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THE TIME COURSE OF ACTION OF SINGLE DOSES OF DIAZEPAM, CHLORPROMAZINE AND SOME BARBITURATES AS MEASURED BY AUDITORY FLUTTER FUSION AND VISUAL FLICKER FUSION THRESHOLDS IN MAN

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The critical threshold for fusion of interrupted random (or " white ") noise stimuli, the auditory flutter fusion threshold (A.F.F.T.), is the auditory equivalent of the visual critical flicker fusion threshold (C.F.F.T.). Both are sensitive in man to the actions of centrally acting drugs (Simonson & Brozek, 1952 ; Besser, Duncan & Quilliam, 1966 ; Besser, 1966, 1967a). The earlier work on the modification of A.F.F.T. by amylobarbitone, chlorpromazine and diazepam suggested that the drug-induced changes in the threshold could be used to determine the time course of action of such compounds, and this paper reports the differential change over 8 hr in the depression of A.F.F.T. and C.F.F.T. induced by chlorpromazine, diazepam and three barbiturates—amylobarbitone, quinalbarbitone and phenobarbitone. A preliminary report of the results with diazepam has been published (Besser, 1967b).

METHODS

Auditory flutter fusion threshold

The technique described previously was used (Besser, 1967a). A random (or " white ") noise stimulus with an intensity of 55 d.B. (re. 0.0002 dyn./cm^2) was used. It could be interrupted at rates between 1.5 and 275 i/sec (with a mark-space ratio of 9:1) and the pulse form was rectangular. The interruptions were switched on and off automatically every 1 sec so that the signal which was presented binaurally to the subject, consisted of alternating 1 sec periods of interrupted and continuous noise. For each test the starting interruption rate was 100 i/sec, well above the critical fusion threshold. The signal was then presented in a sequence of decreasing interruption rates and subjects were asked to report whether or not they could detect the interruptions (that is the " auditory flutter "). The A.F.F.T. was taken to be the fastest interruption rate at which the flutter could be heard and below which flutter responses were consistently obtained.

Critical flicker fusion threshold

A continuously flickering neon light was used, driven by a rectangular pulse generator with a mark-space ratio of 1:1 and which had an illuminated area of 3 cm^2. Subjects were seated with their eyes on a level with the light source and 60 cm from it. The flickering light was presented at a

frequency of 50 i/sec, well above fusion, and then the flicker rate was reduced until the light appeared to the subject to flicker. This was taken to be the C.F.F.T.

Procedure

Each set of observations consisted of 3 A.F.F.T. and 2 C.F.F.T. determinations, starting with an A.F.F.T. determination and then alternating. The end point of the A.F.F.T. determination was the more difficult for the subject to recognize, and, therefore, the first A.F.F.T. determination of the set was used as a practice run and the results were discarded. The means of the two remaining A.F.F.T. and the two C.F.F.T. determinations in each set were used for analysis. A set of measurements was made immediately before drug administration as a baseline, and again at ½, 2, 4, 6 and 8 hr afterwards.

The drugs and doses used were: chlorpromazine 50 mg, diazepam 10 mg, amylobarbitone 100 mg, quinalbarbitone 100 mg, and phenobarbitone 100 mg. The doses are within the range used clinically. Each drug was given by mouth and was used in its normal tablet or capsule form. Each was matched with its own placebo in a double-blind, cross-over trial with balanced treatment orders. Each drug was studied separately in 12 subjects, cross-over between placebo and active treatments occurring at an interval of seven days. In all, 29 subjects particpated (age range 21–35 years) of whom 27 were male. The subjects were volunteers, members of the staff or clinical medical students at this medical college. The experiments started between 9 and 10 a.m., at least ½ hr after a light breakfast. Coffee and tea were not taken before or during the 8 hr experimental session.

Using the A.F.F.T. and C.F.F.T. recorded before tablet administration as a baseline, the changes in the mean thresholds at each time interval afterwards were calculated. The differences between these changes in threshold at each time after the active and after the placebo treatments represent the active drug effects and these were analysed by Students *t* test.

