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RESIDUAL EFFECTS OF FLUNITRAZEPAM

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1 Twelve normal subjects were tested on a large battery of tests after a hypnotic dose of flunitrazepam (1 or 2 mg) and a placebo. Psychological tests were given 12 h after the drug and physiological tests 12, 15 and 18 h after the drug.

2 The tests included self-ratings of hypnotic effects and mood, the electroencephalogram, the auditory electroencephalographic evoked response, skin conductance, tapping, card-sorting and the symbol copying test.

3 Both doses of flunitrazepam were effective hypnotics according to the ratings, with an anxiolytic effect the following day. The EEG was significantly altered up to 18 h after the drug and the behavioural tests showed a motor impairment 12 h after drug administration.

4 Nearly all changes displayed linear dose-related trends.

Introduction

At least one benzodiazepine hypnotic, nitrazepam, appears to be effective clinically and is now extensively prescribed. It is preferable to barbiturates in the treatment of insomnia because it has fewer unwanted effects, is less likely to abuse and is relatively safe in overdosage. However, the physiological and behavioural effects of nitrazepam as assessed the next day are similar to those of the barbiturates (Malpas, Rowan, Joyce & Scott, 1970; Bond & Lader, 1972). Indeed, such residual sedative effects may be inescapable attributes of effective hypnotics. More recently, another benzodiazepine, flurazepam, has been introduced but this also has easily detectable residual effects the next day (Bond & Lader, 1973).

Flunitrazepam (Ro 5-4200) is a new and potent benzodiazepine hypnotic which seems to be similar to nitrazepam in its effects on sleep (Gaillard, Shulz & Tissot, 1973; Kales & Scharf, 1973; Oswald, Lewis, Tagney, Firth & Haider, 1973). As part of its general assessment, the residual effects of this compound were studied in the present experiment: two doses were compared to a placebo on a battery of physiological and psychological tests. It was not compared to any other compounds as nitrazepam, flurazepam and butobarbitone sodium had been tested previously under very similar conditions (Bond & Lader, 1972, 1973) and it was thought that a retrospective comparison between these four drugs was permissible.

Methods

Subjects

Twelve normal, healthy subjects took part in the study. They were six males and six females, post-graduates aged between 23 and 37 years.

Drugs

Two doses of flunitrazepam (1 mg and 2 mg) were compared with a placebo. The subjects were given an identical looking white capsule on each occasion.

Experimental design

Each subject was tested on three separate occasions at intervals of at least one week. The drugs were assigned according to a fully balanced design under double-blind conditions. The subjects were instructed to take the capsule at a prearranged time between 22.00 h and 23 h 30 min and to retire to bed immediately. In the morning, they arose at their normal time, ate their usual breakfast and reported to the laboratory between 09 h 30 min and 11.00 hours. They were tested on the full battery of physiological and behavioural tests 12 h after the drug and on a limited number (the EEG, evoked response and reaction time) 15 h and 18 h after the drug. They were instructed not to drink alcohol on the evenings on which they were to take the sleeping capsules nor on the day of the

test. However, they were allowed their normal intake of caffeine–containing beverages both night and morning but were instructed not to increase their intake if sleepy.

Self rating

Three visual analogue scales of 100 mm were used to measure onset of sleep (very abrupt-very slow), quality of sleep (very bad-very good), and feeling on wakening (very sleepy-very alert). Retrospective ratings were made the next morning. Feeling 12 h after the drug was measured on a series of sixteen analogue scales. This mood rating scale has been subjected to a principal component analysis which yielded three factors (Bond & Lader, 1974). The first factor is alertness and consists of nine of the scales: alert-drowsy, strong-feeble, muzzy-clear-headed, well co-ordinated-clumsy, lethargic-energetic, mentally slow-quick witted, attentive-dreamy, incompetent-proficient and interested-bored. The second factor is one of contentedness and the five scales which group together are: contented-discontented, troubled-tranquil, happy-sad, antagonistic-amicable and withdrawn-gregarious. The third factor calmness, is composed of two scales, calm-excited and tense-relaxed. The subjects rated themselves on the scales by placing a perpendicular mark across each line and the score was measured in mm from the end of the line to the mark. A log transformation was applied to the individual scores on each scale in order to normalize the distributions.

