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A reappraisal of acute doses of benzodiazepines as a model of anterograde amnesia

**Running head:** Benzodiazepines as a model of amnesia

**Keywords:** Benzodiazepines, Amnesia, Memory, Retroactive Interference, Accelerated Forgetting.

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Abstract

Objective: Acute administration of benzodiazepines (BZs) is considered a pharmacological model of general organic anterograde amnesias (OAA). We sought to determine which type of amnesia these drugs best model by comparing the effects of diazepam with those reported in amnesiacs regarding: working memory capacity (WMC), susceptibility to retroactive interference (RI), and accelerated forgetting. Methods: In this double-blind, parallel-group design study, 30 undergraduates were randomly allocated to acute oral treatments with 15 mg diazepam or placebo. WMC and story recall were assessed pre- and post-treatment. Story presentation was succeeded by 10 min of RI (spotting differences in pictures) or minimal RI (doing nothing in a darkened room). Delayed story recall was assessed under diazepam and 7 days later in a drug-free session to assess accelerated forgetting. Results: Recall of stories encoded under diazepam, whether reactivated or not was severely impaired (anterograde amnesia). However, diazepam did not impair WMC, increase susceptibility to RI, or accelerate forgetting. Conclusions: diazepam’s amnestic effects mirror those in patients with probable severe medial temporal damage, mostly restricted to initial consolidation and differ from other OAA (Korsakoff syndrome, frontal, transient epileptic, post traumatic amnesia and most progressive amnesias) in terms of WMC, susceptibility to RI and accelerated forgetting.
1. Introduction

Administration of typical benzodiazepines (BZs) leads to cognitive impairment related to the action of these drugs on the gamma-aminobutyric acid (GABA) inhibitory system (see Tano, Molina, Maldonado & Pedreira, 2009; Zhu et al., 2018). Their acute cognitive effects include small or no change on immediate free-recall of episodic information encoded post-treatment, in contrast to profound impairment in delayed free-recall of this type of content (anterograde amnesia), although delayed cued-recall is less affected (Curran, 1991). These BZ effects are similar among different compounds except that their magnitude can vary depending on dose, pharmacokinetic and pharmacodynamics properties of each drug (Giersch, Boucart, Elliott & Vidailhet, 2010; Pompéia, Manzano, Pradella-Hallinan & Bueno, 2007). Additionally, BZs do not impair short-term memory (e.g. digit span, Curran, Gardiner, Java & Allen, 1993; Hennessy, Kirkby & Montgomery, 1991; Rusted, Eaton-Williams & Warburton, 1991), semantic memory (Nogueira, Pompéia, Galduróz & Bueno, 2006) or implicit long-term memories [see Curran, 1991; although the atypical BZ lorazepam seems to affect priming (Giersch et al., 2010; Pompéia, Gorenstein & Curran, 1996)].

Collectively, acute BZ-induced cognitive effects closely match memory profiles observed in many amnesic patients (Aggleton & Brown, 1999), who show disproportional anterograde amnesia regarding episodic content compared to other cognitive abilities (e.g. general intelligence, immediate free-recall of episodic content and other types of long-term and short-term memory) (Aggleton & Brown, 1999; Parkin & Leng, 2014). The similarity between cognitive effects elicited by BZs and those found in patients with amnesia led some researchers to suggest that this safe (Madhusoodanan & Bogunovic, 2004), transient and reversible drug manipulation could be used as a pharmacological model of the cognitive effects found in organic amnesia (Brown, Lewis, Brown, Horn & Bowes, 1982; Lister, 1985; Curran, 1991; Thomas-Antérion, Koenig, Navez & Laurent, 1999). Retrograde amnesia, present in some amnesiacs (Parkin & Leng, 2014), is not
considered a consistent characteristic in this clinical condition (see Kopelman, 2002). Thus, the fact that BZs do not cause retrograde effects (Muhlert et al., 2011) does not invalidate them as a pharmacological model of amnesia.

What is unclear is which type of amnesia BZs are supposed to model. After all, in the last decades it has become clear that patients with amnesia can have many different cognitive profiles (see Baddeley, 1997; Parkin & Leng, 2014). Therefore, it is timely to reconsider the aptness and boundaries of BZs as a pharmacological model of amnesia. This is especially true considering the renewed interest in manipulations of the BZ receptor system as a potential target for clinical applications that involve changes in memory. Examples are recent findings that some BZ inverse agonists exhibit pro-cognitive (Golovko, Ivanov, Golovko, Dolgo-saburov & Zatsepin, 2018), antianxiety and antidepressant effects (Prevot et al., 2019). Additionally, the possibility of editing unwanted episodic memories with the use of drugs seems to hold great promise (Gravitz, 2019; Phelps & Hofmann, 2019).

Following Parkin and Leng’s (2014) taxonomy of memory disorders, we find that BZs seem to mimic cognitive effects observed in many subtypes of organic, non-material-specific amnesias (Parkin & Leng, 2014; for subtypes, see Table 1 which also includes results of the present study), henceforth referred to, collectively, as organic amnesias. However, these conditions differ regarding cognitive effects that were not assessed in detail in prior BZ studies. Hence, in order to better understand the usefulness of these drugs as a pharmacological model of amnesia, we compared findings from the literature on cognitive profiles of organic amnesiacs with cognitive effects of the typical BZ diazepam on: a) cognitive domains that are well established (immediate and delayed recall and recognition); and b) three yet unclear or unstudied effects of BZs on (i) working memory capacity (WMC), (ii) susceptibility to retroactive interference and (iii) accelerated forgetting, as detailed below.
WMC is a construct that measures the ability to engage attention in a controlled manner, allowing appropriate selection and maintenance of goal-relevant information in mind (Cowan, 2008). WMC is thus involved in encoding (Wang & Morris, 2010) and retrieval of information from long-term memory, potentially influencing both immediate and delayed free-recall of episodic memories (Unsworth, 2016; Unsworth & Engle, 2007). We are aware of only one study that tested acute WMC BZs effects. Performance was unaffected (Reder et al., 2006), mirroring lack of change in most of the transient amnesias (Quinette et al., 2006; Tudesco et al., 2010), medial temporal damage amnesias (e.g. Baddeley & Wilson, 2002) and some MCI patients (Hamdan & Bueno, 2005), while impairment is present in many other amnesia types (see Table 1). This lack of effect of BZs on WMC should be replicated, especially because Reder et al. (2006) used an unconventional WMC task.

Apart from these yet to be confirmed lack of BZ effects on WMC, which could affect encoding and retrieval, it is generally believed that episodic memory deficits in amnesias (Kopelman, 2002) and after acute BZs administration (see Curran, 1986) are due to changes in consolidation of episodic information. Specifically, BZs seem to impair only consolidation related to hippocampal and adjacent regions (see Kirwan & Stark, 2004; Olson, Page, Moore, Chatterjee & Verfaellie, 2006; Yonelinas, Hop, Buonocore, Kroll & Baynes, 2001) and which takes place shortly after encoding (e.g. Brown et al., 1982), called initial, synaptic (Dudai, 2004), or cellular consolidation (Wang & Morris, 2010). During this period, memories are labile and susceptible to interference (Squire, 2009; Tano et al., 2009). In contrast, these drugs seem to spare later consolidation processes (Curran, 1991), or systems consolidation (Squire, 2009; Wang & Morris, 2010), and retrieval mechanisms (Curran, 1991). Indeed, these drugs do not impair recall of information learned pre-treatment (do not cause retrograde amnesia) and, in some cases,
even increase these memories (retrograde facilitation: Curran & Birch, 1991; Hinrichs, Ghoneim, & Mewaldt, 1984).

Regarding consolidation, what is still unclear, which we aim to explore, is whether BZs: a) impair initial consolidation of episodic information anterogradely, as mentioned above and whether this changes in the presence or absence of retroactive interference; and b) disrupt further mnemonic processes anterogradely [later consolidation or active forgetting processes (see Hardt, Nader & Nader, 2013), another of the putative mechanism believed to explain deficits in some organic amnesias (Kopelman, 2002)]. Organic amnesiacs of different types differ in all these respects (see Table 1) so determining the effects of BZs in these consolidation steps/processes may help characterize the type of amnesia that BZs best model in cognitive terms.

