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Benzodiazepine use and brain amyloid load in nondemented older individuals: a florbetapir PET study in the Multidomain Alzheimer Preventive Trial cohort

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ABSTRACT

It remains unclear whether benzodiazepines (BZDs) constitute a risk factor for Alzheimer's disease (AD). In this study, we investigated associations between chronic use of BZDs and brain amyloid load, a hallmark of AD, in 268 nondemented older individuals. F^{18}-florbetapir positron emission tomography scans were performed to assess amyloid load as measured by standardized uptake value ratios, which were compared between chronic BZD users and nonusers using adjusted multiple linear regressions. Short- versus long-acting BZDs were also considered in the analyses. Standardized uptake value ratios were significantly lower in BZD users ($n = 47$) than in nonusers ($n = 221$), independent of multiple adjustments. The effect was stronger for short-acting BZDs than for long-acting BZDs. This is the first large clinical study showing a reduced brain amyloid load in chronic BZD users, especially with short-acting BZDs. Our results do not support the view of BZD use as a risk factor for AD and instead support the involvement of pharmacological mechanisms related to neuronal hyperactivity, neuroinflammation, and sleep quality as potential targets for blocking amyloid accumulation.

1. Introduction

Benzodiazepines (BZDs) are widely used among older adults. Estimates indicate that more than 25% of older adults chronically use BZDs or hypnotic gamma-aminobutyric acid (GABA)ergic non-BZDs in various countries, including France (Breining et al., 2016). BZDs are relatively safe and efficacious for treating symptoms such as anxiety and insomnia if the guidelines for BZD prescription are observed, which include a short duration of use, usually less than 1 month for hypnotics and 3 months for anxiolytics. In contrast, chronic use of BZDs has been associated with multiple side effects, including sedation, fall risk, and functional and cognitive impairment (Airagnes et al., 2016).

Moreover, concerns have recently been raised regarding the increased risk of major neurocognitive disorders (MNCDs), especially Alzheimer's disease (AD), in chronic BZD users. Indeed, several epidemiological studies have found an increased prevalence of

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MNCDs in older adults chronically using BZDs (Chan et al., 2017) (Zhong et al., 2015). A recent meta-analysis of 8 studies found an increased risk of having diagnostic criteria for dementia in BZD users compared with BZD nonusers, with a pooled summary odds ratio of 1.78 (95% CI, 1.33–2.38) (Islam et al., 2016). The likelihood of having MNCD diagnostic criteria may be particularly high with long-acting BZDs, long-term use, and high dosage (Billioti de Gage et al., 2015). However, not all epidemiological studies have found an increased risk of MNCDs with BZDs, especially some large longitudinal studies (Gray et al., 2016) that found no association between BZD use and MNCDs, and it remains unclear whether BZDs constitute a risk factor for MNCDs.

The identification of pathophysiological mechanisms linking BZDs to MNCDs could provide further information on the putative risk of MNCDs in BZD users. However, few studies have investigated the impact of BZDs on brain changes related to MNCDs, and only one small pilot study (Chung et al., 2016) has investigated the association between chronic BZD use and amyloid deposition, a hallmark of the pathophysiology of AD; the latter study examined a group of nondemented older adults from the Alzheimer's Disease Neuroimaging Initiative cohort. The authors of this pilot study found, unexpectedly, that the 15 individuals who were chronically using BZDs had a decreased amyloid load as assessed with F^{18}-florbetapir positron emission tomography (PET) compared with matched BZD nonusers, which challenges the view of BZDs as a risk factor for AD. Nevertheless, this result is consistent with animal studies that have identified neuroprotective properties of BZDs. Indeed, diazepam was found to be protective against hippocampal cell death caused by amyloid in mice. Furthermore, mice treated with diazepam (Tampellini et al., 2010) (Quiroga et al., 2014) or midazolam (Yamamoto et al., 2015) show lower amyloid deposition than control mice. Finally, there may be a contradiction between epidemiological studies, on the one hand, which found an increased risk or no change in the risk of dementia with BZDs, and pathophysiological studies, on the other hand, which suggest neuroprotective effects of BZDs. However, no large pathophysiological study is available in a clinical population, and the few available data in humans require further replication in larger samples.

In our study, we investigated the association between chronic BZD use and amyloid load as assessed by F^{18}-florbetapir PET in a population of 268 nondemented older adults from the Multidomain Alzheimer Preventive Trial (MAPT) study. The primary goal was to test the efficacy of a multidomain intervention and/or omega-3 polyunsaturated fatty acid supplementation on cognitive decline. Based on results from animal studies and a pilot study, we hypothesized that individuals with BZDs would exhibit lower amyloid loads than matched BZD nonusers would. In addition, we investigated whether certain characteristics of BZD use, including dose, duration of use and short- versus long-acting BZDs, specifically influenced amyloid load.

