DRUG RESEARCH AND HUMAN SLEEP 6564

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INTRODUCTION

Psycho-active drugs are used extensively, a clinical situation that justifies the costly contemporary research into brain mechanisms. The human waking brain is at the mercy of a multitude of unpredictable and fluctuating environmental circumstances, immediately present or carried over into any experimental situation in the form of personal prejudices, worries, etc. On the other hand, the sleeping brain offers a period of relative freedom from the problems of daytime life and techniques are now available that permit many simple measurements and allow sleep to be regarded as a valuable tool in the investigation even of drugs whose primary action may not be upon the brain—for example adrenergic β-blockers (1). It has become mandatory that drugs likely to affect sleep, such as new hypnotics or antianxiety drugs, should be investigated using sleep research methods.

I shall here survey some of the practical issues involved in sleep research as a pharmacological tool with no intention to catalog all drug studies that have been reported, but rather to present what may serve as a guide to the nature and uses of sleep research with drugs.

Sleep research requires that patients or volunteers be willing to renounce domestic and some conjugal comforts to sleep in a laboratory on specified nights over a substantial period of time, abstaining from alcohol and all other drugs for several weeks in advance of and throughout the study, leading a regular life that avoids late-night amusements or other causes of sleep deprivation and perhaps submitting not merely to electrophysiological recording but to special procedures such as all-night venous catheterization.

The laboratory on its part must provide the equivalent of expensive hotel accommodations. Investigators can rest during drug studies if subjects can operate an emergency call system from their rooms and if a warning device will waken the investigator should the movement of the recording paper stop. All-night venous catheterization, however, requires the presence of a medical investigator and responsible personnel in attendance all night. Finding and maintaining in study suitable patients or volunteers can be a demanding and costly task.

WHAT IS MEASURED

Electrophysiological recordings for drug studies during sleep should include electroencephalogram (EEG), electro-oculogram (EOG) and submental electromyogram (EMG). On the basis of these simultaneous indices the records are

scored into categories of wakefulness, NREM (orthodox) sleep stages 1, 2, 3, and 4 and into REM (paradoxical) sleep (2). Stage 1 sleep is drowsiness, while stages 3 and 4 are characterized by large, slow EEG waves. The onset of sleep at the beginning of the night is usually taken from the first stage 2 sleep. The recording paper is normally run during the night at a speed of 15 or 10 mm/sec. This means that individual pages represent 20 or 30 sec each, and it is these that form the units of scored measurements. A suitable computer program will convert the scores into convenient data for final analysis. Most all-night electrophysiological records are still scored by eye and hand and it is unlikely, indeed undesirable, that this will ever fully be replaced by computer scoring, since the latter is incapable of noting new and unexpected features in the electrical record.

The total duration of sleep may be decreased by amphetamine derivatives (3), or increased by hypnotics (4–6), but if normal volunteers are used an effect on total sleep time may be seen only in reduction of sleep after drug withdrawal (7). The latency to first sleep onset after lights-out is an unreliable measure owing to inevitable, irregular technical delays in getting subjects to bed and then finally to the point of lights-out. It can, however, be an index of the effectiveness of an hypnotic in insomniac patients who have very long latencies (5).

Intra-sleep restlessness.—An index of the frequency of transitions between stages is a very useful measure, especially one that indicates the frequency of transitions into stage 1 (drowsiness) from other stages of sleep. These transitions are more frequent than spontaneous transitions into wakefulness, and readily give data suited to statistical analysis. Both of these kinds of transitions are usually accompanied by a period of increased muscle potentials or body movements. Measures of actual body-bed movements have, however, not been popular in recent years. In one study of heptabarbital they were found to be of poor discriminative value compared with the EEG stage transitions (4). All the above can be broadly thought of as indices of restlessness and are found to be increased by amphetamine derivatives such as dextro-amphetamine sulphate (8), diethylpropion (9), and fenfluramine (10); by debrisoquine (1); by clinical doses of imipramine, desipramine, or chlorimipramine (11); and they increase during withdrawal reactions following hypnotic drugs (12), or phenelzine (13). The converse effect, namely, reduced restlessness, can be effected by the administration of clinical doses of most hypnotic drugs including barbiturates (3, 10), and has been observed as part of the withdrawal reaction following amphetamine derivatives (10, 14).

