Effect of Flurazepam, Pentobarbital, and Caffeine on Arousal Threshold

Michael H. Bonnet, Wilse B. Webb, and George Barnard

University of Florida, Gainesville, Florida

Summary: After laboratory and procedure adaptation, 6 normal subjects were randomly administered 30 mg flurazepam (twice), 100 mg pentobarbital (twice), 400 mg caffeine (once), and placebo (twice) on nonconsecutive nights. On each night subjects were aroused from standard segments of stage 2 sleep five to eight times with an ascending series of 1,000 Hz tones produced by an audiometer. Arousal threshold and awake threshold after each arousal were measured. Both thresholds were increased by flurazepam and pentobarbital and decreased by caffeine. All of the drugs appeared to modify arousal threshold in a time course fashion such that extreme effects were found during the first half of the night. However, the modifications of waking threshold by caffeine and flurazepam continued throughout the sleep period. The method may be a means of measuring the behavioral time course of drug activity during the sleep period. Key Words: Auditory arousal threshold—Flurazepam—Pentobarbital—Caffeine—Depth of sleep.

The idea that drugs make one sleep more "soundly" can be traced to comments by Monninghof and Presbergen in 1883 in the scientific literature. However, with the exception of a study by Lindsley in 1957 in which the number of button pushes across the night was less after Seconal® than after a control condition in 2 subjects (no sleep stage parameters recorded) and three studies by Itil and co-workers (Itil et al., 1972a; b; 1974) in which subjects were aroused in the morning with a threshold procedure, the relation of drugs to depth of sleep has not been examined. In the Itil et al. studies, subjects were awakened 7 hr after administration of several drugs, but the sleep stage of awakening and possible natural arousals near the time of awakening were not controlled for, and it is possible that the results were a consequence of differential drug effects on sleep stage distribution. Also, the single threshold determination did not allow speculation as to drug time course effects.

Because threshold could serve as a simple and relevant way to monitor the behavioral time course of a drug during the sleep period and because threshold might be one of the keys in the improved sleep reported after hypnotic use, the effects of three common drugs on the depth of stage 2 sleep and on the threshold

Accepted for publication November 1978.
Dr. Bonnet's present address is the Sleep Laboratory (116A-I), Veterans Administration Hospital, 3200 Vine Street, Cincinnati, Ohio 45220.
Address reprint requests to Dr. Webb at the Department of Psychology, University of Florida, Gainesville, Florida 32611.
after arousal across the sleep period were examined. Specifically, it was hypothesized that flurazepam (30 mg) and pentobarbital (100 mg) would increase thresholds in stage 2 in a time course fashion. Caffeine (400 mg) was predicted to decrease thresholds.

METHOD

Six male subjects, aged 21 to 23, were selected from their responses on the Florida Sleep Inventory to have normal sleep habits including an approximate 2330 bedtime and an 8-hr sleep length. Subjects demonstrated good health, minimal use of any drug, and normal auditory thresholds.

Auditory thresholds were determined by a Tracor RA 214 Rudmose screening audiometer which had been modified to produce signals through an earphone insert. The audiometer was built to ANSI 1969 specifications and calibrated by an audio specialist before experiments were begun. Readings reported in this study are sound pressure levels referenced to each subject’s threshold when awake at 2315 on each laboratory night. Other modifications of the audiometer included the addition of a resistor such that signal intensities measured at the hearing aid inserts could be measured between −10 and 102 SPL at 1,000 Hz in 2 or 3 dB steps. Signals were presented in a 3 sec “on,” 3 sec “off” sequence. The 6 sec repetition was chosen so that arousals could be made quickly (about 2 min) before the subject might change stage or produce a body movement. Similar intervals have been used in other studies (Pisano et al., 1966; Zimmerman, 1970), and no significant differences were reported between a total 4 sec interval and a total 10 sec interval (Pisano et al., 1966). Measure reliability and other aspects of methodology are reported elsewhere (Bonnet, 1978; Bonnet et al., 1978).