Regarding interference soon after encountering episodic information (retroactive interference), there is a strong body of data showing that briefly delaying post-acquisition stimulation/interference, by at least 6 minutes of wakeful rest in a quiet, darkened room (minimal retroactive interference: MinRI), dramatically improve delayed recall of episodic content in some amnesics (e.g. Cowan, Beschin & Della Sala, 2004, Cowan, Beschin, Perini & Della Sala, 2005; Dewar, Garcia, Cowan & Della Sala, 2009, Dewar, Della Sala, Beschin & Cowan, 2010; Dewar, Pesallaccia, Cowan, Provinciali & Della Sala, 2012; McGhee, Cowan, Beschin, Mosconi & Della Sala, 2020), although some patients show little or no gains from MinRI (Cowan et al., 2004; Dewar, Pesallaccia, et al., 2012).

Regarding later consolidation processes, forgetting over varying periods of time can be measured, but this has not been investigated in the BZ literature. Accelerated forgetting over longer than 24 hours is only found (see Table 1) in patients presenting anterograde amnesia resulting from Transient Epileptic Amnesia (Elliott, Isaac & Muhlert, 2014), and some MCI patients (Walsh et al., 2014), but not others (Alber, Della Sala & Dewar, 2014; Manes, Cecilia, Calcagno, Cardozo & Hodges, 2008).
Another unstudied BZ effect is whether these drugs interfere with recall of episodic memories learned prior to drug ingestion that have been reactivated, such as when engaging in anterograde delayed recall under the effect of these drugs. Bringing memories back to mind is believed to make them labile and possibly subjected to new consolidation during reencoding opportunities (Scully, Napper & Hupbach, 2017; Squire, 2009) so BZ could also interfere with this process. This is also relevant for those interested in editing previously formed aversive episodic memories by bringing them back to mind, which has had some success using these drugs (see Phelps & Hofmann, 2019; Walsh, Das, Saladin, & Kamboj, 2018), especially in animal studies (Makkar, Zhang & Cranney, 2010). However, this has not been studied in human participants, in whom BZs have only been administered after reminder presentation (reactivation) (e.g. Fernández, Moyano, Radloff, Campos, Allegri, 2017; Rodríguez et al., 2013).

In essence, our study compared the cognitive profile of different types of organic amnesias (reported in the literature) with those of BZs regarding: 1) encoding, retrieval and initial consolidation (including susceptibility to RI and reactivation) of episodic information by healthy participants under the effects of a single oral dose of the typical BZ diazepam, in contrast with performance pre-treatment and/or to placebo-treatment, all assessed on a single day (Day 1); and 2) rates of forgetting after one week (Day 7) of episodic memories encoded under diazepam compared to encoded pre- and placebo-treatment, to assess BZ effects on later consolidation processes.

2. Materials and Methods

2.1. Participants

Participants were physically healthy (18-35 years of age), native Portuguese-speaking volunteers, with body mass index (kg/cm²) between 20-30, who had more than 12 years of schooling. Participants’ depression and anxiety symptoms were within the
normal range (Hospital Anxiety and Depression Scale: Zigmond & Snaith, 1983; adapted for local use by Marcolino et al., 2007; see Supporting information), they did not report any history of learning difficulties (Attention Deficit Hyperactive Disorder, dyslexia, etc.) nor neuropsychiatric or clinical conditions that are related to problems with BZs use cited in the diazepam label (psychosis, drug abuse, glaucoma, allergy to benzodiazepines, pregnancy, lactation, liver, kidney or respiratory problems, hypoalbuminemia, brain damage). They consumed less than 5 units of alcohol per week, did not smoke tobacco or use drugs of abuse such as cannabis and cocaine regularly, and were on no psychoactive medication at the time of the study (excluding hormonal contraceptives with monthly pauses). Women were only tested during menses to standardize their hormonal status and to avoid effects of the active phase of oral contraceptives, which can affect episodic memory (e.g. Mordecai, Rubin & Maki, 2008).

2.2. Test battery

2.2.1. Visual Analogue Mood Scale (VAMS)

Mood was determined to control for subjective effects of BZs such as sedation, which can affect cognitive performance (see Buffett-Jerrott & Stewart, 2002). Mood was assessed by 16 100-mm horizontal ungraded visual-analogue scales, each of which includes opposite mood states at the extremities, such as alert/drowsy (Bond & Lader, 1974), translated for local use by (Guimarães, 1998; Zuardi & Karniol, 1981). Testees were instructed to make a vertical mark along the lines to indicate how they were feeling at that moment. The scores of different scales (number of cm from the extreme left of each line to the vertical mark) were then combined to create scores of mood dimension of “physical sedation”, “mental sedation”, “anxiety”, and “other symptoms” (Guimarães,
1998). This scale was filled in at the beginning of the pre-treatment session and at the beginning and end of the post-treatment session (see Procedure presented next) on the day in which the BZ was administered (Day 1, see Procedure) so as to control for the expected sedative effects of diazepam.

2.2.2. Working memory capacity tasks

2.2.2.1. Counting Span (WMC task)

This is a self-paced, complex span test that includes sequences of screens containing scattered light blue circles, dark blue circles and dark blue squares (based on Engle, Tuholski, Laughlin & Conway, 1999). Participants are asked to count aloud the number of dark blue circles (3 to 9 per screen) on each screen. At the end of the count, participants must repeat the total number counted and immediately pass to the next screen by pressing a key on the keyboard. The procedure is repeated until a screen prompts participants to recall the total number of dark blue circles counted on each of the previous screens, respecting serial position. There were three practice trials before the test commenced. The test itself had three trials each containing 2 to 6 screens. Scores were the 'all-or-nothing load score' (ANL; Conway et al., 2005). This task was carried out at the beginning of the pre-treatment and post-treatment sessions on the day that BZ was administered (Day 1, see Procedure).

2.2.2.2. Running Memory Span (WMC task)

This task is based on Cowan, Elliot, et al. (2005; variant two of the task). Participants heard lists of 10 to 20 spoken digits (from 1 to 9) presented at a rapid pace. The order of digits was pseudorandom: a digit was repeated only after a moving window
of 7 consecutive different digits. To adapt the test to the Portuguese language, in which digits have more syllables/phonemes than in English, pilot studies showed that the duration of each digit should be raised from 250 to 350 ms to allow stimuli to be distinguishable (except for the digit “one”, which was presented for 250 ms because it only contains one phoneme in Portuguese; presenting it for 350 ms led to a phonological distortion). There were 10 practice trials, one for each list length, followed by two test trials for each list length. Participants were instructed to wait until the end of the list, when a screen prompted them to recall as many digits as possible from the end of the list, in the forward order and the same serial position as presented. They were asked to report if they had forgotten any digit within the recalled sequence and point out in which specific positions. Each recalled digit in the correct serial position gained one point. Scores were the mean number of points in all trials. This task was carried out at the same times as the Counting Span task.

### 2.2.3 Prose recall

Six comparable, 25-item prose passages (stories: Bolognani et al., 2015; Martins Bolognani, Pompéia, Bueno & Miranda, 2015) based on the Logical Memory Test (Wechsler, 1987) were used to assess immediate and delayed episodic memory. The stories, recorded in a male voice, were presented only on Day 1. Participants were required to freely-recall them as similarly as possible to the heard content, orally, as soon as the recording ended (immediate free-recall). Immediate free-recall was followed by a 15 min period of manipulation of RI (see below). Delayed recall took place post-treatment on Day 1 and 7 days later (Day 7; see Procedure). Oral recall of the stories was taped for subsequent assessment by the experimenter (first author) and an independent judge, who
was blind to the treatments. Scoring followed that proposed in the Logical Memory Test (Wechsler, 1987) in which order of recalled items is not taken into consideration. Points were awarded for verbatim recall and also a list of synonyms and approximations proposed by a panel of judges (Bolognani et al., 2015). Statistical analysis were carried out separately for data of both raters, but as results of the statistical models were the same, the Results section includes scores determined only by the first author.

For delayed-recall only, when participants failed to freely recall at least one item of each story that had been presented, cues were provided, but only after some insistence by the experimenter that participants try harder to remember. This was done to dissociate impaired storage/consolidation (in which case cues would be unhelpful) from impaired retrieval, which might be affected by BZ-induced lowering of arousal, in which case cues could help bring consolidated information to mind. The first cue was the occupation of the main character. Because Portuguese includes a noun class grammatical gender system, occupation also supplied information on the character’s sex. The second cue was an item that referred to the main problem in the story (e.g. “a story related to a robbery”, as is the case for the Anna Thompson prose passage of the Logical Memory test: Wechsler, 1987). No further cues were offered if participant failed to remember items after the second cue. After cues, only idea units recalled that were different from the cues themselves were scored. Retention (or savings metrics) was calculated by dividing scores in the delayed (free- or cued-) recall by those in immediate free-recall of the same stories. These scores will be detailed in the Results section.