RESULTS

All the drugs tested depressed both the A.F.F.T. and C.F.F.T. and the time course of the effects on the two fusion thresholds were similar. These time courses, however, differed for each drug. After the placebo treatments the mean A.F.F.T. rose over the 8 hr period, but the mean C.F.F.T. remained at or just below the baseline value in all the drug studies—for example, Fig. 1. The baseline starting mean A.F.F.T.s and C.F.F.T.s are shown in Table 1 for each drug. The difference between the change in the threshold from its baseline starting value, following the active and placebo treatments, represents the active drug effect on the threshold. The mean drug effects from 12 subjects are shown for each drug at each time interval in Table 2 (A.F.F.T.) and Table 3 (C.F.F.T.).

TABLE 1

STARTING MEAN AUDITORY FLUTTER FUSION THRESHOLDS (A.F.F.T.) AND VISUAL CRITICAL FLICKER FUSION THRESHOLDS (C.F.F.T.) MEASURED BEFORE PLACEBO AND ACTIVE DRUG ADMINISTRATION

There were 12 subjects in each trial

	A.F.F.T. (i/sec)		C.F.F.T. (i/sec)	
Drug (mg)	Placebo	Active	Placebo	Active
Chlorpromazine 50	43·13	44·58	38·11	38·24
Diazepam 10	41·79	41·00	38·11	37·79
Quinalbarbitone 100	48·08	45·79	38·24	38·36
Amylobarbitone 100	43·58	45·17	36·91	36·69
Phenobarbitone 100	52·46	55·17	40·41	41·17

TABLE 2

AUDITORY FLUTTER FUSION THRESHOLDS AFTER SOME CENTRALLY ACTING DRUGS. THE DIFFERENCES ARE SHOWN (MEAN±STANDARD ERROR) BETWEEN THE CHANGE IN THRESHOLD AFTER THE ACTIVE AND AFTER THE PLACEBO TREATMENTS OVER 8 HR

There were 12 subjects in each drug group.

Time (hr)	Chlorpromazine (50 mg)	Diazepam (10 mg)	Quinalbarbitone (100 mg)	Amylobarbitone (100 mg)	Phenobarbitone (100 mg)
½	—1·88*±0·61	—1·08 ±0·79	—2·63† ±0·82	—0·79±1·74	—2·96*±1·32
2	—2·63 ±1·91	—2·71 ±1·67	—3·04* ±1·29	—2·46±2·29	—4·88*±1·86
4	—3·17†±1·00	—2·17 ±1·33	—2·08 ±1·36	—1·71±2·13	—5·58 ±2·97
6	—1·08 ±1·13	—0·33 ±1·97	—1·42 ±1·68	—1·21±3·50	—6·88*±2·94
8	—1·21 ±1·74	—0·42 ±1·53	—1·17 ±2·14	—0·42±4·16	—8·08*±2·76
Overall means	—1·99†±0·60	—1·34*±0·66	—2·07† ±0·66	—1·32±1·26	—5·68α±1·09

For the difference between active and placebo treatment effects: * $P<0·05$, † $P<0·01$, α $P<0·001$

TABLE 3

CRITICAL FLICKER FUSION THRESHOLDS AFTER SOME CENTRALLY ACTING DRUGS. THE DIFFERENCES ARE SHOWN (MEAN±STANDARD ERROR) BETWEEN THE CHANGE IN THRESHOLD AFTER THE ACTIVE AND AFTER THE PLACEBO TREATMENTS OVER 8 HR

There were 12 subjects in each treatment group

Time (hr)	Chlorpromazine (50 mg)	Diazepam (10 mg)	Quinalbarbitone (100 mg)	Amylobarbitone (100 mg)	Phenobarbitone (100 mg)
½	—0·385 ±0·264	—0·687*±0·299	—1·302†±0·385	—0·344 ±0·350	—0·219 ±0·169
2	—0·688 ±0·476	—1·073†±0·272	—1·104*±0·432	—0·833*±0·300	—0·719 ±0·386
4	—1·260 ±0·589	—0·531 ±0·261	—0·531 ±0·347	+0·094 ±0·345	—0·979*±0·439
6	—0·563 ±0·617	—0·281 ±0·373	—0·927*±0·399	—0·354 ±0·373	—0·979*±0·372
8	—0·323 ±0·729	—0·208 ±0·261	—0·823*±0·342	—0·615 ±0·420	—0·865 ±0·484
Overall means	—0·644*±0·245	—0·556α±0·134	—0·938α±0·169	—0·410*±0·160	—0·752α±0·170