2. Materials and methods

2.1. Participants

The participants were part of the MAPT study (registration: NCT00672685), which was a large 3-year, multicenter, randomized, placebo-controlled superiority trial with 4 parallel groups. The methods and primary results of the MAPT study have been extensively described elsewhere (Andrieu et al., 2017). Briefly, 1680 nondemented older adults with a memory complaint, limitation in at least one instrumental activity of daily living, or slow gait speed were randomly assigned (1:1:1:1) to the multidomain intervention plus omega-3 polyunsaturated fatty acids, the multidomain intervention plus placebo, omega-3 polyunsaturated fatty acids alone, or placebo alone. The primary goal was to assess the efficacy of the interventions in slowing cognitive decline in older adults at risk of MNCDs. A number of participants also underwent florbetapir PET scanning, which was performed as an ancillary study for amyloid assessment. Among the 1680 participants in the MAPT study, n = 1539 were assessed for eligibility at one of the centers with PET imaging facilities during the clinical follow-up (Fig. 1). A total of 1268 participants were excluded from the MAPT F^{18}-florbetapir ancillary study for the following reasons, as shown in Fig. 1: 705 participants were out of the MAPT study at the time of the implementation of the MAPT F^{18}-florbetapir ancillary study (end of follow-up or early discontinuation); 414 participants refused to participate; 81 participants did not attend the PET visit (due to technical problems or personal reasons); and 68 participants were included in another MAPT substudy for positron-emission tomography - fludeoxyglucose assessment. Ultimately, a total of 271 participants participated in the MAPT F^{18}-florbetapir ancillary study.

At baseline, the participants were community-dwelling men and women without dementia, aged ≥70 years, who met at least one of the following criteria: spontaneous memory complaints, limitation in executing ≥1 instrumental activity of daily living or slow gait speed (<0.8 meters/s). Participants with a Mini-Mental State Examination (MMSE) score lower than 24, those with a diagnosis of dementia, and those with any difficulty in basic activities of daily living were excluded, as were those taking polyunsaturated fatty acid supplements at baseline. In the present analysis, 2 participants were excluded because of missing data regarding BZD use duration, and 1 participant was excluded because of a Clinical Dementia Rating (CDR) score of 1 at the time of the PET scan. Thus, a total of 268 participants were included in the analyses (Fig. 1). Both the MAPT and the F^{18}-florbetapir PET study were approved by the French Ethical Committee in Toulouse (CPP SOOM II), and written consent was obtained from all participants.

2.2. Benzodiazepine use and clinical assessments

Participants were identified as chronic BZD users if they had used any type of BZD for at least 1 year at any time before the PET scan. The duration of BZD use was calculated as follows: end date of use (or date of PET scan, if still on medication at PET scan date) minus starting date of use. The BZD dose was standardized by converting the various BZD doses to diazepam dose equivalents. We also distinguished between short- (half-life ≤ 20 hours) and long-acting (half-life > 20 hours) BZDs. We defined this BZD classification according to the official definition recommended by the French National Agency for Drug Safety (https://www.ameli.fr/fileadmin/user_upload/documents/FEGENOR_PIS_RI_Avis1_CT13342.pdf.) because previous epidemiological studies used this classification and found a significant effect of long-acting BZDs but not short-acting BZDs on AD incidence (Shash et al., 2016).

Clinical assessment included age, sex, educational level, cognitive and dementia status assessed with the MMSE and the CDR, MAPT group allocation (4 groups: placebo, multidomain intervention, n-3 PUFA supplementation and multidomain intervention + n-3 PUFA supplementation), depressive symptoms assessed with the 15-item Geriatric Depression Scale (GDS), history of major depressive episodes (MDEs), history of antidepressant intake, and apolipoprotein E ε4 (ApoE ε4) genotype (carriers of at least one ε4 allele vs. noncarriers).

2.3. F^{18}-florbetapir PET analysis

PET scans were realized as close as possible to a clinical visit during the 3 years of follow-up of each patient, as previously described (Vellas et al., 2014) (Del Campo et al., 2016). Participants

