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Benzodiazepine addiction: From lab to street

Edited by
Lais F. Berro, Thiago Marques Fidalgo, James K. Rowlett and Vitor Tardelli

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Benzodiazepine addiction: From lab to street

Topic editors

Lais F. Berro — University of Mississippi Medical Center, United States
Thiago Marques Fidalgo — Federal University of São Paulo, Brazil
James K. Rowlett — University of Mississippi Medical Center, United States
Vitor Tardelli — University of Toronto, Canada

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Yasser Khazaal,
Université de Lausanne, Switzerland

*CORRESPONDENCE
Lais F. Berro
✉ lberro@umc.edu

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Editorial: Benzodiazepine addiction: from lab to street

Lais F. Berro[1]*, Thiago M. Fidalgo[2], James K. Rowlett[1] and Vitor S. Tardelli[2,3]

[1]Department of Psychiatry and Human Behavior, Center for Innovation and Discovery in Addictions, University of Mississippi Medical Center, Jackson, MS, United States, [2]Department of Psychiatry, Universidade Federal de São Paulo, São Paulo, Brazil, [3]Translational Addictions Research Lab, Centre for Addiction and Mental Health, Department of Psychiatry, University of Toronto, Toronto, ON, Canada

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benzodiazepine, Z-drug, abuse, dependence, prescriptions

Editorial on the Research Topic
Benzodiazepine addiction: from lab to street

Benzodiazepine-type drugs (benzodiazepines and newer non-benzodiazepines, such as "Z-drugs") are important therapeutic tools in psychiatry and general medicine. Despite their clinical usefulness, benzodiazepine-type drugs also are associated with several unwanted side effects, including abuse and dependence. In fact, the misuse and abuse of benzodiazepines have increased dramatically in recent years, with overdose deaths on the rise, especially with combinations of benzodiazepines and opioids (1). Given these concerns, the goal of this Research Topic was to highlight novel research examining factors related to the misuse, abuse, and dependence associated with benzodiazepine-type drugs. The success of this Research Topic, which includes 14 published manuscripts ranging from study protocols to review articles, emphasizes the growing interest and importance of this subject to the scientific community. This Research Topic spans multiple topics of investigation relating to benzodiazepine research, including pre-clinical studies, new epidemiology and novel treatment approaches.

Epidemiological studies published in this Research Topic emphasize that benzodiazepine use is on the rise worldwide. Coteur et al. reported an overall increase in benzodiazepine-type drug prescriptions between 2000 and 2019 in Flanders, Belgium. This was manifested as an increase in the number of male patients receiving three or more prescriptions at ages 18–44 and female patients over 65 years of age (Coteur et al.). McHugh et al. reported data on the prevalence of benzodiazepine and Z-drug misuse in the U.S. National Survey on Drug Use and Health from 2015 to 2019. According to their findings, 2% of the population was estimated to have misused a benzodiazepine in the past year, while <0.5% misused Z-drugs. Of note, studies in this Research Topic also corroborate the notion that benzodiazepine use increased due to the COVID-19 pandemic. Perelló et al. conducted a prospective observational study on benzodiazepine prescriptions in Catalonia from March 2020 to December 2021, showing an increase in benzodiazepine prescriptions during that period compared to the previous 2 years.

As evidenced by the studies by Coteur et al. and Perelló et al., the recent increase in benzodiazepine use is partially due to higher benzodiazepine prescription rates. Takeshima et al. reported that physicians are compelled to prescribe benzodiazepine-type drugs frequently despite rating these drugs as unsafe, often choosing efficacy over safety. In fact, the authors describe that physicians often opt to prescribe benzodiazepine drugs over other sleep

aids rated as both safe and effective, such as orexin receptor antagonists (Takeshima et al.). These findings suggest that interventions are needed to reduce benzodiazepine prescription rates and, consequently, the public health burden of benzodiazepine use. To address this issue, Kinney et al. proposed the use of machine learning methods to develop algorithms to classify patients by their likelihood of receiving a benzodiazepine prescription and the number of benzodiazepine prescriptions they are likely to receive at a given patient-physician encounter. Their study showed that support-vector machine and random forest algorithms can accurately classify individuals who are at risk for receiving a benzodiazepine prescription (Kinney et al.), which could ultimately guide clinical practice.

Several other factors also can influence benzodiazepine use. Zandonai et al. describe clinical cases of elite endurance athletes reporting benzodiazepine use to manage insomnia, pain, and to speed up recovery. Of note, the authors emphasize that sports medicine physicians are often unaware of the dangers associated with chronic benzodiazepine use, and benzodiazepine prescription and tapering guidelines are discussed (Zandonai et al.). As part of their physician guidelines, the authors emphasize the need to taper the benzodiazepine dosage while introducing an alternative therapy. In accordance with the International Patient Decision Aid Standards, Aoki et al. developed a decision aid for individuals with anxiety disorders to help with decision-making regarding discontinuation of benzodiazepine treatment. The goal of their approach was to aid patients and healthcare providers in determining whether or not to taper off of benzodiazepines and, if tapering, whether or not to implement cognitive behavioral therapy for anxiety during tapering (Aoki et al.). Their decision aid was well-accepted by both patients and physicians, and could become an important clinical tool.

Greenwald et al. also reported that anhedonia (positive-affective deficit) predicted increased benzodiazepine demand in past-year benzodiazepine users receiving treatment for opioid use disorder. Anhedonia also predicted opioid demand, emphasizing that deficits in the experience and anticipation of reward seem to influence the use of these drugs (Greenwald et al.). In addition to clinical studies, a pre-clinical investigation by Jovita-Farias et al. investigated the relationship between different behavioral effects of the benzodiazepine midazolam in male mice, demonstrating that midazolam preference (i.e. reward) is a multifactorial behavior, and is not dependent solely on the emergence of therapeutic (anxiolytic-like) effects, learning impairments, or on genetic factors. Together, these findings suggest that many factors can interact to influence the decision to use benzodiazepines, both clinically and recreationally, and that further studies are needed to determine factors contributing to benzodiazepine use. To address this gap, Zamboni et al. propose a study protocol using virtual reality to assess the impact of benzodiazepine-associated environmental cues on patient-reported benzodiazepine craving and affective states.

A common theme across several of the publications in this Research Topic was the investigation of Z-drug use as a potential emerging problem. Coteur et al. reported that while alprazolam was the most largely prescribed benzodiazepine in 2000, by 2019 zolpidem had become the most largely prescribed benzodiazepine-type drug in Flanders, Belgium. On the other hand, McHugh et al. reported that Z-drug misuse in the U.S. was less common than benzodiazepine misuse, and those reporting Z-drug misuse presented less concurrent substance use and lower clinical severity. In agreement, Campagnari et al. showed that the use of high doses of zolpidem was not associated with adverse cardiovascular effects (specifically, corrected QT interval elongation), suggesting that zolpidem is a safe drug even when used at higher than recommended doses. Furthermore, Koniuszewski et al. screened the publicly available U.S. FDA adverse event reporting system database for benzodiazepine-type drugs, and their findings suggest that benzodiazepines and Z-drugs differ vastly in adverse event profiles, with benzodiazepines showing a higher incidence of adverse events. Together, these findings suggest that while Z-drug prescription is on the rise, Z-drugs may be safer than conventional benzodiazepines. Further research is necessary to conclusively determine the clinical implications of long-term Z-drug use and misuse.

Of note, Koniuszewski et al. also reported significant sex differences in the rates of adverse events reported for benzodiazepine-type drugs. Specifically, neuropsychiatric adverse events observed for conventional benzodiazepines were more prevalent in females than in males (Koniuszewski et al.). While not directly investigated in their study, the authors emphasize the possibility that steroid hormones may influence the emergence of benzodiazepine-induced adverse events. In fact, Cook et al. demonstrated that, in contrast to their previous study with males, combinations of the conventional benzodiazepine triazolam and the neuroactive steroid pregnanolone induced synergistic reinforcing and sedative effects in female rhesus monkeys. These results corroborate the notion that sex differences exist in benzodiazepine-neuroactive steroid combinations, which could contribute to the different side effect profiles reported between sexes in the study by Koniuszewski et al..

Finally, the thorough review by Engin discussed the mechanisms underlying the abuse/misuse-related effects of benzodiazepine-type drugs. The author reviewed studies suggesting that α1-containing $GABA_A$ receptors may play an important role in benzodiazepine reinforcement, tolerance and dependence. The findings summarized in this review highlight the progress in the field of benzodiazepine research, yet also emphasize the need for further, systematic investigations elucidating the mechanisms underlying benzodiazepine misuse, abuse and dependence. For instance, zolpidem, the most widely prescribed Z-drug (Coteur et al.; McHugh et al.), has selective affinity for α1-containing $GABA_A$ receptors, which, according to the review by Engin, would predict a higher potential for abuse compared to conventional benzodiazepines. However, the study by McHugh et al. shows that, in the U.S., Z-drug misuse is less prevalent than benzodiazepine misuse. Together, these findings suggest that other abuse-related mechanisms also may be at play, including other $GABA_A$ receptor subtypes (e.g., α2-containing $GABA_A$ receptors, see Engin), and highlight the need for further cross-talk between pre-clinical and clinical researchers.

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EDITED BY
Lais F. Berro,
University of Mississippi Medical Center, United States

REVIEWED BY
Kerry Kinney,
University of Mississippi Medical Center, United States
Arghya Pal,
All India Institute of Medical Sciences, India

*CORRESPONDENCE
Kristien Coteur
kristien.coteur@kuleuven.be

†Deceased

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Evolution of benzodiazepine receptor agonist prescriptions in general practice: A registry-based study

Kristien Coteur*, Pavlos Mamouris, Bert Vaes, Marc Van Nuland, Catharina Matheï† and Birgitte Schoenmakers

Department of Public Health and Primary Care, Academic Center for General Practice, KU Leuven, Leuven, Belgium

Background: Contrary to most European guidelines, benzodiazepine receptor agonists (BZRA) are often used continuously at a low dosage, being the most common form of long-term use. In Belgium, BZRA use is monitored by analyzing self-report data about medication use in the last 24 h. This method provides insufficient insight into the terms of use of these psychoactive drugs.

Aim: To describe trends in BZRA prescribing in Flanders, Belgium, between 2000 and 2019.

Design and setting: Population-based trend analysis and a case-control study for the year 2019 were done with data from a morbidity registry in general practice.

Methods: Repeated cross-sectional and joinpoint regression analyses revealed trends in sex- and age-standardized prescription rates among adult patients (18+).

Results: Overall, BZRA prescriptions increased. The highest overall increase was found among male patients 18–44 years old, with an average annual percentage change of 2.5 (95% CI: 0.9, 4.3). Among 65+ female patients, a decrease was found since 2006, with an annual percentage change of −0.7 (95% CI: −1.3, −0.1). In 2019, 12% of registered patients received minimally one prescription, long-term use was observed in 5%, back pain was the most common morbidity significantly associated with a rise in BZRA prescriptions, and zolpidem was the most prescribed BZRA (22%).

Conclusion: Despite some statistically significant decreasing trends, an overall increase in BZRA prescriptions was observed throughout the 19-year study period, especially among long-term users of 18–44 years and 65-plus. Zolpidem became the most prescribed BZRA and warrants more attention.

KEYWORDS

general practice, benzodiazepines, hypnotics and sedatives, public health, inappropriate prescribing

Introduction

In 2018, Belgium reported the highest consumption rate of zolpidem and the third highest consumption rate of benzodiazepines worldwide (1). With 12.73 million packs, or 434.62 million daily defined doses (DDDs) dispensed in ambulatory care, in a country with 11.38 million inhabitants in 2018, the use of benzodiazepine receptor agonists (BZRA) can be perceived as problematic (2).

BZRA are psychoactive drugs that enhance the effect of the neurotransmitter gamma-aminobutyric acid (GABA) in the central nervous system (3–9). Sedating the user, BZRA have both anxiolytic and hypnotic effects. Furthermore, they have anticonvulsive, myo- and vasorelaxant, amnestic and motor skill-impairing effects. The strength of these clinical effects is product specific. In general practice, BZRA are often used to treat insomnia, anxiety, and muscle tension, but also addiction, agitation and neurological disorders (3–5, 10–29).

The majority of BZRA in Belgium is used as hypnotics and anxiolytics, for which multiple expert groups promote a reticent policy. Belgian prescribing guidelines concur with the guidelines in most European countries, stating that BZRA should be used in the lowest possible dose and for the shortest possible duration, i.e., maximally 1–2 weeks for insomnia, and 2–4 weeks for anxiety (3, 30–33). Nonetheless, BZRA are often used continuously at a low, steady dosage, being the most common form of long-term use (12).

Although long-term use may be medically justified for some patients (34), it is associated with serious health problems, such as cognitive impairment, fall risk and resultant hip fractures, insomnia, memory disorders, especially in older populations, and drug dependence (10–18). Therefore, BZRA use should be stabilized or reduced to positively affect public health. Recently, three European registry-based studies have reported positive evolutions, namely an overall decrease in BZRA use. However, two of these studies, in Ireland and Finland, found a decrease in benzodiazepine use but an increase in z-drug use in 2006 and 2005 (34–36). In Belgium, the only published data about the evolution of BZRA use comes from patients' self-reports (37). The most recent survey results suggest a similar evolution, with a decrease in benzodiazepine users from 6.1 to 4.3% between 2004 and 2018 and a slight increase in z-drug users, from 1 to 1.2% (38). However, these results only consider patients' medication use in the last 24 h and do not provide insights into the short-term or long-term use of these psychoactive drugs. Because BZRA are only available upon prescription, the dispensed amounts suggest that patients often receive repeat prescriptions, which are provided by the general practitioner. Generally, half of the prescriptions in ambulatory care are provided by a primary care physician (39). Therefore, we aim to analyze the trends in BZRA prescription rates between 2000 and 2019 using the prescription data of the primary care-based Intego project (40).

Materials and methods

Intego database

Intego, which stands for "integrated computerized network", was established by the Department of General Practice of KU Leuven in Belgium, in 1990. The database of this network contains demographic, clinical, biomedical, and prescription data, which are collected during general practitioners' daily practice. Registration with computer-generated keywords, in the electronic health record, provides a link to classifications such as the International Classification of Primary Care (ICPC-2) for diagnoses, and WHO's Anatomical Therapeutic Chemical (ATC) for medications (Supplementary overviews 1, 2). Participating general practitioners are located in the Flanders region of Belgium, where 58% of the Belgian population resides. The data was found to accurately represent the Flemish population for age and sex (40). In 2019, data was provided by 431 general practitioners, working in 86 practices with an optimal registration performance, meaning that more than 80% of their registered diagnoses were coded. The denominator was the yearly contact group (YCG), which consists of patients who visit the practice at least once in a given year (40).

The ethical committee of KU Leuven Medical School and the Belgian Privacy Commission approved the Intego procedure (ML 1723; SCSZG/13/079).

Study design and population

A population-based trend analysis was done with data collected from 2000 to 2019. Data from the years 2020 and 2021 were excluded to prevent potential bias by COVID-19. A case-control study, in which controls were patients who did not receive BZRA prescriptions, with data from 2019, was performed to contextualize population characteristics. Patients 18 years or older who received minimally one BZRA prescription were selected.

BZRA were defined as the ATC classifications N03AE, N05BA, N05CD, and N05CF. Two groups of patients are compared: patients who received < 3 BZRA prescriptions per year, and those who received three or more prescriptions in 1 year. This prescribing pattern corresponds with the most common definitions of long-term use in interventional trials, being 3–6 months of BZRA use (12, 41–53).

Statistical analysis

A repeated cross-sectional analysis, using a Chi-square trend test with a confidence interval of 95%, was conducted for two time periods (2000 and 2019) to investigate changes in BZRA prescription rates, diagnoses, and prescriptions of other psychoactive medication. This method was also used in the case-control study. Per case, three optimally chosen controls were used. They were matched for practice, age—with a maximum difference of 2 years, and sex. These analyses were performed with R version 4.0.3 and the ccoptimalmatch R package (54, 55).

For the joinpoint regression analyses (JPRA), annual prescription rates were calculated in the total study population and in different groups: male and female, occasional and long-term users, 18–44 years, 45–64 years, and 65 years or older (65+). JPRA is a well-known method for identifying and studying statistically significant trends over time (56). The points with significant changes in prescription rates, join points, are determined by piecewise linear regression. At least four observations between two join points or three observations to the end of the data are needed to map trends. Trends are expressed by two sets of parameters: the annual percentage change (APC) and the average annual percentage change (AAPC). The APC is computed for each trend separately. Trends over the whole period of 2000–2019 were summarized using the AAPC, which is the estimated average of APC per trend weighted by the corresponding trend length. The significance of both parameters is determined with a 95% confidence interval. SEER*Stat package from the Surveillance Research Program of the US National Cancer Institute was used to perform JPRA (57).

Results

Study population

In 2000, the Intego database contained data of 79,600 patients. Of these patients, 9% (n = 7,209) received minimally one BZRA prescription. By 2019, this increased to 12% (N = 206,135; n = 24,962), which corresponded with a 2% rise in the sample of patients with three or more prescriptions in 1 year, and a 1% rise in the sample of patients with <3 prescriptions in 1 year (Table 1). Within both samples, the most often prescribed BZRA changed from lorazepam in 2000 to zolpidem by 2019 (Table 2).

In 2019, 28% of patients with <3 prescriptions in 1 year and 12% of patients with three or more prescriptions in 1 year were 18–44 years old. Only in the latter sample were BZRA most prescribed to patients 65 years or older (55%). Of all patients who received a BZRA prescription, over 60% were female. In 2019, the most common diagnoses in the study population were back pain, hypertension, depression and cancer. Insomnia and anxiety held the sixth and eighth positions. All co-morbidities except depression increased significantly since 2000. Finally, a significant rise of 15% in concomitant opioid prescriptions was observed between 2000 and 2019. Among patients with three or more prescriptions in 1 year, 45% had received a prescription for opioids in 2019. A similar but less pronounced rise was also found for antidepressants, from 38 to 46% (Table 1).

Comparison to control population

From 2000 to 2019, back pain had risen by an average factor of 1.35 in both samples (Table 1). This diagnosis was statistically associated with BZRA prescriptions when compared to the control population. Although depression did not significantly rise in the study population between 2000 and 2019 (Table 1), there was a clear association with BZRA prescriptions (Supplementary Figure S3). All diagnoses under investigation except dementia and concomitantly prescribed psychoactive drugs were significantly associated with BZRA prescribing (Supplementary Figure S3).

Trends in BZRA prescriptions

Patients with <3 BZRA prescriptions in 1 year

In all age categories, a statistically significant increase was found as final trend, starting in 2012 (18–44 years: APC = 4.4; 95% CI: 2.6, 6.1; 45–64 years: APC = 2.6; 95% CI: 4.4, 3.7) and 2016 (65 years and older: APC = 4.7; 95% CI: 0.2, 9.3). In the category 18–44 years, also the overall prescription rate increased, with a significant AAPC of 1.4 (95% CI: 0.6, 2.2). Analyzing the sex-standardized trends, an overall rising trend was found in male patients, with an AAPC of 0.9 (95% CI: 0.2, 1.6). In female patients, a significant rising trend since 2012 was found in all age categories except 65 years or older. Detailed results are shown in Table 3.

Patients with ≥3 BZRA prescriptions in 1 year

Although not all statistically significant, the latest trends in this sample were decreasing trends with APCs ranging from −4.3 (95% CI: −9.4, 1.0) to −0.7 (95% CI: −1.3, −0.1), except among female patients between 18 and 64 years. In the youngest category of female patients, an overall increase was found (AAPC = APC = 1.1; 95% CI: 0.5, 1.7). In the category of 45–64 years, trends fluctuated more (Table 4).

In all categories, significant increases were found before any decreasing trends, resulting in overall rising trends with significant AAPCs ranging from 1.0 (95% CI: 0.1, 1.8) to 2.5 (95% CI: 0.9, 4.3) (Table 4). The fluctuations and strength of these trends are illustrated in Supplementary Figures S4.4–S4.6.

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TABLE 1 Sample characteristics based on BZRA prescription rates in 2000 and 2019.

Characteristic	<3 BZRA prescriptions			≥3 BZRA prescriptions		
	2000 $n = 4,690$ %	2019 $n = 14,380$ %	*p*-value	2000 $n = 2,519$ %	2019 $n = 10,582$ %	*p*-value
Age						
18–44 years	27.8	28.3	0.441	13.2	11.5	0.015
45–64 years	36.9	37.6	0.367	40.0	33.1	<0.001
65+ years	35.2	34.1	0.099	46.8	55.4	<0.001
Sex						
Males	36.7	38.0	0.0853	32.5	33.4	0.303
Females	63.3	61.9	0.0853	67.5	66.6	0.303
(Co-)morbidity						
Insomnia	5.1	14.5	<0.001	7.4	20.7	<0.001
Anxiety	3.1	6.8	<0.001	5.2	10.0	<0.001
Depression	18.6	20.2	0.032	28.0	29.3	0.218
Alcohol	2.2	3.9	<0.001	2.7	9.0	<0.001
Psychiatric problem, other	5.5	17.1	<0.001	7.9	20.8	<0.001
Neurologic	7.5	8.1	0.167	10.1	12.6	<0.001
Dementia	0.6	1.6	<0.001	0.7	1.9	<0.001
Hypertension	17.0	24.1	<0.001	27.6	37.3	<0.001
Cancer	4.8	20.8	<0.001	6.6	25.8	<0.001
Back pain	28.6	39.2	<0.001	34.0	44.6	<0.001
Concomitant medications						
Opioids	21.2	31.3	<0.001	30.1	45.4	<0.001
Antidepressants	25.4	28.9	<0.001	38.4	45.6	<0.001
Antipsychotics	8.8	6.7	<0.001	12.7	12.2	0.349

TABLE 2 Distribution of BZRA prescribed in 2000 and 2019 (*n*: number of prescriptions).

<3 BZRA prescriptions				≥3 BZRA prescriptions			
2000 ($n = 7,147$)	%	2019 ($n = 49,111$)	%	2000 ($n = 16,048$)	%	2019 ($n = 182,815$)	%
Lorazepam	16.3	Zolpidem	20.7	Lorazepam	20.8	Zolpidem	22.4
Alprazolam	14.0	Alprazolam	18.2	Lormetazepam	15.6	Lormetazepam	16.9
Lormetazepam	12.3	Lorazepam	13.8	Alprazolam	13.0	Alprazolam	16.6
Zolpidem	9.8	Lormetazepam	12.2	Bromazepam	11.9	Lorazepam	14.1
Bromazepam	8.2	Diazepam	11.0	Zolpidem	6.8	Clonazepam	6.2
Other	39.4	Other	24.1	Other	31.9	Other	23.8

Discussion

Key findings

Inappropriate BZRA prescribing, at odds with current guidelines, seems to be highly prevalent in Belgium. First, throughout the 19-year study period there was an increase in patients receiving three or more BZRA prescriptions in 1 year, particularly among male patients 18–44 years old and female patients 65 years or older, despite some significant decreasing trends. Second, back pain was the most common diagnosis associated with BZRA prescribing, even though Belgian guidelines recommend against the use of muscle relaxants (58). Diagnoses of anxiety and insomnia, two of the main indications for BZRA use, were rather limited in the study population (on average 8% anxiety and 17.6% insomnia). Nevertheless, the hypnotic zolpidem was the most prescribed BZRA in 2019, accounting for

TABLE 3 Trends in age- and sex-standardized BZRA prescription rates among patients who received <3 BZRA prescriptions in 1 year between 2000 and 2019.

Group	2000 $N = 7,209$	2019 $N = 24,962$	Summary	Trend 1		Trend 2	
	%	%	AAPC	Years	APC	Years	APC
Prescriptions							
BZRA < 3	65.1	57.6	0.6 (−0.1; 1.3)	2000–2019	0.6 (−0.1; 1.3)		
Overall 18–44	18.1	16.3	**1.4 (0.6; 2.2)**	2000–2012	−0.4 (−1.3; 0.6)	2012–2019	**4.4 (2.6; 6.1)**
Overall 45–64	24.1	21.7	0.4 (−0.1; 0.9)	2000–2012	**−0.9 (−1.5; −0.3)**	2012–2019	**2.6 (1.4; 3.7)**
Overall 65+	22.9	19.6	0.5 (−0.2; 1.2)	2000–2016	−0.3 (−0.7; 0.1)	2016–2019	**4.7 (0.2; 9.3)**
Males	23.9	21.9	**0.9 (0.2; 1.6)**	2000–2019	**0.9 (0.2; 1.6)**		
Males 18–44	7.3	6.4	**1.7 (0.8; 2.6)**	2000–2010	−0.1 (−1.6; 1.4)	2010–2019	**3.7 (2.3; 5.1)**
Males 45–64	9.2	8.6	**0.8 (0.2; 1.4)**	2000–2012	−0.6 (−1.3; 0.1)	2012–2019	**3.3 (1.9; 4.6)**
Males 65+	7.3	6.9	**0.5 (0.1; 1.0)**	2000–2019	**0.5 (0.1; 1.0)**		
Females	41.2	35.7	0.4 (−0.4; 1.3)	2000–2019	0.4 (−0.4; 1.3)		
Females 18–44	10.8	9.9	**1.0 (0.1; 1.9)**	2000–2012	−0.9 (−2.0; 0.2)	2012–2019	**4.3 (2.3; 6.3)**
Females 45–64	14.9	13.1	0.1 (−0.5; 0.7)	2000–2012	**−1.0 (−1.7; −0.3)**	2012–2019	**2.0 (0.7; 3.3)**
Females 65+	15.5	12.7	0.4 (−0.3; 1.2)	2000–2016	−0.3 (−0.8; 0.1)	2016–2019	4.8 (0.0; 9.8)

AAPC, average annual percentage change; APC, average percentage change; statistically significant trends (95% CI) in bold.

TABLE 4 Trends in age- and sex-standardized BZRA prescription rates among patients who received ≥3 BZRA prescriptions in 1 year between 2000 and 2019.

Group	2000 $N = 7,209$	2019 $N = 24,962$	Summary	Trend 1		Trend 2		Trend 3	
	%	%	AAPC	Years	APC	Years	APC	Years	APC
Prescriptions									
BZRA ≥ 3	34.9	42.4	0.3 (−1.0; 1.6)	2000–2019	0.3 (−1.0; 1.6)				
Overall 18–44	4.6	4.9	**1.7 (0.6; 2.9)**	2000–2014	**3.0 (1.9; 4.0)**	2014–2019	−1.7 (−5.3; 2.1)		
Overall 45–64	14.0	14.0	0.7 (−0.3; 1.8)	2000–2004	**6.0 (0.9; 11.4)**	2004–2019	**−0.6 (−1.1; −0.1)**		
Overall 65+	16.4	23.5	**1.9 (1.1; 2.8)**	2000–2006	**8.2 (5.5; 11.0)**	2006–2019	**−0.8 (−1.4; −0.3)**		
Males	11.4	14.2	0.5 (−1.0; 2.0)	2000–2019	0.5 (−1.0; 2.0)				
Males 18–44	1.7	2.0	**2.5 (0.9; 4.3)**	2000–2014	**5.1 (3.6; 6.7)**	2014–2019	−4.3 (−9.4; 1.0)		
Males 45–64	4.3	4.8	**1.0 (0.1; 1.8)**	2000–2009	**3.6 (2.0; 5.2)**	2009–2019	**−1.3 (−2.3; −0.4)**		
Males 65+	5.3	7.3	**1.6 (0.8; 2.3)**	2000–2007	**6.4 (4.4; 8.6)**	2007–2019	**−1.2 (−1.8; −0.6)**		
Females	23.6	28.2	0.5 (−1.3; 2.4)	2000–2019	0.5 (−1.3; 2.4)				
Females 18–44	2.9	2.9	**1.1 (0.5; 1.7)**	2000–2019	**1.1 (0.5; 1.7)**				
Females 45–64	9.7	9.2	0.2 (−1.3; 1.8)	2000–2008	**2.3 (0.5; 4.0)**	2008–2012	−4.5 (−10.6; 2.0)	2012–2019	0.7(−1.0; 2.4)
Females 65+	11.0	16.1	**2.1 (1.2; 3.0)**	2000–2006	**8.5 (5.6; 11.6)**	2006–2019	**−0.7 (−1.3; −0.1)**		

AAPC, average annual percentage change; APC, average percentage change; statistically significant trends (95% CI) in bold.

22% of all registered BZRA prescriptions. Finally, comparing characteristics to a matched control sample showed that all diagnoses, except dementia and concomitantly prescribed psychoactive medication, were significantly associated with BZRA prescription.

Context

In comparison to registry-based data from 2014 to 2015, long-term BZRA use, approximated by receiving three or more BZRA prescriptions in 1 year, is one to two percent

more prevalent in Belgium than in European countries such as France and Finland (34, 59). Consistent with previous reports of increased trends in zolpidem use (34, 36, 38), zolpidem became the most prescribed BZRA in Belgium by 2019. This could be due to professionals' perception of z-drugs as more beneficial than benzodiazepines. Their clinical experience with z-drugs overemphasizes the effectiveness in treating insomnia. Moreover, care professionals believe that patients experience significantly fewer side effects when using z-drugs over benzodiazepines (60–62).

Although previous studies have shown that long-term BZRA use is most commonly related to various psychiatric conditions, such as anxiety and depression (12, 35, 63), the most common diagnosis in our study population was back pain. This was followed by hypertension, which was also highly prevalent in the cohort that Torres-Bondia et al. studied (35). Insomnia and anxiety held the sixth and eighth positions, leading us to hypothesize that they are not consistently coded as a diagnosis when being secondary to a somatic disease. This was also suggested by Rosman et al., who found no correlation between the effect of somatic disease diagnoses, and insomnia and anxiety diagnoses, on BZRA prescribing (64). Conversely, BZRA have both myorelaxant and vasodilatory effects so it cannot be ruled out that they are sometimes prescribed for treating the aforementioned conditions.

Finally, in the youngest age category, 18–44 years, all significant findings are rising trends, with an overall increase (AAPC = 1.7; CI: 0.6, 2.9) among patients who received three or more prescriptions in 1 year. Moreover, in 2018, Sidorchuck et al. reported that in Sweden 31% of all 18–24 years old BZRA users received prescriptions to use this medication for more than 6 months (63). These findings highlight the risk of inappropriate long-term prescribing continuing in future generations, as previously described by Cadogan et al. (36).

Strengths and limitations

To our knowledge, this is the first registry-based research that covers a study period of 19 years to describe BZRA prescription trends in primary care. Moreover, comparing trends between two groups of patients, differentiating long-term use from other use, was discussed in only one other recent study (34). Furthermore, we used data from a large real-world study population, representative of the general Flemish population in terms of age and sex (40). Although this is a major strength, this method also brings a few limitations. Data from the Intego project includes coded diagnoses and medication prescriptions, extracted from the electronic health record in general practice. Paper prescriptions, prescriptions by specialists, the dosage, and the frequency of use by the patient were not available. Additionally, data could be influenced by evolutions in coding practices, related to the further development of electronic health record systems and electronic prescribing, or the quality of registration by the general practitioner. Although this may result in an underestimation of prescription rates, it is not expected to affect the direction of the reported trends. Another limitation lies with the exclusion of the data from 2020 to 2021 to prevent potential bias by COVID-19. Although it would be interesting to study the prescribing of hypnotics and anxiolytics during the COVID-19 pandemic, we aimed to map general BZRA prescription trends in primary care. Moreover, an analysis of BZRA prescription trends during COVID-19 would be more interesting in a few years, as we hypothesize that sleep disturbances and anxiety will stabilize to pre-pandemic levels because of patients' resilience.

Finally, during analysis it was difficult to compare the observed prescribing rates with reports from other countries because of differences in the origin of the datasets, patient populations, definition of long-term use, and time periods. However, in their systematic review, Kurko et al. suggest defining long-term use as at least 6 months' use or longer during 1 year, for future research (12). On the one hand, we concur with them and plead for standardization. On the other hand, we did not adhere to this criterion in the current project because the dosage prescribed was not available. Analysis was based on the medication code, patient identifier, and date of prescription. Therefore, it is possible that some prescriptions allowed patients to buy medication for a longer period of time, as is regularly observed in clinical practice when prescribing to chronic users. Therefore, we opted for the minimal threshold of three prescriptions, hypothesizing that this corresponds to minimally 3 months of use. For future projects, we will be able to consider the dosage prescribed by the GP because of a recent update of the Intego database. This will contribute to constructing a more precise proxy for the concept of long-term use.

Implications for research and practice

To improve (de)prescribing practices, the BZRA situation in Belgium demands both interventional and epidemiological studies. First, implementation research to increase non-pharmacological treatment and discontinuation interventions is required. This could be linked to mapping the patients' access to mental health services and accessibility of care. Future interventions could also focus more on empowering patients to discuss their medication use and possible non-pharmacological treatment options. Moreover, policy and guidelines should motivate general practitioners to discontinue long-term BZRA use that is no longer medically justified. Tools that help them regularly review their prescribing practices could be useful in this matter. Second, the significant prevalence of back pain and concomitant opioid prescribing in a BZRA-consuming population warrants further research. Since both BZRA and

opioids have sedating effects for which tolerance is rather quickly developed, the impact of concomitantly prescribing them in this population should be investigated. Third, the Belgian guidelines on the treatment of insomnia recommend a maximum of 1 week of pharmacological treatment, yet 22% of all BZRA prescriptions in the group that received three or more prescriptions per year are for zolpidem, a hypnotic drug. Professionals' attitudes toward z-drugs (60–62) may lead to an inadequate judgment of these drugs' risk-benefit ratio. Since they cause the same adverse side effects as benzodiazepines when used in the long term, including their potential for recreational abuse (65), future campaigns about BZRA discontinuation should explicitly mention and possibly target z-drugs prescribing behavior. Finally, when comparing to a matched control population, all diagnoses except dementia, and concomitant prescriptions of psychoactive drugs were significantly more prevalent in our study population. Further research could clarify whether this comes from the complexity and multimorbidity in BZRA-consuming patients, or inappropriate prescribing or outdated coding.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

CM conceived the study. PM prepared the dataset and performed the cross-sectional and case-control analyses. KC performed the joinpoint regression analysis with guidance of PM. KC wrote the first draft of the article. All authors contributed to the study design and approved the final version of the article.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.1014734/full#supplementary-material

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Erratum: Evolution of benzodiazepine receptor agonist prescriptions in general practice: A registry-based study

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An Erratum on
Evolution of benzodiazepine receptor agonist prescriptions in general practice: A registry-based study

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Due to a production error, there was a mistake in the legend of **Table 4** as published. Both **Tables 3** and **4** were published with the same captions. The correct legend of **Table 4** appears below.

Table 4. Trends in age- and sex-standardized BZRA prescription rates among patients who received ≥3 BZRA prescriptions in 1 year between 2000 and 2019.

Due to a production error, the title of a sub-section was published incorrectly.

A correction has been made to the section **Results**, subsection **Trends in BZRA prescriptions**, paragraph 2. The correct title appears below:

"Patients with ≥3 BZRA prescriptions in 1 year."

The publisher apologizes for these mistakes. The original article has been updated.

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EDITED BY
Lais F. Berro,
University of Mississippi Medical Center, United States

REVIEWED BY
Antonio Mirijello,
Home for Relief of Suffering (IRCCS), Italy
Liana Fattore,
CNR Neuroscience Institute (IN), Italy

*CORRESPONDENCE
Lorenzo Zamboni
lorenzo.zamboni88@gmail.com

†These authors have contributed equally to this work

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Study protocol—Evoked craving in high-dose benzodiazepine users

Lorenzo Zamboni[1,2]*, Silvia Toldo[3], Francesca Fusina[4,5], Matteo Mattiello[1,3], Vanessa Mannari[3], Simone Campagnari[1], Valentina Schiavone[3], Alessio Congiu[1], Giuseppe Verlato[6], Cristiano Chiamulera[3†] and Fabio Lugoboni[1†]

[1]Unit of Addiction Medicine, Department of Internal Medicine, Integrated University Hospital of Verona, Policlinico "G.B. Rossi", Verona, Italy, [2]Department of Neurosciences, University of Verona, Verona, Italy, [3]Department of Diagnostics and Public Health, University of Verona, Verona, Italy, [4]Padova Neuroscience Center, University of Padua, Padua, Italy, [5]Department of General Psychology, University of Padua, Padua, Italy, [6]Diagnostics and Public Health-Unit of Epidemiology and Medical Statistics, University of Verona, Verona, Italy

Benzodiazepine (BDZ) abuse, especially concerning high doses of BDZs, is an impairing substance use disorder (SUD) that is often difficult to treat. Craving and cue reactivity (CR) are two important phenomena that have a prominent role in maintaining addiction and triggering relapses in BDZ abuse; nevertheless, they have rarely been addressed in scientific literature. The present study aims to fill these gaps by implementing a highly innovative virtual reality (VR) design to assess the impact of substance-related environmental cues on BDZ craving, as well as their influence on patients' affective states. Therefore, on one hand, this research will contribute to the assessment of VR feasibility in the study of these phenomena, and, on the other, it will help disentangle the role that CR and craving have on mood and attention, which are equally important factors to consider when treating SUDs. We will recruit a healthy control group and a patient group comprising people seeking treatment for BDZ detoxification. The experimental design will consist of the presentation of three VR scenarios, one neutral, one BDZ-related but without BDZ cues, and another with BDZ cues. The craving will be measured through a virtual analog scale (VAS); the Profile of Mood States (POMS) and Alcohol Attention Scale (AAS) questionnaires in a modified version will also be administered. We will additionally control for VR-induced feelings of sickness by administering the Simulator Sickness Questionnaire (SSQ), and the Presence Questionnaire (PQ) will be used to investigate participants' sense of presence in virtual environments. We expect patients to exhibit higher levels of craving, and that the craving will be higher after exposure to a cue-related virtual environment as compared to a neutral scenario.

KEYWORDS

cue reactivity, benzodiazepine, addiction, virtual reality, abuse

Introduction

Substance use disorders (SUDs) feature craving as one of their most prominent mechanisms and diagnostic criteria (1). Indeed, craving is involved in the long-term maintenance of abstinence in SUDs, as well as having an important impact on the development of the disorder itself and on the course of the treatment (2–4). Craving is defined as an abrupt urge to consume the target substance (5–7), which often escalates into compulsively seeking the substance and other behaviors related to substance use (8, 9).

Benzodiazepines (BDZs) are positive allosteric modulators of the GABA-A (Gamma-Aminobutyric Acid Type A) receptor (10) which are widely prescribed to treat insomnia and anxiety. Despite their widespread use, studies have shown that BDZs should only be employed in specific clinical situations and preferably for short-term use (11–13). Adverse effects and dependence are associated with their long-term use and should be implemented with extreme caution. Clinicians should also consider short or intermittent treatments, which could have important benefits for patients (14).

About 6–76% of total BDZ users are long-term users. Of these, 15–44% present moderate-to-severe withdrawal symptoms, and 3–4% exhibit dependence (15).

High-dose (HD) BDZ dependence is considered a specific SUD (16), and it consistently reduces the quality of life (17, 18). A cross-sectional survey in France, Germany, Italy, and the UK showed that an estimated 0.14% of the general population took higher-than-recommended doses of anxiolytic medications, while 0.06% reportedly abused hypnotics (19). These data are consistent with those reported by a study conducted in Switzerland, which revealed an incidence rate of 0.16% concerning high-dose BDZ use (20) and points toward HD BDZ abusers being around 1.5 million in Europe and 600,000 in the United States.

Long-term BDZ use is particularly problematic because it has been found to be associated with anomalies in cognitive functions such as attention, memory, and learning. It also exposes patients to a higher risk of delirium, cognitive decline, and accidents (21–30).

To alleviate BDZ withdrawal symptoms, which are particularly impairing for patients, gradual tapering of the dosage or substituting the target BDZ with an equivalent dose of another long-acting benzodiazepine and then tapering are the preferred courses of treatment (31, 32).

Furthermore, BDZs are reportedly secondary drugs of abuse for most individuals, with much fewer patients reporting BDZs as primary drugs of abuse. BDZ abuse is mainly associated with the concurrent abuse of opioids (54.2%) and alcohol (24.7%). Jones et al. (33), in their recent review, reported that about one in five people who abuse alcohol are also benzodiazepine abusers.

Cue reactivity (CR) is a hypersensitivity to motivational stimuli and situations (34). It is considered an adaptive response to salient information (cues) that are present in the environment and it can be evaluated by relying on psychological measures (changes in mood and craving ratings), physiological measures (skin conductance and heart rate), and behavioral measures (gestures/actions) (35). CR is particularly relevant in SUDs, in which it increases craving and facilitates relapses: subjects with a history of substance abuse are particularly sensitive to stimuli and situations which have been previously associated with pleasurable substance effects (36). In this respect, CR is an evolutionary response that may be both a risk factor, when cues are present, and a protective one, when cues are absent: for instance, households with no smoking-related cues have been demonstrated to reduce relapses in smokers (37). Likewise, an external environment may present both protective and precipitating elements. In this perspective, studying the characteristics of various contexts and their function as either risk or protective factors is central in treating and preventing abuse-related behaviors by designing motivationally healthy environments (38). Even though the effects of spatial features on affective states and perception have been extensively studied (39), the role of domestic and urban settings in inducing motivated behaviors is still a largely unexplored topic.

Concerning potential research methods, virtual reality (VR) seems a promising technology to implement in CR paradigms (2, 40, 41). VR consists in the simulation of real-life contexts and environments, which are presented in 3D and are multisensory, comprising auditory, olfactory, visual, and/or tactile inputs (42). Such an approach, being more similar to reality, enhances participants' *sense of presence*, that is a state of mind in which virtual environments are perceived as similar to real-world ones, and may therefore be more valid than traditional CR paradigms (e.g., 2D screens, photos, etc.) (43–45).

Higher efficacy may be achieved using technical VR features such as immersion within the VR environment and allowing subjects to actively interact with the system through real-time feedback (46). Other important aspects are the inclusion of substance-related stimuli and the presentation of highly realistic environments (47–49).

To the best of our knowledge, there is currently no literature regarding CR and VR in BDZ abuse. Some studies have addressed CR and alcohol abuse (47, 50, 51) and have highlighted the influence that environmental settings have on craving in alcoholics. This work has been inspired by the study by Ryan et al. (50), especially given the scientific rigor they adopted in their research.

Objectives of the study

General objective

The general objective of the study is the implementation of a VR protocol to identify the causal relationship between environmental features of a specific setting and craving responses in BDZ abusers.

Specific objectives

The primary objective of the study is to identify the causal relationship between exposure to environmental cues related to BDZ use and the degree of BDZ craving in abusers.

Secondary objectives

1. Correlation between the degree of BDZ craving in the various scenarios and measures of mood, affect, attention, sense of presence, and VR malaise in subjects who abuse BDZs.
2. Evaluation of the effectiveness that the three different VR environments have in discriminating between BDZ abusers and control subjects by comparing BDZ craving degree and measures of mood, affect, attention, sense of presence, and VR malaise in the control group vs. those in the experimental group.

Materials and methods

Study design

This research will be an experimental study aiming to measure the degree of BDZ craving induced by VR exposure to environments associated with BDZ use (cues) after immersion in a VR scenario of a bedroom only, and then a bedroom in which BDZ bottles will be present. Every subject will be sequentially exposed to both environments to avoid carry-over effects.

There will be two cohorts of participants. The first group will be the control group and it will comprise subjects that do not suffer from SUD. Participants will be recruited from University students, collaborators, and staff of the University or Hospital. The second group will be the experimental one and will be recruited among BDZ-abusing patients seeking treatment at the Department of Addiction Medicine (Department of Internal Medicine, Integrated University Hospital of Verona) due to their inability to autonomously quit using BDZs. All subjects will be informed regarding the procedures and risks associated with the protocol and experimental design and will be asked to sign an informed consent form before participating in the experiment. Before the experimental session starts, we will collect demographic data and administer a series of questionnaires.

The study will consist of a single session lasting about 45 min. Participants will fill out the Profile of Mood States (POMS) questionnaire before and after the session as a pre-VR baseline measure concerning their mood and affective state. After a 3-min VR baseline, we will administer three scenarios, each lasting 3 min. After the baseline and each of the scenarios, subjects will be required to fill out the VAS to report cravings and a modified version of the Alcohol Attention Scale (AAS) questionnaire. At the end of the experimental session, in addition to the POMS, subjects will also be asked to fill in the Presence Questionnaire (PQ) to assess their sense of presence and the Simulator Sickness Questionnaire (SSQ) to assess the presence of possible adverse effects due to VR exposure.

Materials

Virtual reality instrumentation

HTC-VIVE, which is a VR helmet that facilitates feeling immersed in the proposed virtual scenarios and headphones, enhance auditory immersion as well.

This device allows seeing a virtual world with an optical visor which, thanks to new "room scale" technology, transforms the environment into a 3D space in which the user can freely move. This technology, associated with precise head tracking and controls that simulate hand movements, transforms VR into a particularly immersive experience.

The development platform Unity allows to design and build highly immersive VR scenarios that are compatible with HTC-VIVE.

Procedure

Each subject will be asked to sit in the VR station and will be given all the necessary information regarding the experiment. After signing the informed consent, the subject will give demographic data and will fill out the questionnaires. Before the VR session begins, the participant will be administered the POMS and the VAS on craving. The experimenter will then instruct the participant on how to move in the virtual environment and how to use the HTC-VIVE VR device. The first scenario will be a 3-min baseline simulation during which the subject will familiarize themselves with VR, learn the controls to move around the virtual environment, and practice with the device. In a fixed sequence, the other three scenarios will be shown: house entrance (neutral), bedroom

without BDZs, and bedroom with a medicine bottle similar to commercially available BDZ bottles. The subjects will not have to undertake a specific task but will be allowed to freely explore the environment by using the HTC-VIVE PRO Full Kit directional joystick and by moving their head. At the end of each scenario, the subject will remove the visor and headphones and fill out the VAS to report BDZ craving and the modified AAS scale.

Every scenario, including the baseline, will last 3 min. At the end of the last one, the subject will be administered the VAS craving scale, the POMS, the modified AAS, the SSQ, and the PQ.

Questionnaires

Anamnesis schedule (**Supplementary Appendix 1**) with 10 questions.

VAS scale (**Supplementary Appendix 2**) with a question relative to BDZ craving. The instrument is a single-item visual analog scale with a score ranging from 0 (absent) to 9 (extreme).

The POMS (52) (**Supplementary Appendix 3**) is widely used to assess mood and to identify possibly problematic affective states. It is a self-report questionnaire, and it is mainly used in clinical psychology, psychotherapy, and medicine. It comprises 58 adjectives that define six mood states: tension-anxiety (T), which describes an overt or covert increase in somatic tension; depression (D), which indicates a depressed mood accompanied by a sense of inadequacy, hopelessness, emotional isolation, melancholy, and guilt; aggression-anger (A), which describes anger and dislike toward others; vigor-activity (V), comprising adjectives that suggest exuberance, energy, euphoria, and optimism; tiredness-indolence (TI), which represents boredom, low energy, and physical fatigue; confusion (C), characterized by a sense of disturbance and linked to the organization-disorganization dimension, including anxiety and the feeling of cognitive inefficiency.

The adjectives are rated on a 5-point Likert scale (0 = not at all, 1 = a little bit, 2 = moderately, 3 = quite a bit, and 4 = extremely). A Total Mood Disturbance score (TMD) can be calculated by adding the scores for tension, depression, anger, tiredness, and confusion and then subtracting the score for vigor. The POMS showed good reliability both concerning the TMD score ($\alpha = 0.85$) and the T, D, A, V, S, and C subscales ($\alpha = 0.89$; $\alpha = 0.94$; $\alpha = 0.71$; $\alpha = 0.69$; $\alpha = 0.62$; and $\alpha = 0.77$, respectively).

The SSQ (53) is widely used to measure symptoms of cyber sickness. It comprises 16 items and allows the computation of a total score assessing the severity of the reported symptoms, as well as three subscales for Nausea, Oculomotor Disturbances, and Disorientation (**Supplementary Appendix 4**).

To measure the attention given to BDZ-related cues, a modified version of the AAS questionnaire (54) will be used, with BDZ-themed questions. Responses are given on a Likert scale ranging from 0 to 10 (**Supplementary Appendix 5**).

Participants' sense of presence will be assessed with the PQ (55), comprising 24 items rated on a 7-point Likert scale (**Supplementary Appendix 6**).

Development and creation of the virtual environments

The virtual environments that will be used have been created in photorealistic quality (**Figures 1–4**) and in "cybersickness-free" mode to allow participants to have a comfortable virtual experience, without any unpleasant side effects. Indeed, cybersickness is a feeling of malaise comprising headaches, vomiting, dizziness, and/or nausea, and it is triggered by a mismatch between visual inputs and those responding to actual movements (56). To achieve this, patients will be allowed to move within the virtual environment through "real" steps whose movement will be faithfully reproduced in the virtual environment. Also, subjects may use teleportation to reach distant positions and beyond the play area. Through the joystick, participants will be able to point to the place they wish to reach, with the virtual experience resuming exactly from the desired spot. The VR environments run on the following VR hardware requirements: (a) HTC-VIVE PRO Full Kit (**Figure 5**); (b) Gaming PC, Intel Core i7-9700K—GeForce RTX 2070 8GB–16GB DDR4–480GB SSD—Windows 10—Wi-Fi; and (c) a 49" or 55" TV monitor.

The software has been developed by Hybrid Reality (Padova, Italy[1]), an innovative start-up that developed the scenarios and provides optimization support.

Virtual environments

Figure 2 reported the tutorial scenario. Participants are exposed to this virtual environment for 3 min. The role of this scenario is to increase familiarity with VR and this is the only scenario in which subjects can interact with the experimenter. In this step of the experiment, the experimenter gives the subjects some instructions and tips on how to better interact with the virtual environment.

Figure 3 reported the neutral scenario. Subjects are exposed to this virtual environment for 3 min. This scenario represents a house entryway. In this scenario, the subject can move freely in the virtual environment, but there are no interactive objects. Every interaction between the subjects and the experimenter is forbidden.

Figure 4 reported the No Cue scenario. Subjects are exposed to this virtual environment for 3 min. This scenario represents a bedroom.

1 https://www.hybridreality.it

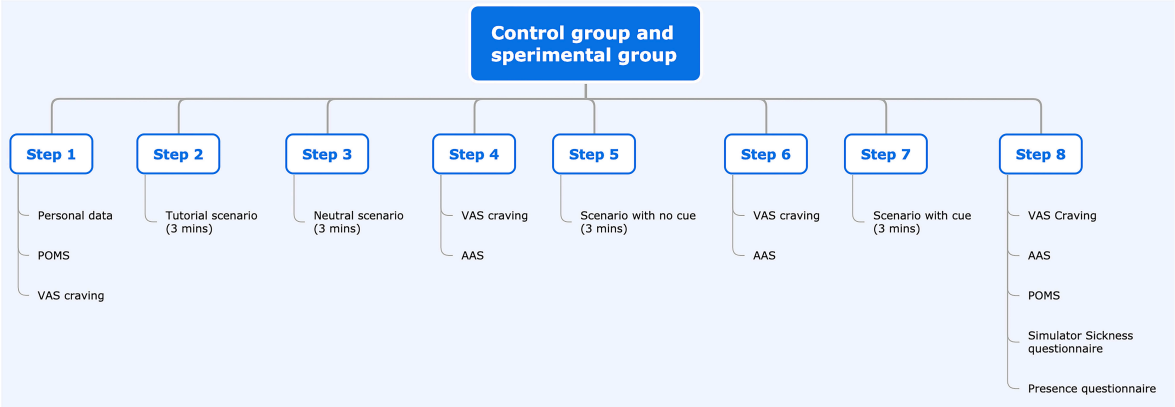

FIGURE 1
Flowchart.

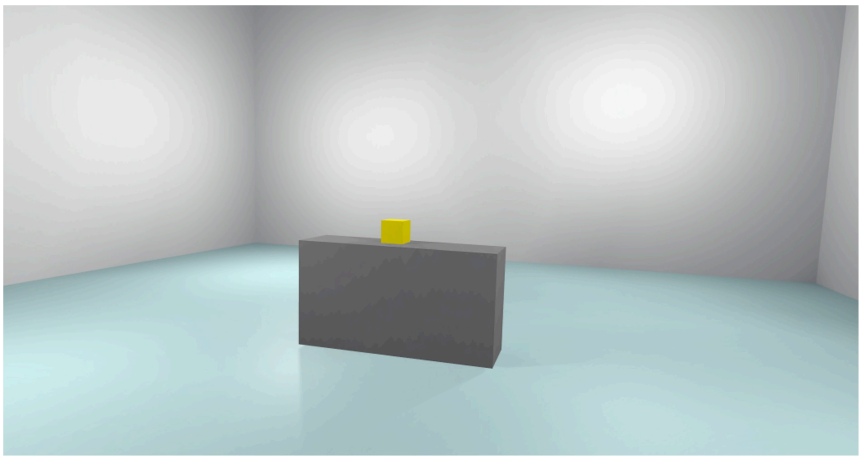

FIGURE 2
Tutorial scenario.

FIGURE 3
Neutral scenario.

FIGURE 4
No cue scenario.

FIGURE 5
Scenario with cue.

In this scenario, the subject can move freely in the virtual environment, but there are no interactive objects. Every interaction between the subjects and the experimenter is forbidden.

Figure 5 reported the Cue scenario. Subjects are exposed to this virtual environment for 3 min. This scenario represents the same bedroom as the No Cue Scenario. In this scenario, the subject can move freely in the virtual environment. There are only BDZ-related interactive objects. Every interaction between the subjects and the experimenter is forbidden.

Given the COVID-19 pandemic, appropriate accessories will also be employed: disposable, non-woven, breathable face masks for HTC-VIVE PRO, waterproof and hygienic replaceable foam rubber for HTC-VIVE, sanitizing spray, hand sanitizer gel, and surgical masks will be used to guarantee appropriate hygiene of the instruments and the patient's safety (Figures 6, 7).

Participants

During the recruitment phase, subjects will be given clear and easy-to-understand information regarding the rationale and purpose of the study, as well as information concerning the possible consequences related to their participation in the study. We will give out informative pamphlets, with detailed information about the research as well as the informed consent, and the subjects will be required to carefully read, fill out, and sign. Both documents will be written in simple language and the name of the person who gave the information to the patient will be listed. The experimenter will further need to sign the informed consent and write the date to validate the document. All procedures will be carried out in accordance with the Declaration of Helsinki.

FIGURE 6
HTC vive.

FIGURE 7
An example of a subject wearing the virtual reality equipment.

Inclusion criteria

Experimental group:

1. Males and females aged 18–65 years.
2. Subjects with BDZ-use disorder asked to be treated at the Addiction Medicine Unit due to their inability to autonomously quit using BDZs.
3. High-dose BDZ abusers. Although the definition of what constitutes a "high dose" is still controversial and no real consensus exists about the appropriate clinical criteria necessary to define it, we will consider a patient a high-dose user if their BDZ intake will be at least five times higher than the maximum daily defined dose (DDD)

Control group:

1. Males and females aged 18–65 years.
2. Subjects without SUDs (including a BDZ-use disorder) according to ICD 10 F10-F19.

Exclusion criteria

At least one of the following:

1. A history of epilepsy or having a first-degree relative with a history of epilepsy.
2. Serious chronic or cardiovascular diseases.
3. Being pregnant.
4. Having a pacemaker or other metal devices on the head and neck, with the exception of piercings and dental braces.
5. Taking psychoactive substances which may interfere with the results of the study.

The presence or absence of each criterion will be assessed by the experimenter before the study begins.

Safety and hygiene measures

To ensure participant safety and hygiene in the experimental setting, we will comply with the guidelines provided by the Italian Superior Institute of Health (Istituto Superiore di Sanità, ISS), which were approved and implemented in the study by Giordano et al. (57). These include the following procedures:

1. Cleaning of the hands. All staff that will handle the VR devices must use an alcohol-based hand sanitizer. Before a user touches a device, they must wash their hands for at least 40 s, following a specific sequence as illustrated on the Ministry of Health's website; also, they must rub their hands with an alcohol-based cleaning gel for at least 20 s. This will be done for both operators and participants.

2. The protective waterproof foam guards must be in place on the visor to ensure sanitization of the device.
3. Inserting the waterproof single-use masks in the device before use and substituting them for each subject or operator.
4. Disinfecting all objects that the participants and operators may touch.
5. Surgical masks will be mandatory at all times.
6. Each hygiene measure must be repeated between one participant and the next. At the end of each daily session, all procedures must be enacted one last time to ensure the correct sanitization of the devices.
7. Hospital cleaning personnel will thoroughly clean the room and hospital environment.
8. Trisept Complex, which is a sanitizing product that is available at the internal pharmacy of the University Hospital, will be used to disinfect the VR devices.

Statistical analyses

Primary endpoint

To evaluate the association between environmental features and craving, we will use the VAS scale (10 levels) in the experimental group after exposure to the three scenarios: neutral, bedroom without BDZ bottles, and bedroom with bottles similar to the ones containing BDZs.

Secondary endpoints

To evaluate the association between BDZ craving and mood, affective state, attention, sense of presence, and VR-induced sickness, we will use the total scores and subscales (if present) of the following questionnaires: VAS, POMS, AAS, SSQ, and PQ as measured at the specific timepoints (see flow chart) in the experimental group. The temporal course of the scores will also be compared between the two groups.

Sample size

The appropriate sample size for this study was computed with the software G*Power 3.1.5.1 (58). We chose to base the computation on the difference between the mean VAS craving scores measured within the subjects after the neutral scenario vs. the BDZ-related scenario. Since we expect this difference to be medium-large, we chose a 0.7 effect size (59). Alpha was set to 0.017 considering multiple comparisons among the three scenarios. Since it will be a pilot study, we choose a two-tailed test with 80% power. The resulting sample size was 25 subjects.

To test if the VR scenarios can appropriately distinguish between BDZ abusers and controls, we will also recruit 25 healthy subjects, bringing the total sample size to 50.