Sleep stage duration.—The relative durations of the different stages of sleep and of periods of wakefulness during the night have often been reported in drug studies. It is important to express these not merely as proportions of the whole night or of some fixed number of hours of sleep, but also in terms of hour by hour distribution. Some drugs will suppress REM sleep in the early night, and then allow a rebound in the late night, with a near-normal whole night value (14, 15). In our Edinburgh laboratory we have seen a similar phenomenon with the frequency of shifts to stage 1 sleep or to wakefulness when administering

sodium amobarbital for long periods. Kleitman and his colleagues 40 years ago reported the same thing for alcohol in its effect on nocturnal motility (16).

The total duration of stage 1 sleep and of periods of wakefulness that intervene in the course of attaining, let us say, the first six hours of sleep, tend to resemble the measures of restlessness already described. Hypnotic drugs reduce them (4, 17) and amphetamine derivatives increase them (3, 9). Morphine and heroin also increase stage 1 sleep specifically (18, 19).

REM sleep.—The relative duration of REM sleep is sensitive to many drugs and has attracted especial attention in research reports. Only a very few drugs that appear on clinical grounds to affect brain function, and are known to enter the brain, appear not to alter REM sleep duration in dosages that have been used. They include trimipramine 150 mg and iprindole 75 mg (11), alpha chloralose 500 mg (20), propranolol 120 mg (1), and caffeine equivalent to three cups of coffee (21).

Some of the drugs used clinically as anti-depressants are outstandingly potent in reducing REM sleep duration, while a host of other drugs suppress REM sleep in smaller degree. The tricyclic anti-depressants imipramine and desipramine, in a single dose of 75 mg at bedtime, reduce REM sleep duration as a percentage of whole night sleep from the normal 20–25 percent to about 5 percent, while the same dose of chlorimipramine will reduce it to zero, although there may be brief episodes of low voltage EEG with a few rapid eye movements but no loss of submental muscle tone (a combination of features that can also occur during MAOI therapy). Continued administration of the tricyclic anti-depressant for a month is associated with a gradual but incomplete return of REM sleep percent to normal (11). Chronic administration of some MAOIs in therapeutic doses, will abolish all signs of REM sleep, even though a 1-3 week delay may ensue before this happens and only after an initial transient increase of REM sleep. The absence of the signs of REM sleep seems to last indefinitely if the MAOI is continued (13, 22–24).

The transient initial increase of REM sleep duration caused by MAOIs may be a small-dose effect[1]. Few other compounds can increase REM sleep but reserpine is notable in that it will do so for at least several days (25–27). In addition 5-hydroxytryptophan (28), chlorpromazine (29), lysergic acid diethylamide (30), or L-tryptophan (31, 32) will increase REM sleep at least under some circumstances.

Numerous other compounds will cause a modest diminution in REM sleep duration: many of the reports have been reviewed by King (33). The compounds range from barbiturates and amphetamines to scopolamine (34) and L-DOPA (35). It is worth emphasizing that REM sleep is a "fragile" state (as Hartmann has aptly called it) which is readily diminished by a wide range of nonspecific noxious conditions, including bodily discomfort, anxiety, or unfamiliarity with laboratory surroundings.

[1] Wyatt, R. J., personal communication.

Finally, Kales has clearly demonstrated (17) that a drug may be capable of reducing the proportion of REM sleep in normal, healthy volunteers, but can lead to an absolute increase in REM sleep duration if it causes a large increase in total sleep time in those patients who otherwise would suffer from severe insomnia.

REM phasic events.—REM sleep is characterized by a variety of phasic events that have been or could be measured in drug studies. Counts indicating the number of rapid eye movements per unit time during REM sleep have been variously termed "profusion" or "density" measures of eye movements. The former term is to be preferred for a phenomenon which, like wild flowers beside a path, are strewn irregularly upon a slowly unfolding vista. It has nothing in common with an index of mass per unit volume in a body of uniform consistency. I make this plea for the term "profusion" as one who was, I believe, the first person to make such counts in relation to drug effects (4).

The profusion of rapid eye movements has been reported to be decreased by administration of a number of hypnotics and enhanced by their withdrawal after continued administration (4, 36–38). Minard & Krausman (39) have described an automatic counter for eye movements and Rechtschaffen et al (40) have described a device for measuring "phasic integrated potentials" from surface electrodes that may actually reflect ponto-geniculo-occipital spikes. Devices such as these may prove useful for measuring phasic activity in future drug research.