Subjects were awakened between five and eight times each night. The first arousal occurred 5 min after initial stage 2 onset. Thereafter the following criteria were imposed for an arousal to be made: at least 5 min of well-defined stage 2 sleep; at least 30 min of continuous sleep; at least 10 min without body movement or muscle artifact greater than 6 sec. The protocol allowed normal cycling of sleep.

Each subject spent 8 nights in the laboratory after earlier adaptation to the laboratory and to the threshold procedure. Nights were 4 to 6 days apart (subjects on Monday night returned on Friday night). Subjects reported to the laboratory at 2200, had electrodes attached so that recording could be made from F1-F7, P1-T5, O2-O2P2, and an eye channel. Subjects entered the sleep room at 2300. They were allowed to insert the earphone into their ear, and a response button was taped into their hand. Waking threshold was measured at approximately 2315. Lights were turned out at 2330.

Subjects had practiced the threshold task by having their threshold measured for at least one 30 min period and on several previous laboratory nights. They were aware that their threshold would be tested between five and eight times during the night. All thresholds were obtained from an ascending stepwise procedure which began at the subject’s threshold when awake at bedtime. Tone intensity was increased before each 3 sec “on” interval in 5 dB steps. If any sign of
tone-evoked response appeared in the ongoing EEG, 2–3 dB steps were used. Subjects had been instructed to press the button whenever they heard the tone and to say “I’m awake” when aroused by the tone. Following the button press and the verbal response, a nightlight was turned on in the subject room and the tone intensity was decreased until the subject stopped pressing the button for each tone and then increased in intensity to measure the subject’s threshold when awake. To eliminate awake threshold values from subjects drifting back to sleep, the stepwise increase and decrease was continued until three continuous awake threshold ascending values were within 5 dB of each other. At this point the nightlight was turned out and the subject was allowed to go back to sleep.

Of the 8 nights, the first was designated as laboratory readaptation. On the last 7 nights, subjects received a pill at 2315 when already in bed. Caffeine was given on either night 2 or night 8 and was only single blind.\(^1\) On the other 6 nights subjects received a numbered uniform pink capsule under double-blind conditions. The order of administration was randomized within-subject such that each subject had flurazepam, pentobarbital, and placebo in one random order on the first 3 nights and in another random order on the second 3 nights.

Statistical Analyses

Both threshold to the verbal response (i.e., when the subject first said he was awake) and threshold when awake after that response were examined by analysis of variance (ANOVA). Two analyses were planned for each variable. The first analysis examined the two administrations of placebo, flurazepam, and pentobarbital (thus having a term for replications). Since caffeine was only given once, a second ANOVA, involving the first administration of each drug and placebo, was done to assess the effects of caffeine on threshold. Following the rule of Paull (1950), interaction terms were pooled to get a single error term to test main effects whenever interaction F-values were less than 2.0. When significant interaction terms were found, pairwise comparisons were made using the Newman-Keuls procedure at the 0.05 level with the 5 degrees of freedom allowed by the Geisser-Greenhouse procedure. Unless otherwise noted, all F-values were checked for significance using Geisser-Greenhouse criteria (1 and 5 df) at the 0.05 level.

RESULTS

An average of more than six stage 2 arousals per night was made. For purposes of analysis, each night was divided into five time segments. All observations for each subject in each time block were averaged to get the value for that block. The time differed in criteria from other arousal periods and were as follows: (1) the first arousal, which occurred 5 min after initial sleep onset and within 60 min of bedtime in all cases; (2) min 61–165; (3) min 166–270; (4) min 271–375; (5) min 376–480. There were a few subjects who did not have an observation in each time

\(^1\) Caffeine was single administration and single blind due to time and packaging constraints. However, subjects had not been informed that they would specifically be taking caffeine, only a “nonprescription medication.”

Sleep, Vol. 1, No. 3, 1979
block for each night. When possible, the missing value was assigned from the other time and night for that condition for that subject. Otherwise, an average value from the surrounding time blocks was used. No analysis had more than five missing observations.