Data analysis

All the variables considered in the study will be analyzed by using their most appropriate descriptive statistic. In particular, we will use mean and standard deviation for normally distributed continuous variables, median and interquartile range for non-normally distributed variables, and frequency distribution for categorical variables.

In the experimental group, we will perform a one-way ANOVA for VAS craving at each study timepoint, meaning after exposure to each scenario.

After the repeated-measures ANOVA, we will conduct Bonferroni's multiple comparison correction to compute *post-hoc* comparisons and test significant contrasts among the various timepoints for VAS craving. Should the ANOVA assumptions be violated, a Friedman test will be performed. In the experimental group, we will also compute Pearson's correlations or Spearman's rank coefficients among VAS, POMS, AAS, SSQ, and PQ scores measured at the same timepoints. We will also explore the correlations between VAS score variations in the three scenarios (Δ VAS neutral—bedroom without BDZ; Δ VAS neutral—bedroom with BDZs; Δ VAS bedroom without BDZs—bedroom with BDZs) and the POMS, AAS, SSQ, and PQ score variations (Δ).

Finally, we will use multilevel linear models to assess group differences in the VAS, POMS, AAS, SQ, and PQ scores as repeatedly measured in the same subjects at specific timepoints. All statistical analyses will be conducted using the PRISM6 software (GraphPad, CA, USA).

Study plan

Flow chart of the study for the experimental and control groups.

Ethics statement

Approval for the research was obtained from the Ethics Committee for Clinical Trials (CESC) of the Provinces of Verona and Rovigo based at the Integrated University Hospital of Verona, Italy (approval code: 3624CESC with Protocol No. 16883 of 09-03-2022). The latest revision of the Declaration of Helsinki as well as the Oviedo Declaration is the basis for the ethical conduct of the study. The study protocol is designed and will be conducted to ensure adherence to the principles and procedures of Good Clinical Practice and to comply with Italian law, as described in the following documents and accepted,

by signature, by the study investigators: ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996; Directive 91/507/EEC, The Rules Governing Medicinal Products in the European Community; D. L.vo n. 211 of 24 June 2003; D. L.vo n. 200, 6 November 2007; Ministerial Decree of 21 December 2007; AIFA Determination, 20 March 2008. All essential clinical records will be retained to demonstrate the validity of the study and the integrity of the data collected. The promoter of this study, in accordance with the responsibilities required by the rules of good clinical practice (Legislative Decree 211/2003) and in accordance with the laws and regulations regarding data protection (including the European Regulation on the protection of personal data 2016/679), will process the personal data that will be collected exclusively for the implementation of the study and for device surveillance.

Discussion

Benzodiazepines are among the most widely used psychotropic medications worldwide, but the chronic use of BDZs can cause several deficits. The risk of dependence after long-term use has been widely reported, and abrupt withdrawal of the drug causes several unpleasant symptoms. In addition to subjects that begin using BDZs to treat anxiety and insomnia and end up using them inappropriately, some subjects deliberately abuse BDZs. In this case, BDZs are taken to counter anxiety or to enhance the effects of other drugs, such as alcohol or opioids, in what becomes a polydrug use pattern (15, 60). Withdrawal syndrome, even from therapeutic doses of BDZs, can be severe and, in some cases, may preclude the patient from ceasing the use of the drug (32, 61). Notwithstanding the important presence of BDZs in clinical practice, no studies have analyzed CR in the context of BDZ addiction yet. VR is a promising research tool since it creates a state of immersion closer to reality, but that still allows the measure of neuropsychological and behavioral responses in a more controlled way (62). For this reason, VR has been extensively used in addiction to drugs and tobacco, for example, to explore smoking withdrawal, craving, and cue reactivity (62). These VR reports, while confirming the findings that were demonstrated in previous, traditional laboratory studies (i.e., cues presented as pictures or videos), still need to better characterize VR-triggered cue reactivity. Environmentally induced craving has been described for various SUDs, and especially for alcohol and tobacco, in which the subjects who were exposed to abuse-related VR stimuli manifested increased craving (47, 63, 64). This has not been investigated in BDZ dependence, but looking at literature concerning other substances, we expect that CR may also be involved in BDZ addiction. Therefore, we expect increased craving in BDZ abusers exposed to BDZ-like stimuli in VR settings, and also significant differences in craving between the experimental group and the control group, with the former exhibiting higher levels of overall craving. One of the most critical issues regard the design of complex and personalized experimental sessions that would also allow measuring and standardizing the variables and parameters of interest (65).

Conclusion

Studies on BDZ abuse are not many, especially concerning high-dose abusers. There are still many relatively unknown variables that would nevertheless be important to investigate, such as craving.

BDZ craving is clinically characterized as an incontrollable urge to take the target substance when it is not readily available. Unlike what has been done for alcohol and tobacco, environmentally induced craving is often considered absent in BDZ abuse and, therefore, it has not yet been investigated in BDZ users. Its role in BDZ abuse, however, has never been tested in scientific research, and neither has its actual presence or absence in this SUD. Virtual reality, therefore, enables the study of this phenomenon without exposing the subjects to real risks. This study protocol aims to fill the current gaps in the scientific literature concerning BDZ-evoked craving in high-dose BDZ users and to better characterize the possible role of the environment in this important mechanism.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee for Clinical Trials (CESC) of the Provinces of Verona and Rovigo based at the Integrated University Hospital of Verona, Italy (approval code: 3624CESC with Protocol No. 16883 of 09-03-2022). The patients/participants provided their written informed consent to participate in this study.

Author contributions

LZ, CC, and FL: conceptualization and writing review and editing. LZ: data curation and investigation. GV: statistical analysis. CC and LZ: methodology. FL and CC: supervision. SC, FF, ST, AC, VM, MM, and VS: writing original draft. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2022.956892/full#supplementary-material

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EDITED BY
James Rowlett,
University of Mississippi Medical Center, United States

REVIEWED BY
Bradford Fischer,
Cooper Medical School of Rowan University, United States
Roberta Tittarelli,
University of Rome Tor Vergata, Italy

*CORRESPONDENCE
Lorenzo Zamboni
lorenzo.zamboni88@gmail.com

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Case Report: High doses of Zolpidem and QT interval lengthening: Is there a relationship? A case series

Simone Campagnari [1], Lorenzo Zamboni[1,2]*, Francesca Fusina [3,4], Rebecca Casari [1] and Fabio Lugoboni [1]

[1]Unit of Addiction Medicine, Department of Internal Medicine, G.B. Rossi Hospital, Verona, Italy, [2]Department of Neuroscience, Biomedicine and Movement, University of Verona, Verona, Italy, [3]Department of General Psychology, University of Padua, Padua, Italy, [4]Padova Neuroscience Center, University of Padua, Padua, Italy

Zolpidem is indicated in cases of severe insomnia in adults and, as for BDZs, its assumption should be limited to short periods under close medical supervision. Since several drugs cause corrected QT interval (QTc) elongation, the authors investigated whether high daily doses of Zolpidem could cause QTc elongation. The study was conducted in the Addiction Medicine Unit of the G.B. Rossi University Hospital in Verona. The data were collected from hospitalizations carried out between January 2015 and February 2020 and refer to a total of 74 patients, 38 males and 36 females, who were treated for detoxification from high doses of Zolpidem with the "Verona Detox Approach With Flumazenil." One patient out of 74 had QTc elongation (479 ms). The patient was male and took a daily dose of 50 mg of Zolpidem; he did not take concomitant therapies that could cause QTc lengthening. He had no electrolyte alterations, no contemporary or previous intake of barbiturates, heroin, cocaine, THC, alcohol, NMDA or nicotine which could cause an elongation of the QTc interval. The present study highlights the low risk of QTc elongation due to high dosages of Zolpidem; however, if, on one hand, we can affirm that Zolpidem is a safe drug, on the other, the widespread use of high dosages of this drug for prolonged periods of time is problematic and worrying.

KEYWORDS

Zolpidem, abuse, high dose, addiction, QTc, Z-drug

Introduction

Benzodiazepines (BDZs) and Z-drugs (Zolpidem, Zaleplon, Zopiclone) are among the most prescribed drugs in Western countries and, while there has been a downward trend in annual BDZ prescriptions, Z-drug prescriptions have instead increased exponentially. This is probably due to a widespread opinion among doctors that the

latter don't involve the problems associated with chronic use of BDZs, while, in fact, Z-Drug (ZD) use requires the same safeguards that apply to BDZs (1–4).

The use of ZDs is indicated in cases of severe insomnia in adults and, as for BDZs, it should be limited to short periods under close medical supervision. The pharmacokinetic differences between the molecules allow for some important considerations with respect to the selection criteria of these drugs. Zolpidem and Zopiclone, being eliminated more slowly, are more suitable for the treatment of insomnia with central awakenings, unlike Zaleplon, which has a rapid elimination and is thus best suited for the management of initial insomnia (5). The main contraindications to the use of Z-drugs are the following: drug hypersensitivity, myasthenia gravis, severe respiratory insufficiency, Obstructive Sleep Apnea Syndrome (OSAS) and advanced hepatic insufficiency. The use of ZDs is contraindicated in children.

ZDs, like BDZs, are characterized by high manageability, and starting from their introduction on the market it was initially thought that they could offer advantages in terms of efficacy and safety compared to classic BDZs. However, over time it was shown that even this class of drugs can lead to important side effects, such as cognitive impairment, worsening of psychomotor performance, and increasing the risk of falls and fractures, especially if taken at higher doses or in association with other psychoactive substances such as alcohol. (6–9).

Zolpidem and Zopiclone abuse are primarily described in patients with a history of drug addiction, alcoholism or psychiatric disorders and, minimally, also in patients not belonging to these categories. Their use should, therefore, reflect careful indications concerning posology (10, 11).

Zolpidem is an imidazopyrimidine and, although it is not structurally connected to BDZs, it has a similar mechanism of action: it enhances the inhibitory effect of GABA on nerve transmission, binding the related receptor with consequent increases in permeability to chlorine ions.

Although early reports highlighted a profile of low abuse risk for Zolpidem, in recent years an important increase in Zolpidem dependence has been detected; for this reason, Zolpidem was transferred to Schedule IV of the 1971 Convention (i.e., for drugs inducing dependence such as BDZs) in 2001 (12–14).

High-dose Zolpidem use (600-2000 mg/die) has been associated with psychostimulant effects, such as feelings of well-being, euphoria ("high"), energy, alertness, sociability, talkativeness, delusions and psychotic experiences, sleepwalking, falling asleep while driving, sleep-related eating disorders or engaging in other activities while not fully awake (15–17).

It is also interesting to note that, in a large number of hypnotic drug abusers, a selective preference for Zolpidem was reported by subjects that were positive at screening tests for adult Attention Deficit/Hyperactivity Disorder (18, 19).

Finally, intense craving, inability to stop use and withdrawal were associated with long-term high dose Zolpidem consumption. Through the analysis of adverse reaction data provided by the European Medicines Agency and after assessing the potential for abuse and dependence of ZDs, it has been shown that Zolpidem is more frequently involved in both abuse and withdrawal problems.

The QT interval begins with the beginning of the QRS complex and ends with the end of the T wave and has an inverse relationship with heart rate (HR). A rate-related (or corrected) QT interval (QTc) according to Bazett's formula (20) can be calculated as:

$$QTc = \frac{\text{QT interval}}{\text{cardiac cycle insecond}}$$

The upper limit of a normal QTc interval is 470 ms in males and 480 ms in females. As for the lower limits of the QTc, they have not been well established, but sometimes values between 330 and 360 ms are mentioned. (21).

Long QT syndrome (LQTS) is a myocardial repolarization disorder characterized by a prolonged QT interval on the electrocardiogram (ECG). The main symptoms in patients presenting LQTS include palpitations, syncope, seizures and sudden cardiac death. This syndrome can be congenital or acquired. The acquired form is related to drug therapy and the presence of hypokalemia and hypomagnesaemia may accentuate the risk of drug-induced LQTS development. One of the main risks of LQTS is that it generates polymorphic ventricular tachycardia, i.e., a ventricular rhythm greater than 100 bpm with frequent changes in the QRS axis, its morphology, or both (22, 23).

Torsades de pointes (TdP) is a form of polymorphic ventricular tachycardia which derives from either acquired or congenital QT interval prolongation, and manifests with a heart rate between 160 and 250 bpm. These variations take the form of a progressive, sinusoidal and cyclical evolution of the QRS axis; the peaks of the QRS complexes appear to "twist" around the isoelectric line of the recording. This condition tends to spontaneously regress; however, multiple episodes can occur in rapid succession and may degenerate into ventricular fibrillation and sudden cardiac death. Determining the absolute and comparative risk of many drugs associated with QT interval prolongation is difficult, as most of the available data comes from case reports or small series of observations. Furthermore, the incidence of QT prolongation without torsades de pointes (Tdp) is much higher than the incidence of TdP itself.

The pathophysiological mechanism underlying drug-induced TdP is the development of abnormal depolarizations of the cell membrane in the final part of the action potential, defined as early post-depolarization (EAD), or during diastolic repolarization, termed late post-depolarization (DAD).

Almost all drugs that cause LQTS cause blockage of the potassium channel, thereby inhibiting the rapid outward flow of potassium ions and, therefore, cellular repolarization (24, 25).

Furthermore, lower heart rates result in a smaller potassium output from the cell during repolarization, as there are fewer repolarization events; also, the reduction of extracellular potassium increases the degree of inhibition induced by the drug on the rapid potassium current, consequently increasing the QT interval. (25).

Most patients with drug-induced LQT have one or more risk factors for the condition.

In a review of the literature that included 249 patients with non-cardiac drug-associated QT prolongation, 97% had at least one risk factor and 71% had at least two. These included: female sex in 71%, history of heart disease in 41%, concomitant use of another QT prolonging drug in 39%, hypokalemia in 28%, a high dose of the drug in 19%, and a previous history of LQTS in 18%.

The most common risk factor for drug-induced LQTS is being female. In a review of the literature of 332 patients with cardiovascular drug-associated Torsades de pointes (Tdp), 70% were women. Compared to males, females have a longer QTc and a greater response to drugs that block the rapid potassium channel, favoring Tdp, possibly due to the effect of sex steroids on ion channel expression. Estrogen potentiates the prolongation of the QT interval induced by bradycardia and the development of arrhythmia. Conversely, androgens reduce the QT interval and make it less sensitive to drugs. (26–28).

In scientific literature, benzodiazepines and Z-Drugs are considered safe concerning LQTS (29); to date, however, there are no studies which address the abuse of high dosage of Z-drugs and QTc elongation risk.

Since several drugs cause QTc elongation, with this case series we want to analyze if QTc lengthening occurs on a total of 74 patients admitted to the Addiction Medicine Unit in Verona, Italy for daily use of high doses of Zolpidem.

Patients and methods

The study was conducted in the Addiction Medicine Unit of the G.B. Rossi University Hospital in Verona. The data were collected from hospitalizations carried out between January 2015 and February 2020 and refer to a total of 74 patients, of which 38 were males and 36 were females, that were being treated for detoxification from high doses of Zolpidem with "Verona Detox Approach With Flumazenil" (30, 31).

The criterion of dependence on high doses of ZDs was defined on the criteria established by the DSM IV-TR, which provide for the presence of continuous use for a period greater than 6 months, a daily intake of Zolpidem greater than at least five times the maximum recommended daily dose and problematic use of ZDs, such as mixing various molecules, increasing dosages, using for pleasure, obtaining them through illegal means or deriving negative social consequences. The dosage assumed was obtained from a drug use history assessment performed by the staff doctors.

The variables examined were demographic ones, that considered age and sex, and clinical ones, that considered the following: type of BDZ used; DDDE (mg); heart rate (HR; bpm); QY (ms); QTc (ms); Na+; K +; Cl-; additional therapy other than the BDZ.

Upon admission, blood samples were taken to assess electrolyte concentration and an electrocardiogram was performed in order to obtain HR and the QT interval. QT was corrected with HR using Bazett's formula : QTc = QT/Vrr.

Results

A total of 74 patients including 38 males and 36 females (**Table 1**) that were hospitalized, between January 2015 and February 2020, to the Addiction Medicine Unit of the G.B. Rossi University Hospital in Verona, Italy.

The average age of these patients was 44 years (SD 11,77) with a minimum of 23 and a maximum of 74 years; 38 were workers, 25 were unemployed and 11 were students (**Table 2**).

The mean daily dose of Zolpidem was 402.36 mg/day (SD ± 368.88) (**Table 3**), with a minimum of 20 mg/day and a maximum of 2,250 mg/day, while the mean DDD is 50.21 mg/day.

Assuming that the threshold value of QTc is 470 ms in males and 480 in females, we recorded a single case exceeding these threshold values (32). The patient was a male presenting a QTc of 479 ms and taking 50 mg/day of Zolpidem (25 mg of Diazepam equivalents daily dose); he also did not have concomitant therapies that could have caused QTc lengthening, no electrolyte alterations (Na 140 mmol/L, K + 3.75 mmol/L, Cl- 105 mmol / L), no contemporary or previous intake of barbiturates, heroin, cocaine, THC, alcohol, NMDA or nicotine.

It should also be noted that the QTc value of one of the 74 patients was not recorded.

TABLE 1 Gender.

Gender	Frequency	Percentage
Male	38	51.35
Female	36	48.65
Total	74	100

TABLE 2 Status.

Status	Frequency	Percentage
Worker	38	51.35
Unemployed	25	33.78
Other (student, retired)	11	14.86
Total	74	100

TABLE 3 Drug dosage.

	N	Minimum	Maximum	Mean	SD
Dosage mg (mean)	74	20	2,250	402.36	368.88
DDD	74	2	300	50.21	58.61

Discussion

Zolpidem is a non-benzodiazepine receptor modulator used in short-term treatment of insomnia aimed at patients with difficulty starting sleep; it improves measures of sleep latency, sleep duration, and reduces the number of awakenings in patients with transient insomnia. It also improves sleep quality in patients with chronic insomnia and can act as a minor muscle relaxant. (33–35).

The main thing to consider when starting patients on Zolpidem is the possibility of addiction and the development of withdrawal symptoms following drug dismission if the drug has been taken for a long time. An interprofessional team approach comprising healthcare professionals such as a pharmacist, a therapist, a nurse, and a clinician is critical; patients should also be educated on possible withdrawal effects resulting from the medication. Because Zolpidem has the potential to cause dependence, it should not be prescribed for long periods of time.

Moreover, long term use of Z-drugs causes cognitive impairments: several case control studies report that benzodiazepine or Z-drug use approximately doubles the risk of being involved in a motor vehicle accident (7, 36). Other effects may include dependence (37), and next-day cognitive, memory, psychomotor and balance impairments (38).

There is a mistaken belief that Z-drugs have advantages both in terms of efficacy and safety compared to BDZs, and, for this reason, many countries have seen a lower prescription of BDZs in favor of an increase in prescriptions of Z-drugs, especially Zolpidem. However, even this class of drugs can have important side effects, such as reduced functionality of cognitive abilities and worsening of psychomotor performances (1–4, 6, 8, 9).

Inadequate prescribing (non-therapeutic high-dosage administration, long duration of the treatment, association with different drugs, absence of monitoring) and poor overall assessment of the risks associated with their prescribing carries many potential risks to patients, including the risk of developing addiction.

Several drugs have been proven or suspected to cause QT prolongation; many of these medications are frequently used in the intensive care unit (ICU), such as different types of anesthetics, sedatives, antibiotics, antimycotics, antidepressants and antipsychotics. The mechanism behind drug-induced QT prolongation is primarily the blockade of potassium ion channels (Ikr) in myocytes, causing prolonged cardiac repolarization. A secondary mechanism is the blockade of hepatic drug degradation because of inhibition of the cytochrome P450 enzyme CYP3A4. Awareness on the many medications known to cause drug-induced LQTS is imperative, especially when the drugs are combined in the same patient (39, 40).

In literature there is no study that correlates the use of high doses of Z-drugs, in particular Zolpidem, with the analysis of QTc on the ECG. With this study we analyzed whether there was a correlation between the intake of high doses of Zolpidem and QTc prolongation.

The data collected showed that 1.35% of Zolpidem abusers developed QTc elongation, which corresponds to only one subject of the 74 total (with the exception of the single patients for whom we didn't record QTc data). We could deduce that Zolpidem does not represent a significant cause of this side effect, but this is the only study in literature that treats this problem with very selective criteria. Regarding the population that was taken into consideration, we can affirm that QTc lengthening can be explained by the simultaneous intake of other drugs that present this problem among their side effects (41, 42).

With the information we have, we are unable to formulate a hypothesis that explains this phenomenon. Perhaps other studies focusing on gender differences of patients taking high doses of BDZs or Z-drugs might be more insightful.

Limitation

This study presents several limitations: it's a retrospective study, Zolpidem dosage was reported by subjects, no follow-up was included and the sample size is small.

Conclusion

The present study shows a low risk of QTc elongation caused by high daily doses of Zolpidem; however, it must be considered that this is the only study currently present in literature and it could be useful to expand the data by analyzing a larger sample. Z-drugs and Zolpidem, proved to be safe drugs considering the risk of QTc lengthening, however the problem of abuse and dependence of this drug represents a growing phenomenon which is often underestimated by healthcare professionals.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the ethics committee of the Verona University Hospital (approval code 683CESC). The patients/participants provided their written informed consent to participate in this study.

Author contributions

SC, LZ, RC, and FL were responsible for the study concept and design. SC, FF, and LZ drafted the manuscript. LZ was responsible for the study methodology. All authors critically reviewed the content and approved the final version of the manuscript for publication.

Conflict of interest

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Publisher's note

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EDITED BY
Benjamin Rolland,
Université Claude Bernard Lyon 1, France

REVIEWED BY
Bernard Angerville,
Université Paris-Saclay, France
Udo Bonnet,
Castrop-Rauxel Evangelical Hospital, Germany

*CORRESPONDENCE
Thomas Zandonai
✉ tzandonai@umh.es

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Benzodiazepines in sport, an underestimated problem: Recommendations for sports medicine physicians' practice

Thomas Zandonai[1,2,3]*, Ana María Peiró[1,2,4,5], Francesca Fusina[6,7], Fabio Lugoboni[8] and Lorenzo Zamboni[8]

[1]Department of Pharmacology, Paediatrics and Organic Chemistry, Miguel Hernández University of Elche, Alicante, Spain, [2]Neuropharmacology on Pain and Functional Diversity, Institute of Health and Biomedical Research of Alicante (Instituto de Investigación Sanitaria y Biomédica de Alicante), Alicante, Spain, [3]Department of Social and Developmental Psychology, Sapienza University of Rome, Rome, Italy, [4]Pain Unit, Department of Health of Alicante, University General Hospital, Alicante, Spain, [5]Clinical Pharmacology Unit, Department of Health of Alicante, University General Hospital, Alicante, Spain, [6]Padova Neuroscience Center, University of Padova, Padua, Italy, [7]Department of General Psychology, University of Padova, Padua, Italy, [8]Unit of Addiction Medicine, Department of Internal Medicine, Integrated University Hospital of Verona, Policlinico "G.B. Rossi", Verona, Italy

In the last years, only few studies in literature have focused on the use and abuse of benzodiazepines (BZDs) in sport. Benzodiazepine-related problems include misuse, addiction, driving impairments, and morbidity and mortality related to overdose and withdrawal. Two clinical cases regarding elite endurance athletes evidenced that they had started to use BZDs to counteract insomnia, to recover faster from training sessions and to manage muscle pain. One of the important points that emerged from their stories was that their sports doctors did not recognize the drugs' addictive properties, and did not intervene to gradually reduce the dosage. Experts have previously provided recommendations for BZD therapy management in clinical practice. In this article, we would like to address sports medicine physicians specifically and provide guidelines to help them manage situations involving BZD prescription, the recognition of addiction, and intervention strategies.

KEYWORDS

addiction, sleep, insomnia, anxiety, drugs, guidelines

Introduction

Sleep is vital for health and well-being and it is important for cognitive functioning, mood, mental health, and cardiovascular, cerebrovascular, and metabolic health (1). The need to guarantee adequate sleep to elite athletes has increasingly prompted researchers to investigate the most important factors influencing athletes' sleep quality. Recent studies have addressed the effects of overtraining during preparation (2) and the benefits of interventions that both assess and manage travel fatigue and jet lag (3). Additionally, these studies proposed strategies for enhancing sleep quality as well as tools for practitioners who manage and optimize athletes' sleep (2, 3).

Poor sleep may diminish athletic performance, impair recovery, and increase the risk of injuries (2, 4). Moreover, it compromises athletes' abilities to maintain both good performance levels and a positive mood. A possible risk factor for sleep disturbances in athletes is also anxiety (2, 5, 6). Indeed, a recent meta-analysis on current elite athletes showed that 34% of them had symptoms of anxiety/depression (7). For this reason, occasionally, the loss of this balance could induce them to turn to sleeping medications as a solution. Over the past few years, research has tried to direct the attention of the sporting community to the use and abuse of benzodiazepines (BZDs) in sport (8–10).

Benzodiazepines are among the most commonly prescribed medications for insomnia and anxiety, and they are extensively used in clinical practice. BZDs act as positive allosteric modulators of the GABA-A (Gamma-Aminobutyric Acid Type A) receptor (11). BZDs can be subdivided into different groups based on their chemical structure and pharmacokinetic properties, resulting in different associated mechanisms of action and consequent clinical effects. Long-term BZD use is generally avoided due to their potential in the development of addiction (12, 13). Indeed, several studies have evidenced that BZDs should be considered a suitable treatment for specific clinical situations and for short-term use only (2–4 weeks) (14).

Despite clinical recommendations, long-term BZD users range from 6 to 76% of total users. Fifteen to forty-four percentage of them present moderate-to-severe withdrawal symptoms, and 3–4% have a full-fledged addiction (15). These drugs present several dangerous side effects. Among these, some of the most important are: multifocal cognitive dysfunction (16); the fact that BZDs could impair information acquisition, with additional adverse effects on anterograde memory processes (17); and, finally, the increase in cognitive decline incidence in the elderly, especially when they make long-term use of BZDs (18, 19). Regarding driving, a recent study showed that the impairing effects of benzodiazepine hypnotics on driving may mitigate over time following long-term use (i.e., 3 years or more), although the BZD-related neurocognitive impairments may remain (20). BZDs inhibit transmission on the postsynaptic γ-aminobutyric acid (GABA) neurons inducing a decrease in muscle spasms through alterations of central nervous system conduction. One of their effects is induced muscle weakness, which could put serious strain on the joints and back especially during intensive sport efforts, causing possible injuries. However, quality studies on this issue are needed to support this (21, 22).

Moreover, BZDs used in association with other drugs could increase difficulties in treatment (23); BZD use is associated with increased risk of car accidents (24); long- and short-acting BZDs increase the risk of falling, especially in elderly patients and children (25–27), as well as accident-related consequent hospital admissions (28, 29). In pregnant women, BZDs could contribute to neonatal morbidity and some congenital malformations (30). Lastly, long-term BZD use has been found to reduce the quality of life (31).

Benzodiazepines in sport

A few years ago, some studies tried to encourage the debate on the use of BZDs in sports (32, 33), but only in recent years does interest seem to have been rekindled. Indeed, two clinical cases were reported in literature regarding elite endurance athletes, both addicted to such high doses of BZDs that they needed detoxification even after a week of hospitalization (8, 9) using a slow infusion of flumazenil (Verona approach) (34). The first of the two cases was that of a 38-year-old professional cyclist. He used high doses of caffeine, and subsequently cocaine, to improve his performance. This led him to develop insomnia, which he tried to manage with lormetazepam, to which he became addicted; furthermore, he continued his caffeine and cocaine use, especially in the morning, to counteract the effects of lormetazepam, in what became a vicious cycle of addiction (8). His medical history showed long-term lormetazepam use in the form of an oral solution, starting from 2.5 mg/ml and leading to 20 ml 1/4 50 mg, the equivalent of 250 mg of diazepam per day.

In the second case, a 30-year-old female elite marathon runner was similarly in treatment for lormetazepam detoxification. She too reported increasing her daily intake of lormetazepam to sleep better and to increase her training performances. As a result, her lormetazepam intake reached a total of 360 ml (900 mg) of lormetazepam per day, which is the equivalent of 4500 mg/day of diazepam.

According to their anamnesis, they had started to use lormetazepam to counteract insomnia, to recover faster from exercise sessions and to manage muscle pain. Regarding this last issue, studies which investigated the effects of BZDs on pain management found limited utility and conflicting results (35–37). In addition, athletes likely experience pain and pain treatment differently than people undertaking general exercise, and they may be more likely to use different types of analgesic drugs, incurring in more risks than benefits (38, 39). Like all medications, BZDs have the potential for both harm and benefit. For this reason, physicians should help patients consider these factors appropriately, and develop a treatment plan that is safe and effective for them (40). However, one of the most striking points that emerged from these two cases was that their sports doctors did not recognize the drugs' addictive properties, and no intervention to reduce the therapy was implemented.

Given that there is a widespread conviction among athletes that taking drugs will improve their performance, the question arises whether there is evidence that BZDs, which are not prohibited in sport, are indeed beneficial to exercise performance. According to our latest literature review, it seems that BZDs have no ergogenic effect on exercise performance

and potentially even have a negative effect on exercise. The ten studies reviewed by the authors used relatively small sample sets of participants and heterogeneous methodologies with regards to exercise test type, participant type, BZD types and the doses administered. The therapeutic use of BZDs seems unquestionable, but another source of uncertainty is that most of the studies failed to report if BZDs actually improved sleep quality or reduced pain. The review highlights that the drugs' active mechanisms are still unknown, that further studies are needed in order to increase the number of participants, and that female participants must be included to discern gender's influence on the drugs' effects (10). Moreover, the risk of misuse is still largely underestimated by clinicians and institutions around the world and chronic users of BZDs may develop deficits in working memory, learning, and attention. Depression, injurious falls, and traffic accidents are other common complications related to chronic use of BZDs (12).

Recommendations for sports medicine physicians

The above-mentioned data are required in order to enable the creation of evidence-based policies and guidelines for treatment. Experts have previously provided recommendations for the management of benzodiazepine therapies in the clinical practice (14, 41), but we would like to make the following recommendations to sports medicine physicians specifically to help them in future situations which entail benzodiazepine prescription, the recognition of addiction, and intervention strategies.

Sports medicine physicians should:

1. examine the specific benzodiazepine's likely benefits and risks in each individual case early in treatment and, in the case of prescription, closely monitor any behavior which could indicate misuse/abuse;
2. have knowledge concerning any concomitant use by the athlete of other drugs (such as analgesics) or non-pharmacological treatments, if necessary;
3. evaluate alternative treatments to benzodiazepines, especially if the treatment is expected to last more than a month;
4. we also suggest that they conduct a complete psychological assessment with an accurate anamnesis in order to ensure prescription of the best treatment for the patient;
5. calculate the potential duration of the treatment well in advance, considering any need for long-term drug therapy for insomnia and anxiety disorders; consider conducting psychological evaluations and multimodal medical assistance;
6. maintain close contact with patients that use BZDs and constantly monitor them to promptly address misuse/abuse behavior, specifically checking whether the patient:
   - takes therapeutic (low) doses for more than 3 months;
   - needs to take benzodiazepines to carry out normal sports activities every day;
   - continues using the drug although the original therapeutic indication has ceased to be necessary;
   - has difficulty stopping use of the drug or reducing the dosage due to withdrawal symptoms;
   - develops symptoms of anxiety between doses or, in the case of short-term benzodiazepine use, develops cravings for the next dose;
   - maintains regular contact with his/her sports medicine physicians to request repeated prescriptions;
   - displays elevated anxiety levels if the next prescription is not fulfilled rapidly;
   - increases their dosage compared to the original prescription and if they ask for prescriptions more frequently;
   - shows continued symptoms of anxiety, panic, agoraphobia, insomnia, depression, or physical symptoms despite the extended use of benzodiazepines;
7. gradually reduce the drug dosage after introducing a more specific therapy;
8. reconsider the diagnosis if the patient does not respond to therapy, or if the drug is taken for a longer duration or at higher doses than were originally foreseen.
9. It is important to remember that benzodiazepines are generally safe drugs, but that they could become very dangerous if combined with other substances, such as alcohol or opioids.

Recently, the International Olympic Committee (IOC) and the National Collegiate Athletics Association (NCAA) have addressed sleep as a major contributor to athletic performance and as a fundamental feature of athletes' mental health. These statements represent the increased awareness of the importance of sleep health among athletes (42).

Conclusion

In this article, we would like to address sports medicine physicians specifically and provide guidelines to help them manage situations involving BZD prescription, the recognition of addiction, and intervention strategies. We believe that these recommendations could also help to athletes and warn them of the possible effects that BZD can induce if taken without medical supervision.

Finally, it is important that sports medicine physicians keep in mind that BZDs are potentially addictive,

particularly fast-acting BZD typologies. Therefore, due to the lack of evidence about clinical issues regarding misuse criteria, additional information about the complicated relationship between BZDs and exercise is required, possibly involving larger samples.

Data availability statement

The original contributions presented in this study are included in this article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

TZ and LZ substantially contributed to the conception, drafting of the manuscript, and critical revision of the manuscript for important intellectual content. All authors reviewed the manuscript, provided significant input, read, and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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EDITED BY
James K. Rowlett,
University of Mississippi Medical Center,
United States

REVIEWED BY
Miroslav M. Savic,
University of Belgrade, Serbia
Dai N. Stephens,
University of Sussex, United Kingdom

*CORRESPONDENCE
Elif Engin
✉ eengin@mclean.harvard.edu

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$GABA_A$ receptor subtypes and benzodiazepine use, misuse, and abuse

Elif Engin[1,2]*

[1]Stress Neurobiology Laboratory, Division of Basic Neuroscience, McLean Hospital, Belmont, MA, United States, [2]Department of Psychiatry, Harvard Medical School, Boston, MA, United States

Benzodiazepines have been in use for over half a century. While they remain highly prescribed, their unfavorable side-effect profile and abuse liability motivated a search for alternatives. Most of these efforts focused on the development of benzodiazepine-like drugs that are selective for specific $GABA_A$ receptor subtypes. While there is ample evidence that subtype-selective $GABA_A$ receptor ligands have great potential for providing symptom relief without typical benzodiazepine side-effects, it is less clear whether subtype-selective targeting strategies can also reduce misuse and abuse potential. This review focuses on the three benzodiazepine properties that are relevant to the DSM-5-TR criteria for Sedative, Hypnotic, or Anxiolytic Use Disorder, namely, reinforcing properties of benzodiazepines, maladaptive behaviors related to benzodiazepine use, and benzodiazepine tolerance and dependence. We review existing evidence regarding the involvement of different $GABA_A$ receptor subtypes in each of these areas. The reviewed studies suggest that α1-containing $GABA_A$ receptors play an integral role in benzodiazepine-induced plasticity in reward-related brain areas and might be involved in the development of tolerance and dependence to benzodiazepines. However, a systematic comparison of the contributions of all benzodiazepine-sensitive $GABA_A$ receptors to these processes, a mechanistic understanding of how the positive modulation of each receptor subtype might contribute to the brain mechanisms underlying each of these processes, and a definitive answer to the question of whether specific chronic modulation of any given subtype would result in some or all of the benzodiazepine effects are currently lacking from the literature. Moreover, how non-selective benzodiazepines might lead to the maladaptive behaviors listed in DSM and how different $GABA_A$ receptor subtypes might be involved in the development of these behaviors remains unexplored. Considering the increasing burden of benzodiazepine abuse, the common practice of benzodiazepine misuse that leads to severe dependence, and the current efforts to generate side-effect free benzodiazepine alternatives, there is an urgent need for systematic, mechanistic research that provides a better understanding of the brain mechanisms of benzodiazepine misuse and abuse, including the involvement of specific $GABA_A$ receptor subtypes in these processes, to establish an informed foundation for preclinical and clinical efforts.

KEYWORDS

benzodiazepines (BDZs), drug abuse, $GABA_A$ receptor, withdrawal, tolerance, reward, dependence

1. Introduction

Benzodiazepines (BDZs) have been in use since 1960s and are still prescribed at high rates with over 90 million prescriptions dispensed in the US alone each year (1). In 2015, one in eight US adults reported BDZ use within the past year, further illuminating the widespread use of BDZs. Studies from other countries indicate comparable rates of prescribed or non-medical BDZ use despite some variation in rates and in the specific subpopulations (e.g., the elderly) where BDZ use is most common (2–8).

BDZs achieve their therapeutic effects through the allosteric modulation of gamma amino butyric acid type A receptors (GABAARs). GABAARs are postsynaptic pentameric complexes, with the subunits comprising the pentamere drawn from a subunit repertoire of at least 19 subunits (α1-6, β1-3, γ1-3, δ, ε, θ, π, ρ1-3). Most GABAARs in the brain are composed of 2 α, 2 β and one γ or δ subunit, with the specific subunit composition influencing receptor kinetics, subcellular localization, and anatomical distribution of the receptor in the brain, as well as its pharmacological properties with regards to its modulation by different drug classes (9–12). GABA binding to binding sites at the interface of α and β subunits leads to the opening of the chloride channel at the center of the pentamere, allowing chloride movement between the intracellular and extracellular spaces. In the adult brain, this usually results in chloride influx to the cell and hyperpolarization, while in the immature brain [and possibly in the mature brain under certain pathological conditions; (13)], the opening of the channel leads to chloride efflux and depolarization. BDZ binding sites are distinct from the GABA-binding site and are located at the interface of the α and γ subunits on GABAARs containing the α1, α2, α3, or α5 subunits (α1GABAAR, α2GABAAR, α3GABAAR, and α5GABAAR). Thus, BDZs bind a subset of GABAARs, at a site distinct from the GABA-binding site, and their effect is to increase the frequency of chloride channel opening at a given GABA concentration, causing a leftward shift in the GABA dose-response curve without altering the maximal response.

BDZs have anxiolytic, sedative, hypnotic, amnestic, anticonvulsant, myorelaxant effects (9). While this heterogenous effect profile has made it possible for BDZs to be used for a wide range of indications and in different settings, the desired effects in one setting are often viewed as undesired side-effects in another setting (e.g., sedation and anterograde amnesia are highly desirable effects when BDZs are used in a peri-surgical setting but are highly undesirable when they are used as anxiolytics in the treatment of generalized anxiety disorder).

Considering the apparent functional relevance of the subunit composition of GABAARs to receptor properties and anatomical location, it was postulated that the different behavioral effects of BDZs may be mediated by their positive modulation of different GABAAR subtypes. Findings from early studies indeed indicated that BDZ modulation of α1GABAARs is required for the sedative effects (14), while BDZ modulation of α2GABAARs is required for the anxiolytic-like effects of BDZs (15). Continued work in this area not only confirmed and further expanded the association of specific behavioral effects with specific GABAAR subtypes (16–25), but uncovered new, previously unappreciated indications for subtype-selective GABAAR modulation (26–30).

The above studies, many of which were carried out in genetically modified mice due to a lack of subtype-specific pharmacological agents, demonstrated the possibility of developing subtype-specific agents that would have efficacy for specific indications without the undesirable effects of BDZs. Efforts to develop subtype-selective GABAAR modulators have yielded a large number of drugs in the last 30 years [For recent reviews, see (31, 32)]. While no truly subtype-specific drug has been developed to date, several compounds with subtype-selective affinity or subtype-selective efficacy have been investigated in preclinical studies for their behavioral effects, with a few of them also making it to clinical trials. The below sections aim to answer the question of whether these subtype-selective compounds would have reduced abuse and dependence liability compared to classical BDZs by summarizing relevant findings from preclinical studies.

2. Benzodiazepine abuse and misuse

DSM-5-TR (33) criteria for Sedative, Hypnotic, or Anxiolytic Use Disorder (pp. 620–621) focus on a number of problematic drug-related behaviors many of which can also be studied in preclinical work. The criteria can be roughly categorized as those that indicate *loss of control over use* (i.e., using the drug in larger doses or for a longer time than intended, continuing use despite negative consequences, failed attempts to reduce or stop use), *expenditure of significant time and effort for drug related activities, often at the expense of other desirable activities* (e.g., time/effort/money spent in acquiring the drug, recovering from drug effects, giving up on other activities in favor of using the drug, not being able to focus on other activities due to craving, failure to fulfill major obligations at work, home, school, other social settings, due to drug use), *risky drug use* (e.g., recurrent use in physically hazardous situations such as driving under the influence, taking risks to acquire the drug), and *pharmacological criteria* (i.e., development of tolerance and withdrawal). Having only 2 of the 11 listed symptoms is sufficient for diagnosis, with the presence of 2–3 symptoms considered "mild", 4–5 symptoms "moderate", and 6 or more symptoms "severe".

While the DSM criteria outline the typical *behavioral presentations* of BDZ abuse and misuse, research on medical and non-medical use of BDZs reveals the most common *reasons* underlying BDZ abuse and misuse.

While some recreational users of BDZs use BDZs alone for their alcohol-like euphoric effects, BDZs are more often abused in combination with other drugs, most commonly opioids, to supplement the high (34–36). These users typically use BDZs at higher doses than the common therapeutic range (37) and as suggested by the recent popularity of fast-acting designer BDZs in illicit drug markets, they may prefer faster and shorter acting BDZs (38). Another common use of BDZs among illicit polydrug users is to use the BDZs as a way of managing the anxiety and irritability commonly experienced as a part of the withdrawal from the primary drug when regular access is disrupted, or managing anxiety experienced due to co-occurring psychiatric conditions (34, 39).

Misuse of BDZs in medical settings involves the use of BDZs for different indications, at different doses, and/or for longer periods of time than recommended. Off-label prescription of BDZs, particularly

for indications such as post-traumatic stress disorder, obsessive-compulsive and related disorders, and mood disorders is common (40–43). While this is a concern, it should be noted that off-label prescription of medications for different indications than those approved is common practice for many drugs and is not specific to BDZs.

The second concern with BDZ misuse is patients using BDZs at higher doses that recommended, particularly when used long-term. While well-documented development of tolerance to the effects of BDZs would support the expectation that patients would escalate dose with long-term use, there has been relatively little empirical evidence to support consistent escalation of BDZ dose, even among long-term BDZ users (44, 45). This may be due to the fact that tolerance develops primarily to the sedative effect of BDZs, which is often viewed as an undesirable side-effect by individuals who take BDZs for anxiety-related indications, while tolerance to the anxiolytic effect is either small and delayed or non-existent in humans (46).

While off-label use or dose escalation do not seem to be major concerns for BDZ misuse, extended use is a significant issue. Current recommended length of treatment with BDZs is 2–4 weeks, with no BDZ approved for use for more than 4 months. Yet, many patients are prescribed BDZs for months, years, decades, sometimes indefinitely (47–53). More alarmingly, while the number of new BDZ prescriptions remained stable between 2005 and 2015, there was a 50% increase in renewed prescriptions during the same period, suggesting a specific increase in this problematic, longer-term use (54). Aside from continued need for therapeutic relief, withdrawal symptoms are the primary reason for long-term BDZ use.

In 2020, FDA issued a requirement to update the Boxed Warning on BDZs, indicating that following chronic use of BDZs over several days or weeks, abrupt cessation or dose reduction of BDZs can cause severe withdrawal symptoms, including seizures (1). Indeed, studies indicate that withdrawal symptoms can continue for months, even years (55). In a recent Internet study, 60–85% of individuals reported having moderate to very severe symptoms in different life domains while tapering off BDZs, with 54% of them reporting suicidal thoughts (55). The challenges involved in discontinuing BDZs were present even when tapering was done in a clinical setting where the withdrawal symptoms were closely managed (56, 57).

In summary, two major reasons for BDZ abuse and misuse are the reward-related effects of BDZs, mainly related to abuse, and physical dependence, as defined by the presence of a withdrawal syndrome upon discontinuation, which is the primary underlying factor for misuse, with likely involvement in abuse as well. As noted earlier, the efforts to develop GABAAR subtype selective compounds have been motivated by the idea of developing GABAergic therapeutics without the unfavorable side-effect profile of classical BDZs. Thus, a highly significant question is whether GABAAR subtype-specific compounds, if developed, would have the same abuse and misuse liability as BDZs. To start answering this question, we review evidence regarding the involvement of specific GABAAR subtypes in behaviors relevant to the 3 main domains of DSM-5-TR criteria for Sedative, Hyponotic, and Anxiolytic Use disorder: Reward-related effects of BDZs which support persistent drug-seeking, development of maladaptive behaviors associated with BDZ use, and development of BDZ tolerance and withdrawal.

3. GABAAR subtypes and reward-related effects of BDZs

Based on the DSM criteria provided above, it is possible to inquire into the rewarding effects of BDZs at multiple levels. For any compound to be used by choice or abused, it should first serve as a reinforcer, that is, its administration should increase the likelihood of behaviors that preceded it and/or were causally linked to it. The simplest form of this would be a preference for BDZs over alternatives when the two come at equal and negligible cost. For instance, rodents drink more from the bottle containing the water-soluble BDZ midazolam, when midazolam and water are provided in a two-bottle choice setup in their home-cages (24, 58–60). A related concept is drug-seeking behavior: BDZs support associative learning in a conditioned place preference paradigm where animals spend more time in the BDZ-associated chamber of a two-chamber apparatus during the drug-free test session (61). The second level would be the question of willingness to expend effort to acquire the drug. BDZs are self-administered in tests where animals have to engage in operant behaviors (e.g., press a lever) to receive the drug (34, 62) and increase the level of effort the animals are willing to expend to receive a brain stimulation reward in intracranial self-stimulation (ICSS) studies [i.e., reward enhancement; (24, 63, 64)]. These two levels are linked to the value of BDZs as reinforcers and thus, the question of reward (see Section 3.3 for possible issues with this interpretation). However, DSM criteria go further than this and include many maladaptive consequences of BDZ abuse, including the devaluation of natural reinforcers (e.g., food, sex) and giving these up in favor of BDZs, engaging in risky behaviors under the influence of or in order to acquire BDZs, and the neglect of responsibilities (e.g., poor parental behavior) due to BDZ abuse. As many of these behaviors may depend on the reinforcing value of the drug, with stronger reinforcers causing more maladaptive behaviors, we will be covering maladaptive behaviors under the general heading of reward-related behaviors. However, it should be noted that interactions with specific properties of drugs may influence each of these categories differentially. For instance, alcohol and stimulant use have different effects on the disinhibition of sexual behaviors and risk-taking (65).

At the level of simple preference, the preference of rodents for the midazolam-containing liquid in two-bottle choice experiments has been shown to depend on midazolam binding to α1 and α2GABAARs (24, 59). These studies employed mice with point mutations that make the targeted subunit insensitive to BDZs (14–16, 18, 19). While mice with point mutations on the α3 or α5 subunits continued to prefer midazolam-containing solution, this preference was abolished in mice with mutated α1 or α2 subunits.

In support of the integral role of α1 modulation in the pleasurable effects of BDZs, α1-preferring compound zolpidem is self-administered by non-human primates (NHPs) and has higher reinforcement value than non-selective BDZs, such as diazepam or midazolam, in self-administration tests (66–69). Comparison between zolpidem and midazolam is particularly relevant, as early studies suggest that short-acting BDZs act as stronger reinforcers than longer acting BDZs [(66, 70, 71); see (72) for a comparison of pharmacokinetic properties of commonly used BDZs]. Zolpidem, as a rapidly eliminated BDZ modulator, might owe its reinforcing value to its fast action as well as its receptor selectivity. Thus, a comparison with a rapidly eliminated non-selective BDZ, such as

midazolam, isolates the role of receptor selectivity as a determinant of reinforcement value.

Self-administration of zolpidem demonstrates that α1-binding may be sufficient to sustain self-administration. Another relevant question is whether α1-binding is necessary. For instance, the 2-bottle choice experiments above indicate that α1-binding might be necessary for midazolam preference. Some studies (73) indeed suggest that α1-sparing compounds do not maintain self-administration in NHPs, agreeing with the necessity of α1-binding. Others (68, 69) suggest that sparing α1 is not sufficient to eliminate self-administration. Shinday et al. (69) elucidate the importance of drug history in this process, where α1-sparing compounds maintained self-administration in animals trained with midazolam, but not in animals trained with cocaine. As subjective stimulus properties of BDZs were shown to be primarily mediated by α1 in drug discrimination tests (67), this finding is unlikely to be a result of the subjective similarities between the effects of an α1-sparing drug and the training compound midazolam. Drug history was found to be important in the reinforcing effects of BDZs in humans as well, where non-selective BDZs were found to be more reinforcing in individuals with histories of sedative use and moderate alcohol consumption (34). It is possible that previous chronic exposure to GABAergic compounds causes changes in the expression and trafficking of GABAAR subtypes (74) and/or plasticity involving other systems (75, 76), such that α1-sparing compounds can activate brain circuitry involved in the experience of reward at a level that can maintain self-administration (see below for a more detailed discussion of plastic changes following long-term exposure to GABAergic drugs).

In cases where α1-sparing compounds are self-administered, efficacy at α2/3 seems critical for the maintenance of self-administration, based on reports that BDZ self-administration in NHPs is not influenced by the co-administration of an α5-selective negative modulator (77) and that compounds with reduced efficacy at α2/3 do not maintain self-administration (73). A role for α2GABAARs in reinforcing properties of BDZs has also been substantiated by ICSS studies in mice, where mice with mutated α2 subunits that render this subunit insensitive to the effects of BDZs no longer showed the reward-facilitating effects of diazepam or midazolam (24, 63). Similar to findings with self-administration of α1-sparing compounds by NHPs, Schwienteck et al. (78) reported that low-efficacy positive allosteric modulators with some selectivity for α2/3 lead to weak reward-facilitation in ICSS, suggesting that high-potency modulation of α2GABAARs might be both necessary and sufficient for self-administration and reward-facilitation effects. The demonstration of a role for α1GABAARs in ICSS has been less straightforward. While some studies suggested that α1-binding may be necessary (24) and sufficient (78), others noted negligible involvement of α1GABAARs in reward-facilitation effects (63). The differences in findings may be due to variability in dose ranges employed in different studies, as highly sedative compounds such as zolpidem can non-selectively reduce responding in ICSS giving the impression of reduced reward-facilitation, as well as to the variability in the drug histories of the animals in each study, as the studies involve sequential testing with multiple drugs. Finally, while the lack of α2- or α3-specific agents prevents conclusions regarding the individual contribution of each subtype to BDZ reward in pharmacological studies, the gene-targeted mouse studies suggest a possible involvement of α3GABAARs in the reward-enhancing effects of diazepam in ICSS (63), while such involvement was not found for reward-facilitation by midazolam (24), leaving the question of α3 involvement unresolved.

In summary, there is evidence that α1, α2, and possibly α3 subunits contribute to the reward-related effects of BDZs, with no involvement of α5GABAARs (77).

3.1. Maladaptive behaviors linked to BDZ use and GABAAR subtypes

The main maladaptive behaviors noted in DSM for Sedative, Hypnotic, and Anxiolytic Use Disorder can be categorized as those that represent abandoning natural rewards or responsibilities in favor of the drug and those that represent risky behaviors while using or to acquire the drug.

Devaluation of natural rewards (e.g., food, sex, caring for one's offspring, socializing) is a common consequence of drug addiction and has been investigated through animal models for different classes of drugs of abuse (79–83), often comparing drug responses to responses to palatable foods, such as sucrose. These experiments usually take the form of providing a sucrose solution while the animals are anticipating a drug reward. This leads to a comparison of the stronger drug reward with the now weaker, devalued natural reward. The effects of BDZs in this commonly used natural reward devaluation task have not been investigated. However, some early studies found a paradoxical role of drugs of abuse, including BDZs, in conditioned taste aversion (CTA) tasks (83). CTA tasks involve the pairing of a new, palatable food (e.g., a sucrose or saccharin solution) with an illness-inducing agent, such as lithium chloride. After this, animals avoid the consumption of the illness-associated stimulus. If the illness-associated stimulus is delivered intra-orally without operant behavior on the part of the animal, it is accompanied by suppressed ingestion responses, as well as active rejection responses such as gaping (84). The fact that preceding a palatable gustatory stimulus with a drug of abuse that is regularly self-administered by animals leads to reduced consumption of this stimulus was perplexing. Moreover, in the intra-oral delivery setting, the animals suppressed ingestion but showed no active rejection responses in this case, suggesting that the gustatory stimulus was not necessarily considered "aversive". This type of suppression of response to natural reward has instead been considered a form of natural reward devaluation, where the animals show reduced interest in the natural stimulus that was previously linked with a BDZ or other drug of abuse, because the stimulus is now considered less rewarding (i.e., is devalued) compared to the greater reward of the drug (83). This reduction of interest in palatable gustatory stimuli due to BDZ pairing cannot be attributed to an aversive effect of BDZs, as these compounds are readily self-administered, or to an overall suppression of appetite, as BDZs are otherwise known to increase food intake (85), further supporting the likelihood of a natural reward devaluation due to reward comparison effect.

Caring for offspring can be conceived of as a natural reward and as a translational measure of carrying out responsibilities. While evidence suggests that acute or sub-chronic administration of BDZs causes impairments in maternal behavior and fragmented care for the offspring (86, 87), no studies to our knowledge investigated the question of maternal care in a free choice setting where the dams are

provided with a choice to self-administer BDZs or care for offspring. As noted, studies also used acute or brief administration of BDZs which does not represent a drug use disorder scenario.

Overall, there is some support for the idea that BDZs might lead to devaluation of natural rewards, however, this question has not been systematically studied. Moreover, there is no information about the specific GABAAR subtypes that might be involved in this process to clarify whether the targeting of the specific GABAAR subtype might reduce the liability of natural reward devaluation compared to non-selective BDZs.

Acute administration of BDZs causes behavioral disinhibition and increased sensitivity to recent rewards, leading to increased risky decision-making (86, 88, 89). Strikingly, the facilitatory effects of BDZs on risky decision-making seemed limited to individuals with drug abuse histories and to relatively high doses of BDZs (89, 90), characteristics often observed in recreational BDZ users. Indeed, there is some evidence that polydrug users who also abuse BDZs engage in more risky behaviors compared to non-BDZ-using polydrug users (91, 92). Thus, there is some evidence that BDZ use may be associated with increased risk-taking behaviors, however, the brain mechanisms of BDZ-induced risk-taking are mostly unknown. One study showed that administration of lorazepam was linked to reduced activation of the amygdala and the medial prefrontal cortex and increased activation of the insular cortex during risky decision making [i.e., choosing of risky options over safe ones; (90)]. However, the study involved the administration of low doses of lorazepam which did not cause changes in risk-taking behaviors, which complicates the interpretation of the changes in brain activity. There have also been no studies to date investigating the involvement of different GABAAR subtypes in the promotion of risk-taking by BDZs. As all BDZ-sensitive GABAARs are expressed in the cortex and the amygdala, the findings from the Arce et al. (90) study also do not provide any clues as to which subtype(s) may be critical for the observed risk-promoting effects of BDZs. More relevant translationally is also the question of whether these acute effects are exacerbated upon chronic use, as is the case in the DSM definitions of Sedative, Hypnotic, and Anxiolytic Use Disorder, and how they might promote a cycle of risk-taking and drug use.

Overall, BDZs are self-administered and have been shown to facilitate reward effects in different species and a few studies investigated which GABAAR subtypes may be involved in these effects. However, maladaptive behavioral patterns observed in Sedative, Hypnotic, and Anxiolytic Use Disorder have not been studied in animal models, despite the availability of validated models from studies of other drugs of abuse. Thus, the question of whether specific GABAAR subtype(s) may play a central role in the progression of BDZ use from self-administration to a cycle of self-destructive behaviors remains open.

3.2. Brain mechanisms of BDZ reward

Drugs of abuse achieve their rewarding effects similarly to natural rewards, by increasing dopaminergic neurotransmission from the ventral tegmental area (VTA) to its mesolimbic target structures. While unexpected natural rewards initially cause increased dopamine firing in the VTA, after repeated presentation, the firing shifts to predictive cues from the reward itself (93). Importantly, drugs of abuse continue to cause increased firing even after repeated presentations, counter to the normal functioning of the brain reward system (60). Another important property of drugs of abuse is that they can induce long-lasting plasticity after even a single exposure (94). While the specific type of plasticity observed in the VTA depends on the mechanism of action of the specific drug of abuse, the overall effect is to cause increased dopamine release into the nucleus accumbens (NAc) and a priming of the VTA dopamine system that makes it more likely to respond to similar stimuli in the future.

BDZ actions on the mesocorticolimbic dopamine system are similar to other drugs of abuse. Specifically, BDZs increase dopamine release from the VTA onto target mesolimbic structures through a disinhibition mechanism, where BDZ binding to the GABAARs expressed on the VTA GABAergic interneurons leads to inhibition of the interneurons and the subsequent increased activation of the dopaminergic projection neurons (59, 60). Such a disinhibition-based mechanism is shared by some other drugs of abuse, such as opioids (95). In addition, like other drugs of abuse, a single injection of BDZs can cause VTA synaptic plasticity in the form of increased ratio of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) to *N*-methyl-D-aspartate (NMDA) receptor-mediated excitatory currents in the VTA for at least 3 days post-injection (96). BDZ binding to α1GABAARs seems to be both necessary and sufficient to induce BDZ-induced disinhibition and excitatory plasticity of the VTA dopamine neurons, as these effects were abolished in α1H101R mice that have BDZ-insensitive α1 subunits and the same effects could be induced by the α1-preferring GABAAR modulator zolpidem (59, 96). These physiological data provide a mechanistic explanation for the above behavioral findings noting self-administration of α1-preferring compounds and reduced ability of α1-sparing compounds to sustain self-administration.