Physiological measures

Background EEG The EEG was recorded from two bipolar saline pad electrodes (Bond & Lader, 1972). Thirty-two standardized click stimuli of moderate intensity were presented through a loudspeaker placed below and behind the subject's head. The stimuli were presented with a random interval of 8-12 s and the subject was instructed to respond as quickly as possible. The EEG was analysed through four broad wave-band filters 0.1 dB down at the following upper and lower frequencies: 1 : 2.4-4 Hz; 2 : 4-7.5 Hz; 3 : 7.5-13.5 Hz; 4 : 13.5-26 Hz. The outputs of these four filters were fed into four analog-to-digital converter inputs of a PDP-12A computer which sampled each wave-band for 5 s epochs commencing 1 s after each click and calculated the mean rectified voltage in each wave-band. These mean voltages were added together to give the mean voltage in the total frequency band of 2.4-26.0 Hz, and each individual wave-band voltage was also expressed as a percentage of this total.

Evoked response The computer averaged 500 ms epochs of the electroencephalographic wave forms following each of the thirty-two clicks. The averaged evoked response was displayed on the oscilloscope of the computer and cursors were set after identifying four peaks in each evoked response: 1: a positive peak with a mean latency of 72 ms (P_1); 2: a negative peak, mean latency 127 ms (N_1); 3: a positive peak at about 200 ms (P_2); 4: a negative peak, mean latency 284 ms (N_2). Three peak-to-peak amplitudes were also measured in microvolts: 1: P_1-N_1; 2: N_1-P_2; 3: P_2-N_2.

Skin conductance The palmar skin resistance (sweat gland activity) was recorded on a polygraph (Bond & Lader, 1972) and also fed into the computer via another analog channel. The computer read the resistance (in kΩ) at the time of each click, converted it to the log reciprocal (in log μmhos), i.e. to the corresponding log skin conductance value (Lader & Wing, 1966), and calculated the mean log conductance level. The other skin conductance variable consisted of counting the number of spontaneous fluctuations per minute greater than a certain criterion (0.003 log μmhos; Lader & Wing, 1966).

Psychological measures

Auditory reaction time Simple auditory reaction time to the clicks was measured during the course of the EEG recordings and the reciprocal transformation applied.

Tapping rate Using the first two fingers of his preferred hand, the subject tapped a key as quickly as possible. The key was connected to a resettable mechanical counter at the back of the machine which recorded the number of key depressions made during 60 seconds.

Cancellation of 4s A simple cancellation of 4s task was constructed in which the frequency of 4s was forty in four hundred. The time to complete the task and the number of errors made each time was recorded.

Card sorting test This comprised of three tasks of matching cards into two, four or eight categories and equivalent 'motor' sorts in which the cards were merely dealt into two, four or eight piles without matching (Bond & Lader,

1972). The times taken for each of the six tasks and the number of errors were recorded.

The digit symbol substitution test (DSST) This is a subtest of the Wechsler Adult Intelligence Schedule (W.A.I.S.) and consists of a coding task in which symbols are substituted for numbers. The instructions were given according to the W.A.I.S. manual (Wechsler, 1955) and the score was the number of items correct in 90 seconds.

The symbol copying test (SCT) This utilises the same symbols as in the digit symbol substitution test but the subject has only to copy and not code them (Kornetsky, Vates & Kessler, 1959). The score was the number of items correct in 90 seconds.

Analysis of data

Most of the experiment was run on-line, in real-time, using a PDP 12A computer, the data being stored on digital magnetic tape. The additional data from the pencil and paper tests were transcribed on to the same magnetic tape and all variables were analysed using a Fortran II system. A split plot analysis of variance between subjects and drugs was calculated for those variables measured only 12 h after the drugs, differences between drugs being estimated against within-subject variance; and a split-split plot analysis of variance, the main sources of variance being subjects, drugs and times was calculated for those variables measured 12, 15 and 18 h after the drugs. Order effects were also extracted. Differences between drugs were obtained also from the drugs x times interaction and were estimated against within-subject within-occasion error variance. Tukey's test was computed for the difference between means (Winer, 1962). A trend analysis using orthogonal polynomials was also calculated to test for rectilinear dose-effect curves (Winer, 1962).

Results

Self-rating

Onset of sleep was rated as significantly faster and quality of sleep as significantly better after both doses of flunitrazepam (Figure 1). Both these measures followed a significant linear dose-related course. Although the subjects felt more sleepy on wakening after flunitrazepam, this was not significant.