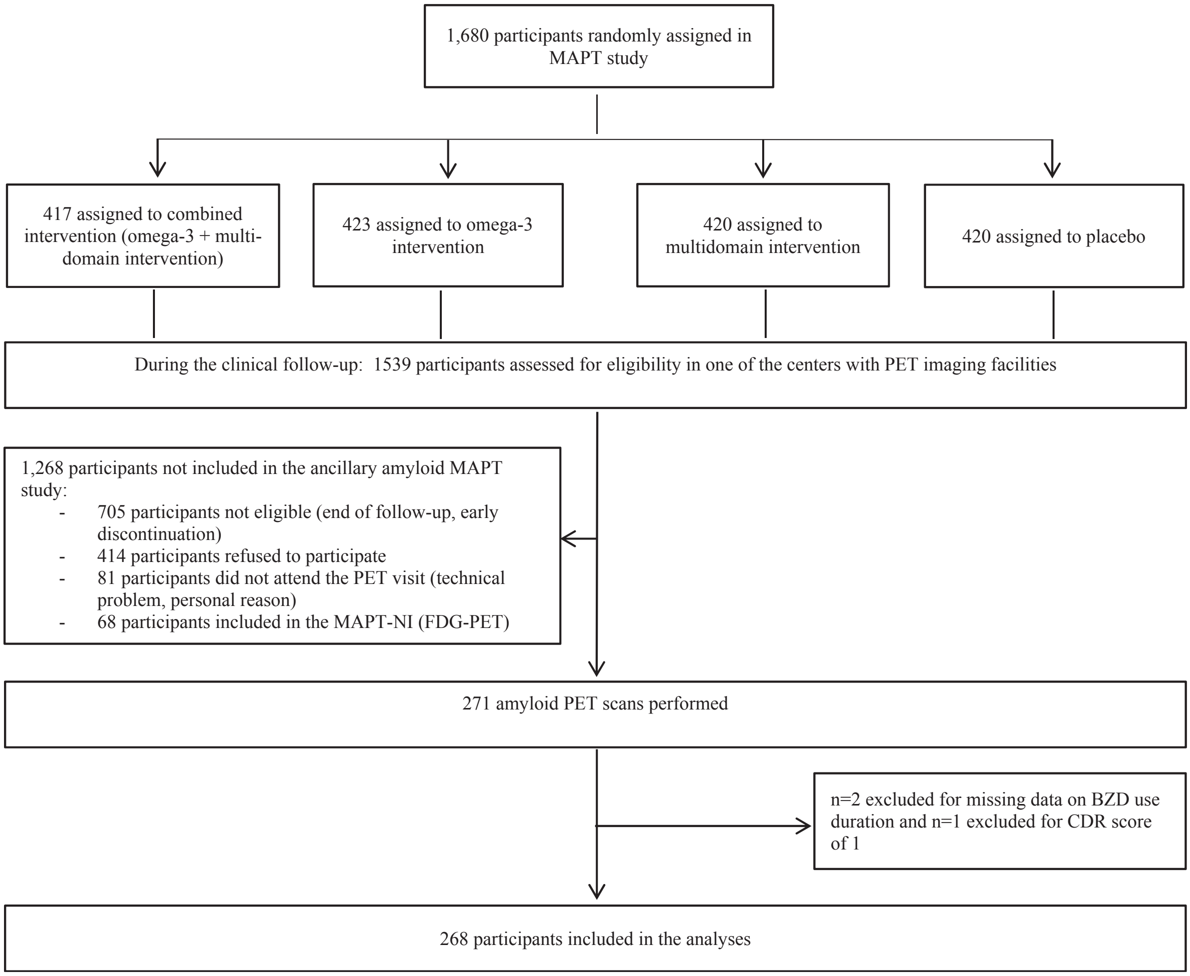

Fig. 1. Flowchart of the MAPT F^{18}-florbetapir substudy. Abbreviations: PET, positron emission tomography; BZDs, benzodiazepines; CDR, Clinical Dementia Rating; MAPT, Multidomain Alzheimer Preventive Trial.

were examined using 5 different hybrid PET-CT scanners, including one PET CT 690 (GE Healthcare), one Discovery RX VCT (General Electric), 2 True Point HiRez (Siemens Medical Solutions), and one Biograph 4 Emission Duo LSO (Siemens Medical Solutions). All tomographs operated in 3D detection mode. All PET sinograms were reconstructed with an iterative algorithm, with corrections for randomness, scatter, photon attenuation, and decay, which produced images with an isotropic voxel of $2 \times 2 \times 2$ mm^3 and a spatial resolution of approximately 5-mm full width at a half maximum at the field of view center. The acquisition data were processed using the standard package delivered with each acquisition system. All cerebral emission scans began 50 minutes after a mean injection of 4 MBq/kg weight of F^{18}-florbetapir. For each subject, 10- or 15-minute frames were acquired to ensure movement-free image acquisition.

A semiautomated quantitative analysis (cortical to cerebellar regional mean standardized uptake value ratio [SUVR]) was applied using the mean signal of 6 predefined anatomically relevant cortical regions of interest (frontal, temporal, parietal, precuneus, anterior cingulate, and posterior cingulate) with the whole cerebellum used as the reference region as previously described (Clark et al., 2011; Fleisher et al., 2011). In this procedure, the F18-florbetapir PET images were coregistered to the F18-florbetapir template provided by AVID company. Quality control based on the semiquantification process was also provided by AVID Lab.

In addition to the SUVR calculation, the PET scans were visually analyzed in the MAPT cohort and classified as either amyloid positive or negative. The methods applied for the visual analyses have been described in detail elsewhere (Payoux et al., 2015), but briefly, the F18-florbetapir PET images were visually assessed by a panel of 3 independent observers, who were specialists in molecular imaging and blinded to all clinical and diagnostic information; the observers used a binary scale to classify each scan as "negative" if there was no significant F18-florbetapir cortical retention or "positive" if there was some significant F18-florbetapir cortical retention; the final consensus allowed each PET scan to be classified as either positive or negative. The mean (standard deviation) SUVR associated with a positive PET scan was 1.35 (0.17), indicating that SUVRs associated with positive PET scans were generally higher than the threshold found in the literature (1.17) (Clark et al., 2011; Fleisher et al., 2011), whereas the mean (standard deviation) SUVR in the negative group was 1.08 (0.1). In our report, we also compared the proportions of positive and negative PET scans between the BZD users and nonusers.

2.4. Statistical analysis

Because the MAPT F^{18}-florbetapir ancillary study was implemented secondarily in the MAPT cohort, the PET scans may have been performed at different time points during the participants' follow-up. Analyses were therefore performed using clinical scores from the follow-up visit closest to the PET scan. Clinical variables were described and compared according to the use of BZDs.