Studies in rodents also point to the possibility of α2GABAAR involvement in BDZ-induced reward. α2GABAARs are expressed at negligible levels in the VTA, suggesting their involvement in BDZ reward may be through a different node in the brain reward system. Due to the high expression of α2GABAARs in the NAc, one possibility is that α2GABAARs mediate BDZ reward not by influencing dopamine release from VTA to target structures, but by modulating the effects of dopamine on those target structures such as the NAc. Viral-mediated knockdown of α2GABAARs in the NAc was indeed sufficient to abolish midazolam preference in a two-bottle choice drinking task (24). As α2GABAARs are expressed on both D1+ and D2+ medium spiny neurons (MSNs) of the NAc (97), it is difficult to speculate on an exact mechanism by which α2GABAARs of NAc regulate BDZ reward. Recent work suggests that α2GABAARs on D2+ NAc MSNs may be involved in the regulation of stress resiliency (29). As the effects of stress and subjective reward from BDZs seem to be closely linked (58, 98), it is possible that the α2GABAAR inhibitory regulation of D2+ MSNs plays a role in BDZ reward as well. Furthermore, α1GABAARs are expressed at high levels in the parvalbumin positive (PV+) interneurons of the NAc, which have been shown to play a significant role in motivated behaviors and the effects of drugs of abuse (99). The role of α1GABAARs in regulating the activity of this pivotal cell population indicates a second possible venue through which α1GABAARs might be involved in the reward-related effects of BDZs.

3.3. Issues related to interpretation and translation of findings from animal studies of BDZ reward

Studies using animal models provide a rich opportunity to understand pharmacological and brain mechanisms far beyond what could be achieved through studies in humans alone. However, like every modeling attempt, they come with certain possible confounds and alternative explanations that complicate the interpretation of findings within each model. Moreover, it is not clear whether findings from animal models can be directly translated to humans and caution should be exercised when drawing translational conclusions.

In animal models, the multitude of behavioral effects induced by BDZs often complicate the interpretation of results as purely reward-related. For instance, the two-bottle choice experiments where the rodents are presented with a bottle of water and a bottle of midazolam mixture may be affected by the sedative and amnestic effects of BDZs as well as their pleasurable subjective effects. Sedation may place a limit on drinking from the midazolam-containing bottle, as midazolam is fast-acting and highly sedative. The bottle placement is randomized every 24-h in these types of experiments, but amnestic effects may make it difficult for mice to learn which bottle has the pleasure-inducing liquid within the 24 h where the bottles remain put. Drugs affecting certain combinations of GABAARs may appear more preferred compared to other combinations due to increased pleasurable effects, or due to a reduction in sedative and/or amnestic effects, or when pleasurable effects and sedation and/or amnestic effects are mediated by the same receptor subtype, the pleasurable effects might be masked by the other effects. Similarly, while findings from the conditioned place preference test are often interpreted as drug-seeking behavior, they depend on the animal's ability to associate the context with the subjective effects of the drug during the training sessions and then retrieve this memory during the test session. Drugs with amnestic effects may interfere with this process. Drugs affecting specific receptor subtype combinations with reduced amnestic effects may look like they induce more drug-seeking behavior, purely due to better memory rather than increased reward, or again, reward-like effects might be masked by amnestic effects. ICSS, on the other hand, can be sensitive to the anticonvulsant effects of BDZs (100), as electrical stimulation of the forebrain can induce seizure activity. As anticonvulsant effects of BDZs are largely mediated by α1GABAARs (9), sparing binding to this subunit could increase ICSS thresholds (i.e., reduce apparent reward-facilitation by the compound) because of increased seizure susceptibility independent of any reward-related effects.

Self-administration studies often involve training with a drug that easily supports the acquisition of the operant behavior (e.g., cocaine), and then the ability of different drugs to maintain self-administration is tested. However, as noted in the above sections, for most drugs of abuse, even a single exposure can lead to long-lasting plastic effects in the brain reward circuitry. Moreover, we have noted that although there are points of convergence in the overall effects of drugs of abuse on the brain, the specific nature of these plastic effects depends on the properties of the drug. Based on this information, perhaps it is not surprising that Shinday et al. (69) found that the drug history of the animal determines whether an α1-sparing compound will maintain self-administration behavior or not. Thus, whether the animals received other compounds prior to testing and the specific properties of these compounds have the potential to affect outcomes and mask or supplement reward-related properties of the BDZs or subtype-selective compounds.

A final significant point is the comparability of the findings across species, and ultimately, the translatability of the findings to humans. Studies suggest many cross-species similarities in the expression of different GABAARs in brain areas relevant for the experience and processing of reward. For instance, high levels of α2 and α4, moderate-to-high levels of α1, and low-to-moderate levels of α3 expression in the striatum is observed in rodents (101–103), NHPs (104, 105), and humans (106). However, while α5 expression is undetectable in the striatum in rodents (101–103), studies report high levels of α5 in the NHP (105) and human (106) striatum. In the prefrontal cortex, while α5 expression is largely limited to layer 5, with low expression in other layers in rodents (101), the expression is more diffuse across layers in humans, with high expression in layers 4, 5 and 6, moderate expression in layers 2 and 3, and low expression in layer 1 (107). Thus, through strong expression in the striatum and more pronounced expression in the prefrontal cortex, α5 is more likely to have a role in reward processes in NHPs and humans than in rodents. In this sense, the finding that the co-administration of an α5-selective negative allosteric modulator did not influence triazolam self-administration in rhesus monkeys is highly relevant, suggesting that this subunit does not play an integral role in the maintenance of self-administration despite its dense expression in relevant brain areas in this species.

Based on the above-noted differences in GABAAR expression in different species, it is important to reemphasize here is that while all of the two-bottle choice, CPP, and ICSS studies reviewed above were conducted in rodents, all of the self-administration studies were conducted in NHPs. This adds another layer of complexity to comparative interpretation of the findings where differing task demands of different behavioral paradigms is also combined with possible species differences. Unfortunately, data on GABAAR expression in other relevant brain areas, such as the VTA, is missing in NHPs and humans, further adding to the uncertainty of the translatability of findings.

4. Tolerance to BDZ effects, BDZ withdrawal, and GABAAR subtypes

4.1. BDZ tolerance

Tolerance occurs at different rates for the different behavioral effects of BDZs, with rapid development of tolerance to the sedative and hypnotic effects, followed by the anticonvulsant effects (46, 57, 108–113). Tolerance to the anxiolytic effects is delayed and inconsistent in animal studies (114–118) and seems to be rare or non-existent in humans (46, 109, 110, 119, 120). Similarly, amnestic effects of BDZs do not seem to be attenuated during chronic treatment (111, 121–124). Lack of tolerance to amnestic effects can be considered a disadvantage, as amnestic effects are an undesirable side-effect of BDZs in most of their uses, particularly in case of elderly patients who take BDZs long-term, often for sleep problems (125–127).

A few studies addressed the question of whether the chronic modulation of specific GABAAR subtypes would lead to the same type of tolerance to specific behavioral effects as non-selective BDZs. Vinkers et al. (118) investigated the sedative, anxiolytic, and hypothermic effects of acute diazepam in mice treated chronically

with diazepam, bretazenil [partial, non-selective GABAAR positive allosteric modulator (PAM)], zolpidem (α1-preferring PAM), or TPA023 (α2/3 preferring PAM). Tolerance was observed to all three effects in chronic dizepam treated animals. In bretazenil treated mice, cross-tolerance to anxiolytic and hypothermic effects were observed, although there was no tolerance to sedative effects. Most strikingly, zolpidem-treated mice showed full tolerance only to the hypothermic effects of diazepam, while no tolerance to any of the effects was observed in TPA023-treated mice.

At first sight, the finding that zolpidem did not lead to sedative tolerance is particularly surprising, as the sedative effects of BDZs are mediated primarily by the α1GABAARs, raising the expectation that sedative tolerance would also be observed with a compound that is selective for α1GABAARs. However, studies conducted on mice with mutations that render specific GABAAR subunits BDZ-insensitive indicate that BDZ-binding to α5GABAARs is required for the development of tolerance to the sedative effects of BDZs (128). In wild-type mice, the development of tolerance to the sedative effects of diazepam was associated with a decrease in the expression of α5 subunits in the dentate gyrus. In the context of these findings, lack of tolerance to zolpidem's sedative effects can be attributed to its lack of affinity for the α5GABAARs. In line with this, chronic treatment with a non-selective BDZ can cause cross-tolerance to the sedative effects of zolpidem (129), presumably due to the fact that chronic BDZ exposure has led to changes in dentate gyrus α5GABAAR expression during this time. Chronic BDZ treatment also causes a reduction in the expression of α1GABAARs in cortex (74, 130), which may also play a role in sedative tolerance.

Overall, studies suggest that α1-preferring compounds may cause little or no sedative tolerance compared to non-selective BDZs, however, findings are far from unequivocal (66, 74, 131–135). Moreover, as α1-preferring compounds are used long-term primarily for their hypnotic effects, the more clinically relevant question is whether tolerance develops to their hypnotic effects through chronic use. While some animal studies suggest that tolerance develops to the sleep-promoting effects of zolpidem over chronic administration (136), clinical work suggests less tolerance to the hypnotic effects of zolpidem compared to non-selective BDZs, at least at lower doses (137, 138).

Additionally, despite demonstrating tolerance to the anxiolytic-like properties of diazepam, the Vinkers et al. (118) study suggests that α2/3-selective compounds may provide anxiety relief even chronically, without any apparent tolerance to the anxiolytic effects. As α1-sparing compounds also do not cause sedation, this would be the ideal scenario for a long-term, effective anxiolytic. However, while previous studies suggested that α1GABAARs are required for the sedative effects of BDZs, recent preclinical work suggests that at high occupancy levels, BDZ binding to α3GABAARs may be sufficient to produce sedation (25). These preclinical findings also help to explain clinical findings that MK-409, a compound with selective efficacy at the α2/3GABAARs caused sedation in healthy volunteers (139).

The most relevant aspect of tolerance development to BDZ misuse and abuse would be the escalation of dose over use in order to attain the previous levels of pharmacological effect. However, studies show that escalation to higher doses over long-term use is rare with BDZs (44, 45, 140, 141). In summary, tolerance to specific effects of BDZs does not constitute a major problem from the perspective of BDZ misuse and there is some evidence that at least sedative tolerance can be circumvented through the use of α1GABAAR-selective compounds.

4.2. BDZ withdrawal

Tolerance and dependence are often viewed as related phenomena, both stemming from compensatory changes in the affected receptors and systems over prolonged exposure. However, experimental evidence suggests that the development of BDZ tolerance is not an indication that the individual will experience physical dependence to BDZs. On the contrary, BDZ tolerance and withdrawal seem to be independent phenomena where withdrawal symptoms can be observed in behavioral domains where no tolerance was observed and vice versa (64, 142). This behavioral distinction between tolerance and withdrawal is accompanied by distinct molecular effects of long-term exposure to BDZs vs. discontinuation of treatment [e.g., (143)].

Common BDZ withdrawal symptoms include agitation, anxiety, mood swings, muscle tension and spasms, feeling of "pins and needles", perceptual sensitivity to light and sound, and seizures. Severe withdrawal can involve hallucinations and paranoid delusions, depersonalization, and can be fatal (57, 144, 145). Withdrawal symptoms appear within 2–3 days of cessation for short-acting BDZs and 5–10 days for longer-acting BDZs (137). Severe symptoms can mostly be avoided by gradual discontinuation over 6–8 weeks. However, even with managed discontinuation, it is estimated that up to half of the patients develop some level of withdrawal symptoms (145). For instance, in a study where patients were withdrawn from BDZs with individually calculated and managed withdrawal parameters over 2 weeks, with clinical monitoring every 48 h including physical examination and intensive psychological support and psychoeducation, 6 out of 9 long-term lorazepam users failed to discontinue the drug (57), demonstrating the significant challenge imposed by withdrawal symptoms to discontinuation of BDZs. Not surprisingly, particularly for patients who have been using BDZs long-term (i.e., more than 6 months) or at high doses (e.g., equivalent of 100 mg diazepam per day or more), hospitalization during the withdrawal period and pharmacological management of the symptoms is recommended (145, 146).

Overall, withdrawal symptoms upon BDZ discontinuation are common and serious. Withdrawal symptoms are the main driver of BDZ misuse and can contribute to abuse where users may start using BDZs primarily for their positive effects as outlined above, but are drawn into an abuse cycle as the primary motivator behind use switches to the avoidance of withdrawal symptoms (147).

Despite the clear significance of withdrawal symptoms and the availability of tools, such as gene-targeted mouse models and some pharmacological compounds with at least some selectivity for specific GABAAR subtypes, the role of specific GABAAR subtypes in BDZ withdrawal symptoms has been addressed in only a few studies. Work in NHPs has demonstrated withdrawal signs after the discontinuation of α1-preferring compounds and the recapitulation of flumazenil (non-selective BDZ antagonist) precipitated withdrawal by α1-selective antagonists (73, 113, 129). However, these studies included measurement of only a small subset of typical BDZ withdrawal symptoms and it is not clear whether α1-preferring agents might engender only a subset of withdrawal symptoms. Similarly, the

duration or severity of withdrawal symptoms were not evaluated systematically in comparison to non-selective BDZs, leaving open the possibility that withdrawal from α1-preferring compounds might be milder, at least on certain symptoms, and/or briefer than that from non-selective BDZs. Finally, there is some evidence that discontinuation of α2/3-selective compounds may not result in a BDZ-like withdrawal syndrome (73, 129).

4.3. Brain mechanisms of BDZ tolerance and withdrawal

While it is tempting to assume that tolerance and withdrawal result simply from a compensatory mechanism whereby the cell-surface expression of the targeted receptor is reduced, BDZ tolerance and withdrawal seem to involve not only changes in GABAAR expression and function, but more complicated mechanisms that go beyond the GABAergic system.

Starting with the GABAergic changes, several studies reported changes in the expression levels of mRNAs for GABAAR receptor subunits upon chronic BDZ administration and discontinuation. As these changes have been thoroughly reviewed elsewhere (148) and seem to be complex and dependent on the brain area investigated, the specific BDZs employed, length and dose of administration, and whether the measures are taken at the end of the chronic administration period or following withdrawal, we will provide only a brief synopsis of the most common findings here.

The most common changes following chronic administration of BDZs are in expression of the α1 and α4 subunits (74, 149–154). While the findings have been mixed in terms of the presence of an effect, where effects were found, they were often in the direction of a reduction in α1 expression and an increase in α4 expression. Reduction in α1 expression in the cortex and the hippocampus has also been reported following withdrawal from chronic BDZs (124, 154). In experiments conducted in rat cerebellar granule cells, 5-day exposure of the cells to diazepam resulted in a decrease in α1 expression similar to the above *in vivo* studies (143). Withdrawal of diazepam, however, led to both a decrease in α1 and an increase in α4, suggesting discrete effects of chronic exposure and tolerance on GABAAR subunit expression. Withdrawal from zolpidem, an α1-preferring compound, led to similar changes in α1 and α4 expression as diazepam exposure *in vitro* (155). Similar reductions in α1 expression (in addition to α3 expression) were observed in the somatosensory cortex of mice following chronic exposure to zolpidem *in vivo* (74). An important conclusion of these findings is that the changes observed in GABAAR subunit expression are not limited to the subunits that are modulated by a given drug. We observe changes in the expression of the α4 subunit, which BDZs do not bind, following BDZ exposure and withdrawal, as well as changes in the α3 and α4 subunits following chronic exposure to an α1-preferring compound (74, 143, 155). Thus, for chronic exposure or withdrawal following a subtype-specific compound, we cannot assume that the GABAAR changes will be limited to the GABAAR subtype that is affected by this compound.

In addition to the above complex changes taking place in the GABAARs, BDZ tolerance and withdrawal involve other neurotransmitter systems in the brain. The glutamatergic system and synaptic plasticity involving NMDA and AMPA receptors, for instance, are causally involved in the development of a withdrawal syndrome following the cessation of chronic BDZ treatment (156). When the drug is withdrawn at the end of chronic BDZ treatment, there is often an asymptomatic refractory period of 3 to 5 days before the symptoms begin. Even for longest-acting BDZs, this refractory period is too long to be explained by the gradual clearance of the drug. During this refractory period, glutamatergic synapses go through a number of plastic changes with the insertion of AMPA receptors into the synapse and their subsequent phosphorylation, leading to increased AMPA/NMDA transmission ratio (157–163). Treatment with AMPA (but not NMDA) receptor antagonists during the refractory period abolishes the development of the withdrawal syndrome (164–167), demonstrating the causal involvement of this type of plasticity in excitatory synapses in the development of the withdrawal symptoms. A reduction in NMDA receptor expression and function is observed secondary to this enhancement of AMPA-mediated conductance (167) and the administration of NMDA receptor antagonists during the symptomatic portion of the withdrawal period can ameliorate symptoms (164). Even more strikingly, it was demonstrated that the co-administration of an NMDA receptor antagonist during chronic lorazepam administration can abolish tolerance to the anticonvulsant effects of lorazepam, although an overall reduction of BDZ-binding sites was observed in NMDA antagonist administered animals similar to controls (109), suggesting that glutamatergic mechanisms may be more important for the development of tolerance and dependence than changes in GABAAR expression.

The involvement of other systems and receptors [e.g., nitric oxide, (168); adenosine, (169); neuropeptide systems (170)] in the development of BDZ tolerance and/or withdrawal has been suggested, however, it is not clear whether the changes in these systems are essential for tolerance/dependence development or secondary to the observed changes in the glutamatergic and GABAergic systems.

Despite the well-established essential role of excitatory synaptic plasticity in the development of BDZ tolerance and withdrawal and close interactions between the glutamatergic and GABAergic systems, it is not known whether chronic modulation of specific GABAAR subtypes may lead to more rapid or enhanced glutamatergic plasticity. An understanding of these interactions would be essential for predicting dependence liability of subunit-specific GABAAR modulators. Similarly, it is not clear how GABAAR subtypes may interact with other neurotransmitter systems in a way that might exacerbate the observed tolerance and dependence symptoms, even if those neurotransmitter systems are not causally involved in the development of BDZ tolerance or BDZ withdrawal syndrome.

4.4. Issues related to interpretation and translation of findings from animal studies of BDZ tolerance and withdrawal

While hippocampal plasticity, which has been the focus of most studies related to BDZ withdrawal, is likely to be involved in the development of several withdrawal symptoms, it is highly likely that the development of tolerance to different behavioral

effects of BDZs and the development of different withdrawal symptoms following BDZ discontinuation involve different brain areas. Similarly, different GABAAR subtypes might be involved in different withdrawal symptoms. Thus, behavioral studies covering all common withdrawal symptoms and systematically investigating the development of each following chronic modulation of a specific GABAAR subtype followed by drug discontinuation are needed. If only specific symptoms develop following discontinuation of a GABAAR subtype-specific modulation, this can also be used as an opportunity to study the brain mechanisms of specific withdrawal symptoms. The studies reviewed above, while informative, have not undertaken a detailed study of the withdrawal phenomenon and its mechanisms, and during a time other areas of neuroscience and neuropharmacology research have seen an explosion of new findings with unprecedented detail, our understanding of BDZ withdrawal has progressed relatively little since the early studies conducted in 1990s and early 2000s.

5. GABAARs in alcohol and other substance use disorders

GABAARs are expressed heavily in most brain regions involved in the effects of drugs of abuse and modulate the activity of brain circuits involved in the behavioral effects of drugs (171). As such, it is not surprising that different GABAARs have been implicated in the effects, use, and abuse of other drugs. Of these, alcohol is arguably the most relevant for discussion here due to its shared GABAergic mechanism.

Similar to BDZs, alcohol achieves most of its behavioral and subjective effects through positive allosteric modulation of GABAARs. Unlike BDZs, however, at high concentrations, alcohol modulates all GABAARs in an unselective manner, whereas at low concentrations (i.e., "social" drinking), synaptic GABAARs are mostly insensitive to alcohol's effects, whereas extrasynaptic, BDZ-insensitive GABAARs containing the δ subunits are highly sensitive to these low alcohol concentrations (172, 173). With chronic exposure, extrasynaptic responsiveness to ethanol decreases while synaptic responsiveness increases, with a concurrent relocation of α4GABAARs from extrasynaptic to synaptic locations (174). Changes in the expression and trafficking of other GABAARs, some of them similar to those observed with BDZ exposure, are also observed following chronic exposure to ethanol in animal models (175–178). In humans, several studies identified associations between GABRA2 gene (encoding the α2 subunit of the GABAAR) variations and alcohol use disorder (179–184). However, GABRA2 single nucleotide polymorphisms (SNPs) failed to reach significance on genome-wide association studies (GWAS) using more conservative analysis methods (185, 186). Still, GABRA2 gene expression was reduced in the hippocampi of alcohol dependent individuals in postmortem analyses (187). Others have found associations between polymorphisms in GABRA1 and GABRA6 genes and alcohol dependence (188, 189), however, again, these genes were not hits in GWAS studies.

Polymorphisms in the GABRA2 gene have also been implicated in stimulant (cocaine) and opioid (heroin) use disorders, particularly in interaction with early life adversity (190, 191). In cocaine-dependent individuals, GABRA2 SNPs were associated with cocaine cue reactivity (192). The involvement of α2GABAARs in some, but not all, effects of cocaine has also been confirmed in rodent studies (191, 193, 194). Finally, long-term exposure to cocaine was found to cause changes in the expression of α2GABAARs in the hippocampi of rodents (195), however, this finding was not confirmed in postmortem studies of hippocampi from individuals with cocaine use disorder (187). Others found that cocaine use disorder was associated with disruptions in several GABA-related genes in the postmortem dorsolateral prefrontal cortex, including GABRA1 and GABRA4. Interestingly, no changes were observed in genes related to glutamate signaling, emphasizing the special role of GABARs in the pathophysiology of substance use disorders (196).

6. Conclusions and directions

As seen, our knowledge regarding the involvement of specific GABAAR subtypes in all areas relevant to BDZ misuse and abuse, that is, reward processes, drug-related maladaptive behaviors, tolerance, and withdrawal, is characterized by gaps and a lack of systematic and mechanistic studies. Due to its central role in both BDZ misuse and BDZ abuse, an understanding of the mechanisms of BDZ withdrawal and how each GABAAR subtype is involved in the initiation and continuation of the withdrawal syndrome is particularly important. Research so far suggests that α1-sparing compounds would be highly desirable as anxiolytics, as they have the potential to provide anxiolysis without sedation and seem to have reduced abuse and misuse liability due to the apparent role of α1GABAARs in both the reward-related effects of BDZs and the development of a BDZ withdrawal syndrome upon cessation. However, some studies suggest the possible involvement of other GABAAR subtypes in these processes as well and it is not clear whether abolishing action at the α1GABAARs is sufficient to overcome potential for abuse and misuse. Considering the increasing burden of BDZ abuse, the common practice of BDZ misuse resulting in severe BDZ dependence in many patients, and the current efforts to produce subtype-specific GABAAR modulators as alternatives to classical BDZs, there is an urgent need for systematic and mechanistic research in this area.

Author contributions

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Conflict of interest

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Publisher's note

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EDITED BY
James K. Rowlett,
University of Mississippi Medical Center,
United States

REVIEWED BY
Richard De La Garza,
University of California, Los Angeles,
United States
Carolina Haass-Koffler,
Brown University, United States
Sally Huskinson,
University of Mississippi Medical Center,
United States

*CORRESPONDENCE
Mark K. Greenwald
✉ mgreen@med.wayne.edu

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Anhedonia modulates benzodiazepine and opioid demand among persons in treatment for opioid use disorder

Mark K. Greenwald[1]*, Tabitha E. H. Moses[1], Leslie H. Lundahl[1] and Timothy A. Roehrs[1,2]

[1]Substance Abuse Research Division, Department of Psychiatry and Behavioral Neurosciences, School of Medicine, Wayne State University, Detroit, MI, United States, [2]Sleep Disorders Center, Henry Ford Health System, Detroit, MI, United States

Background: Benzodiazepine (BZD) misuse is a significant public health problem, particularly in conjunction with opioid use, due to increased risks of overdose and death. One putative mechanism underlying BZD misuse is affective dysregulation, *via* exaggerated negative affect (e.g., anxiety, depression, stress-reactivity) and/or impaired positive affect (anhedonia). Similar to other misused substances, BZD consumption is sensitive to price and individual differences. Although purchase tasks and demand curve analysis can shed light on determinants of substance use, few studies have examined BZD demand, nor factors related to demand.

Methods: This ongoing study is examining simulated economic demand for alprazolam (among BZD lifetime misusers based on self-report and DSM-5 diagnosis; n = 23 total; 14 male, 9 female) and each participant's preferred-opioid/route using hypothetical purchase tasks among patients with opioid use disorder (n = 59 total; 38 male, 21 female) who are not clinically stable, i.e., defined as being early in treatment or in treatment longer but with recent substance use. Aims are to determine whether: (1) BZD misusers differ from never-misusers on preferred-opioid economic demand, affective dysregulation (using questionnaire and performance measures), insomnia/behavioral alertness, psychiatric diagnoses or medications, or urinalysis results; and (2) alprazolam demand among BZD misusers is related to affective dysregulation or other measures.

Results: Lifetime BZD misuse is significantly ($p < 0.05$) related to current major depressive disorder diagnosis, opioid-negative and methadone-negative urinalysis, higher trait anxiety, greater self-reported affective dysregulation, and younger age, but not preferred-opioid demand or insomnia/behavioral alertness. Alprazolam and opioid demand are each significantly positively related to higher anhedonia and, to a lesser extent, depression symptoms but no other measures of negative-affective dysregulation, psychiatric conditions or medications (including opioid agonist therapy or inpatient/outpatient treatment modality), or sleep-related problems.

Conclusion: Anhedonia (positive-affective deficit) robustly predicted increased BZD and opioid demand; these factors could modulate treatment response. Routine assessment and effective treatment of anhedonia in populations with concurrent opioid and sedative use disorder may improve treatment outcomes.

Clinical trial registration: https://clinicaltrials.gov/ct2/show/NCT03696017, identifier NCT03696017.

KEYWORDS

benzodiazepine, demand, anhedonia, affective dysregulation, opioid use disorder

1. Introduction

Although the opioid overdose epidemic continues to generate unprecedented numbers of deaths, medical, and epidemiological data clearly indicate these adverse outcomes are not solely due to over-consumption of opioids but often involve use of multiple substances (1–6). The Food and Drug Administration recognizes the health dangers of opioid/benzodiazepine (BZD) polysubstance use, and issued labeling changes for prescribing BZDs and opioids (7). However, the impact of such changes is minimal when people take a prescribed drug inconsistent with its labeling or use someone else's prescription [e.g., (8)].

There has been limited systematic research on mechanisms underlying BZD/opioid polysubstance misuse [for review, (9)]. Although BZDs are often co-prescribed with opioids (10–13), there is substantial co-occurring use and misuse of opioids and BZDs (14–16). Whereas BZD misuse alone can be harmful, when combined with opioids, BZD misuse contributes dose-dependently to health-risk behaviors, poor treatment outcomes, overdoses and deaths (16–26).

Interpreting BZD misuse and consequences, particularly in the context of opioid misuse, is challenging. First, temporal patterns of opioid/BZD consumption are highly variable, ranging from simultaneous use (co-administration) to sequential use (one drug used within several hours before the other) to concurrent use (both drugs consumed during a broader temporal window, e.g., within a few days/weeks of one another). The behavioral mechanisms underlying these different co-use patterns are likely to differ. In fact, persons who co-use BZDs with opioids report several motives including managing anxiety, enhancing the drug "high," promoting sleep, and suppressing opioid withdrawal (27–30). Second, BZD/opioid polysubstance use rarely occurs in isolation, i.e., persons using BZDs and opioids often use other psychoactive substances such as nicotine, alcohol, cannabis, and psychostimulants. Also, it is important to separate the psychopharmacological effects and consequences of BZD use from those of alcohol use, as these are highly comorbid (5, 31–33) and share similar reinforcing properties (34) and neurochemical mechanisms of action. A third interpretive challenge is that there are demographic differences in BZD/opioid polysubstance use. For example, BZD use and misuse is more common among women than men (26, 35), whites than other racial/ethnic groups (36), and among injection opioid users (26, 37); notably, the latter two factors are correlated, as some prior research has found opioid injectors are more likely to be white than black (38–40). Finally, several types of comorbidities can potentially modulate BZD/opioid use including anxiety-related symptoms/diagnoses (41–43), and sleep problems (9, 44, 45). A common assumption is that exaggerated negative affect plays a pivotal role in motivating BZD use to "self-medicate" anxious or depressive symptoms [i.e., negative reinforcement; (46)], however, this may not be the only functional relationship between psychiatric conditions and the reinforcing effects of BZDs.

Several theories of substance use disorders have outlined a central role of affective dysregulation and stress-reactivity (47–51). The present research builds on a dual-deficit theory of reward deficiency and stress surfeit in addiction (52). Our working hypothesis is that BZD/opioid polysubstance misuse may be perpetuated by a dual-deficit in hedonic regulation (difficulties modulating emotional reactions relative to the context and the person's long-term goals). From the standpoint of clinical practice (which we emphasize more than etiological issues), we propose that this dual-deficit *maintains* polysubstance misuse and makes treatment more challenging. Further, we propose this dual-deficit biases motivated behaviors (predominantly guided by negative reinforcement processes), such that polysubstance use acutely blunts aversive states and directs actions away from natural rewards.

Benzodiazepine seeking/consumption, as for other misused substances, is sensitive to economic price. This process can be studied using self-administration (actual consumption) or hypothetical purchase tasks (simulated) and applying demand curve analysis to examine the intensity and elasticity of demand (53, 54), which can also be conceptualized as amplitude and persistence of demand, respectively (55). Alprazolam is a rapid-onset BZD that is frequently misused (56–59). Studies of rhesus monkeys have demonstrated that BZDs are self-administered, however, economic demand for BZDs is complexly related to a compound's selectivity and intrinsic efficacy at α1 subunit-containing $GABA_A$ receptors, as well as the animal's baseline history of self-administration (60–64). Therefore, it is reasonable to use a standard, often-misused BZD such as alprazolam to investigate individual difference in BZD demand. Recently, it was shown that alprazolam functioned as a reinforcer in three of six monkeys tested. For two of those three alprazolam self-administering animals, alprazolam enhanced self-administration of fentanyl whereas for the other monkey alprazolam self-administration suppressed fentanyl intake (65). These data highlight the importance of individual differences in the reinforcing effects of BZDs and opioids; however, we presently have limited understanding of the reasons underlying these differences.

To our knowledge, only three clinical studies have used hypothetical purchasing tasks to investigate BZD demand, although none specifically with alprazolam. Petry and Bickel (66) studied

40 persons undergoing treatment for heroin use disorder. Among several price and income manipulations, they found that diazepam (the only BZD studied) substituted for heroin, whereas heroin purchases were independent of diazepam prices, suggesting an asymmetrical substitution effect. This indicates that diazepam is reinforcing in persons addicted to heroin but does not specify for what reason(s). In a separate study, Petry (67) also reported that diazepam demand was price-elastic among individuals with DSM-IV alcohol abuse/dependence and a history of polysubstance use. Recently, Schwartz et al. (68) studied 52 persons in outpatient opioid agonist treatment for opioid use disorder at a baseline visit and a 6-month follow-up visit; they found that demand intensity for BZD pills (not specified) increased across time points and was predictive of BZD-positive urine samples.

In summary, we lack data on factors that influence BZD demand, alone and especially in the context of opioid use disorder. Importantly, group factors can be included in demand curve analyses to examine individual difference variables that modulate BZD consumption. Accordingly, the present study aims to investigate: (1) among persons in treatment for opioid use disorder, whether lifetime or past-year BZD misusers differ from never-misusers on measures of simulated opioid demand (co-primary outcome), affective dysregulation (e.g., psychiatric diagnoses, anhedonia, distress tolerance), and insomnia/daytime sleepiness; and (2) in the subgroups of lifetime and past-year BZD misusers, whether simulated BZD demand (co-primary outcome) is specifically associated with affective dysregulation, controlling for other factors.

2. Materials and methods

2.1. Study context

The local IRB approved all research procedures. This ongoing study is being conducted according to the Declaration of Helsinki and is registered at ClinicalTrials.gov (NCT03696017). All participants provided informed consent.

2.2. Participant selection

This study assesses patients currently in treatment (baseline visit) for their opioid and potentially other substance use disorder(s) who are not presently clinically stable, which we defined *a priori* as early (first 6 months) in treatment or in treatment longer but self-report having used opioids during the past month. As this programmatic research is thematically focused on BZD/opioid polysubstance use, we attempted to recruit a sample enriched with individuals with a history of BZD misuse in addition to their opioid misuse; however, we did not explicitly require a history of, or current, use of BZDs to be enrolled in this study.

First, we defined *BZD misuse history* based on two lifetime factors, either: (1) any BZD misuse based on a "yes" response to the question, "Have you ever used sedatives/hypnotics not as prescribed intending to get high," on the Drug History and Use Questionnaire DHUQ (described in Section "2.3.4. Substance use"), or (2) diagnosis of sedative use disorder involving a BZD based on the SCID diagnostic interview (described in Section "2.3.5. Psychopathology and affective dysregulation"). Any participant meeting at least one of these two criteria was classified as a lifetime BZD misuser, and any participant not meeting either criterion was classified as a BZD never-misuser. Importantly, any participant who reported using BZDs as exactly prescribed for them throughout their lifetime, and denied misuse, was classified as a never-misuser. Second, to account for possible temporal variation in the effects of BZD misuse or abstinence, we defined differences in *recency* of BZD misuse as either (1) more than 1 year ago, or (2) within the past year, relative to the date of the initial screening visit. Participants who reported BZD misuse more than 1 year prior, or met DSM-5 criteria for partially remitted or past sedative use disorder were classified as misusers more than a year ago. Participants who reported BZD misuse within the past year, or met DSM-5 criteria for current (past-year) sedative use disorder involving a BZD, were classified as past-year misusers. Thus, we formed three distinct groups for analyses: (1) never misuse, (2) misuse > 1 year ago, and (3) past-year misuse of BZDs.

All participants are adults, ages 18–70 years old enrolled in a substance use disorder treatment program (outpatient or residential) in the Detroit metropolitan region. Exclusion criteria were estimated IQ < 80, expired breath alcohol > 0.02% breath alcohol concentration, neurological disorders that affect cognition, and current psychosis or suicidality. This study is also approved to re-contact participants (in-person or remotely) for 3-month follow-up assessment; these follow-up data will be reported elsewhere.

2.3. Experimental assessments

2.3.1. Hypothetical opioid and benzodiazepine purchase tasks

A simulated *Opioid Purchasing Task* is tailored to each participant's preferred opioid and route of administration (e.g., injected, snorted, oral) based on screening self-report. Of the 59 total participants, 46 reported using heroin (22 snorted, 23 injected, 1 smoked), 1 snorted fentanyl, 10 took oral hydrocodone, and 2 took oral oxycodone. The purchasing task is modeled after extant purchasing tasks for various substances [e.g., (69–71)], but personalizing the task for specific opioids/routes is novel. Participants are asked to imagine a typical day, with no access to other opioids unless they buy the preferred opioid at the listed prices. Participants make purchasing choices based on instructions that the amount purchased at each unit price (independent observations) must be consumed within 24-h (i.e., no saving or stockpiling drug). Prices per morphine 10-mg equivalent dose are $0 (free; no constraint) and 20 non-zero unit prices of $0.01, $0.10, $0.50, $1, $3, $5, $7.50, $10, $12.50, $15, $20, $25, $30, $35, $40, $45, $50, $60, $80, and $100. The participant indicates on a standard form how many unit doses s/he would purchase (dependent variable) at each unit price (independent variable).

A parallel simulated *BZD Purchasing Task* uses similar instructions and unit prices for alprazolam (0.25-mg equivalent oral dose): $0 (free), and $0.01, $0.10, $0.50, $1, $3, $5, $7.50, $10, $12.50, $15, $20, $25, $30, $35, $40, $45, $50, $60, $80, and $100. The participant indicates on a standard form how many unit doses s/he would purchase at each unit price. Among the 37 BZD misusers in this sample (11 of whom endorsed a prior prescription), 15 reported misuse of two or more BZDs across their lifetime (concurrent past-month misuse of multiple BZDs was infrequent): 25 endorsed ever misusing alprazolam (Xanax^{TM}), 13 endorsed misusing diazepam

(Valium™), 11 endorsed misusing clonazepam (Klonipin™), and 6 endorsed misusing lorazepam (Ativan™), and 3 (who misused in the past month) did not identify the specific BZD(s) by name. All participants reported misuse of these BZDs only *via* the oral route of administration (e.g., no snorting or injection). Thus, use of an oral alprazolam purchasing task was appropriate in this participant sample.

2.3.2. Demographics

Information on age, educational level/degree, and self-identified sex, race, and ethnicity are obtained *via* self-report. Estimated verbal intelligence is obtained by administering the Shipley Institute of Living Scale (72).

2.3.3. Type of treatment

Standardized forms are used to collect information on type of treatment facility (acute or longer-term residential, transitional, day program, or other outpatient), type of medication for opioid use disorder [grouped as agonist therapy (methadone and buprenorphine) vs. no agonist therapy (naltrexone and no medication)], and other non-substance use disorder treatment medications (e.g., for anxiety, depression, sleep, pain).

2.3.4. Substance use

Substance use is evaluated with a comprehensive *Drug History and Use Questionnaire* developed in our laboratory (available on request); it is used (either *via* paper/pencil or Qualtrics administration) to assess lifetime substance use (e.g., onset of use, regular use of opioids and BZDs and other substances, adverse consequences of substance use, number of quit attempts). This instrument also is used to determine the relative timeline of opioid and BZD use (prescribed or not), misuse and progression.

Biomarkers of recent substance use include alcohol breath testing and urine drug screening. Participants must provide a supervised alcohol-free breath sample (<0.02% BAC; AlcoSensor Intoximeter). A urine sample is collected into multi-test cups with temperature strips (CLIA Waived; temperature must be 92–96° F). Samples are tested for opioids, methadone, cocaine metabolites, benzodiazepines, amphetamines, barbiturates (negative cutoff < 300 ng/ml), and THC (negative cutoff < 50 ng/ml). After the study began, we initiated fentanyl urinalysis using test strips; however, at this time, too few participants have data for this measure.

2.3.5. Psychopathology and affective dysregulation

The *Semi-Structured Clinical Interview for DSM-5* [SCID; (73, 74)] is used to evaluate lifetime and current psychiatric and substance use disorders. The SCID is administered by a trained clinical psychology masters level student, supervised by co-author LHL.

Anhedonia, the reduced experience or anticipation of pleasure (75, 76) linked to dopamine-mediated reward dysfunction and drug craving (77–80), is measured with the validated 14-item *Snaith-Hamilton Pleasure Scale* [*SHAPS*; (81, 82)]. Individuals are asked about their agreement with 14 statements; example items include: "I would be able to enjoy my favorite meal" (food/drink), "I would enjoy seeing others' smiling faces" (social interaction), "I would be able to enjoy a beautiful landscape or view" (sensory experience), and "I would find pleasure in my hobbies and past-times" (interest/past-times), which is consistent with a recent conceptualization of anhedonia as having multiple domains, although these are not yet well understood (83). Each statement receives a score of either 0 (definitely agree or agree) or 1 (definitely disagree or disagree). High scores reflect the participant's disagreement with the item statement (i.e., inability to experience pleasure from the event). Notably, most healthy individuals score < 2 (low anhedonia), whereas psychiatric patient samples often score 2 or higher.

The *Beck Depression Inventory-II (BDI-II)* is a gold-standard, 21-item clinical measure of current (past 2-week) depression symptoms validated against the original version (84) and in low-income African-Americans (85) and substance users (86). Guidelines for BDI-II cutoff scores are that: 0–13 indicates no or minimal depression; 14–19 indicates mild to moderate depression; 19–28 indicates moderate to severe depression; and 29–63 indicates severe depression (87).

The *State-Trait Anxiety Inventory (STAI)* (88) is a well-validated 40-item measure that differentiates symptoms of state anxiety (Y1 scale) from chronic trait anxiety (Y2 scale) by evaluating agreement with each item on a four-point Likert scale.

The 14-item *Perceived Stress Scale (PSS)* (89) measures the degree to which the subject views past-month life situations as stressful. It is reliable and correlates with self-report and behavioral criteria.

The 36-item *Difficulties with Emotion Regulation Scale (DERS)* (90) measures six empirically valid constructs related to emotion dysregulation: Non-acceptance of emotional responses, Difficulties in engaging in goal-directed behavior, Impulse control difficulties, Lack of emotional awareness, Limited access to emotion regulation strategies, and Lack of emotional clarity.

The 20-item *Alcohol and Drug Use Self-Efficacy Scale (ADUSE)* (91) assesses self-efficacy and responses to high-risk situations that can trigger substance use. Items are grouped into negative affect, social positive withdrawal/urges, and physical/other concerns; subjects indicate how "tempted" and "confident" they would be in each situation.

Distress tolerance, defined as the perceived capacity to tolerate distress and interpreted here as the ability to remain drug abstinent in the face of difficulties (92–94) is measured with the *Distress Tolerance Scale* which has 15 items with good construct validity and reliability (95).

We include two performance measures putatively related to affective dysregulation. The *Paced Auditory Serial Addition Task (PASAT)* (96) is a mental arithmetic task that measures processing speed and flexibility during which participants must add each new digit to the one presented immediately prior. We used three trial blocks of increasing difficulty such that the presentation rate of numbers that must be held in memory and added increases within each trial block. Participants can quit performing the task during trial block three; performance accuracy and latency to task termination are outcome measures. In the *Emotional Stroop Test* (97), words presented (in different colors) vary in their affective meaning: neutral, pleasant, negative, aggressive. The participant is instructed to identify (by key-pressing) the color of the printed word; response accuracy and latency (ms) are outcome measures.

2.3.6. Insomnia and behavioral alertness

The 7-item *Insomnia Severity Index (ISI)* asks about problem severity of sleep-onset, sleep-maintenance, early morning awakening, sleep satisfaction, interference with daily function, perceived impairment, and level of distress from insomnia. It has good internal consistency and concurrent validity (with polysomnography, sleep diaries, and clinician or significant-other reports), making it a valid and reliable measure of perceived sleep disturbance (98, 99).

The 8-item *Epworth Sleepiness Scale (ESS)* measures "sleep propensity," i.e., recent likelihood of dozing or falling asleep (rather than just feeling tired) in several situations (100). It is reliable and some items correlate with the gold-standard Multiple Sleep Latency Test.

The *Psychomotor Vigilance Task (PVT)* (101, 102) is a computerized, adaptive task (reaction time to a visual stimulus presented at random inter-trial intervals) that is used to assess attentional lapses; this objective, validated measure of sleepiness will complement the ISI and ESS measures.

2.4. Data analysis

Economic demand curve analysis is used to estimate the amounts of each participant's preferred-opioid and, for lifetime BZD misusers, alprazolam consumed across increasing unit prices. Specifically, we measure each participant's demand *intensity* (amplitude at low prices) and *elasticity* (resistance to price increases) based on the number of opioid $10 units purchased/consumed, in relation to opioid unit prices ranging from $0.01–$100.00. For participants with lifetime BZD misuse history, we also measure demand intensity and elasticity for alprazolam 0.25 mg units in relation to alprazolam unit prices, also ranging from $0.01–$100.00.

Hypothetical purchase task data were screened for unsystematic responses. Two curves that were unsystematic (one opioid, one BZD) were removed from analyses; this low proportion of data removal is similar to rates reported in prior studies. Each participant's hypothetical purchase data were entered into a GraphPad Prism template[1]. Consumption values were transformed using the inverse hyperbolic sine transform (IHS; Equation 1 below) which is approximately log-equivalent for consumption values > 5 and for values < 5 converges to zero, such that zero consumption values can be included in analyses. Curves were fit with both non-normalized and normalized versions of the zero-bounded exponential model of demand (103):

$$IHS(Q) = IHS(Q_0) * (e^{-[\alpha \div IHS(Q_0)]Q_0 x})$$

$$\text{where } IHS(Q_0) = log_{10}(0.5Q_0 + \sqrt{0.25\,Q_0{}^2 + 1}.$$

In this model, Q is consumption, Q_0 is consumption at unit price = 0 (demand *intensity*), x is unit price, and α is a free parameter that indexes the rate of change of the curve slope. This model accounts for these data which included many instances of reported zero consumption, and preserves the log-like scaling that represents relative changes in consumption with relative changes in unit price, i.e., the definition of *elasticity*.

Each model (opioid and BZD) was first used to estimate intensity of demand (Q_0) and demand elasticity (α) and curve fit (r^2), separately for each participant, in GraphPad Prism. The model also automatically calculates "essential value" [*EV*; (54)], which is proportional to the inverse of α [$EV = 1/(100 \times \alpha)$], and easily communicates the rate of change in elasticity, namely, higher *EV* reflects greater resistance to the (typical consumption-decreasing) effect of increasing unit prices.

1 https://ibrinc.org/behavioral-economics-tools/

As these study participants were in substance use disorder treatment, it is unsurprising that some individuals indicated no demand for opioids (n = 15 of 58) or alprazolam (n = 7 of 23) by providing all-zero consumption values across unit prices (i.e., non-participation). For these curves, Q_0 and *EV* were recoded as 0; in these cases, the α parameter was treated as undefined/missing because it was infinitely high, reflecting low demand (104), and we used the *EV* parameter instead of α to retain a larger sample size for analysis.

For both opioid and alprazolam purchasing tasks, the binary variable "participation" (i.e., making non-zero vs. all-zero responses) and continuous parameters r^2, Q_0, α, and *EV* from demand modeling for each participant were exported into SPSS v27 to examine subgroup differences. Zero-bounded exponential modeling in GraphPad Prism was also used to generate subgroup-average demand curves for plotting (see figure captions for results of group-average curve fits).

For Aim 1, ANOVAs and chi-square tests were used to examine BZD misuse history group (never, >1 year ago, and past-year) differences in demographic, opioid use disorder treatment type, psychiatric diagnoses/medications, urinalysis results, medications, experimental opioid demand, affective dysregulation, and sleep-related measures. For Aim 2, ANOVAs, correlations, and multiple linear regression were used to examine associations of affective dysregulation and other measures with experimental BZD demand metrics.

3. Results

Figure 1 presents the CONSORT diagram for participant flow through the experimental procedures.

3.1. Aim 1: Differences between BZD misusers and never-misusers

Table 1 presents characteristics for the overall sample (n = 59) and by subgroups of participants who denied lifetime BZD misuse (n = 22), who misused BZDs > 1 year ago (n = 17) and who misused BZDs within the past year (n = 20), based on self-report from the *Drug History and Use Questionnaire* and *SCID* interview-based diagnosis of sedative disorder (see Section "2.2. Participant selection" for details). The subgroups significantly ($p < 0.05$) differed on several measures. Relative to never-misusers, lifetime BZD misusers (past-year and >1 year groups did not differ) were younger, more likely to be diagnosed with current major depressive disorder (with trends toward more depression symptoms on the BDI-II and likelihood of taking an antidepressant medication), and to present a urine sample that was opioid-negative and methadone-negative (with a trend toward more cocaine-negative samples).

Relative to never-misusers, lifetime BZD misusers reported significantly higher scores for trait anxiety (STAI Y2 scale) and emotion regulation problem (DERS). Unexpectedly, lifetime BZD misusers had more correct responses and were less likely to quit task performance under cognitive duress (PASAT), and had faster response latencies during positive and negative affective interference trials (Emotional Stroop task). However, covariance analyses (ANCOVA) with age–which differed between BZD-misuser and never-misuser groups (see **Table 1**), found that these group

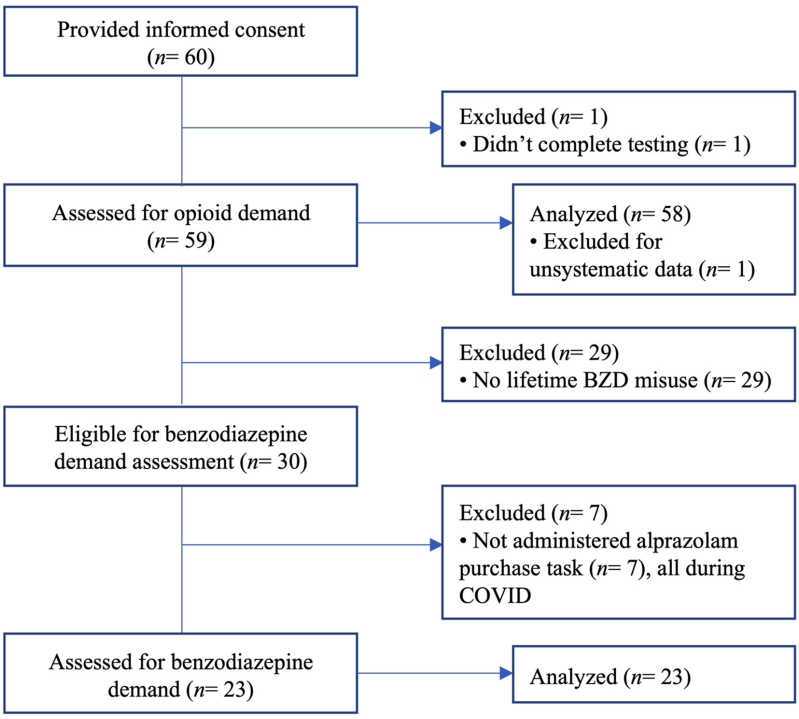

FIGURE 1
CONSORT diagram illustrating the flow of participants through the experimental procedures.

differences in task performance were no longer significant, i.e., older age more parsimoniously explained longer response latencies (Stroop) and less accurate performance and more task quitting (PASAT). There were no other BZD misuse group differences on other measures, and presenting a BZD + urine sample was not associated with these measures.

Opioid demand curve fits were very high: 54 of 58 participants had r^2 values > 0.80. Table 1 indicates that, based on the primary SPSS analysis of parameters that were computed from each participant's demand curve (i.e., units of analysis), opioid demand intensity and essential value did not significantly differ for lifetime BZD misusers (>1 year ago or past-year) vs. never-misusers.

In contrast, higher SHAPS anhedonia scores were significantly positively correlated with higher intensity of opioid demand (Q_0, $r = 0.59$, $p < 0.001$), but not essential value ($r = 0.19$, $p = 0.160$). To refine the interpretation of these effects, participants were stratified into three groups based on SHAPS total scores (0, 1, or 2+), consistent with previous clinical studies and the observed distribution of scores in the present sample. Table 2 (upper section) and Figure 2A illustrate that those participants with SHAPS scores ≥ 2 had significantly higher opioid demand intensity, but not essential value, compared to subgroups with lower SHAPS scores. SHAPS scores and BDI-II scores were significantly correlated in the overall sample ($r = 0.56$, $p < 0.001$). Compared to SHAPS scores, BDI-II depression symptom scores showed a similar but weaker positive association with opioid demand intensity ($r = 0.39$, $p = 0.002$) and were not significantly associated with essential value ($r = 0.20$, $p = 0.139$).

Figure 2B illustrates that those participants with BDI-II depression symptom scores ≥ 14 (i.e., mild or greater depression severity) exhibited higher levels of opioid demand than those with lower BDI-II scores [$\leq$13 indicates no clinical concern (87)]. SHAPS scores significantly correlated with several other measures of affective dysregulation (Table 3), however, these other measures were not related to opioid demand.

A multiple stepwise linear regression model with these two predictors found that only SHAPS anhedonia scores significantly predicted opioid demand intensity (standardized *beta* = 0.593, $t = 5.46$, $p < 0.001$) and explained 34.0% of the variance (adjusted r^2), $F(1,55) = 29.79$, $p < 0.001$. SHAPS scores significantly correlated with younger age ($r = -0.31$, $p = 0.018$) and lower scores on the DTS ($r = -0.42$, $p = 0.001$), and with higher scores on STAI Trait Anxiety ($r = 0.56$, $p < 0.001$), and DERS ($r = 0.45$, $p < 0.001$), ISI ($r = 0.45$, $p < 0.001$), and PSS ($r = 0.33$, $p = 0.008$). Importantly, SHAPS scores singularly and significantly predicted opioid demand intensity when controlling for all these covariates, although adjusted r^2 decreased to 23.3%, standardized *beta* = 0.497, $t = 4.13$, $p < 0.001$, $F(1,52) = 17.06$, $p < 0.001$.

In exploratory analyses, opioid demand metrics did not significantly differ when comparing males ($n = 36$) vs. females ($n = 21$), opioid injection users ($n = 23$) vs. non-injection users ($n = 34$), participants on opioid agonist therapy (methadone or buprenorphine, $n = 41$) vs. no agonist therapy (naltrexone or no medication, $n = 17$), participants in outpatient treatment ($n = 44$) vs. residential treatment ($n = 9$), nor participants with positive vs. negative urinalysis results, or presence/absence of substance use disorder and mental health diagnoses.

3.2. Aim 2: Differences within lifetime BZD-misusing subgroups

Among lifetime BZD misusers, alprazolam demand curve fits were very high: 22 of 23 participants had r^2 values > 0.80. SHAPS

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TABLE 1 Participant characteristics [mean (SD) or percent (n)], stratified by BZD misuse group.

Measure	Total sample (N = 59)	Never misused BZD (n = 22)	BZD misuse > 1 year ago (n = 17)	BZD past-year misuser (n = 20)	Group χ^2 or F (p)
Demographics					
Sex (M, F)	38, 21	14, 8	10, 7	14, 6	0.51 (0.775)
Race (B, W, other, missing)	31, 21, 3, 4	15, 4, 2, 1	7, 8, 0, 2	9, 9, 1, 1	7.08 (0.314)
Age	43.83 (13.27)	**52.25** (12.25)	**33.53** (7.65)	**43.00** (13.27)	**13.14 (<0.001)**
Education	12.03 (2.03)	12.36 (2.28)	11.35 (1.58)	12.25 (2.05)	1.37 (0.262)
Estimated IQ	105.48 (9.71)	107.57 (8.21)	101.65 (8.02)	106.45 (11.83)	2.00 (0.145)
Treatment facility					7.40 (0.494)
Acute residential	2% (1)	0% (0)	0% (0)	5% (1)	
Longer-term residential	14% (8)	5% (1)	19% (3)	21% (4)	
Transitional care	5% (3)	5% (1)	6% (1)	5% (1)	
Day program	47% (27)	45% (10)	44% (7)	53% (10)	
Other outpatient	32% (18)	45% (10)	31% (5)	16% (3)	
Diagnoses [current (past-year)]					
Sedative use disorder	19% (11)	**0%** (0)	**0%** (0)	**55%** (11)	**25.22 (<0.001)**
Alcohol use disorder	19% (11)	14% (3)	31% (5)	15% (3)	2.04 (0.360)
Stimulant use disorder	48% (27)	43% (9)	47% (7)	55% (11)	0.63 (0.732)
Cannabis use disorder	30% (17)	19% (4)	44% (7)	30% (6)	2.65 (0.266)
Anxiety disorder	21% (12)	10% (2)	25% (4)	30% (6)	2.55 (0.280)
Post-traumatic stress disorder	27% (15)	25% (5)	27% (4)	30% (6)	0.13 (0.937)
Major depressive disorder	26% (14)	**5%** (1)	**33%** (5)	**40%** (8)	**7.13 (0.028)**
Bipolar disorder	13% (7)	5% (1)	13% (2)	20% (4)	2.06 (0.358)
Urinalysis results (+)					
BZD	15% (9)	9% (2)	12% (2)	25% (5)	2.28 (0.320)
Cocaine	20% (12)	36% (8)	12% (2)	10% (2)	5.58 (0.061)
Opioids	29% (17)	**50%** (11)	**18%** (3)	**15%** (3)	**7.71 (0.021)**
Methadone	53% (31)	**82%** (18)	**47%** (8)	**25%** (5)	**13.85 (<0.001)**
THC	10% (6)	5% (1)	12% (2)	15% (3)	1.32 (0.517)
Medications (non-BZD)					
MOUD agonist	71% (42)	86% (19)	71% (12)	55% (11)	5.03 (0.081)
Antidepressant	27% (16)	9% (2)	35% (6)	40% (8)	5.87 (0.053)
Analgesic	13% (7)	0% (0)	19% (3)	21% (4)	4.41 (0.110)
Preferred-opioid demand					
Participation (non-zero values)	75% (44)	77% (17)	77% (13)	70% (14)	0.34 (0.845)
Curve fit (r^2)	0.93 (0.10)	0.89 (0.14)	0.95 (0.06)	0.94 (0.05)	1.74 (0.186)
Q_0, non-normalized	19.33 (36.72)	14.31 (27.21)	26.85 (45.28)	18.22 (38.20)	0.55 (0.578)
a, non-normalized	0.1527 (0.8644)	0.3812 (1.4465)	0.0086 (0.0126)	0.0418 (0.0917)	0.81 (0.451)
Essential value, non-normalized	5.53 (12.70)	6.09 (18.66)	6.33 (9.17)	4.25 (6.64)	0.15 (0.861)
a, normalized	0.1423 (0.7607)	0.3170 (1.2621)	0.0354 (0.0529)	0.0498 (0.0635)	0.87 (0.427)
Essential value, normalized	3.80 (6.84)	3.94 (9.14)	4.09 (5.50)	3.38 (5.15)	0.06 (0.946)
Affective dysregulation					
SHAPS (anhedonia)	1.46 (2.15)	0.73 (1.35)	2.12 (2.96)	1.70 (1.92)	2.29 (0.111)
BDI-II (depression)	17.57 (11.44)	13.32 (10.60)	20.88 (11.60)	19.53 (11.16)	2.68 (0.078)

(Continued)

TABLE 1 (Continued)

Measure	Total sample (N = 59)	Never misused BZD (n = 22)	BZD misuse > 1 year ago (n = 17)	BZD past-year misuser (n = 20)	Group χ^2 or F (p)
STAI Y1 (state anxiety)	48.12 (6.69)	47.48 (10.40)	48.29 (3.08)	48.65 (3.41)	0.16 (0.852)
STAI Y2 (trait anxiety)	43.77 (13.02)	**36.50** (10.70)	**48.35** (10.01)	**47.15** (14.63)	**5.65 (0.006)**
PSS (perceived stress)	30.03 (4.69)	28.81 (4.47)	31.12 (3.37)	30.40 (5.71)	1.24 (0.297)
ADUSE temptation	53.48 (18.79)	46.57 (20.12)	56.12 (15.72)	58.50 (18.44)	2.41 (0.099)
ADUSE confident	59.28 (20.63)	56.29 (24.22)	65.88 (15.88)	56.80 (19.77)	1.25 (0.295)
DERS (emotion dysregulation)	76.68 (28.66)	**61.01** (26.15)	**87.38** (26.33)	**85.35** (26.47)	**6.31 (0.003)**
DTS (distress tolerance)	3.28 (1.04)	3.60 (1.21)	3.24 (0.93)	2.96 (0.83)	2.06 (0.137)
PASAT accuracy (# correct)	88.19 (61.58)	**58.27** (51.49)	**105.53** (51.05)	**106.35** (69.26)	**4.66 (0.013)**
PASAT quit %	20% (12)	**36%** (8)	**6%** (1)	**15%** (3)	**6.03 (0.049)**
Stroop positive latency (ms)	769 (636)	**959** (824)	**558** (136)	**584** (266)	**3.21 (0.049)**
Stroop negative latency (ms)	811 (767)	**1,034** (934)	**608** (214)	**600** (281)	**3.43 (0.040)**
Sleep/Behavioral alertness					
Epworth Sleepiness Scale	8.98 (4.37)	8.18 (4.95)	8.88 (4.26)	9.95 (3.76)	0.86 (0.429)
Insomnia Severity Index	13.19 (7.42)	12.45 (7.47)	13.35 (6.47)	13.85 (8.36)	0.19 (0.831)
Psychomotor Vigilance Task					
#attentional lapses	9.85 (11.28)	13.36 (12.57)	6.94 (7.70)	8.45 (11.83)	0.81 (0.448)
Mean lapse reaction time (ms)	1,884 (9,275)	666 (875)	927 (2,389)	4,037 (15,798)	1.84 (0.169)
#false starts	9.12 (12.72)	9.14 (14.48)	8.12 (11.05)	9.95 (12.56)	0.09 (0.912)

M, male; F, female; B, black; W, white; BZD, benzodiazepine; THC, Δ^9-tetrahydrocannabinol; MOUD, medications for treating opioid use disorder; SHAPS, Snaith-Hamilton Pleasure Scale; BDI-II, Beck Depression Inventory-II; STAI, State Trait Anxiety Inventory (Y1 = trait, Y2 = state); PSS, Perceived Stress Scale; ADUSE, Alcohol and Drug Use Self-Efficacy Scale; DERS, Difficulty in Emotion Regulation; DTS, Distress Tolerance Scale; PASAT, Paced Auditory Serial Addition Test; Stroop, Emotional Stroop task. Sedative use disorder diagnosis (DSM-5) and self-report of BZD misuse were to create the groups in this table, so this represents a manipulation check. Only non-BZD medications are reported because reasons for prescription are not being collected for overlapping indications involving BZDs (e.g., anxiety vs. insomnia). Bolded values indicate a significant overall group difference.

