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Flunitrazepam-induced changes in neurophysiological, behavioural, and subjective measures used to assess sedation

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Abstract

Introduction: Certain features of event-related potentials (ERPs), electroencephalographic (EEG), and behavioural measures vary with differing states of alertness and/or sedation. *Purpose*: This study was conducted to investigate changes in several measures usually viewed as reflecting states of sedation/sleepiness associated with the use of a range of doses of the hypnotic benzodiazepine (BZD) flunitrazepam (FNZ). *Methods*: This was a double blind, independent group design study of the effects of acute oral doses of FNZ in young healthy volunteers. Forty-eight subjects were randomly allocated to one of four groups—FNZ (0.6, 0.8, and 1.0 mg) and placebo (PLAC)—and tested prior to treatment and then in a posttreatment session close to the theoretical peak plasma concentration. ERP latencies and amplitudes were measured at midfrontal (Fz), midcentral (Cz), and midparietal (Pz) using a standard auditory oddball paradigm. EEG changes were assessed at Pz. Behavioural measures included the digit–symbol substitution test (DSST), a cancellation task (CT), and subjective ratings of alertness and attentiveness by the subjects (SUB) and the experimenter (EXP). *Results*: FNZ led to psychomotor impairments and decreased alertness and attention; these effects were consistent with previous findings. A progressive, dose-related increase in P3 latency occurred in Fz, Cz, and Pz, and there was an increase in N1 (Fz, Cz) and N2 (Fz). N2–P3 amplitude decreased in Fz. EEG power bands beta 1 increased for the two highest doses, but no significant differences were noted in theta, delta, and alpha bands. P3 latencies, experimenter-rated levels of alertness, and DSST scores differentiated all three doses of FNZ from PLAC. *Conclusion*: The most sensitive measures used were P3 latencies of the ERPs (which varied with FNZ dose), DSST, and the experimenter-rated levels of alertness. However, we found no evidence for the assumption that one single phenomenon was reflected in all measures and different mechanisms were probably involved. Further experiments will be needed for more in-depth probing of the finer mechanisms underlying sedation/sleepiness and how they affect behavioural and eletrophysiological measures of the central nervous system (CNS) function.

Keywords: Benzodiazepines; EEG; Event-related potentials; ERP; Flunitrazepam; P300; Sedation

1. Introduction

Benzodiazepines (BZDs) present a well-known spectrum of action on the central nervous system (CNS), which includes sedative–hypnotic, muscle relaxant, anxiolytic, and anticonvulsive effects, as well as cognitive impairment (e.g., psychomotor, subjective effects, and memory). Although it seems relatively well established that the amnestic effects of BZDs are not entirely secondary to sedation/sleepiness (Curran, 2000; Veselis et al., 2001), other effects such as psychomotor impairment and subjective changes have been traditionally equated with BZD-induced sedation

Abbreviations: BZD, Benzodiazepine; CNS, central nervous system; CT, cancellation task; DSST, digit–symbol substitution test; EEG, electroencephalogram; ERP, event-related potentials; FNZ, flunitrazepam; PLAC, placebo; PCA, principal component analysis; SUB, subjective ratings performed by the subjects; EXP, subjective ratings performed by the experimenter; STAI, State–Trait Anxiety Inventory; VAS, Visual Analogue Scales.

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or sleepiness (Curran, 2000). As the latter are nonobjective measures, they may be altered by motivation and learning (Laurijssens and Greenblatt, 1996). Electroencephalogram (EEG) and event-related potentials (ERPs) may be additionally used as objective means of detecting these changes (Kutas and Dale, 1997). In addition, the value of combinations of behavioural and neurophysiological measures has been recently stressed (Gevins et al., 2002).

The effects of sedative or hypnotic drugs on EEG include decreases in the power of alpha bands and increases in theta and delta activity (Engelhardt et al., 1992; Wauquier, 1999). These effects have been found after administration of BZDs. However, discrepancies in EEG findings have been reported with this class of drugs. For instance, decreases in theta power have also been shown after BZD ingestion (Manmaru and Matsuura, 1989), although an increase in this effect is expected when alertness is decreased (Coull, 1998). In addition, BZDs are potent activators of beta activity (van Leeuwen et al., 1995; Urata et al., 1996). This activating effect is considered paradoxical because an increase in EEG fast frequencies is usually interpreted as cortical alertness (van Leeuwen et al., 1995; Coull, 1998).