Quantitative variables were described as the means and standard deviations and compared using Student's t-test. Qualitative variables were described as counts and percentages and compared using the chi-squared test.

We used multiple linear regression to estimate the effect of BZD use (the independent variable) on SUVR (the dependent variable). The model included adjustments for age, sex, educational level, MMSE, GDS, history of MDEs, history of antidepressant intake, ApoE ε4 genotype, group allocation in the trial, and duration from baseline to PET scan to account for the potential confounding effects of these factors. In case of missing data on the adjustment covariates, we performed multivariate imputation by chained equations (van Buuren and Groothuis-Oudshoorn, 2011), with 5 imputations. The same analysis was used for each cortical region: frontal, temporal, parietal, anterior cingulate, and posterior cingulate. The use of BZDs was first considered as a binary variable (BZD users vs. BZD nonusers) and then as a 3-category variable (short- vs. long-acting BZDs vs. BZD nonuse). BZD nonusers were used to define the reference level of the variables. In the subgroup of BZD users, we used univariate linear regressions to estimate the link between SUVR and BZD dosage and then between SUVR and BZD duration. Analyses were performed using R 3.2.3 (R Development Core Team. R: A language and environment for statistical computing).

3. Results

3.1. Sample characteristics

The clinical and demographic characteristics of the study participants are shown in Table 1. Forty-seven participants of 268 were identified as chronic BZD users, and the BZDs included in our analyses were as follows: bromazepam (n = 12), clonazepam (n = 6), and prazepam (n = 1) as long-acting GABAergic BZD derivatives; alprazolam (n = 3), lorazepam (n = 3), lormetazepam (n = 4), and oxazepam (n = 1) as short-acting GABAergic BZD derivatives and zolpidem (n = 9) and zopiclone (n = 8) as short-acting GABAergic non-BZD derivatives.

There was no difference between chronic BZD users and BZD nonusers in age, sex, educational level, MMSE scores, CDR score, ApoE ε4 status, and group allocation. The GDS score, history of MDE, and antidepressant intake were increased in chronic BZD users.

3.2. Associations between benzodiazepine use and brain amyloid load

The results of multiple linear regression analyses are illustrated in Figures 2–4. The first model (Fig. 2) includes amyloid SUVR in the total cortex and in 5 brain regions as the dependent variables and chronic BZD use, age, sex, educational level, MMSE, CDR, GDS, history of MDE, antidepressant intake, ApoE ε4 status, group allocation, and duration from baseline to PET scan as explicative variables. In the regression analysis of total cortex SUVR, we found significant effect of chronic BZD use (beta = −0.06, $p = 0.023$), ApoE ε4 status (beta = 0.11, $p < 0.001$), and multidomain intervention (beta = −0.09, $p < 0.001$). SUVRs were significantly lower in BZD users than in nonusers in the total cortex and all the studied brain regions, except the frontal lobe.

In the second model (Fig. 3), where we distinguished short- and long-acting BZDs, we found significant effects of short-acting BZDs on amyloid load (beta = −0.10, $p = 0.005$ for the total cortex SUVR), whereas we found no significant effect of long-acting BZDs (beta = −0.02, $p = 0.6$ for total cortex SUVR). SUVRs were significantly lower in short-acting BZD users compared with nonusers in the total cortex and all the studied brain regions, with the most robust differences found for the parietal cortex and the anterior cingulate.

Table 1
Demographic and clinical characteristics of the population

Demographic and clinical characteristics	BZD users (n = 47)	BZD nonusers (n = 221)	*p* Value
Age (y)	75.7 (3.8)	76.3 (4.5)	0.347
Sex (% female)	31 (66%)	130 (59%)	0.458
Education			0.514
No diploma or primary school certificate	12 (26%)	66 (30%)	
Secondary education	17 (36%)	62 (28%)	
High school diploma	7 (15%)	32 (14%)	
University level	10 (21%)	68 (31%)	
ApoE ε4 carriers	7 (17.9%)	58 (29.9%)	0.186
MMSE	28.2 (1.5)	28.2 (1.6)	0.844
CDR 0.5	21 (44.7%)	120 (54.3%)	0.299
GDS	4.4 (3.7)	2.7 (2.3)	0.004
History of MDE	21 (44.7%)	28 (12.7%)	<0.001
Antidepressant intake	22 (46.8%)	30 (13.6%)	<0.001
Group allocation			0.404
Multidomain intervention	14 (29.8%)	54 (24.4%)	
n-3 PUFA supplementation	8 (17.0%)	52 (23.5%)	
Multidomain intervention and n-3 PUFA supplementation	16 (34.0%)	57 (25.8%)	
Placebo	9 (19.1%)	58 (26.2%)	
Duration from baseline to PET scan (d)	517 (253)	499 (235)	0.631
BZD dosage (mg, diazepam equivalent)	7.8 (9.5)	-	
BZD duration of use (mo)	83.9 (96.8)	-	

Characteristics closest to the PET scan are shown. Values are expressed as the mean (standard deviation) or n (%). Comparisons were performed with t-tests (quantitative data) and chi-squared tests (qualitative data).
Key: PET, positron emission tomography; BZDs, benzodiazepines; ApoE ε4, apolipoprotein E ε4; MMSE, Mini–Mental State Examination; CDR, Clinical Dementia Rating; GDS, Geriatric Depression Scale; MDE, major depressive episode; n-3 PUFA, omega-3 polyunsaturated fatty acid.