Sleep cycle periods.—The REM sleep episodes recur about every 90 minutes, reflecting, it now seems fairly certain, a basic cycle detectable by day as well. Several authors have examined their data for evidence of an acceleration or a slowing of the cyclic appearance of REM sleep under the influence of a drug. The difficult mathematical procedures involved have proved a handicap. Suitable techniques and computer programs will probably become available and accepted in the next few years. So far reserpine and L-tryptophan have been found to cause an acceleration of the cycle (41).

NREM sleep.—Stage 1 sleep has been discussed above. Stage 2 seems at present to occupy the humble position of a filler-in, so that if Stages 3 and 4 sleep are decreased by drugs, Stage 2 sleep occupies the time, as is also true if REM sleep has been diminished by some drug. Stages 3 and 4 sleep, with their large slow waves, are commonly grouped together for purposes of duration measurement in drug studies. Outstanding among drugs that reduce their duration are the benzodiazepines, so that flurazepam 30 mg causes a major and long-lasting reduction (17). Fenfluramine in chronic use can cause sustained high levels of Stages 3 + 4 in some individuals and low levels in others (10). Thyroxine can restore Stages 3 + 4 to myxedematous patients in whom it is absent (42). MAOIs and tricyclic anti-depressants also have been noted to cause irregular or transient enhancement of Stages 3 + 4 duration (11, 13, 43). The

amplitude and frequency of the EEG slow waves have not been quantified by sleep researchers, though unquantified enhancement has been noted (10).

Electrodermogram.—There is a naturally-occurring difference of electrical potential between the palm and the dorsum of the forearm, which changes in response to sudden emotion. Sporadic changes of this kind occur spontaneously in man at rest, especially in anxiety states, and they diminish with relaxation. They also occur sporadically in sleep, but rather surprisingly occur most often in Stages 3 and 4 sleep (44). Daytime mental stress has been associated with increase of this spontaneous, nocturnal electrodermogram activity (45) and conversely the sedative drugs secobarbital 200 mg, amobarbital 200 or 400 mg, and nitrazepam 10 or 20 mg have been found to reduce it (46, 47).

EEG rhythms.—The EEG itself is altered in appearance by some drugs. Best-known is "barbiturate fast" at about 18–20 c/sec, superimposed on the other EEG rhythms, most marked anteriorly, and seen in Stage 1 and in REM sleep. It is a feature that appears in greater or lesser degree with many sedative drugs (48), depending on dose, and may be missed unless EEG recordings are made without high frequency filters in operation. Enhancement of amplitude and duration of the normal 12 c/sec EEG sleep spindles can occur with some benzodiazepines, and with tricyclic anti-depressants (49). MAOIs are associated with prominent rhythms of 8–9 c/sec superimposed on the other rhythms and with abnormally prolonged EEG K-complexes (13). All these features, and especially the sedative-induced fast activity, can be useful in indicating the persistence of a drug in the brain for several days after the last or only dose (12).

Blood constituents.—In the last few years it has been found that sleep is the most important stimulus for human growth hormone (HGH) secretion and that Stages 3 and 4 sleep especially are associated with this secretion (50, 51). It has thus become common in many sleep laboratories to take blood samples at frequent intervals throughout the night by means of an indwelling venous catheter simultaneously with continuous electrophysiological recording of sleep. Takahashi et al (52), one of the first exponents of this technique, described abolition of the HGH peak in four subjects following imipramine 50 mg but no effect of diphenylhydantoin, phenobarbital, chlordiazepoxide, or isocarboxazid. In recent work in our Edinburgh laboratory, conducted chiefly by Dr. O. O. Ogunremi, we have found that the plasma cortisol in sleep (mainly at the end of the night) is significantly reduced by clinical doses of sodium amobarbital or of benzoctamine (Tacitin®) and rises above normal as part of a withdrawal reaction. Whether the profound suppression of Stages 3 and 4 sleep by benzodiazepines is accompanied by change of plasma HGH is not yet known.

BEHAVIORAL CORRELATIONS

Drugs used in sleep research are generally psychoactive drugs and it is therefore important to look for correlations between the electrophysiological or hormonal effects of a drug and its effects on psychic experience and overt

behavior. The delay in abolition of the signs of REM sleep during MAOI treatment roughly correlates with the delay in clinical improvement of depressed patients as judged by visual analog self-ratings (13) or nurses' ratings based on observation of the patient in ignorance of the electrophysiological changes (23). Self-ratings of mood and appetite have similarly revealed parallels with sleep data during treatment with the slimming-drug, fenfluramine (53).