Arousal Threshold

The summarized dual replication ANOVA for the arousal threshold data can be found in Table 1. The Drug by Time block interaction was nonsignificant ($F = 0.50$). There were significant main effects for Drug ($F = 14.0, p < 0.025$) and for Time across the night ($F = 25.6, p < 0.005$). An examination of the drug totals revealed that mean thresholds were significantly higher than placebo ($43.2$ dB) after administration of both pentobarbital ($51.7$ dB) and flurazepam ($57.2$ dB). In the Time block data, thresholds were lower on first arousal (5 min after initial sleep onset) than at any other time and lower on early morning arousal (min $376-480$) than on the arousals during the middle of the night (min $61-270$).

A significant Time of night by Drug interaction was found when the data from the first administration of caffeine, pentobarbital, flurazepam, and placebo were analyzed. Because the interaction $F$-value ($F = 2.90$) was significant ($p < 0.01$) with the normally allowed 12 and 56 degrees of freedom but not with the 1 and 5 degrees of freedom allowed by Geisser-Greenhouse criteria, $\epsilon$ was calculated from the variance-covariance matrices to estimate true degrees of freedom (Winer, 1971). For the interaction, the $\epsilon$ value of 0.48 allowed 6 and 27 degrees of freedom, and at this level, the Drug by Time interaction remained significant ($p < 0.05$). The Drug by Time means can be found in Table 2 and seen in Fig. 1.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>$p$</th>
<th>Geisser-Greenhouse $p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td>5</td>
<td>14755</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time block</td>
<td>4</td>
<td>4655</td>
<td>25.6</td>
<td>0.001</td>
<td>0.005</td>
</tr>
<tr>
<td>Drugs</td>
<td>2</td>
<td>2545</td>
<td>14.0</td>
<td>0.001</td>
<td>0.025</td>
</tr>
<tr>
<td>Replications</td>
<td>1</td>
<td>605</td>
<td>3.32</td>
<td>0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Pooled error</td>
<td>164*</td>
<td>182</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Drug means
- Placebo: $43.2$ dB
- Pentobarbital: $51.7$ dB
- Flurazepam: $57.2$ dB
- Placebo less than both drugs*

Time block means

<table>
<thead>
<tr>
<th>Block</th>
<th>Arousal (dB)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34.3</td>
<td>$&lt; all^b$</td>
</tr>
<tr>
<td>2</td>
<td>62.0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>60.2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>53.0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>45.8</td>
<td>$&lt; 2,3^b$</td>
</tr>
</tbody>
</table>

a Three missing observations.

b These differences are significant as tested with the Newman-Keuls test and Geisser-Greenhouse degrees of freedom ($df = 5$).
FIG. 1. Top: Time course of flurazepam, pentobarbital, placebo, and caffeine as measured by arousal threshold from stage 2 sleep (referenced to prior evening threshold when awake) in replication 1. Bottom: Time course of flurazepam, pentobarbital, placebo, and caffeine as measured by threshold when awake (referenced to prior evening threshold when awake) in replication 1.
TABLE 2. Drug by Trial means for caffeine, placebo, flurazepam, and pentobarbital in replication 1

<table>
<thead>
<tr>
<th>Trial</th>
<th>Caffeine</th>
<th>Placebo</th>
<th>Flurazepam</th>
<th>Pentobarbital</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22.0 dB</td>
<td>30.8</td>
<td>50.3</td>
<td>31.0</td>
</tr>
<tr>
<td>2</td>
<td>34.9</td>
<td>49.3</td>
<td>32.8</td>
<td>65.0</td>
</tr>
<tr>
<td>3</td>
<td>26.7</td>
<td>57.6</td>
<td>63.7</td>
<td>65.8</td>
</tr>
<tr>
<td>4</td>
<td>49.2</td>
<td>53.5</td>
<td>53.5</td>
<td>52.3</td>
</tr>
<tr>
<td>5</td>
<td>32.8</td>
<td>44.5</td>
<td>49.2</td>
<td>48.7</td>
</tr>
</tbody>
</table>

The noted differences were found with the Newman-Keuls test at the 0.05 level based on 27 df.