While EEG parameters indicate continuous variations in electrical potentials generated by the brain, ERPs measure the same type of activity but time-locked to sensory, motor, or cognitive events (Timsit-Berthier and Gerono, 1998). The most frequently used paradigm to evoke ERPs is the auditory "oddball" task in which subjects have to detect occasional target signals randomly interspersed among more frequent standard stimuli (Picton, 1992). The averaged ERPs elicited by standard tones are characterised by evoked N1 and P2 components whereas ERPs elicited by target tones contain these two early exogenous components as well as later sequences of endogenous components, N2 and P3 (Iragui et al., 1993). The major strength of ERP techniques is that they enable the monitoring of ongoing electrophysiological changes starting at stimulus presentation and ending after execution of a response (Münte et al., 1996).

Alterations in late components such as P3 have been considered a sensitive method of detecting subtle BZD-induced differences in vigilance (Milligan et al., 1989; Engelhardt et al., 1992). However, others have concluded that P3 latency and amplitude changes cannot be interpreted as being the consequences of sedation alone (Ray et al., 1992; Pang and Fowler, 1994). Authors such as Urata et al. (1996) and Hayakawa et al. (1999) who studied the effects of only a small dose of triazolam on auditory oddball paradigm scores claimed that BZDs could cause cortical changes without distinct general sedation or subjective drowsiness. The latter authors, however, employed only one dose of the drug, and it would be important to evaluate the effects of various doses of a BZD on this and other measures of sedation to compare their dose–response curves and determine whether or not they are measuring the same phenomenon (Weingartner et al., 1993).

The effect of sedation on ERPs is a particularly important issue because the P3 or P300 has long been used as a measure of cognitive processing (Polich, 1994; Pooviboonsuk et al., 1996), although the exact nature of this type of processing, as well as the physiological processes underlying its generation is unknown (Kügler et al., 1993). If we are to posit that the P3 does indeed constitute a measure that can be of use in understanding the cognitive effects of psychotropic drugs (e.g., BZDs), then the extent to which sedation influences ERP changes must also be investigated.

The aim of the present study was to investigate changes in a number of measures that are usually viewed as reflecting sedation/sleepiness associated with the use of a range of doses of the hypnotic BZD flunitrazepam (FNZ). For this purpose, the authors studied the ERP (N1, P2, N2, and P3) and EEG effects of three levels of dosage of FNZ. Classical psychometric tests (digit–symbol substitution test [DSST] and cancellation task [CT]) and subjective measures of sedation and attentiveness, as rated by the experimenter (EXP) and the subjects (SUB) themselves, were also employed. FNZ was selected because it is a full BZD agonist with typical cognitive effects (Pompéia et al., 1996a,b; 2000). The range of doses of FNZ employed here was determined following results of previous studies on a large number of cognitive measures (psychomotor, subjective, and memory; see Pompéia et al., 1996a,b; 2000).

2. Methods

2.1. Subjects

Forty-eight normal volunteers (24 men), aged 18–36 years (24.8 ± 5.1 years), with average body mass index (weight/height^2, 22.1 ± 2.1), more than 12 years of schooling and normal trait anxiety (STAI, 37.4 ± 7.9; see Gorenstein et al., 1995). Participants met the usual exclusion criteria for clinical trials (e.g., pregnancy, allergy, chronic clinical, or psychiatric disorders), had no history of drug abuse or heavy alcohol drinking, consumed less than five units (Miller et al., 1991) of alcohol per week, did not smoke or regularly use drugs of abuse such as cannabis and cocaine and were on no medication at the time of the study. The volunteers had no hearing impairment and were able to discriminate high- from low-frequency tones with ease.

2.2. Procedure

This was an independent group design study using single oral BZD doses. Subjects were randomly allocated, apart from perfect balancing by sex to one of four treatments (12 subjects in each group): placebo (PLAC) and 1.0 (FNZ 1.0), 0.8 (FNZ 0.8), and 0.6 mg (FNZ 0.6) of FNZ. The Ethics Committee of the institution at which the experiment was conducted (UNIFESP) approved the protocol, and all subjects signed informed consent forms. Subjects were

instructed to abstain from alcohol or other drugs for 24 h before and after the experiment.

The volunteers arrived at the laboratory at 0800 h. They received a light breakfast and were tested immediately after (pretreatment). Following these tests (approximately at 0900 h), they received the treatment (PLAC, FNZ 0.6, 0.8, or 1.0 mg). A second session occurred near theoretical peak plasma concentration of FNZ (1.5 h; see Mattila and Larni, 1980). Subjects were familiarised with all pretreatment tests before performance was assessed. Different versions of DSST and CT were counterbalanced across subjects and experimental sessions (pre- and posttreatment). In the post-treatment session, the following order of tests was employed: ERPs (15 min), EEG (3 min), psychomotor (10 min), and subjective measures (10 min).