2.2.3.1. Retroactive interference manipulation
Immediately after immediate free-recall of stories, participants were subjected to one of two interference conditions that lasted 15 min, based on Alber et al. (2014):

a. Immediate retroactive interference condition (RI): participants carried out a spot-the-difference task for 15 min, which consisted of finding differences in pairs of pictures (images of real life scenes, such as landscapes, animals, buildings) which remained side by side onscreen for 28 s and were identical except for two subtle differences. Participants were instructed to find and indicate with the mouse the location of differences between each pair of pictures for 23 of the 28 s. Talking was not allowed during this task. After the 23 s exposure, circles around the two differences were shown for 5 s to indicate where they had been. An additional difference was included in one of the images, so that the instruction was to find “up to three differences”. This was done to ensure that participants would continue doing the task upon finding the second difference. The number of detected differences was measured.

b. Immediate minimal retroactive interference condition (MinRI): in this condition, participants were asked to keep to their chairs, relax but not sleep, not use mobile phone or read and do nothing for 10 min in a darkened room. During this time, they were continuously supervised from an adjacent room. In the rare cases in which participants did not comply with the instructions, the experimenter would interfere to remind them. Next, they carried out the spot-the-difference task (described above) for 5 min, which ensured that the cognitive demands that preceded the next task were equated in both MinRI and RI conditions (see Dewar, Alber, Butler, Cowan, Della Sala, 2012).

2.3. Drug-treatment
Oral treatments were formulated in identical capsules: placebo (talcum) or diazepam, a long acting BZ (see Mandelli, Tognoni, Garattini, 1978; Riss, Cloyd, Gates & Collins, 2008) in a dose (15 mg) which elicits severe transient anterograde amnesia (Gorissen, Curran, & Eling, 1998; Gorissen, Eling, Van Luijtelaar, & Coenen, 1995; Unrug-Neervoort, Van Luijtelaar, & Coenen, 1992).

2.4. Procedure

The Ethics Committee of the institution where the experiment was conducted (UNIFESP) approved the study protocol. All participants provided informed consent to take part in a study about “cognitive effects of a benzodiazepine in comparison to placebo” that would occur on two occasions, 7 days apart. At recruitment, no details were provided regarding the activities to be conducted on the 7-day delay session (Day 7). The study followed a parallel-group, double-blind design in which participants were randomly allocated to the placebo of diazepam treatments.

Participants were instructed to abstain from alcohol and other drugs for 24 hours before and after the experimental sessions. They were tested on two days (Day 1 and Day 7; Fig. 1): Day 1 included a pre- and a post-treatment session, both of which took place in the morning after a light breakfast containing no caffeine. Upon arrival, participants were asked to report baseline mood/sedation using the VAMS. After filling out the VAMS, WMC was determined using two tasks in random order (Counting Span and Running Memory Span). A pair of prose passages was then presented, each followed by immediate free-recall that was succeeded by 15 min of one of two interference conditions based on the study by Alber et al. (2014): a) spot-the-difference task (RI condition); or b)
immediate unfilled delay (MinRI condition). At the end of this session participants received the oral treatment (diazepam or placebo).

The post-treatment session on Day 1 started 60 min after drug administration, which is the time taken for diazepam to reach theoretical peak-plasma concentration in most healthy young individuals (Mandelli et al., 1978). Between treatment administration and the beginning of the post-treatment session, participants mainly talked to the experimenter and accessed messages on their mobile phones. The post-treatment session began with the VAMS to control for BZ-induced sedation, followed by the WMC tasks and then by the presentation of another pair of stories using the same procedure as in the pre-treatment session. We will refer to these stories as having been presented at the beginning of the post-treatment session. After the end of the 15 min that succeeded the second of this pair of stories, participants were submitted to a surprise delayed free-recall of all presented stories (four stories: a pair from the pre-treatment and a pair from the beginning of the post-treatment sessions). Participants were instructed to recall these stories in any order and in as much detail as they could remember with no time limit to do so.

The protocol was the same as that of Alber et al. (2014) regarding story presentation and recall, except that we: a) presented two instead of only one pair of stories; b) provided up to two cues when participants reported that they could not recall the stories at all, so that possible retrieval failures due to the sedative effects of the drug could be dissociated from effects on consolidation (see Buschke, 1984; Ivanoiu et al., 2005; Tulving, 1974); and c) presented a final pair of stories at the end of the post-
treatment session, followed only by immediate free-recall and MinRI or RI, but not delayed free-recall so that they would not be reactivated under the effects of diazepam and placebo (the reason for this is presented later).

At the end of post-treatment session, participants filled out a last VAMS to determine whether diazepam-induced subjective sedation was still apparent. They were then told that they should return for an extra test session 7 days later for a “surprise recognition task” involving pictures used in the spot-the-difference task (see Supporting information). It was not mentioned that delayed recall of the stories would be assessed. Participants were then transported home and asked not to drive or operate machinery that could lead to injury on that day.

The session that took place on Day 7 was treatment-free. Participants carried out an intelligence quotient (IQ) test (short form of Raven’s Advanced Progressive Matrices adapted by Arthur, Tubre, Paul & Sanchez-Ku, 1999; See Appendix S1) to ensure groups were equivalent in this respect. - As there is no reason to suppose that IQ changes over a period of one week, it was measured on this drug-free occasion because doing so on Day 1 would have increased fatigue unnecessarily. Participants then undertook a surprise 7-day delay free-recall of all stories presented on Day 1 and were then asked if they had rehearsed or thought about the stories during the previous week. Note that of the 6 stories presented during the first experimental day, only the last two had not been subjected to delayed free-recall at the post-treatment session (not reactivated). This was done so that we could: 1) determine the persistence of possible immediate MinRI effect 7 days later when participants did not reactivate stories by means of delayed free-recall during the post-treatment session (control condition not included in Alber et al., 2014; Dewar, Pesallaccia, et al., 2012, Dewar, Alber, Cowan & Della Sala, 2014); and 2) assess the anterograde impact of diazepam on forgetting over different time spans at the first delayed
recall opportunity (not reactivated): short delay, reflected by retention of stories encoded and recalled post-treatment on Day 1; long delay, retention of stories encoded at the end of the post-treatment session on Day 1 and recalled on Day 7. Lastly, a recognition test of the pictures from the spot-the-difference task was undertaken, which was what participants expected to occur on this occasion (see the Supporting information for these results). Participants were reimbursed for transport and meal expenses.

All randomizations in this study were carried out using www.randomizer.org. It was, however, not possible to fully counterbalance all factors. Instead, we constructed 15 random orders of tasks conforming to the structure shown in Fig. 1, each used once for a diazepam participant and once for a placebo participant. To construct these 15 orders, at each phase, counting and running spans were put in random order. Each time a participant was presented with a pair of stories, one had a male character and one had a female character, in random order, with stories randomly selected without replacement from the available pool of such stories. The order of RI and MinRI retention intervals was also randomized at each juncture.

2.5. Statistical analyses

Data bank is fully available (Segura et al., 2020). The adopted level of significance was p<0.05. Chi-square was used to compare the proportion of men and women in each experimental group. General Linear Models (GLMs) followed by Bonferroni post hoc test, when applicable, were used to analyze the remaining data. The analyses of demographic data were conducted with group (diazepam and placebo) as a factor. Analyses of memory variables had many factors (described below) and full factorial interactions were considered. When factors interacted, only higher order effects are
described. Additionally, all models were run with and without physical and mental sedation scores of the VAMS (in separate models) as covariates. Scores above or below three standard deviations in their respective treatment groups were considered outliers and excluded from the analyses (reported in the Results section when applicable).

The models used to analyse working memory capacity tasks performance included the between-participant factors treatment (placebo vs. diazepam) and within-subject factor session (pre- vs. post-treatment). The GLM used to analyse immediate free-recall of stories had the between subject factor treatment and within-subject factors encoding moment (three levels: pairs of stories presented pre-treatment, beginning and end of the post-treatment session) and interference (RI vs. MinRI), even though the interference manipulation occurred only after immediate free-recall. The latter factor was included to determine whether immediate recall of the different versions of the stories were comparable and adequately randomized.

