep. Sleep is an "active" process (*18*). Since hypnotics inhibit neuronal metabolism and can produce coma in high dosage, it seems plausible that these drugs might reduce the rate at which sleep produces its as yet unspecified restorative effects. The "spreading-out" of the same number of delta waves over a longer sleep period may be a correlate of this reduced rate. Thus, NREM stages 2, 3, and 4 may represent increasing intensities of the same biological process (*10*); a longer period of less intense (stage 2) activity might produce biological effects equal to those of a shorter period of more intense (stage 4) sleep. Of course, hypnotics increase sleep by other actions as well, lowering arousal level so that sleep onset is more rapid and diminishing the number of awakenings during sleep (*1–3*).

Although other investigators (*3, 19*) have used computers to investigate the effects of benzodiazepines on sleep, none (to our knowledge) have reported a separate analysis of number, amplitude, and density of delta waves as we have. Our techniques distinguish the individual contributions of these variables to variations in amount of visually scored stage 4 sleep. They provide a powerful new tool for the analysis of human sleep patterns. Elsewhere we have demonstrated that these computer measures of delta sleep possess high night-to-night reliability (*6*) and are extraordinarily sensitive to the effects of age (*6, 20*).

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17. This finding provides further evidence that the time of onset of the first REM period may depend upon the state of NREM systems rather than solely upon REM "pressure" [compare with I. Feinberg, M. Braun, R. L. Koresko, F. Gottlieb, *Arch. Gen. Psychiatry* **21**, 262 (1969)].
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19. The computer techniques used by J. R. Smith, I. Karacan, B. P. Keane, M. Yang [*Electroencephalogr. Clin. Neurophysiol.* **41**, 587 (1976)] most closely resemble our own. They studied the effects of clorazepate dipotassium (7.5 mg three times per day) on the sleep of medical student volunteers and reported a reduction in total time occupied by 0- to 3-hertz activity, as well as substantial reductions in amplitude and density of 0- to 3-hertz activity. However, their analysis [J. R. Smith, W. F. Funke, W. C. Yeo, R. A. Ambuehl, *ibid.* **38**, 435 (1975)] sets a high-amplitude criterion (35 μv peak-to-peak) for delta recognition, so that change in number and duration of delta waves is confounded by change in amplitude. Our measure of delta-wave activity is amplitude-free, and it seems almost certain that it was this difference which produced the discrepant results. Other possible causes are pharmacologic differences between the drugs and the differences in schedule of administration.
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