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Effects of a benzodiazepine on free recall of semantically related words

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Although it is widely known that benzodiazepines impair episodic memory, few studies have investigated their effects upon specific processes involved in free recall. This study evaluated the acute effects of flunitrazepam (1.0 mg; 1.3 mg) and placebo in healthy volunteers on immediate and delayed free recall of word lists considering serial positions as well as semantic relations between words inserted in the middle of the lists (e.g. milk-cheese-butter). Flunitrazepam promoted a global amnestic effect, impairing recall in all serial positions except the last words (recency effect). Primacy and recency effects were preserved as indexed, respectively, by larger recall of the first and last words in relation to adjacent items. Facilitation in recall of semantically related words was not impaired by the drug when compared to recall in adjacent positions, in spite of a dose-dependent diminution of the number of words recalled also in mid-list positions. Flunitrazepam-induced deficits were interpreted as impairment in the formation of new associations between items, or groups of items in the case of related words, and context. Copyright © 2006 John Wiley & Sons, Ltd.

KEY WORDS — benzodiazepines; episodic memory; semantic facilitation; serial position curve

INTRODUCTION

Benzodiazepines (BDZ) lead to anterograde impairment of episodic memory (Buffett-Jerrott and Stewart, 2002; Curran, 1991, 2000), an effect most often assessed by free recall tasks.

Free recall of word lists is a task widely used in evaluating episodic memory which is made up by the conscious storage and retrieval of meaningful events. Word lists are presented in a given context, that is they occur in a specific locale, time and circumstance, constituting thus a definite episode, registered in the episodic memory according to the theoretical framework advanced by Tulving (1972, 1983). The lists are usually built up with common words, that is words that bear a certain meaning shared by the linguistic community. These meaningful words are stored in semantic memory, consisting of cultural facts common to most people in that community.

Semantic network theories assume that concepts (as those expressed by the lexicon) are represented as nodes in a network; highly related words are deemed to be located close together. When a node is activated, activation spreads automatically through the semantic network reaching other nodes more or less easily depending on the distance between them (Collins and Loftus, 1975).

BDZ effects on verbal free recall were often evaluated using lists of non-related words (e.g. Brown *et al*., 1994; Ghoneim *et al*., 1984; Unrug *et al*., 1997), permitting the assessment of processing of isolated items or between items and context. However, mnemonic processes involved in the facilitating effects of semantically associated words in free recall after BDZ ingestion were little investigated and their mechanisms are unknown. Ghoneim and Mewaldt (1975), in a study in which four categorised words of four different categories were randomly distributed in 16-item lists, found that diazepam-treated subjects were able to use categorization effectively to enhance their recall. However, this conclusion is weakened by the fact that, in this experiment, recall after diazepam did not differ from that after placebo. Another study

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(Frith *et al*., 1984) compared recall of lists in which all items were related or unrelated and concluded that the recall impairment produced by diazepam was reduced when the to-be-remembered words were related to one another. However, recall of lists of words in which all items are related are not directly comparable with that of lists in which all items are unrelated, because in lists of related words the category(ies) that defines them can serve as clue(s) for recall, making them cued and not free recall tasks (Tulving and Pearlstone, 1966). Also, in lists of related words the previous knowledge of the category(ies) can influence the generation of items that do not necessarily belong to the list, producing false memories (intrusions of the same category) (Mintzer and Griffiths, 2000; Roediger and McDermott, 2000; Huron *et al*., 2001), or subjects may increase their scores by guessing, without the involvement of episodic memory, a phenomenon rarely observed during recall of lists of unrelated items (Roediger and McDermott, 2000). Hence, the use of lists in which semantically related and unrelated items are included might help in the understanding of BDZ amnestic effects because in this case it is possible to assess the effects of these drugs on the activation of established relations between items. In order to make recall of related and non-related words comparable, we employed in the present study a paradigm used by several groups (Andrade *et al*., 2003; Craik and Levy, 1970; Tulving and Patterson, 1968) that consists of the introduction of semantically related words (e.g. milk-cheese-butter) only in the middle positions of otherwise unrelated words lists. The recall of these related words can then be compared to that of non-related words in other lists in the same input positions, reducing the confounding of category cues and excluding the possible interference of factors involved in primacy and recency effects (Kelley and Nairne, 2001). This manipulation also permits the assessment of the efficiency of item processing, as well as the processing of relations between items and of relations between items and context.

The serial position curve, which reflects free recall of lists of words taking into consideration the order in which stimuli are presented, shows a predominant recall of the first and last words in comparison to the middle ones, characterising primacy and recency effects, respectively (Capitani *et al*., 1992; Deese, 1957; Murdock, 1962).