For the differences between active and placebo treatment effects: * $P<0·05$, † $P<0·01$, α $P<0·001$

Diazepam 10 mg (Fig. 1 and Fig. 2)

Diazepam 10 mg induced a significant overall depression of both thresholds (for A.F.F.T. $P<0.05$, C.F.F.T. $P<0.001$). The drug-induced change was apparent at ½ hr, maximal at 2 hr and had virtually disappeared by 6 hr. The changes in the thresholds from the baseline values following the placebo and the active treatments are shown separately in Fig. 1. The difference between the change in threshold after the diazepam and after the placebo treatment (that is, the effect due to the active treatment) is shown in Fig. 2, where the placebo condition is represented by the zero line.

Chlorpromazine 50 mg (Fig. 2)

Following 50 mg chlorpromazine there was a significant overall depression of the two thresholds (A.F.F.T. $P<0.01$, C.F.F.T. $P<0.05$). The effect was apparent ½ hr after administration, reached a maximum at 4 hr and had largely but not completely worn off by 8 hr.

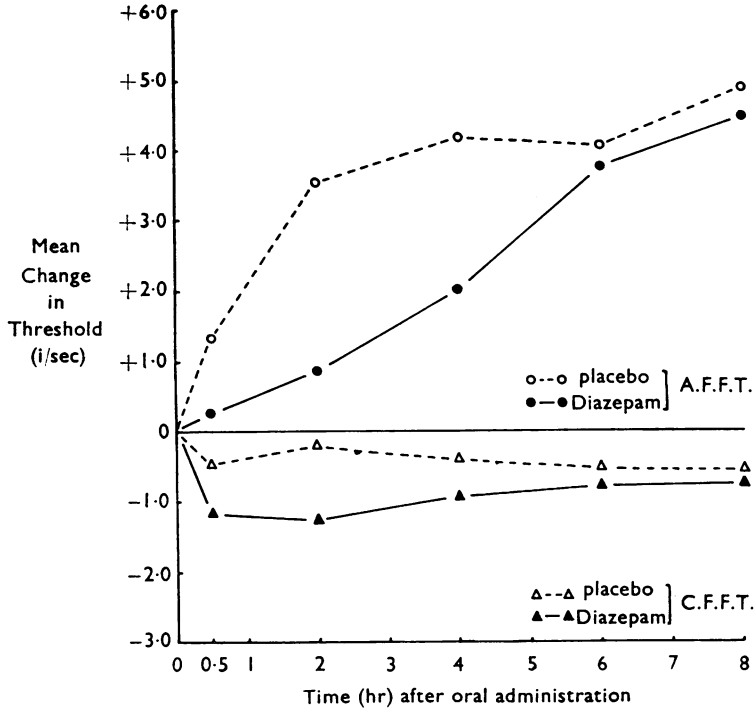

Fig. 1. The changes in A.F.F.T. (circles) and C.F.F.T. (triangles) after diazepam 10 mg (continuous line) and placebo (interrupted line) in 12 subjects over 8 hr. The pre-drug administration thresholds are given in Table 1. The overall differences between diazepam and the placebo effects are significant (Tables 2 and 3).