For feeling at testing 12 h after the drug, the mood rating scale was used.

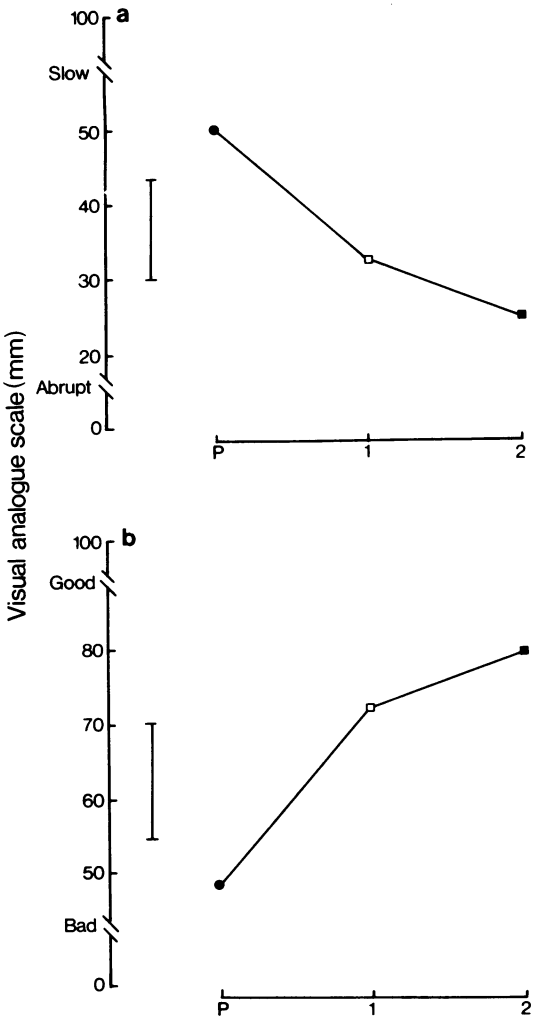

Figure 1 Mean (n = 12) scores of self-ratings of onset of sleep (a) and quality of sleep (b), rated 12 h after placebo (P, ●), flunitrazepam (1 mg) (1, □) and flunitrazepam (2 mg) (2, ■). The vertical bar represents the 0.05 critical difference, i.e. means further apart than this value are significantly different at the 0.05 level of confidence at least.

Factor 1 scales Two of the scales loading on this factor showed some significant results:

Alert-Drowsy. The subjects rated themselves as significantly more drowsy after the higher dose of flunitrazepam compared to both placebo and the low dose (Figure 2).

Proficient-Incompetent. The subjects rated themselves as significantly more incompetent