The primacy effect may derive from a larger amount of rehearsal that one is able to devote to the first items compared to the following items of the list (Atkinson and Shiffrin, 1971; Rundus, 1971). Primacy is attenuated or eliminated by experimental manipulations that render such rehearsal more difficult or impossible (Glenberg *et al*., 1980).

In some circumstances, the recency effect is mediated by short-term memory (STM), reflecting the temporary maintenance of information in working memory phonological loop (Basso *et al*., 1982) and is evidenced when recall occurs immediately after list presentation, being reduced or eliminated with the introduction of a delay or interference task before retrieval (Capitani *et al*., 1992; Glanzer and Cunitz, 1966; Postman and Phillips, 1965). The recency effect also reflects a tendency of first recalling the last items in a sequence and is abolished if subjects are dissuaded from using this strategy (Baddeley, 2000).

The beginning and end of a sequence of items are salient aspects of such a sequence, thus providing reference points or markers on which memory can be based (Henson, 1998). This means that, both primacy and recency can be mediated by markers of temporal and spatial distinction in the context of a list, in addition to rehearsal and short-term memory, respectively, suggesting that intrinsic contextual clues also determine better recall of stimuli in these positions. Therefore, primacy and recency represent the involvement of many factors and different encoding and retrieval strategies (Baddeley, 1997, 2000).

Most of the studies aimed at investigating BDZ effects on free recall of word lists do not analyse input position nor consider memorisation of the first and last items of each list in order to avoid interference of primacy and recency effects. These studies tend to focus on recall of middle items because they reflect the ability to store new information in long-term memory regardless of anchoring and rehearsal processes involved in primacy and recency. In the few studies that did evaluate BDZ effects considering serial position input, the recency effect was shown to be unaffected (Curran *et al*., 1987; Ghoneim and Mewaldt, 1975; Ghoneim *et al*., 1981; Unrug *et al*., 1997), in consonance with the lack of effects of BDZs on STM functioning as evaluated by digit span forward test (Curran *et al*., 1987; Gentil *et al*., 1989; Lister and File, 1984). In contrast, there are reports of reduction of the primacy effect induced by BDZs (Ghoneim *et al*., 1984; Unrug *et al*., 1997). These findings are based on comparative analysis between BDZ- and placebo- or methylphenidate-treated groups (Unrug *et al*., 1997). However, as mentioned, primacy is in fact defined as the enhanced recall of initial items in comparison to the following ones in the same experimental group (Gershberg and Shimamura, 1994; Glenberg *et al*., 1980). Moreover, only recall of one or a few lists were reported in these studies.

Therefore, it is possible that primacy was lower only in comparison to the control condition, and that the memorisation pattern evidenced by the shape of the serial position curve was maintained. This would suggest that the enhancement in recall brought about by the initial list marker or by extra rehearsal is not directly affected by BDZs and that the reduction in recall of the first items vis-à-vis the control levels simply reflects their general amnestic effect.

Therefore, the analysis of several components of the free recall task allows the study of several aspects of mnemonic processes that may clear the nature of BDZ amnestic effects, and conversely, BDZ effects on these components may provide clues about the functioning of the phenomenon of memory.

The purpose of the present study was to investigate the acute effects of a BDZ agonist, flunitrazepam, on free recall of words with or without semantic relations in the middle positions of the lists, as well those effects at the beginning and end of lists, namely the primacy and recency effects. In addition to free recall, other tests sensitive to BDZs effects were performed to ensure that the doses used led to BDZ classical cognitive effects.

MATERIALS AND METHODS

Subjects

Sixty healthy, native Portuguese speaking University students (27 men), aged 23.47 ± 4.14 years (mean $\pm$ SD), with normal body mass index and trait anxiety (Biaggio and Natalício, 1979) served as volunteers. Subjects met the usual exclusion criteria for clinical trials (e.g. pregnancy, allergy, chronic clinical disorder), and did not have a history of drug abuse or heavy alcohol use. They were requested to have a light breakfast on the testing day and were asked to avoid alcohol or any other psychoactive drug from 24 h before the testing day, except caffeine and nicotine. Written informed consent was given by all volunteers prior to their inclusion in this study, which was approved by the local Ethics Committee (UNIFESP).

Treatment. A double-blind, between-subjects design was used to compare the effects of flunitrazepam 1.0 mg (Flu1.0; $n = 20$), 1.3 mg (Flu1.3; $n = 20$) and placebo ($n = 20$), administered orally. Subjects were randomly allocated to each one of the treatment groups, apart from balancing by sex.