In addition, we found that the proportion of negative PET scans in the BZD users was significantly greater than that in the nonusers (n = 37, 79% vs. n = 140, 64%, respectively, $\chi^2_1 = 4.085$, $p = 0.043$), and notably, 23 (85%) negative PET scans were found in the short-acting BZD group.

3.3. Effect of dose and duration of benzodiazepine use on brain amyloid load

We found no significant associations between SUVR in the total cortex and dose (beta = 0, $p = 0.896$) or duration of BZD use (beta = 0, $p = 0.625$) in the subgroup of BZD users.

4. Discussion

We found that nondemented older adults who chronically use BZDs had a reduced brain amyloid load compared with BZD nonusers, independent of potential confounding factors such as cognitive impairment and history of depression and antidepressant intake. In addition, we found that short-acting BZDs showed the strongest effect, whereas we found no effect of dose or duration of BZD use on brain amyloid load. Our results are consistent with a previously published pilot study (Chung et al., 2016) that found reduced brain amyloid in chronic BZD users from the Alzheimer's Disease Neuroimaging Initiative cohort. In addition, our results are consistent with animal studies showing that administration of BZDs reduces brain amyloid deposition.

BZDs act as positive allosteric modulators of GABA-A receptors and depress neuronal activity via the increase in intracellular chlorine ions through chloride channels, hyperpolarizing the cell and decreasing its probability of firing. Neurodegeneration and reduction in neuronal and synaptic activity are key features of the

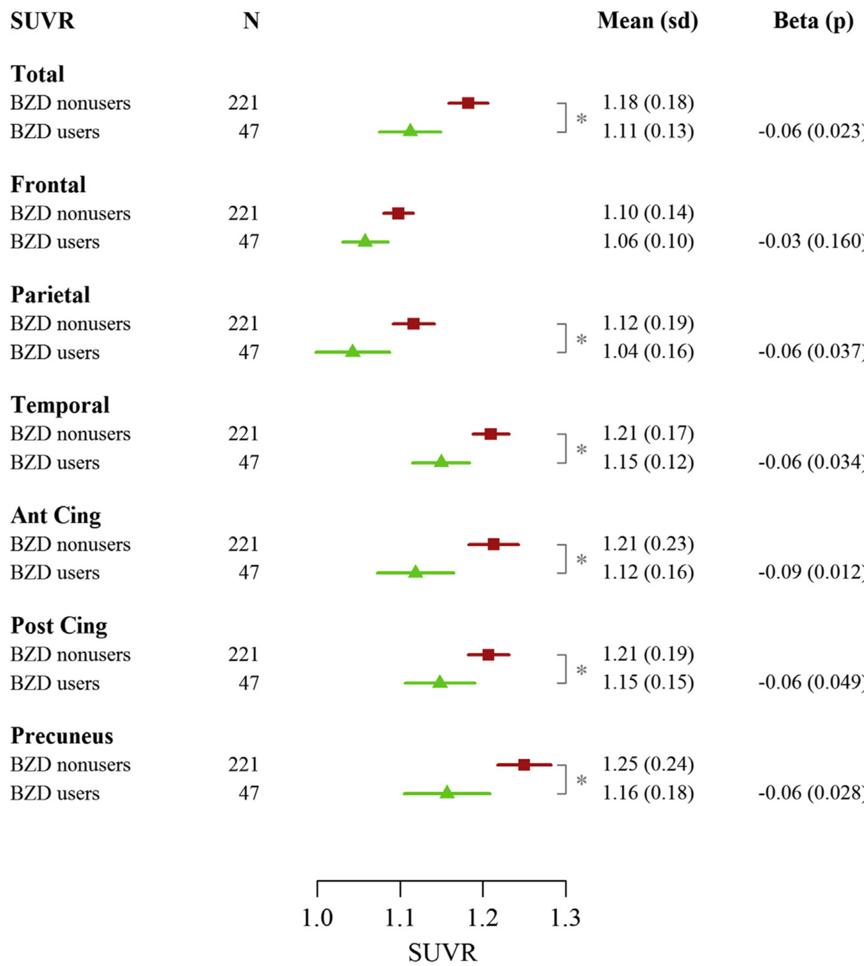

Fig. 2. Multiple linear regressions of the associations between benzodiazepine use (benzodiazepine users in green triangles, benzodiazepine nonusers in red squares) and brain amyloid load as assessed with F^{18}-florbetapir PET in total cortex and 6 brain regions. The amyloid load was significantly lower in BZD users compared with BZD nonusers in the total cortex and all the studied regions except the frontal cortex after controlling for age, sex, educational level, MMSE, CDR, GDS, history of MDE, antidepressant intake, ApoE ε4 status, group allocation and duration from baseline to PET scan. * indicates a *p*-value < 0.05. Abbreviations: PET, positron emission tomography; SUVR, standardized uptake value ratio; Ant Cing, anterior cingulate; Post Cing, posterior cingulate; BZDs, benzodiazepines; ApoE ε4, apolipoprotein E ε4; MMSE, Mini–Mental State Examination; CDR, Clinical Dementia Rating; GDS, Geriatric Depression Scale; MDE, major depressive episode. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