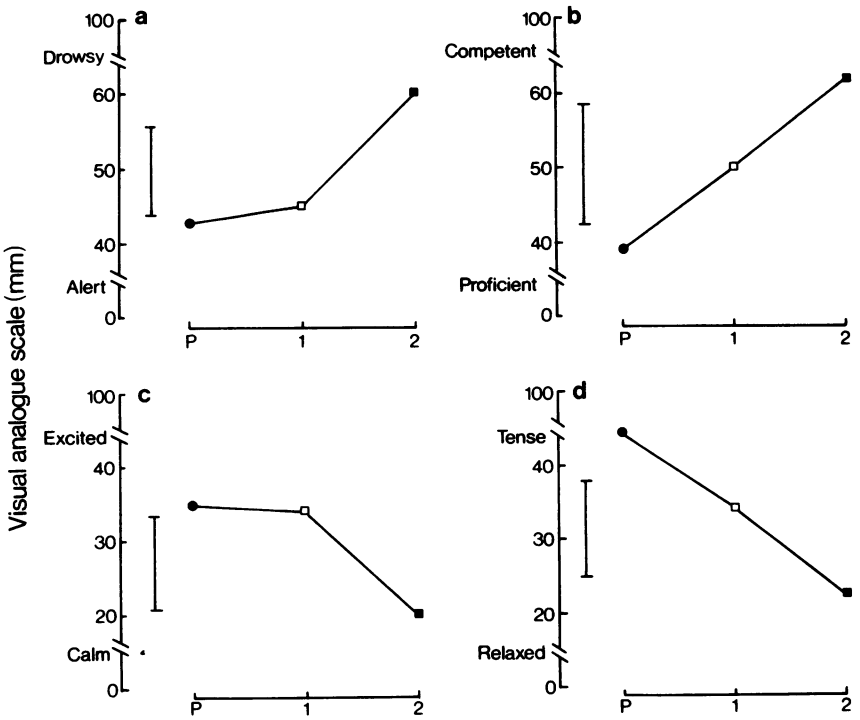

Figure 2 Mean (n = 12) scores of self-ratings on continua: alert-drowsy (a), proficient-incompetent (b), calm-excited (c) and relaxed-tense (D) rated 12 h after placebo (P, ●), flunitrazepam (1 mg) (1, □) and flunitrazepam (2 mg) (2, ■). The vertical bar represents the 0.05 critical difference, i.e. means further apart than this value are significantly different at the 0.05 level of confidence at least.

after the higher dose of flunitrazepam than after placebo (Figure 2).

Factor 2 scales None of the scales loading on factor 2 showed statistically significant drug effects.

Factor 3 scales Both scales loading on this factor showed some statistical significance.

Calm-Excited. The subjects rated themselves as significantly more calm after the upper dose of flunitrazepam compared to both placebo and the low dose (Figure 2).

Relaxed-Tense. The subjects rated themselves as significantly more relaxed after the upper dose of flunitrazepam than after placebo (Figure 2).

All these measures showed significant linear dose-related trends.

Physiological measures

Broad waveband analysis of the EEG Both doses of flunitrazepam tended to decrease the slow wavebands, 2.4-4.0 Hz and 4.0-7.5 Hz, and increase the fast waveband, 13.5-26.0 Hz, up to 18 h after the drug but these results were statistically significant for the higher dose only (Table 1). There were no significant effects on the 7.5-13.5 Hz waveband nor on the total voltage. The proportion of activity in each waveband showed highly significant differences which were still present up to 18 h after administration and therefore showed no alteration with time (D x T interaction not significant, Table 1). The higher dose significantly decreased the proportion of activity in the three slower wavebands and increased it in the fast waveband compared to placebo. The low dose had similar but less significant effects (Table 1). All the variables which showed a significant difference from placebo also showed a significant linear drug trend. The means for the most significant EEG variable, the percentage of fast activity, are plotted in Figure 3.

Averaged evoked response The latencies of the evoked response were not consistently altered by

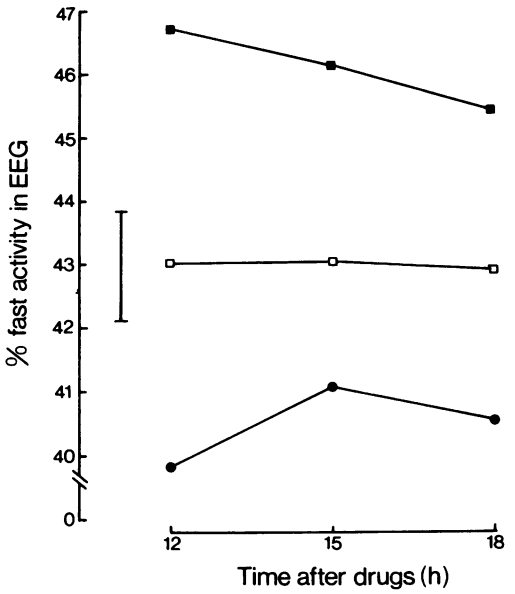

Figure 3 Mean ($n = 12$) scores for percentage of fast activity (13.5-26.0 Hz) in the EEG 12, 15 and 18 h after placebo (•), flunitrazepam (1 mg, □) or flunitrazepam (2 mg, ■). The vertical bar represents the 0.05 critical difference, i.e. means further apart than this value are significantly different at the 0.05 level of confidence at least.

the drug. All three potentials were, however, decreased in amplitude throughout the day. The P_1-N_1 component was significantly diminished by both doses of flunitrazepam ($F = 5.85$; $P < 0.01$) and the N_1-P_2 component was significantly diminished by the upper dose ($F = 3.49$; $P < 0.05$). Both showed a significant linear dose-related trend. The P_2-N_2 component was not significantly altered.