2.3. Treatment

Formulated in identical capsules containing the active principle (FNZ) and talc (PLAC).

2.4. Test battery

2.4.1. Event-related potentials

Subjects were seated in comfortable chairs that allowed them to adjust the position of the head to reduce muscular artefacts. During the experiment, they were instructed to fixate on a point located at a distance of approximately 2 m. ERPs were elicited by a series of binaural tones at 70 dB nHL, with a 10-ms rise/fall and a 100-ms plateau time. The interstimulus interval was 1 s. The tones were presented in a random sequence with a 1500-Hz tone (rare target) occurring 20% of the time and the 800-Hz standard tone occurring 80% of the time. Volunteers were instructed to press a button in response to each target tone as quickly and as accurately as possible with their dominant hand. Stimuli were presented until a total of 15 artefact-free responses to target tones were recorded. Then a second block of 15 artefact-free responses to target tones was obtained to ensure replication of the morphological structure of the average ERPs and to facilitate component identification.

ERPs were recorded with Ag/AgCl electrodes from the midfrontal (Fz), midcentral (Cz), and midparietal (Pz) locations according to the international 10–20 system and were referenced to linked mastoids with a forehead ground. The electrode impedance was checked and adjusted until it was lower than 5 kΩ. The bandpass filter was 0.1–50 Hz (3 dB down, 12 dB/octave slope). The electrooculogram (EOG) was recorded from electrodes above the right eyebrow and just lateral to the outer canthus of the left eye.

The evoked electroencephalographic activity was digitised at 3 ms/point for 1000 ms (starting 100 ms before stimuli onset) and averaged on line by Neuropack equipment (MEB5508B—Nihon Kohden, Tokyo, Japan) that also controlled stimulus presentation and automatic artefact rejection on each channel. Latencies were determined following Iragui et al. (1993): N1 was identified as the most negative point between 50 and 150 ms poststimulus; P2 as the maximum positive point between 125 and 230 ms. N1 and P2 latencies were measured in both standard and target tones. N2 was identified as the most negative point between 175 and 400 ms and P3 as the most positive point between 250 and 500 ms. N1 and P2 latencies were measured after both standard and target tones; N2 and P3 latencies were measured after target tones only. Peak-to-peak amplitude measures were N1–P2, P2–N2, N2–P3.

2.4.2. Electroencephalogram

Subjects were instructed to relax and close their eyes for a couple of minutes. Twelve EEG epochs (1024 points, 250 samples/s intervals) were recorded at Pz, which were referenced to the right earlobe (A2) and bandpass filtered from 0.5 to 100 Hz. The power spectrum was estimated by the Welsh method of averaging modified periodograms using a Hanning window (Oppenheim and Schafer, 1989). To achieve extra smoothing, there was an overlap of 256 samples between adjacent data segments. The 12 power spectra were averaged resulting in the final power spectrum of the total EEG recording. Five specific frequency bands were chosen for analysis: 0.5–3.5 Hz (delta), 4.0–7.5 Hz (theta), 8–12 Hz (alpha), 12.5–21 Hz (beta 1), and 21.5–30 Hz (beta 2) (see van Leeuwen et al., 1995).

2.4.3. Psychomotor/attention tests

2.4.3.1. Cancellation test. CT (Bond and Lader, 1972) is a paper-and-pencil measure of focused attention at speed, scored for the time taken to cross out the number 4 that appeared at a frequency of 40 in 400 random digits. One second was added to the score for each error of omission committed.

2.4.3.2. Digit–symbol substitution test. DSST (Wechsler, 1955) is a paper-and-pencil test involving coding skills. Subjects were required to substitute digits for symbols for 90 s. Scores were the total number of correct substitutions.

2.4.4. Subjective ratings

Visual Analogue Scales (VAS) (Bond and Lader, 1974; Gorenstein and Gentil, 1983) were used as the subjective measure. Rating is performed by marking a point in a line of 100 mm that represented the full range of a particular dimension. Subjects (SUB; Bond and Lader, 1974) and the experimenter (EXP; Gorenstein and Gentil, 1983) rated the participant's level of arousal (from "very alert" to "very sleepy") and attention (distracted–attentive). Scales were analysed individually.