The analyses of the anterograde and retrograde BZ effects measured at the post-treatment session (Day 1) regarding stories followed by RI and MinRI included retention scores as dependent variables. Retention was calculated dividing the number of items recalled in delayed free-recall (post-treatment) by the number of story units immediately freely recalled of the corresponding stories (pairs presented pre- and at the beginning of the post-treatment session). The GLMs included the factors treatment, encoding moment (pre- vs. beginning of the post-treatment session) and type of interference (RI vs. MinRI). The same models were run to determine retention considering scores after providing cues at delayed recall, which were necessary on Day 1 mainly for stories presented post-treatment to the diazepam group. The scores used were the summed number of items remembered after one and two cues, excluding items provided in the cues themselves.
because many diazepam treated participants did not recall any items even after one cue (this was also done for all the other cued recall analyses described below).

For the analyses of retention assessed on Day 7 of reactivated prose on Day 1 (post-treatment) we ran three models. The first involved only free-recall on Day 7 of stories encoded pre-treatment and recalled (reactivated) post-treatment. This analyses would indicate if retention of information learned pre-treatment and reactivated under diazepam or placebo, either followed by RI or MinRI, would be differently recalled after 7 days. The factors were treatment and interference. We could not run a similar model with free-recall including encoding moment (pre- vs. post-treatment) because many drug-treated participants failed to freely recall any items encoded under the drug (severe anterograde amnesia; see Table 3), which preclude calculating retention (recall divided by zero). Hence, the other two models considered cued-recall retention of stories presented at two moments, pre-treatment and at the beginning of the post-treatment session, all of which were reactivated post-treatment. Each of these GLMs used one of the following metrics: 1) the number of idea units of stories recalled on Day 7 before cues (7-day delay free-recall) divided by the total number of idea units recalled after cues post-treatment on Day 1; and; 2) the number of idea units of stories recalled on Day 7 after cues divided by the total number of idea units recalled after cues (cued recall) post-treatment on Day 1. These GLMs included factors treatment, interference and encoding moment (pre-treatment vs. beginning of the post-treatment session).

To determine the extent to which diazepam anterogradely affected forgetting over a short and long retention delay we ran a model with the factors treatment, interference and duration of delay considering: retention of the first pair of stories presented post-treatment and recalled post-treatment on Day 1 (short delay for forgetting), versus retention of the last pair of stories presented at the end of the post-treatment session.
subjected to delayed recall only on Day 7 (longer delay, with no reactivation on Day 1). In this analysis, retention was calculated by dividing delayed cued-recall of stories presented at the beginning of the post-treatment session by immediate free-recall of these stories on the same session, versus delayed cued-recall on Day 7 of stories presented at the end of the post-treatment session on Day 1 (not reactivated), divided by immediate free-recall in the post-treatment session on Day 1.

3. Results

Groups did not differ in terms of demographics (proportion of men/women, age, estimated IQ). Changes in sedation/mood were not observed in the placebo group while diazepam ingestion led to higher physical and mental sedation post-treatment that remained stable until the end of the experiment, indicating participants were under the drug until the very end of the post-treatment session (for more details see the Supporting information).

The measures of sedation were used as continuous predictors in all memory recall analyses and did not change the pattern of results (data not shown), so findings described below do not include this control for sedation as it would decrease statistical power that could mask possible effects of interest.

To facilitate interpretation of our results: a) Table 1 summarizes all investigated memory effects of diazepam in contrast to those found in the literature on patients with different types of organic amnesias according to the classification of Parkin and Leng (2014); b) Table 2 lists the Tables and Figures in which all types of memory effects are displayed.
3.1. Effects on Working Memory Capacity

The analysis of the Counting Span scores yielded no effect of treatment [F(1,28)=0.03, p=0.86, pη²=0.001], session [F(1,28)=0.66, p=0.42, pη²=0.02], nor their interaction: [F(1,28)=1.32, p=0.26, pη²=0.04] (Fig. 2A), showing that diazepam did not affect performance. Likewise, there was no effect of treatment [F(1,28)=0.70, p=0.41, pη²=0.02] nor an interaction of treatment and session [F(1,28)=3.27, p=0.08, pη²=0.10] in the Running Memory Span task (Fig. 2B), but a session effect was apparent [F(1,28)=4.30, p=0.047, pη²=0.13]: performance declined slightly on the post-treatment session. Visual inspection of Figure 2B suggests that this was due to a reduction in performance in the diazepam group, but an exploratory post hoc analysis for the insignificant interaction (p=0.08) failed to show significant contrasts.

3.2. Effects on Immediate free-recall

The interaction between treatment and session on immediate free-recall of prose was significant [F(2,54)=7.49, p=0.001, pη²=0.22] (Fig. 3; means ± SD are presented in the Supporting information S2 Table). The post hoc analysis showed no differences between groups at baseline, but the diazepam group recalled less idea units of stories presented at the beginning of the post-treatment session compared to recall in this same group at the other moments and immediate recall of the placebo group at all moments (ps<0.05). Immediate free-recall of the last pair of stories, however, were only marginally
lower in the diazepam than the placebo group (p=0.07). There was no effect of interference [F(1,27)=0.11, p=0.74, \( \eta^2=0.004 \)], nor did this factor interact with treatment [F(1,27)=0.17, p=0.68, \( \eta^2=0.006 \)], encoding moment [F(2,54)=0.46, p=0.63, \( \eta^2=0.017 \)], nor with treatment and encoding moment [F(2,54)=0.09, p=0.91, \( \eta^2=0.003 \)], which was expected because this manipulation only occurred after immediate free-recall. This indicated the stories were balanced in terms of immediate recall difficulty.

3.3. Effects on Delayed recall

3.3.1. Analysis of the anterograde and retrograde effects measured at the post-treatment session (Day 1).

The analyses of retention without providing cues (delayed free-recall) on Day 1 revealed an interaction of treatment and encoding moment [F(1,28) = 96.71, p<0.001, \( \eta^2 = 0.77 \)]. The diazepam group retained less items of stories presented post-treatment than of stories presented pre-treatment and post-treatment by the placebo group (ps<0.001). In other words, we showed clear anterograde amnesia for material encoded under the drug but no retrograde amnesia for content presented prior to treatment (Fig. 4A), as expected. Additionally, this interaction showed that, in the placebo group, retention of stories presented post-treatment was higher than that of stories encoded pre-treatment (p<0.01). Differently, for the diazepam-treated individuals, retention was higher for stories presented pre-treatment than by the placebo group (ps<0.001), that is, diazepam led to retroactive facilitation, which is common in BZ studies (see the Discussion section).

Interference effects were not significant [F(1,28) = 0.001, p<0.99, \( \eta^2 = 0.001 \)], did not
interact with treatment \( [F(1,28) = 0.80 \ p<0.38, \ \eta^2 = 0.028] \), encoding moment \( [F(1,28) = 1.93 \ p<0.18, \ \eta^2 = 0.18] \), nor with both the latter factors \( [F(1,28) = 0.52 \ p<0.48, \ \eta^2 = 0.02] \).

<INCLUDE FIGURE 4 AND TABLE 3 NEAR THIS POINT>

The results after cues (Fig. 4B) followed exactly the same pattern [interaction of treatment vs. session \( F(1,28) = 56.56 \ p<0.001, \ \eta^2 = 0.67 \) with the same post hoc significant contrasts], showing that the drug effects did not merely result from difficulty in retrieving information, but greatly impaired storage/consolidation that was not accessible even with reminders. Again, there was no significant effect of interference \( [F(1,28) = 3.05, \ p<0.09, \ \eta^2 = 0.10] \), a factor that also failed to interact with encoding moment \( [F(1,28) = 1.09, \ p<0.30, \ \eta^2 = 0.04] \) and treatment \( [F(1,28) = 0.06, \ p<0.81, \ \eta^2 = 0.002] \), and both the latter in a full cross interaction \( [F(1,28) = 0.43, \ p<0.52, \ \eta^2 = 0.015] \).