Skin conductance Flunitrazepam produced a reduction in mean skin conductance level which showed a significant linear trend. However, the difference between the upper dose and placebo only just reached statistical significance. The higher dose significantly reduced the number of fluctuations compared to both placebo and the low dose ($F = 4.87$; $P < 0.05$) and this variable also showed a significant linear dose-related course.

Psychological measures

Auditory reaction time Reaction time was slightly increased by the drugs but this was not significant.

Tapping rate Both doses of flunitrazepam significantly slowed tapping rate ($F = 11.30$; $P < 0.001$) and there was a highly significant linear trend.

Cancellation of 4s The time taken to complete the cancellation task was increased by the drug but this failed to reach statistical significance.

Card sorting test Sorting into two categories was significantly slowed by the higher dose of flunitrazepam compared to both placebo and the low dose ($F = 4.77$; $P < 0.05$). There was also a significant linear trend. The other two category sorts were not significantly affected.

The motor sorts showed more effect. All three

Table 1 *F*-ratios and *t*-values for broad waveband analysis of the EEG

Waveband	*Drugs*				*Drugs x time interaction*
	F	t	t	t	F
		Placebo-Flunitrazepam (1 mg)	*Placebo-Flunitrazepam (2 mg)*	*Flunitrazepam (1 mg)-Flunitrazepam (2 mg)*	
2.4-4.0 Hz	4.71*	1.40	3.06**	1.66	1.17
4.0-7.5 Hz	6.90**	1.65	3.71**	2.06	0.17
7.5-13.5 Hz	2.46	–	–	–	0.52
13.5-26.0 Hz	4.04*	1.12	2.82*	1.70	1.64
Total Hz	1.07	–	–	–	0.89
% 2.4-4.0 Hz	3.93*	1.00	2.77*	1.77	1.22
% 4.0-7.5 Hz	18.55***	2.25*	6.03***	3.78**	0.92
% 7.5-13.5 Hz	8.04**	2.36*	3.98***	1.62	0.95
% 13.5-26.0 Hz	22.25***	2.92**	6.65***	3.73**	1.84

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

showed a significant linear dose-related effect although sorting into two piles did not quite show a significant drug effect. Sorting into four piles was significantly slowed by the upper dose of flunitrazepam ($F = 5.61$; $P < 0.05$) and sorting into eight piles was significantly slowed by both doses compared to placebo with the higher dose producing correspondingly more impairment than the lower ($F = 15.96$; $P < 0.001$).

The symbol copying test Significantly fewer items as compared with placebo were completed on this test after flunitrazepam ($F = 4.78$; $P < 0.05$) but the effect was somewhat greater for the lower than for the higher dose.

The digit symbol substitution test Fewer items of this test were completed after the drug but this effect just failed to reach statistical significance.

Discussion

Flunitrazepam showed clear dose-related effects on almost all the measures, i.e., in those significantly altered by the drug, there was a corresponding significant rectilinear drug trend. This also seemed to occur with nitrazepam (Bond & Lader, 1972) but it was not clearly demonstrated with flurazepam (Bond & Lader, 1973). Both doses of flunitrazepam exerted a hypnotic effect which the subjects recognized as a speedier onset of sleep as well as its superior quality. They did not report excessive sleepiness on wakening after flunitrazepam but in conflict with this, they reported feeling more drowsy and incompetent 12 h after the higher dose. Thus, the subjects seemed only able to clearly distinguish the difference between drug and placebo at a time when they were normally alert, i.e. mid-morning. At this time they were also aware of the anxiolytic effect of flunitrazepam as characterized by a definite increase in ratings of calmness and relaxation on the mood rating scale. This rather pleasant feeling of calmness and relaxation has also been reported after flurazepam (Bond & Lader, 1973).

Two components of the evoked response were significantly diminished by flunitrazepam up to 18 h after administration. This result showed a very similar pattern to flurazepam (Bond & Lader, 1973), but a slightly different one from nitrazepam which also diminished the P_2-N_2 wave (Bond & Lader, 1972).