Test battery

Control tests. Cancellation Test (CT; Bond and Lader, 1974): a measure of focused attention at speed, scored for the time taken to cross out a digit which appeared at a frequency of 40 in 400 random digits. Time was corrected for number of errors (for each error 1 s was added to the total score), according to the procedure established by Pompéia et al. (1996).

Digit-Symbol Substitution Test (DSST; Wechsler, 1955): a subtest of the Wechsler Adult Intelligence Scale involving coding skills, scored for the number of substitutions correctly performed in 90 s.

Subjective ratings (VAS; Bond and Lader, 1974): composed of 12 visual analogue scales which were grouped in 3 factors (Guimarães, 1998): mental sedation, physical sedation and anxiety.

Prose Recall: immediate and delayed (about 30 min) recall of a story with 14 ''idea items'' presented orally (Correa and Gorenstein, 1988a,b). Scores were based on number and precision of items recalled (1 = perfect recall; 0.5 = partial recall or synonym). Evaluated post-treatment only.

Word lists. Twenty lists including 15 common and concrete Portuguese nouns were employed. Ten lists included semantically unrelated words, and the other 10 lists were made up of unrelated words except semantically related items inserted in the middle serial positions (positions 7, 8 and 9) (e.g. milk-cheese-butter). The words were presented sequentially on a computer screen for 250 ms (inter-item interval of 4 s). Subjects were instructed to process each word semantically (imaging).

Procedure

Subjects arrived at the laboratory at 9:00 am at which time they carried out baseline (pre-treatment) measures of CT, DSST and VAS, followed by treatment. Testing began 1.5 h after substance intake (at theoretical peak plasma concentration of the drug: Jochemsen *et al*., 1983). At the beginning of the post-treatment session, subjects completed the VAS, and heard the story, which they immediately had to recall. The 20 word-lists were then presented. At the end of each list, subjects were required to freely recall all the words seen, in any order. Half the lists contained semantically related words in the 3 middle positions (7-8-9) and in half of each type of lists free recall was delayed by a distracting task (DSST or CT), yielding 4 types of lists: related with and without delayed recall, and unrelated, with or without delayed recall. Presentation of the type of list was random and counterbalanced between groups. Thus, CT and DSST were not applied in a fixed order. However, the first CT and DSST conducted post-treatment were carried out

within 10 min of theoretical peak plasma concentration of flunitrazepam and the last, within 10 min of the end of the experiment. After the 10th list, subjects were required to recall the story (delayed prose recall). After recall of the last list, subjects were required to fill in a last VAS.

Statistical analysis

Groups were initially compared in terms of age, body mass index and trait anxiety using one-way analysis of variance (ANOVA) with group as factor, followed by *post hoc* Tukey *t*-test for comparisons of means. Differences between groups in VAS, CT and DSST were investigated using analysis of covariance (ANCOVA) with group and time (first and last measures post-treatment) as factors and considering pre-treatment scores as the covariant. ANOVAs followed by Tukey *t*-tests were also used in the free recall analysis and will be described along with the results. For analysis purposes, recall in the 15 serial positions were grouped into threes; the primacy portion reflected the number of words recalled in the first 3 positions (positions 1-2-3), the recency portion, recall of the last three words (13-14-15), and the effect of semantic relation was reflected by the recall of words 7-8-9 Primacy, recency and effects of semantic relations were considered by comparisons of recall in these positions in relation to that in adjacent positions (Dunlosky and Matvey, 2001), also grouped into threes (4-5-6 and 10-11-12). The significance level was set at 5%. Effects not mentioned below were not significant.

RESULTS

Six subjects (Flu1.0 = 2; Flu1.3 = 4) were excluded due to excessive somnolence. The analysis were, therefore, conducted for: Flu1.0 = 18; Flu1.3 = 16; placebo = 20.

No differences among groups were observed in age, body mass index or trait anxiety.

Cancellation test and digit-symbol substitution test

On the CT and DSST there were group ($F_{2,50} = 43.87$, $p < 0.0001$; $F_{2,49} = 13.68$, $p < 0.0002$) and time effects ($F_{1,51} = 61.34$, $p < 0.0001$; $F_{1,50} = 12{,}16$, $p < 0.001$), but no interaction. Groups Flu1.0 and Flu1.3 were impaired in relation to placebo ($p < 0.01$); dose-dependent effects were only observed on CT ($p < 0.002$). Performance was better at the last post-treatment measure than at the first ($p < 0.001$). On CT, the errors were from omission; commission errors were not found.