later stages of AD. In contrast, recent findings tend to suggest that neuronal hyperactivity is one of the earliest dysfunctions in the pathophysiological cascade of AD, with neuronal hyperactivity preceding the formation of amyloid plaque in animal studies (Busche and Konnerth, 2016). Indeed, a positive longitudinal correlation has been found between neuronal activity and amyloid accumulation, which suggests that regulation of neuronal activity may modulate amyloid production (Bero et al., 2011) (Cirrito et al., 2008). Interestingly, although neuronal hyperactivity is associated with increased amyloid accumulation, a reduction in neuronal activity results in decreased amyloid production, as well as reduced axonal dystrophy and synaptic loss in areas near amyloid plaques (Yuan and Grutzendler, 2016). In agreement with this concept, Cirrito et al., 2005 found that a greater neuronal firing rate increased extracellular amyloid deposition in mice, whereas inhibition of neuronal activity decreased the amyloid load. Consistent results have been found in vitro using hippocampal slices, in which increased neuronal activity has been found to promote amyloid deposition by modulating beta-secretase processing of amyloid precursor protein (APP) (Tampellini et al., 2009). Preclinical findings from sleep induction (Boespflug and Iliff, 2018) and from epilepsy (Sen et al., 2018) also suggest a similar relationship between neuronal activity and amyloid production. Some human studies tend to be consistent with this relationship, especially a recent study that found that cognitively normal older adults with the greatest activation on functional magnetic resonance imaging during a memory task showed the highest accumulation of amyloid plaque 2 to 6 years later (Leal et al., 2017). These results suggest that individuals who require additional neuronal activation to perform in the memory task may be at an increased risk of amyloid accumulation, whereas those with low neuronal activation in the memory task, although performing well, may have a lower risk of amyloid production. Based on these accumulating findings, some authors have proposed that targeting neuronal hyperactivity with pharmacological treatment such as antiepileptic drugs may attenuate amyloid progression and ultimately prevent the development of AD (Haberman et al., 2017). Consistently, the reduced amyloid load observed in the BZD users in our study may be related to the GABAergic effect of BZDs by which BZDs decrease neuronal excitability.

The other possible explanations of our findings are related to the effect of BZDs on translocator protein (TSPO) 18 kDa, which was formerly known as peripheral BZD receptor, and mechanisms related to neuroinflammation as a potential risk factor for cognitive impairment. TSPO is an outer mitochondrial membrane protein involved in steroid metabolism and other mitochondrial functions,

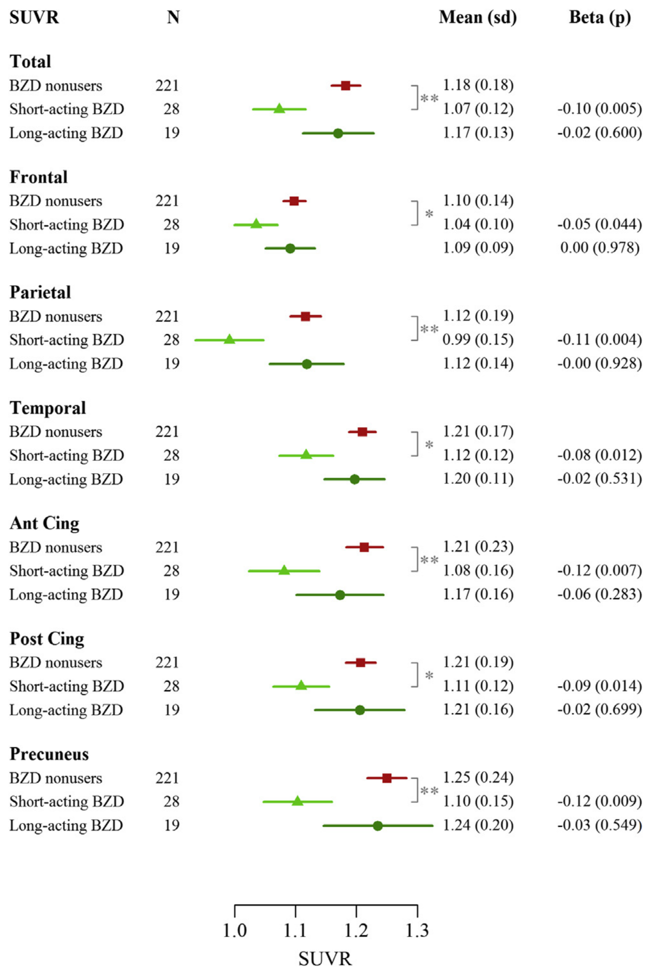

Fig. 3. Multiple linear regressions of the associations between benzodiazepine use, divided according to short-acting benzodiazepines (light green triangles), long-acting benzodiazepines (dark green circle), and benzodiazepine nonusers (red squares) and brain amyloid load as assessed with F^{18}-florbetapir PET in total cortex and 6 brain regions. The amyloid load was significantly lower in short-acting BZD users compared with BZD nonusers in the total cortex and all the studied regions after controlling for age, sex, educational level, MMSE, CDR, GDS, history of MDE, antidepressant intake, ApoE ε4 status, group allocation, and duration from baseline to PET scan. In contrast, there were no significant differences between long-acting BZD users and nonusers in brain amyloid load. * indicates a p-value < 0.05, ** indicates a p-value < 0.01. Abbreviations: PET, positron emission tomography; SUVR, standardized uptake value ratio; Ant Cing, anterior cingulate; Post Cing, posterior cingulate; BZDs, benzodiazepines; ApoE ε4, apolipoprotein E ε4; MMSE, Mini–Mental State Examination; CDR, Clinical Dementia Rating; GDS, Geriatric Depression Scale; MDE, major depressive episode. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