The broad waveband analysis of the EEG showed the characteristic changes produced by benzodiazepines but these were much more marked after the upper dose. The amount of activity in the slow wavebands was decreased and that in the fastest waveband increased and these effects had not diminished 18 h after the drug. The proportion of activity in the 13.5-26.0 Hz waveband once again proved to be one of the most sensitive indicators of drug action. The results on this variable for flunitrazepam were compared with the results for nitrazepam, flurazepam and butobarbitone in two previous studies (Bond & Lader, 1972, 1973) and are displayed in Figure 4. It can be seen that the three benzodiazepines produced similar dose related effects. Flunitrazepam (2 mg) has by far the greatest effect on the EEG followed by nitrazepam (10 mg) and flurazepam (30 mg). Roughly speaking, flunitrazepam (1 mg) is equipotent to nitrazepam (6.5 mg) and flurazepam (19.5 mg). The barbiturate, 150 mg, has a greater effect than the 200 mg dose in the previous experiment which emphasizes the need for caution in comparing between experiments involving relatively small numbers of subjects.

The reductions in mean skin conductance level and in the number of fluctuations in the tracing produced by flunitrazepam 12 h after administration indicate a diminished level of arousal or alertness. This may reflect either the calmness or the drowsiness produced by the drug or both and supports the subjects' self-reports of these effects.

The behavioural effects showed a similar pattern to those found after nitrazepam. In general, it was the motor tasks which were affected most. Tapping rate and the motor part of the card-sorting test were greatly slowed and only the motor part of the DSST, represented by the SCT, was significantly impaired by the drug.

In conclusion, it seems that the new benzodiazepine hypnotic, flunitrazepam, has similar behavioural and physiological effects to nitrazepam and the latter last for at least 18 h after drug administration. Its subjective effects are mainly pleasant ones of an anxiolytic nature, similar to those of flurazepam. However, unlike flurazepam, it shows clear dose effect curves and a good hypnotic action in both doses tested. It may be a useful compound for patients requiring an effective hypnotic with some anxiolytic action the following day. In single doses of 1 mg the residual effects are not excessive but the 2 mg dose undoubtedly has definite behavioural, physiological and subjective effects the next day. Nevertheless, great caution should be exercised in extrapolating this result to anxious patients receiving regular hypnotics. Drug effects are much less marked in these clinical circumstances (Malpas, Legg & Scott, 1974; Tansella,

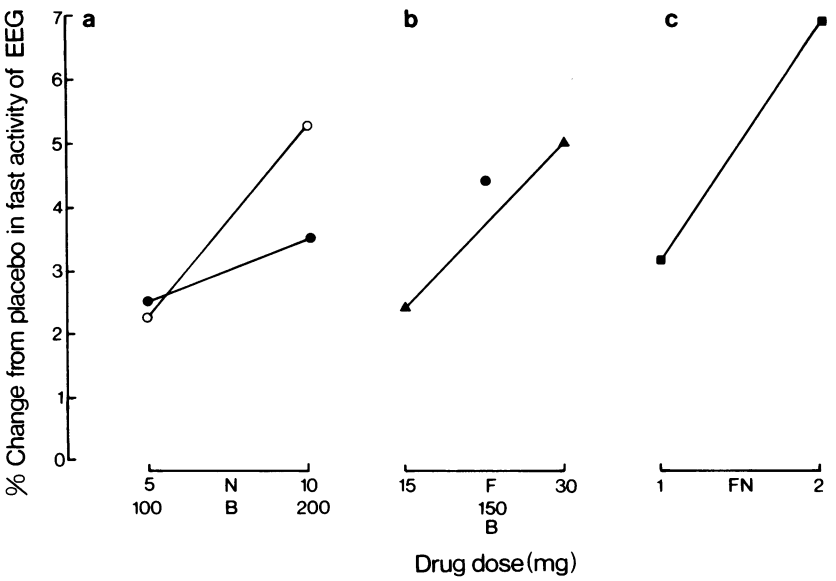

Figure 4 Mean (n = 12) scores for increases in percentage of fast activity (13.5-26.0 Hz) induced in the EEG 12 h after (a) nitrazepam (N, 5 mg and 10 mg, ○) and butobarbitone (B, 100 mg and 200 mg, ●) (Bond & Lader, 1972), (b) flurazepam (F, 15 mg and 30 mg, ▲) and butobarbitone (B, 150 mg, ●) (Bond & Lader, 1973), and (c) flunitrazepam (FN, 1 mg and 2 mg, ■) (present study), all compared with placebo.

Zimmerman-Tansella & Lader, 1974) probably reflecting complex relationships between anxiety, sedative drug effects and chronic drug usage.

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