Subjective ratings

On mental and physical sedation, significant differences were observed between groups ($F_{2,50} = 10.01$, $p < 0.0002$; $F_{2,50} = 6.85$, $p < 0.002$) and time ($F_{1,51} = 7.97$, $p < 0.007$; $F_{1.51} = 23.78$, $p < 0.001$). Subjects in groups Flu1.0 and Flu1.3 reported more sedation than placebo ($p < 0.004$) and higher sedation was reported in the last than the in the first measure post-treatment ($p < 0.01$). Dose-dependent effects were only observed for physical sedation ($p < 0.01$). In terms of anxiety there was only an effect of time ($F_{1.51} = 3.99$, $p = 0.05$), more symptoms being reported at the end of the experiment ($p = 0.05$).

Prose recall

Differences between groups were found on immediate ($F_{2,51} = 7.82$, $p < 0.001$) and delayed ($F_{2,51} = 5.44$, $p < 0.01$) recall of prose. In both cases, Flu1.0 and Flu1.3 groups were impaired in relation to placebo ($p < 0.03$), but there were no differences between doses. The forgetting rate (i.e. difference in number of items after delayed and immediate recall) for both active doses was equivalent to placebo's.

Word free recall task

A four-way ANOVA with repeated measures was conducted: group (placebo, Flu1.0, Flu1.3) versus delay (immediate or delayed) versus relations (unrelated or related) versus serial position grouped into threes. Groups differed in the total number of words recalled ($F_{1,51} = 101.67$, $p < 0.0001$) (mean ± SD: placebo = 145.9 ± 44.6; Flu1.0 = 108.5 ± 28.6; Flu1.3 = 83.2 ± 23.2), which was lower after Flu1.0 and Flu1.3 than after placebo ($p < 0.001$). Dose-dependent effects were also observed ($p < 0.02$).

A significant interaction occurred between group, delay and input position ($F_{8,204} = 2.92$, $p < 0.004$). All groups showed primacy effect when recall in grouped positions 1-2-3 was compared to that in adjacent positions (4-5-6), both after immediate and delayed recall in lists with related and unrelated words ($p < 0.001$). Also, recency effects (higher recall in positions 13-14-15 than in positions 10-11-12) were observed after immediate recall in all groups ($p < 0.007$) but the distracting task abolished this effect for all treatments ($p < 0.0001$). When the recall test was immediately after presentation, Flu1.3 group

recalled fewer words than placebo in all grouped serial positions ($p < 0.0002$), except the last three words. Thus, albeit preservation of primacy effects (superior recall of first words in relation to the following items), Flu1.3 impaired recall in all serial positions including the first words in relation to placebo, except in the recency portion. Considering delayed recall, the Flu1.3 group recalled less than the placebo-treated subjects in all grouped positions including the end of the list ($p < 0.04$), and the Flu1.0 group recalled less than those who took placebo in positions 1-2-3 and 13-14-15 ($p < 0.005$). Differences between active doses were observed in positions 4-5-6 and 7-8-9 ($p < 0.04$) in lists with immediate recall and in positions 7-8-9 and 10-11-12 when recall was delayed ($p < 0.02$) (figure 1). Hence, Flu1.3 impaired the recall of words in position 7-8-9, which included both semantically related and unrelated words, in comparison to both placebo and Flu1.0 in the immediate or delayed conditions (see secondary analysis below for details of the effect of Flu1.3 on semantic facilitation).

The four-way ANOVA mentioned above revealed also an interaction between word relatedness and serial position ($F_{4,204} = 84.03$, $p < 0.0001$) showing that regardless of group, recall in positions 7-8-9 in lists containing related words was increased when compared to lists containing only unrelated words, as well as increased compared to adjacent serial position (4-5-6) in related lists only ($p < 0.001$). This finding indicates that semantic facilitation occurred for all groups in the same manner. Thus, flunitrazepam did not prevent the enhancement in recall produced by semantic relationship between mid-list words. Recall in positions 1-2-3, 10-11-12 and 13-14-15 was not influenced by semantic relatedness, while that in position 4-5-6 was lower in lists with semantic relations than in all-unrelated words ($p < 0.004$). This latter effect was not influenced by the drug, as indicated by the lack of interaction between position, word relatedness and treatment factors.