including cell proliferation and differentiation, mitochondrial respiration and regulation of cytochrome C release, caspase activation, and apoptosis (Veenman et al., 2007). Ligands of TSPO elicit pleiotropic neuroprotective effects including reduction of amyloid accumulation (see Arbo et al., 2015 for review). Specifically, the effect of BZDs on amyloid as ligands of TSPO may be mediated by modulation of neuroinflammation via the synthesis of neurosteroids, which has been found to modulate amyloid pathology (Minter et al., 2016).

We found that users of short-acting BZDs but not users of long-acting BZDs had lower amyloid loads than BZD nonusers. Epidemiological studies tend to find that long-acting BZDs but not short-acting BZDs are associated with an elevated risk of MNCDs (Billioti de Gage et al., 2015), although no clear explanation has been found yet. Pharmacological differences between short- (especially Z-drugs) and long-acting BZDs include differences in alpha 1 receptor affinity, with short-acting BZDs showing higher alpha 1 affinity, which results in a stronger soporific effect (Nutt and Stahl, 2010). Interestingly, recent evidence suggests that poor sleep quality promotes amyloid pathology, and conversely, individuals with good sleep quality may be at reduced risk of brain amyloid accumulation (Boespflug and Iliff, 2018). Therefore, in our study, the reduced amyloid load in short-acting BZD users may be explained by superior sleep quality in these individuals. However, only a few participants in the MAPT cohort were assessed for sleep quality, and we were unable to reliably test this hypothesis.

The most robust region-specific associations were observed in our study for the anterior cingulate and the parietal cortices, similar to findings from the previously published pilot study (Chung et al., 2016). However, because we found that the total amyloid load was smaller in BZD users, it is likely that BZDs have a global effect on brain amyloid. The most significant differences observed in the anterior cingulate and parietal cortices may be related to recent findings showing that early accumulation of amyloid occurs primarily in these regions (Grothe et al., 2017). Indeed, the nondemented individuals in our study are likely to show early progression of amyloid pathology, and differences between BZD users and nonusers may be at a maximum in these regions of early amyloid deposition, whereas amyloid deposition may be less significant in other brain regions with smaller differences between BZD users and nonusers.

We found no association between the amyloid load and dosage or duration of BZD use, which is consistent with a previously published pilot study (Chung et al., 2016). A possible explanation of this result is related to the potential ceiling effect of BZDs on amyloid, suggesting that after a certain dose or time of use, no additional benefit in lowering the amyloid load is achieved with BZDs. Another complementary explanation could be that the maximum lowering effect of BZDs occurs with a relatively short usage and that longer usage has no further benefit on the amyloid load. This hypothesis is consistent with preclinical studies showing that the in vitro effects of BZDs on amyloid formation are rapid and occur within hours of exposure (Yamamoto et al., 2015). In our study, we have limited the scope of the investigation to chronic BZD use lasting longer than one year, and it remains unclear whether a shorter duration of use could also have an impact on amyloid load, which may be investigated in further studies.

We found no significant difference in the MMSE score between the BZD users and nonusers despite the lower amyloid load in the BZD users. A greater amyloid load has been associated with lower cognitive performance, and individuals with a lower amyloid load are expected to exhibit greater cognitive performance. However, previous studies have suggested that the MMSE may be inappropriately sensitive in reflecting cognitive deficits related to amyloid deposition, especially in nondemented individuals with high MMSE scores and low interindividual variations, such as those included in our study (Lim et al., 2016). In addition, we may hypothesize that the greater cognitive performance observed in the BZD user group due to the lower amyloid load could have been counteracted by the well-known negative effect of BZDs on cognitive performance, resulting in a similar cognitive impairment to that observed in the BZD nonusers with a higher amyloid load.

Limitations of our study include that the MAPT study was not primarily designed to assess the effect of BZD use on the brain amyloid load, and our results were derived from a secondary analysis, which may limit the strength of our conclusions. Moreover, the use of a cross-sectional design to examine the association between BZD use and amyloid load prevents inference of causality; the possible causal relationship between reduced amyloid load and BZDs remains to be confirmed. In addition, no measure of