2.4.5. Statistical analysis

Change scores (posttreatment minus pretreatment) were used in the statistical analysis. One-way ANOVAs with treatments as factor, followed by Tukey *t* tests, were used

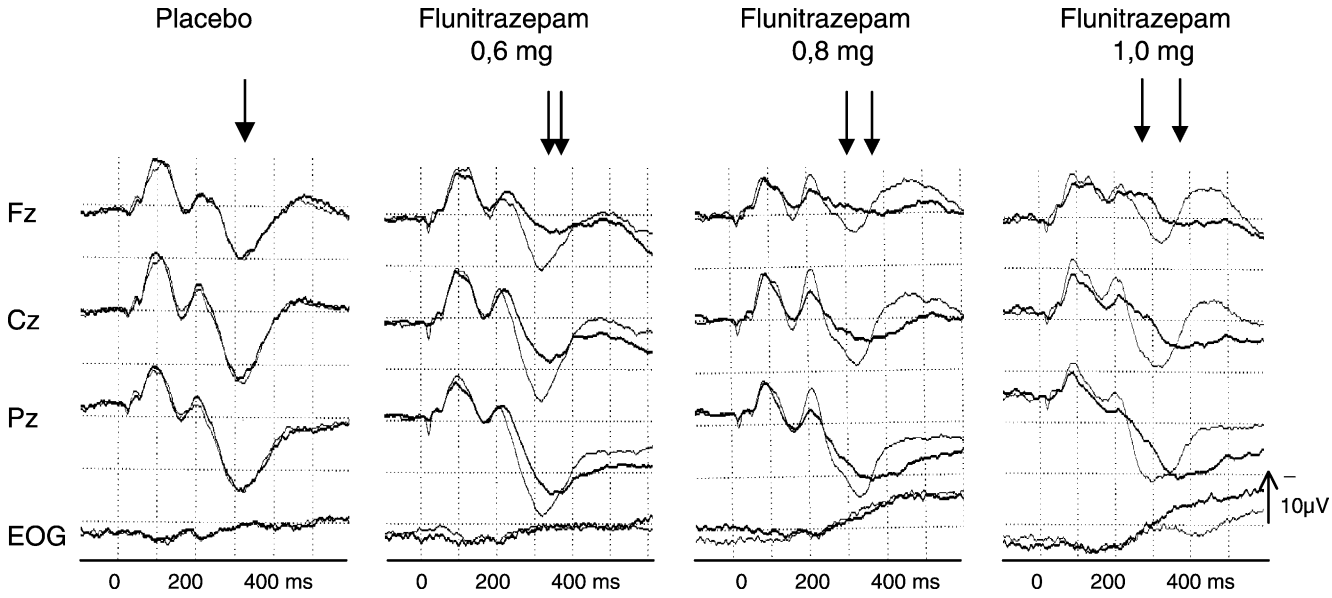

Fig. 1. Treatment grand average ERPs evoked by targets. Pretreatment (thin lines) and posttreatment (thick lines) curves are superimposed. Recordings began 100 ms before stimulus. Calibration: 100 ms/horizontal division and 20 = B5V/vertical division.

to analyse data from the CT, DSST, and subjective measures. ERPs and EEGs were analysed through nonparametric tests (Kruskal–Wallis H test followed by Mann–Whitney U tests). Spearman linear correlations were calculated for all tasks that showed significant drug effects. The significance level of 5% was adopted for all statistical comparisons except for correlations, for which Bonferroni corrections were employed. For exploratory purposes, the principal component analysis (PCA) was performed (Johnson and Wichern, 1998) with a special focus on the first two principal components. Four variables were preselected for the PCA since the size of the sample was insufficient for an analysis with all of the variables (Bryand and Yarnold, 1994). The criterion for choosing three of the variables (P3 latency, DSST, and alertness rated by the experimenter) was that they showed treatment effects with all doses of FNZ. The beta 1 frequency band was also selected as an important variable because it showed treatment effects with the two highest doses and its effect is largely associated to BZDs.

3. Results

No differences among groups were observed for age, body mass index, and trait anxiety. One subject from the FNZ 1.0 group was replaced because he could not be kept awake during posttreatment session. Measures not mentioned below did not show treatment effects.

3.1. Event-related potentials

Fig. 1 (grand averages of ERPs of 12 subjects) is shown as an illustration; no statistical analysis based on the grand average was carried out.