TABLE 2 Parameter estimates for opioid and alprazolam demand, stratified by Snaith-Hamilton Pleasure Scale (SHAPS) anhedonia total scores.

Measure (Mean, SD)	SHAPS = 0	SHAPS = 1	SHAPS = 2+	Group χ^2 or F (p)
Preferred-opioid demand	(n = 29)	(n = 11)	(n = 18)	
Participation (non-zero values)	**69%** (20)	**55%** (6)	**94%** (17)	**6.48 (0.039)**
Curve fit (r^2)	0.92 (0.11)	0.93 (0.13)	0.93 (0.05)	0.28 (0.973)
Q_0 (intensity)	**9.70** (19.29)	**7.18** (9.54)	**42.28** (55.17)	**6.02 (0.004)**
a, non-normalized	0.3055 (1.2846)	0.0087 (0.0097)	0.0328 (0.0847)	0.53 (0.592)
Essential value, non-normalized	4.19 (15.85)	3.62 (7.73)	8.86 (8.66)	0.90 (0.412)
a, normalized	0.2492 (1.073)	0.0618 (0.0627)	0.0192 (0.0477)	0.58 (0.566)
Essential value, normalized	2.75 (7.83)	3.04 (5.43)	5.93 (5.60)	1.30 (0.282)
Alprazolam demand	(n = 8)	(n = 7)	(n = 8)	
Participation (non-zero values)	75% (6)	43% (3)	88% (7)	3.69 (0.158)
Curve fit (r^2)	0.93 (0.06)	0.98 (0.04)	0.90 (0.13)	1.35 (0.282)
Q_0 (intensity)	5.82 (6.77)	2.30 (3.31)	36.81 (61.19)	2.11 (0.148)
a, non-normalized	0.0362 (0.3918)	0.0676 (0.0389)	0.0429 (0.1029)	0.18 (0.839)
Essential value, non-normalized	**0.77** (1.07)	**0.08** (0.12)	**4.62** (5.26)	**4.58 (0.023)**
a, normalized	0.0452 (0.0518)	0.0908 (0.0461)	0.0406 (0.0677)	1.77 (0.197)
Essential value, normalized	**0.90** (1.10)	**0.16** (0.12)	**3.72** (4.50)	**3.63 (0.045)**

Bolded values indicate a significant overall group difference.

anhedonia and BDI-II depression symptom scores, which were correlated in the overall sample, remained significantly correlated in this subgroup (r = 0.61, p < 0.001). In bivariate analyses, alprazolam demand intensity (Q_0) significantly correlated with SHAPS scores (r = 0.69, p < 0.001) and marginally with BDI-II scores (r = 0.40, p = 0.058); and alprazolam essential value (EV)

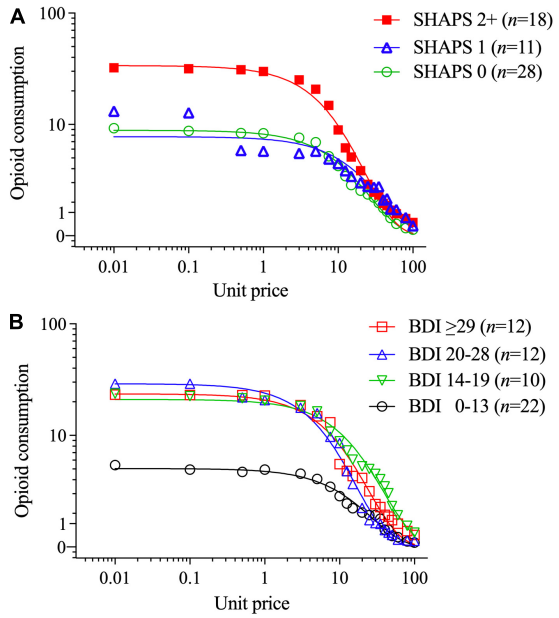

FIGURE 2
Opioid demand stratified by **(A)** Snaith-Hamilton Pleasure Scale (SHAPS) anhedonia total scores (0 vs. 1 vs. 2+) and **(B)** Beck Depression Inventory-II (BDI-II) scores (using clinical cutoff values). The primary SPSS analysis found significant SHAPS anhedonia group differences in opioid demand (see text, Section "3.1. Aim 1: Differences between BZD misusers and never-misusers"), and parameters computed in GraphPad Prism from group-average curves in Figure 1A confirm that the subgroup with SHAPS scores ≥ 2 compared to scores of 1 or 0 had higher opioid demand intensity (Q_0 = 33.7 vs. 7.64 vs. 8.75, respectively), and essential value (*EV* = 7.75 vs. 2.98 vs. 2.36, respectively), $F(1,18) = 84.2$. The primary SPSS analysis did not find a significant BDI-II group difference in opioid demand intensity or essential value; however, parameters computed in GraphPad Prism from group-average curves in Figure 1B found that BDI-II scores indicating mild or greater depression severity (≥14) were associated with higher opioid demand intensity (Q_0 = 4.9, 21.0, 29.0 and 23.5 for groups with scores of 0–13, 14–19, 20–28, and ≥ 29, respectively) and essential value (*EV* = 1.32, 8.24, 4.91, and 5.75, respectively), $F(1,18) = 115$.

significantly correlated with both SHAPS scores ($r = 0.46$, $p = 0.027$) and BDI-II scores ($r = 0.44$, $p = 0.034$). SHAPS scores significantly correlated with several other measures of affective dysregulation in the subgroup of past-year BZD users (Table 3), however, these other measures were not related to alprazolam demand intensity or essential value. Although participant age was related to measures of affective dysregulation, age was not significantly related to BZD demand intensity or essential value. As we did for opioid demand, the same clinical cut-points were used to form SHAPS and BDI-II subgroups.

Table 2 (lower section) and Figure 3 illustrate that those participants with SHAPS scores ≥ 2 (Figure 3A) and BDI-II scores ≥ 20 (indicating moderate to severe depression levels; Figure 3B) exhibited differences in alprazolam demand. A multiple stepwise linear regression model with these two predictors found that only SHAPS scores significantly predicted alprazolam demand intensity (standardized *beta* = 0.691, $t = 4.38$, $p < 0.001$) and explained 45.2% of the variance (adjusted r^2), $F(1,21) = 19.14$, $p < 0.001$. A multiple stepwise linear regression model with these two predictors found that only SHAPS scores significantly predicted alprazolam essential value (standardized *beta* = 0.462, $t = 2.39$, $p < 0.027$) and explained 17.6% of the variance (adjusted r^2), $F(1,21) = 5.70$, $p < 0.027$.

Alprazolam demand metrics did not significantly differ when comparing males ($n = 14$) vs. females ($n = 9$), injection opioid users ($n = 11$) vs. non-injection users ($n = 12$), participants on opioid agonist therapy ($n = 13$) vs. no agonist therapy ($n = 10$), those in outpatient treatment ($n = 14$) vs. residential treatment ($n = 8$), nor participants with positive vs. negative urinalysis results. Notably, presenting a BZD + urine sample ($n = 7$), reflecting recent use, was not significantly related to alprazolam demand. Although presence/absence of substance use disorder diagnoses and some mental health diagnoses was unrelated to alprazolam demand, there were two exceptions. First, presence ($n = 8$) vs. absence ($n = 14$) of major depressive disorder diagnosis was associated with greater alprazolam essential value (mean *EV* = 4.04 vs. 0.80), $F(1,20) = 4.50$, $p = 0.047$, with a trend toward higher demand intensity (mean Q_0 = 36.5 vs. 4.5), $F(1,20) = 3.90$, $p = 0.062$, as well as higher symptom scores on SHAPS anhedonia (M = 3.75 vs. 0.57), $F(1,20) = 18.70$, $p < 0.001$, and BDI-II depression (M = 29.3 vs. 14.1), $F(1,20) = 12.96$, $p = 0.002$. Second, presence ($n = 7$) vs. absence ($n = 15$) of PTSD diagnosis was associated with greater alprazolam demand intensity (mean $Q_0 = 41.8$ vs. 4.2), $F(1,20) = 5.35$, $p = 0.032$, and higher symptom scores on SHAPS anhedonia (M = 3.43 vs. 0.93), $F(1,20) = 7.56$, $p = 0.012$, and BDI-II depression (M = 31.3 vs. 14.2), $F(1,20) = 17.84$, $p < 0.001$.

Benzodiazepine and opioid demand intensities were highly positively correlated ($r = 0.98$, $p < 0.001$; Figure 4A), as were BZD and opioid essential values ($r = 0.86$, $p < 0.001$; Figure 4B) and choice participation ($\chi^2 = 27.00$, $p < 0.001$). Regression slopes within each panel of Figure 4 indicate that opioid demand metrics were proportionally greater for the preferred-opioid than alprazolam. Repeated measures ANOVAs found that demand intensity was non-significantly higher for the preferred-opioid than alprazolam (Q_0 = 16.1 vs. 15.5), $F(1,22) = 3.98$, $p = 0.085$; whereas, essential value was significantly higher for the preferred-opioid than alprazolam (EV = 4.10 vs. 1.90), $F(1,22) = 6.83$, $p = 0.016$. Figure 4C illustrates average demand curves (in the BZD-misusing group) for both the preferred-opioid and alprazolam.

4. Discussion

This ongoing study of persons being treated for opioid use disorder, and with polysubstance misuse histories, is examining factors that modulate economic demand for a standard BZD that is frequently misused (alprazolam) and each participant's preferred misused opioid. The primary novel finding from this analysis is that participants who report multiple symptoms of anhedonia–a deficit in the experience and anticipation of pleasure–manifest significantly increased economic demand for both alprazolam and opioid drugs.

The first aim of the study was to determine whether BZD misuse history is related to affective dysregulation, opioid economic demand, and other clinically relevant measures. Based on systematic self-report and psychiatric diagnosis of sedative use disorder (involving a BZD), more than half of the sample (37 of 59 participants) were classified as having misused BZDs during their lifetime and over half of those (20 of 37) misused BZDs during the past year, whereas the remaining participants denied lifetime BZD misuse (22 of 59; comparison group). Relative to never-misusers, BZD misusers (both

TABLE 3 Correlations between selected measures of affective dysregulation, insomnia severity and age in the overall sample (N = 59), and in parentheses, the subgroup of lifetime benzodiazepine misusers (n = 23).

	1	2	3	4	5	6	7	8	9	10
SHAPS (anhedonia)										
BDI-II (depression)	**0.56 (0.61)**									
STAI Y2 scale (state anxiety)	**0.57 (0.56)**	**0.86 (0.91)**								
PSS (perceived stress)	**0.31** (0.28)	**0.61 (0.69)**	**0.56 (0.71)**							
ADUSE temptation (to use drugs)	0.25 **(0.42)**	**0.41 (0.60)**	**0.62 (0.65)**	**0.42 (0.51)**						
DERS (emotion regulation problems)	**0.45 (0.56)**	**0.64 (0.74)**	**0.73 (0.84)**	**0.44 (0.62)**	**0.47 (0.68)**					
DTS (distress tolerance)	**−0.45 (−0.56)**	**−0.71 (−0.61)**	**−0.71 (−0.65)**	**−0.42 (−0.48)**	**−0.39 (−0.45)**	**−0.55 (−0.76)**				
ISI (insomnia severity)	**0.43 (0.59)**	**0.54 (0.53)**	**0.47 (0.50)**	**0.44 (0.43)**	**0.27** (0.29)	**0.50 (0.57)**	**−0.43 (−0.53)**			
PASAT accuracy (# items correct)	0.17 (0.24)	0.21 (0.23)	0.20 (0.29)	0.12 (0.05)	0.21 (0.31)	0.17 **(0.39)**	0.12 (−0.24)	−0.01 (0.25)		
PASAT quit (yes = 1)	**−0.29** (−0.14)	**−0.23** (−0.20)	−0.19 (−0.13)	−0.11 (0.04)	−0.10 (−0.23)	−0.12 (−0.01)	0.11 (0.04)	−0.08 (−0.09)	**−0.31** (−0.16)	
Age (years)	**−0.31** (−0.31)	**−0.42 (−0.64)**	**−0.45 (−0.62)**	**−0.51 (−0.50)**	**−0.49 (−0.48)**	**−0.43 (−0.58)**	0.15 (0.30)	**−0.29** (−0.37)	**−0.46 (−0.44)**	**0.40** (0.27)

Correlations in bold font are significant ($p < 0.05$). All correlations are Pearson r except PASAT quit (Kendall tau).

lifetime and past-year subgroups) were: higher on trait anxiety and emotion regulation problems and more likely to meet criteria for current major depressive disorder (consistent with our hypothesis); more likely to present opioid-negative and methadone-negative urine samples; and younger in age. In general, lifetime and past-year BZD misusers did not significantly differ on any of these measures; the only observed differences were between BZD misusing subgroups and the never-misuser group.

Surprisingly, BZD misusers did not significantly differ from never-misusers on several symptom measures of affective dysregulation including anhedonia (SHAPS), depression (BDI-II), state anxiety (STAI), distress tolerance (DTS), perceived stress (PSS), nor self-efficacy to resist substance use (ADUSE). Also, BZD misusers and never-misusers did not differ on current anxiety disorder, PTSD or bipolar disorder diagnoses (although the latter was infrequent) that are commonly linked to problems of affective dysregulation, nor did the groups differ on current non-sedative substance use disorder diagnoses.

Interestingly, BZD misusers and never-misuser groups did not significantly differ in experimental opioid demand. To our knowledge, this is the first clinical study to examine opioid demand in relation to differences in BZD-misuse history. By comparison, Petry and Bickel (66) examined simulated demand for heroin or the BZD diazepam in persons with a heroin-use history; most reported histories of injection use and all reported polysubstance use. Although all participants were in outpatient treatment and most were maintained on buprenorphine, all were instructed to imagine drug purchases while *not* receiving medication treatment (whereas such an instruction was not given in the present study). Those authors found that demand for heroin modestly decreased (i.e., was inelastic or relatively insensitive) in response to increases in its experimental price. In a separate assessment, it was found that as heroin price increased, diazepam purchasing increased. Thus, diazepam functioned as an economic substitute for heroin; yet, heroin purchases were found to be independent of diazepam prices, indicating asymmetrical substitution. In a recent study of persons in outpatient opioid agonist treatment for opioid use disorder, Schwartz et al. (68) found at a baseline visit that intensity of demand for BZD pills, which was lower than for heroin or cocaine in their sample, predicted the proportion of BZD-positive urine samples over a 6-month follow-up interval; thus, demand metrics were found to have clinical predictive value, which has also been demonstrated for treatment of tobacco use disorder (105), alcohol use disorder (106), and cocaine use disorder (107). Notably, Schwartz et al. (68) observed that opioid demand intensity (but not essential value) was significantly greater than for BZD pills, whereas the present study found that essential value for opioid vs. alprazolam significantly differed, with a trend for demand intensity. It is possible that (a) differences in the participant samples from the two studies, and/or (b) assessment of demand using the participant's preferred opioid and route in the present study, played a role in these slightly discrepant findings.

The second aim of the present study was to investigate whether measures of affective dysregulation would modulate BZD demand within the subgroup of past-year BZD users. Anhedonia, which was associated with significantly greater opioid demand, was also found to significantly increase BZD demand. Therefore, in this sample, elevated anhedonia was a common predictor of increased drug demand. Interestingly, current depression symptom levels (BDI-II) showed similar, but slightly weaker, effects than anhedonia on both opioid and BZD demand. This raises an important question of behavioral specificity. Anhedonia symptoms measured with the SHAPS represents a more narrow phenotype (positive hedonic deficit) than depression symptoms measured with the BDI-II. Although the Beck Depression Inventory has three items (#4, loss of pleasure; #12, loss of interest; #21, loss of interest in sex) that have been proposed to measure anhedonia (75) the majority of items focus on negative-affective symptoms and neurovegetative signs of affective

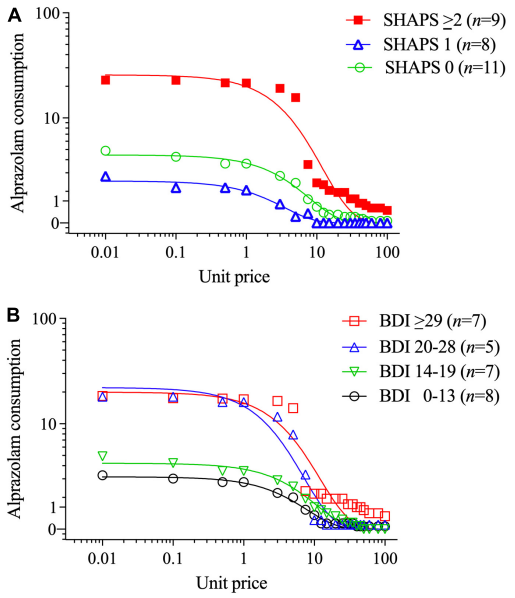

FIGURE 3
Alprazolam demand stratified by **(A)** Snaith-Hamilton Pleasure Scale (SHAPS) anhedonia total scores (0 vs. 1 vs. 2+), and **(B)** Beck Depression Inventory-II (BDI-II) depression total scores (using clinical cutoff values). The primary SPSS analysis found that participants with higher SHAPS anhedonia scores (≥2) and higher BDI-II depression symptom scores had significantly higher alprazolam demand intensity (see Section "3.2. Aim 2: Differences within lifetime BZD-misusing subgroups"). Parameters computed in GraphPad Prism from group-average curves in **Figure 2A** confirm that the subgroup with SHAPS scores ≥ 2 compared to scores of 1 or 0 had higher alprazolam demand intensity (Q_0 = 25.7 vs. 2.11 vs. 4.22, respectively), and essential value (*EV* = 3.11 vs. 0.074 vs. 0.34, respectively), $F(1,18) = 78.9$. The primary SPSS analysis did not find a significant BDI-II group difference in alprazolam demand intensity or essential value; however, parameters computed in GraphPad Prism from group-average curves in **Figure 2B** found that progressively increasing BDI-II scores (0–13, 14–19, 20–28, and ≥ 29) were associated with monotonically increasing alprazolam demand intensity (Q_0 = 1.92, 5.98, 15.7 and 19.9, respectively) and essential value (*EV* = 0.26, 0.37, 1.26, and 2.40, respectively), $F(1,18) = 74.7$.

disorder. Thus, anhedonia more precisely captures impairment of positive reinforcement. Interestingly, anhedonia but not depression was found to predict cocaine use in a clinical trial (108), in support of its distinct construct and predictive validity.

Anhedonia has been associated with impaired reinforcement learning (82, 109, 110). Thus, for persons with higher (vs. lower) anhedonia, repeated drug use and conditioning may strengthen drug demand to a greater degree so that it becomes more intense (at low prices, Q_0) and resistant to price increases (inelastic, or higher essential value). This phenotype maps onto some proposed sub-domains of anhedonia, e.g., approach motivation, reward valuation, effort valuation/willingness to work, and habit formation (111, 112). Although anhedonia might generally increase drug demand (as we found for opioid and alprazolam), it is conceivable that anhedonia might also interact differently across misused drugs [but see (113) for interpretive complexities]. Notably, laboratory animal models of drug self-administration have found that GABAergic agents including BZDs and alcohol can produce anti-conflict effects, i.e., they disinhibit punished behaviors (114–117) and this could enhance the expression of risky behaviors (118). For people who have experienced adverse consequences of opioid and sedative polydrug use (119, 120), i.e., such use has been punished or suppressed, BZDs (and alcohol) may interfere with efforts to abstain. Further research might explore whether this BZD anti-conflict effect could be enhanced in persons with higher anhedonia and may also interact with the behavioral cost of the drug.

The neural substrate for anhedonia is hypothesized to involve disruptions to a cortical/subcortical neural circuit whereby elevated prefrontal cortical excitability leads to decreased striatal dopamine activation (79, 121–123). Interestingly, opioid withdrawal-related anhedonia in rats (increased intracranial electrical self-stimulation) was associated with reduced vulnerability to subsequent morphine self-administration (124). However, in samples of patients with opioid use disorder, anhedonia has been variously found to correlate with recent opioid use during medication treatment but not during long-term abstinence (125, 126) as well as drug-cue or natural reward cue-reactivity during opioid abstinence (127–129) but not in all studies (130); these mixed findings imply that elevated anhedonia may be a dissociable phenotype from opioid or other substance use/abstinence [cf. (131)].

In a preclinical study, rats withdrawn from BZD exposure exhibited reduced preferences for both a cage compartment that had been paired with a sexual odor cue and for a context previously paired with amphetamine–a pattern of attenuated reward-seeking behaviors suggestive of increased anhedonia (132). In humans, there are very few studies of BZDs and anhedonic symptoms. Use of BZDs among patients with major depressive disorder was found to be associated with increased anhedonia but not anxiety or depression symptom levels (perhaps because BZDs mitigated anxiety) and anhedonia was the strongest predictor of BZD use in that study (133). A recent clinical study of repeated ketamine infusions in 42 patients with treatment-resistant major depression found that ketamine (an NMDA receptor antagonist) significantly reduced anhedonia (SHAPS scores) after each infusion but only among the subgroup of patients who did not use BZDs (134). Although the present study was not designed to examine BZD withdrawal and anhedonia, we did not find any significant difference in anhedonia scores between BZD misusers and never-misusers, nor between participants whose urine samples tested BZD-positive vs. BZD-negative. Further research is needed to understand the relationship between BZD use/discontinuation and anhedonia.

Benzodiazepines modulate activity to varying degrees at $GABA_A$ receptor subtypes which differentially correlate with their reinforcing, sedative/hypnotic and myorelaxant properties (60, 63, 135, 136). In persons with opioid use disorder, BZDs might also (e.g., *via* GABA interneurons on *mu*-opioid receptors) indirectly modulate *mu*-opioid receptor function (137), potentially leading to altered sensitivity to drug reinforcement (138). In the present study, neither past-year BZD misuse nor BZD-positive urine samples were related to opioid demand; however, only 15% of the overall sample had BZD-positive samples so there is likely insufficient statistical power to detect an effect. Unfortunately, we lack systematic data on the precise temporal pattern of BZD and opioid use (e.g., simultaneous vs. concurrent), which could potentially influence these results.

We also note that several measures were not significantly related to BZD misuse group, anhedonia or depression, nor opioid or BZD demand. These include some demographic factors (e.g., notably, no sex differences), psychiatric comorbidities other than sedative use disorder, and several measures of negative affective dysregulation. Absence of effects of insomnia and negative affective disturbance–which are often related (44, 139)–was surprising. However, we did

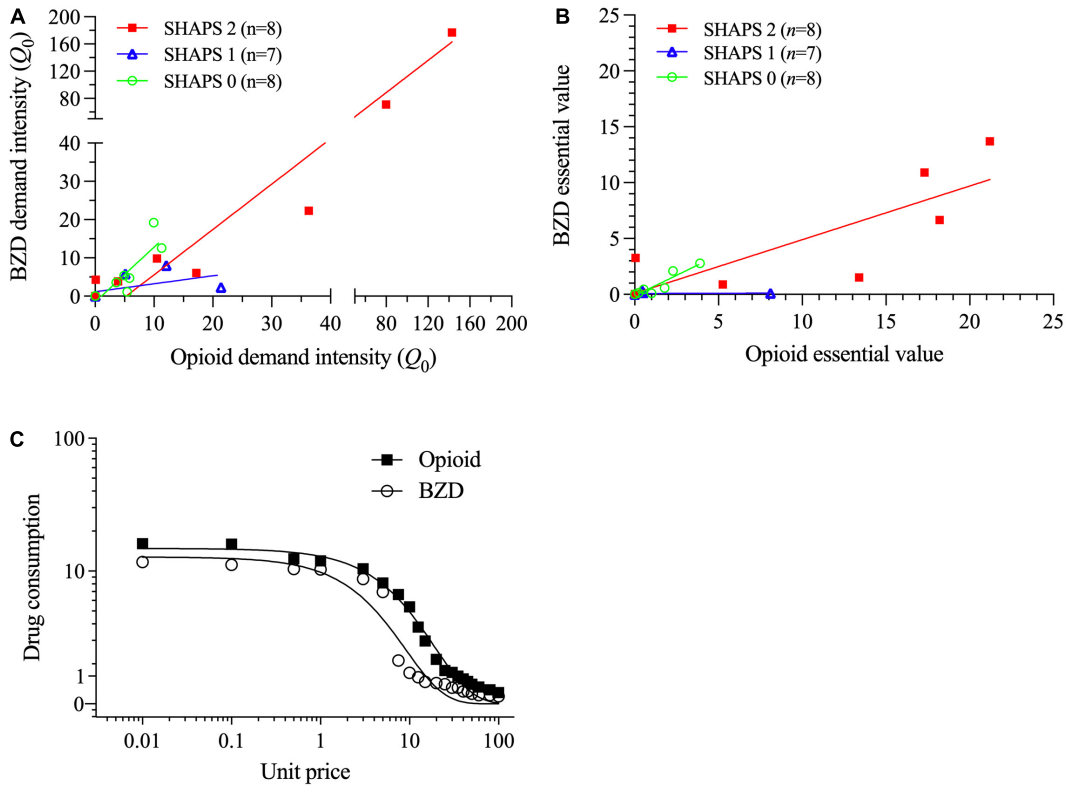

FIGURE 4
Within the subgroup of lifetime benzodiazepine (BZD) users, correlations of **(A)** opioid and benzodiazepine demand intensities (split axes enable better data visualization at low values) and **(B)** opioid and benzodiazepine essential values. Each of these two panels shows values separately for subgroups with 0, 1 or ≥ 2 Snaith-Hamilton Pleasure Scale (SHAPS) anhedonia scores. **(C)** In the primary SPSS analysis, opioid essential value (but not demand intensity) was significantly greater for the preferred-opioid than alprazolam (see Section "3.2. Aim 2: Differences within lifetime BZD-misusing subgroups"). In the secondary GraphPad Prism analysis, the preferred-opioid and alprazolam curves significantly differed (Q_0 = 18.7 vs. 11.6; *EV* = 4.83 vs. 1.03), $F(1,18) = 68.0$, $p < 0.01$.

find that younger age was significantly related to several measures of affective dysregulation (SHAPS, BDI-II, STAI, ADUSE, DTS, DERS, PASAT), both in the overall sample and within the past-year BZD-use group (see Table 3), but age was not significantly related to BZD demand metrics.

This study has several limitations. First, there is a relatively small sample size, although our sample is not smaller than others' comparable work (66, 68). Notably, our planned enrollment is expected to be up 120 participants, so we will have ample power to examine these and other effects in greater detail. Second, we conducted sensitivity analyses that excluded individuals with zero participation in the purchase task (leading to reduction in group size); these analyses suggest that alprazolam demand metrics should be cautiously interpreted, whereas censoring of participants with zero participation did not significantly alter opioid demand metrics. Third, we are recruiting individuals from various treatment settings/modalities to increase the heterogeneity and population representativeness of the sample with regard to polysubstance use and types of interventions; although this introduces variance that may complicate interpretation of the findings, we believe it can improve the generalizability of findings to treatment settings and prompt new hypotheses for investigation. Fourth, it is presently not feasible to collect reliable data on medication treatment doses, which could affect opioid demand and perhaps BZD demand. Notably, it has been shown in laboratory animal models that acute pretreatment with morphine, buprenorphine or naltrexone can increase fentanyl demand elasticity, i.e., decrease essential value (140). Fifth, unlike Petry and Bickel (66), we did not examine cross-price elasticity between the preferred-opioid and alprazolam in this study; although we designed such a manipulation, this was ultimately excluded due to the length of the overall assessment battery (several additional measures in this battery are not reported here). Finally, consistent with the work by Schwartz et al. (68), we are interested in whether these demand measures can predict longer-term outcomes. In the present study, we are collecting 3-month follow-up measures; however, at this time, these data are too sparse for meaningful analysis. However, it should be noted that purchasing "participation" (i.e., making non-zero drug choices at any price) in an in-treatment population may indicate the presence of a relapse risk. Thus, in future research, it could be useful to include participation as well as demand intensity and essential value metrics when reporting results with samples of patients.

In conclusion, this study identifies increased anhedonia as a shared factor for greater economic demand of opioid and BZD drugs in persons with histories of polysubstance use who are being treated for opioid use disorder. Anhedonia, which has commonly received attention in psychiatric disorders especially major depressive disorder [e.g., (75, 77, 141)], has a biological basis partly independent of depressive symptoms (142–144). Anhedonia has been observed during acute and protracted drug abstinence and may be related to drug craving (78). Thus, anhedonia may play an important predictive role in substance use disorder treatment outcome. Based on our

findings that anhedonia can modulate drug demand, along with recent findings that experimental demand can predict treatment outcome in substance use disorders (68), we believe that it could be useful to routinely include assessments of anhedonia and hypothetical drug demand in clinical settings to monitor the progress and recovery of persons with these disorders.

Future directions are to understand the multidimensional nature of affective dysregulation in this population, develop improved biomarkers/phenotypes to predict clinical outcomes and, from this improved understanding, develop behavioral, medication and neuromodulation interventions to reduce anhedonia and improve treatment efficacy (111, 141, 145–147).

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Human Investigation Committee, Wayne State University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

MG acquired funding, designed the study, oversees data collection, conducted the analyses, and drafted the manuscript. TM, LL, and TR contributed to study design and assessments and edited the manuscript. TM conducted psychiatric interviews and collected data. LL supervised psychiatric assessments. All authors taken responsibility for the content of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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EDITED BY
Thiago Marques Fidalgo,
Federal University of São Paulo, Brazil

REVIEWED BY
Marianna Mazza,
Agostino Gemelli University Polyclinic (IRCCS), Italy
David Neubauer,
School of Medicine, Johns Hopkins University, United States
Michihiro Takahashi,
Takahashi Psychiatric Clinic, Japan

*CORRESPONDENCE
Yoshikazu Takaesu
✉ takaesuy@med.u-ryukyu.ac.jp

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Physicians' attitudes toward hypnotics for insomnia: A questionnaire-based study

Masahiro Takeshima[1], Yumi Aoki[2,3], Kenya Ie[4], Eiichi Katsumoto[5], Eichi Tsuru[6], Takashi Tsuboi[3], Ken Inada[7], Morito Kise[8], Koichiro Watanabe[3], Kazuo Mishima[1] and Yoshikazu Takaesu[3,9]*

[1]Department of Neuropsychiatry, Akita University Graduate School of Medicine, Akita, Japan, [2]Psychiatric and Mental Health Nursing, St. Luke's International University, Tokyo, Japan, [3]Department of Neuropsychiatry, Kyorin University School of Medicine, Tokyo, Japan, [4]Department of General Internal Medicine, St. Marianna University School of Medicine, Kawasaki, Japan, [5]Katsumoto Mental Clinic, Osaka, Japan, [6]Department of Neurosurgery, Munakata Suikokai General Hospital, Fukuoka, Japan, [7]Department of Psychiatry, Kitasato University School of Medicine, Sagamihara, Japan, [8]Centre for Family Medicine Development, Tokyo, Japan, [9]Department of Neuropsychiatry, Faculty of Medicine, University of the Ryukyus, Nishihara, Japan

Introduction: Benzodiazepines and non-benzodiazepines are still widely prescribed despite safety concerns and the introduction of novel hypnotics (orexin receptor antagonists [ORA] and melatonin receptor agonists [MRA]), which may be influenced by physicians' attitudes toward hypnotics.

Methods: A questionnaire survey was administered to 962 physicians between October 2021 and February 2022, investigating frequently prescribed hypnotics and the reasons for their selection.

Results: ORA were the most frequently prescribed at 84.3%, followed by non-benzodiazepines (75.4%), MRA (57.1%), and benzodiazepines (54.3%). Compared to non-frequent prescribers of hypnotics, a logistic regression analysis showed that frequent ORA prescribers were more concerned with efficacy (odds ratio [OR]: 1.60, 95% confidence interval [CI]: 1.01–2.54, $p = 0.044$) and safety (OR: 4.52, 95% CI: 2.99–6.84, $p < 0.001$), frequent MRA prescribers were more concerned with safety (OR: 2.48, 95% CI: 1.77–3.46, $p < 0.001$), frequent non-benzodiazepine prescribers were more concerned with efficacy (OR: 4.19, 95% CI: 2.91–6.04, $p < 0.001$), and frequent benzodiazepine prescribers were more concerned with efficacy (OR: 4.19, 95% CI: 2.91–6.04, $p < 0.001$) but less concerned with safety (OR: 0.25, 95% CI: 0.16–0.39, $p < 0.001$).

Discussion: This study suggested that physicians believed ORA to be an effective and safe hypnotic and were compelled to prescribe benzodiazepine and non-benzodiazepine frequently, choosing efficacy over safety.

KEYWORDS

benzodiazepine, melatonin receptor agonist, non-benzodiazepine, orexin receptor antagonist, questionnaire survey

1. Introduction

Benzodiazepine (BZ) and non-benzodiazepine (NBZ) increase the risk of dependence with long-term use (1). In recent years, novel hypnotics, such as melatonin receptor agonists (MRA) and orexin receptor antagonists (ORA), with safety profiles have been introduced (2–4), but BZ and NBZ are still commonly prescribed for insomnia in real clinical practice (5, 6). In a study using a large Japanese claims database, 59.5% were reportedly prescribed BZ, and 36.8%

were prescribed NBZ as the first hypnotic for insomnia treatment between January 2012 and December 2016 (5).

Several guidelines provide several recommended individual hypnotics for insomnia. Academy of Sleep Medicine (AASM) Clinical Practice guidelines recommended triazolam, zaleplon, and ramelteon for sleep onset insomnia, suvorexant, and doxepin for sleep maintenance insomnia, and temazepam, zolpidem, and eszopiclone for both sleep onset and sleep maintenance insomnia; Clinical Practice Guideline by the American College of Physicians recommended eszopiclone, zolpidem, and suvorexant (7–10). Although there are many types of BZ, BZ recommended in insomnia guidelines are limited to a few drugs, such as triazolam and temazepam, while NBZ, MRA, and ORA seem commonly recommended in many guidelines, despite their small variety (7–10). However, while the AASM Clinical Practice guidelines and Korean Clinical Practice Guideline recommended each hypnotic based on the type of insomnia (7, 9), other guidelines did not clearly show recommended hypnotics according to characteristics of the patients (e.g., the severity of insomnia, physical comorbidity) (8, 10). Further, these guidelines did not provide strategies for when those hypnotics are not effective (7–10).

In this current situation, where the evidence for insomnia treatment is insufficient, physicians' prescribing behavior for insomnia may be influenced by clinicians' attitudes, such as preferences toward and beliefs about hypnotics based on their clinical experience. In 2004, National Institute for Clinical Excellence recommended short-acting BZ for insomnia from a cost perspective due to the lack of solid evidence distinguishing between short-acting BZ and NBZ at the time. Yet, NBZ prescriptions increased, and BZ prescriptions decreased in the UK (11, 12). To clarify this, a previous study examined general practitioners' (GPs) attitudes toward prescribing BZ and NBZ (12). The study showed that GPs believed NBZ was superior to BZ in efficacy and safety. The research team concluded that these GPs' attitudes might explain the increase in NBZ prescriptions (12). To determine why BZ and NBZ, which have safety concerns, are still commonly prescribed even with the advent of novel hypnotics, it is necessary to investigate recent physicians' attitudes toward prescribing hypnotics.

To clarify this, we conducted a questionnaire survey to examine recent physicians' attitudes toward prescribing hypnotics, including MRA and ORA.

2. Materials and methods

2.1. Study design and participants

This study is an unpaid questionnaire survey of physicians to examine the factors associated with each frequently prescribed class of hypnotic. We sent questionnaires between October 22, 2021 and February 1, 2022, to physicians affiliated with the Japanese Primary Care Association (JPCA) and the All Japan Hospital Association (AJHA) by e-mail, and the Japanese Association of Neuro-Psychiatric Clinics (JAPC) by letter. Members of the JPCA consist of primary care physicians and other healthcare professionals engaged in primary care. Members of the AJHA are representatives of hospitals who have joined the association in agreement with its purpose of contributing to the improvement of public health and the development of local communities by conducting surveys, research, and other activities necessary for the progress and development of hospitals and the fulfillment of their missions. Members of the JAPC are physicians with at least 5 years of clinical experience in psychiatry who manage a clinic with psychiatry as its primary advocacy department or equivalent.

2.2. Survey items

The survey items consisted of physician attributes (age groups: 20s, 30s, 40s, 50s, 60s, 70s, and 80s and over), specialty (psychiatric or otherwise), frequently prescribed hypnotics (e.g., BZ and NBZ hypnotics, MRA, and ORA), and reasons for selecting frequently prescribed hypnotics (e.g., effectiveness, appropriate duration of action, safety, familiarity, recommended, and drug price). Questions regarding frequently prescribed hypnotics and the reasons for their use were multiple-choice with no rank order. The questionnaire sent to participants is shown in **Supplementary Table 1**.

2.3. Details of hypnotics

Supplementary Table 2 shows the details of hypnotics that can be prescribed under insurance coverage at the time of the study. Japanese physicians can prescribe all the hypnotics listed in **Supplementary Table 2** to patients with insomnia, regardless of whether they are board-certified specialists. BZ was launched between 1967 and 1999, NBZ between 1989 and 2012, MRA between 2010 and 2020, and ORA between 2014 and 2020. Daily drug prices at the maximum dose were roughly less than 50 yen for drugs marketed before 2000 except quazepam, 50–100 yen for drugs marketed between 2000 and 2010, and more than 100 yen for drugs marketed after 2010.

2.4. Statistical analysis

Categorical variables are expressed as numbers (%). To examine factors associated with each frequently used drug for insomnia, a binary logistic regression analysis was performed comparing age group, specialty, and reasons for choosing frequently used drugs. P-values < 0.05 (two-sided) were considered significant. All statistical analyses were performed with SPSS Statistics 28.0 (IBM Corp., Armonk, NY, USA).

2.5. Ethics

The Ethics Committee of St. Luke's International University (2021-604) approved this study. Informed consent was obtained from the participants in written or electronic form before answering the questionnaire. The study was conducted in accordance with the Declaration of Helsinki.

3. Results

In this survey, the response rate from JPCA, AJHA, and JAPC and the overall response rate was 4.73% (251/5,306), 6.62% (168/2,537),

TABLE 1 Characteristics of the subjects.

Item	Number (%)
N	962 (100%)
Age group	
20s	12 (1.2%)
30s	85 (8.8%)
40s	180 (18.7%)
50s	272 (28.3%)
60s	284 (29.5%)
70s	109 (11.3%)
80s or more	18 (1.9%)
Affiliated organizations	
JPCA	251 (26.1%)
AJHA	168 (17.5%)
JAPC	543 (56.4%)
Specialty	
Non-psychiatry	390 (40.5%)
Psychiatry	572 (59.5%)

Categorical variables are expressed as numbers (%). AJHA, All Japan Hospital Association; JAPC, Japanese Association of Neuro-Psychiatric Clinics; JPCA, Japanese Primary Care Association.

32.1% (543/1,690), and 10.1% (962/9,533), respectively. Table 1 shows the characteristics of the subjects in this study. Most subjects were middle-aged or older, with 29.5% in their 60s, 28.3% in their 50s, and 18.7% in their 40s. On the other hand, a small number of subjects were young adults, with 8.8% in their 30s and 1.2% in their 20s. Among 962 subjects, 26.1% belonged to JPCA, 17.5% to AJHA, and 56.4% to JAPC. For the medical specialty of the subjects, 40.5% specialized in non-psychiatry and 59.5% in psychiatry.

Figure 1 shows the results of the survey. Regarding frequently prescribed hypnotics, 84.3% of subjects frequently prescribed ORA, 75.4% of subjects frequently prescribed NBZ, 57.1% of subjects frequently prescribed MRA, and 54.3% of subjects frequently prescribed BZ. Regarding the reason for selecting medications often used for insomnia: 76.2% of subjects answered safety, 62.3% familiarity, 48.1% efficacy, 40.7% appropriate duration of action, 8.0% drug price, and 5.7% recommendation.

Table 2 shows the results of the logistic regression analysis examining factors associated with the frequent prescribing of each hypnotic. Compared to non-frequent BZ prescribers, frequent BZ prescribers were associated with psychiatrist (odds ratio [OR]: 2.67, 95% confidence interval [CI]: 1.83–3.90, <0.001), considering important for efficacy (odds ratio: 4.19, 95% CI: 2.91–6.04, $p < 0.001$), appropriate duration of action (OR: 2.26, 95% CI: 1.56–3.27, $p < 0.001$), familiarity (OR: 3.74, 95% CI: 2.65–5.29, $p < 0.001$), drug price (OR: 3.87, 95% CI: 1.91–7.82), and considering not important for efficacy (OR: 0.25, 95% CI: 0.16–0.39, $p < 0.001$) when selecting medications for insomnia. Compared to non-frequent NBZ prescribers, frequent NBZ prescribers were associated with psychiatrist (OR: 2.43, 95% CI: 1.62–3.67, $p < 0.001$), considered important for efficacy (odds ratio: 4.19, 95% CI: 2.91–6.04, $p < 0.001$), appropriate duration of action (OR: 4.93, 95% CI: 3.11–7.82, $p < 0.001$), and familiarity (OR: 2.29, 95% CI: 1.63–3.22, $p < 0.001$), but not associated with safety, recommended, and drug price when selecting medications for insomnia. Compared to non-frequent MRA prescribers, frequent MRA prescribers were associated with non-psychiatrist (OR: 0.43, 95% CI: 0.31–0.59, $p < 0.001$), considering the appropriate duration of action important (OR: 2.19, 95% CI: 1.58–3.03, $p < 0.001$), safety (OR: 2.48, 95% CI: 1.77–3.46, $p < 0.001$), and familiarity (OR: 1.35, 1.00–1.82, $p = 0.047$), but not associated with age group, efficacy, and recommended when selecting medications for insomnia. Compared to non-frequent ORA prescribers, frequent ORA prescribers were associated with being a psychiatrist (OR: 2.823, 95% CI: 1.83–4.35, $p < 0.001$), considered important for efficacy (OR: 1.602, 95% CI: 1.01–2.54, $p = 0.044$), safety (OR: 4.52, 95% CI: 2.99–6.84, $p < 0.001$), and considering not important for drug price (OR: 0.39, 95% CI: 0.21–0.73, $P = 0.003$) but not associated with age group, efficacy, appropriate duration of action, familiarity, and recommended when selecting medications for insomnia.

4. Discussion

This is the first study to examine attitudes toward choice regarding medication for insomnia, including novel hypnotics such as MRA and ORA. The most frequently used medicines for insomnia were ORA, followed by MRA, NBZ, and BZ. Additionally, this study found that frequent ORA prescribers were more concerned with efficacy and safety, frequent MRA prescribers were more concerned with safety, frequent NBZ prescribers were more concerned with effectiveness, and frequent BZ prescribers were more concerned with efficacy but less concerned with safety.

Orexin receptor antagonists was the hypnotic with the highest percentage of frequent prescribers, and frequent ORA prescribers believe ORA is efficacious and safe but expensive. In a study using a large Japanese claims database, 0.4% were prescribed ORA as the first hypnotic drug for insomnia between January 2012 and December 2016 (5). Although this study did not examine prescription frequency by class of hypnotics, this study suggests that ORA prescriptions have expanded rapidly over the past several years in treating insomnia. An American AASM Clinical Practice Guideline weakly recommended suvorexant for sleep maintenance insomnia based on the quality of evidence, the balance of benefits and harms, and patient values and preferences (7). In addition, a recent network meta-analysis (NMA) reported that both suvorexant and lemborexant were significantly superior in efficacy and had no difference in safety compared to placebo and concluded that lemborexant is one of the drugs with a favorable profile (13).

Interestingly, this study was performed before this NMA was published, yet the results were consistent in efficacy and safety. Regarding drug price, this study shows that frequent ORA prescribers were less concerned with drug price. The result is understandable because the highest drug price for hypnotics was that of lemborexant, followed by suvorexant at the time of this study. Although ORA drug prices are indeed high, a previous study conducted in Japan showed that lemborexant was superior to zolpidem in terms of cost-effectiveness (14). Therefore, many physicians may prescribe ORAs frequently because of their efficacy and safety despite the high cost of ORA. This study found that frequent ORA prescribers were likely to be psychiatrists rather than non-psychiatrists. Insomnia is a common comorbidity in patients with psychiatric disorders (10) and a factor that anticipates suicide-related events in patients with psychiatric disorders (15, 16). In

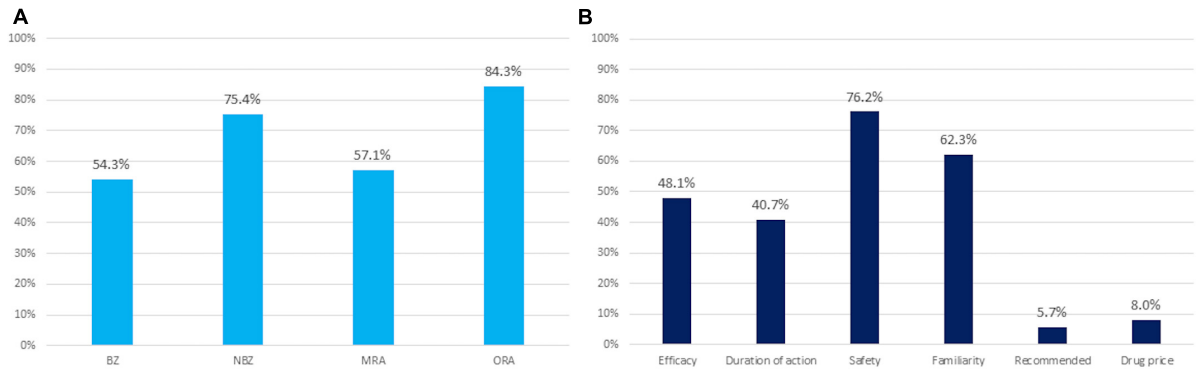

FIGURE 1
Results of the survey. **(A)** Medications frequently used for insomnia. **(B)** Reasons for selecting medications frequently used for insomnia. Values are expressed as a percent. BZ, benzodiazepine; NBZ, non-benzodiazepine; MRA, melatonin receptor agonist; OR, odds ratio; ORA, orexin receptor antagonist.

addition, patients with psychiatric disorders are associated with long-term use of benzodiazepine receptor agonists (BzRA) (17, 18) and are thus considered a high-risk group for BzRA side effects. For these reasons, psychiatrists look for effectiveness and safety in hypnotics.

Non-benzodiazepine was the hypnotic with the second-highest percentage of frequent prescribers. Frequently, NBZ prescribers believe NBZ is efficacious, has an appropriate duration of action, and is familiar but do not believe it is safe. In a 2005 survey conducted at West Lincolnshire Primary Care Trust in the United Kingdom, which examined GPs' attitudes toward prescribing BZ and NBZ, GPs believed that NBZ was more effective and safer compared to BZ in treating insomnia (12). However, when the study was conducted, novel hypnotics such as MRAs and ORAs without dependency concerns had not been developed (2–4). A recent NMA reported that eszopiclone, zopiclone, and zolpidem were more effective but had more side effects compared with a placebo in terms of treating insomnia (13). This NMA also reported that although no significant difference was noted in dropout due to adverse events between the eszopiclone and placebo groups, zolpidem and zopiclone had significantly more dropouts due to adverse events than placebo (13). Furthermore, previous studies have reported that NBZ was associated with side effects such as increased risk of falls (19, 20), balance dysfunction (21, 22), and increased risk of road traffic crashes, as noted with BZ (19, 22, 23). In addition, a study in Israel reported that NBZ was associated with an increased risk of long-term use of hypnotics compared with BZ (24). With the advent of novel hypnotics with fewer side effects and with an accumulation of research on the side effects of NBZ, physicians prescribing hypnotics probably no longer believe that NBZ is safe. Frequent NBZ prescribers were more concerned with familiarity than non-frequent NBZ prescribers, probably because NBZ is the second oldest hypnotic after BZ.

Melatonin receptor agonists was the hypnotic with a third of the percentage of frequent prescribers, and frequent MRA prescribers believed MRA to be safe, with an appropriate duration of action, and familiar, but did not believe it efficacious. Given that the safety of MRA has been confirmed by various studies (2, 13, 25), it can be inferred that MRAs are often prescribed by safety-conscious physicians. In fact, this study showed that frequent MRA prescribers were more common among non-psychiatrists, probably because non-psychiatrist insomniacs are more likely to have physical comorbidity than psychiatrist insomniacs. Regarding efficacy, a 2017 meta-analysis reported that ramelteon reduced sleep latency by 9 min compared to placebo (7), but a recent NMA reported that ramelteon did not differ in efficacy from placebo and concluded that ramelteon showed no material benefit for insomnia (13). This lack of robustness of the effect of ramelteon on insomnia may have led to the results of this study. Frequent MRA prescribers believe that MRA has an appropriate duration of action terms of duration of action. Unlike BZ and NBZ, few drugs are classified as MRAs, only ramelteon for insomnia in adults and melatonin for insomnia in children. Nevertheless, one possible reason the duration of action of MRAs was considered adequate may be that ramelteon has no hangover effect (2).

Benzodiazepine was the hypnotic with the lowest percentage of frequent prescribers. Frequently BZ prescribers believe BZ to be unsafe but think it is practical, with an appropriate duration of action, familiarity, and inexpensive. These findings are understandable given that BZ is more effective but less safe than placebo (13), an old and familiar drug, inexpensive drug, and available in various action drugs. Interestingly, approximately half of the physicians prescribe BZ frequently, although they realize the safety issues associated with BZ. Although this is only speculation because this study did not examine pharmacotherapy strategies for insomnia, for patients whose insomnia did not remit with hypnotics other than BZ, BZ may often be changed from or added to the hypnotics. A recent NMA reported that in a head-to-head comparison, short-acting BZ was more effective than lemborexant, suvorexant, and ramelteon in short-term treatment (13).

This study showed that frequent BZ and NBZ prescribers were more concerned with efficacy but not safety. It is not possible to conclude from this survey whether frequent prescribers of these drugs are using them because they do not value safety or whether they were compelled to prescribe them frequently out of necessity, with an understanding of safety issues and an expectation of efficacy. This conclusion is only speculation, but given the repeated warnings about the safety of BZ and NBZ (26), physicians may prescribe BZ and NBZ frequently because insomnia has not improved with other safety hypnotics.

This study has some limitations. First, the survey had a low response rate, especially from JPCA and AJHA, whose members

are predominantly non-psychiatrists and were surveyed *via* e-mail. In addition to the low overall response rate, the difference in the response rates between psychiatrists and non-psychiatrists might have affected the results of this study. Second, this study did not examine individual hypnotics frequently used by clinicians. The study results may contain heterogeneity, given that hypnotics classified in the same class may differ in their effects, side effects, and duration of action. Third, because this study used a multiple-choice method of surveying frequently used hypnotics, it was impossible to directly link the factors that the subjects considered important when selecting a hypnotic different from the formula often prescribed. Some subjects may have frequently been prescribed one class of hypnotics for effectiveness and frequently used another for safety. However, the results of this study are generally consistent with the recent NMA regarding efficacy and safety (13), the results regarding familiarity also reflect the timing of the launch of each class of hypnotics in Japan, and the results regarding drug prices reflect the prices of hypnotics at the time of the study, the impact of the lack of a direct link between frequently prescribed hypnotic and the reason for their choice would not be significant. Fourth, the study did not consider comorbidities in patients for whom hypnotics were prescribed. The prescription of one class of hypnotic may be affected by comorbidities. Psychiatrists may avoid prescribing BzRA to patients with schizophrenia, mood disorders, anxiety disorders, and alcohol use disorders since these comorbidities have been reported to be predictors of long-term prescribing of BzRA (17). Further, non-psychiatrists may avoid prescribing suvorexant to patients with antifungals and antivirals or immunocompromised

TABLE 2 Logistic regression analysis examining the factors associated with frequent prescribing of each hypnotic.

	BZ (*N* = 522)		NBZ (*N* = 725)		MRA (*N* = 549)		ORA (*N* = 811)	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Age group								
20s	Reference		Reference		Reference		Reference	
30s	8.04 (1.15–56.26)	0.036*	14.37 (2.94–70.23)	<0.001*	0.45 (0.053–3.87)	0.469	0.68 (0.072–6.42)	0.736
40s	9.37 (1.40–62.76)	0.021*	27.03 (5.67–128.82)	<0.001*	0.26 (0.032–2.14)	0.211	0.32 (0.037–2.81)	0.306
50s	9.20 (1.39–61.02)	0.021*	26.12 (5.55–122.82)	<0.001*	0.22 (0.028–1.81)	0.160	0.29 (0.033–2.46)	0.254
60s	11.77 (1.77–78.43)	0.011*	21.65 (4.58–102.4)	<0.001*	0.19 (0.023–1.54)	0.120	0.21 (0.025–1.84)	0.160
70s	27.11 (3.82–192.22)	<0.001*	36.61 (7.09–188.97)	<0.001*	0.22 (0.026–1.81)	0.157	0.20 (0.022–1.80)	0.150
80s and more	9.88 (1.053–92.72)	0.045*	12.55 (1.88–83.89)	0.009*	0.15 (0.014–1.49)	0.104	0.13 (0.012–1.42)	0.094
Specialty								
Non-psychiatry	Reference		Reference		Reference		Reference	
Psychiatry	2.67 (1.83–3.90)	<0.001*	1.22 (0.84–1.80)	0.298	0.43 (0.31–0.59)	<0.001*	2.82 (1.83–4.35)	<0.001*
Reasons for selecting medications frequently used for insomnia								
Efficacy								
No	Reference		Reference		Reference		Reference	
Yes	4.19 (2.91–6.04)	<0.001*	2.43 (1.62–3.67)	<0.001*	0.73 (0.53–1.02)	0.068	1.60 (1.01–2.54)	0.044*
Appropriate duration of action								
No	Reference		Reference		Reference		Reference	
Yes	2.26 (1.56–3.27)	<0.001*	4.93 (3.11–7.82)	<0.001*	2.19 (1.58–3.03)	<0.001*	1.135 (0.74–1.75)	0.568
Safety								
No	Reference		Reference		Reference		Reference	
Yes	0.25 (0.16–0.39)	<0.001*	0.68 (0.44–1.07)	0.094	2.48 (1.77–3.46)	<0.001*	4.52 (2.99–6.84)	<0.001*
Familiarity								
No	Reference		Reference		Reference		Reference	
Yes	3.74 (2.65–5.29)	<0.001*	2.29 (1.63–3.22)	<0.001*	1.35 (1.00–1.82)	0.047*	1.16 (0.77–1.75)	0.476
Recommended								
No	Reference		Reference		Reference		Reference	
Yes	0.94 (0.45–1.93)	0.860	2.21 (0.98–4.99)	0.055	1.63 (0.86–3.08)	0.134	1.92 (0.76–4.83)	0.167
Drug price								
No	Reference		Reference		Reference		Reference	
Yes	3.87 (1.91–7.82)	<0.001*	1.12 (0.55–2.28)	0.759	0.65 (0.39–1.09)	0.101	0.39 (0.21–0.73)	0.003*

Categorical variables are expressed as numbers (%). *P*-values with significant results (<0.05) are labeled with an asterisk. BZ, benzodiazepine; NBZ, non-benzodiazepine; MRA, melatonin receptor agonist; OR, odds ratio; ORA, orexin receptor antagonist.

patients because suvorexant is contraindicated in Japan with these comorbidities. Fifth, because this study was conducted on Japanese physicians, caution should be exercised when generalizing the results to physicians in other countries with different healthcare systems or environments. Japan has a universal health insurance system, which allows citizens to easily access medical care and receive treatment with a low financial burden. Therefore, Japanese patients may be more accepting of new, expensive ORA than patients in other countries. Regarding the healthcare environment, Japan was the first country in the world in which ORA was approved, and Japanese physicians may be more familiar with ORA than physicians in other countries. Despite the fact that Japan has a medical system and environment conducive to prescribing ORA, about 3/4 of the physicians prescribed NBZ, and about 1/2 prescribed BZ frequently in this survey. This indicates the limitations in treating insomnia with ORA alone, and these findings may be useful to physicians in countries with different health care systems and environments than Japan. Although the most frequently used medications for insomnia were the newest and most expensive ORA in this study, countries with different healthcare systems or environments may have obtained different results from this study. Sixth, this study lacked data on participants' actual prescriptions during the study period. Thus, it was not possible to compare participants' responses with their actual prescriptions.