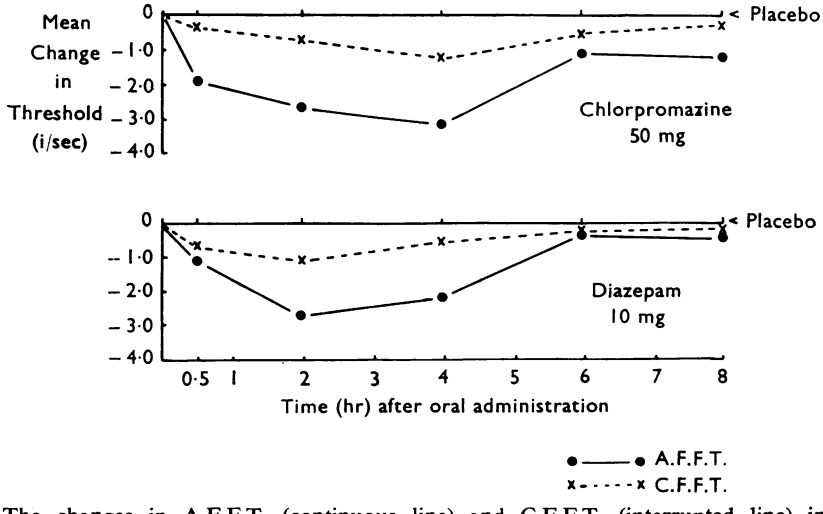

Fig. 2. The changes in A.F.F.T. (continuous line) and C.F.F.T. (interrupted line) induced by diazepam 10 mg (lower diagram) and chlorpromazine 50 mg (upper diagram) over 8 hr, each in 12 subjects. The threshold values after the placebos are represented by the zero baselines, the pre-drug means are given in Table 1. The overall differences between the effects of the active treatments and the placebos are significant (Tables 2 and 3).

Barbiturates (Fig. 3)

(1) *Quinalbarbitone 100 mg.* The overall threshold depression was significant (A.F.F.T. $P<0.01$, C.F.F.T. $P<0.001$). The effect had a rapid onset since the depression was almost as great at $\frac{1}{2}$ hr as at 2 hr when it was maximal for A.F.F.T., while it was slightly greater at $\frac{1}{2}$ hr than at 2 hr for C.F.F.T. The depression gradually decreased from 2 hr onwards but had not completely disappeared at 8 hr.

(2) *Amylobarbitone 100 mg.* The depression of the mean C.F.F.T. after amylobarbitone was significant overall ($P<0.05$). The depression of A.F.F.T. was consistently present