Hypnotic drugs have been the subject of a number of studies of psychomotor efficiency the day after a single bed-time dose and secobarbital (54), sodium amobarbital, and nitrazepam (55) have, for example, been found to impair performance the day after a single clinical dose. There is a need for chronic studies of the effects of such drugs on performance, and these could well run in conjunction with sleep studies to determine, for example, whether manifestations of "tolerance" and withdrawal parallel one another.

THE NECESSITY FOR CHRONIC STUDIES

Single-dose studies of a drug can often give useful information. In clinical practice, however, drugs may be taken over long periods, and experience of sleep studies shows that the results of single-dose studies usually give an incomplete picture and sometimes a quite misleading answer.

The first dose of a drug may affect sleep less than does repeated dosage. MAOIs are outstanding examples (13). Flurazepam 30 mg provides another example because the decrease it causes in Stage 4 sleep may be trivial on the first night of use, but profound after two weeks (5, 56).

The advantages offered by chronic studies include the opportunity to observe diminution of drug effect with time (a "tolerance" effect) and eventual withdrawal abnormalities, usually opposite in direction to the original drug effects and constituting a "rebound" that often continues for weeks. Fig. 1 illustrates these phenomena for desipramine 75 mg nightly for one month. In the study concerned there was a significant negative correlation between degree of deviation from the baseline and successive days of administration, and there was a rebound lasting a month following withdrawal.

What conclusions can be drawn from a time course such as that shown in Fig. 1? One may conclude that even if clinical response is often delayed for three weeks, modest oral doses of desipramine powerfully and immediately affect the human brain (indeed the effect is present in the first two hours). One may conclude that some sort of homeostatic mechanisms operate to bring the measured variable back towards normal on a time scale of weeks. One may further conclude from the rebound that the presence of desipramine can lead to modification of some brain constituents that have a life span of the order of a month, as may be judged by their rate of decay. One may further speculate that these slow-changing constituents, presumably of a protein nature, may parallel other brain constituents that are equally slow-changing under the drug's influence and which relate to the slow therapeutic response.