The study findings suggest that physicians were compelled to prescribe BZ and NBZ frequently for efficacy, disregarding safety. In the future, it is hoped that cognitive-behavioral therapy for insomnia, which has been established to be effective and safe (27, 28), will become more widely used and that evidence will be accumulated regarding treatment strategies for patients who fail to respond to novel hypnotics with a safety profile.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of St. Luke's International University (2021-604). The patients/participants provided their written informed consent to participate in this study.

Author contributions

KIe, KIn, KW, and YT: conceptualization and project administration. YA and YT: methodology. MT: software, resources, and visualization. MT, YA, and YT: formal analysis. YA, KIe, EK, ET, TT, KIn, and MK: investigation. YA, KIe, EK, ET, TT, and MK: data curation. MT and YA: writing—original draft preparation. KIn, KW, and KM: supervision. KM and YT: funding acquisition. All authors read and agreed to the published version of the manuscript.

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Conflict of interest

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2023.1071962/full#supplementary-material

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EDITED BY
Lais F. Berro,
University of Mississippi Medical Center,
United States

REVIEWED BY
Fatjona Kamberi,
University of Vlorë, Albania
James K. Rowlett,
University of Mississippi Medical Center,
United States

*CORRESPONDENCE
Francisco José Pérez-Cano
✉ franciscoperez@ub.edu

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Changes in prescription drug abuse during the COVID-19 pandemic evidenced in the Catalan pharmacies

Maria Perelló[1,2], Karla Rio-Aige[2,3], Pilar Rius[1], Guillermo Bagaría[1], Anna M. Jambrina[2,4], Montse Gironès[1], Francisco José Pérez-Cano[2,3]* and Manel Rabanal[2,3,4]

[1]Council of the Pharmacist's Association of Catalonia, Barcelona, Spain, [2]Physiology Section, Department of Biochemistry and Physiology, Faculty of Pharmacy and Food Science, University of Barcelona, Barcelona, Spain, [3]Institute of Research in Nutrition and Food Safety (INSA), Santa Coloma de Gramenet, Spain, [4]Directorate-General for Healthcare Planning and Regulation, Ministry of Health, Government of Catalonia, Barcelona, Spain

Introduction: The impact of a pandemic on the mental health of the population is to be expected due to risk factors such as social isolation. Prescription drug abuse and misuse could be an indicator of the impact of the COVID-19 pandemic on mental health. Community pharmacists play an important role in addressing prescription drug abuse by detecting signs and behaviors that give a clearer indication that a drug abuse problem exists.

Methods: A prospective observational study to observe prescription drug abuse was conducted from March 2020 to December 2021 to compare with data obtained in the previous 2 years, through the Medicine Abuse Observatory, the epidemiological surveillance system set up in Catalonia. Information was obtained through a validated questionnaire attached on a web-based system and data collection software. A total of 75 community pharmacies were enrolled in the program.

Results: The number of notifications during the pandemic period (11.8/100.000 inhabitants) does not indicate a significant change compared with those from pre-pandemic period, when it was 12.5/100.000 inhabitants. However, the number of notifications during the first wave when lockdown was in place stood at 6.1/100,000 inhabitants, significantly lower than in both the pre-pandemic and the whole of the pandemic periods. Regarding the patient's profile, it was observed that the proportion of younger patients (<25 and 25–35) rose in contrast to older ones (45–65 and >65). The use of benzodiazepines and fentanyl increased.

Conclusions: This study has made it possible to observe the impact of the pandemic caused by COVID-19 on the behavior of patients in terms of use of prescription drugs through analysis of the trends of abuse or misuse and by comparing them with the pre-pandemic period. Overall, the increased detection of benzodiazepines has pointed out stress and anxiety generated by the pandemic.

KEYWORDS

COVID, medicine abuse, observatory, drug, benzodiazepines, community pharmacy, pandemics

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified in Wuhan (China) in December 2019 as the cause of the illness designated as COVID-19 (1). With almost 7 million confirmed cases and more than 89,000 deaths by January 5, 2022, Spain remains one of the European countries most severely affected by the ongoing COVID-19 pandemic (2–4).

In Spain, the first virus case was detected on January 31, 2020, and several weeks later, on February 25, 2020, the first case in Catalonia (a northeastern region in the country) was identified after a 36-year-old woman visited Italy from February 12 to 22, 2020 (5).

From then on, the adoption of lockdown measures by the national and regional governments steadily increased over time, from the recommendation of preventive measures in late February and early March to increasingly stricter social distancing measures. On March 13, a nationwide lockdown was announced and on March 15 it was enforced. In addition, a strengthened lockdown was rolled out with the closure of all non-essential economic activities on March 31 (6).

On April 25, Spain started to ease its lockdown with a gradual lifting of restrictions due to decreasing trends in confirmed cases, hospitalizations, and daily deaths. Hence, the "lifting lockdown" process began and the state of emergency ended on June 21. Although the situation had stabilized by the summer period (July-September) and a significant rise in the number of COVID-19 cases was not observed, the Government of Catalonia forbade gatherings of more than 10 people in public or private premises and advised people to stay at home unless strictly necessary.

In October, the second wave started and the state of emergency was rolled out again. This new one ran from October 25, 2020, to May 9, 2021. As in the first state of emergency, the level of restrictions varied depending on the successive waves of COVID-19 cases: The second wave was from October to early December; the third wave from January to March, and the mild fourth wave during April and May. Soon after the first COVID-19 vaccine (BNT162b2 mRNA) was approved in December 2020, Spain started its mass immunization campaign.

In this context, an impact of the pandemic on the mental health of the population is to be expected due to risk factors such as social isolation, uncertainty over disease status, and economic and housing problems (7–9). The pandemic also affects the control of chronic disease (10). Additionally, a number of studies have shown that fear of COVID-19 infection was associated with high levels of emotional stress, especially in women, while an increase in anxiolytics use during lockdown was observed (7). Furthermore, it is reported that being young had a positive association with depression and anxiety (7, 8, 11). Although the impact of the COVID-19 pandemic on mental health cannot be quantified yet, there is the suggestion of a wave of mental illness associated with the consequences of the pandemic (12) and a rise in the consumption of benzodiazepines to cope with these disorders (7, 11, 13–16).

Concerns were also reported by several sources and some experts described an increased availability after the lockdown period of diverted prescription opioids, such as tramadol, buprenorphine and methadone (17).

As a vital part of the healthcare system, pharmacies play an important role in providing medicines, therapeutics, vaccines, and critical health services to the public. Moreover, pharmacists have knowledge about the safe and effective use of medications and about the adverse effects of their inappropriate use. In addition, pharmacists do more point-of-care work to help take the pressure off doctors. Patients have also turned more often to the pharmacist to request chronic medication and/or that necessary to tackle the situation generated by the pandemic (18–20).

Equally, prescription drug abuse and misuse, defined as the intentional use of a medication without a prescription or in a way other than as prescribed, or for the experience or feeling its causes, could be a possible indicator of the COVID-19 pandemic's impact on the behavior of the public due to effects on mental health. Furthermore, the inappropriate use, can be also unintentional, such as when it is due to ignorance or cognitive impairment (21).

According to the National Survey on Drug Use and Health (NSDUH), in 2021, an estimated 9.6% of past year users of drugs other than alcohol (or 10.2 million people) perceived that they used these drugs "a little more or much more" during the COVID-19 pandemic than they did before, which include prescription pain relievers, tranquilizers, stimulants, or sedatives (22). As well, in Europe, it is estimated that at least 5 800 overdose deaths, involving illicit drugs, occurred in the European Union in 2020, this represents an estimated mortality rate due to overdoses of 17.4 deaths per million for the adult population. Most of these deaths are associated with polydrug toxicity, which typically involves combinations of illicit opioids, other illicit drugs, medicines and alcohol. In some countries, benzodiazepines are commonly mentioned (23).

Given this situation, the aim of the work was to identify trends of medicine abuse in Catalonia and study whether there has been any change in the pattern with respect to previous years, through report of community pharmacies.

For this purpose, the information was obtained from the Medicine Abuse Observatory (MAO), that has been operating in community pharmacies of Catalonia since 2017, and allows to observe and analyze the behavioral patterns of the population with respect to this phenomena (24).

2. Material and methods

2.1. Community pharmacy framework

As community pharmacists have a key role in carrying out epidemiological surveillance and should be committed to promoting the safe and effective use of medicines, in 2017 the Medicine Abuse Observatory (MAO) was set up in Catalonia. The MAO was settled as a project supported by the Catalonia Pharmacists Council and the Ministry of Health of the Government of Catalonia. It makes it possible to observe and analyze trends about the most diverted drugs and the behavioral patterns of the population with respect to this issue *via* community pharmacies. In this context, we conducted a prospective observational study from March 2020 to December 2021, taken as the COVID-19 period, in order to compare it with data from 2 years prior (July 2017-February 2020), taken as pre-COVID-19 period (22).

2.2. Enrolled pharmacies

Data was obtained from community pharmacies enrolled in surveillance for the detection of suspected cases of drug abuse and

misuse project. There were 60 of them during the pre-COVID period and this number rose up to 75 for the COVID period. In both periods, selected pharmacies belonged to the Catalan sentinel pharmacy network (Catalan Sephanet) (25). The pharmacies are scattered throughout the region, based in 3 phases. First, a selection phase, to determine the minimum number of sentinel pharmacies and the location of these to obtain the greatest possible representativeness. This phase includes a cluster and population analysis, and adjustment for strata and population. Second, a voluntariness and random selection phase of the pharmacies that belong to the selected area, to mitigate bias in the reporting depending on the degree of motivation. Third, a training by the Barcelona College of Pharmacists, in order to standardize data collection. The main topics covered were the basis of the method and operational procedures coupled with a theoretical framework furnishing information about the phenomenon. The training sessions were performed regularly to resolve issues and clarify questions about screening procedures (24, 25).

2.3. Data report

A validated questionnaire (Abuse Drug Questionnaire, ADQ) was created. It was based on Finch's criteria, that enables to identify signs and behaviors that give a clearer indication that a drug abuse exists. These elements, which Finch described in 1993, are: pattern of calling for refills after hours and/or repeatedly needing early refills, prescriptions from multiple physicians, frequent visits to emergency rooms, strong preference and knowledge for a particular drug and incongruence between severity of the complaint and the physical presentation. Based on this theoretical framework, the situations that would arise in community pharmacies, such as repeated requests for medicine, or the request for a prescription medicine with a false prescription or without it, would be indicators of suspected misuse or abuse of these medicines.

The questionnaire consisted of an anonymous multiple-choice test containing 10 closed and two open-ended questions categorized in four different parts. Pharmacist identification (questions 1 and 2). Patient demographic variables as age, sex, and origin, are included in questions 3 to 5. The substance involved and how it is requested, was required in questions 6 to 9. In this sense, it is considered "does not require a prescription" for over the counter (OTC) medicines and "requested with prescription" for prescriptions needing frequent refills and/or from multiple physicians. We consider "probably forged prescription" as a counterfeit prescription (copies) or any falsification made on a right prescription form. Finally, in question 10 and the 2 open-ended questions, pharmacist management is enquired. The aim of these three questions is to know in which cases the medicines are dispensed and to study the reasons for which these medicines are dispensed.

The pharmacist filled out the questionnaire when a patient that requested a medicine presented two or more of the defined signs and behavioral symptoms and was suspected of being a medicine abuser. Patient information was obtained anonymously by observation during the interview and neither verbal nor written consent were needed. Otherwise, the substances to follow up were chosen taking into account the evidence from scientific literature and data from our environment (22). In this sense, a list to select the type of benzodiazepines was included and also an item entitled "others" that allows pharmacists to report any medicine.

In order to ease the reporting, the ADQ collected during the studied period were passed by a web-based survey. Data collection software called Typeform (Typeform SL, Barcelona, Spain) was embedded in the Barcelona College of Pharmacists' website, which is the principal online work tool for pharmacists in this area of Spain. This software transformed the ADQ electronic data into an Excel spreadsheet to operate them.

2.4. Statistical analysis

To categorize the number of notifications of both periods, the number of inhabitants corresponding to the enrolled pharmacies for each one was considered: 146,335 for the data coming from the 60 community pharmacies in the pre-COVID period and 218,701 for the data coming from the 75 community pharmacies in the COVID period. Likewise, the categorical variables obtained for the COVID period were analyzed as percentages and compared with those obtained in the pre-pandemic period. The X^2 test was used for this purpose and a p-value < 0.05 was considered statistically significant. Additionally, quantitative analysis was also performed for some items such as benzodiazepines. The analyses were conducted with SPSS software, version 18 (SPSS Inc., Chicago, IL, USA).

Multiple correspondence analysis (MCA) was also performed to find similarities in the individual profiles simultaneously by R version 4.1.2 (R Foundation, Austria) (https://www.R-project.org/) using the packages FactoMineR (https://cran.r-project.org/web/packages/FactoMineR/index.html) for the analysis and factoextra for the visualization (https://cran.r-project.org/web/packages/factoextra/index.html). In this analysis, two categories that present high coordinates and are close in space are directly associated with each other. When the cos2 value for one variable category is close to one, this indicates it is well represented by two dimensions. This MCA analysis made it possible to find similarities between customers in terms of their characteristics and behavior and establish the relation and the degree of association between different variables.

3. Results

3.1. Number of notifications

The number of notifications during the pandemic period was 11.8/100,000 inhabitants in the Catalonian region and this proportion was not significantly different when compared to the pre-pandemic period (12.5/100,000 inhabitants, $p = 0.39$). It should be borne in mind that the pandemic period consisted of a number of periods in which only the first wave included total lockdown. Thus, the number of notifications during the first wave was 6.1/100,000 inhabitants, significantly lower than in both the pre-pandemic period and the whole pandemic periods ($p < 0.05$).

All the validated notifications under study allow to characterize the patient's profile, the substances involved, the drug requested, and the supply of medicine. First, the participant's features were multi-parametrically approached to analyze similar profiles between the individuals in the study and to evaluate associations between

variable categories (Figure 1) by multiple correspondence analysis (MCA). The variance obtained was 10.2% for Dim-1 and 7.9% for Dim-2 in the pre-COVID period, while the explained variance for the COVID period was 11.1% for Dim-1 and 8.1% for Dim-2 (Supplementary Figures 1A, B, respectively). The variables had similar correlations with the first two dimensions in both periods, where the REQUEST variable was the most correlated with Dim-1 and the second most correlated with Dim-2, followed by the DRUG variable, the most correlated with Dim-2 (Supplementary Figures 1C, D). This finding was in line with the visualization of the results in Figure 1 in which it can be observed that some REQUEST categories had the highest cos2 value, "Probably forged prescription" in the pre-COVID period (Figure 1A) and "Does not require a prescription" (Figure 1B) in the COVID period, indicating that these factors were important in explaining the variability in the dataset. Moreover, it can also be seen that "Probably forged prescription" and "Benzodiazepines" were very close and appear on the negative y-axis (Dim-2) in the pre-COVID MCA (Figure 1A), indicating a strong correlation between them. On the other hand, these categories were less representative in the COVID MCA, where the categories "Does not require a prescription" and "Dextromethorphan" gained strength on the positive y-axis (Dim-2) (Figure 1B). In line with these results, when individuals were colored by variables, the REQUEST variable tended to cluster the subjects (Supplementary Figure 2). These overall differences between periods can be better observed when they are quantitatively stratified by factors.

3.2. Change in the patient profile during the pandemic period

The proportion of male and female involved in the notifications was similar between the two periods studied, with male in the majority (∼65% male: ∼35% female) (Figure 2A).

In relation to age, in both periods the highest proportion of notifications was from patients in the 25–35 years range (∼30%) followed by those aged 36–45 and 46–65, both with very similar values (22–27%) (Figure 2B). The lowest proportion of prescription drug users was found in the youngest and the oldest age intervals (<13% in both cases and periods). When both periods were compared, we observed that during the pandemic there was a significant rise in the proportion of users in the youngest group (<25 years) at the expense of those in the 36–45 years range ($p < 0.05$).

The combination of age and sex data makes it possible to better characterize the profile of the patients showing a differential predominance pattern between male and female. In the most prevalent age range (25–35 years) as in the <25 years and 36–45 years ranges, there are more male than female (∼80% vs. 20%). In addition, this pattern changes and the proportion of female increases in the oldest group (>65 years). The 46–64 years age group has similar proportions of male and female (∼50%). However, no significant differences were observed between the pandemic and pre-pandemic periods (Figure 2C).

Also, in relation to whether the notifications concerned a native or non-native patient, and although in both cases there were more native patients involved (∼60–70%), in the pandemic period there was a rise in native patients asking for an item considered drug abuse in pharmacies ($p < 0.05$) (Figure 2D).

3.3. Pandemic effect on the involved substances and drug requests

The data obtained in both the pre-pandemic and pandemic periods show that the substances involved are quite variable albeit with a similar preliminary pattern (Figure 3A). The main drug class involved was benzodiazepines (∼30–40%), followed by codeine (∼20%), tramadol (∼7%), and methylphenidate (∼5%), while the rest of the requested prescriptions are present in the questionnaire at <2%. However, other not explicitly requested drugs were also involved in an overall proportion of ∼25%. Even so, there was a significant increase during the pandemic in the detection of benzodiazepines and fentanyl ($p < 0.05$). In addition, a different pattern was found in some drugs stated in the "others" section. For instance, pseudoephedrine was requested during the pandemic by 4.9%, pregabalin by 1.8%, and cycloplegic drops by 0.2% whereas in the pre-pandemic period these values were about 0.8, 5.8, and 4.4%, respectively ($p < 0.05$). This differential shift in patients' behavior on drug substances is clearly illustrated by the MCA in Figure 3B.

Regarding benzodiazepines, the group of prescription drugs most frequently detected, it was observed that the number of notifications intended to increase during the pandemic and it rose from 3.95/100,000 inhabitants to 4.93/100,000 inhabitants ($p = 0.07$). The most detected benzodiazepine was clonazepam (44.2%) followed by alprazolam (22.5%), lorazepam (17.4%) and diazepam (10.5%).

The sex variable also shows no difference between periods and they were higher in males in both periods.

In terms of age, benzodiazepines represent the most detected group of medicines in >65 years (56.3% of notifications) with no difference between periods. Despite this, the <25 group had a significant increase in incidence ($p < 0.05$) and, in contrast, the 25–35-year-old group showed a decrease ($p < 0.05$). Benzodiazepine use by the rest of the age ranges did not vary due to the pandemic (data not shown).

With reference to the drug request or the way users tried to get the medicine, some changes due to COVID were observed (Figure 4A). In both the pre-pandemic and the pandemic periods, most of the notifications were due to drugs requested without a prescription (∼50%) and forged prescriptions (∼30%), whereas notifications coming from over the counter (OTC) drugs and formal prescription drugs were less frequent. The pandemic triggered a significant rise in requests with a prescription and also with forged prescriptions ($p < 0.05$). In addition, in the COVID period the number of notifications of medicines that required a prescription or which were requested without one was also lower ($p<0.05$). Notifications of OTC drugs behaved similarly in both periods.

The benzodiazepine request pattern remains practically the same in both periods, where ∼50% of people try to get the medicine by using a forged prescription, ∼27% by requesting without a prescription, and ∼20% through a formal prescription (data not shown).

3.4. Changes in request frequency and supply of medicines

The request for the medicine was sometimes performed insistently, and this frequency pattern was similar in both periods

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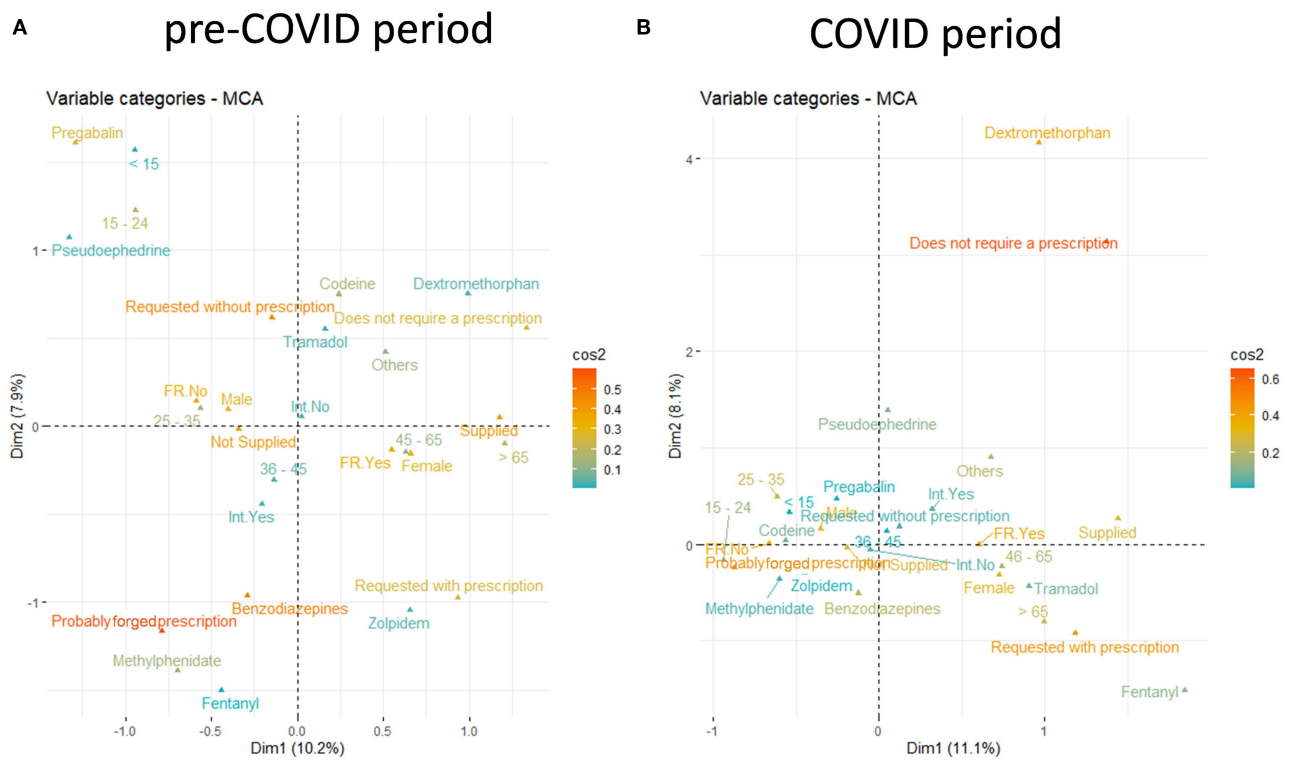

FIGURE 1
Two-dimensional MCA plots for the pre-COVID period **(A)** and the COVID period (**B**). Cos2 measures the degree of association between variable categories and axial categories. If the variable category is well represented by the dimensions, values for cos2 are close to one.

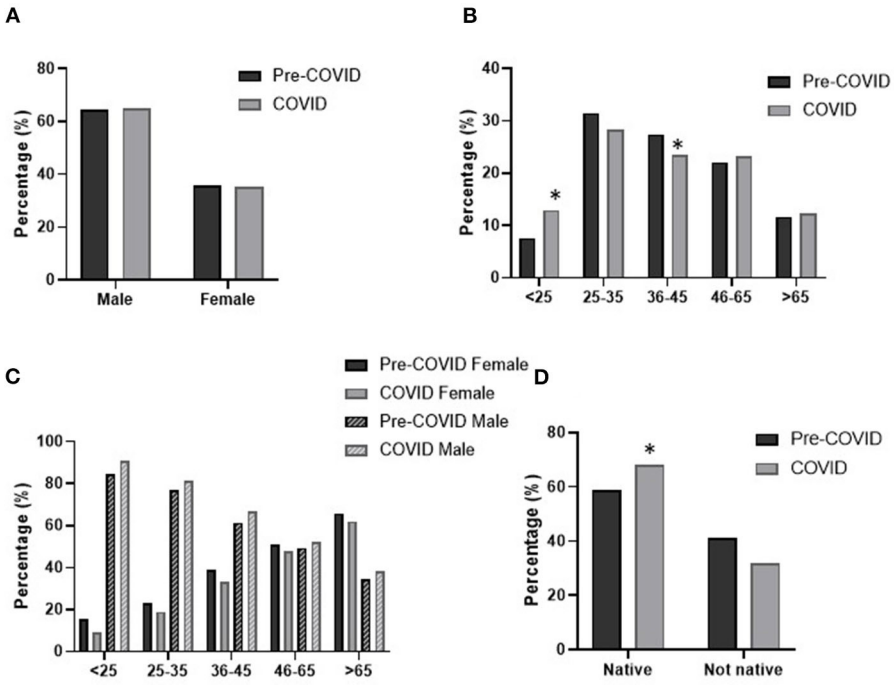

FIGURE 2
Patient profile across pre-pandemic and pandemic periods. Distribution of participants by **(A)** sex, **(B)** age, **(C)** age and sex and **(D)** origin. Statistical differences: *$p < 0.05$ pandemic vs. pre-pandemic.

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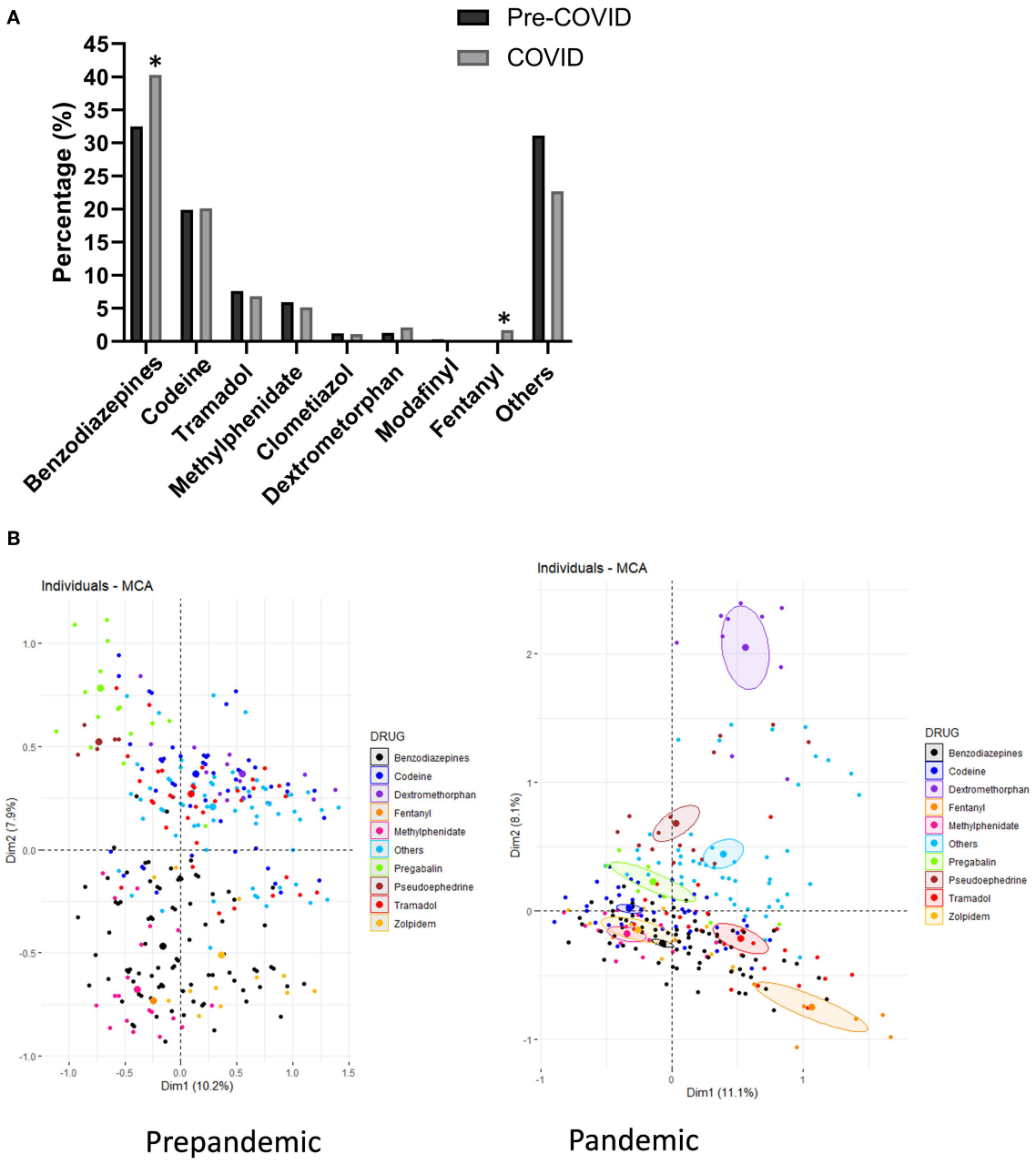

FIGURE 3
Description and proportion of type of drug according to **(A)** both periods and **(B)** depiction by MCA analysis. Statistical differences: *$p < 0.05$ pandemic vs. pre-pandemic.

(Figure 4B). In certain cases, the drug was asked for using intimidation. It should be noted that a trend to increase (from ~11 to ~14%) was observed during the pandemic period vs. the pre-pandemic one, but it was not statistically significant ($p = 0.10$) (Figure 4C).

The management and attitude of pharmacists when addressing these requests was similar in both periods and in ~80% of cases dispensation did not occur. However, the supply percentage was slightly lower during the pandemic period ($p < 0.05$) (Figure 4D).

Behavior related to benzodiazepines showed no difference between periods and request frequency was around 50% in both. In addition, no difference related to intimidation was detected either. It could be observed that nearly half of notifications with intimidation involved benzodiazepines (50.7% for the pre-pandemic and 46.9% for the pandemic period).

As to the management of pharmacists when abuse or misuse was under suspicion, during the pandemic period fewer supplies were delivered ($p < 0.05$) and 60.6% of them were so to people aged 46–65 and >65 years, who made requests with higher frequency ($p < 0.05$ for the 46–65 and >65 years groups vs. the other age-groups), and mainly were anxiolytics and opioid painkillers (fentanyl, codeine, and tramadol). However, there was overall a decrease in the dispensation of benzodiazepines ($p < 0.05$) to people in this age range.

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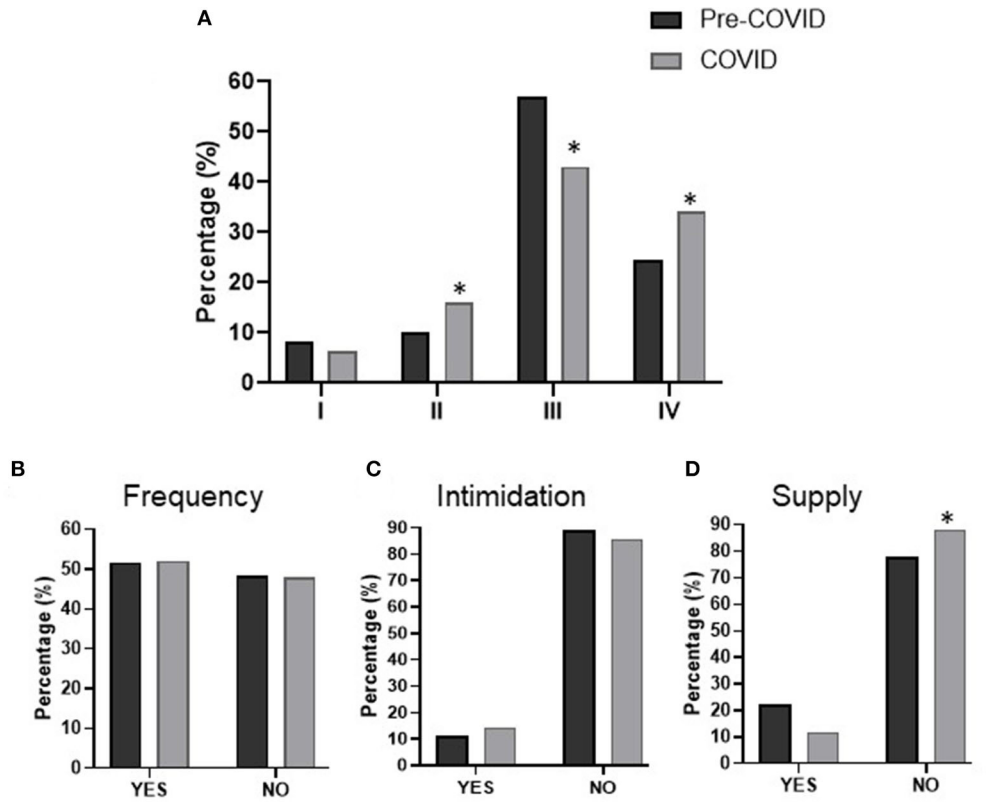

FIGURE 4
Drug-requesting behavior of patients. Distribution of participants according to **(A)** type of request, **(B)** frequent request, **(C)** use of intimidation, and **(D)** supply of medicine. Requesting types are as follows: I. prescriptions corresponding to an over-the-counter (OTC) drug; II. formal prescription; III. without prescription; IV. Forged prescriptions. Statistical differences: *$p < 0.05$ pandemic *vs*. pre-pandemic.

3.5. Waves of the pandemic period

Although the global period of the pandemic has been analyzed as a whole, we are aware that each wave within this period had a different restriction pattern in which the first wave was the most rigorous as there was total lockdown of the population. Thus, after analyzing the patterns in each wave, we found similar behavior in some aspects, but some trends and statistical differences appeared too, especially with respect to age distribution (Figure 5).

On the one hand, as noted above, the number of notifications per inhabitant was similar across waves, with the exception of the first wave, the only one including total lockdown and showing significantly lower numbers (6.1/100,000 inhabitants) than the rest of the pandemic periods, which were even lower than the pre-pandemic period ($p < 0.05$). Besides, the patient profile did not change regarding sex, and the male-female interval was similar across waves (from 55–45% to 74–26%). However, when the influence of age in the notifications was studied, a pattern over the course of the pandemic waves ($p < 0.05$) was found, whereby younger patients (<25 and 25–35) rose for some variables at the cost of older ones (45–65 and >65) (Figure 5).

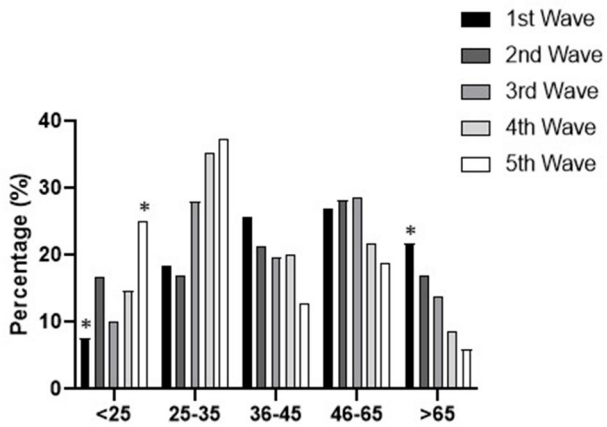

FIGURE 5
Patient age distribution profile according across waves. Statistical differences: *$p < 0.05$ vs. the value in the total pandemic.

No changes were found between waves from the native/non-native patient point of view, where the maximum differential proportion was 82.4%/17.6% in the first wave, shifting to 62.2%/37.8% in the last wave ($p < 0.05$). As for drug requests and patient intimidating behavior, a similar pattern was also observed over the course of the pandemic. However, the request frequency proportion was different across the waves of the pandemic, inasmuch as request notifications were more frequent in the first three waves (64.9, 56.4, and 62.6%) than in the last two (44.4 and 38.9%) ($p < 0.05$). Nonetheless, pharmacist supply did not change

during the COVID period analyzed. As for the type of drug, the contribution of each one was variable among waves, with no clear pattern during the whole pandemic period, and in all cases, there were higher contributions of benzodiazepine and fentanyl prescription than during the pre-pandemic period ($p < 0.05$).

4. Discussion

This study describes changes in prescription drug abuse trends during the COVID pandemic. Results are in line with preliminary findings which, despite not supporting a rise in drug use during the early stages of the COVID pandemic (16, 26), do show an increase in some prescription drugs such as benzodiazepines (14–17). This may be accounted for by users wishing to combat the concerns and anxiety experienced in response to the COVID-19 pandemic and the resulting lockdown measures. The rise in prescription medicines used to cope with increased mental health issues during the COVID-19 pandemic is also highlighted by the European Monitoring Center for Drugs and Drug Addiction (EMCDDA), which refers to loneliness, feeling depressed and stress as the main reasons for increased use of benzodiazepines (27).

In addition, the COVID-19 pandemic has affected drug markets (supply shortages of drugs as well as price increases). These problems, combined with a general economic loss, can lead to changes to prescription or OTC medicines that are mixed with cheaper drugs (14, 26–28).

Equally, primary healthcare assistance reduced mental health services during the national lockdown due to the COVID-19 outbreak and there was a decrease in follow-up and control of chronic diseases and adult vaccination coverage (10). The possible consequences of these deprivations include an increase in admissions to emergency departments for mental illness and drug and alcohol use and also a rise in suicide risk (15). In fact, according to the European Drug Report, an increase in emergency room visits related to benzodiazepines was detected in a sample of surveillance hospitals in 2020 compared to 2019 (29).

Although the consequences in terms of increases in dependence on substances such as alcohol or prescription drugs may only become visible with time, our study makes it possible to observe the behaviors and medicines that have been most identified in relation to this phenomenon in community pharmacies.

First of all, in terms of age, and even though the highest proportion of users were patients in the 25–35 years range, there was a rise in those under 25 years of age and also in those over 65. This increase in the extreme ages can be explained, firstly, by the change in the behavior of drug markets as noted above, and secondly, by the lack of monitoring of the health status of the older adults by primary care services. This is consistent with data in a study carried out in the United States, where it was reported that early adolescent substance use during the pandemic was associated with increased use of nicotine and misuse of prescription drugs (30), and also with concerns about possible increases in drug-related deaths in some countries, mostly among young people and due to intake of benzodiazepines (16).

Turning to sex, in this analysis males asked for abuse drugs more often than females, which is in line with the published evidence on this phenomenon. The combination of age and sex data in our study shows that males are more frequent in the 25–35 years age group, while women are more common in the oldest group. Also, if we consider the medicines involved and the sex of patients, there's an association pattern for the COVID period between tramadol and fentanyl with females. This contrasts with the data obtained in the previous study during the pre-pandemic period, where no such association was observed, probably because women and young people had more mental health issues during lockdown (7).

There was an increase in the use of benzodiazepines, the most common substance, in all age groups, but it is especially remarkable in the group over 65 years of age. Our results are in line with the massive increase in reports of abuse during the pandemic, which seem to point at social isolation as one of the greatest risk factors for their drug abuse (31). Indeed, a recent study about the use of drugs and health resources by patients with pre-existing mental disorders (depression or anxiety) pointed out that there was a remarkable increase in the number of daily doses per inhabitant (DDI) of some benzodiazepines such as alprazolam or lormetazepam during the six months following the lockdown end in a regional health service in Spain (13). These data tie in with those published by the Spanish Agency of Medicines and Medical Devices, which reported that in the first quarter of 2020, the DDI of these substances was higher than in the last quarter of 2019 (57.19 DDI *vs.* 55.51 DDI) (13).

In this approach, tramadol was most commonly detected as an abuse drug substance in the 46–65 age group, and Z-drugs in older age groups. In contrast, the substance that was widely detected in the 25–35 years of age group was pseudoephedrine, which has sympathomimetic properties and whose acute effects include stimulant effects such as euphoria, lower sense of fatigue, anorexia, accelerated thinking, and psychotic symptoms with auditory and visual hallucinations. Oral and intravenous use has been recorded in misuse cases (32). It is also used as a slimming agent and in sports as an ergogenic agent. Moreover, there is a growing interest in preparations containing pseudoephedrine related to their use for recreational purposes, especially by adolescents and young adults, and for the production of psychoactive substances—the synthesis of methamphetamine and methcathinone (ephedrone) used as designer drugs (33). This high demand needs to be closely monitored and scrutinized in terms of its health and social consequences. Here Project STOP, a decision-making tool for pharmacists developed in Australia, allows community pharmacists to verify pseudoephedrine requests, and since its implementation there has been a downward trend in pseudoephedrine sales. This program is a multi-strategic approach to reducing inappropriate supplies through community pharmacies (34). This study also notes that community pharmacists are healthcare professionals who can prevent the potential misuse and abuse of medicines and also provide the option to monitor trends in this phenomenon.

Experts working in harm-reduction services in some EU Member States have also suggested that the use of methamphetamine may have become more popular in some user groups (35).

In the case of codeine, the same trend has remained and has been more commonly reported for younger (<25 years) people. However, in this age group, there has also been an increase in the notifications of benzodiazepines, which is related to the higher involvement in the detection of this phenomenon in young people by community pharmacies. The rising trend of misuse of benzodiazepines as well as of other substances that indicate illicit use needs to be monitored (16).

Apart from these substance-related data, the positive impact of the lockdown imposed due to the state of health emergency in order to curb the disease reduced people's mobility, including their access to pharmacies. Therefore, the large difference between the number of requests made by native patients vs. non-native patients can be associated with this situation and the restrictions enforced which reduced the percentage of non-native patients involved (36). As a result, the number of notifications per 100,000 inhabitants is lower during the pandemic lockdown period compared to the pre-pandemic period.

Thus, the pandemic meant that people could not seek medical attention whenever needed. This prompted the Health Ministry to allow pharmacies to supply the medicine even when the prescription had expired (10). At the same time, patients tried to abuse some medicines because they did not have enough, meaning that they were consuming more than in the pre-pandemic period. This may reinforce the data obtained related to the request type, in which there was an increase for some medicines with a prescription. This situation was especially common in the older age groups, who were also more associated with requests involving intimidation. In fact, in the early stages of the pandemic, verbal intimidation in requesting drugs rose up to significantly different values compared to pre-pandemic levels. Although as the pandemic went on this behavior tended to revert to its usual pattern, there was nevertheless a clear trend in threatening behaviors, which might have been related to stress and fear due to the circumstances. By contrast, young patients did not confront pharmacists but rather tried to get the drug through forged, copied or altered prescriptions or by going to different pharmacies.

Here it should be noted that pharmacists deliver services that have been shown to improve patient outcomes by providing information on COVID-19 and dispensing medications to maintain continuity of healthcare along with other services, so they remain the most accessible healthcare provider. However, pharmacists also experienced an increase in the number of patients seen during the pandemic. It is up to pharmacists to reassure patients and provide care while taking into account their mental health. In addition, the increase in the burden of pharmacist duties may also have led to a decrease in the pharmacist's mental wellbeing, as some studies suggest (37, 38). However, and in spite of this, pharmacists have continued to provide good healthcare to patients and detect patterns of probable abuse or misuse of medicines. Consequently, they have refused to supply these medicines when not indicated, and the number of dispensations made even decreased compared to the pre-pandemic period.

Community pharmacists play a vital role in supporting local communities, providing reliable information to patients, and relieving pressure on the rest of the healthcare system. Nonetheless, progress needs to be made in other demands not related to SARS-CoV-2 infection and it is essential to reestablish all care activity that has been interrupted as soon as possible. Identifying mental health-related needs is a priority along with regular assessment of substance use and suicidal ideation to evaluate the prevalence of psychological distress over time (10, 14, 39). This is in line with what Nora Volkow, the Director of the National Institute on Drug Abuse (NIDA), has said: "Clinicians should monitor for signs of substance misuse or use disorders in their patients, given the unprecedented stresses, fears, or even grief they may be facing" (40).

Regarding the various waves that took place during the pandemic, the greater detection and involvement of older people in the first period and the higher prevalence in frequently requesting some medicines probably reflects their concerns about the situation. They may have feared running out of medication, bearing in mind that specifically in the first wave the population was completely locked down. So they sought to have sufficient treatment to cope with the distress and nervousness produced by the pandemic. Hence as the pandemic progressed and restrictions and accesses were mitigated, a decrease in the involvement of the older adults was observed contrasting with higher detection of young people seeking to get medicines, most likely for the reasons already discussed above. Thus, the last period showed an increase in requests for medicines that do not need a formal prescription, specifically dextrometorphan and some sympathomimetic agents used as decongestants. This trend is related to this younger age group who also tried to get other drugs using forged prescriptions or by asking for them with no prescription.

5. Conclusions

This study made it possible to observe the impact of the pandemic caused by COVID-19 as shown on the behavior of patients regarding the use of prescription drugs through analysis of the trends of drug abuse or misuse and comparing them with the pre-pandemic period.

Overall, the first wave was the period with the highest impact, and a rise in benzodiazepines points to stress and anxiety generated by the pandemic and evidencing use of substances to cope with those feelings. A higher frequency of younger and older age groups showed that the needs of these population segments were greater, since published data suggests the pandemic aftermath has affected them the most. Efforts and resources need to be provided where they are due so that they are beneficiaries of the best healthcare.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

Conceptualization: MP, MR, and FP-C. Methodology: MP, MR, FP-C, and MG. Software: KR-A. Validation: GB, PR, and AJ. Formal

analysis: MP, KR-A, and FP-C. Investigation: MP and MR. Resources: MP, PR, and MR. Data curation: MP and KR-A. Writing—original draft preparation: MP, FP-C, MR, and KR-A. Writing—review and editing: MP, PR, and AJ. Visualization: MG and PR. Supervision: MR and FP-C. Project administration: MR, PR, and GB. Funding acquisition: MR. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1116337/full#supplementary-material

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EDITED BY
Scott E. Hemby,
High Point University, United States

REVIEWED BY
Mark Andrew Smith,
Davidson College, United States
Elif Engin,
McLean Hospital, United States

*CORRESPONDENCE
Lais F. Berro
✉ lberro@umc.edu

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Individual differences in the effects of midazolam on anxiety-like behavior, learning, reward, and choice behavior in male mice

Caio Jovita-Farias[1], Meagan E. Follett[2], Behaim C. Dias-Junior[1], Yasmim A. Serra[1], Natali D. Kisaki[1], Thaísa Barros-Santos[1], Nailton M. S. de Jesus[1], Isa R. S. Rodrigues[1], Larissa E. L. Macedo[1], Elena L. A. Malpezzi-Marinho[3], Alexandre J. Oliveira-Lima[1], Eduardo Ary Villela Marinho[1], James K. Rowlett[2] and Lais F. Berro[1,2]*

[1]Department of Health Sciences, Universidade Estadual de Santa Cruz, Ilhéus, BA, Brazil, [2]Department of Psychiatry and Human Behavior, University of Mississippi Medical Center, Jackson, MS, United States, [3]Department of Biological Sciences, Universidade Estadual de Santa Cruz, Ilhéus, BA, Brazil

Introduction: The aim of the present study was to investigate the behavioral effects of the benzodiazepine midazolam in male mice, in models of anxiolysis, learning, and abuse-related effects.

Methods: In a first set of experiments, male Swiss mice were submitted to the training session of a discriminative avoidance (DA) task on the elevated plus maze to evaluate anxiety-like behavior and learning after vehicle or midazolam (1, 2 or 5 mg/kg, i.g.) administration. The same animals were submitted to a conditioned place preference (CPP) protocol with midazolam (1, 2 or 5 mg/kg, i.g.). In a second experiment, outbred (Swiss) and inbred (C57BL/6) male mice were submitted to a two-bottle choice (TBC) oral midazolam drinking procedure. Animals were exposed to one sucrose bottle and one midazolam (0.008, 0.016 or 0.032 mg/ml) plus sucrose bottle.

Results: Midazolam (1 and 2 mg/kg) induced anxiolytic-like effects, and all doses of midazolam prevented animals from learning to avoid the aversive closed arm during the DA training session. Assessment of midazolam reward *via* the CPP procedure and choice *via* the TBC procedure showed notable variability. A 2-step cluster analysis for the CPP data showed that midazolam data were well-fitted to 2 separate clusters (preference vs. aversion), albeit with the majority of mice showing preference (75%). Correlational and regression analyses showed no relationship between midazolam reward and anxiolytic-like effects (time spent in the open arms in the DA test) or learning/memory. Two-step cluster analysis of the TBC data also demonstrated that, regardless of strain, mice overall fell into two clusters identified as midazolam-preferring or midazolam-avoiding groups. Both midazolam preference and avoidance were concentration-dependent in a subset of mice.

Discussion: Our findings show that midazolam preference is a multifactorial behavior, and is not dependent solely on the emergence of therapeutic (anxiolytic-like) effects, learning impairments, or on genetic factors (inbred vs. outbred animals).

KEYWORDS

benzodiazepine, midazolam, elevated plus maze, self-administration, conditioned place preference, mice

1. Introduction

Benzodiazepines are among the most widely prescribed psychiatric medications, with more than 8% of the adult U.S. population reporting benzodiazepine use (1). This widespread use is partially driven by benzodiazepine prescriptions for one of their many therapeutic uses, predominantly anxiety and sleep disorders. However, benzodiazepine misuse also has increased in recent years, with nearly 20% of individuals who use benzodiazepines reporting misuse in the U.S. (2, 3). This has prompted a growing public health concern, particularly due to increasing rates of benzodiazepine-related overdose deaths and emergency department visits in recent years (4).

Decades of research have helped elucidate the pharmacological mechanisms underlying the behavioral effects of benzodiazepines (5). However, many questions still remain unanswered regarding their abuse-related behavioral effects, particularly due to inconsistencies in the literature. The positive reinforcing effects of benzodiazepines have been shown more consistently in studies using intravenous drug self-administration (5, 6). On the other hand, many pre-clinical self-administration studies using the oral route showed no or low reinforcing effects of benzodiazepines (7–9). Similarly, benzodiazepines have been shown to exert rewarding effects in the conditioned place preference (CPP) model in some studies (10–12), but not others (13, 14). This discrepancy is particularly relevant given that studies show that benzodiazepines can increase (15, 16), decrease (17, 18), or even not alter (19, 20) extracellular dopamine brain levels, depending on the study or protocol.

In addition to these inconsistencies, the relationship between the anxiety-decreasing (anxiolytic) effects and the abuse potential of benzodiazepines remains poorly understood. Anxiety is a risk factor for sedative, hypnotic, or anxiolytic use disorder (21), and studies have shown that greater anxiety sensitivity is associated with increased rates of non-medical benzodiazepine use (22, 23). Epidemiological studies also show that anxiety is associated with higher rates of benzodiazepine misuse and use disorder [for review see (4)]. However, whether experiencing an anxiolytic effect is associated with and/or necessary for the emergence of the positive reinforcing effects of benzodiazepines remains unknown.

The aim of the present study was to investigate the behavioral effects of the benzodiazepine midazolam in male mice, with a focus on its rewarding effects and self-administration. The rewarding effects of midazolam were evaluated using CPP, and were compared with the anxiolytic effects of this drug using an elevated plus maze-discriminative avoidance task (24). Self-administration of midazolam was evaluated using a two-bottle choice (TBC) model. Inbred and outbred mice were used in the TBC model to investigate potential broad genetic determinants of midazolam preference/avoidance.

2. Methods

2.1. Animals

Three-month-old Swiss male mice from the breeding colony of Universidade Estadual de Santa Cruz (UESC) and 3-month-old C57BL/6 male mice obtained from Harlan/Envigo were used. The first set of experiments (Swiss mice; discriminative avoidance task and CPP) was performed at UESC, and animals were group housed (8 per cage) in polypropylene cages (41 × 34 × 16.5 cm). Rodent chow (Nuvilab, Quimtia SA, Colombo, PR, Brazil) and water were available *ad libitum* throughout the experiments. The TBC experiments were performed at UESC (Swiss mice) and at the University of Mississippi Medical Center (UMMC, C57BL/6 mice), and subjects were individually housed in polypropylene caging (30 × 19 × 13 cm) with food available *ad libitum* and fluids restricted to those available within the context of the experiment 24 h per day. All animals were maintained under controlled temperature (22–23°C) and light (12 h light, 12 h dark; lights on at 06h45 at UESC and at 07h00 at UMMC) conditions.

Animals were maintained according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (8th Edition, revised 2011) and in accordance with the Brazilian Law for Procedures for Animal Scientific Use (#11794/2008). The Institutional Animal Care and Use Committees of UESC (protocol #006/2017) and UMMC (protocol #1395) approved the experimental procedures.

2.2. Drugs

For the discriminative avoidance and CPP experiments, midazolam (Roche®) was diluted in sterile saline, and administered intragastrically (gavage) at a volume of 10 ml/kg. For the TBC experiments, midazolam (Roche®) was diluted in a 4% sucrose solution in water to various concentrations (0.008–0.032 mg/mL).

2.3. Elevated plus maze-discriminative avoidance task

The elevated plus maze-discriminative avoidance task model was developed to allow for the investigation of several behaviors and behavioral effects of drugs in the same model. This model allows for the simultaneous investigation of learning/memory, anxiety-like behavior and locomotor activity (24–26). The elevated plus maze-discriminative avoidance task has been validated with the use of several different drug classes, including anxiolytic drugs such as benzodiazepines (24, 27–29) and ethanol (30, 31), stimulants (24, 26, 32–34) and opioids (35).

The behavioral sessions are performed in a modified elevated plus maze, in which the animal explores two adjacent closed arms (28.5 x 7 x 18.5 cm), one of which is aversive (aversive stimuli: 100 watts light and 80 dB noise when the animal enters the arm), and two adjacent open arms (28.5 x 7 cm) lacking the light and noise stimuli. For this experiment, a test session is performed 24 h after a training session. During the 10-min training session, animals are placed individually in the center of the apparatus with free access to the 4 arms. The aversive stimuli are activated each time the animal entered the aversive closed arm, and were interrupted when the animal leaves this compartment. The test session lasted 3 min, during which the animal was again placed in the center of the apparatus. However, during the test session the aversive stimuli were not activated when the animal entered the aversive closed arm,

although the inactive lamp was still present over the closed aversive arm as an environmental cue.

All experimental sessions were filmed for later quantification of the time spent in each of the arms of the device (aversive closed arm, non-aversive closed arm, and open arms), as well as immobility time (indirect measure of sedative-motor effects) using the ANY-maze® software (version 5.1, Stoelting). Immobility was calculated as the total time spent immobile during the training session. Learning and memory were assessed by quantifying the difference in the percentage of time spent in the aversive closed arm compared to the non-aversive closed arm during the training and test sessions, respectively. Anxiolytic-like effects were measured by the percentage of time spent in the open arms of the device during the training session (longer time spent in the open arms = decrease in anxiety-like behavior). Percent time spent in the open arms was calculated according to the equation: time spent in the open arms / total session time * 100. Percent time spent in the closed arms was calculated according to the equation: time spent in the arm of interest / total time spent in the closed arms * 100.

2.4. Conditioned place preference

The CPP apparatus consisted of two conditioning compartments of equal size (40 × 20 × 20 cm): compartment A, with black and white vertical lines on the walls and a black wooden floor, and compartment B, with black and white horizontal lines on the walls and a dark (red) smooth floor. The two main compartments were connected by a central compartment (40 × 10 × 15 cm) that was accessible by sliding doors. Test sessions were filmed, and the time spent in each compartment was measured using the ANY-maze® software (version 5.1, Stoelting). The CPP procedure consisted of the following phases:

Habituation (Day 1): Animals were placed in the center of the apparatus with free access to all compartments for 10 min. No treatments were administered.

Pre-conditioning test (Day 2): Animals were placed in the center of the apparatus with free access to all compartments, and behavior was recorded for 15 min. No treatments were administered.

Conditioning (Days 3–14): An unbiased design was used because animals showed no preference for either of the compartments in the pre-conditioning test. Therefore, animals were randomly assigned to an experimental group and to a "midazolam-paired compartment" in a counterbalanced manner. The conditioning sessions were performed during 12 consecutive days, during which the doors remained closed and animals were confined to one of the conditioning compartments. On odd days, animals received an intragastric administration of midazolam. On even days, animals received an intragastric administration of saline. Ten minutes after midazolam or saline administrations, animals were confined to the assigned drug- or saline-paired compartment for 10 min.

Post-conditioning test (Day 15): Animals were placed in the center of the apparatus with free access to all compartments, and behavior was recorded for 15 min. No treatments were administered.

2.5. Two-bottle choice

Subjects were initially habituated to two 15 ml bottles of water for 3 days, followed by habituation to two 15 ml bottles containing 4% sucrose for another 3 days. Following this initial habituation phase, subjects had 24-h access to two 15 ml drinking bottles in their individual home cages, one containing 4% sucrose, and the other containing 4% sucrose plus midazolam (0.008, 0.016 or 0.032 mg/ml). All subjects were exposed to each concentration of midazolam for 14 days, and bottle sides were switched every 7 days. Consumption from each bottle was measured once every 24 h, at which time all subjects were weighed and bottles were refilled. In order to ensure data were not affected by liquid loss due to bottle leaks, for each cohort (Swiss vs. C57BL/6 cohorts) two bottles were left in an empty cage for 1 week, during which time liquid loss was measured and found to be <0.1 ml/day.

2.6. Experimental design

2.6.1. Experiment 1. Evaluation of the anxiolytic-like, cognitive and rewarding effects of the benzodiazepine midazolam

Forty-eight Swiss male mice were randomly distributed into four groups and submitted to the elevated plus maze-discriminative avoidance task procedure, as described previously. On the training day, animals received an intragastric administration (gavage) of vehicle solution (n = 18) or midazolam at doses of 1 (MDZ 1, n = 12), 2 (MDZ 2, n = 18) or 5 (MDZ 5, n = 12) mg/kg. Ten minutes after administration, animals were placed individually in the center of the apparatus and had free access to all arms of the apparatus for 10 min. Twenty-four hours after the training session, all animals were submitted to a 3-min drug-free test session.