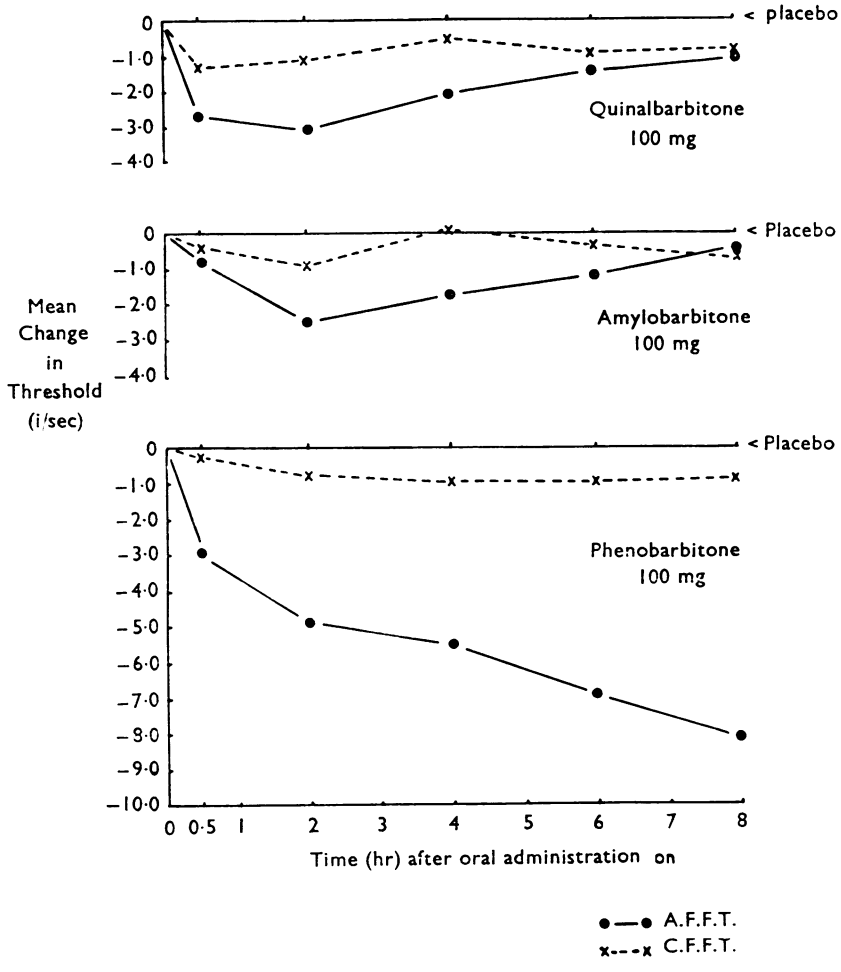

Fig. 3. The changes in A.F.F.T. (continuous line) and C.F.F.T. (interrupted line) induced by quinalbarbitone 100 mg (upper diagram), amylobarbitone 100 mg (middle diagram) and phenobarbitone 100 mg (lower diagram) over 8 hr, each in 12 subjects. The threshold values after the placebos are represented by the zero lines; the starting means are given in Table 1. The overall differences between active and placebo effects are significant, except in the case of amylobarbitone on A.F.F.T. (Tables 2 and 3).

over the 8 hr and was similar to that seen with C.F.F.T., though it did not reach statistically significant levels on *t* tests owing to an unusually large standard error of the overall mean (Table 2). The drug-induced effects were apparent at ½ hr, maximal at 2 hr and had almost disappeared at 8 hr after administration.

(3) *Phenobarbitone 100 mg.* The overall means of both thresholds were significantly depressed ($P<0.001$). With both A.F.F.T. and C.F.F.T. drug effects were present at ½ hr. The depression of C.F.F.T. reached its maximum at 4 hr, and was maintained at the same level at 6 and 8 hr after administration. The A.F.F.T. depression was greater at 4 hr than at 2 hr but then it appeared to increase further at 6 and 8 hr. This apparently increased depression of the threshold suggested by the last two values, however, is probably fortuitous since the mean rise in the A.F.F.T. of this group of subjects after the placebo was unusually great at 6 and 8 hr.

The action of phenobarbitone was thus slow to reach its maximum, but this was maintained up to 8 hr after drug administration.

Comparison of A.F.F.T. and C.F.F.T.

In all cases the depression induced in A.F.F.T. was greater than that in C.F.F.T. However, the standard errors were also greater for the flutter than the flicker fusion thresholds as can be seen in Tables 2 and 3. These tables also show that the standard errors tended to increase with time.

DISCUSSION

The previous study of the alteration in A.F.F.T. following single doses of amylobarbitone, diazepam and chlorpromazine over 3 hr, suggested that amylobarbitone and diazepam differed from chlorpromazine in their time course of action (Besser, 1967a). Chlorpromazine induced a depression in A.F.F.T. which was greater at 3 hr than at 1½ hr, while amylobarbitone and diazepam produced a threshold depression which was as great at 90 min as at 180 min. These differences have been confirmed in the present study in which the time of observation has been extended to 8 hr and the changes in C.F.F.T. have also been determined.

All three drugs produced some depression of both A.F.F.T. and C.F.F.T. at ½ hr but the maximum time of action of chlorpromazine (50 mg) was at 4 hr whereas after diaezpam (10 mg) and amylobarbitone (100 mg) it was at 2 hr. The duration of action also differed in that chlorpromazine still showed some depression of both thresholds at 8 hr but the diazepam effect had virtually worn off by 6 hr and the amylobarbitone curve had almost returned to the placebo baseline at 8 hr.

These results with chlorpromazine accord with those of Kornetsky, Vates & Kessler (1959); DiMascio, Havens & Klerman (1963) and Latz & Kornetsky (1965) who showed that there was significant depression of a number of intellectual and motor functions after 100–200 mg of chlorpromazine. The maximal action was seen at 3½–4 hr after oral administration. DiMascio *et al.* (1963) continued their studies up to 7 hr and at that time still showed impairment of function, while Kornetsky *et al.* (1959) reported some impairment of tapping and digit-symbol substitution and digit copying at 14–15 hr after 100–200 mg of the drug.

The time course of action of diazepam does not appear to have been reported. Schwartz, Koechlin, Postma, Palmer & Krol (1965), using tritiated diazepam in two subjects, showed that the peak blood levels of radioactivity occurred at 2 and 4 hr after oral administration.