3.1.1. Latencies

As shown in Table 1, change scores of P3 latencies showed differences among groups at Fz ($H = 23.41$, $P < .00001$, $df = 3$), Cz ($H = 23.55$, $P < .00001$, $df = 3$), and Pz ($H = 23.49$, $P < .00001$, $df = 3$), for which PLAC had shorter latencies than those of all three doses of FNZ ($Ps < .004$). For N2 at Fz ($H = 8.95$, $P < .03$, $df = 3$) and N1 at Fz ($H = 13.66$, $P < .004$, $df = 3$) and at Cz ($H = 9.60$, $P < .03$, $df = 3$), relative to rare stimuli, change scores for PLAC's latency were shorter than FNZ 1.0's ($Ps < .02$).

Table 1
Change scores (posttreatment minus pretreatment; mean ± S.E.) of latencies in ERP per treatment (FNZ and PLAC) according to electrode location (LOC), type of stimuli (STIM) and potential (POT)

LOC	STIM	POT	FNZ (1.0 mg)	FNZ (0.8 mg)	FNZ (0.6 mg)	PLAC
Fz	Rare	N1	22 ± 19	8 ± 22	9 ± 18	− 14 ± 28
		P2	11 ± 32	9 ± 21	1 ± 15	− 3 ± 15
		N2	51 ± 56	49 ± 55	29 ± 40	5 ± 20
		P3	82 ± 63	69 ± 46	46 ± 38	0 ± 7
	Frequent	N1	1 ± 27	2 ± 31	0 ± 18	0 ± 15
		P2	− 2 ± 16	1 ± 52	− 8 ± 21	5 ± 23
Cz	Rare	N1	17 ± 20	1 ± 14	4 ± 22	− 9 ± 12
		P2	19 ± 22	10 ± 19	4 ± 19	5 ± 13
		N2	56 ± 61	37 ± 53	28 ± 27	7.5 ± 15
		P3	83 ± 61	67 ± 45	42 ± 38	− 1 ± 7
	Frequent	N1	− 7 ± 17	− 14 ± 21	− 5 ± 15	1 ± 23
		P2	− 4 ± 21	− 11 ± 27	− 6 ± 21	9 ± 19
Pz	Rare	N1	16 ± 23	− 8 ± 12	3 ± 27	− 6 ± 12
		P2	25 ± 26	1 ± 14	7 ± 17	7 ± 16
		N2	57 ± 59	40 ± 52	37 ± 46	2 ± 11
		P3	90 ± 67	58 ± 41	36 ± 50	6 ± 17
	Frequent	N1	− 2 ± 20	− 7 ± 17	− 8 ± 15	6 ± 16
		P2	− 5 ± 25	− 7 ± 31	− 6 ± 21	9 ± 20

N1 and N2 = negative potentials; P2 and P3 = positive potentials.

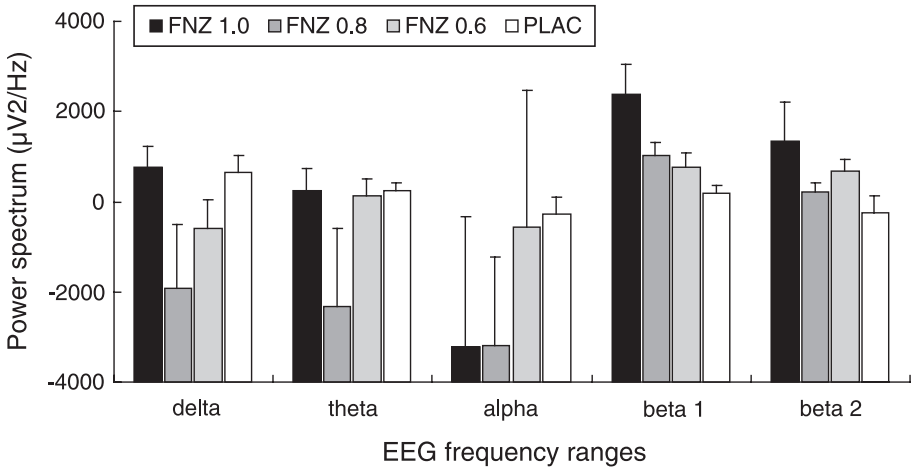

Fig. 2. Difference (posttreatment minus pretreatment) in the EEG power ranges (mean ± S.E.) per group: FNZ (followed by dose in mg) and PLAC.