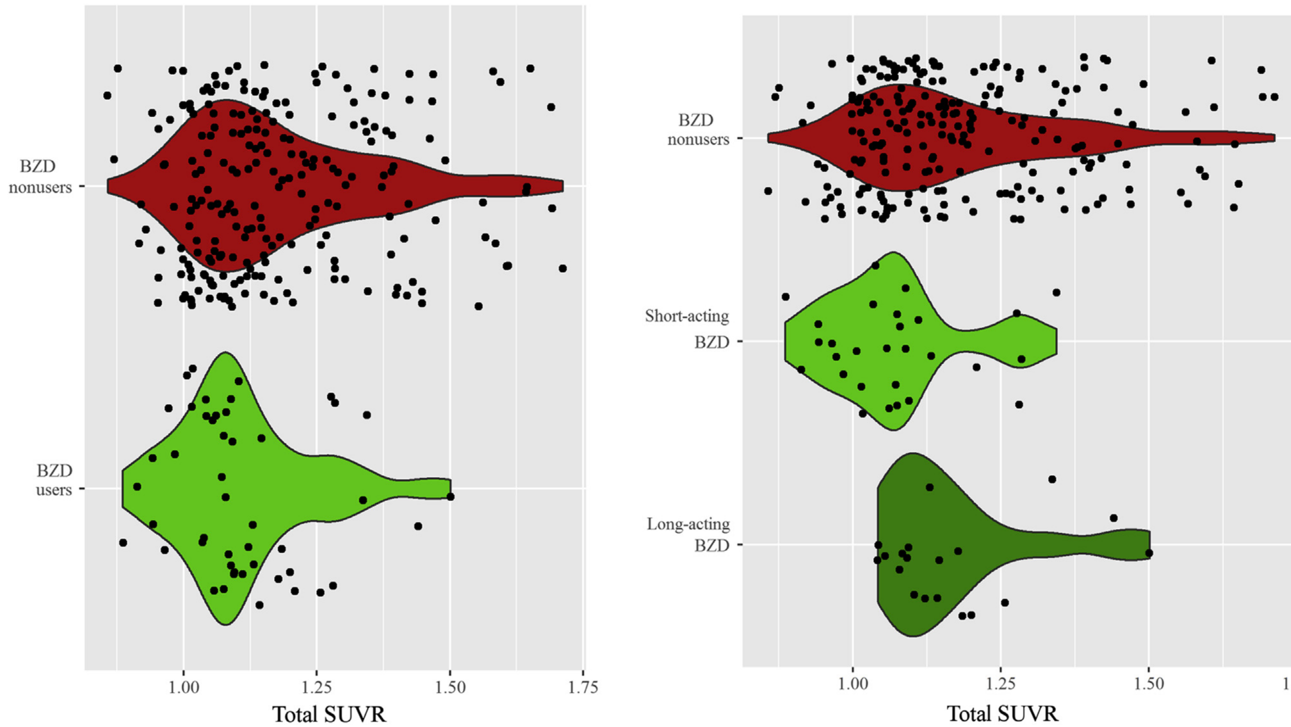

Fig. 4. Scatterplots of the individual total SUVR in BZD users and nonusers (left graph) and in BZD nonusers and short-acting BZD users and long-acting BZD users (right graph). Abbreviations: BZD, benzodiazepine; SUVR, standardized uptake value ratio.

anxiety was available in the MAPT study, and only a few participants had sleep quality assessments. These potential confounding factors may have influenced our results because anxiety and sleep quality have been previously associated with changes in amyloid load. Participants also had PET scans at different time points during follow-up, which may constitute a limitation. However, controlling for duration from baseline to PET scans in our regression models did not influence the associations between BZD use and amyloid load. Regarding the distinction between short- and long-acting BZDs in our analyses, we had relatively small sample sizes; thus, the predominant effect of short-acting BZDs on brain amyloid should be considered with caution. Finally, the effect of BZDs on the amyloid load was relatively small in our study, and we could not exclude the possibility that the observed differences were derived from a subgroup of individuals among the BZD nonusers with a total SUVR greater than 1.5. Nevertheless, most BZD users had SUVRs located within the SUVRs of the visually rated negative PET scans, and compared to the nonusers, we found a greater proportion of negative PET scans among the BZD users, suggesting a potentially clinically relevant effect of BZDs on brain amyloid.

In conclusion, our results do not support the view of BZDs as risk factors for AD and instead suggest that pharmacological mechanisms related to reduction in neuronal activity, neuroinflammation, and/or sleep quality may be involved in the reduction of amyloid pathology. In fact, and although epidemiological studies found conflicting results, a recent well-controlled study found that, after controlling for multiple confounding factors including psychiatric disorders, BZD use was associated with a reduced incidence of AD (Imfeld et al., 2015). However, we do not intend to suggest that BZDs should be used to prevent AD specifically because the chronic use of BZDs has several side effects that certainly overcome the potential benefits on amyloid pathology. Guidelines for BZD prescription should be carefully observed, including the duration of use, which may not exceed 1 month as a hypnotic and 3 months as an anxiolytic. Moreover, reducing amyloid pathology does not necessarily lead to a decreased incidence of AD, which involves multiple other pathophysiological mechanisms, such as tau pathology and vascular disorders. Interestingly, a recent animal study found that lowering neuronal activity may indeed prevent amyloid formation but simultaneously promotes tau pathology and causes detrimental effects on synapses in AD transgenic mice (Akwa et al., 2018). Nevertheless, our paper suggests that further investigations of GABA- and/or TSPO-related mechanisms involved in neuronal excitability, neuroinflammation, and sleep quality may allow the identification of novel pathophysiological pathways in AD and provide pharmacological targets to reduce amyloid formation.

Disclosure

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