Because the relatedness effect did not interact with group and delay, the comparison of recall in positions 7-8-9 and adjacent positions would do little to reveal the effects of flunitrazepam on semantic facilitation. In order to further investigate these effects a secondary set of analysis was conducted to determine whether

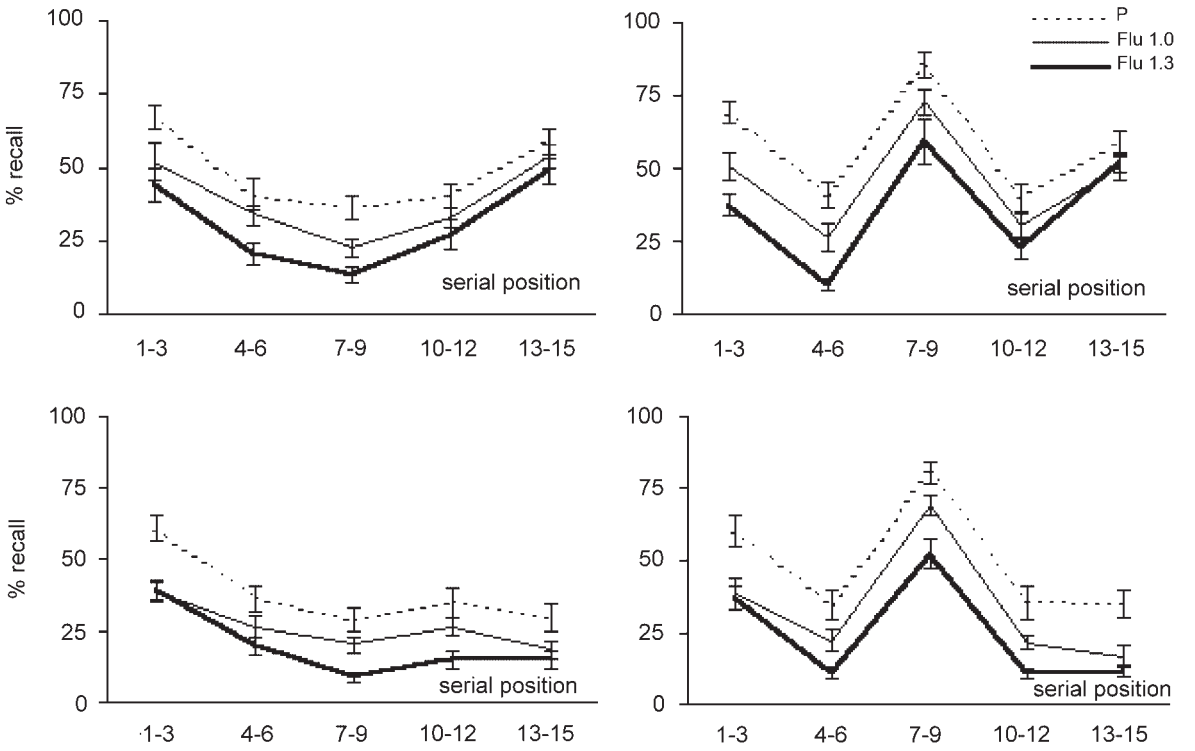

Figure 1. Serial position curves of unrelated words lists (left panel) and lists with semantically related words inserted in the middle (right panel). Immediate (upper panel) and delayed recall (bottom panel). Treatments: placebo (P), flunitrazepam 1.0 mg (Flu1.0) and flunitrazepam 1.3 mg (Flu1.3); (mean ± SE)

groups differed in recall of words in positions 7-8-9. For a first analysis, a one-way ANOVA was used to compare groups regarding the recall increment of related items in positions 7-8-9 (i.e. difference of recall between the lists that contained related and unrelated items). Since there was no interaction between the type of list and time of recall, immediate or delayed, in the previous four-way ANOVA, the present analysis considered conjointly lists of immediate and delayed recall. This analysis showed that recall increment was proportionally equal for all groups ($F_{2,51} = 0.45$, $p = 0.64$).

Following this analysis, groups were compared in terms of the number of related and unrelated lists in which no items were remembered. This analysis had group and semantic relatedness as factors and was conducted separately from cases in which one or more of the three words in serial positions 7-8-9 were freely recalled, because, when no words are generated, subjects may have no cues that lead them to recall other words. This analysis showed list type ($F_{1,51} = 103.57$, $p < 0.0001$) and group ($F_{2,51} = 29.13$, $p < 0.0001$) effects. Semantic relations decreased the probability of zero words being recalled ($p < 0.0002$), irrespective of group. When both list types were considered jointly (group effect), zero recalls after Flu1.3 and Flu1.0 occurred more often than after placebo ($p < 0.003$) and more after Flu1.3 than Flu1.0 ($p < 0.001$) (mean $\pm$ SD: placebo = 31.5 ± 20.1; Flu1.0 = 48.9 ± 16.8; Flu1.3 = 68.1 ± 13.3 for unrelated lists; P = 8.5 ± 11.4; Flu1.0 = 18.9 ± 12.8; Flu1.3 = 32.5 ± 19.1 for related lists). The lack of interaction indicated a generalised dose dependent amnesic effect of flunitrazepam.