(top). Significant differences (27 df) are noted in Table 2. Drug effects were limited to the first three trials. Flurazepam appeared to increase thresholds mainly during the first and second time periods (first 3 hr of sleep) and pentobarbital during the second and third periods (1 to 4.5 hr after bedtime). The largest effect for caffeine was seen in the first 4.5 hr of sleep.

Threshold of Subjects When Awake

Because threshold values were referenced to waking threshold at 2315, all threshold values taken during the night from awake subjects would be expected to be zero. Therefore, positive values reflect deficits with respect to 2315 threshold and negative values reflect improvements.

The dual replication ANOVA for threshold when awake is summarized in Table 3. The Drug by Time interaction was nonsignificant (F = 1.05). Significant main effects were found for Drug and Replication, while Time effect narrowly missed significance. As can be seen from the table, waking threshold after placebo was

TABLE 3. Summarized waking threshold ANOVA for two replications of placebo, flurazepam, and pentobarbital

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>p</th>
<th>Geisser-Greenhouse p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td>5</td>
<td>843.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time block</td>
<td>4</td>
<td>140.5</td>
<td>6.54</td>
<td>0.001</td>
<td>0.1</td>
</tr>
<tr>
<td>Drugs</td>
<td>2</td>
<td>420.8</td>
<td>19.6</td>
<td>0.001</td>
<td>0.01</td>
</tr>
<tr>
<td>Replications</td>
<td>1</td>
<td>174.0</td>
<td>8.11</td>
<td>0.01</td>
<td>0.05</td>
</tr>
<tr>
<td>Pooled error</td>
<td>163*</td>
<td>21.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug means</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td>0.15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pentobarbital</td>
<td></td>
<td>3.74</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flurazepam</td>
<td></td>
<td>5.31</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug less than</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pentobarbital</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flurazepam</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Four missing observations.

b These differences are significant as tested with the Newman-Keuls test and Geisser-Greenhouse degrees of freedom (df = 5).

Sleep, Vol. 1, No. 3, 1979
very close to zero (0.15 dB) as expected. However, thresholds were elevated significantly by both flurazepam (5.31 dB) and pentobarbital (3.74 dB) as compared to placebo. Thresholds were significantly lower in the second replication than they were in the first.

In the analysis including caffeine, the Drug by Time interaction was again nonsignificant (F = 1.00). Main effects were found for Drug (F = 26.5, p < 0.005) but not for Trial (F = 3.33, NS). The caffeine mean (−2.54 dB) was significantly lower than the placebo and hypnotic means. The Drug by Trial data were plotted (Fig. 1, bottom), so that comparison could be made with arousal threshold effects seen in the top of the figure.

DISCUSSION

All three drug substances significantly altered both arousal threshold from stage 2 sleep and the auditory threshold when awake after the sleep arousal. Flurazepam and pentobarbital increased thresholds and caffeine lowered them. Although the Time of night by Drug interactions did not always reach a level of statistical significance, it does appear that the drug effects were most marked in the early part of the night.

From Fig. 1 (top), it can be seen that the highest flurazepam thresholds are in the second hour of sleep. An early effect of this drug is supported by an EEG frequency analysis after flurazepam and placebo (Itil et al., 1974), by the relatively larger performance decrements found by Roth et al. (1977) 3.5 hr after drug administration than 10 hr after drug administration, and by a concurrent but independent study of auditory arousal thresholds after flurazepam administration (Johnson et al., 1979). In the study by Johnson et al., a very similar time course for flurazepam was seen (although data were taken from several sleep stages), but significantly increased thresholds were seen only at the point corresponding to the highest increases in the present study.