3.1.2. Amplitudes

The change scores for peak-to-peak amplitudes P2–N2 for rare stimuli at Cz ($H = 16.00$, $P < .002$, $df = 3$) showed treatment effect and were greater for PLAC compared to all active treatments (Ps < .04); FNZ 0.8 was also greater than FNZ 0.6 ($P < .02$). N2–P3 for rare stimuli also showed treatment effect at Pz ($H = 12.59$, $P < .006$, $df = 3$), and were smaller for FNZ 0.8 than all other groups ($P < .05$).

3.2. Electroencephalogram

Only the beta 1 frequency band showed a treatment effect ($H = 10.30$, $P < .02$, $df = 3$), and this was greater for FNZ 1.0 and FNZ 0.8 than for PLAC (Ps < .02; see Fig. 2).

3.3. Psychomotor tasks

Cancellation time [$F(3,41) = 4.21$, $P < .01$] was faster for PLAC than FNZ 0.8 and FNZ 1.0 (Ps < .05). DSST [$F(3,41) = 10.00$, $P < .00004$] was impaired by the three active doses in relation to PLAC (Ps < .0004; see Fig. 3).

3.4. Subjective ratings

Alertness as measured by the subjects [$F(3,43) = 6.63$, $P < .0009$] and the experimenter [$F(3,43) = 6.48$, $P < .001$] differentiated treatments. Subjects in the PLAC group referred less sedation than those who took the two highest doses of FNZ (Ps < .006), the same occurring for the experimenter scale (EXP) for all active treatments (Ps < .05). In terms of attention, subjects [$F(3,43) = 3.49$, $P < .03$] in the FNZ 1.0-mg treatment rated higher distraction than those in the PLAC group ($P < .03$).

3.5. Statistical analysis

Correlations between the selected variables that showed treatment effects are given in Table 2.

The PCA based on the four preselected variables showed a balanced description of the first principal component suggesting that those four variables have an equivalent relevance in terms of the representation of the data. The second component, however, showed a different description, with a strong weight of the P3 latency and alertness as

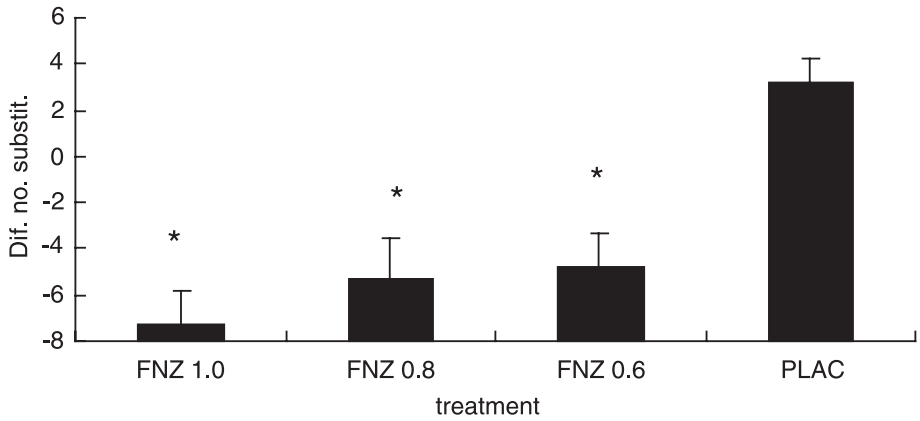

Fig. 3. Difference (posttreatment minus pretreatment) in the number of correct substitutions in the DSST per group: FNZ (followed by dose in mg) and PLAC.

Table 2
Spearman correlation coefficients (r) between selected measures that showed significant treatment effects (after Bonferroni corrections)

	P3 (Pz)	DSST	Beta 1	EXP (alert)
P3 (Pz)	–	−.55	.51	.52
DSST	−.55	–	−.57	−.56
Beta 1	.51	−.55	–	−.16
EXP (alert)	.52	−.56	−.16	–

P3 (Pz) = P3 latency at Pz; DSST = digit–symbol substitution test; Beta 1 = beta 1 EEG frequency band; EXP (alert) = subjective assessment of volunteer's alertness by experimenter.

measured by the experimenter. These two components, for the FNZ 1.0 compared with control, accounted for 76% of the total variance of the data, the first responsible for 48% and the second 28%.