The three barbiturates studied, quinalbarbitone, amylobarbitone and phenobarbitone, have sometimes been classified into short, intermediate and long-acting drugs (Goodman & Gilman, 1955 ; Wilson & Schild, 1959, but not Goodman & Gilman, 1965). The onset of action of quinalbarbitone was rapid, virtually reaching its maximum at $\frac{1}{2}$ hr after medication. Amylobarbitone although having a small effect at $\frac{1}{2}$ hr, showed its maximum action at 2 hr but after phenobarbitone the depression, present at $\frac{1}{2}$ hr, was not at its peak until 4 hr. The effects of amylobarbitone and quinalbarbitone decreased over the period between 2 and 8 hr, although they had not completely disappeared at the end of this time. After phenobarbitone, however, the maximum effect was maintained up to 8 hr after administration.

Kornetsky *et al.* (1959) and Latz & Kornetsky (1965) studied quinalbarbitone and showed that after a dose of 100 mg the impairment of tapping, symbol copying and digit-symbol substitution tests which was maximal at $\frac{1}{2}$–1 hr was still present at 14–15 hr. Hinton (1961) studied the actions of amylobarbitone sodium, quinalbarbitone and butobarbitone, in 200 mg doses, on sleep. While each significantly reduced movements of the subjects in bed and gave longer and less broken sleep, compared with a placebo, there was no difference between the drugs in their onset or duration of hypnotic action, nor on the incidence of hangover effects.

From the present studies it would appear that apart from the quicker onset of action of quinalbarbitone, there is no difference in the time course of quinalbarbitone and amylobarbitone.

The greater depression of auditory flutter fusion thresholds compared with the critical flicker fusion thresholds must not necessarily be taken to imply that the A.F.F.T. is more sensitive than the C.F.F.T. to the actions of these drugs. Since the variances were also greater with A.F.F.T. than C.F.F.T. it follows that for a given drug a greater change in mean threshold must be obtained with flutter than with flicker fusion if statistical significance is to be achieved. It seems desirable to measure both thresholds simultaneously since alterations in one mean can be confirmed in the other.

The tendency for the standard errors of the means of the drug-induced depressions of the threshold to be greater towards the end of the 8 hr period than earlier, suggests that there is a smaller variation between subjects in the speed with which the drug action appears than there is in the time taken for it to pass off.

Simultaneous measurement of the drug-induced changes in the auditory flutter fusion threshold and the visual flicker fusion threshold at intervals following the administration of centrally acting drugs, provides a sensitive technique for establishing their time course of action in man.

SUMMARY

1. The time course of the action of single doses, within the therapeutic range, of diazepam (10 mg), chlorpromazine (50 mg), amylobarbitone (100 mg), quinalbarbitone (100 mg) and phenobarbitone (100 mg) have been studied by measuring the drug-induced

depression of the auditory flutter fusion threshold (A.F.F.T.) and the visual critical flicker fusion threshold (C.F.F.T.) at ½, 2, 4, 6 and 8 hr following their administration by mouth. Each drug was investigated separately in a double-blind, cross-over study in 12 normal subjects.

2. The drugs had similar effects on A.F.F.T. and C.F.F.T. but each drug had a characteristic time course.

3. The actions of all the drugs were detectable ½ hr after administration. Diazepam had its maximum action at 2 hr, after which the depression of the thresholds gradually diminished and had virtually worn off by 6 hr. Following chlorpromazine administration the maximum effect occurred at 4 hr, after which the effect decreased but had not completely disappeared at 8 hr.

4. Among the barbiturates the action of quinalbarbitone appeared earliest, being almost at its maximum at ½ hr. After amylobarbitone the action was only slight at ½ hr but maximal at 2 hr. The effects of these two drugs decreased steadily over the period from 2 to 8 hr but had not completely disappeared at the end of this time. The action of phenobarbitone was slow to develop but was fully maintained up to 8 hr.

5. Simultaneous measurement of drug-induced changes in A.F.F.T. and C.F.F.T. at intervals after drug administration provides a sensitive technique for the study of the time course of the actions of centrally acting drugs in man.

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