Data was further investigated by analysing group differences independently of this amnesic effect. This analysis considered possible effects of flunitrazepam on altering subjects' capacity of using generated words as cues to remember the others words that formed the set of three words in positions 7-8-9. This could only be conducted if groups were equated in terms of cases in which at least one of the three words was generated, so data was corrected to the percentage of lists in which one, two or three middle words were recalled. Factors were group, list type (related or unrelated) and percentage in which three, two, or only one word were generated (what we called ''clustering effect''). The three factors interacted ($F_{4,102} = 5.83$, $p < 0.0003$): for all groups, there were more recalls of a single than two or three middle words in the unrelated lists ($p < 0.006$). Single recalled words also occurred more often in unrelated lists than in related lists ($p < 0.007$). This latter finding suggests that remembering at least one word of the related set increased the probability of the others being recalled, but this did not occur when this word was not related to the other two. An inverse pattern was observed in the conditions where recall of two or three words took place. Recall of two or three words was higher in related than unrelated lists ($p < 0.05$), except in the placebo group, which did not exhibit differences regarding the type of list as to recall of two words, suggesting that the placebo group was capable of doing inter-item associations more often, even when words were not semantically associated. Conversely, Flu1.3 led to more single word recalls than placebo in unrelated lists ($p < 0.04$), demonstrating that the BDZ reduced the ability of making inter-item associations between unrelated items. All groups displayed more recalls of two words in lists of related items than lists of unrelated items ($p < 0.006$). Placebo and Flu1.0 groups recalled three more than two words in lists of related items ($p < 0.006$) (Table 1), but this difference was not seen in the Flu1.3, despite the fact that this dose led to higher recall of two and three related than unrelated words.

In order to verify if flunitrazepam sedative effects affected the memory scores (free and prose recall tasks), ANCOVAs were performed employing as covariates mental and physical sedation scores of the VAS scale (post- minus pre-treatment). The significant differences remained in both free recall and prose recall when mental ($F_{2,50} = 18.96$, $p < 0.001$; $F_{2,50} = 7{,}45$, $p < 0.01$, respectively) as well physical sedation ($F_{2,50} = 19.39$, $p < 0.001$; $F_{2,50} = 6.84$, $p < 0.01$, respectively) were covaried, showing that flunitrazepam amnesic effects were significant and independent of sedation.

Many interlist intrusions (defined as recall of words seen in previous lists) were found. Firstly, we conducted a two-way ANOVA (group vs. relatedness) to compare the probability of related and unrelated

Table 1. Percentage (SD) of words recalled in the middle serial positions (7-8-9) according to clustering adjusted for proportion of lists recalled per subject

	Clustering	*p*	Flu1.0	Flu1.3
Unrelated words	1	65.2 (23.7)	79.3 (17.6)	92.5 (17.3)
	2	29.4 (19.7)	16.7 (15.3)	7.5 (17.3)
	3	5.3 (12.4)	4.0 (8.0)	0.0 (0.0)
Related words	1	3.7 (5.9)	2.5 (4.9)	10.7 (12.4)
	2	29.2 (15.9)	34.1 (16.5)	45.3 (22.2)
	3	67.1 (20.2)	63.4 (17.2)	44.0 (22.5)

Treatments: placebo (P), flunitrazepam 1.0 mg (Flu1.0) and flunitrazepam 1.3 mg (Flu1.3).

words appearing in later lists. Because there were 270 unrelated words in all lists and only 30 related words, proportional recall was used in this analysis. Results showed a group effect ($F_{2,51} = 3.39$, $p = 0.04$): more intrusions were found in the Flu1.3 group than in the placebo and Flu1.0 treatments ($p = 0.05$). In order to compare these errors directly between groups that differed in overall recall we calculated the proportion of related and unrelated words remembered by each subject and then determined the proportion of these related and unrelated words that appeared as intrusions in later lists. A two-way ANOVA (group vs. relatedness) was then applied to this data and again a group effect was shown ($F_{2,51} = 6.22$, $p < 0.003$), having the Flu1.3 group made more inter-list intrusions errors than the Flu1.0 and placebo groups ($p < 0.005$), and a relatedness effect ($F_{1,51} = 18.17$, $p < 0.0001$), unrelated words having more often constituted intrusions than related words ($p < 0.0002$). No interaction was revealed.