Itil et al. (1972b; 1974) reported a significant effect of flurazepam on arousal threshold in their early morning awakening in only one of two studies. Johnson et al. (1979) did not find a significant effect of flurazepam in their early morning arousal. To examine the late effects of flurazepam more thoroughly in the present data, a separate test was made on the flurazepam and placebo data from the last trial of the night. Thresholds after flurazepam were not significantly higher than after placebo on the first administration of flurazepam and placebo as implied by the top of Fig. 1. However, thresholds were higher after flurazepam than after placebo on the second night flurazepam was given, and remained significantly higher when the data from the 2 nights were averaged (\( \bar{x}_f = 50.6, \bar{x}_p = 39.2, t = 2.729, p < 0.05 \)). Four of six studies of performance 8 hr or more after flurazepam administration have found effects of the drug on at least some tasks: Bond and Lader (1973), Veldkamp et al. (1974), Bixler et al. (1976), and Roth et al. (1977) found effects; Siegler et al. (1966) and Bixler et al. (1973) found none. In total, evidence seems to favor at least marginal continued action of flurazepam throughout the sleep period.

After administration of pentobarbital, thresholds were highest in the second and
third time periods (Fig. 1) but then diminished to placebo levels. The increase in threshold over the first 2 hr of the sleep period agrees well with evidence that the EEG effects of pentobarbital are seen as early as 30 min after ingestion but become more prominent 2 hr after drug administration (Elliot et al., 1975). The time course of action (about 270 min during the sleep period) agrees well with the drug company estimate of drug time course of about 4½ hr.\(^2\)

Caffeine appeared to have effects on threshold for about 4½ hr also. There appears to be no other evidence in the literature on the time course effects of caffeine except the observation that sleep latencies after caffeine administration remain longer than placebo latencies throughout more than 4½ hr of the night (Bonnet, 1978).

A significant effect of time of night was found in the sleep arousal threshold data but not in the auditory threshold of subjects when awake. As noted from the placebo condition (Table 2 and Fig. 1, top), this effect appeared primarily in the first arousal, which was routinely taken 5 min after stage 2 onset and 6–10 min after initial sleep onset. The value was probably low because 30 min of sleep were required before any other arousal and proximity to an arousal or even a body movement lowered thresholds (Kleitman, 1963; Bonnet et al., 1978).

The analysis of variance for the waking threshold data showed absolutely no evidence for a Drug conditions by Trial interaction and comparatively little evidence for an overall Trials effect. Visual examination of the Drug condition by Trial data (Fig. 1, bottom) was consonant with the finding of overall changes particularly with flurazepam and caffeine. However, some decline of the effects of pentobarbital is indeed seen after 4½ hr, as in the arousal threshold data. In the placebo condition, thresholds were remarkably constant across the night and directly comparable to presleep threshold.

While the arousal threshold and auditory threshold data from waking subjects are not in perfect agreement, drug effects on both are obvious and time course effects are probable. Clearly, however, a 6 subject study can only be suggestive and must leave a more explicit statistical description of drug behavioral time course during sleep to further studies.

On a more theoretical level, the present data add to a long list of findings that strike at the validity of EEG as a single descriptor of ongoing behavior. EEG was validated as a measure largely through its close correlation with threshold. To the extent that threshold varies significantly within EEG state (Williams et al., 1964), a more comprehensive behavioral index is indicated.

ACKNOWLEDGMENT

The authors would like to thank Dr. Laverne C. Johnson for his critical reading and comments during the writing of this paper. This research was performed at the University of Florida and supported by a grant from Hoffmann-La Roche, Inc., Nutley, New Jersey.

---

\(^2\) Duration of action of pentobarbital is estimated at 3–6 hr in the pentobarbital sodium (Nembutal\(^8\)) package insert supplied by Abbott Pharmaceuticals, Inc., April 1975 revision.
REFERENCES


Bonnet MH. The reliability of a depth of sleep measure and the effects of flurazepam, pentobarbital, and caffeine on depth of sleep. Dissertation Abst Int (B) 38:5632, 1978.


Sleep, Vol. 1, No. 3, 1979