One week after the test day, the animals treated with midazolam during the discriminative avoidance task experiment were submitted to the CPP protocol, as described previously. Animals were maintained in the same midazolam groups, receiving the same dose of midazolam during the discriminative avoidance and the CPP protocols. Animals were submitted to the habituation and pre-conditioning test sessions. During the conditioning phase, animals received intragastric administration (gavage) of midazolam (1, 2 or 5 mg/kg, groups MDZ1, MDZ 2 and MDZ 5, respectively; n = 12 per group) on odd days and were confined to the drug-paired compartment for 10 minutes. On even days, all animals received intragastric administration (gavage) of saline and were confined to the opposite compartment for 10 min.

Twenty-four hours after the last day of the conditioning protocol, the post-conditioning test was performed, and the time spent in each of the main compartments was recorded. Expression of drug-induced CPP or conditioned place aversion was determined using the "score" measure (time spent in the drug-paired compartment minus time spent in the saline-paired compartment). A longer time spent in the compartment associated with the drug compared to the compartment paired with saline (positive score) was considered as indicative of the development of midazolam-induced CPP, while a negative score

indicated midazolam-induced place aversion. A score of 0 indicates no preference.

2.6.2. Experiment 2. Evaluation of midazolam choice behavior

Twenty-nine Swiss (outbred) male mice and 43 C57BL/6 (inbred) male mice were submitted to the habituation and TBC protocols, as previously described. Consumption of sucrose and midazolam plus sucrose solutions were averaged for the last 3 days of self-administration of each midazolam concentration (0.008, 0.016 or 0.032 mg/ml). Preference or aversion for the midazolam bottle over the sucrose only bottle was assessed for each midazolam concentration by calculating the consumption of the midazolam bottle / total consumption of the two bottles * 100.

2.7. Statistical analyses

The behavioral data from each experiment (Discriminative avoidance: % time spent on each arm of the device or immobility time; CPP: score; TBC: % preference) were analyzed using one- or two-way analysis of variance (ANOVA), with or without repeated measures (specific analyses described in the results section for each experiment). For all analyses, Bonferroni *t*-tests were used as the *post-hoc* test. In addition to the dependent measures listed above, three derived scores were calculated, including: (1) CPP score = time spent in the drug-paired compartment–time spent in the non-drug-paired compartment during the post-conditioning test session; (2) Learning score = time spent in the non-aversive closed arm–time spent in the aversive closed arm during the EPM training session; and (3) Memory score = time spent in non-aversive closed arm–time spent in the aversive closed arm during the EPM test session (24 h after training). The behavioral data from each experiment were analyzed using one- or two-way analysis of variance (ANOVA), with or without repeated measures (specific analyses indicated in the results section for each experiment). For all analyses, Bonferroni *t*-tests were used for multiple comparison tests. These analyses, as well as all graphical representations, were performed using the GraphPad Prism software (version 9).

Initial analyses of the data for the CPP studies revealed considerable variance for the CPP score, with distributions of scores predominantly positive (i.e., above zero, or no preference) but with negative scores (i.e., aversion) of relatively high magnitude. We proposed that mice showed either a significant preference or aversion to the midazolam-paired chamber, which represent diametrically opposed predictions for a drug reported consistently to have rewarding effects. Similarly, the TBC studies showed considerable variance for the percentage of preference for the midazolam-containing bottle, leading to a related prediction that mice either preferred or avoided consumption of midazolam. To test these possibilities, we conducted 2-step cluster analysis using CPP score and TBC preference measures. Two-step cluster analysis is a hybrid approach that first calculates a distance measure (centroids) to separate groups, followed by a probabilistic approach to choose an optimal subgroup (36, 37). Distance measures were determined by the log-likelihood criterion and cluster numbers were determined by the Schwarz Bayesian Criterion, with a default of 15 clusters iterations total.

Cohesion and separation of clusters was evaluated using the silhouette coefficient. Internal validity was evaluated further by comparing clusters using unpaired *t*-tests, Fisher's exact tests (categorical data), ANOVA and planned Bonferroni *t*-tests (to test for dose-associated effects), as well as conducting repeated clustering ($n = 15$) with newly randomized order of data for each analysis (cluster results can depend on order of data entered). External validation presented more difficulties, because of the lack of available data and analytic approaches providing construct validity associating either concurrent or mechanistic measures of midazolam reward. This study tested two hypotheses that addressed external validation: Midazolam reward is mediated by (1) reduction of anxiety (anxiolysis) and (2) associative learning and memory processes. To assess these hypotheses, correlation (Pearson r) and regression analysis were performed with CPP Score as a predictor of time in open arm (anxiolysis), learning and memory score. In addition, concepts of preference and aversion in CPP procedures are conceptually related to preference and avoidance in the TBC procedure, although as with our hypotheses, there are no available data to address these comparisons directly. Regardless, a general concordance between CPP and TBC with regards to number of clusters (i.e., preference vs. aversion/avoidance) would provide external validation. Cluster analyses were performed using IBM SPSS Statistics software (version 28). For all analyses, family-wise error rate (alpha) was constrained to $p \leq 0.05$.

3. Results

3.1. Experiment 1. Evaluation of the anxiolytic-like, cognitive and rewarding effects of the benzodiazepine midazolam

3.1.1. Elevated plus maze-discriminative avoidance task

Results from the training session of the discriminative avoidance experiment are illustrated in Figure 1, including analyses of anxiety-like (Figure 1A), sedative-motor (Figure 1B) and learning (Figure 1C) behaviors. One-way ANOVA of the % time spent in the open arms showed a statistically significant difference between groups [$F_{(3, 56)} = 4.792$; $p < 0.01$]. The two lowest doses of midazolam (1 and 2 mg/kg, $p < 0.01$ and $p < 0.05$, respectively, Bonferroni tests) significantly increased the % time spent in the open arms compared to vehicle. One-way ANOVA of immobility time also showed a statistically significant difference between groups [$F_{(3, 44)} = 6.093$; $p < 0.01$], with Bonferroni tests showing a significant increase in immobility time for the two highest doses of midazolam (2 and 5 mg/kg, $p < 0.05$ and $p < 0.001$, respectively) compared to vehicle. A two-way repeated measures ANOVA showed a significant interaction between compartment (aversive closed arm vs. non-aversive closed arm) and treatment (vehicle vs. midazolam) for the % time spent in the closed arms [$F_{(3, 55)} = 12.52$; $p < 0.0001$]. Bonferroni *post-hoc* analyses showed that only the vehicle group spent a significantly greater % time in the non-aversive closed arm compared to the aversive closed arm (*p*

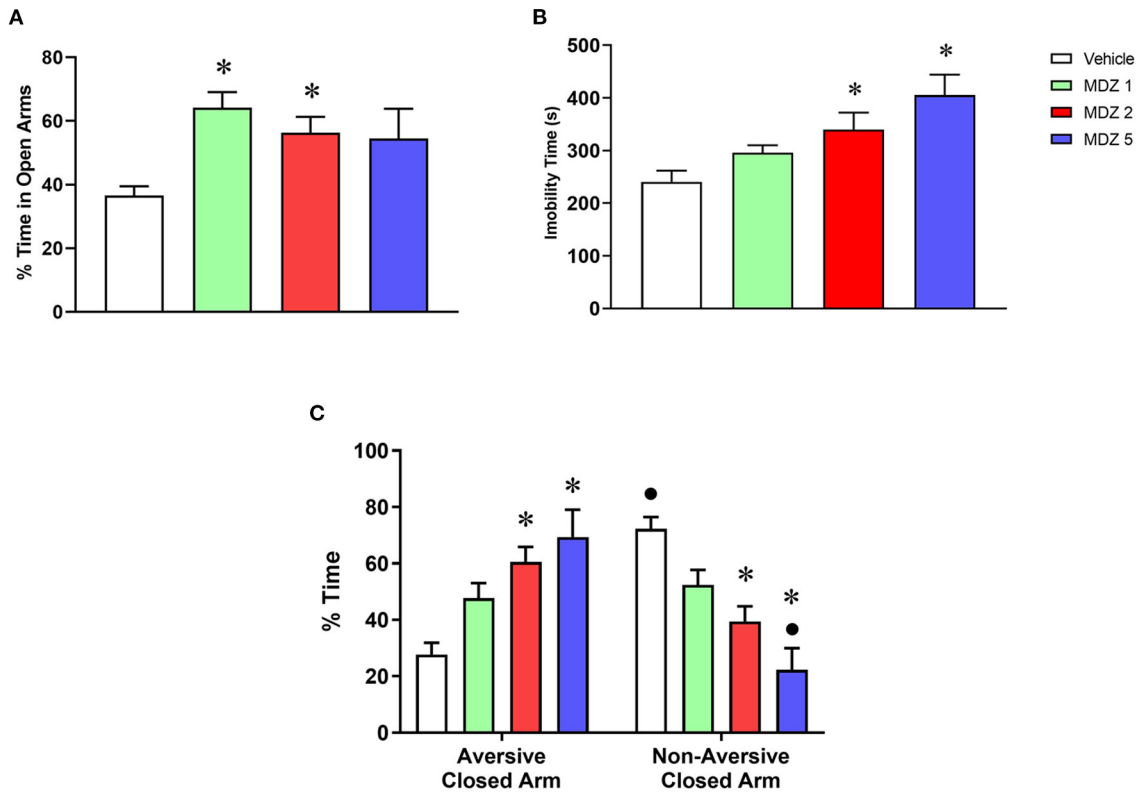

FIGURE 1
Results from the training session of the elevated plus maze-discriminative avoidance task following i.p. administration of vehicle ($n = 18$) or midazolam at the doses of 1 (MDZ 1, $n = 12$), 2 (MDZ 2, $n = 18$) or 5 (MDZ 5, $n = 12$) mg/kg. **(A)** Time spent in the open arms (anxiety-like behavior); **(B)** immobility time (sedative-motor effects); **(C)** time spent in the aversive vs. non-aversive closed arms (learning). Data are shown as mean ± SEM. *$p < 0.05$ compared to Vehicle within the same parameter; •$p < 0.05$ compared to time spent in the aversive closed arm within the same group.

< 0.001), demonstrating that animals learned to avoid the aversive closed arm. No significant differences were observed in the % time spent in the closed arms for the groups treated with the lowest doses of midazolam (1 and 2 mg/kg), and animals treated with the highest dose of midazolam (5 mg/kg) spent a significantly lower % time in the non-aversive closed arm compared to the aversive closed arm ($p < 0.01$), suggesting that all doses of midazolam impaired learning. In agreement, animals treated with 2 and 5 mg/kg midazolam were also significantly different from the vehicle group for both % time spent in the closed aversive arm ($p < 0.001$ and $p < 0.0001$, respectively) and % time spent in the non-aversive closed arm ($p < 0.001$ and $p < 0.0001$, respectively).

Results from the memory measures during the test session of the discriminative avoidance experiment are illustrated in Figure 2. For the analysis of the % time spent in the closed arms, two-way repeated measures ANOVA showed a significant interaction between compartment (aversive closed arm vs. non-aversive closed arm) and treatment (vehicle vs. midazolam) [$F_{(3, 55)} = 3.968$; $p < 0.05$]. Only the vehicle group spent a significantly greater % time in the non-aversive closed arm compared to the aversive closed arm ($p < 0.01$), indicating that animals learned the association between aversive and non-aversive arms during the training session. No significant differences were observed between the % time spent in the aversive vs. non-aversive closed arms for midazolam-treated animals. Animals treated with midazolam spent a significantly higher % time in the aversive closed arm ($p < 0.05$ for 2 mg/kg) and a significantly lower % time in the non-aversive closed arm ($p < 0.05$ for 2 and 5 mg/kg) compared to the vehicle group.

3.1.2. Conditioned place preference

CPP results, measured as the difference in time spent in the midazolam- and saline-paired side (CPP score), are shown as a function of pre-conditioning and post-conditioning in Figure 3. During pre-conditioning, CPP scores for individual mice tended to aggregate near zero, with variability contributed by 2–4 mice at each dose condition. However, a more distributed set of CPP scores at each dose was observed in the post-conditioning tests. Two-way repeated measures ANOVA showed no significant differences of dose or conditioning phase [e.g., dose x conditioning phase interaction: $F_{(2, 33)} = 0.593$, $p = 0.558$].

Because the CPP score represents a dichotomous variable, with positive numbers indicating preference and negative numbers indicating aversion, we explored the possibility of mice falling into distinct categories. Two-step clustering analysis was conducted separately for each dose of midazolam (1.0, 2.0, 5.0 mg/kg). Schwarz Bayesian Criterion (BIC) reached acceptable clustering with two centroids for all three doses. Figure 4 shows the results of this cluster analysis, with cluster 1 = aversion, i.e., negative numbers, and cluster 2 = preference, i.e., positive numbers. With some

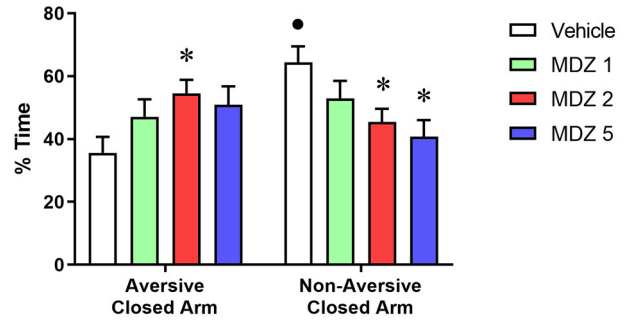

FIGURE 2
Results from the test session of the elevated plus maze-discriminative avoidance task 24-h after the training session, during which animals received i.p. administration of vehicle ($n = 18$) or midazolam at the doses of 1 (MDZ 1, $n = 12$), 2 (MDZ 2, $n = 18$) or 5 (MDZ 5, $n = 12$) mg/kg. No drugs were administered before the test session. Time spent in the aversive vs. non-aversive closed arms (memory retrieval). Data are shown as mean±SEM. *$p < 0.05$ compared to Vehicle within the same parameter; •$p < 0.05$ compared to time spent in the aversive closed arm within the same group.

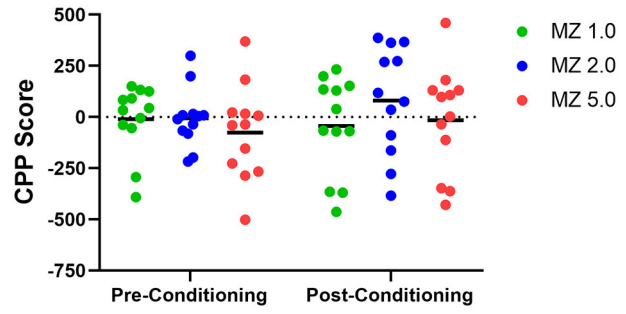

FIGURE 3
Conditioned place preference (CPP) and place aversion (CPA) in male Swiss mice ($n = 12$ per group) following midazolam doses (1.0, 2.0, and 5.0 mg/kg, i.g.). Data are CPP scores (time spent in drug-paired compartment−time spent in non-drug-paired compartment) after pre-exposure to the chambers (Pre-Conditioning) and after pairings with midazolam (Post-Conditioning). Individual points represent individual subjects, and horizontal bars represent mean values.

exceptions at 1.0 and 5.0 mg/kg, all CPP scores fell above or below zero, depending on the cluster. Based on the analysis, 66.7–75% of mice were grouped into the preference category (Figure 4, top panels), with frequency distributions showing the majority of subjects with CPP scores above zero and low degrees of overlap (Figure 4, middle panels). Silhouette analysis of cluster cohesion and separation showed index scores of 0.79 to 0.82, indicating "good" cluster quality (Figure 4, bottom panels). Repeated analyses with randomized data sets did not alter results. Importantly, we performed the same 2-step cluster analysis for the pre-conditioning data, based on the assumption that no clustering would be possible prior to any drug conditioning. Single clusters were obtained for the pre-conditioning phase for 2.0 and 5.0 mg/kg groups, and while two clusters were obtained for the 1.0 mg/kg group, the iterative process identified two outliers (CPP scores of −293 and −392) and resulted in a silhouette score categorized as "poor." Interestingly, the two mice with the outlier scores remained at negative numbers for the post-conditioning test, indicating that they fell into the "aversion" cluster. Because these two subjects did not change to the "preference" cluster (which would result in a substantial change in CPP scores) and this pattern was not evident at the other two doses, we did not exclude the mice from any of the analyses.

We performed additional internal validation tests that also provided information on dose-dependency (Figure 5). For these analyses, the clusters were analyzed with separate repeated measures ANOVAs. For cluster 1 (aversion; Figure 5 top panel), the ANOVA demonstrated no effects of dose [dose main effect: $F_{(2, 7)} = 1.469$, $p = 0.293$; dose x conditioning phase interaction: $F_{(2, 7)} = 0.677$, $p = 0.539$] but a significant main effect of conditioning phase [$F_{(1, 7)} = 9.296$, $p = 0.019$], indicating that a significant aversion occurred, irrespective of dose. To test specifically for dose-related effects, Bonferroni t-tests were conducted within the doses pre- and post-conditioning; and no significant differences were evident (p's > 0.05).

For cluster 2 (preference, Figure 5 bottom panel), the repeated measures ANOVA showed no significant effects related to dose [dose main effect: $F_{(2, 23)} = 2.118$, $p = 0.143$; dose x conditioning phase interaction: $F_{(2, 23)} = 0.672$, $p = 0.520$] but a significant main effect of conditioning phase [$F_{(1, 22)} = 7.866$, $p = 0.010$]. Planned Bonferroni t-tests showed that an increase in time spent in the drug-paired side occurred for the 2.0 and 5.0 mg/kg doses of midazolam (adjusted $p = 0.031$ and 0.023, respectively), indicating significant dose-dependent CPP with midazolam for cluster 2.

Based on the cluster analysis, the majority of mice showed preference for the midazolam-paired compartment; an effect off-set by mice showing aversion. It was notable that there was variance in the pre-conditioning phase, with one group even displaying 2 clusters, raising the likelihood that the mice demonstrated preference or aversion in the absence of drug conditioning. To evaluate the nature of change in CPP score from pre- to post-conditioning, we first coded mice with three numbers according to the following categories: −1.0, mice showing positive scores in pre-conditioning and negative in post-conditioning; 0, mice that stayed either positive or negative in pre-conditioning and post-conditioning; +1.0, mice showing negative scores in pre-conditioning and positive scores in post-conditioning. A frequency histogram was plotted (Figure 6), showing that for each dose, the majority of mice did not change from pre- to post-conditioning, i.e., if they showed a negative pre-conditioning score, they showed a negative post-conditioning score. The next highest frequency was the mice showing a change from negative to positive CPP scores after conditioning, with a smaller number of mice (25% for all three doses) demonstrating a shift from positive to negative CPP scores.

3.1.3. Comparison of parameters from conditioned place preference and elevated plus maze-discriminated avoidance tasks

In order to obtain information regarding potential behavioral mechanisms underlying midazolam-induced conditioned place preference, we conducted additional analyses to determine the extent to which CPP score in mice showing place preference could

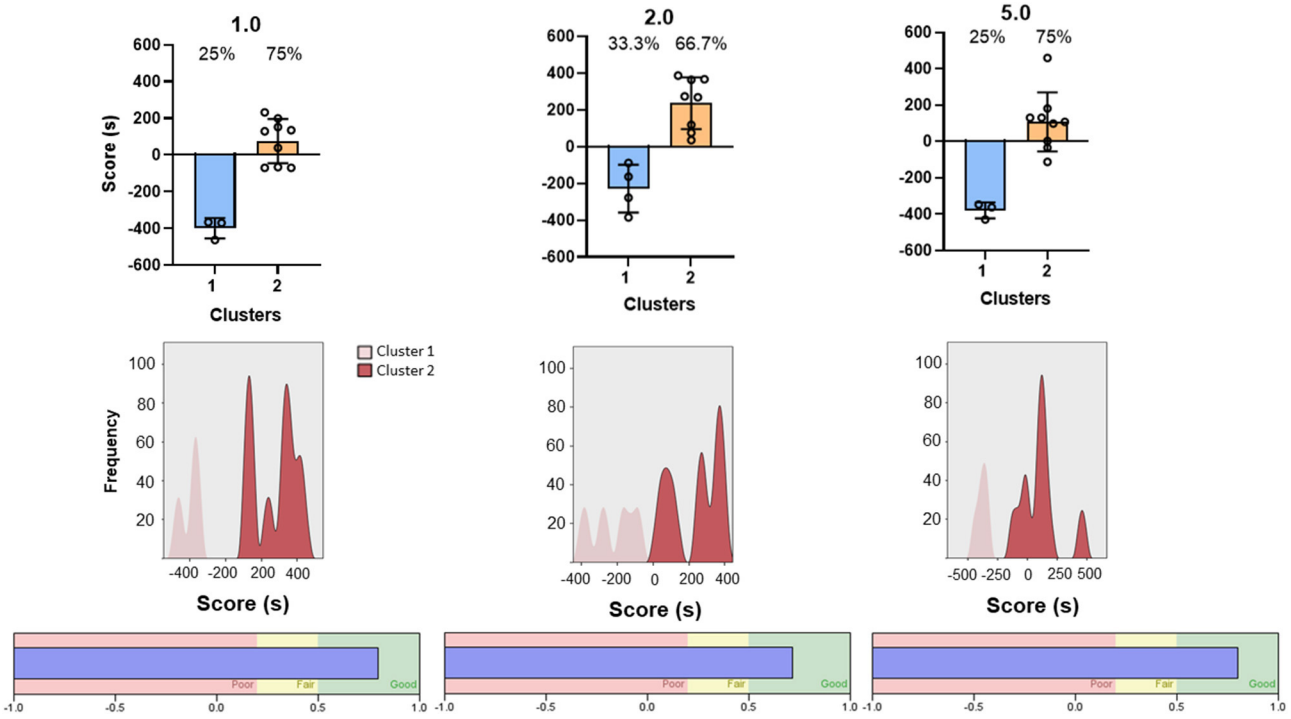

FIGURE 4
Two-step cluster analysis of midazolam-induced conditioned place preference in Swiss mice ($n = 12$ per dose). Each column represents analysis performed on each of three doses of midazolam, indicated at the top (1.0, 2.0, and 5.0 mg/kg, p.o.). **(Top)** panels are CPP scores (time spent in drug-paired compartment−time spent in non-drug-paired compartment) as a function of the 2 identified clusters (cluster 1−aversion, cluster 2−preference). Data are individual subjects, with bars representing mean values and error bars showing SEM values. Numbers in the panels represent percentages of mice in each cluster. **(Middle)** panels are frequency distributions (relative percentage) for each dose. **(Bottom)** panels show corresponding silhouette analysis for cohesion and separation of the clusters.

predict effects in the EPM (time spent in the open arm, i.e., anxiolytic-like effects; learning and memory scores). Correlation matrices (Pearson r) are shown in Table 1 for the 4 measures, conducted within each dose of midazolam. As evident from the table, no correlations were significant with CPP score, although significant positive correlations were obtained for time in open arms for memory score at 1.0 mg/kg midazolam and learning score at 5.0 mg/kg of midazolam.

To test specifically if CPP score was a reliable predictor of EPM-DA parameters, individual linear regression analyses were conducted for the cluster 2 (preference) mice (Table 2). In every case, the regression parameter values were not significantly different from zero, with relatively low goodness-of-fit values (R2). Therefore, no evidence to support the hypotheses that CPP reflects anxiolytic or learning and memory associated processes were obtained for this data set.

3.2. Experiment 2. Evaluation of midazolam choice behavior

Results from TBC tests with both Swiss and C57BL/6 mice cohorts are shown in Figure 7. For Swiss mice (Figure 7, left panel), ANOVA revealed a significant effect of concentration [$F_{(2, 56)} = 4.383$, $p = 0.030$]; however, no multiple comparisons were significant (p's > 0.05, Bonferroni t-tests). For C57BL/6 mice, the overall ANOVA was not significant [$F_{(2, 84)} = 0.099$, $p = 0.899$]. However, as with CPP in Swiss mice, midazolam preference was highly variable, with mice showing both preferences (above 50%) and avoidance (below 50%). We used 2-step cluster analyses to evaluate the extent to which mice in these studies could be parsed into those that preferred midazolam above 50% levels and those that avoided consuming the drug. For Swiss mice, all three concentrations resulted in 2 clusters (Figure 8). In general, the distribution of mice into the two clusters was approximately equal, with very little overlap among clusters (Figure 8, top and middle panels). The differences in percent midazolam preference between clusters was confirmed by unpaired t-tests [0.008 mg/ml: $t_{(27)} = 14.01$, $p < 0.0001$; 0.016 mg/ml: $t_{(27)} = 8.596$, $p < 0.0001$; $t_{(27)} = 7.556$, $p < 0.0001$]. For all three doses, the silhouette scores were in the "good" range (i.e., >0.5; Figure 8, bottom panels).

For C57BL/6 mice, two of the concentrations resulted in similar clustering to the Swiss mice (0.016 and 0.032 mg/ml; Figure 9, top panels). Two-step cluster analysis of the lowest concentration, however, resulted in 3 clusters, with a cluster predominantly above equal preference (cluster 1, mean = 71.9 %, "preference"), a cluster near equal preference (cluster 2, mean = 44.7%, "indifference"), and a cluster well below equal preference (cluster 3, mean = 17.7%, "avoidance"). ANOVA performed on these data was significant [$F_{(2, 40)} = 113.4$, $p < 0.0001$] and multiple comparisons confirmed that all clusters were significantly different from one another

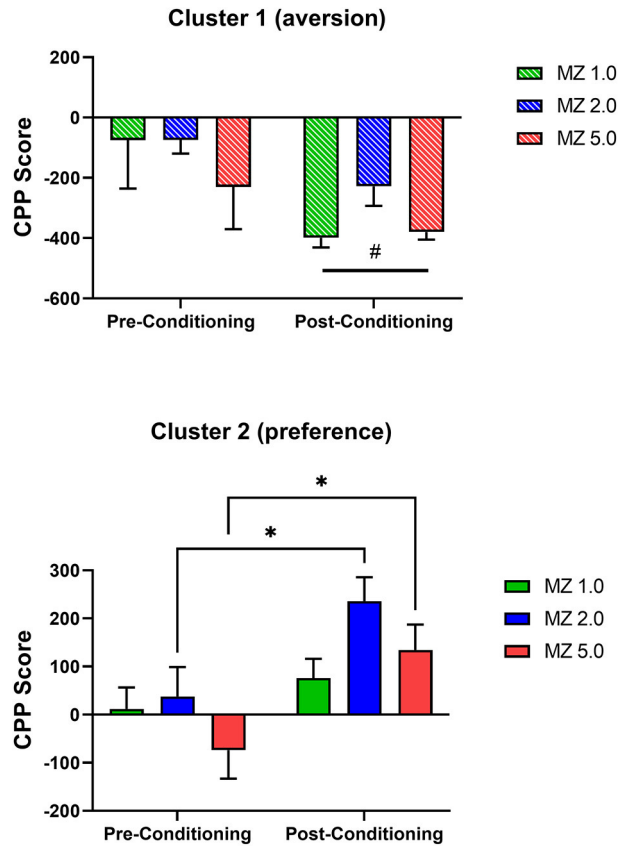

FIGURE 5
Conditioned place preference (CPP) results with midazolam (MZ) per clusters, determined by 2-step cluster analysis, in male Swiss mice. Data are expressed as mean +/- SEM CPP Score (time spent in drug-paired compartment−time spent in non-drug-paired compartment) for $N = 36$ mice total. Results are from pre-conditioning tests performed prior to midazolam−saline pairings and post-conditioning tests conducted after training sessions. The **(Top)** panel shows results from cluster 1 (aversion); note that $\#p < 0.05$, main effect of conditioning phase, ANOVA. The **(Bottom)** panel shows results from cluster 2 (preference); note that $*p < 0.05$, Bonferroni *t*-tests.

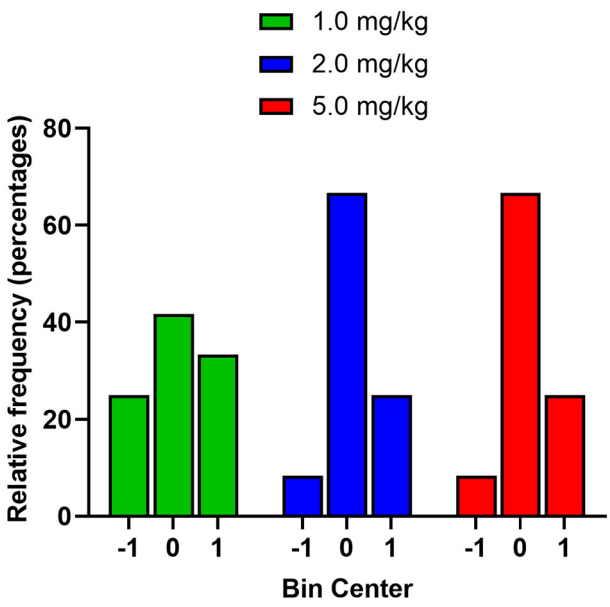

FIGURE 6
Frequency distribution of male Swiss mice based on change from pre- to post-conditioning in the CPP tests ($n = 12$ per dose). Mice were coded with three numbers according to the following categories: −1.0, mice showing positive scores in pre-conditioning and negative in post-conditioning; 0, mice that stayed either positive or negative in pre-conditioning and post-conditioning; +1.0, mice showing negative scores in pre-conditioning and positive scores in post-conditioning.

(Bonferroni tests, adjusted p's< 0.0001). As with the Swiss mice, the two higher concentrations resulted in clustering into two groups that were nearly evenly distributed: 0.016 mg/ml, cluster 1 (preference) = 41.9%, cluster 2 (avoidance) = 58.1%; 0.032 mg/ml, cluster 1 (preference) = 58.1%, cluster 2 (avoidance) = 41.9%. Statistical comparisons verified the differences: 0.016 mg/ml, $t_{(41)} = 10.04$, $p < 0.0001$; 0.032 mg/ml, $t_{(41)} = 10.98$, $p < 0.0001$. In addition, silhouette scores for all three analyses fell between 0.5 and 1.0, indicating good cohesion and separation of clusters (Figure 9, bottom panels).

A noteworthy characteristic of the TBC data is the observation that different mice were in different clusters across the concentrations. This is expected, because many mice showed mixtures of preference and avoidance depending on the concentration, as is a fundamental characteristic of self-administration data over all drug classes and most procedures. To quantify this phenomenon, we coded each mouse as "same" of "mixed" effects. "Same" indicated a mouse for which all three concentrations were either above 50% preference or ≤50% preference. "Mixed" indicated a mouse for which at least one concentration differed from the other concentrations. For example, a mouse with preference above 50% preference for 0.016 mg/ml but below 50% preference for the other two concentrations was coded as "mixed." For the two strains, we compared the two clusters by conducted Fisher's exact tests. As shown in Figure 10, for Swiss mice, both same and mixed categories were observed about equally and did not differ between the clusters (Fisher's exact test, $p > 0.05$). Interestingly, for C57BL/6 mice, cluster 1 (preference) mice were predominantly in the mixed category, whereas cluster 2 (avoidance) mice were infrequently coded as mixed (Fisher's exact test, $p = 0.0002$). This analysis indicates that for Swiss TBC results, effects dependent on dose accounted for half of the subjects in both clusters, whereas with C57BL/6 mice, effects were dependent on dose predominantly for the mice showing preference for the midazolam solutions.

4. Discussion

Despite the clear and growing concern over benzodiazepine misuse, investigating the abuse-related effects of these drugs has not been as straightforward as researchers might expect. The pre-clinical literature on the effects of benzodiazepines in animal models has been filled with contradictory findings, and establishing models to investigate benzodiazepine

TABLE 1 Correlation matrices (Pearson's r values) for Swiss mice in the conditioned place preference and elevated plus maze-discriminative avoidance tasks (mice from cluster 2 only).

1.0 mg/kg Midazolam	CPP score	Time in open arms	Learning score	Memory score
CPP score	1.000	0.392	0.303	0.030
Time in open arms	0.392	1.000	0.060	*0.745**
Learning score	0.303	0.060	1.000	0.083
Memory score	0.030	*0.745**	0.083	1.000
2.0 mg/kg Midazolam	**CPP score**	**Time in open arms**	**Learning score**	**Memory score**
CPP score	1.000	0.105	−0.211	0.456
Time in open arms	0.105	1.000	0.529	0.383
Learning score	−0.211	0.529	1.000	0.150
Memory score	0.456	0.383	0.150	1.000
5.0 mg/kg Midazolam	**CPP score**	**Time in open arms**	**Learning score**	**Memory score**
CPP score	1.000	0.451	0.287	−0.550
Time in open arms	0.451	1.000	*0.789**	−0.228
Learning score	0.287	*0.789**	1.000	−0.157
Memory score	−0.550	−0.228	−0.157	1.000

*$p < 0.05$, Pearson's r correlation.
CPP score (CPP test) = time spent in drug-paired compartment–time spent in non-drug-paired compartment.
Time Open Arms (EPM) = total time spent in the open arms during training session.
Learning score (EPM training session) = time spent in non-aversive closed arm–time spent in the aversive closed arm.
Memory score (EPM test session) = time spent in non-aversive closed arm–time spent in the aversive closed arm.

TABLE 2 Linear regression analysis for Conditioned Place Preference as a predictor of Elevated Plus Maze-Discriminative Avoidance task performance (*n* = 12 Swiss mice per dose, from cluster 2 only).

	Regression parameter value (SEM)		
1.0 mg/kg Midazolam	**Time in open arms**	**Learning Score**	**Memory score**
Y-intercept	279.93 (31.60)	−17.34 (37.31)	−3.06 (12.78)
Slope	0.26 (0.23)	0.23 (0.27)	0.007 (0.094)
R^2	0.15	0.09	0.01
2.0 mg/kg Midazolam			
Y-intercept	242.30 (72.49)	−38.92 (72.27)	−64.84 (40.09)
Slope	0.07 (0.27)	−0.14 (0.27)	0.19 (0.15)
R^2	0.01	0.21	0.04
5.0 mg/kg Midazolam			
Y-intercept	194.19 (66.85)	−215.41 (101.63)	−3.75 (11.95)
Slope	0.48 (0.36)	0.43 (0.55)	−0.11 (0.06)
R^2	0.20	0.08	0.30

Dependent variable was CPP score (CPP test) = time spent in drug-paired compartment–time spent in non-drug-paired compartment.
Time Open Arms (EPM) = total time spent in the open arms during training session.
Learning score (EPM training session) = time spent in non-aversive closed arm–time spent in the aversive closed arm.
Memory score (EPM test session) = time spent in non-aversive closed arm–time spent in the aversive closed arm.

reward and reinforcement has been a challenge. Specifically, studies have shown opposite effects for benzodiazepine self-administration (5–9), benzodiazepine-induced CPP (10–14) and changes in brain dopamine levels induced by benzodiazepines (15–20).

In the present study, assessment of midazolam reward *via* the CPP procedure and choice *via* the TBC procedure showed notable variability, with evidence that mice developed CPPs or conditioned place aversions (CPAs) with midazolam exposure and, similarly, preferred or avoided midazolam in the TBC model. We evaluated the extent to which mice could be divided into broadly different categories, i.e., midazolam-preferring vs. midazolam non-preferring, using 2-step cluster analysis. This approach was used because it does not require a priori choice of number of

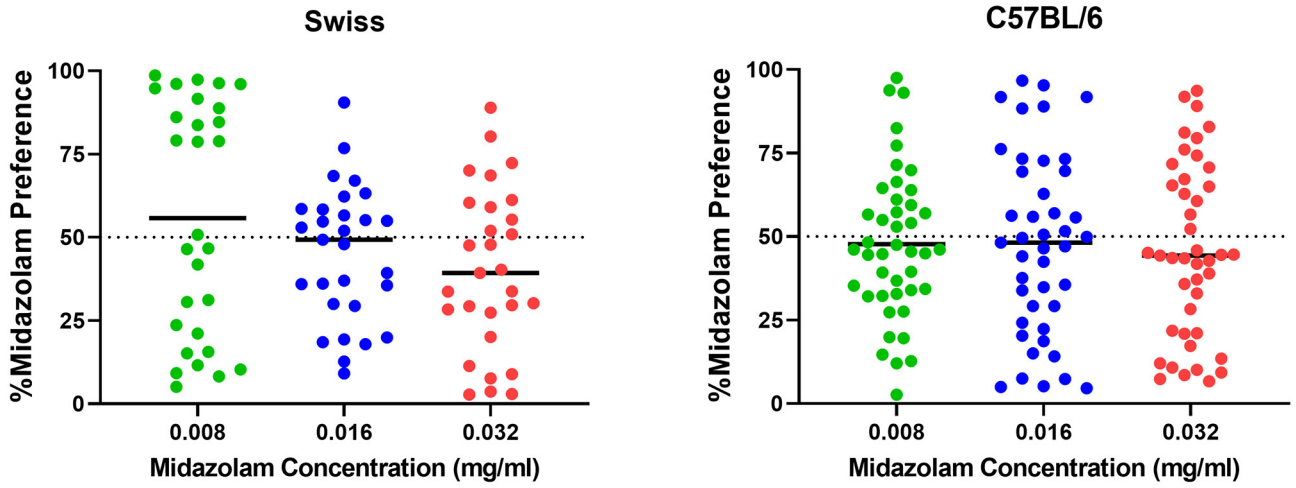

FIGURE 7
Two-bottle choice results following midazolam concentrations (0.008, 0.016, 0.032 mg/ml) in male Swiss mice ($N = 36$) and male C57BL/6 mice ($N = 43$). Data are percentage of midazolam preference, calculated as the volume consumed from the midazolam bottle/total volume consumed, multiplied by 100. Note that values above 50% indicate preference for midazolam, whereas values below 50% indicate avoidance of the midazolam bottle. Individual points represent individual subjects, and horizontal bars represent mean values.

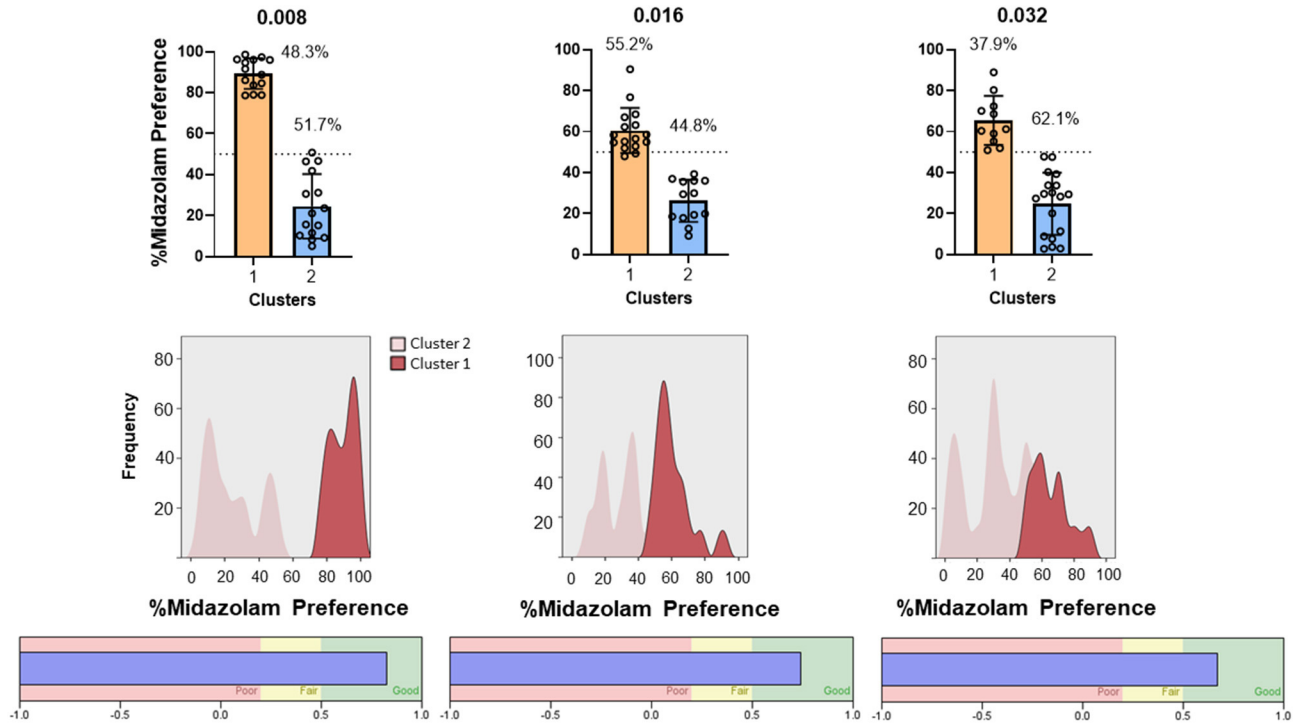

FIGURE 8
Two-step cluster analysis of midazolam preference in the two-bottle choice procedure in male Swiss mice ($N = 36$ mice total). Each column represents analysis performed on each of three concentrations of midazolam, indicated at the top (0.008, 0.016, 0.032 mg/ml). **(Top)** panels are percent midazolam preference (the volume consumed from the midazolam bottle/total volume consumed, multiplied by 100) as a function of the 2 identified clusters (cluster 1–aversion, cluster 2–preference). Data are individual subjects, with bars representing mean values and error bars showing SEM values. Numbers in the panels represent percentages of mice in each cluster. **(Middle)** panels are frequency distributions (relative percentage) for each dose. **(Bottom)** panels show corresponding silhouette analysis for cohesion and separation of the clusters.

possible clusters, opening up the possibility for identification of other sub-groups (e.g., mice demonstrating indifference to the midazolam solutions). Regarding the CPP data, midazolam data were well-fitted to 2 separate clusters, albeit with the majority of mice showing preference (75%). When analyzed separately, the 2.0 and 5.0 mg/kg doses engendered significant preference

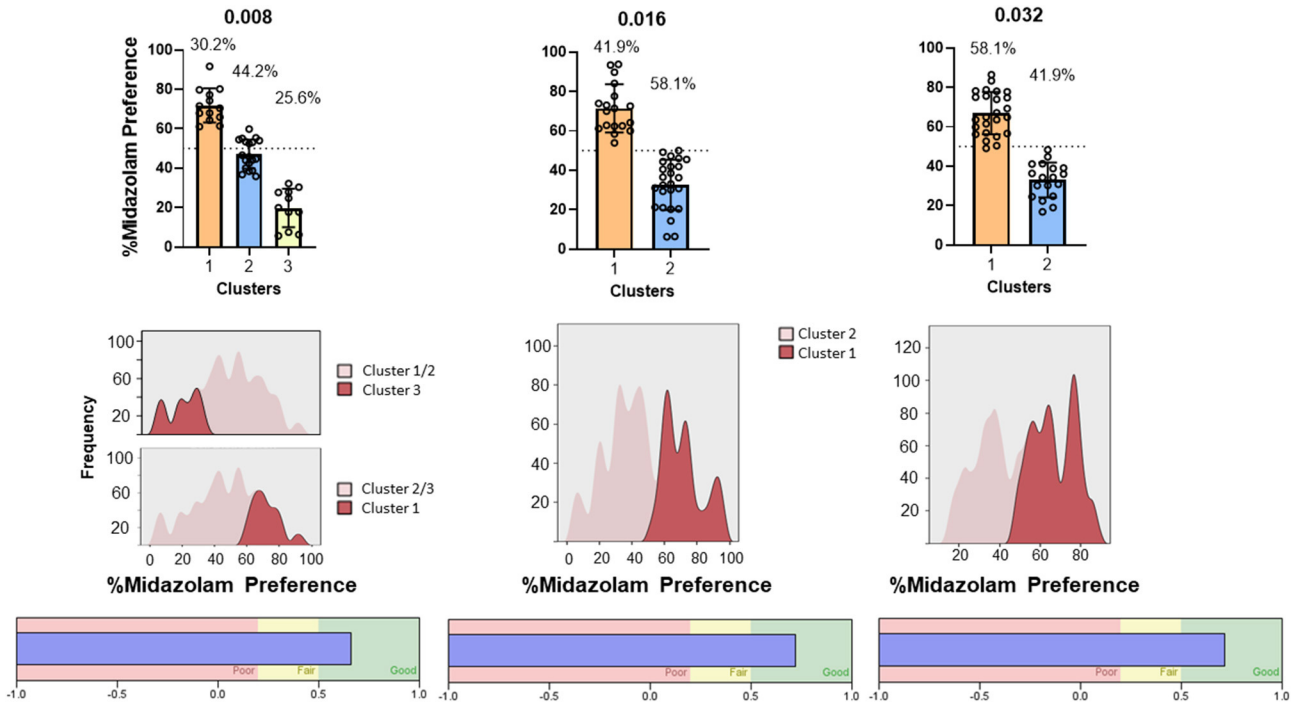

FIGURE 9
Two-step cluster analysis of midazolam preference in the two-bottle choice procedure in male C57BL/6 mice ($N = 43$ mice total). Each column represents analysis performed on each of three concentrations of midazolam, indicated at the top (0.008, 0.016, 0.032 mg/ml). Other details as in Figure 8.

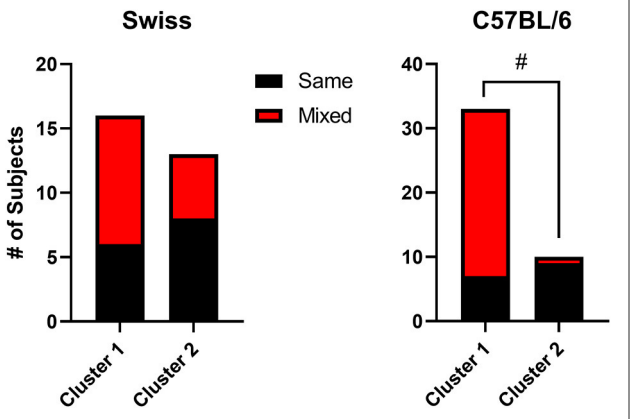

FIGURE 10
Stacked bar charts showing the number of mice coded as "same" (indicating a mouse for which all three concentrations were either above 50% preference or ≤50% preference) or "mixed" (indicating a mouse for which at least one concentration differed from the other concentrations). Data are separated by cluster 1 (preference) and cluster 2 (avoidance) for the two strains (Swiss and C57BL/6 male mice). Note that #$p < 0.05$, Fisher's exact test.

in the preference cluster, whereas the aversion cluster generally showed robust aversions with no dose-related effects. The majority of mice demonstrated CPP by an increase in time spent in the midazolam-paired compartment, mostly by increasing time spent in the particular chamber vs. shifting preference from one chamber to another. This latter observation suggests that mice already showing an aversion to a drug-paired chamber may not be likely to change to a preference, however, the mice in the aversion cluster mostly showed increased time in the non-drug-paired side instead of no change from pre-conditioning tests.

The distinct clusters observed for the CPP experiment allowed us to assess the relationship between reward and other characteristic effects of benzodiazepines. In this regard, midazolam had anxiolytic-like effects in mice, increasing the time spent in the open arms of the modified EPM apparatus, consistent with previous studies (38, 39). To investigate the relationship between the anxiolytic-like and rewarding effects of midazolam, we conducted correlational analysis as well as regressed CPP scores vs. time spent in the open arms of the EPM. These analyses showed no relationship between midazolam reward and this measure of anxiolytic-like effects, suggesting that the emergence of anxiolytic-like effects is not sufficient to guarantee the expression of rewarding effects. Interestingly, strong positive correlations were shown for learning and memory scores vs. time in the open arms, suggesting that a stronger anxiolytic-like effect was associated with a higher degree of learning and memory impairment. In fact, midazolam reducing the aversiveness of the open arm may play a key role in any learning/memory impairment associated with this particular task.

The finding that midazolam impaired learning and memory of a discriminative avoidance task is consistent with previous pre-clinical studies with midazolam (28) and other benzodiazepine-type drugs (24, 29, 40). Because the CPP model relies on associative learning, we also investigated

a potential correlation between the rewarding (CPP) and cognitive-impairing (discriminative avoidance task) effects of midazolam. As with anxiolytic-like measures, we found no significant relationships between these two measures, suggesting that the rewarding and aversive effects of midazolam emerged despite significant learning deficits induced by this drug.

In addition to testing the hypotheses that midazolam reward is associated with its anxiolytic and cognitive-impairing effects, these comparisons potentially provided tests of external validity for the 2-step cluster approach. Clearly these findings did not provide external support for the clustering, with lack of a relationship between CPP and cognitive effects perhaps the most perplexing. However, it is critical to note that the effects of midazolam in the learning and memory components of the discriminative avoidance task were to impair these processes, whereas CPP and CPA involve forming associative pairings. Moreover, learning to avoid an open arm may represent a form of fear conditioning, as opposed to reward learning represented by CPP, which was the result of 75% of the mice, and while neural circuits mediating aversive and reward learning may overlap, there likely are distinct functional differences [e.g., (41)]. Regarding anxiolysis, the hypothesis that the expression of reward may reflect reductions in anxiety is based primary on self-report data from human subjects identifying motives for taking benzodiazepines [e.g., (4)], rather than data from laboratory animal studies. Collectively, these observations do not provide external validity for the clustering but also are insufficient to discount the clustering approach, given that anxiolysis and learning/memory were components of hypothesis testing and not empirical conclusions *per se*.

External validation of mice being categorized as midazolam-preferring vs. midazolam-averse comes primarily from the TBC experiments. Two-step cluster analysis demonstrated that two different strains of mice overall fell into two clusters identified as midazolam-preferring or midazolam-avoiding groups, with only one exception being the lowest concentration of midazolam tested in C57BL/6 mice, which resulted in an additional (third) cluster characterized as indifference (i.e., equal distribution of drinking from midazolam + sucrose and sucrose alone bottles). Both midazolam preference and avoidance were concentration-dependent in a subset of mice, with some showing preference at some concentrations but avoidance at others. However, there was a trend for this pattern to occur more frequently in the midazolam-preferring Swiss mice and a statistically significant difference between midazolam-preferring and midazolam-avoiding C57BL/6 mice, suggesting that mice in the midazolam-avoiding groups tended to only show avoidance regardless of the concentration of drug.

Wild type laboratory mice can be divided into two main genetic categories: inbred and outbred (42). Inbred mice, such as the C57BL/6 mouse strain, are genetically homogeneous, and there is little genetic variation within this strain, which can reduce experimental variability and allow for the evaluation of genetic influences on specific behavioral phenomena. Outbred mice, such as the Swiss mouse strain, are bred specifically to maximize genetic diversity and heterozygosity within a population and, in theory, there are no two genetically identical outbred subjects. Therefore, the use of genetically heterogeneous and homogeneous strains allowed us to assess whether genetic factors could influence the expression of midazolam preference vs. avoidance. Our findings showed that both inbred and outbred mice demonstrated a strikingly similar pattern of preference and avoidance in the TBC experiments, even with the two TBC studies conducted at separate facilities. These studies ruled out a potential influence of genetic factors in our findings, raising the possibility that midazolam preference vs. avoidance groupings may develop in mice due to epigenetic factors.

The present findings corroborate a recent study in non-human primates showing that only half of the subjects self-administered the benzodiazepine alprazolam intravenously, although that study was conducted in rhesus monkeys with a history of opioid self-administration (43). These findings are also in agreement with a choice study in humans showing that, while diazepam was always preferred over placebo, placebo was preferred over oxazepam in nearly 22% of choice tests by recreational benzodiazepine users (44). The mechanisms underlying these contrasting findings within a study remain unknown. However, the unique pharmacokinetic properties of midazolam and other benzodiazepines may have contributed to these results. Due to its pharmacokinetic and pharmacodynamic properties, midazolam induces hysteresis, which results in a delay between the peak drug serum concentrations and the peak drug behavioral effects (45). Hysteresis indicates that the relationship between drug concentration vs. drug effects is not a straightforward, direct relationship, but may have an inherent delay and imbalance, which may be a result of active metabolites, or a consequence of changes in pharmacodynamic properties (45). Importantly, studies have shown that hysteresis influences benzodiazepine self-administration in rats (46). Of note, hysteresis also has been reported for both alprazolam (47) and oxazepam (48). Although further studies are needed to understand how this specific effect could affect some animals but not others, these pharmacokinetic and pharmacodynamic mechanisms may have influenced our findings.

Overall, our findings show that midazolam preference is a multifactorial behavior, and is not dependent solely on the emergence of anxiolytic-like effects, or on genetic factors (inbred vs. outbred animals). Also, the rewarding effects of midazolam in the CPP model emerged even at doses that induced significant learning deficits in mice. The protocols established in the present study can be used in future research to evaluate the neuropharmacological mechanisms involved in the different behavioral effects of benzodiazepine drugs within the same group of animals. Of note, important limitations of our study include the lack of sex differences investigation, with the possibility that different results would have been obtained for female mice. Also, the sample size in our CPP studies limited some of our analyses, and future studies should consider including multiple cohorts of animals to increase sample size in order to better capture benzodiazepine-induced CPP vs. CPA in mice. Regardless, our data emphasize the importance of considering interindividual variability within a sample, and suggest that variability may be an inherent phenomenon to the study of the abuse-related behavioral effects of benzodiazepines. Embracing variability may provide new avenues of study and a better understanding on how and why benzodiazepine drugs are abused.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This animal study was reviewed and approved by the Institutional Animal Care and Use Committees of UESC (protocol #006/2017) and UMMC (protocol #1395).

Author contributions

AO-L, EM, JR, and LB were responsible for the study concept and design. CJ-F, MF, BD, YS, NK, TB-S, NJ, IR, and LM contributed to data acquisition. CJ-F, EM-M, AO-L, EM, JR, and LB assisted with data analysis and interpretation of findings. LB and JR drafted the manuscript. All authors agree to be accountable for all aspects of the work, provided critical revision of the manuscript for important intellectual content, and approved the final version for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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EDITED BY
Thiago Marques Fidalgo,
Federal University of São Paulo, Brazil

REVIEWED BY
Kevin Xu,
Washington University in St. Louis, United States
Perry Menzies,
St. Michael's Hospital, Canada

*CORRESPONDENCE
R. Kathryn McHugh
✉ kmchugh@mclean.harvard.edu

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Prevalence and correlates of the misuse of z-drugs and benzodiazepines in the National Survey on Drug Use and Health

R. Kathryn McHugh[1,2]*, Victoria R. Votaw[3,4], Emma W. Trapani[1] and Megan D. McCarthy[1]

[1]Division of Alcohol, Drugs, and Addiction, McLean Hospital, Belmont, MA, United States, [2]Department of Psychiatry, Harvard Medical School, Boston, MA, United States, [3]Department of Psychology, University of New Mexico, Albuquerque, NM, United States, [4]Center on Alcohol, Substance Use, and Addictions, University of New Mexico, Albuquerque, NM, United States

Background: Benzodiazepines and non-benzodiazepine hypnotics (z-drugs) are commonly prescribed for their anxiolytic and hypnotic properties, though they can also be misused. In studies examining the epidemiology of prescription drug misuse, these medication classes are commonly combined, rendering inadequate knowledge of their patterns of misuse. The objective of this study was to characterize the population prevalence, conditional dependence, and sociodemographic and clinical correlates of the misuse of benzodiazepines and z-drugs.

Methods: Data from the National Survey on Drug Use and Health from 2015 to 2019 were used to estimate population-level prevalence and characteristics of benzodiazepine and z-drug misuse. Groups were derived based on past-year misuse of benzodiazepines alone, z-drugs alone, or both drug types. Unadjusted regression analyses were used to compare groups on characteristics of interest.

Results: Exposure to benzodiazepines and/or z-drugs *via* prescription or misuse was common; however, only 2% of the population was estimated to have misused a benzodiazepine in the past year, and less than 0.5% misused z-drugs. People who misused only z-drugs were generally older, more likely to have health insurance, more educated, and had less severe psychiatric symptoms. This group was also more likely to report misuse to cope with sleep difficulty. Although concurrent substance use was highly prevalent in all groups, people who misused z-drugs alone generally reported less concurrent substance use than the other groups.

Conclusion: The misuse of z-drugs is less common than benzodiazepines, and people who misuse only z-drugs appear to generally have lower clinical severity. Nonetheless, a substantial subgroup of people exposed to z-drugs report concurrent, past-year use of other substances. Further research on z-drug misuse, including consideration of whether it should be grouped with other anxiolytic/hypnotic drugs, is needed.

KEYWORDS

benzodiazepines, z-drugs, sedatives, prescription drug misuse, sedative/anxiolytic use disorder

1. Introduction

Benzodiazepines and non-benzodiazepine hypnotics (also referred to as z-drugs) are commonly prescribed for their anxiolytic and/or hypnotic effects. In addition to their therapeutic potential, these medications also have reinforcing properties (1–3) and thus can be misused (i.e., used at a dose or frequency greater than prescribed, without a prescription, or for reasons other than their therapeutic effect). Although these drugs have only modest reinforcing properties (2, 4–6), their misuse is common (7). The prevalence of misuse may be attributable—at least in part—to the high levels of population exposure to these drugs *via* prescription (8). Misuse of benzodiazepines and other sedatives can lead to an array of adverse consequences, such as the development of sedative/anxiolytic use disorder (9), and these medications are often present in drug overdose deaths, such as opioid overdoses (10).

Despite these public health impacts, little is known about differences in the misuse of benzodiazepines and z-drugs. The National Survey on Drug Use and Health (NSDUH), conducted annually by the Substance Abuse and Mental Health Services Administration (SAMHSA), is the largest epidemiological survey on substance use trends in the United States (U.S.). Within the NSDUH, sedatives (e.g., z-drugs and benzodiazepines with hypnotic effects) and tranquilizers (e.g., benzodiazepines with anxiolytic effects and non-benzodiazepine tranquilizers) are assessed separately, but these drugs are commonly combined into a single category of tranquilizing/sedating drugs in studies examining the epidemiology of prescription drug misuse (11–13). Similarly, even in studies outside of the NSDUH, investigators commonly assess the use and misuse of drugs producing anxiolytic and/or hypnotic effects as one category (14). Given differences in the mechanisms of action, therapeutic effect, and access to these medications, understanding differences in the populations at risk for misusing these medications as well as patterns of and reasons for misuse may help to support risk stratification and ultimately can begin to inform interventions for reducing misuse of these medications.