Another analysis explored the effect of flunitrazepam on the proportion of between-lists intrusion errors, considering group, type of list, and immediate or delayed recall, resulting in significant effects for all the three factors (respectively, $F_{2,51} = 4.56$; $F_{1,51} = 12.61$; $F_{1,51} = 12.33$; $p < 0.01$), and no interaction between them. Again, the Flu1.3 group made more intrusions than the other groups ($p < 0.01$). Also, intrusions were more frequent on delayed recall as well as on recall of unrelated lists ($p < 0.001$), independently of drug treatment.

DISCUSSION

Results in the control tasks (CT, DSST, VAS and Prose Recall) revealed lower performance of Flu1.0 and Flu1.3 in comparison to placebo, indicating that the doses used in the present study were high enough to corroborate previous findings of cognitive effects of BDZ (psychomotor, attentional, subjective and amnesic) (Buffett-Jerrott and Stewart, 2002; Curran, 2000; Pompéia *et al*., 2000, 2003). Memory impairment was evident in free recall of words and prose even though normal forgetting rate was observed in recall of prose after flunitrazepam ingestion. In the lists of words flunitrazepam produced a global amnesic effect evidenced both by lower overall free recall as more cases in which zero items were recalled in lists of unrelated words. The former effect was reflected by a clear dose-dependent reduction in the number of words recalled in all input positions, except in the final positions of immediately recalled lists (discussed below) showing that despite the general amnestic effects of flunitrazepam, the shapes of the serial position curves did not change.

Regarding the first positions, our analyses showed that, in spite of a reduction in the absolute number of words in comparison with placebo, the words in these positions continued to be recalled more often than the words in adjoining positions in both flunitrazepam groups, indicating that the primacy effect was proportionally preserved. In previous studies on BDZ effects on serial position curves, it was claimed that this class of drug reduces primacy effect due to impairment of active rehearsal (Unrug *et al*., 1997). However, this does not appear to completely explain BDZ effects on this phenomenon (Curran, 2000; File and Lister, 1982). In our case, reduced rehearsal would tend to lead to a dose-dependent disappearance of primacy effect in relation to the adjoining positions, which did not occur. It is more likely that the preservation of the primacy effect in flunitrazepam-treated groups, though reduced by a general amnestic effect, was due to a preservation of the ability to use inside-list cues that make the first items of the list more distinct.

In the recency portion of lists there was no sign of drug-induced alteration, in agreement with previous reports (Brown *et al*., 1994; Ghoneim *et al*., 1984; Unrug *et al*., 1997), and also in accord with the lack of BDZ effects on short-term memory (Curran *et al*., 1987; Gentil *et al*., 1989). The delay interposed between the end of presentation of a list and recall attempt abolished the recency effect in all groups, supporting the view that, in the conditions of the present study, subjects were not using the lists end-marker to obtain the recency effect, but rather their strategy was indeed that of using short-term memory. After the delay drug-treated subjects exhibited an impaired recall of the last items as compared to placebo.

Regarding the semantically related words, a similar effect was observed: the drug did not impair the facilitation of recall of the related items because regardless of the group, there was more memorisation of these items both in relation to the adjoining positions in lists of related words as in relation to recall of words in the same positions in unrelated lists even though we observed a dose-dependent decrease of the number of words recalled in mid-list positions, which reflected the general amnestic effect of the drug mentioned above. The preservation of the beneficial effect of semantically related words in the flunitrazepam groups shows that the strategy used to

memorise related items, whatever it may be, was not altered by this drug beyond its general amnestic effect.

The salience of the related stimuli in the middle of a series of unrelated ones may account, at least in part, for their enhanced recall since distinctive events, defined as events that are incongruent with the prevailing context, are better remembered than are congruent events because they are more salient (Hunt and Lamb, 2001). Learning a list of words involves the processing of individual words (item-specific processing) and a relational processing by which one seeks common features among them (Engelkamp and Zimmer, 1994; Hunt and Einstein, 1981). As a result of the conjoint processing of both similarities and differences among the discretely presented words, subjects may process the mid-list related words in a distinctive manner, that is these words might be perceived as distinct from the rest of the list (Hunt and Lamb, 2001).

In support of the notion that distinctiveness has a role in determining the facilitation in recall of the related items, an effect sometimes observed in experiments using salient elements to be memorised is that they provoke impoverished memory for the adjoining items (Dunlosky *et al*., 2000), possibly because they succeed in taking limited attentional resources away from these items (but see Schmidt, 1985). This effect was noted in the slight decrease of recall of words in positions 4–6 in all groups.