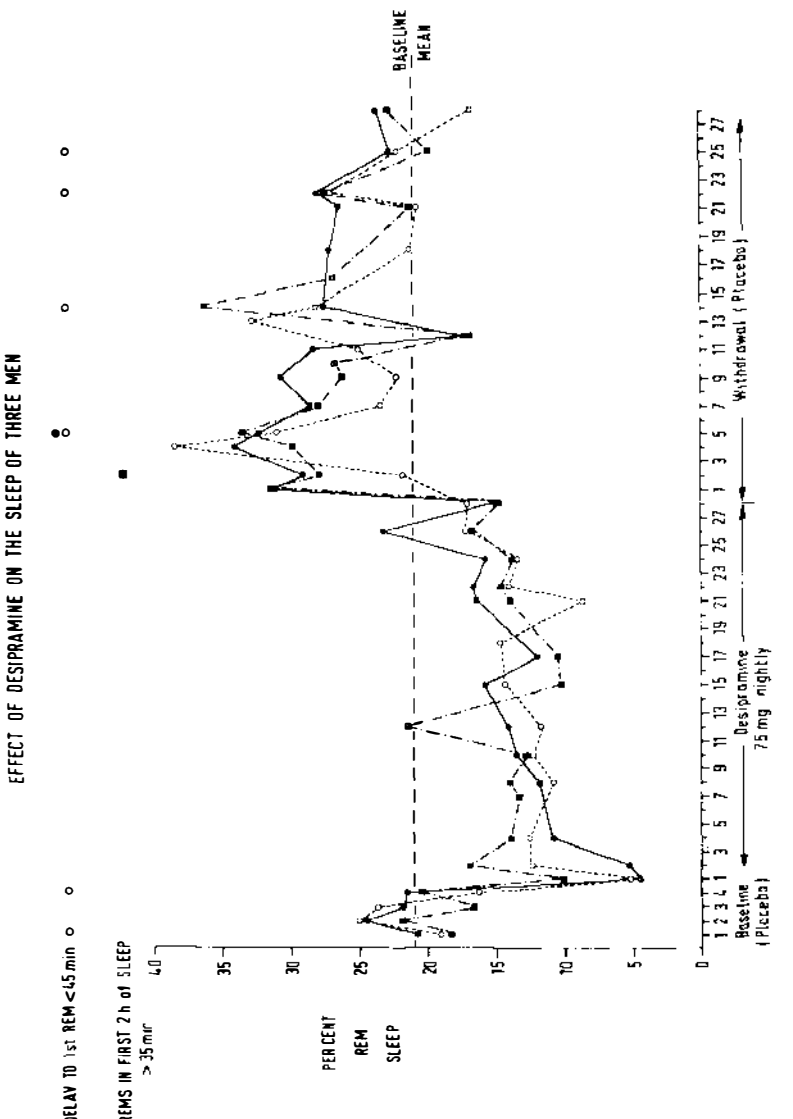

FIG. 1. The proportion of sleep spent in the REM (paradoxical) phase during the whole night's sleep by three men and the effect on it of giving desipramine for four weeks (total sleep duration was not affected). Reproduced with permission from the *British Journal of Psychiatry* (11).

Rebounds.—It has earlier been emphasized that hour-by-hour events of each night should be examined. In the rebound of REM sleep there is an altered distribution, with abnormally large amounts of REM sleep often appearing abnormally soon after sleep onset, as the upper part of Fig. 1 indicates.

The rebound in Fig. 1 appears to reach a maximum after about four days. The time to the rebound peak is roughly related to the time taken to eliminate the drug. It can be four days after fenfluramine (10), three weeks after a very large dose of phenobarbital that takes three weeks to eliminate (12), ten days after heroin when a similar period is needed to clear products from the urine (19), and similar time relations have been reported for nitrazepam (12) and for large doses of tricyclic drugs (49).

A lapse of several days between withdrawal and maximum rebound abnormalities lends some support to the view that slow drug elimination and a consequent gradual let-down may be associated with lower clinical abuse potential. Phenobarbitone and chlordiazepoxide are examples of drugs with relatively low abuse potential. Fenfluramine, where withdrawal mood depression is, like the peak of REM sleep rebound, delayed for four days, provides another example of a drug with low abuse potential despite the fact that it is an amphetamine derivative (57).

REM sleep is not the only sleep variable that may show a rebound. Intrasleep restlessness and Stages 3 + 4 sleep rebounds have also been observed (10).

How long should a chronic study continue? No dogmatism is possible but experience indicates that five weeks are needed to follow the time course of withdrawal from many drugs and a similar period should be required to follow the converse changes of tolerance. Ideally a late baseline series of nights should complement pre-drug baseline nights. Limitations of time and money may dictate a briefer or pilot study, and indeed the principal features of a drug's effects and the presence or absence of rebound abnormalities can sometimes be detected by a sequence of only three drug nights and two withdrawal nights (15).

Design Problems.—Conventionally-designed drug experiments, involving single doses of varying size, in some planned order in which placebo is also included, are appealing in their neatness and readily susceptible to statistical evaluation. Unfortunately they usually make the incorrect assumption that rebounds do not exist and they bear no relation to normal clinical usage which involves long-term administration.

Chronic sleep studies are laborious and costly and hence only a few subjects are used in each study unless the drug is of unusual importance. These small numbers militate against dose-response studies, and both the small numbers and the gradual evolution of effects with time do not suit conventional statistical methods. Chronic studies with only a few subjects nevertheless permit the demonstration of consistent patterns of response over time among different subjects and do not preclude calculations of probability of observed events.

If one has studied only three subjects for some fifty nights each and has predicted that their lowest REM sleep percentages will occur in the first drug

week and that their highest will occur in the first withdrawal week, and if, for example, the four lowest and the four highest values for each man occur in the predicted weeks then statistics are superfluous.

Another approach is to consider the means for each single drug night and each withdrawal night and to determine if any of these depart by more than two standard deviations from the baseline mean at a time and in a direction earlier predicted (10, 57). Alternatively one can design a chronic study with perhaps three subjects in blocks of, for example, four baseline, four early drug, four late drug, four early withdrawal, and four late withdrawal nights and then use analysis of variance to assess the significance of differences between the means of these blocks. This is a conservative approach that may miss the information that a graphical display can provide, particularly if, for example, the first two recorded withdrawal nights differ little from baseline but are on a rising curve that is only really manifest on the fourth recorded withdrawal night.

CONCLUSION

Sleep research has come of age in pharmacology, and chronic studies can give useful information about initial and late drug effects, about tolerance and withdrawal phenomena, and about the time-course of the drug's action. The methods are laborious and expensive, and suited only to the detailed investigation of intrasleep features in small numbers of subjects.

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