3.3.2. Analyses of retention assessed on Day 7 of reactivated prose on Day 1

The model comparing retention on Day 7 only of stories presented pre-treatment and freely recalled post-treatment on Day 1 yielded an effect of treatment \( [F(1,24) = 6.04, \ p<0.022, \ \eta^2 = 0.20] \), showing that after 7 days the diazepam group no longer showed the retention advantage (retrograde facilitation) over placebo in recalling the stories presented pre-treatment that was observed post-treatment on Day 1, but presented a smaller retention of episodic information. Put simply, reactivation of prose under the effects of
the drug interfered with memorization of the content. Interference had no effects \[F(1,24) = 0.02, p<0.88, \eta^2 = 0.001\] and did not interact with treatment \[F(1,24) = 0.24, p<0.63, \eta^2 = 0.01\]. This could, however, have been due to diazepam-induced retrieval difficulties so we also took cued-recall into account in the follow-up analysis. First, the model with retention using *free*-recall on Day 7 in relation to *cued* delayed recall on Day 1 (Fig. 5A) excluded three participants from the diazepam group [two recalled no items of stories presented post-treatment, even after cues on Day 1, and one was an outlier (score 3 SD above mean)]. This model showed an effect of treatment on retention \[F(1,25) = 37.32, p = 0.001, \eta^2 = 0.60\], diazepam having reduced retention in comparison to placebo. This analysis also showed less retention, irrespective of groups, when encoding took place post-treatment than pre-treatment [effect of encoding moment: \(F(1,25) = 11.94, p=0.002, \eta^2 = 0.32\)]. There was no interaction of encoding moment with treatment \[F(1,25) = 3.52, p = 0.07, \eta^2 = 0.12\], nor with interference \[F(1,25) = 1.48, p = 0.23, \eta^2 = 0.06\]. This indicated that despite the diazepam-induced initial increase in post-treatment delayed recall of stories encoded pre-treatment compared to placebo on Day 1 (retrograde facilitation), the anterograde memorization of this reactivated information under the effects of the drug was indeed impaired and not an effect of retrieval difficulties. Interference had no effects \[F(1,25) = 1.43, p = 0.24, \eta^2 = 0.05\] and did not interact with treatment \[F(1,25) = 0.15, p = 0.70, \eta^2 = 0.006\], nor moment and treatment \[F(1,25) = 0.04, p = 0.83, \eta^2 = 0.001\].

In the same model, but now considering retention based on *cued*-recall on both Day 1 and Day 7 (Fig. 5B), we excluded the same three diazepam-treated participants
mentioned above. This model showed the same effects [treatment: F(1,25) = 25.99, p<0.001; η² = 0.51; encoding moment: F(1,25) = 5.29, p=0.03; η² = 0.17; interaction: F(1,25) = 5.26, p=0.03; η² = 0.17, but with no significant post hoc contrast (ps>0.08)], providing evidence that the diazepam-induced effects were not due to retrieval difficulties.

3.3.3. Analyses of forgetting over a short delay (encoding and recall/reactivation post-treatment on Day 1) and long delay (encoding post-treatment on Day 1 and recall on Day 7)

The analysis comparing cued-recall retention considering different delay periods [short delay (immediate free-recall in the beginning of post-treatment and delayed cued-recall post-treatment on Day 1); long delay (immediate free-recall in the end of post-treatment on Day 1 and delayed cued-recall on Day 7) showed that diazepam impaired retention compared to placebo [treatment effect: F(1,26) = 70.57, p < 0.001, η² = 0.73] including impaired recall of stories presented at the end of the post-treatment session on Day 1. Also, irrespective of treatment, retention decayed more over the long than the short delay [effect of duration of delay: F(1,26) = 75.85, p < 0.001, η² = 0.74] (Fig. 6). Importantly, treatment did not interact with duration of delay [F(1,26) = 0.35, p < 0.56, η² = 0.013], showing that although diazepam disrupted memorization soon after encoding, it did not enhance forgetting over 7 days despite being a long acting BZ compound. Hence, diazepam did not alter memory decay, or rate of forgetting differently from placebo. Interference, again, had no effects [F(1,26) = 0.005, p < 0.94, η² = 0.000] and did not interact with the other factors (interference and treatment [F(1,26) = 0.06, p < 0.81, η² = 0.002]; duration of delay and interference [F(1,26) = 0.000, p < 0.99, η² =
Overall diazepam effects were similar to those found in medial temporal lobe amnesia with severe hippocampal atrophy and MCI probable converters (Table 1).

4. Discussion

In the present study, the acute oral treatment with 15 mg of diazepam induced cognitive effects that corroborated our predictions that typical BZs cause profound anterograde amnesia for episodic content (Gorissen et al., 1998; Gorissen et al., 1995; Unrug-Neervoort et al., 1992). As expected these effects were not explained by diazepam-induced physical and mental sedation (Buffett-Jerrott & Stewart, 2002; Curran, 1991; Curran & Birch, 1991; Mintzer & Griffiths, 2007; Sarasin, Ghoneim & Block, 1996), because controlling for them did not change the pattern of results. However, we found that BZs do not affect all stages of the formation of episodic memories, which were not clearly separated in prior studies in the literature. Encoding seems not to be considerably affected as participants under diazepam presented only the usual minor reductions in immediate recall (see Curran, 1991) when compared to delayed recall, and led to no WMC impairment (as suggested in Reder et al., 2006). Retrieval processes were also not affected, as BZ-treated participants could freely recall pre-treatment presented stories (no retrograde amnesia), in agreement with prior findings (e.g. Hinrichs et al., 1984). Furthermore, the drug did not accelerate forgetting (see Geurts, Geurts, van der Werf & Kessels, 2015; Kopelman & Stanhope, 1997) over a period of one week compared...
with the placebo group (a novel finding). Hence, BZs seem to impair consolidation that takes place shortly after encoding, which we found to occur both for newly encoded information and reactivated episodic memories (new finding). This consolidation process under diazepam was also not facilitated by minimization of retroactive interference (see Dewar et al., 2009), which was also not assessed in the preceding literature. Each of these results will be detailed below. We will argue that, together, these findings suggest that BZs are not an adequate model of all non-material specific organic amnesias (e.g. Curran, 1991; Lister, 1985; Thomas-Antérion et al., 1999).

Regarding immediate free-recall of memory for prose, our findings were equivalent to the pattern of effects found for other BZs (Curran, 1986, 1991; Curran & Birch, 1991; Ghoneim, Mewaldt, Berie & Hinrichs, 1981) and in the types of organic amnesias that we contrasted BZs effects with (e.g. Baddeley and Wilson, 2002; Dewar et al., 2010): diazepam reduced scores, but to a much smaller extent than the impairment in delayed free-recall. Although a significant reduction in immediate free-recall was observed at peak-plasma concentration of the drug, by the end of the post-treatment session scores of diazepam-treated participants were only marginally and non-significantly worse (p=0.07) than that of participants treated with placebo, even though the sedative effects of diazepam remained constant until the experiment ended on Day 1. This can be explained by adjustments to some drug effects, or specific acute tolerance to memory effects (see Cittadini & Lader, 1991; Ellinwood, Linnoila, Easler & Molter, 1983), indicating that people are able to overcome these symptoms, which were much subtler than those on delayed recall.

Hence, the slight reduction in immediate free-recall of diazepam-treated participants at the beginning of the post-treatment session cannot be wholly ascribed to sedation, nor to difficulties in encoding and active search and retrieval of recently
activated long-term memories, since the drug did not impair WMC (see Unsworth, 2016; Unsworth & Engle, 2007). Apart from attention limited working memory, automatic linguistic processes are also at play in immediate prose-recall (Jefferies, Lambon Ralph, Baddeley, 2004). However, it is unlikely that the latter were affected by diazepam which, like most BZ compounds, do not impair verbal short-term nor implicit memory processes (Curran, 1991; except for lorazepam: see Giersch et al., 2010).

Lack of BZ-induced WMC deficits also shows that these drugs do not mimic amnesias which have been shown to have lowered WMC, such as reports in Korsakoff (Parkinson, 1980), frontal (Roussel, Dujardin, Hénon & Godefroy, 2012), Post-Traumatic (McAllister, Flashman, Sparling & Saykin, 2004), AD and some MCI amnesias (Hamdan & Bueno, 2005; Gagnon & Belleville, 2011). In this respect, BZs seem to elicit effects that are more related to those found in amnesiats with altered medial temporal functioning (e.g. Allen, Vargha-Khadem & Baddeley, 2014; Baddeley & Wilson, 2002; Kopelman & Stanhope, 1997; Leng & Parkin, 1989; Parkinson, 1980; Quinette et al., 2006, 2003). This requires confirmation, however, because few studies have investigated this construct in amnesiats and we found none that adequately evaluated these effects in Post-Electroconvulsive Amnesia (see Table 1).