The overarching objective of this study was to characterize and compare the prevalence of z-drug and benzodiazepine misuse, as well as clinical correlates, past-year concurrent substance use, and motives for misusing medications utilizing annual population survey data from the NSDUH. Our first aim was to characterize the past-year prevalence of z-drug and benzodiazepine use and misuse. We also aimed to characterize the conditional misuse and dependence rates of these medications, which we defined as the proportion of people with *any* use of z-drugs or benzodiazepines in the past year (including use as prescribed or misuse) who misused these drugs or reported symptoms of a sedative/anxiolytic use disorder, respectively. Our second aim was to compare the sociodemographic and clinical characteristics of people who misused z-drugs and/or benzodiazepines in the last year. Our third aim was to characterize the past-year prevalence of other drug use and the extent of concurrent substance use, which we defined as the use of multiple substances over a defined period (15), among people who misused z-drugs and/or benzodiazepines in the last year. Finally, we aimed to compare motives for misuse among people who misused z-drugs and/or benzodiazepines. This was an exploratory, hypothesis-generating study.

2. Materials and methods

This secondary data analysis was preregistered on the Center for Open Science Framework.[1] We analyzed data collected as part of the NSDUH between the years 2015 and 2019. The NSDUH is an annual population survey in the United States that assesses substance use and related health variables. The NSDUH assesses benzodiazepine and z-drug use and misuse separately and can be used to estimate population prevalence and associated characteristics.

The NSDUH is an independent, multistage probability sample for each of the 50 states and Washington, DC. Each year, approximately 70,000 individuals are asked to complete a screening survey. To be eligible to participate, individuals must be above 12 years of age and reside in the United States. Selected participants then move to an interview phase in which data are collected. Participants are compensated $30 for completing the interview. By aggregating data from 2015 to 2019, our sample includes 282,768 participants.

2.1. Measures

All variables were assessed using a standardized assessment administered by the Substance Abuse and Mental Health Services Administration. Details about the assessment are available at: https://www.samhsa.gov/data/data-we-collect/nsduh-national-survey-drug-use-and-health.

The NSDUH assesses the prevalence of use and misuse of four categories of prescription drugs, including opioid pain relievers, psychostimulants, tranquilizers, and sedatives. Although benzodiazepines are included in both the tranquilizer and sedative categories, they are also combined into a separate category of "any benzodiazepine," which was used for these analyses. The z-drugs, including zolpidem, eszopiclone, and zaleplon (both generic and brand name), are exclusively assessed within the sedative category. To derive variables for z-drug use and misuse, we combined each of the assessed z-drugs into one category.

Binary (yes/no) indicators of any past-year use (including use as prescribed and misuse) and misuse only were used to define three groups: benzodiazepines only, z-drugs only, and combined benzodiazepines and z-drugs. The NSDUH collects data on any use, including use as prescribed and misuse, for certain benzodiazepines, including alprazolam products, lorazepam products, clonazepam products, diazepam products, temazepam products, flurazepam, or triazolam; if a participant reports past-year misuse of any additional benzodiazepine, they were also coded as reporting any past-year use. These data are collected by displaying the names and pictures of various benzodiazepines and asking respondents to indicate which medications they have used in the past year in any form. NSDUH also collects data on misuse, which is defined as the use of prescription drugs "in any way that a doctor did not direct you to use them. . .including taking someone else's prescription, or taking one's own prescription in any way other than prescribed (e.g.,

1 https://osf.io/vm658

taking a larger quantity, taking more often, or taking for a longer duration than prescribed)"(16).

To assess the prevalence of sedative/anxiolytic use disorder (the substance use disorder corresponding to the problem use of benzodiazepines or z-drugs), we used a binary (yes/no) indicator of the presence of a past-year *Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV)* (17) diagnosis of abuse or dependence for sedatives or tranquilizers (i.e., anxiolytics). Of note, the NSDUH assessed *DSM-IV* abuse and dependence symptoms separately. We combined these in the present analysis, roughly consistent with the *DSM-5* (18) diagnosis of sedative, hypnotic, or anxiolytic use disorder.

In addition to demographic data provided by the NSDUH, indicators of mental health, overall health, and functioning were also included to characterize differences in drug type groups. To assess suicidal thinking and behavior, all participants over 18 years of age were asked if, in the past year, they had serious thoughts of killing themselves (suicidal thinking), made a plan to kill themselves (suicide plan), or attempted to kill themselves (suicide attempt) (19). Participants were asked to provide a binary (yes/no) response. The Kessler-6 Distress Scale was used to measure psychological distress. Participants received a score from 0 to 24, with higher scores reflecting greater psychiatric distress (20). The World Health Organization Disability Assessment Schedule (WHODAS) was used to assess impaired functioning in various domains such as cognition, mobility, and self-care (21). The possible total scores range from 0 to 24, with higher scores reflecting more severe impairment. A single item assessing self-reported overall health, ranging from "poor" to "excellent," was used as an indicator of health status.

2.2. Analysis

Unadjusted population prevalence was estimated for the misuse of z-drugs and/or benzodiazepines first in the full sample and in the subgroup of people who reported *any* use (prescribed or misused) of these drugs in the past year. The unadjusted prevalence of sedative/anxiolytic use disorder was also estimated in both the full sample and the subgroup who reported past-year use of z-drugs and/or benzodiazepines. In addition, multinomial logistic regression analyses were used to compare the likelihood of reporting misuse or a use disorder across three groups of respondents with *any* z-drug or benzodiazepine use (z-drug use only, benzodiazepine use only, use of both z-drugs and benzodiazepines).

Multinomial logistic regressions were used to compare sociodemographic and clinical characteristics among the three groups (z-drug misuse only, benzodiazepine misuse only, and misuse of both z-drugs and benzodiazepines), with group status as the dependent variable. These characteristics included: gender, health insurance status, age, race, education, suicidal thinking and behavior (suicidal thinking, suicide plan, suicide attempt), overall health, functional impairment, and psychiatric distress.

Unadjusted multinomial logistic regression analyses also were used to compare misuse groups with respect to the presence of past-year use of other substances, including alcohol, tobacco, heroin, cocaine (combined crack and powder cocaine), methamphetamine, hallucinogens, inhalants, misused opioid analgesics, and misused stimulants, as well as the count of the total number of substances used.

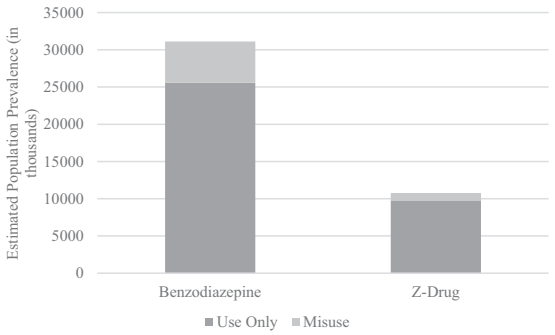

FIGURE 1
Estimated prevalence of prescribed use only and misuse of benzodiazepines and z-drugs (in thousands).

Finally, unadjusted multinomial logistic regression was used to compare misuse groups with respect to each motive for the last episode of tranquilizer and/or sedative misuse. As multiple motives could be reported (i.e., categories were not mutually exclusive), separate regressions were conducted for each motive.

Consistent with prior investigations using this dataset, we used an alpha of 0.05 for significance testing. A false discovery rate procedure (22) adjusted the *p*-value for multiple testing for each outcome. All analyses accounted for the complex survey design of the study (i.e., oversampling of young adults and racial/ethnic minority populations) and the use of combined years of survey data following the recommendations from SAMHSA.

3. Results

Any use of benzodiazepines or z-drugs (including use as prescribed and misuse) was highly prevalent, with an estimated 13.7% of the population using one of these medications in the previous year. Benzodiazepine use was more than twice as common as z-drug use, with an estimated 11.4% of the population using or misusing a benzodiazepine and 4% using or misusing a z-drug.

3.1. Misuse prevalence estimates

The past-year prevalence of benzodiazepine and z-drug misuse is depicted in Figure 1. An estimated 2% of the population engaged in past-year misuse of a benzodiazepine, and less than 0.4% misused a z-drug. The prevalence was much higher among people exposed to these medications in the past year (including legitimate use as prescribed), with an estimated 17.7% of all people who used benzodiazepines misusing them and 9.2% of people who used z-drugs misusing them.

The proportion of people with a sedative/anxiolytic use disorder was similar between drug types, with 2.3% of people who used benzodiazepines and 2.1% of people who used z-drugs reporting a past-year sedative/anxiolytic use disorder.

Among people who reported past-year misuse of benzodiazepines, 12.3% met criteria for a sedative/anxiolytic use disorder, and 13.6% of people who reported past-year z-drug misuse met criteria for a disorder.

When considering subgroups of participants based on whether, in the past year, they exclusively used benzodiazepines, exclusively used z-drugs, or used both, the prevalence of misuse and use disorder varied. Any past-year use of *both* z-drugs and benzodiazepines was associated with the highest likelihood of misuse (compared to benzodiazepines alone: OR = 1.36, 95% CI = 1.23, 1.51; compared to z-drugs alone OR = 3.64, 95% CI = 3.09, 4.29), followed by benzodiazepines alone (compared to z-drugs: OR = 2.67, 95% CI = 2.23, 3.07), and finally z-drugs alone.

This same pattern of findings was observed for sedative/anxiolytic use disorder. Specifically, people who used both drug types were more likely to meet criteria for a sedative/anxiolytic use disorder than those who used benzodiazepines alone (OR = 2.42, 95% CI = 1.91, 3.07) or z-drugs alone (OR = 13.89, 95% CI = 7.78, 24.79); benzodiazepine use alone was also associated with higher odds of a use disorder than z-drugs alone (OR = 5.74, 95% CI = 3.18, 10.36).

3.2. Sociodemographic and clinical characteristics

The sociodemographic and clinical characteristics of the three groups (past-year z-drug misuse only, benzodiazepine misuse only, both z-drug and benzodiazepine misuse) are presented in Table 1. These analyses indicated significant overall differences among groups in age, education, and health insurance status, but not gender. In general, these results demonstrated a pattern of older age for people with z-drug use only (approximately 40% of people who reported only misusing z-drugs were 50 or older). The z-drug-only group also generally had higher levels of education, partly due to the low base rate in school-aged adolescents, which was less than half of the adolescent prevalence rate of the benzodiazepine-only and combined groups. Finally, the z-drug-only group had very high rates of health insurance, with over 94% reporting health insurance, compared to 85% of benzodiazepine use only and 86% in the combined group.

Descriptive data are presented for race in Table 1; however, the regression results were not interpreted due to low base rates for some combinations of race and substance use resulting in quasi-complete separation in the regression models.

With respect to clinical characteristics, model effects were found for suicidal ideation, suicide plan, and suicide attempt, as well as psychiatric distress and disability, but not for overall health status. Suicidal thinking and behavior were consistently most common in the combined group (24.9% estimated to have suicidal ideation, 11% suicide plan, 7% suicide attempt), followed by benzodiazepines only (18.3% suicidal ideation, 7.3% suicide plan, 3.7% suicide attempt) and z-drugs only (10.6% suicidal ideation, 3.4% suicide plan, 0.9% suicide attempt). This is consistent with results of the psychiatric distress (Kessler-6) and disability (WHODAS) results, which found that scores were highest in the combined group, followed by the benzodiazepine-only group, and finally, the z-drug-only group.

3.3. Other drug use and concurrent substance use

Other drug use was highly prevalent in all three groups. Drug use also varied between groups, with some variability in the magnitude of effects (Table 2). The benzodiazepine and z-drug group consistently reported more drug use than the z-drug alone group for all substances except alcohol, which was common (>85%) in all three groups. The combined group also was more likely to report past-year use of all drugs than the benzodiazepine-only group except alcohol, tobacco, and cannabis. The benzodiazepine-only group reported more past-year substance use for all substances than the z-drug-only group, except for alcohol and inhalants.

Consistent with these findings, more concurrent substance use (count of substances used, including sedatives and tranquilizers) was greater in the combined group (estimated population mean = 6.03 drugs, 95% CI = 5.70, 6.37) than in the z-drug-only group (mean = 3.19, 95% CI = 3.05, 3.33) and the benzodiazepine-only group (mean = 4.32, 95% CI = 4.25, 4.39), and greater in the benzodiazepine-only group than in the z-drug only group.

3.4. Motives for misuse

Motives for the most recent misuse also varied significantly across groups (see Figure 2). People who misused z-drugs only were less likely than both the combined group and the benzodiazepine-only group to report all motives except for sleep. Misusing for sleep was significantly more common in the z-drug group than in the benzodiazepine group only (OR = 13.80, 95% CI = 9.98, 19.08) and was not significantly different from the combined group (OR = 1.96, 95% CI = 1.23, 3.13).

4. Discussion

The findings of the present analysis indicate that more than 1 in 10 people in the U.S. are exposed to either benzodiazepines or z-drugs annually. Although most of those exposed to these medications do not misuse them, a substantial subgroup reported misuse (approximately 9% of those exposed to z-drugs and almost 18% of those exposed to benzodiazepines), and a small subgroup reported misuse at the severity of a substance use disorder (approximately 2% of people exposed to either drug). Of note, the sedative/anxiolytic use disorder rates among those with any benzodiazepine use reported herein were similar to those previously estimated using 2015–2016 NSDUH data (9), but, to our knowledge, use disorder estimates have not been previously reported for those with z-drug use.

Our estimate for the prevalence of benzodiazepine misuse is similar to prior reports in the literature (9), indicating that approximately 2% of the U.S. population engages in misuse each year. Z-drug misuse has been less well-characterized, and notably, its prevalence was low in the general population (<0.05%). Nonetheless, more than 9% of people who reported any past-year use of a z-drug reported misuse, suggesting that misuse among people exposed to z-drugs is not uncommon.

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TABLE 1 Population estimates of sociodemographic characteristics of people with past-year benzodiazepine and/or z-drug misuse.

	Benzodiazepine misuse only		Z-drug misuse only		Benzodiazepine and z-drug misuse	
	Estimate	(95% CI lower, upper)	Estimate	95% CI	Estimate	95% CI
Age[a]						
12–17 years old	7.5%	(6.9, 8.1%)	3.5%	(2.5, 4.9%)	7.4%	(5.0, 11.0%)
18–25 years old	30.3%	(28.8, 31.7%)	12.8%	(10.4, 15.5%)	23.5%	(19.5, 28.0%)
26–34 years old	23.3%	(21.9, 24.7%)	19.9%	(16.3, 24.2%)	22.1%	(17.2, 28.0%)
35–49 years old	18.8%	(17.6, 20.0%)	24.0%	(18.7, 30.4%)	21.0%	(15.6, 27.6%)
50–64 years old	15.0%	(13.2, 17.0%)	28.5%	(22.6, 35.3%)	21.2%	(14.4, 30.1%)
65 or older	5.2%	(4.1, 6.7%)	11.3%	(7.3, 17.1%)	4.8%	(1.9%, 11.5%)
Gender						
Male	50.3%	(48.6, 51.9%)	43.5%	(37.9, 49.3%)	49.7%	(44.0, 55.4%)
Female	49.7%	(48.1, 51.4%)	56.5%	(50.7, 62.1%)	50.3%	(44.6, 56.0%)
Race[b]						
White (non-Hispanic)	73.3%	(71.2, 75.3%)	75.6%	(70.2, 80.2%)	82.9%	(78.0, 86.9%)
Black/African American (non-Hispanic)	7.2%	(6.5, 8.0%)	5.9%	(3.5, 10.0%)	3.2%	(1.6, 6.2%)
Native American/Alaska native (non-Hispanic)	0.3%	(0.3, 0.5%)	0.5%	(0.2, 1.6%)	0.6%	(0.2, 2.3%)
Native Hawaiian/other Pacific Islander (non-Hispanic)	0.3%	(0.2, 0.5%)	*	*	0.1%	(0.0, 1.1%)
Asian (non-Hispanic)	1.8%	(1.3, 2.5%)	3.1%	(1.5, 6.2%)	1.4%	(0.7, 2.8%)
More than 1 race (Non-Hispanic)	2.5%	(2.1, 3.0%)	3.0%	(1.7, 5.2%)	2.1%	(1.3, 3.5%)
Hispanic	14.5%	(12.9, 16.3%)	11.9%	(7.9, 17.7%)	9.7%	(6.4, 14.3%)
Education[a]						
Less than high school	10.7%	(9.5, 12.0%)	5.1%	(3.4, 7.5%)	8.8%	(5.5, 13.6%)
High School grad	21.5%	(20.0, 23.0%)	15.1%	(11.5, 19.6%)	22.4%	(17.8, 27.7%)
Some college/associates degree	36.7%	(35.0, 38.4%)	35.4%	(29.5, 41.8%)	26.8%	(21.5, 32.7%)
College graduate	23.6%	(22.2, 25.1%)	40.9%	(35.4, 46.6%)	34.7%	(28.6, 41.2%)
12–17 years olds	7.5%	(6.9, 8.1%)	3.5%	(2.5, 4.9%)	7.4%	(5.0, 11.0%)
Health insurance[a]						
Yes, respondent is covered by health insurance	84.8%	(83.5, 86.0%)	94.1%	(91.0, 96.2%)	86.1%	(80.4, 90.3%)
No, respondent is not covered by health insurance	15.2%	(14.0, 16.5%)	5.9%	(3.8, 9.0%)	13.9%	(9.7, 19.6%)
Serious suicidal ideation[a]						
No	81.7%	(80.2, 83.1%)	89.4%	(85.6, 92.3%)	75.1%	(69.3, 80.1%)
Yes	18.3%	(16.9, 19.8%)	10.6%	(7.7, 14.4%)	24.9%	(19.9, 30.7%)
Suicide plan[a]						
No	92.7%	(91.7, 93.6%)	96.6%	(94.0, 98.1%)	89.0%	(83.8, 92.7%)
Yes	7.3%	(6.4, 8.3%)	3.4%	(1.9, 6.0%)	11.0%	(7.3, 16.2%)
Suicide attempt[a]						
No	96.3%	(95.6, 96.9%)	99.1%	(98.5, 99.5%)	93.0%	(89.2, 95.6%)
Yes	3.7%	(3.1, 4.4%)	0.9%	(0.5, 1.5%)	7.0%	(4.4, 10.8%)
Kessler-6 score	10.67	(10.38, 10.95)	8.52	(7.80, 9.23%)	11.70	(10.41, 12.99)
WHODAS score	8.45	(8.14, 8.76)	7.40	(6.62, 8.19%)	9.93	(8.72, 11.14)
Overall health						
Excellent	15.7%	(14.2, 17.2%)	21.0%	(16.6, 26.3%)	21.0%	(15.5, 27.6%)
Very good	36.7%	(34.9, 38.5%)	40.8%	(34.8, 47.1%)	33.9%	(27.1, 41.5%)
Good	32.3%	(30.5, 34.1%)	25.5%	(20.1, 31.9%)	24.9%	(19.1, 31.8%)
Fair/poor	15.4%	(14.0, 16.9%)	12.7%	(8.9, 17.7%)	20.2%	(14.9, 26.8%)

[a]Overall model was statistically significant using false discovery rate correction ($p \leq 0.009$). [b]Significance not reported due to small cell sizes, *data not available. Although a small number of participants in the z-drug-only group reported past-year use of non-benzodiazepine tranquilizers, data on the source for those drugs are not presented. WHODAS, World Health Organization Disability Assessment Schedule.

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TABLE 2 Estimated population prevalence of past-year substance use among people reporting past-year benzodiazepine and z-drug misuse.

	Benzodiazepine misuse only		Z-drug misuse only		Benzodiazepine and z-drug misuse	
	Estimate	(95% CI lower, upper)	Estimate	95% CI	Estimate	95% CI
Alcohol	88.7%	(87.0, 90.2%)	85.3%	(80.6, 88.9%)	87.2%	(81, 91.5%)
Tobacco[a]	65.6%	(63.7, 67.4%)	40.5%	(34.8, 46.5%)	73.8%	(63.3, 82.1%)
Marijuana[a]	63.7%	(61.8, 65.5%)	37.0%	(32.1, 42.2%)	67.9%	(59.9, 74.9%)
Heroin[a]	5.4%	(4.6, 6.2%)	1.1%	(0.5, 2.2%)	12.0%	(8.7, 16.4%)
Cocaine or crack[a]	3.7%	(3.0, 4.4%)	1.0%	(0.3, 3.5%)	8.2%	(5.2, 12.8%)
Hallucinogens[a]	21.6%	(20.4, 22.9%)	7.5%	(6.1, 9.3%)	29.5%	(24.6, 35.0%)
Inhalants[a]	5.4%	(4.6, 6.3%)	4.1%	(2.0, 8.1%)	9.7%	(7.3, 12.9%)
Methamphetamine[a]	8.2%	(7.4, 9.2%)	1.1%	(0.4, 2.9%)	14.1%	(10.8, 18.3%)
Pain relievers[a]	42.2%	(40.3, 44.0%)	28.5	(23.2, 34.5%)	65.2%	(58.8, 71.1%)
Stimulants[a]	24.9%	(23.6, 26.3%)	10.9%	(8.1, 14.6%)	38.2%	(31.7, 45.0%)

[a]Overall model was statistically significant using false discovery rate correction ($p \leq 0.002$).

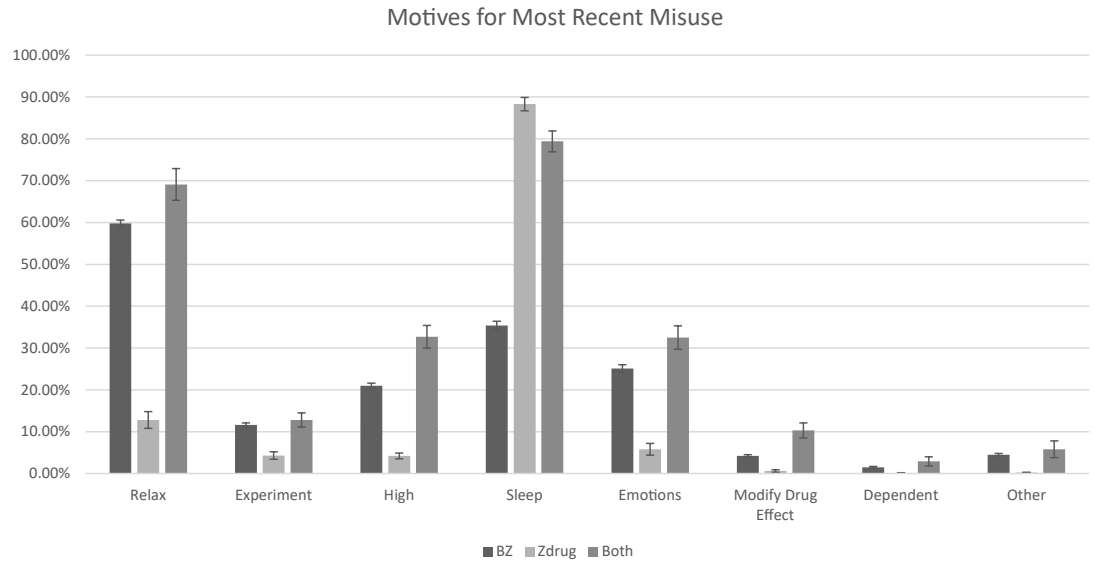

FIGURE 2
Motives for most recent misuse (*modify drug effect* refers to attempts to increase or decrease the effects of another substance; *dependent* refers to physiological dependence). All model effects statistically significant using false discovery rate correction ($p \leq 0.002$). BZ, benzodiazepine only group.

Importantly, several differences were observed in the characteristics of people who misused these two drugs. When comparing people who misused benzodiazepines only and those who misused z-drugs only, people who misused z-drugs were generally older, more highly educated, and had substantially less severe psychiatric symptoms. The z-drug-only group was also less likely to report the use of most other drugs and had less concurrent substance use (i.e., fewer drug types used in the past year) than the benzodiazepine-only group. Previous research has also demonstrated that benzodiazepines have greater misuse liability among those with histories of alcohol use disorder (23) and increase the reinforcing effects of opioids when they are taken in combination (24), consistent with our findings that those with benzodiazepine misuse had higher rates of other drug use.

Yet, it is of note that the use of other drugs was common—and higher than general population base rates—in all three groups, including people who misused z-drugs alone. This pattern of concurrent substance use is concerning, given the potential for adverse events when these drugs are combined with other depressants. We are limited in our conclusions regarding the level of risk associated with concurrent substance use identified in our analysis, given data are not available on co-use or simultaneous use (e.g., using benzodiazepines and opioids at the same time), which is particularly risky for overdose. It has been thoroughly documented that simultaneous use of other substances, particularly opioids, is common among those who misuse benzodiazepines (14). However, future research on co-use among those who misuse z-drugs will be needed to better understand the prevalence of risky co-use patterns in this population.

Another future direction concerning polysubstance use might include characterizing co-occurring substance use disorders among those with z-drug misuse. We chose to focus on concurrent substance use broadly, given the risks associated with combining central nervous system depressants and the aim of our manuscript to inform risk stratification and methodological decisions regarding the measurement of benzodiazepine and z-drug misuse. However, there is evidence that other substance use disorders are highly prevalent among those with benzodiazepine misuse (9), and that benzodiazepine misuse is associated with poorer substance use disorder treatment outcomes (14). It is currently unclear the extent to which z-drug misuse co-occurs with other substance use disorders and impacts treatment outcomes.

The most common motive for the misuse of z-drugs—by far—was to sleep (over 88% of participants reported this motive). In contrast, misuse of benzodiazepines, or both benzodiazepines and z-drugs, was associated with a broad array of motives, such as to relax, to sleep, to manage emotions, to get high, to experiment, or to modify the effects of other drugs (e.g., to increase or decrease an effect of another substance). These differences are consistent with the mechanism of action and pharmacological properties of benzodiazepines and z-drugs. Z-drugs bind preferentially to $GABA_A$ receptors containing α1-subunits, which modulate sedation and amnesia (25, 26). In contrast, benzodiazepines bind non-selectively to sites that contain α 1-, α 2-, α 3-, or α5-subunits, with the α2- and α3-subunits implicated in anxiolytic effects (25, 26).

Nevertheless, it is notable that the most common motives for both drug classes were consistent with *relief* motives rather than *reward* motives for misuse. A large body of literature indicates that negative reinforcement motives are associated with the progression of substance use severity (27, 28), and therefore assessing reasons for misuse of benzodiazepines and z-drugs might help identify individuals at risk of developing symptoms of a use disorder and target interventions addressing underlying problems with sleep and anxiety. Psychometric and qualitative research on motives for benzodiazepine and z-drug misuse are needed to further this line of work, given limited work in this area (14) and a lack of wide-spread measures of motives for benzodiazepines and z-drugs, such as is available for alcohol use motives [e.g., Drinking Motives Questionnaire (29)]. The assessment of motives for benzodiazepines and z-drug misuse likely requires unique considerations; for example, it is unclear the extent to which specific motives for misuse of these medications are distinct, given the overlap between items such as "to sleep" and "to relax."

The results of the present analysis (e.g., differences in conditional misuse rates, motives) may be attributable to differences in the reinforcing properties of these two drug classes. Yet, few studies have directly compared the reinforcing properties of benzodiazepines and z-drugs, and extant studies were conducted over two decades ago, primarily enrolled men, and have produced equivocal findings. These human laboratory studies indicate that benzodiazepines (i.e., triazolam, alprazolam) and z-drugs (i.e., zolpidem, zopiclone) have similar reinforcing properties, as indicated by subject-rated measures (e.g., drug liking, street value) and drug choice paradigms (30–32). However, in these studies, z-drugs were more likely than benzodiazepines to produce adverse side effects (e.g., dizziness) and less likely to be identified as barbiturates, benzodiazepines, or alcohol in drug discrimination paradigms (30–32). Using a proposed algorithm to address the misuse liability of hypnotic drugs based on human laboratory findings in combination with other factors (e.g., half-life, actual misuse prevalence rates, severity of withdrawal), Griffiths and Johnson (5) concluded that the evaluated benzodiazepines generally had higher misuse liability than the evaluated z-drugs. Future research should directly compare the reinforcing properties of commonly misused benzodiazepines and z-drugs in more diverse samples.

Taken together, the results of the present analysis may help inform the decision on whether to combine anxiolytic and hypnotic drugs in future research and surveillance efforts. Our results suggest differences between z-drugs and benzodiazepines, ranging from conditional misuse and dependence rates to indicators that the groups that misuse z-drugs alone vs. benzodiazepines (with or without z-drugs) are less clinically severe, more likely to misuse for the drug's indication (sleep), and report less concurrent substance use. Accordingly, studies that combine z-drugs and benzodiazepines may lead to underestimates of conditional liability and clinical severity of benzodiazepine misuse. This is not to say that z-drugs are free of potential harm, as they increase the risk of overdose in high-risk populations (33), but accurately characterizing the population of those most likely to misuse z-drugs might help inform preventative efforts to reduce such harm. Ultimately, the decision whether to combine these drug types will depend on the question or interest and may be informed by statistical power (particularly for studies of z-drug misuse), we recommend that studies combining these drug types also include sensitivity analyses examining whether results of the combined group hold for each subpopulation.

5. Limitations and future directions

Several methodological limitations impact the interpretation of the present findings. First, this analysis is subject to the general limitations of the NSDUH, including the inability to generalize to groups un- or underrepresented (e.g., incarcerated people, unhoused people) and sampling biases (34). Several methodological features of the NSDUH (e.g., not assessing certain variables, not assessing variables over a past-year time frame) limited the variables we could examine in the present analysis. Several unexamined factors in the present analysis, such as co-use of substances, frequency of misuse, and source of prescription medications for misuse, would undoubtedly aid in the clarification of differences between those who use and misuse benzodiazepines and/or z-drugs. We also combined responses across tranquilizers and sedatives for several substance-specific variables, such as motives and use disorder, thus rendering conclusions about the unique effect of medication classes challenging. This is particularly relevant for the group who reported misuse of benzodiazepines and z-drugs, for which we cannot determine if use disorder was secondary to one or both of these substances and if those in this subgroup reported different motives for the misuse of different medication classes. Similarly, non-benzodiazepine tranquilizers and sedatives

could contribute to participants' responses to questions about motives and sedative/anxiolytic use disorder if a participant reported misuse of both benzodiazepine and non-benzodiazepine tranquilizers and/or sedatives, but given the low base rates of non-benzodiazepine products (35), this is likely exceedingly rare. We also decided to combine *DSM-IV* sedative/anxiolytic abuse and dependence, consistent with a DSM-5 approach to diagnosis. Nevertheless, this approach may have conflated complex persistent benzodiazepine dependence with sedative/anxiolytic use disorder, despite different treatment needs for these presentations (36). We recommend that future analyses of NSDUH data leverage latent variable mixture models to understand subtypes of sedative/anxiolytic use disorder symptoms (37), as well as item response theory to assess the validity of this diagnosis (38). Lastly, although the NSDUH 2020 public use data file is currently available, we excluded this data from our analysis, given substantially different substance use disorder prevalence rates in 2020 compared to previous years, which is likely attributable to the introduction of DSM-5 criteria and/or COVID-19 impacts on data collection procedures (39).

6. Conclusion

Overall, this study indicated that misuse is not uncommon in people exposed to benzodiazepines and z-drugs and should be monitored in people prescribed these medications. People who misuse both drug types appear to have significant clinical severity concerning psychiatric severity and other drug use. Those with concurrent substance use may be of particular concern for overdose, given the risks of combining substances, as well as elevated suicidal thinking and behavior. The populations of people misusing and motives for misuse further suggest differences between the misuse of benzodiazepines and z-drugs that may benefit from consideration, where possible, as separate categories.

Data availability statement

Publicly available datasets were analysed in this study. This data can be found here: SAMHSA (datafiles.samhsa.gov).

Author contributions

RM, VV, and MM planned the study aims and analyses. RM conducted the data analysis. All authors contributed to the draft of the manuscript and edited and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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EDITED BY
Benjamin Rolland,
Université Claude Bernard Lyon 1, France

REVIEWED BY
Jakub Nalepa,
Silesian University of Technology, Poland
Juliana Gomes,
Federal University of Pernambuco, Brazil

*CORRESPONDENCE
Kerry L. Kinney
kkinney@umc.edu

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Predicting benzodiazepine prescriptions: A proof-of-concept machine learning approach

Kerry L. Kinney[1,2]*, Yufeng Zheng[2,3], Matthew C. Morris[1,2], Julie A. Schumacher[1,2], Saurabh B. Bhardwaj[1,2] and James K. Rowlett[1,2]

[1]Department of Psychiatry and Human Behavior, University of Mississippi Medical Center, Jackson, MS, United States, [2]Center for Innovation and Discovery in Addictions, University of Mississippi Medical Center, Jackson, MS, United States, [3]Department of Data Science, University of Mississippi Medical Center, Jackson, MS, United States

Introduction: Benzodiazepines are the most commonly prescribed psychotropic medications, but they may place users at risk of serious adverse effects. Developing a method to predict benzodiazepine prescriptions could assist in prevention efforts.

Methods: The present study applies machine learning methods to de-identified electronic health record data, in order to develop algorithms for predicting benzodiazepine prescription receipt (yes/no) and number of benzodiazepine prescriptions (0, 1, 2+) at a given encounter. Support-vector machine (SVM) and random forest (RF) approaches were applied to outpatient psychiatry, family medicine, and geriatric medicine data from a large academic medical center. The training sample comprised encounters taking place between January 2020 and December 2021 (N = 204,723 encounters); the testing sample comprised data from encounters taking place between January and March 2022 (N = 28,631 encounters). The following empirically-supported features were evaluated: anxiety and sleep disorders (primary anxiety diagnosis, any anxiety diagnosis, primary sleep diagnosis, any sleep diagnosis), demographic characteristics (age, gender, race), medications (opioid prescription, number of opioid prescriptions, antidepressant prescription, antipsychotic prescription), other clinical variables (mood disorder, psychotic disorder, neurocognitive disorder, prescriber specialty), and insurance status (any insurance, type of insurance). We took a step-wise approach to developing a prediction model, wherein Model 1 included only anxiety and sleep diagnoses, and each subsequent model included an additional group of features.

Results: For predicting benzodiazepine prescription receipt (yes/no), all models showed good to excellent overall accuracy and area under the receiver operating characteristic curve (AUC) for both SVM (Accuracy = 0.868–0.883; AUC = 0.864–0.924) and RF (Accuracy = 0.860–0.887; AUC = 0.877–0.953). Overall accuracy was also high for predicting number of benzodiazepine prescriptions (0, 1, 2+) for both SVM (Accuracy = 0.861–0.877) and RF (Accuracy = 0.846–0.878).

Discussion: Results suggest SVM and RF algorithms can accurately classify individuals who receive a benzodiazepine prescription and

can separate patients by the number of benzodiazepine prescriptions received at a given encounter. If replicated, these predictive models could inform system-level interventions to reduce the public health burden of benzodiazepines.

KEYWORDS

benzodiazepine, prescriptions, machine learning, support vector machine, random forest

1. Introduction

Benzodiazepines are the most commonly prescribed psychotropic medications in the U.S. (1), with approximately 12.5% of U.S. adults reporting past-year benzodiazepine use (2). They are known for their anxiolytic, sedative, hypnotic, relaxant, and anticonvulsant effects, and they are primarily indicated for short-term use in anxiety and sleep disorders (3, 4). Benzodiazepine use is associated with risk of serious adverse effects, such as psychomotor impairment, cognitive decline, falls, accidents, opioid overdose, substance use disorders, and death (5, 6), suggesting benzodiazepines pose a significant public health burden. Moreover, simultaneous receipt of multiple benzodiazepine prescriptions is considered a suboptimal and potentially high-risk prescribing pattern, as it can lead to increased plasma concentrations and risk of toxicity (7, 8), but there is a paucity of research on the correlates of multiple benzodiazepine prescription receipt. The present study aims to develop an algorithm to predict whether a patient is likely to receive a benzodiazepine prescription and the number of benzodiazepine prescriptions they are likely to receive at a given medical encounter, which could reduce the public health burden of benzodiazepine use and misuse by connecting patients with evidence-based treatments for anxiety or sleep disorders before they receive a prescription.

Research suggests access to benzodiazepines differs by demographic factors such as race, sex, and age. Indeed, multiple studies have found that in the U.S., White individuals are more likely than other racial groups to receive a benzodiazepine prescription (9, 10). Differences in the need for anxiety or insomnia treatment is unlikely to explain the variation in benzodiazepine prescriptions by race (11). Although the discrepant nature of benzodiazepine prescription rates by race may safeguard individuals from minoritized backgrounds from the risks associated with benzodiazepine use, they are also indicative of underlying disparities in screening for and treating anxiety and insomnia. Benzodiazepine rates have also been shown to differ by insurance status, such that individuals who are insured are more likely to receive a benzodiazepine prescription compared to patients without insurance coverage (12, 13). Another long-standing finding with benzodiazepine use is that women are more likely to use benzodiazepines than men (14), and female gender is associated with higher mean cumulative dosage of benzodiazepines (15). Moreover, male prescribers are more likely to prescribe benzodiazepines to female compared to male patients (15), which could indicate physician bias (e.g., male physicians may view their female patients as more anxious and in greater need of medication to treat their distress). Age is also associated with the likelihood of receiving a benzodiazepine prescription, with older patients more commonly receiving a benzodiazepine prescription (12, 16). Thus, a machine learning approach to identifying who is likely to receive a benzodiazepine prescription could not only help hospital systems begin to develop strategies to reduce the public health burden of benzodiazepines, but also identify disparities in the identification and treatment of anxiety and sleep disturbance by raising awareness of non-clinical factors that play a role in prescription prediction.

Additional research suggests individuals who are at the greatest risk of adverse benzodiazepine-related outcomes have an increased likelihood of receiving a benzodiazepine prescription (12). For example, patients with depression, schizophrenia, or a substance use disorder are prescribed benzodiazepines at higher rates than those without these conditions (12, 17–19). Similarly, individuals who are prescribed an antidepressant are more likely to be prescribed a benzodiazepine than those who do not use antidepressants (12). Individuals with a comorbid psychiatric or substance use disorder are at elevated risk of misusing benzodiazepines and of negative outcomes related to benzodiazepine use compared to the general population (17, 20, 21). Indeed, research suggests benzodiazepines are associated with new onset and worsening of depression symptoms (22) and that concurrent use of benzodiazepines with alcohol or opioids is associated with increased risk of emergency department visits, injury, overdose, and death (23–28). Furthermore, one study found that individuals with more severe chronic obstructive pulmonary disease (COPD) were more likely to receive multiple benzodiazepine prescriptions compared to those with less severe COPD (8). This finding is especially concerning given benzodiazepines' respiratory depressant effect (29). As far as we are aware, no research has examined other clinical predictors of receipt of multiple benzodiazepine prescriptions. Developing an algorithm to predict who is likely to receive a benzodiazepine prescription and to stratify patients by the number of benzodiazepine prescriptions they are likely to receive at a given encounter represents an important first step toward reducing benzodiazepine prescriptions in these vulnerable populations.

To our knowledge, there is no predictive algorithm that exists to classify patients by their likelihood of receiving a benzodiazepine prescription or to stratify patients by the number of benzodiazepine prescriptions they are likely to receive at a given encounter. Machine learning uses computational modeling to learn from existing data, thereby improving predictive performance (30). The emergence of electronic medical records has led to the creation of large, rich sources of data that are ripe for health-related

analyses which use machine learning to answer clinical questions more efficiently than traditional approaches (31). Specifically, machine learning methods can efficiently handle large numbers of predictors; capture complex, multidirectional, and non-linear relationships between variables; and classify clinically important populations (32). Prior research suggests machine learning can be used to predict patients' risk for a variety of negative health outcomes (30, 31, 33), including sustained opioid prescription (34) or opioid overdose (35). Importantly, such an approach may help hospital systems begin to address issues of disparities in access to treatment for anxiety and sleep disorders and the use of potentially inappropriate prescriptions by raising awareness of non-clinical factors that are related to prescribing. The current study aims to apply machine learning methods to develop algorithms for stratifying patients by the likelihood of receiving a benzodiazepine prescription and the number of benzodiazepine prescriptions they are likely to receive at a given encounter.

2. Materials and methods

2.1. Data source

Electronic health record data were obtained from a research data warehouse at an academic medical center (36). Data for this study were de-identified and date-shifted and thus did not include any protected health information. The data warehouse compiles data from the electronic records system, Epic, based on encounters at all of the institution's hospitals and clinics. The present study included data from encounters in three specialties: family medicine, outpatient psychiatry, and geriatric medicine, as benzodiazepines are most commonly prescribed in these settings (14). All patients who identified their race as either Black/African-American or White/Caucasian were included; all other races were excluded, as only 4.2% of encounters in the training dataset and 3.6% of encounters in the test dataset were with patients who identified as another race (see **Figure 1** for a diagram of included encounters). This proof-of-concept study builds on prior research by using machine learning to identify demographic and clinical factors to improve prediction of benzodiazepine use in patients seen at Mississippi's only academic medical center.

2.2. Features and outcomes

The following sets of features were selected: anxiety and sleep diagnoses (four features: primary anxiety disorder diagnosis, any anxiety disorder diagnosis, primary sleep disorder diagnosis, any sleep disorder diagnosis), demographic characteristics (three features: age, gender, race), medications (four features: opioid prescription at encounter, number of opioid prescriptions at encounter, antidepressant prescription at encounter, antipsychotic prescription at encounter), other clinical variables (four features: any mood disorder diagnosis, any psychotic disorder diagnosis, any neurocognitive disorder diagnosis, prescriber specialty), and insurance status (two features: any insurance, type of insurance). Breathing-related sleep disorder diagnoses were excluded because benzodiazepines are contraindicated for these disorders (6). The outcomes of interest were whether a benzodiazepine was prescribed at the encounter (yes/no) and the number of benzodiazepine prescriptions given to the patient at the encounter (0, 1, 2+). Benzodiazepine prescriptions included: alprazolam, clonazepam, diazepam, chlordiazepoxide, clorazepate, lorazepam, midazolam, and temazepam.

2.3. Analytic approach

We used the Statistical Package for Social Sciences (SPSS, version 28.0) for data management and MATLAB R2020b for analysis. We used multiple machine learning approaches to determine how to optimize prediction of benzodiazepine prescriptions. Specifically, we applied support vector machine (SVM) and random forest approaches to the de-identified electronic health record data for encounters in psychiatry, family medicine, and geriatrics at an academic medical center. To train and test the prediction models, two separate datasets were collected. The training dataset was collected between January 2020 and December 2021, while the test dataset was collected between January 2022 and March 2022. Overall patient-level sample characteristics are presented for the training and test datasets in **Supplementary Table 1**. No cross validations (e.g., k-fold) were applied to our analyses, as the test dataset was completely separated from the training dataset. All prediction results reported in the following tables and figures were derived from the test dataset. To compare the performance of different algorithms, the true positive rate (i.e., the ratio of values that are predicted to be positive and are actually positive to all positive values), the true negative rate (i.e., the ratio of values that are predicted to be negative and are actually negative to all negative values), and the overall accuracy were calculated. Some research suggests the area under the receiver operating characteristic curve (AUC) is a better measure for evaluating the predictive ability of machine learning algorithms compared to accuracy (37); therefore the AUC was also calculated for the models predicting benzodiazepine receipt. The AUC was not calculated for models predicting the number of prescriptions, as receiver operating characteristic curves are not suitable for multi-class classifications. The AUC is a function of both sensitivity and specificity and can be interpreted such that a value of 1.0 is a perfect test of classification, 0.90–0.99 is considered excellent, 0.80–0.89 is considered good, 0.70–0.79 is considered fair, 0.51–0.69 is a poor test, and a value of 0.5 corresponds with no improvement in prediction over chance (38, 39). A total of 17 features were selected *a priori* based on existing literature. Given the small number of features and the large sample size, we opted to manually combine different sets of features to test the classification accuracy, which can be more easily interpreted than using data-driven approaches to feature selection. A model building approach was used to determine which sets of features would maximize predictive accuracy. The models tested were as follows:

Model 1: Anxiety and Sleep Diagnoses Only.

Model 2: Anxiety and Sleep Diagnoses + Demographic Characteristics.

Model 3: Anxiety and Sleep Diagnoses + Demographic Characteristics + Co-Prescriptions.

Model 4: Anxiety and Sleep Diagnoses + Demographic Characteristics + Co-Prescriptions + Other Clinical Variables.

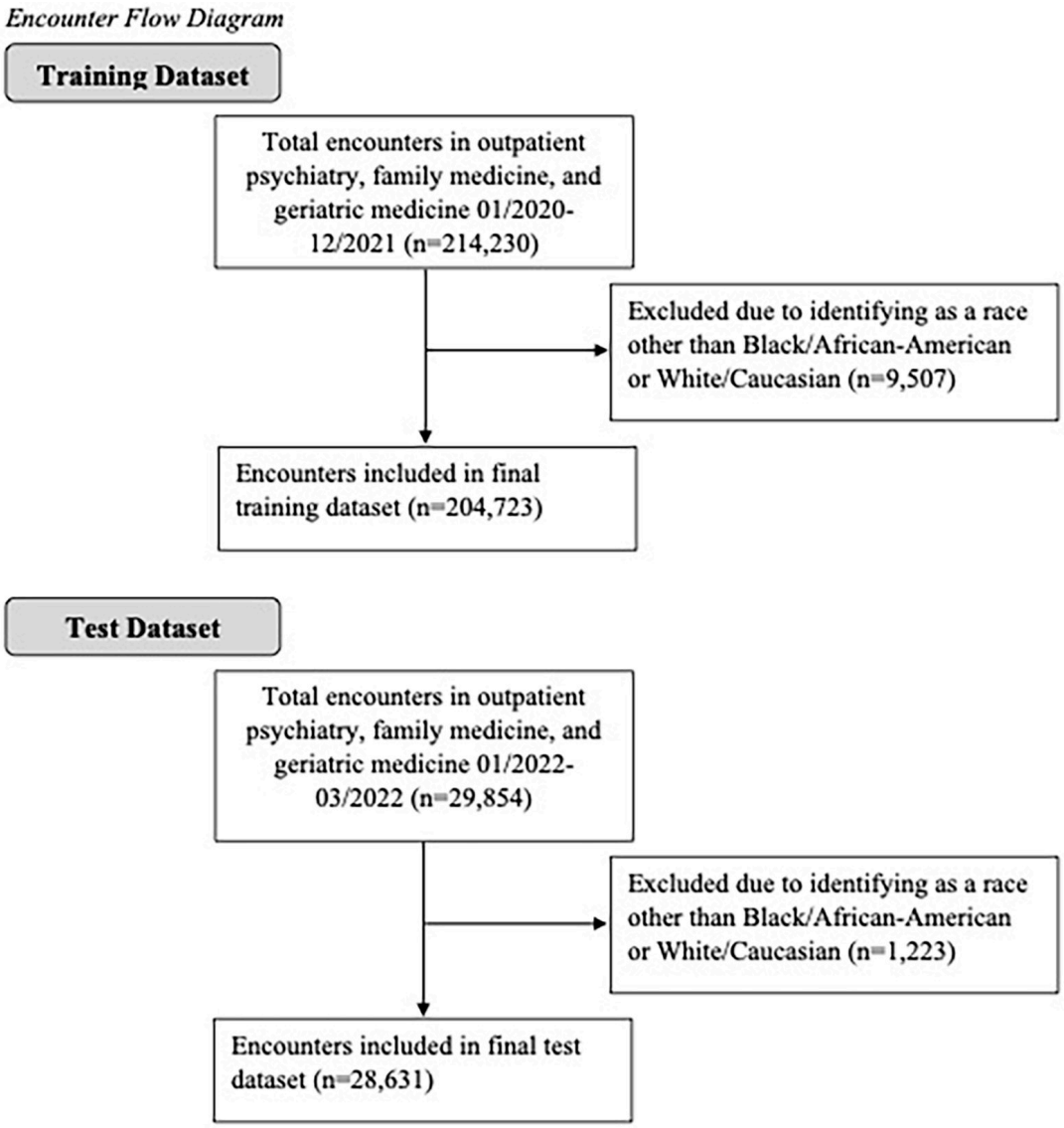

FIGURE 1
Encounter flow diagram.

Model 5: Anxiety and Sleep Diagnoses + Demographic Characteristics + Co-Prescriptions + Other Clinical Variables + Insurance.

All training samples were used for training. Undersampling was employed on the training dataset to avoid bias given the unequal distribution of negative responses (i.e., did not receive a benzodiazepine prescription) compared to positive responses (i.e., received a benzodiazepine prescription). Suppose there are m samples of benzodiazepine prescription (yes), and n (typically $n > m$) samples of non-benzodiazepine prescription (no). We randomly selected n' ($= m$) samples from n samples. Each model was repeated 30 times, yielding 30 different n' samples. A multivariate analysis of variance (MANOVA) was performed for each model to compare whether mean performance differed between the random forest and SVM approaches. The SVM and random forest algorithms were implemented on the Matlab R2020b platform using default settings, except where noted otherwise.

2.3.1. Support vector machine

Support vector machine is a supervised learning model that analyzes data and performs non-linear classification (40). When provided a set of training data, in which each observation is coded as belonging to a group, an SVM training algorithm uses the data to build a model that can assign new data points to a specific category. An SVM creates a hyperplane (or set of hyperplanes), or a separating line between data belonging to different classes, for classification. It seeks to identify the optimal hyperplane by maximizing the distance between the hyperplane and the closest data points in each class. By maximizing the distance between the hyperplane and the nearest data points in each class, the SVM model minimizes the generalization error of the classifier (41).

For the present analyses, in the SVM method, we used a Gaussian kernel function and a one-versus-one coding design, which yields two (or three) binary learners and for two (or

three) classes. To create a receiver operating characteristic curve, we transformed SVM classification scores to class posterior probabilities, which are obtained by predicting the maximum class posterior probability at each point in a grid.

2.3.2. Random forest

Random forest is another supervised learning model that can be used for classification. It uses ensemble learning, meaning it combines multiple models to solve complex problems, rather than using an individual model (32). The random forest algorithm relies on bagging or bootstrap aggregating to improve accuracy. It uses random subsets of a training dataset to generate individual decision trees for each subsample. Each decision tree will produce an output (i.e., a classification). The final output is chosen based on "majority voting;" in other words, the random forest output is the class that is chosen by the most trees. The random forest approach can reduce the effects of overfitting in individual decision trees (42).

For the present study, in the random forest model, we trained an ensemble of 100 classification trees using the entire training dataset. A random subset of predictors was used at each decision split. The selection of the split predictors aims to maximize the split-criterion gain over all possible splits of all predictors. The number of candidate predictors considered for each tree (i.e., mtry) differed for each model such that $mtry = roundup\left(\sqrt{\#Features}\right)$. Random subsets of the training dataset were sampled with replacement. The final classifications are the combined results of all trees.

2.3.3. Feature selection

It should be noted that a model with few predictors is preferred, as it is less costly and time-consuming to use (43). To address this concern, many choose to employ data-driven feature selection approaches, e.g., (44) to remove non-informative features from models. Methods for data-driven feature selection include wrapper methods, which evaluate multiple models by adding and/or removing features to optimize model performance, and filter methods, which assess the relevance of features separately from the predictive models and only include predictors that meet specified criteria in the final model (43). However, both approaches have disadvantages. Wrapper methods involve the evaluation of many models, which significantly increases computation time, and it can increase the risk of over-fitting the model (43). In contrast, filter methods are more computationally efficient, but they involve using selection criteria that are not necessarily related to the optimization of the model. Moreover, because each feature is evaluated separately, it is possible that redundant features are selected for the final model, while interactions between features are not quantified during the feature selection process (43).

In addition, tree-based algorithms, such as random forest, conduct feature selection automatically. For instance, during the construction of a tree, if a feature is not employed in any split, the model is effectively independent of the feature (43). In fact, prior research suggests tuning random forest models can reduce the effect of non-informative features (45), precluding the need for feature selection in random forest approaches. Conversely, random forest is a powerful classifier because it can utilize weak features, which may be suppressed by methods such as principal component analysis, to boost the classification performance.

In the present study, we used relatively few features (i.e., 17 features), which were selected *a priori* based on existing literature. We have previously employed this approach (46), resulting in improved accuracy when compared to data-driven feature selection. Given the small number of features and the large sample size, we opted to manually combine different sets of features to test the classification accuracy, which can be more easily interpreted than using data-driven approaches to feature selection.

3. Results

In the training dataset, collected between January 2020 and December 2021, there were a total of 204,723 encounters taking place at outpatient psychiatry, family medicine, or geriatric medicine (involving 37,979 patients); there were 4,424 encounters at which a patient received at least one benzodiazepine prescription, while there were 200,299 encounters where a patient received no such prescription. Of these, there were 3,988 encounters where a patient received one benzodiazepine prescription and 436 encounters where a patient received two or more benzodiazepine prescriptions. Patient-level characteristics for the training dataset are presented by benzodiazepine prescription status in **Supplementary Table 2**.

In the test dataset, collected between January 2022 and March 2022, there were a total of 28,631 encounters (involving 14,404 patients); there were 842 encounters at which a patient received at least one benzodiazepine prescription and 27,789 where a patient received no such prescription. In the test data, there were 792 encounters where a patient received one benzodiazepine prescription and 50 encounters where a patient received two or more benzodiazepine prescriptions. Because the number of "positive" observations (i.e., received a benzodiazepine prescription) is significantly lower than the number of "negative" (i.e., did not receive a benzodiazepine prescription) observations, the number of positive and negative observations were balanced prior to model training in order to avoid bias. Patient-level characteristics for the test dataset are presented by benzodiazepine prescription status in **Supplementary Table 3**.

All prediction results (e.g., accuracy, AUC) reported in the following tables and figures were derived from the test dataset.

3.1. Prescription receipt

Table 1 and **Figure 2** display the results for the models predicting whether a patient received a benzodiazepine prescription at a given encounter (yes/no). As depicted in **Figure 2A**, for the SVM approach, overall accuracy did not improve after including the first set of features (i.e., anxiety and sleep diagnoses). For the random forest approach, Model 2 maximized overall accuracy when predicting whether a patient received a benzodiazepine prescription at a given encounter (yes/no), and the random forest model slightly outperformed the SVM model (Random Forest benzodiazepine prescription receipt Model 2 accuracy = 0.887; SVM benzodiazepine prescription receipt Model 1 accuracy = 0.883, $F(1, 58) = 52.892$, $p < 0.001$).

TABLE 1 Benzodiazepine prescription receipt (yes/no) prediction results.

		Support vector machine		Random forest		Comparison	
	Number of features	M (SD)	95% CI	M (SD)	95% CI	*F*	*p*
Model 1	4					37.940	<0.001
Accuracy		0.883 (0.003)	0.882–0.884	0.874 (0.005)	0.873–0.876	58.935	<0.001
TPR		0.828 (0.008)	0.824–0.831	0.850 (0.014)	0.845–0.855	58.935	<0.001
TNR		0.884 (0.003)	0.883–0.886	0.875 (0.006)	0.873–0.877	58.935	<0.001
AUC		0.864 (0.016)	0.858–0.870	0.877 (0.0001)	0.877–0.878	21.428	<0.001
Model 2	7					374.600	<0.001
Accuracy		0.883 (0.002)	0.882–0.884	0.887 (0.002)	0.886–0.887	52.892	<0.001
TPR		0.826 (0.006)	0.824–0.829	0.843 (0.003)	0.842–0.844	187.103	<0.001
TNR		0.885 (0.002)	0.884–0.886	0.888 (0.002)	0.887–0.889	34.678	<0.001
AUC		0.869 (0.022)	0.860–0.877	0.917 (0.001)	0.916–0.917	140.144	<0.001
Model 3	11					832.822	<0.001
Accuracy		0.868 (0.0003)	0.868–0.869	0.860 (0.003)	0.859–0.861	251.494	<0.001
TPR		0.882 (<0.0001)	0.882–0.882	0.925 (0.004)	0.924–0.927	3184.364	<0.001
TNR		0.868 (0.0003)	0.868–0.868	0.858 (0.003)	0.857–0.859	320.672	<0.001
AUC		0.909 (0.017)	0.902–0.915	0.938 (0.001)	0.938–0.939	95.037	<0.001
Model 4	15					571.875	<0.001
Accuracy		0.868 (0.0004)	0.868–0.869	0.872 (0.004)	0.871–0.874	34.529	<0.001
TPR		0.882 (<0.0001)	0.882–0.882	0.924 (0.006)	0.922–0.927	1,534.352	<0.001
TNR		0.868 (0.0004)	0.868–0.868	0.871 (0.004)	0.869–0.872	15.506	<0.001
AUC		0.924 (0.008)	0.920–0.927	0.951 (0.001)	0.951–0.952	341.328	<0.001
Model 5	17					767.678	<0.001
Accuracy		0.869 (<0.0001)	0.869–0.869	0.875 (0.003)	0.874–0.876	163.709	<0.001
TPR		0.882 (<0.0001)	0.882–0.882	0.924 (0.005)	0.922–0.926	1,970.827	<0.001
TNR		0.868 (<0.0001)	0.868–0.868	0.873 (0.003)	0.872–0.874	101.359	<0.001
AUC		0.923 (0.013)	0.918–0.928	0.953 (0.001)	0.953–0.953	156.308	<0.001

Results of the one-way multivariate analysis of variance (MANOVA) comparing prediction performance between support vector machine and random forest approaches for each model. TPR, true positive rate; TNR, true negative rate; AUC, area under the receiver operating characteristic curve.

However, as shown in Figure 2B and Figure 3, when examining the AUC, Model 4 maximized the AUC for the SVM approach when predicting benzodiazepine prescription receipt at an encounter (SVM benzodiazepine prescription receipt Model 4 AUC = 0.924), while Model 5 maximized the AUC for the random forest approach when predicting whether a patient received a benzodiazepine prescription (Random Forest benzodiazepine prescription receipt Model 5 AUC = 0.953).