4. Discussion

Changes in ERPs, psychometric tests, and subjective scales in the present study confirm that acute doses of FNZ can modify various cognitive aspects when assessed at a time close to theoretical peak plasma concentration (Pompéia et al., 1996a,b, 2000). The question that is raised is whether these effects are secondary to the sedative–hypnotic action of this BZD or primarily linked to other effects of the drug. Hypnotic effects are classically considered to involve more pronounced depression of the CNS than sedation, and this can typically be achieved by increasing the dose of sedative–hypnotic drugs (Tobler et al., 2002), that is, sedative and hypnotic effects act in a continuum of effects of the same nature. The time that the drug was given, i.e., in the morning, likely attenuates its sedative effects. However, to perform all the tests, it was undesirable that the volunteers fall asleep during the examination. Indeed, one subject from the FNZ 1.0-mg treatment, who could not be kept awake during the posttreatment session, was replaced. Moreover, lack of time-of-day effect on P300 has been reported (Polich, 1994).

4.1. Electroencephalogram

No increase in the powers of theta or delta EEG activities nor reduction of alpha power was detected. This lack of EEG effects corroborates some BZD studies using similar quantitative EEG methods (Urata et al., 1996; Bauer et al., 1997) but contradicts others (Matjek, 1982; Engelhardt et al., 1992). It could be claimed that the oral dose of triazolam (0.125 mg) employed by Urata et al. (1996) and Hayakawa et al. (1999) and the dose of midazolam (10 μg/kg iv) used by Bauer et al. (1997) were too small to elicit somnolence and consequently EEG changes. However, the FNZ dosage used in this study cannot be considered too low to cause sedation, since 1.0 mg of FNZ is the therapeutic dose of this drug, which is marketed as a hypnotic. Nevertheless, it led to subjective sedation, as rated both by the subjects and by the experimenter, but produced no changes in EEG indicative of sedation/ sleepiness.

An alternative interpretation for the absence of somnolence-related EEG changes concerns the methodology employed. It is possible that the duration of the EEG recording may account for the differences reported. In our and other studies (Urata et al., 1996; Bauer et al., 1997), EEG was recorded for less than 3 min, while other research groups that have shown alpha, delta, and theta changes recorded for longer periods ranging from 3 to 14 min (Matejek, 1982; Saletu et al., 1986). Furthermore, short acquisition periods make it less likely that subjects will fall asleep, thus decreasing the possibility of changes taking place in alpha, theta, and delta, which are indicators of progressive drowsiness (Niedermeyer, 1999). Although it is a well-known fact that the all-night spectral analysis of EEG after BZD is different from nonpharmacologically induced sleep, involving a reduction in low-frequency activity (Borbély, 1995), we did not find differences in alpha either. In fact, our subjects, as seen in the EEG, did not fall asleep, either in Stage 1 or somnolence, since there was no reduction in the power of alpha activity, which is an indicator of somnolence.

The utility of beta EEG activity in detecting BZD effects is described at length in the literature, and enhancement of this frequency band is encountered in both sleep and waking states (Veselis and Reinsel, 1992; Tobler et al., 2002). The effect was also observed in this study, with changes in beta 1 after the two largest doses of FNZ. Although some authors describe a close relationship between increased beta and BZD sedation (Veselis and Reinsel, 1992), others suggest that the sedative and beta EEG effects of BZD site ligands are mediated by different molecular and neural substrates (Tobler et al., 2002). Thus, increased beta in EEG can be considered an intrinsic BZD effect rather than a sedation-associated linked effect.

This study found that EEG recording, as an objective method, did not show sedative effects. All the other measures showed effects that may have been due partly to sedation and partly to intrinsic effects of BZD.

4.2. ERP latencies

The dose-dependent increase in P3 latency is in agreement with various studies using other BZDs such as diazepam (Ray et al., 1992), triazolam (Pang and Fowler, 1994; Urata et al., 1996; Hayakawa et al., 1999), clonazepam (Rockstroh et al., 1991), and lorazepam (Pooviboonsuk et al., 1996).

P3 is considered to reflect cognitive processing (Kutas and Dale, 1997) and attention (Coull, 1998), and its latency considered to represent the relative duration of evaluation/ classification during multiprocessing of the stimuli (Coles et al., 1985). It therefore seems that FNZ impairs these faculties, even at doses too low (0.6 mg) to lead to

subject-rated sedation and in the absence of changes suggestive of sedation in the spectral analysis of the EEG.

Increases in N1 latency are considered indicative of decreased attention (Coull, 1998) and of changes in early feature analysis (Iragui et al., 1993). Only the highest dose of FNZ caused impairment of these functions as confirmed by subject-rated attentiveness, whereas the smaller doses (0.6 and 0.8 mg) appeared to be insufficient to affect either measure. However, the DSST, as mentioned above, which measures sustained attention (Lezak, 1995), showed impairment with all doses. CTs are also a measure of the ability to focus attention quickly (Bond and Lader, 1972) and showed impairment for the two highest doses.