Possible hippocampal-dependent changes at encoding could also be related to the fact that delayed recall of information encoded under BZs improved slightly after cues were provided. This suggests that contextual memory traces at encoding in diazepam-treated participants may not have been as well integrated with their episodes (see Kopelman, 2002) as may have occurred in those who took placebo, leading these memories to be slightly harder to recall later on without cues (cue-dependent forgetting: Tulving, 1974). This, however, does not wholly explain the BZ-induced anterograde amnesia, as restoring access to this information by providing cues still indicated that
consolidation was severely disrupted by the drug (trace-dependent forgetting: Tulving, 1974; see also Shimmerlik, 1978). This confirms that BZs mainly affect consolidation of episodic information (Curran, 1991) that take place soon after encoding, between immediate and delayed free-recall (Curran, 1986; Gorissen et al., 1998). This is consistent with the effects found in patients with all types of anterograde amnesia that we contrasted BZ effects with (see Aggleton & Brown, 1999; Alber et al., 2014; Baddeley & Wilson, 2002; Hirst, Johnson, Kim, Risse & Phelps, 1986; Quinette et al., 2006; Tulving, 2002).

Higher retention in the placebo group of the stories presented at the beginning of the post-treatment session, compared to that of the stories encoded pre-treatment, can be explained by a series of factors: 1) the shorter lag since the post-treatment story presentation (less time to decay: see Hinrichs et al., 1984); 2) a recency effect (Baddeley & Hitch, 1993); and/or 3) non-immediate retroactive interference resulting from the presentation of new to-be-remembered material from the second pair of stories (Dewar et al., 2010; Hinrichs et al., 1984), or even from facts and activities involved in taking part in the experiment. - Note that this type of retroactive interference differs from the one manipulated in the RI paradigm used here, in which interference or lack of interference (MinRI) happen immediately after encoding/immediate free recall (Dewar et al., 2009) and not after longer delays-.

In contrast, the diazepam group showed higher retention of stories presented pre-treatment than the placebo-treated individuals, or retroactive facilitation (Curran, 1991; Ghoneim, Hinrichs & Mewaldt, 1984; Hinrichs et al., 1984), which rules out that BZs impair retrieval per se, corroborating prior findings (Coenen & van Luitelaar, 1997; Curran, Schiwy & Lader, 1987; Delgado, Izquierdo & Chaves, 2005). Naturally, retrograde facilitation cannot be verified in organic amnesic patients because the onset of their conditions can only be established retrospectively, so this does not add information
regarding the suitability of diazepam as a pharmacological model of a specific type of clinical anterograde amnesia. However, this finding speaks to other issue regarding BZs effects which will be discussed next: state-dependent learning, time course of effects on consolidation and susceptibility to interference.

Firstly, retrograde facilitation allows the exclusion of state-dependent effects having interfered with results (see Lister, 1985). Secondly, in BZs studies, retrograde facilitation (Hinrichs et al., 1984) is only observed when new information is presented after drug administration, possibly because this new information that does not immediately follow encoding interferes retroactively with content learned before in placebo-treated participants, but not in those who ingest BZs, who retain less of the new information (see Curran, 1991; Ghoneim et al., 1984). In effect, BZs seem not to affect consolidation mechanisms that are already underway (see Brown et al., 1982; Fiebig & Lansner, 2014), from a prior drug-free session, nor active forgetting processes (see Hardt et al., 2013). This confirms that these drugs only anterogradely impair initial consolidation processes (Wang & Morris, 2010) that take place soon after encoding (Fiebig & Lansner, 2014). In fact, we show that these processes may be so disrupted that there can be no benefit from MinRI, which acts by protecting memory from overloading (see Dewar, Pesallaccia, et al., 2012). Alternatively stated, BZs seem not to anterogradely spare “residual retention” abilities (Dewar, Pesallaccia, et al., 2012) just after encoding, which can benefit from MinRI effects.

Although diazepam elicited profound anterograde amnesia to a similar extent to that found in most deeply organic amnesic patients, many of the latter benefit from MinRI (Cowan et al., 2004; Cowan, Beschin, et al., 2005; Dewar et al., 2009, 2010; Dewar, Alber, et al., 2012; Dewar, Pesallaccia, et al., 2012). This suggests that diazepam affects consolidation similarly only to the subgroup of amnesiacs who also fail to show such
benefits. The reasons why they do not are still unclear, but might be related to more severe hippocampal atrophy (Cowan et al., 2004; Dewar, Pesallaccia, et al., 2012). It can therefore be hypothesized that processes related to the hippocampus and adjacent areas, which are essential for initial consolidation (Dudai, 2004; Mednick, Cai, Shuman, Anagnostaras & Wixted, 2011; Wang & Morris, 2010), are affected in these clinical conditions and in the amnesia induced by acute BZ administration. Functioning of these areas, apart from relating to consolidation (Squire, 2009), is also involved in other abilities that may explain the small anterograde diazepam-induced impairment in immediate free-recall. These structures are important for a particular type of association between items at encoding (conjunction working memory: Olson et al., 2006; see also Hannula, Tranel & Cohen, 2006) that is not measured in the WMC tasks that we employed, and can also alter the ability to recruit episodic long-term memory that can support immediate episodic recall (Jeneson & Squire, 2011; Verfaellie & Keane, 2017). This must be confirmed in studies designed for this particular purpose.

The fact that MinRI did not increase recall in placebo-treated controls is not surprising, as this manipulation has been found not to improve memory retention in many studies with young (Martini, Riedlsperger, Maran & Sachse, 2017; Martini, Zamarian, Sachse, Martini & Delazer, 2018; Varma et al., 2017) and elderly (Dewar et al., 2010) people with optimum cognitive abilities, particularly when tested with prose memory tasks. In samples such as these, consolidation may be so efficient that it is not possible to measure benefits of minimizing interference, except in very specific experimental conditions which differ from the spot-the-difference task used here (see Varma, Daselaar, Kessels & Takashima, 2018). These conditions involve autobiographical thinking, which can interfere with formation of new episodic memories (see Andreasen et al., 1995; Craig, Della Sala & Dewar, 2014; Varma et al., 2018).
It is of note that the anterograde amnesia elicited by BZs does not seem to depend on the tasks or activities that follow immediate free-recall of episodic memories. This is fortunate because no attention has been given in the literature to what activities were carried out immediately after presentation of the to-be-remembered stimuli in BZ-treated individuals. In contrast, activities carried out immediately following presentation of episodic content potentially affect delayed recall in amnesics whose clinical conditions were shown to be potentially susceptible to RI [resulting from brain injury due to anoxia, head injury, stroke, AD and MCI (Cowan et al., 2004; Cowan, Beschin, et al., 2005; Dewar et al., 2009, 2010; Dewar, Pesallaccia, et al., 2012)]. This has been overlooked and deserves attention when assessing these types of patients.

We also found that BZs may not only anterogradely affect initial consolidation, but also initial (re)consolidation after reactivation of information by bringing previously encoded memories to mind. Although pre-treatment stories were better freely delayed recalled in the post-treatment session by those who took diazepam than placebo-treated participants (retrograde facilitation), this reactivation of memories under the drug led to retention on the 7-day drug-free session that was lowered and comparable to that following placebo. Reactivation is believed to return memory traces to a labile state (Nader, Schafe & Le Doux, 2000; Sara, 2000; Scully et al., 2017), making them more susceptible to interference (McKenzie & Eichenbaum, 2011) by factors such as amnestic drugs. The fact that both consolidation of new and reactivated memories are impaired by BZ corroborates that consolidation and reconsolidation may be mediated, at least in part, by the same physiological mechanisms (Dudai & Eisenberg, 2004; McKenzie & Eichenbaum, 2011; for a different view, see Lee, 2010). This diazepam-induced effect on reactivation is relevant for those interested in potential ways of altering prior memories in psychopathologies such as post-traumatic stress disorder and phobias (Dunbar &
Taylor, 2016; Gravitz, 2019; Phelps & Hofmann, 2019). Acute BZs effects may aid in this respect, but we have no data to compare our results with. Prior studies in humans only administered these drugs after reactivation (Fernández et al., 2017; Rodríguez et al., 2013) and did not test changes in reactivated memories under their effects, as done here.