The further from the beginning and end of lists the smaller the capacity to discriminate individual items that compose that list, rendering them more susceptible to interference from outer-list items (King *et al*., 2002), a fact that may lead to increase in the number of intrusions. The presence of related words in the middle of lists breaks their homogenous character, thus decreasing their potential for interference, and, therefore, to the number of intrusions, as in fact occurred in the present study. In addition, larger retention intervals increase the subjective similarity between elements of a list favouring potential interference and an increase of intrusion errors, as was also verified here in all treatments. The higher dose of flunitrazepam increased the number of intrusions, a result that seems to indicate that the drug enhanced the subjective similarity between stimuli. This effect may provide an ancillary explanation for the general amnesic effect of the drug. It should be noted, however, that the effect of Flu1.3 did not interact with the effect of delay nor the presence of related stimuli in the lists, suggesting that the drug-induced increase of intrusion errors is an independent effect appearing only with larger doses, due perhaps to sedation.

As the related words seemed to be perceived as more distinct and hence better recalled than the remainder one could conclude that flunitrazepam did not affect the capacity of relational-nor of item-specific processing. That is this class of drugs does not seem to impair the apprehension of specific features of each item nor the presence or absence of common features among several items of a list. But the BDZ may have altered the capacity for *formation* of new associations both between the to-be-remembered items and between items (or groups of items, in the case of related words) and situational or extrinsic context. According to Gorissen *et al*. (1998), BDZs impair new associations to be formed, either between two or more items in a list or between items and context. The hippocampus has been suggested as subserving neural mechanisms for binding together the array of features associated with a specific event into an integrated memory trace (Davachi and Wagner, 2002; O'Reilly and Rudy, 2001). A strong support for this line of reasoning lies in the recent report by Sperling *et al*. (2002) that the BDZ lorazepam decreases the extent and magnitude of activation (assessed by functional MRI) within the hippocampal area, as well as in the inferior prefrontal cortex, another cerebral structure thought to be involved in the encoding of relational information, that is in the formation of new associations (e.g. Badgaiyan *et al*., 2002), in a face-naming associative learning task.

Assuming that such a binding system is affected by BDZ, the general amnesic effect on remembering the list can be explained. The mid-list related words are already bound together in the semantic memory system and activation of semantic networks is considered to be an automatic process, as revealed for instance in studies of conceptual priming (see Smith *et al*., 1994), and not altered by BDZ (Bishop and Curran, 1998). But, even these related words were not fully recorded compared to the placebo condition. This latter aspect can be accounted for by the binding impairment between the group formed by the related words and the situational context.

In free recall tasks, contrary to cued recall (most frequently used paradigm to investigate this facilitation; for example Gorissen *et al*., 1998), there are no external cues to guide retrieval, and so subjects have to generate their own cues. Generating at least one word previously seen during the test phase makes it possible to use it as a cue to recall other items. Our results indicate that albeit flunitrazepam's generalised amnestic effect that leads to overall difficulty in recalling items, once one word of the semantic triplet is

remembered the drug does not seem to interfere with recall of the others. In fact, clustering of these related items was observed in placebo as well as in flunitrazepam treated subjects: recall of two and three of the set of related words was larger in all groups in comparison with unrelated words in the same serial positions; the inverse occurred also, recall of only one of the related words was lower than recall of two and three of the related words.

In summary, the method used helped the investigation of several aspects of flunitrazepam mnemonic effects in one single free recall paradigm. The results show that flunitrazepam-induced amnesia did not interfere with the processes involved in primacy effect (the most likely explanation being that distinctive beginning cues continue to be effective in promoting this effect), with recency effect (probably due to preserved short-term memory), with distinctiveness among the several items comprising the lists, nor with activation of semantic networks, a requisite for the facilitation of recall of semantic related words. The general amnesic effect seems to be related to difficulty in forming new associations between extrinsic context and the elements comprised by the event. From the standpoint of memory theorising, the present results evidence distinct processes that act together in the formation of a memory trace. From a clinical standpoint it should be pointed out that semanticaly related events occur often in daily life. As long as semantic facilitation seems to be, at least partially, preserved after BDZ intake, benzodiazepine ingestion may not have such a dramatic effect on memory as is usually indicated by laboratory studies, in which memory tests are usually designed to tap episodic memory for unrelated events or stimuli. However, future studies must determine whether semantic facilitation is altered in a similar manner in patients who take these drugs who differ in age, schooling and medical history from healthy University students.

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