As in the case of N1, increased N2 latency was also observed only for the higher dose. This measure may reflect higher cognitive processing such as stimulus categorisation during the decision-making process itself (Iragui et al., 1993) and/or the duration of the processes of perceptual analysis (Timsit-Berthier and Gerono, 1998) and stimulus discrimination (Iragui et al., 1993). BZD-induced decreases of N1, P2, and N2 have been considered as a generalised decrease of neuronal activity related to sedation (Allen et al., 1991). Modification of the amplitude and/or latency of N2 have also been ascribed to the sedative effect of BZD (Rockstroh et al., 1991). A different conclusion was drawn by Pooviboonsuk et al. (1996), who claimed that changes in the amplitude of N1, but not in N1 latency could reflect the sedative effects of these drugs, and by Abduljawad et al. (2001), who showed that the attenuation of the N1/P2 complex after BZD ingestion cannot be simply equated with sedation.

4.3. ERP amplitudes

Overall, ERP amplitude decreases revealed FNZ effects similar to those observed for ERP latencies. Our results for ERP amplitude lend support to BZD impairment of (a) attention, through changes in peak to peak amplitudes including N1 and P2 (Picton et al., 1974) and through amplitude changes including the P3 component (Coull, 1998), and (b) target discrimination at an early stage of stimulus analyses (Münte et al., 1996) and registration of target features (Iragui et al., 1993), through changes in amplitudes involving N2.

4.4. Task sensitivity

In terms of task sensitivity, the P3 latencies, the experimenter-rated level of alertness, and the DSST were the most sensitive measures because they differentiated all three doses of the BZD from PLAC. These measures were followed by the beta 1 frequency band, CT, and subject-rated alertness, which showed effects for the two highest doses. Finally, the last set of sensitive measures was the subject-rated attentiveness and N1 and N2 latencies, which only differentiated PLAC from FNZ 1.0.

It is of interest that the P3 latency was a more sensitive marker of BZD activity in comparison to changes in the actual amount of beta 1 in the frequency spectrum of the EEG. This may occur because ERPs enable the monitoring of a continuum of electrophysiological changes starting at stimulus presentation and ending after execution of a response (Münte et al., 1996), so that if any of the intervening processes is affected, P3 latency is changed.

4.5. Correlations between measures

DSST performance correlated with both beta 1 frequency band and P3 latency. P3 latencies showed moderate correlations with alertness (EXP). The beta 1 EEG frequency band correlates with DSST and not to alertness (EXP and SUB).

The absence of high linear correlations among EEG, ERP, psychomotor, and subjective measures obtained from various FNZ dosage levels may be seen as contradicting the hypothesis that all these variables reflect the same phenomenon, i.e., sedation and/or sleepiness. However, changes in alertness probably contribute to BZD effects, but are not solely responsible for them. On the other hand, as Laurijssens and Greenblatt (1996) pointed out, although correlations between several different parameters have been reported, this does not necessarily mean that they are measuring the same phenomenon.

The modest correlations observed probably simply indicate that effects become more marked as doses are increased and this would be expected in measures that are sensitive to BZD effects such as the ones used here. In fact, a qualitative inspection of the data for most variables indicates that dose-dependent effects, i.e., significant differences between active treatments, would probably have been observed if a larger sample had been used (see Fig. 3). Hence, it seems more sensible to view the variables the authors used as measures of sedation as also reflecting distinct intrinsic effects of BZD rather than epiphenomena related solely to changes in arousal. The PCA results were compatible with that statement, as two components, with different structures involving four selected major variables, explained the greatest part (76%) of variance of the data.

5. Conclusion

The most sensitive measures used in this study were the P3 latencies of the ERPs (which varied with FNZ dosage), the DSST, and the experimenter-rated levels of alertness. Overall, FNZ impaired subjective and objective measures of attention and psychomotor activities in the absence of classical objective effects associated with sedation/sleepiness. These latter effects were not present or had only a limited influence on the other measures used here. Therefore, these changes are probably due to other intrinsic effects of BZD, rather than closely related to the effects of

sedation/sleepiness. In fact, after comprehensive analyses of ERP, EEG, behavioural measures, and their correlations, we found no evidence suggesting that one single phenomenon was being measured. Further research is needed for more in-depth probing of the finer mechanisms underlying sedation and how they affect behavioural and electrophysiological measures of the CNS function.

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