In contrast to initial consolidation effects later consolidation processes that take place over hours or days (Fiebig & Lansner, 2014; Tano et al., 2009; Wang & Morris, 2010) were not further impaired by the drug, as diazepam failed to accelerate forgetting (Geurts et al., 2015). That is, we found the same rate of decay in retention in the placebo and diazepam groups for the pair of stories presented and recalled post-treatment (short delay on Day 1) compared to the decay of the last pair of stories encoded at the end of post-treatment section on Day 1 (not followed by delayed recall in that session) and recalled after a longer delay (7 days). Admittedly, this may have been due to a floor effect considering the very low performance in the diazepam group on delayed recall on Day 7. However, if this finding is confirmed, this would rule out that BZs model Transient Epileptic Amnesia, which are associated with accelerated forgetting (Elliott et al., 2014; Lah Mohamed, Thayer, Miller & Diamond, 2014) (see Table 1). Interestingly, in this clinical condition, hippocampal volume is negatively associated with anterograde amnesia but does not relate to accelerated forgetting (Butler et al., 2009), which is associated to other diffuse temporal pathologies (Muhlert et al., 2011). This endorses findings that specific hippocampal damage in this type of patient impairs earlier consolidation phases, but is less involved in memory decay over longer periods of time (Lah et al., 2014).

In sum, the data indicate that acute oral BZ administration: 1) slightly impairs encoding, which is not related to altered WMC, found to be preserved; 2) spares episodic memory retrieval; and 3) reduces initial consolidation processes (of newly encoded and
reactivated information), but not later consolidation processes because diazepam did not accelerate forgetting. Furthermore, the impairment in initial consolidation is not ameliorated by minimizing retroactive interference.

5. Conclusions

Unlike prior publications (Lister, 1985; Thomas-Antérion et al., 1999), which compiled data from various studies to point to the similarity of BZ-induced cognitive effects with various types of patients with anterograde amnesia, our findings were all obtained in the same experiment and extended our knowledge of the cognitive effects of the typical BZ diazepam. With this approach, we conclude that acute doses of BZs adequately model the cognitive profile of rare cases of stable and progressive amnesias in which it has been claimed that hippocampal areas seemed to be particularly affected. However, the lack of studies that assessed a possible advantage of minimizing retroactive interference in transient amnesias (Transient Global Amnesia and Post-Electroconvulsive Amnesia) preclude us from determining the similarity of their effects with those of BZs, even though they also do not seem to accelerated forgetting nor alter WMC.

6. Practical applications

Using BZs to model cognitive effects observed in amnesiacs with severe hippocampal and adjacent damage can be useful to: 1) explore the neurobiology of memory; 2) allow testing of manipulations that may improve episodic memory in these patients (Golovko et al., 2018), who are unlikely to be available in sufficient number to enable controlled experimental designs; and 3) manipulate priorly consolidated memories (see Makkar et al., 2010; Walsh et al., 2018) brought to mind (reactivated) under BZ
effects, especially memories related to anxiety and depression symptoms which are modulated by the BZ receptor system (see Prevot et al., 2019).

7. Limitations

Similarly to other pharmacological studies, our experiment included a relatively small sample due to the ethical restrictions in administering drugs to healthy volunteers. Participants were selected to be as homogenous as possible in representing individuals with optimum cognitive performance in order to reduce variability and increase statistical power: they were all young, healthy, highly schooled, and as hormonally homogenous as possible when testing male and female participants. Randomization of individuals to the experimental treatment was successful in assuring similarity in the placebo and diazepam groups regarding baseline immediate free-recall of prose, WMC, IQ and mood. The expected subjective and amnestic effects of diazepam were observed, showing that the selected diazepam dose and time of post-treatment sessions were adequate. Our results are therefore not extendable to participants with other demographic and clinical characteristics. Additionally, our findings regarding memory decay may have differed had we used different long-term memory delays and ensured overlearning before delays (e.g. Walsh et al., 2014). Having BZ-treated participants recall episodic memories related to emotional and not neutral content could also have led to other effects (Walsh et al., 2018). Regarding the type of to-be-remembered information and MinRI/RI paradigm used here, although there is evidence that susceptibility to RI occurs irrespective of type of interference (Dewar et al., 2010) and episodic content (Alber et al., 2014; Craig et al., 2014; Craig, Dewar, Harris, Della Sala & Wolbers, 2016), since the present study was designed it has come to light that there are specific paradigms that are successful in
showing MinRI improvement in retention in healthy adults (Varma et al., 2018). Therefore, because the parameters that influence these effects are still emerging, lack of benefit from MinRI in BZ-treated individuals should be further explored, preferably with more than one dose of the drug so that dose-dependent effects can be established.

Data Availability Statement

The data that support the findings of this study are openly available in Mendeley Data at http://dx.doi.org/10.17632/2mcz32jdp4.2,

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List of figures

Fig. 1. **Experimental design.** The experiment took place on two days. On Day 1 there was a baseline (pre-treatment) and a post-treatment session. In the pre-treatment session, a pair of stories was presented, each of which was followed by immediate free-recall succeeded by a 15-minute long interval which involved either: a) RI: carrying out a spot-the difference (STD) task as immediate retroactive interference); or b) MinRI: 10 min rest in a dark room as immediate minimal retroactive interference) followed by 5 min of the STD task to prevent continuous active maintenance in working memory. The participants then received an oral treatment (random allocation to a placebo or diazepam 15 mg group). The post-treatment session began 60 min after treatment, when another pair of stories was presented following the same procedure as pre-treatment. This was followed by a surprise delay recall in any order of all 4 stories presented so far plus another pair of stories at the end of the session followed by RI and MinRI, which were only subjected to immediate free-recall (no reactivation in this session). Subjective sensations, such as sedation, was assessed with Visual-Analogue Mood Scales (VAMS) at the beginning of
the pre- and post-treatment sessions and at the very end of the experiment. Working Memory Capacity (WMC) was measured at the beginning of the pre- and post-treatment session just after completion of the VAMS. In the 7-day delay session (Day 7) there was no administration of treatment or presentation of new content. This session consisted of a surprise delay recall of the 6 stories presented in any order. The order of WMC tasks and MinRI vs. RI were balanced between participants, sessions and treatments, as were the different versions of these tasks. See text for details.

Fig. 2. Scores on working memory capacity tests. Individualized (dots and squares) and mean (± SE) scores (histograms with error bars) on the Counting Span task (A: all or nothing load scores: ANL) and Running Memory Span task (B; mean number of recalled digits in the correct serial order), per treatment group (placebo or diazepam) and session on Day 1.

Note: The only significant effect was an effect of session for the Running Memory Span (pre-treatment>post-treatment: p<0.05).
Fig. 3. **Immediate free-recall.** Individualized (dots and squares) and mean (± SE) idea units (histograms with error bars) recalled during immediate free-recall of stories presented at different test moments (pre-treatment; begin. post = beginning of the post-treatment session, end of the post-treatment session) per treatment group (placebo or diazepam) and type of interference (MinRI = Minimal Retroactive Interference and RI = Retroactive Interference).

Note: Each story contained 25 idea units; the interference manipulation occurred only after immediate recall, which explains why it was not significant. Diazepam impaired performance at the beginning of the post-treatment session (p<0.05) and showed a trend towards significance (p=0.07) at the end of the post-treatment session, but there were no effects of MinRI vs. RI, or interactions with this factor (see section 3.4 for details).
Fig. 4. Retention at delayed recall (Day 1). Individualized (dots and squares) and mean (± SE) retention scores (histograms with error bars) assessed in delayed post-treatment free-recall (A) and after providing cues (B) of stories presented pre-treatment and at the beginning of the post-treatment sessions, by treatment group (placebo or diazepam) and type of interference (MinRI = Minimal Retroactive Interference and RI = Retroactive Interference).

Note: Retention (savings) was obtained by dividing the number of story idea units remembered during delayed free- (A) or cued-recall (B) by the number of idea units remembered during immediate free-recall. Treatment and encoding moment interacted for both free- and cued-recall (see section 3.5.1 for post hoc results).

Fig. 5. Retention on Day 7. Individualized (dots and squares) and mean (± SE) retention scores (histograms with error bars) of stories on Day 7 [(A) free-recall, before cues; (B) cued-recall] in relation to the delayed cued-recall in the post-treatment session of stories encoded pre-treatment and at the beginning of the post-treatment session on Day 1, per treatment group (placebo or diazepam) and type of interference (MinRI = Minimal Retroactive Interference and RI = Retroactive Interference).
Note: Savings using delayed free-recall at the post-treatment session is not pictured because most diazepam-treated participants had total anterograde amnesia for stories encoded post-treatment. Effects of treatment (lower in the diazepam group) and encoding moment (lower when encoding took place post-treatment on Day 1) were observed in A and B. See section 3.5.2 for statistical details.

Fig. 6. Retention of story items over a short delay (encoding and retrieval post-treatment on Day 1) and a long delay (encoding post-treatment on Day 1 and recall on Day 7). Individualized (dots and squares) and mean (± SE) retention scores (histograms with error bars) post cues in the first delayed recall opportunity of stories encoded post-treatment, per treatment group (placebo or diazepam) and type of interference (MinRI = Minimal Retroactive Interference and RI = Retroactive Interference).

Note: Encoding and recall on Day 1: retention was calculated by dividing delayed cued-recall at the post-treatment session by immediate free-recall of stories presented at the beginning of the same session; encoding on Day 1 and recall on Day 7: retention
calculated by dividing delayed cued-recall of stories presented at the end of the post-treatment session at the 7-day delay by immediate free-recall during the post-treatment session. Delayed-cued recall was used because free-recall resulting in too many zero scores in the diazepam group. Delayed free-recall at the post-treatment session was not used because most diazepam-treated participants did not recall any idea of the stories encoded post-treatment in that same session.
List of tables

Table 1. Summary of characteristics of different types of patients with organic non-material-specific anterograde amnesia and effects of acute diazepam (15 mg) oral administration on the differential diagnostic criteria based on the cognitive constructs under investigation.

<table>
<thead>
<tr>
<th>Type of patients according to aetiology/affected brain area</th>
<th>Working memory capacity</th>
<th>Benefit from minimal retroactive interference (MinRI)</th>
<th>Episodic memory decay</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Permanent amnesias</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Korsakoff</td>
<td>Impaired(^1)</td>
<td>-</td>
<td>Typical(^2)</td>
</tr>
<tr>
<td>Medial-temporal and other non-frontal lesions #</td>
<td>Nonimpaired(^3)</td>
<td>No (severe hippocampal atrophy)(^4)</td>
<td>Typical(^2;6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes (other lesion)(^4;5)</td>
<td></td>
</tr>
<tr>
<td><strong>Frontal Amnesias</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Impaired(^7)</td>
<td>Mixed results for pure frontal and mixed lesions(^4;5)</td>
<td>Typical(^8)</td>
</tr>
<tr>
<td><strong>Progressive Amnesias</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alzheimer’s Disease (AD)</td>
<td>Impaired(^8;10)</td>
<td>Yes</td>
<td>Typical(^12;13)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No (decreases in more severe AD)(^11)</td>
<td></td>
</tr>
<tr>
<td>Mild Cognitive Impairment (MCI) #</td>
<td>Nonimpaired(^9)</td>
<td>Yes(^11;14)</td>
<td>Accelerated(^15)</td>
</tr>
<tr>
<td></td>
<td>Impaired(^16)</td>
<td>No (low benefit in AD probable converters)(^11)</td>
<td>Typical(^12;17)</td>
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<tr>
<td><strong>Transient amnesias</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transient Epileptic Amnesia</td>
<td>Nonimpaired(^18)</td>
<td>-</td>
<td>Accelerated(^19;20)</td>
</tr>
<tr>
<td>Transient Global Amnesia</td>
<td>Nonimpaired(^21)</td>
<td>-</td>
<td>Typical(^22)</td>
</tr>
<tr>
<td>Post-Traumatic Amnesia</td>
<td>Impaired(^23)</td>
<td>-</td>
<td>Typical(^24)</td>
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<tr>
<td>Post ECT Amnesia</td>
<td>-</td>
<td>-</td>
<td>Typical(^25)</td>
</tr>
<tr>
<td>Benzodiazepine (acute diazepam, 15 mg) #</td>
<td>Nonimpaired(^26)</td>
<td>No(^27)</td>
<td>Typical(^26)</td>
</tr>
</tbody>
</table>
Note: # Similar pattern of effects; ECT: Electroconvulsive Therapy; 1Parkinson (1980); 2McKee & Squire (1992); 3Baddeley, Jarrold, & Vargha-khadem (2011); 4Cowan et al. (2004)*; 5Dewar et al. (2010)*; 6Geurts et al. (2015); 7Roussel et al. (2012); 8Janowsky, Shimamura, & Squire (1989); 9Hamdan & Bueno (2005); 10Kensinger, Shearer, Locascio, Growdon, & Corkin (2003); 11Dewar, Pesallaccia, et al. (2012); 12Alber et al. (2014); 13Hart, Kwentus, Taylor, Harkins, & Commonwealth (1987); 14Cowan et al. (2005a); 15Walsh et al. (2014); 16Gagnon & Belleville (2011); 17Manes et al. (2008); 18Tudesco et al. (2010); 19Elliott et al. (2014); 20Lah et al. (2014); 21Quinette et al. (2003); 22Hodges & Warlow (1990); 23McAllister et al. (2004); 24Levin, High and Eisenberg (1988); 25Lewis & Kopelman (1998); 26Present study. *patients with other types of lesions were also included.
Table 2: Summary of the changes in memory for stories after acute diazepam oral ingestion compared to treatment with placebo investigated in the present study according to the expected and tested (previously unstudied) effects, the interpretation of findings and the Tables and Figures in which the effects can be observed.

<table>
<thead>
<tr>
<th>Memory effects of diazepam</th>
<th>Interpretation*</th>
<th>Table/Figure</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Expected BZ effects</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Slightly impaired immediate recall post-diazepam (Day 1)</td>
<td>- Possible slight impairment in associative memory (between items at encoding)</td>
<td>Figure 3 Table S2</td>
</tr>
<tr>
<td>- Impaired savings of information presented post-diazepam at delayed recall after short (Day1) and long delay (Day7)</td>
<td>- Anterograde amnesia</td>
<td>Figure 4 and 5 Table S2</td>
</tr>
<tr>
<td>- Increased savings at delayed recall post-diazepam (Day 1) of information encoded pre-treatment</td>
<td>- Lack of retrograde amnesia / retrograde facilitation</td>
<td>Figure 4 Table S2</td>
</tr>
<tr>
<td><strong>Previously unstudied BZ</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Preserved performance on running memory span and counting span tasks post-diazepam (Day 1)</td>
<td>- Preserved Working Memory Capacity</td>
<td>Figure 2</td>
</tr>
<tr>
<td>- No difference in savings post-diazepam between minimal retroactive interference (MinRI) and retroactive interference (RI) on Day 1 and Day 7</td>
<td>- No facilitation of recall after MinRI</td>
<td>Figure 4 and 5 Table S2</td>
</tr>
<tr>
<td>- Equivalent amount of decay from Day 1 to Day 7 in savings post-diazepam</td>
<td>- No accelerated forgetting</td>
<td>Figure 6 Table S2</td>
</tr>
<tr>
<td>- Retrograde facilitation post-diazepam on Day 1 is no longer observed after a long delay (Day 7)</td>
<td>- Impaired reconsolidation</td>
<td>Figure 5 Table S2</td>
</tr>
</tbody>
</table>

Note: Day 1 included two sessions, pre- and post-treatment, in both of which stories were presented followed by MinRI and RI with immediate subsequent immediate recall, although delayed recall was carried out only post-treatment (see Fig. 1); Day 7 involved a drug-free session with delayed recall of all stories. *See Results and Discussion sections for detailed explanation.
Table 3. Number of participants who scored zero (did not recall any idea units from the stories: total anterograde amnesia) during delayed recall post-treatment on Day 1 and Day 7 for free-recall (0 cues) and after one and 2 cues (cued-recall) according to moment in which stories were encoded and interference conditions (MinRI: Minimal Retroactive Interference; RI: Retroactive Interference).

<table>
<thead>
<tr>
<th>Encoding session</th>
<th>Recall</th>
<th>Cues</th>
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<th>Placebo</th>
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<tr>
<td></td>
<td></td>
<td>Cues</td>
<td>MinRI</td>
<td>RI</td>
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<td></td>
<td><strong>Post-treatment session</strong></td>
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<tr>
<td></td>
<td><strong>Beginning of the post-treatment session</strong></td>
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<td><strong>(subjected to delayed recall on Day 1)</strong></td>
<td><strong>(subjected to delayed recall on Day 1)</strong></td>
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</tbody>
</table>

Note: There were no zero scores for immediate free-recall.