3.2. Number of prescriptions

Table 2 and Figure 4 display the results for the models predicting the number of benzodiazepine prescriptions received at a given encounter (0, 1, 2+). As demonstrated in Figure 4, Model 2 maximized overall accuracy when predicting how many benzodiazepine prescriptions a patient received at an encounter (0, 1, 2+), with the random forest model slightly outperforming the SVM model (Random Forest number of benzodiazepines Model 2 accuracy = 0.878; SVM number of benzodiazepines Model 2 accuracy = 0.877, $F(1, 28) = 0.808$, $p = 0.372$), though this difference was not statistically significant.

4. Discussion

Benzodiazepines, which are associated with risk of serious adverse effects (5, 6), represent a significant public health burden (2). Research suggests certain clinical (12, 17–19) and demographic factors (9, 10, 12, 14–16) are associated with benzodiazepine use. However, to our knowledge there is no predictive algorithm which exists that can classify patients by whether they are likely to receive a benzodiazepine prescription and the number of benzodiazepine prescriptions they are likely to receive at a given encounter. The present study used SVM and random forest approaches to develop an algorithm to predict whether a patient is likely to receive a benzodiazepine prescription at a given encounter and how many benzodiazepine prescriptions they are likely to receive at a given encounter, which could facilitate efforts to reduce the public health burden of benzodiazepine use and misuse. We

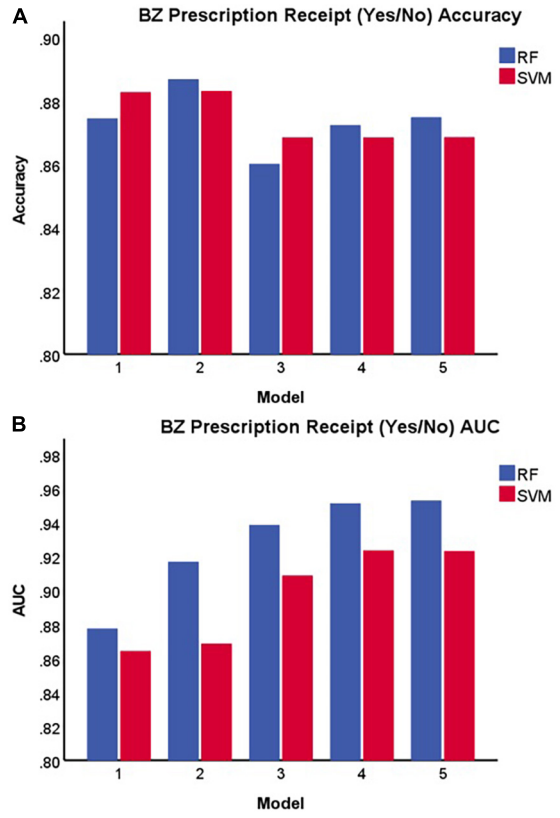

FIGURE 2
Accuracy **(A)** and area under the receiver operating characteristic curve **(B)** of each model in predicting benzodiazepine prescription receipt at a given encounter (yes/no). BZ, benzodiazepine; SVM, support vector machine; RF, random forest; AUC, area under the receiver operating characteristic curve.

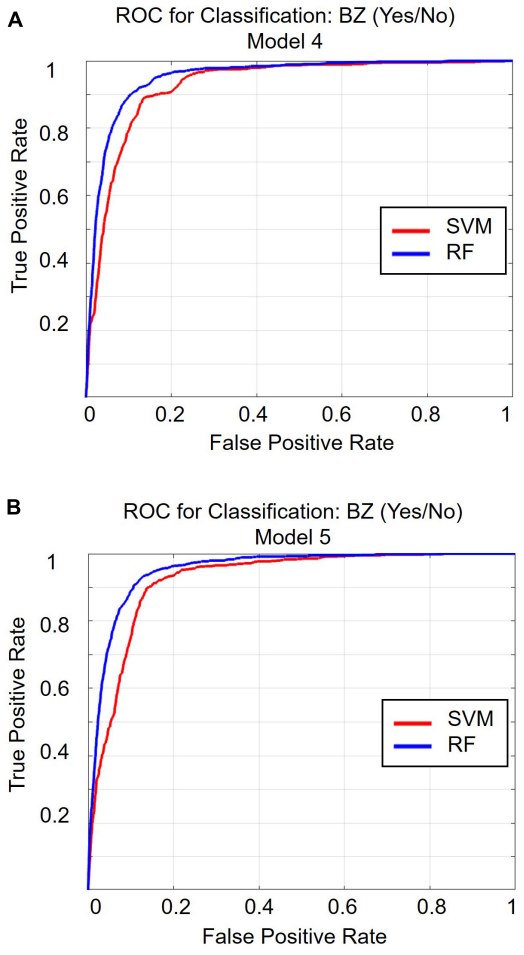

FIGURE 3
Receiver operating characteristic curve for classification Model 4 **(A)** and for classification Model 5 **(B)**. ROC, receiver operating characteristic curve; BZ, benzodiazepine; SVM, support vector machine; RF, random forest.

took a step-wise approach to developing a prediction model in order to determine which categories of features are needed to predict benzodiazepine prescriptions accurately. Based on this analysis, both SVM and random forest algorithms may accurately classify individuals who receive a benzodiazepine prescription and can separate patients by the number of benzodiazepine prescriptions received, though there are some differences in performance between the approaches. This proof-of-concept study demonstrates the potential of machine learning approaches in identifying individuals to target for prevention efforts to reduce the burden of benzodiazepine use and inadequately treated anxiety and sleep disorders.

For the SVM approach, overall accuracy did not improve beyond Model 1 (i.e., anxiety and sleep disorder diagnoses), while for the random forest approach, Model 2 (i.e., anxiety and sleep disorder diagnoses and demographic characteristics) maximized overall accuracy when predicting whether a patient received a benzodiazepine prescription at a given encounter (yes/no). For both machine learning approaches, Model 2 maximized overall accuracy when predicting the number of benzodiazepine prescriptions received at an encounter (0, 1, 2+). The random forest model slightly outperformed the SVM model for both outcomes of interest. Of note, including additional groups of features beyond anxiety and sleep diagnoses and demographic characteristics did not improve overall accuracy and, in fact, decreased accuracy slightly. This runs counter to prior research suggesting co-prescriptions, comorbid conditions, and insurance status are important predictors of receiving a benzodiazepine prescription (12). It is possible that the predictive value of those factors is better accounted for by sleep and anxiety disorder diagnoses or patients' demographic characteristics (i.e., race, age, or gender).

It should be noted that although overall accuracy did not improve when more categories of features were added, including co-prescribed medications in both the SVM and the random forest models improved the true positive rate for benzodiazepine prescription receipt, as well as the number of benzodiazepines prescribed, at a given encounter. Furthermore, including other clinical variables (i.e., any mood disorder diagnosis, any psychotic disorder diagnosis, any neurocognitive disorder diagnosis, prescriber specialty) and insurance status (i.e., whether the patient has insurance, type of insurance) improved the true positive rate for two or more benzodiazepine prescriptions. This suggests that decisions about which categories of features to include in a model may be driven by whether the system

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TABLE 2 Number of benzodiazepine prescriptions (0, 1, 2+) prediction results.

		Support vector machine		Random forest		Comparison	
	Number of features	M (SD)	95% CI	M (SD)	95% CI	*F*	*p*
Model 1	4					2.167	0.085
Accuracy		0.877 (0.006)	0.875–0.879	0.876 (0.007)	0.874–0.879	0.189	0.666
TPR1		0.806 (0.024)	0.800–0.815	0.819 (0.024)	0.810–0.828	4.340	0.042
TPR2		0.081 (0.048)	0.064–0.099	0.051 (0.039)	0.036–0.066	7.223	0.009
TNR		0.880 (0.006)	0.878–0.883	0.879 (0.008)	0.876–0.882	0.346	0.558
Model 2	7					51.346	<0.001
Accuracy		0.877 (0.006)	0.874–0.879	0.878 (0.004)	0.877–0.880	0.808	0.372
TPR1		0.805 (0.024)	0.796–0.814	0.727 (0.020)	0.719–0.734	182.135	<0.000
TPR2		0.084 (0.046)	0.067–0.102	0.220 (0.055)	0.199–0.241	106.706	<0.000
TNR		0.880 (0.007)	0.878–0.883	0.884 (0.004)	0.882–0.885	4.859	0.031
Model 3	11					212.277	<0.001
Accuracy		0.864 (0.006)	0.862–0.867	0.846 (0.004)	0.844–0.847	192.047	<0.001
TPR1		0.851 (0.021)	0.843–859	0.741 (0.021)	0.734–0.749	403.404	<0.001
TPR2		0.071 (0.053)	0.051–0.091	0.307 (0.039)	0.293–0.322	391.945	<0.001
TNR		0.866 (0.007)	0.864–0.869	0.850 (0.005)	0.848–0.851	130.430	<0.001
Model 4	15					44.441	<0.001
Accuracy		0.861 (0.006)	0.859–0.864	0.853 (0.007)	0.850–0.855	25.549	<0.001
TPR1		0.732 (0.019)	0.725–0.739	0.783 (0.013)	0.778–0.788	147.144	<0.001
TPR2		0.371 (0.018)	0.365–0.378	0.380 (0.040)	0.365–0.395	1.248	0.269
TNR		0.866 (0.007)	0.863–0.868	0.856 (0.007)	0.853–0.858	32.896	<0.001
Model 5	17					39.007	<0.001
Accuracy		0.861 (0.006)	0.859–0.863	0.852 (0.007)	0.849–0.854	31.096	<0.001
TPR1		0.732 (0.023)	0.723–0.741	0.789 (0.013)	0.784–0.794	135.405	<0.001
TPR2		0.372 (0.013)	0.367–0.377	0.313 (0.041)	0.298–0.329	54.608	<0.001
TNR		0.866 (0.006)	0.864–0.868	0.855 (0.008)	0.852–0.857	38.426	<0.001

Results of the one-way multivariate analysis of variance (MANOVA) comparing prediction performance between support vector machine and random forest approaches for each model. TPR, true positive rate; TNR, true negative rate; TPR1, true positive rate for 1 benzodiazepine prescription; TPR2, true positive rate for 2 or more benzodiazepine prescriptions.

employing these machine learning methods is motivated primarily by maximizing sensitivity or specificity. For example, given that an intervention to reduce or prevent benzodiazepine prescribing represents a low risk to the patient, some hospital systems may prefer to use a prediction model that maximizes sensitivity, as false positives would not be a major concern. In contrast, if a hospital system is extremely resource-limited, they may prefer to maximize specificity.

In light of interpretation guidelines (38, 39), all of the SVM and random forest models predicting whether a patient received a benzodiazepine prescription at a given encounter (yes/no) tested in the present study demonstrate good to excellent predictive ability. Model 4 yielded the maximum AUC value for the SVM approach, suggesting that including the most relevant diagnoses, demographic characteristics, co-prescribed medications, and other clinical variables maximizes the predictive value for SVM. However, when using the random forest approach, Model 5 yielded the maximum AUC, suggesting insurance status offers additional predictive value. Both of these approaches yielded AUC values in the excellent range, with random forest slightly outperforming SVM. Thus, employing a random forest approach that utilizes all of the categories of features tested in the present study yields the maximum predictive value when evaluated *via* AUC.

One finding of note in the present study is that although overall accuracy is high for both the prediction of whether a patient will receive a benzodiazepine prescription at a given encounter and the number of benzodiazepines received, the true positive rate for identifying patients who received two or more benzodiazepine prescriptions at a given encounter was relatively low for both the SVM and random forest approach. This may be due to the relatively low base rate of patients receiving multiple benzodiazepine prescriptions at an encounter. To account for this obstacle, in the present study the number of positive and negative observations were balanced prior to model training in an attempt to avoid bias. However, despite low base rate questions being widely recognized as a concern in machine learning, the best method for accounting for this imbalance remains an open question (47). Further research is needed to determine

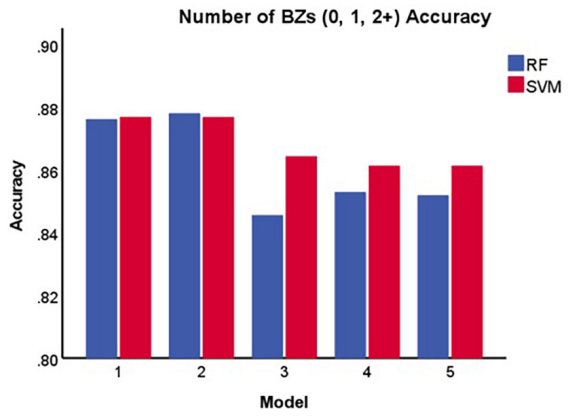

FIGURE 4
Accuracy of each model in predicting the number of benzodiazepines a patient receives at a given encounter (0, 1, 2+). BZ, benzodiazepine; SVM, support vector machine; RF, random forest.

how to best predict the likelihood of receiving two or more benzodiazepine prescriptions.

The present proof-of-concept study suggests that we can predict whether an individual is likely to receive a benzodiazepine prescription at a given encounter and how many benzodiazepine prescriptions they are likely to receive based on information from their electronic health record, with good to excellent predictive ability. Future research is needed to determine whether these predictive models could be useful in a clinical context by alerting providers to a patient's classification and offering suggestions for how to proceed in light of the risks benzodiazepines can pose to patients' health (5, 6). For example, if the predictive models used in the present study were employed by a hospital system, a message could be triggered by the algorithm in a patient's chart that informs a provider of the patient's risk, provides information on first-line treatments for anxiety and sleep conditions, and makes treatment recommendations. This may include suggesting that the provider refer the patient to cognitive behavioral therapy for anxiety or sleep disturbance (48–51), attempt treatment with a selective-serotonin reuptake inhibitor for anxiety (52), and/or offer the patient educational materials on sleep hygiene and coping skills. Further research is needed to determine whether such an intervention reduces the public health burden of benzodiazepine use and inadequately treated anxiety and sleep disorders. Moreover, the same machine learning methods used in the present study could be applied to examine who is likely to convert to higher risk use (e.g., long-term or high-dose use) (5) if provided a benzodiazepine prescription. Similar methods have been successfully applied to the prediction of opioid use disorder onset (53), sustained opioid prescription (34), and opioid overdose (35). In addition, future research should investigate the utility of employing these machine learning models in longitudinal follow-up data to identify patients who, when prescribed a benzodiazepine, are at elevated risk of side effects or other complications. This would allow for prevention efforts to be targeted at patients who are at the greatest risk of suffering the negative consequences of benzodiazepine use.

The present findings should be interpreted in light of the study's limitations. First, due to the approach used in the present study, we were unable to ascertain which specific features had the best predictive value. Additionally, the models used in the present study did not provide information on the direction of the relationship between features and the likelihood of receiving a benzodiazepine prescription, although the extant literature provides clues. Moreover, we did not control for benzodiazepine prescription history. Therefore, it is possible that a patient had already received a benzodiazepine prescription prior to the encounters examined in the current study or that patients received benzodiazepine prescriptions by other providers not captured in the current dataset. Finally, there may be additional features that were not included in our models but have value in predicting benzodiazepine prescriptions.

Taken together, the present study suggests SVM and random forest predictive models based on anxiety and sleep diagnoses and demographic characteristics can accurately classify individuals who receive a benzodiazepine prescription and can separate patients by the number of benzodiazepine prescriptions received, with random forest slightly outperforming SVM approaches. Moreover, including additional features can improve the AUC. If results are replicated, machine learning approaches may be useful in determining who to target for prevention efforts to reduce the public health burden of benzodiazepine use and misuse.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: The data warehouse from which the information for this study is derived has been de-identified and date-shifted so that it does not include any protected health information (PHI). Pursuant to 45 CFR 46, use of this database does not meet the definition of human subjects' research and does not require IRB review. UMMC Faculty, Staffs, and Students can access this dataset. Requests to access these datasets should be directed to Center for Informatics and Analytics, cia@umc.edu.

Author contributions

KK designed the study in consultation with all co-authors and drafted the manuscript. YZ conducted the machine learning analyses and provided feedback on the methods and results sections. MM, JS, and JR participated in the design of the study. SB provided consultation on clinicians' benzodiazepine prescription decision-making processes. All authors provided feedback on the manuscript and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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Vitor Tardelli,
University of Toronto, Canada

REVIEWED BY
Riccardo Maccioni,
The Scripps Research Institute, United States
Gerasimos Konstantinou,
University of Toronto, Canada

*CORRESPONDENCE
Yoshikazu Takaesu
✉ takaesuy@med.u-ryukyu.ac.jp

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Development and acceptability of a decision aid for anxiety disorder considering discontinuation of benzodiazepine anxiolytic

Yumi Aoki[1,2], Yoshikazu Takaesu[2,3]*, Ken Inada[4], Hiroki Yamada[5], Tomohiko Murao[6], Toshiaki Kikuchi[7], Masahiro Takeshima[8], Masayuki Tani[9], Kazuo Mishima[8] and Tempei Otsubo[10]

[1]Department of Psychiatric and Mental Health Nursing, Graduate School of Nursing, St. Luke's International University, Tokyo, Japan, [2]Department of Neuropsychiatry, Kyorin University School of Medicine, Tokyo, Japan, [3]Department of Neuropsychiatry, Graduate School of Medicine, University of the Ryukyus, Okinawa, Japan, [4]Department of Psychiatry, Kitasato University School of Medicine, Sagamihara, Kanagawa, Japan, [5]Department of Psychiatry, Showa University Northern Yokohama Hospital, Yokohama, Kanagawa, Japan, [6]Yutaka Clinic, Kanagawa, Japan, [7]Department of Neuropsychiatry, Keio University School of Medicine, Tokyo, Japan, [8]Department of Neuropsychiatry, Akita University Graduate School of Medicine, Akita, Japan, [9]Department of Psychiatry, Oouchi Hospital, Tokyo, Japan, [10]Department of Psychiatry, Tokyo Women's Medical University Adachi Medical Center, Tokyo, Japan

Aim: We aimed to develop a decision aid (DA) for individuals with anxiety disorders who consider tapering benzodiazepine (BZD) anxiolytics, and if tapering, tapering BZD anxiolytics with or without cognitive behavioral therapy (CBT) for anxiety. We also assessed its acceptability among stakeholders.

Methods: First, we conducted a literature review regarding anxiety disorders to determine treatment options. We cited the results of the systematic review and meta-analysis, which we conducted previously, to describe the related outcomes of two options: tapering BZD anxiolytics with CBT and tapering BZD anxiolytics without CBT. Second, we developed a DA prototype in accordance with the International Patient Decision Aid Standards. We carried out a mixed methods survey to assess the acceptability among stakeholders including those with anxiety disorders and healthcare providers.

Results: Our DA provided information such as explanation of anxiety disorders, options of tapering or not tapering BZD anxiolytics (if tapering, the options of tapering BZD anxiolytics with or without CBT) for anxiety disorder, benefits and risks of each option, and a worksheet for value clarification. For patients (n=21), the DA appeared to be acceptable language (86%), adequate information (81%), and well-balanced presentation (86%). The developed DA was also acceptable for healthcare providers (n=10).

Conclusion: We successfully created a DA for individuals with anxiety disorders who consider tapering BZD anxiolytics, which was acceptable for both patients and healthcare providers. Our DA was designed to assist patients and healthcare providers to involve decision-making about whether to taper BZD anxiolytics or not.

KEYWORDS

anxiolytic, anxiety disorder, benzodiazepine, decision aid, shared decision making

1. Introduction

Anxiety disorders are common mental disorders characterized by emotional and stress reactions to a threat or anticipation of future concern (1), leading to a significant effect on a person's physical and social functioning. Previous research revealed that individuals with anxiety disorders are associated with significant impairment to personal life (2) and quality of life (3), suicidal ideation and suicide attempts (4), and high care costs (5). Therefore, continued improvement in the care of people with anxiety disorders is important.

Benzodiazepine (BZD) anxiolytics are one of the treatment choices that are frequently used worldwide for the acute phase of anxiety disorders. However, the long-term BZD anxiolytic use is not recommended because of its disadvantages, including dependence (6), decline in cognitive functions (7), hip fractures associated with falls (8, 9), and impaired driving ability (10). Consequently, most anxiety disorder guidelines recommend that BZD anxiolytics should be used for only a short period (11–15). Moreover, some guidelines do not recommend the use of BZD anxiolytics, even for short-term periods, except in critical situations (16, 17).

Despite the evidence-based recommendations described above, BZD anxiolytics are commonly used worldwide for anxiety disorders (18, 19). Therefore, the safe discontinuation or tapering of BZD anxiolytics for anxiety disorders is essential. Thus, the establishment of treatment strategy against long-term BZD use for anxiety disorders may be warranted in clinical settings.

To address this issue, the evidence that psychological therapy is effective in reducing symptoms for anxiety disorders should be considered (20). Particularly, cognitive behavioral therapy (CBT) is an effective psychological intervention for anxiety disorders (21, 22). Several current guidelines recommend CBT as a first-line therapy because of its effectiveness in improving anxiety symptoms and comparatively fewer risks than BZD anxiolytics (11, 12, 17). Several trials assessing strategies for BZD discontinuation, such as gradual tapering or adding CBT, have reported the effectiveness of adding CBT in the short term (23). On the other hand, CBT has certain disadvantages, such as the lack of a fast-acting effect, longer consultation time, and high cost (24). Therefore, individuals with anxiety disorders deliberating on further non-medication treatment might face the advantages and disadvantages of CBT.

Approaches of treatment decision-making have shifted from the so-called paternalistic approach, where doctors take initiative in the decision-making, to patient-centered communication. In this type of approach, strategies such as "shared decision making" (SDM) have been emphasized, which focus on a patient's value-based discussion that involves a two-way communication between the patient and their clinician about the positive and negative aspects of each treatment option (25, 26).

In relation to the SDM process, decision aids (DAs) have recently gained attention as patient-centered communication tools that promote two-way conversation between patients and healthcare providers during specific medical or mental conditions that require further treatment planning (27). DAs are intended to support individuals participating in the decision-making process by aiding them to make well-informed, preference-based choices when choosing their treatment options (27). DAs provide related information regarding the available options and aid people to solidify their own preferences, which are associated with different characteristics of each option (27). DAs can promote a patient's involvement and increase concordance between their choices, preferences, and values during the decision-making process (28).

Various DAs, most of which were for decision-making during treatment initiation, have been developed in many areas including the somatic and psychiatric fields (28). Moreover, we developed several DAs for decision-making about whether the treatment should be continued or discontinued such as DA for depression remission (29) and DA for insomnia remission (30). Ramos-García et al. developed a Spanish version of DA for patients with generalized anxiety disorder (31), based on their needs that patients with GAD preferred an active and collaborative role in decision-making (32). However, to our best knowledge, there is no Japanese version of a DA for patients with anxiety disorders who are receiving BZD anxiolytics and considering further pharmacology treatment.

The aim of this study was to develop a Japanese version of DA for patients with anxiety disorders who are considering whether to discontinue BZD anxiolytics as well as whether to taper them with CBT or without CBT, if discontinuing BZD. The stakeholder's acceptability of the DA were also examined. We have translated the DA into English so that many more people can utilize it.

2. Methods

2.1. Study design and conceptual framework

The Ottawa Decision Support Framework (33) and International Patient Decision Aid Standards (IPDAS) were used to systematically develop the DA (34) (Figure 1). The IPDAS is one of the evidence-based frameworks that was established to standardize the development process and elements of DAs (35). The development process is as follows: (1) deciding the target people and assessing their decision-making needs, (2) establishing a steering committee made up by mental health professionals, (3) performing a literature review to decide the treatment options and related evidence-based outcomes, (4) creating a prototype of the DA, (5) assessing the acceptability of the prototype among stakeholders including patients and healthcare providers, (6) correcting the DA using the results of acceptability tests to create a final version of the DA, and (7) testing the developed DA for its effectiveness in clinical environment (35).

2.2. Determining the target population

The target people of the DA in this study was those who had been diagnosed with anxiety disorders, such as social anxiety disorder, generalized disorder, and panic disorder, and showed improvements in their symptoms and health conditions following treatment with BZD anxiolytics. Patients who were on medication but still experiencing symptoms were not targeted by the DA. The steering group expect that the DA would be useful in both primary care clinics and psychiatric outpatient clinics.

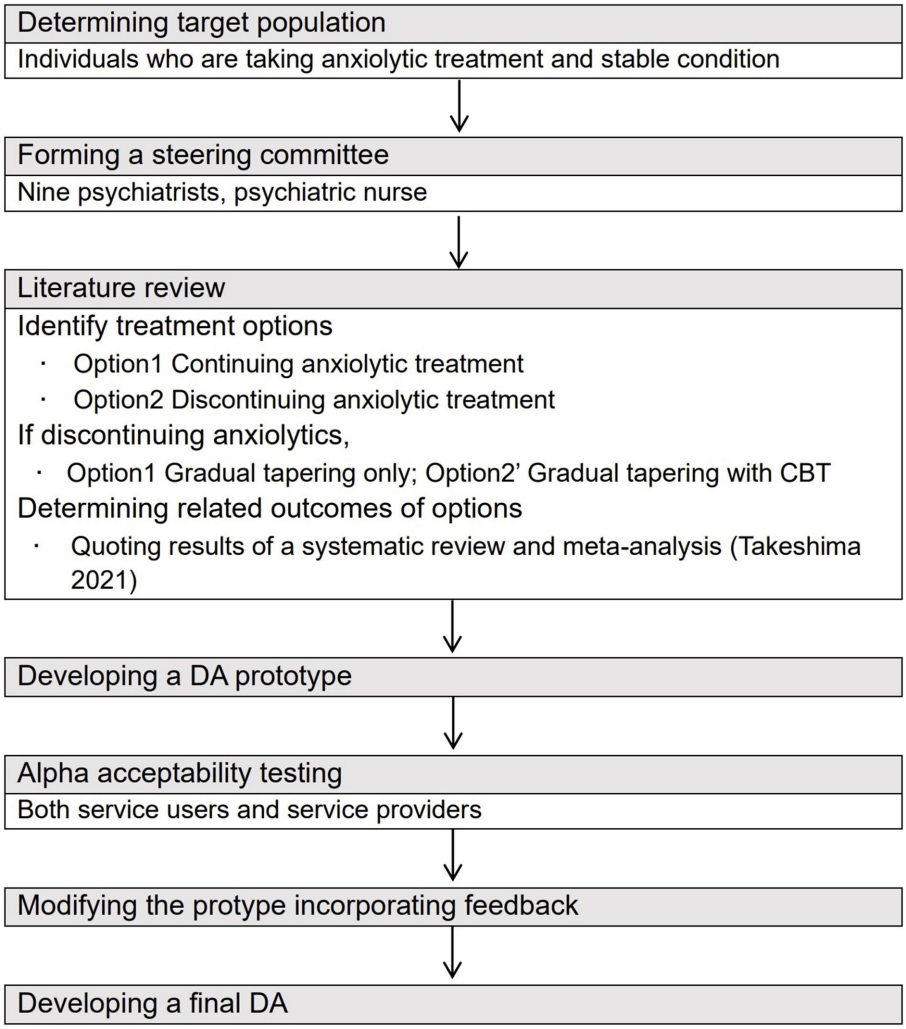

FIGURE 1
Process of developing a DA for those with anxiety disorder who consider tapering anxiolytics based on the approach of Coulter et al. (2013) (34).

2.3. Assembling a steering committee members

The authors established a steering committee consisting of mental health professionals on anxiety disorders and DA methodology. The group was consisted of nine psychiatrists who regularly saw people with anxiety disorders and a psychiatric nurse who was familiar with SDM literature in the mental health field (36) and had experience creating DAs for mood disorders (29, 37), insomnia (30), and attention deficit hyperactivity disorder (38).

2.4. Literature review for exploring the related outcomes of each treatment option

The steering committee members examined the relevant published articles that explained anxiety disorders as a target disease and explored the advantages and disadvantages of the following treatment options: (1) continuing BZD anxiolytics, (2) tapering BZD anxiolytics, if tapering (3) gradually tapering BZD anxiolytics without CBT, and (4) gradually tapering BZD anxiolytics with CBT.

For the outcomes of the last two options, the committee referred to the results of a systematic review and meta-analysis that the authors had conducted and reported in detail elsewhere earlier (39). The meta-analysis indicated that CBT might be effective for stopping BZD anxiolytics, both in the short term (≤3 months) and long term (12 months) (39). Furthermore, references regarding the lifestyle changes that individuals with anxiety disorders can implement in daily life as self-management were also searched.

2.5. Developing the DA prototype

The committee members created a DA prototype according to the quality criteria of the IPDAS (33), citing the results of our literature review described above (39). DAs are basically of two types: one DA is for preparation for discussion with healthcare providers (designed to be used by patients at home) and the other DA is for conversation

between patients and health care professionals to share decisions during clinical consultations (designed to encourage patients to be actively involved in conversations) (40). Our DA included both of those functions: preparation aid before consultation and conversation aid during consultation. For the preparation aid, the DA prototype provided queries to be selected by putting a check mark (worksheet for value clarification) and a box for any additional comments to be completed at home, which would be shared and discussed with their doctors during consultation. DAs should be understood by people who are unfamiliar with medical knowledge and therefore should be developed using eighth-grade level language (41). Considering this, the committee attempted to use simpler expressions. Moreover, in accordance with previously published evidence-based DAs, we described the outcome probabilities using pictograms, which showed how many people out of 100 would experience an event so that it could be easily understood by people with any literacy level (42).

2.6. Acceptability testing

We conducted acceptability testing of the DA prototype by surveying stakeholders. We adopted a mixed-methods survey.

Following a validated acceptability scoring measurement that assess the comprehensiveness of the DA in terms of its length, amount of information, balance of provided information, and ability to target decisions (43). This is the common DA development process that ensures the quality of the final version of the DA in accordance with stakeholder evaluation.

We recruited patients from the psychiatric outpatient departments of our university hospitals. Outpatients were approached if they fulfilled the following conditions: (i) aged ≥20 years, (ii) using BZD anxiolytics for at least 3 months, and (iii) showing improvements in their symptoms and health condition due to treatment with BZD anxiolytics. Furthermore, health care providers who regularly provided consultation to patients with anxiety disorders from the same department as those used by the outpatients were recruited. Approximately 20 individuals from each group were included in this study. The sample size was determined following the methods used in previous studies on DA development and acceptability testing (29, 30). Both the individuals with anxiety disorder and healthcare professionals were asked to read the DA prototype and participate in the survey. Finally, we modified and improved the DA prototype to create a final version using the results of acceptability testing.

3. Results

3.1. Components of the DA prototype

Our DA prototype was a 32-page A5 booklet, which contained a description of the target people, instruction on how to use this tool, and an explanation of anxiety disorders. The prototype next provided the options of continuing (option 1) or tapering BZD anxiolytics (option 2), the advantages and disadvantages of each option, and a worksheet for value clarification. The booklet further prepared a box for those with anxiety disorders to put down any queries or comments to their clinicians, which could be asked in the next consultation on whether to continue or taper BZD anxiolytics. Additionally, for the tapering current anxiolytics option, the DA prototype showed additional options for gradually tapering BZD anxiolytics without CBT (option 1′) or with CBT (option 2′). For each option, the DA prototype recommended gradual tapering which involved reducing the dose by ≤25% over 4–8 weeks to prevent rebound anxiety, based on the current guidelines for BZD (15). Next, our DA described the advantages and disadvantages of these two options, along with a worksheet of value clarification for each option. The outcomes of each option were cited according to the outcomes of the meta-analysis that the authors had previously conducted, which found that gradual tapering with CBT was more effective than gradual tapering without CBT for success of stopping BZD anxiolytics both in the short-term (≤3 months) and long-term (12 months) (39). We described this evidence in the DA prototype using pictorial diagrams consisting of 100 faces, in which the number of colored faces meant the proportion of individuals who were predicted to experience the outcomes (Figure 2). Moreover, the DA prototype had a box for additional comments or queries to their clinicians, which could be asked in the next consultation on whether to taper BZD anxiolytics with CBT or without CBT. Supplementary material S1 showed the detailed information of the DA prototype.

3.2. Acceptability testing

3.2.1. Patients

Twenty-one patients with anxiety disorders, such as general anxiety disorder (GAD) with sleep disorder ($n = 6$), GAD ($n = 2$), panic disorder (PD) ($n = 2$), PD with sleep disorder ($n = 1$), depression with GAD and sleep disorder ($n = 1$), depression with PD and sleep disorder ($n = 1$), PD with social anxiety disorder and sleep disorder ($n = 1$), and unknown ($n = 7$) participated in the DA acceptability testing. Ten patients (48%) were taking antidepressants as well as benzodiazepine anxiolytics, 4 (19%) were not, and 7 (33%) were unknown. Ten patients were taking hypnotics besides benzodiazepine anxiolytics, 4 (19%) were not taking them, and 7 (33%) were unknown whether to take them. Among the 21 patients, 14 (67%) have no CBT experience, while 7 (33%) were unknown. The mean age of the participants was 48.0 (±9.2) years, among which 14 (67%) were women, 5 (24%) were men, and 2 (10%) were unknown. Nine participants (43%) had a high school degree or lower level of education, 4 (19%) had vocational college level education, and 8 (38%) were university graduates.

Table 1 shows that the results of the patients' feedback. The length of explanation or instruction was reported to be "just right" in 18 of 21 participants (86%). The amount of provided information was judged as "just right" in 17 of 21 participants (81%). The presentation of both options was rated as not biased but well balanced in 20 of 21 participants (95%). The DA was considered to be useful for decision-making about whether to taper anxiolytic drug or not in 17 of 20 patients (85%). A total of 14 of 20 patients (70%) thought that they could foresee their chance of successful stopping of current anxiolytics using the DA. Finally, 17 of 19 participants (89%) reported that the DA enabled easy decision making, while 18 of 21 participants (86%) thought that the DA had enough information to support to decide whether to continue or taper anxiolytics.

In the comments from the participants, overall positive feedback on the DA prototype were observed. Some quotations are shown below.

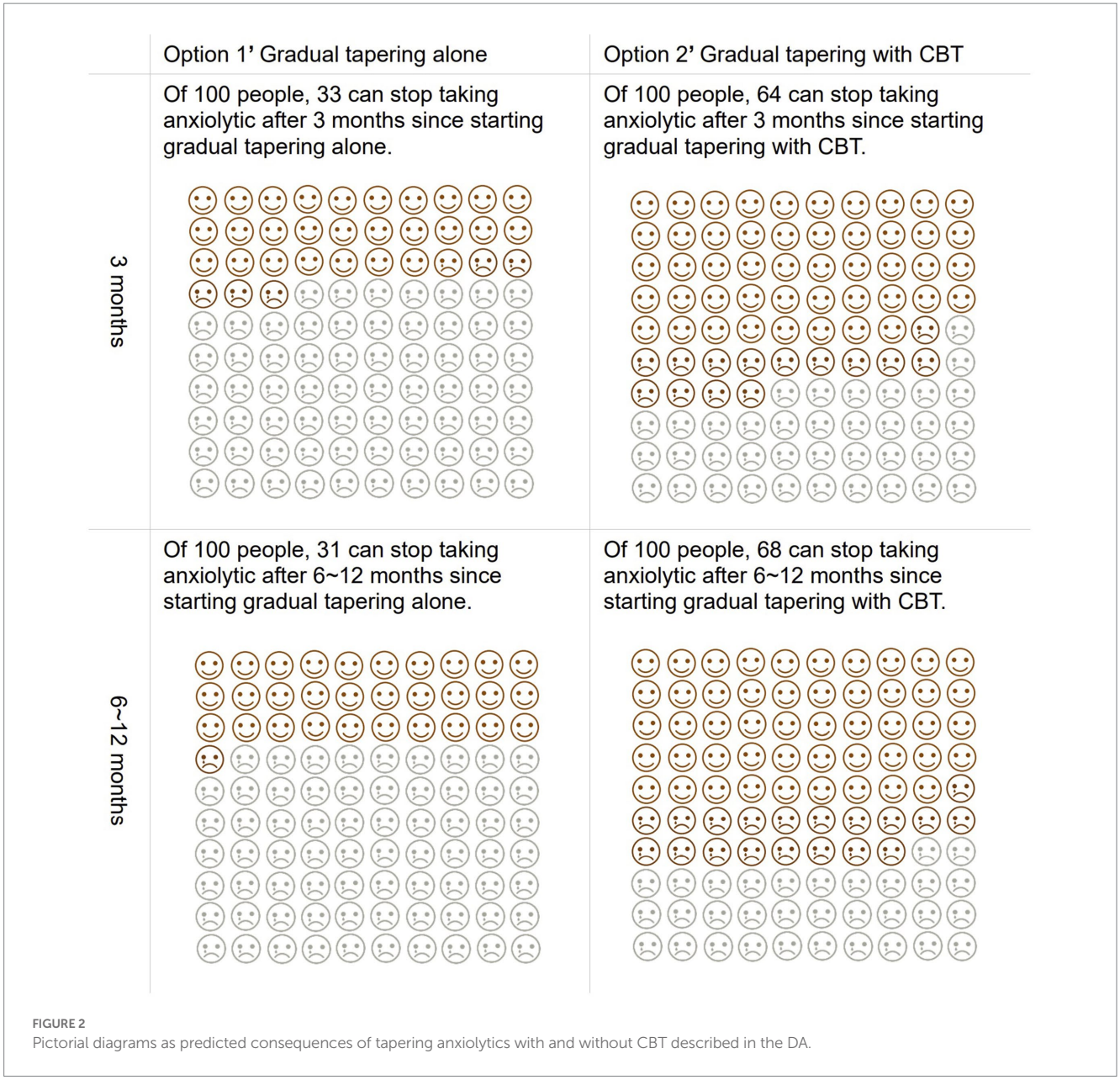

FIGURE 2
Pictorial diagrams as predicted consequences of tapering anxiolytics with and without CBT described in the DA.

TABLE 1 Patient assessment on the way information is presented in each section of the prototype (n=21).

	Mean	SD
About this booklet/Instructions on use	3.00	0.63
What is anxiety disorder?	3.29	0.56
Further treatment options	3.10	0.62
Comparing pros and cons of each option	3.10	0.77
Value clarification	3.19	0.81
Preparation for SDM	3.14	0.85
Appendices	3.43	0.51

SD, Standard Deviation. Rating system: four-point Likert scale from 1 to 4, 4 being excellent, 3 for good, 2 for fair, and 1 for poor.

> *"I thought it was a good way to discuss and decide together."* (Patient 8).

> *"This is a good opportunity to fully think about anxiety disorder and my current treatment."* (Patient 10).
>
> *"I liked that it was explained in a way that made it easy for my family members who do not have a good knowledge about anxiety disorder to be able to read and understand it."* (Patient 11).
>
> *"This is good because I had felt that my doctors had not given me much detailed information about my treatment so far."* (Patient 17).
>
> *"I could understand my current condition. This booklet gave me an indication of what stage of treatment I was at."* (Patient 19).
>
> *"I thought it was good to be able to organize my thoughts and concerns in advance for the consultation."* (Patient 20).

Furthermore, suggestions were provided to include additional explanations of some terms.

TABLE 2 Healthcare providers' perceptions of the DA prototype (n=20).

	Mean	SD
It will be easy for me to use.	4.10	0.74
It is easy for me to understand.	4.30	0.48
It will be easy for me to experiment with using the strategy before making a final decision to adopt it (n = 19)	3.89	0.33
The results of using the strategy will be easy to see.	4.10	0.74
This strategy is better than how I usually go about helping patients decide about continuing or stopping anxiolytics.	4.20	0.79
This strategy is compatible with the way I think things should be done (n = 19)	4.33	0.71
The use of this strategy is a more cost-effective than my usual approach to helping patients decide about continuing or stopping anxiolytics	3.50	0.85
Compared with my usual approach, this strategy will result in my patients making more informed decisions.	4.70	0.48
Using this strategy will save me time.	3.80	1.14
This strategy is a reliable method of helping patients make decisions about continuing or stopping anxiolytics	4.40	0.52
Pieces or components of the strategy can be used by themselves.	3.70	0.67
This type of strategy is suitable for helping patients make value laden choices.	4.20	1.03
This strategy complements my usual approach.	3.70	1.16
Using this strategy does not involve making major changes to the way I usually do things.	3.90	0.57
There is a high probability that using this strategy may cause/result in more benefit than harm.	4.30	0.48

SD, Standard Deviation. Scored range from 1 = strongly disagree to 5 = strongly agree.

3.2.2. Healthcare providers

Ten clinicians participated in the DA acceptability testing. The mean age of the clinicians was 37.3 (±10.1) years, and they included 2 (20%) women and 8 (80%) men.

The overall reaction of the DA prototype was preferable (Table 2). The comments from the clinicians contained several positive aspects of the DA prototype, including the concept of shared decision-making, visualization and friendly illustration, simple wording, and presentation of not biased either option.

The examples of comments from clinicians are provided below.

> *"I found the explanations with illustrations on how to taper off medication easy to understand."* (Clinician 1).
> *"I wanted to use it immediately in my clinic."* (Clinician 4).
> *"I did not know that I could make use of this kind of booklet before, so it's a novelty."* (Clinician 5).
> *"It is nice that patients can gain basic knowledge about anxiety disorders and its treatment, which would help them to develop their own preferences and take the initiative in discontinuation decision-making."* (Clinician 6).
> *"I like that it describes alternative methods, such as breathing and relaxation techniques, along with medicines."* (Clinician 9).
> *"A detailed explanation of how this is used would be helpful."* (Clinician 10).

3.3. Correcting the prototype incorporation stakeholder's comments

The committee assembled and shared the results of the stakeholder's acceptability test described above. We fully discussed and deliberated the results to utilize them to modify the DA prototype.

3.4. Developing the final DA

Our final DA was developed (Supplementary material S2) to ensure a high-quality decision support tool (Table 3). The final DA fulfilled all the IPDAS qualifying criteria (six of six), which were required for consideration as a DA (35), as well as all the IPDAS certification criteria (six of six), which judged the DA to contain a low risk of harmful bias (35). Moreover, the DA covered most IPDAS quality criteria (19 of 23), which added strength to the DA but whose lack did not mean a high risk of harmful bias (35). The status of the IPDAS criteria fulfilled by the final DA was considered higher than other Ottawa DAs that target other healthcare treatments or health screenings (44).

Additionally, the healthcare professionals who will be utilizing this DA will be required to be familiar with this tool. Therefore, the committee also created a DA manual for healthcare professionals that presented a detailed explanation of how to use the DA during decision-making in the clinical setting (Supplementary material S3).

4. Discussion

This is the first study to develop and assess the acceptability of a Japanese/English version of the DA for individuals with anxiety disorders for considering whether to continue BZD anxiolytics and whether CBT for anxiety should be added, if BZD is being discontinued.

The acceptability testing results suggested that the DA was well acceptable and favored by both patients and clinicians. This indicates that the DA was confirmed by stakeholders who were expected to use our DA. The strong point of the DA is that the committee systematically developed this tool using evidence-based criteria, in which both patients and clinicians, who were not involved in the development process, confirmed the DA. This implies that DA can

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TABLE 3 International patient decision aid standards criteria met by current decision aid (30).

Item	1. Qualifying criteria	2. Certification criteria	3. Quality criteria
Information	Describes the health condition or problem for which decision is required [a]	Shows the negative and positive features of options with equal detail [a]	Describes the natural course of the health condition or problem if no action is taken [a]
	Explicitly states decision that needs to be considered [a]		Makes it possible to compare the positive and negative features of available options [a]
	Describes the options available for the index decision [a]		
	Describes positive features of each option [a]		
	Describes negative features of each option [a]		
Probabilities			Provides information about outcome probabilities associated with the options [a]
			Specifies the defined group of patients for whom the outcome probabilities apply [a]
			Specifies the event rates for outcome probabilities [a]
			Allows the user to compare outcome probabilities across options using the same time period [a]
			Allows the user to compare outcome probabilities across the same denominator [a]
			Provides more than 1 way of viewing the probabilities (e.g., words, numbers, diagrams) [a]
Values	Describes what it is like to experience consequence of the options [a]		Asks patients to think about which positive and negative features of options matter most to them [a]
Guidance			Provides a step-by-step way to make a decision [a]
			Includes tools like worksheets or lists of questions to use when discussing options with a practitioner [a]
Development			Development process included a needs assessment with clients or patients [a]
			Development process included a needs assessment with health professionals [a]
			Development process included review by clients/ patients not involved in producing the decision support intervention [a]
			Development process included review by professionals not involved in producing the decision support intervention [a]
			Field tested with patients who were facing the decision [b]
			Field tested with practitioners who counsel patients who face the decision [b]
Evidence		Provides citations to the evidence selected [a]	Describes how research evidence was selected or synthesized [a]
		Provides a production or publication date [a]	Describes the quality of the research evidence used [a]
		Provides information about the update policy [a]	
		Provides information about the levels of uncertainty around the event or outcome probabilities [a]	
Disclosure		Provides information about the funding source used for development [a]	Includes authors'/developers' credentials or qualifications [a]
Plain Language			Reports readability levels [a]

(Continued)

TABLE 3 (Continued)

Item	1. Qualifying criteria	2. Certification criteria	3. Quality criteria
Evaluation		Describes what the test is designed to measure [b]	Evidence improved match between preferences of the informed patient and the option chosen [b]
			Evidence patient decision aid helps patients improve their knowledge about options' features [b]

[a]Criteria met by the developed decision aid. [b]Criteria to be met with effectiveness testing, not applicable for the current decision aid

be used in clinical settings. Ramos-García et al. also reported that their Spanish DA for patients with generalized anxiety disorder was easy to use, virtually appealing, and accepted by patients and clinical experts (31). These studies supported the suitability of DAs for anxiety-related disorders. Given that most people are highly motivated in contributing to the decision-making about their own treatment (32), these novel DAs could address the needs of patients with anxiety disorders.

The discontinuation of BZD anxiolytics has several advantages and disadvantages. The advantages include avoidance of adverse events, such as falls, drowsiness, and cognitive decline, whereas the disadvantages include worsening of anxiety and possible withdrawal symptoms. Thus, even if the patients desire to discontinue their medication, they may face conflicts between the advantages and disadvantages. Our DA might possibly reduce this conflict, since this tool successfully provides the evidenced-based characteristics of each option and asks the patients to clarify their own preferences. Using our DA with healthcare providers might also help patients to deliberate on further treatment courses with less conflict.

Several studies have been conducted to develop and assess psychosocial interventions for dealing with the risks of BZD use thus far (23). Heather et al. (45) reported that individuals with insomnia who received a letter warning about the harms of long-term use of BZD hypnotics showed larger reductions in BZD consumption than those who did not receive such a letter (23, 43). Thus, the presentation of not only the advantages but also the disadvantages of anxiolytic use to patients might lead to successful medication reduction. Our DA included both advantages and disadvantages of anxiolytics in a well-balanced manner. Moreover, our DA succeeded in supplying daily activities and relaxation techniques to reduce anxiety, which individuals with anxiety disorder could adopt in their everyday lives. In these regards, our DA contributes to the current literature, which suggests useful psychosocial interventions focusing on the prevention of the adverse aspects of long-term anxiolytic use. Furthermore, the uniqueness of our DA is that we have created a framework that allows patients to discuss and decide their options together with their clinicians, rather than unilaterally providing them with related information.

This study has some limitations. First, although our DA fulfilled most IPDAS quality criteria (35), some items should be covered in the future to improve the quality. Those items include field-testing and providing evidence of the intervention. To address this issue, the steering committee plans to conduct beta field-testing during the decision-making process of whether to discontinue BZD anxiolytics in a clinical setting. Second, there may be differences in the level of acceptance and appreciation among the patients who were shown their diagnosed disorder through the DA. Therefore, we plan to examine the differences between the diagnoses in beta field-testing. Third, patients with anxiety disorders often take antidepressant and BZD including some participants in this study. Therefore, there may be differences in the difficulties of discontinuing BZD if an antidepressant was also taken. We then plan to examine the differences between those on antidepressants and those who were not on antidepressants, in the beta field test. Forth, CBT for anxiety disorders include different elements and unique skills are required for each anxiety disorder. Our DA provided only non-specific general information of CBT for anxiety disorders, which is a limitation of this study. Additionally, the intervention effects of this DA need to be verified in a clinical setting.

5. Conclusion

This study described the development process and acceptability of a DA for the tapering BZD anxiolytics for anxiety disorders. The developed DA was acceptable to all stakeholders. The results could help in the treatment decisions of both individuals with anxiety disorder and their clinicians who are deliberating on the discontinuation of anxiolytic therapy.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by The Ethics Board of Kyorin University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

YA: study design, drafting and revising the DA prototype, data analysis and interpretation, revising the DA, and drafting the manuscript. KI, MTak, and TO: study design, revising the DA prototype, data collection, data analysis and interpretation, revising the DA, drafting, and editing the manuscript. HY, TM, TK, and MTan: study design, revising the DA prototype, data interpretation, revising the DA, and editing the manuscript. YT and KM: study design, revising the DA prototype, data collection and interpretation, revising the DA, editing the manuscript, and funding acquisition. All authors made substantial contributions to conception and design, acquisition

of data, or analysis and interpretation of data, took part in drafting the article or revising it critically for important intellectual content, agreed to submit to the current journal, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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Conflict of interest

YA received speaker's honoraria from Sumitomo Pharma, Meiji Seika Pharma, Viatris Pharmaceuticals Japan. YT received a lecture sponsorship from Takeda Pharmaceutical, Sumitomo Pharma, Otsuka Pharmaceutical, Meiji Seika Pharma, Kyowa Pharmaceutical, Eisai, MSD, and Yoshitomi and re-search funding from Otsuka Pharmaceutical, Meiji Seika Pharma, MSD, and Eisai. KI has received personal fees/grant support from Eisai, Eli Lilly, Janssen, Meiji Seika Pharma, Mitsubishi Tanabe Pharma, Mochida, MSD, Novartis, Otsuka, Shionogi, Sumitomo Pharma, and Yoshitomiyakuhin in the last three years. HY received lecture fees from Takeda Pharmaceutical, Lundbeck Japan, Sumitomo Pharma, Otsuka Pharmaceutical, Meiji Seika Pharma, Janssen Pharma, Kyowa Pharmaceutical, Eisai, MSD, Yoshitomiyakuhin, Mochida Pharmaceutical and Viatris in the last three years. TM declares no interest of conflict. TK has received speaker's honoraria from Takeda Pharmaceutical, Sumitomo Pharma, Viatris Pharmaceuticals Japan, MSD, Eisai, Ltd., and Yoshitomi Pharmaceutical. MTak has received speaker's honoraria from Takeda Pharmaceutical, Otsuka Pharmaceutical, Daiichi Sankyo Company, Sumitomo Pharma, Meiji Seika Pharma, Viatris Pharmaceuticals Japan, MSD, Eisai, Ltd., and Yoshitomi Pharmaceutical, and research grants from Otsuka Pharmaceutical, Eisai, Shionogi and the Japanese Ministry of Health, Labour and Welfare (R3-21GC1016) outside the submitted work. MTan declares no interest of conflict. KM received speaker's honoraria from EISAI Co., Ltd., Nobelpharma Co., Ltd., Takeda Pharmaceutical Co., Ltd., MSD Inc. and research grants from Eisai Co., Ltd., Sumitomo Pharma Co., Ltd., Takeda Pharmaceutical Co., Ltd., AMED (JP21dk0307103KM), the Japanese Ministry of Health, Labour and Welfare (19GC1012, 21GC0801). TO has received speaker's honoraria from IQVIA, Takeda Pharmaceutical, Otsuka Pharmaceutical, Daiichi Sankyo Company, Sumitomo Pharma, Meiji Seika Pharma, Viatris Pharmaceuticals Japan, MSD, Eisai, Ltd., Kyowa Pharma, Lundbeck Japan, Lily, and Yoshitomi Pharmaceutical.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2023.1083568/full#supplementary-material

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EDITED BY
Matthew S. Ellis,
Washington University in St. Louis,
United States

REVIEWED BY
Esa R. Korpi,
University of Helsinki, Finland
Mark Andrew Smith,
Davidson College, United States

*CORRESPONDENCE
James K. Rowlett
✉ jrowlett@umc.edu

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Behavioral effects of triazolam and pregnanolone combinations: reinforcing and sedative-motor effects in female rhesus monkeys

Jemma E. Cook, Donna M. Platt, Daniela Rüedi-Bettschen and James K. Rowlett*

Department of Psychiatry and Human Behavior, Center for Innovation and Discovery in Addictions, University of Mississippi Medical Center, Jackson, MS, United States

Introduction: Benzodiazepines (BZs) are prescribed as anxiolytics, but their use is limited by side effects including abuse liability and daytime drowsiness. Neuroactive steroids are compounds that, like BZs, modulate the effects of GABA at the $GABA_A$ receptor. In a previous study, combinations of the BZ triazolam and neuroactive steroid pregnanolone produced supra-additive (i.e., greater than expected effects based on the drugs alone) anxiolytic effects but infra-additive (i.e., lower than expected effects based on the drugs alone) reinforcing effects in male rhesus monkeys, suggestive of an improved therapeutic window.

Methods: Female rhesus monkeys (n=4) self-administered triazolam, pregnanolone, and triazolam-pregnanolone combinations intravenously under a progressive-ratio schedule. In order to assess characteristic sedative-motor effects of BZ-neuroactive steroid combinations, female rhesus monkeys (n=4) were administered triazolam, pregnanolone, and triazolam-pregnanolone combinations. Trained observers, blinded to condition, scored the occurrence of species-typical and drug-induced behaviors.

Results: In contrast to our previous study with males, triazolam-pregnanolone combinations had primarily supra-additive reinforcing effects in three monkeys but infra-additive reinforcing effects in one monkey. Scores for deep sedation (i.e., defined as atypical loose-limbed posture, eyes closed, does not respond to external stimuli) and observable ataxia (any slip, trip, fall, or loss of balance) were significantly increased by both triazolam and pregnanolone. When combined, triazolam-pregnanolone combinations had supra-additive effects for inducing deep sedation, whereas observable ataxia was attenuated, likely due to the occurrence of robust sedative effects.

Discussion: These results suggest that significant sex differences exist in self-administration of BZ-neuroactive steroid combinations, with females likely to show enhanced sensitivity to reinforcing effects compared with males. Moreover, supra-additive sedative effects occurred for females, demonstrating a higher likelihood of this adverse effect when these drug classes are combined.

KEYWORDS

benzodiazepine, neuroactive steroid, self-administration, sedation, rhesus monkey (*Macaca mulatta*)

1. Introduction

Benzodiazepines (BZs) are positive allosteric modulators of γ-aminobutyric acid type A ($GABA_A$) receptors and facilitate the modulation of chloride conductance by GABA (1–3). These drugs are among the most widely prescribed medications for the treatment of anxiety- and sleep-related disorders, but BZs have unwanted side effects including sedation and motor impairment, as well as the potential for abuse (1, 4). Considerable effort has been directed towards developing strategies to improve the therapeutic window for these drugs by augmenting the therapeutic effects and reducing or eliminating unwanted side effects.

In addition to the benzodiazepine modulatory site, $GABA_A$ receptors possess a number of other distinct modulatory sites (5). One such site binds neuroactive steroids. Neuroactive steroids are endogenous (or synthetic) compounds that can function as positive allosteric modulators and can produce many of the same behavioral effects as BZs, including anxiolysis, analgesia, sedation, reinforcing and anticonvulsant effects (6–10). Importantly, neuroactive steroids can modulate a subpopulation of $GABA_A$ receptors that are insensitive to classical BZs (11, 12). The similarity in effects produced by BZs and neuroactive steroids despite their different modulatory sites and distinct $GABA_A$ receptor populations raises the possibility that combining a neuroactive steroid with a BZ could produce clinically-beneficial effects with lower doses of both the BZ and the neuroactive steroid. Lower doses of the component drugs presumably would produce fewer side effects.

Several groups have investigated the behavioral effects of BZ-neuroactive steroid combinations in assays of both therapeutic and abuse-related effects. In these interaction studies, combinations of BZs and neuroactive steroids engender unique profiles, although the exact nature of the interaction appears to be dependent on the drugs under study, the doses tested, and the behavioral measure. For example, using isobolographic analysis, Chuang and Reddy (13) found supra-additive (i.e., potencies of combinations greater than predicted based on the effects of the drugs alone) antiseizure effects in mice with mixtures of the BZ midazolam and the synthetic neuroactive steroids brexanolone or ganaxolone. Likewise, combinations of the BZ triazolam and the neuroactive steroid pregnanolone produced supra-additive effects in a rhesus monkey conflict model of the anxiolytic-like effects of drugs (14). Similar findings of supra-additive anxiolytic-like effects also were observed in rats with an elevated zero maze procedure (15). In contrast, infra-additive effects (i.e., potencies of combinations less than predicted based on the effects of the drugs alone) were observed for mixtures of triazolam and pregnanolone in rhesus monkeys responding under a progressive-ratio schedule of i.v. drug self-administration, an assay that measures the reinforcing effects of drugs (14). In other studies in monkeys and rats trained to discriminate midazolam or triazolam, respectively, combinations of BZs and neuroactive steroids typically produced BZ-like discriminative stimulus effects that were additive in nature [i.e., potencies of combinations were as predicted based on the effects of the drugs alone; (15, 17)]. Collectively, the results of these studies would suggest that the combination of a BZ and a neuroactive steroid does, in fact, improve the therapeutic window (i.e., supra-additive therapeutic effects with additive or infra-additive abuse-related effects).

Although the finding of infra-additive reinforcing effects of a BZ-neuroactive steroid combination in monkeys is compelling, these data were obtained with male monkeys only (14). Importantly, sex-specific behavioral and physiological effects of progesterone and progesterone-based neuroactive steroids are well documented in both human and non-human subjects (18, 19). Therefore, the present study sought to determine the extent to which infra-additive reinforcing effects of triazolam-pregnanolone combinations would be observed in a cohort of female monkeys tested under the same conditions as those used by Fischer and Rowlett (14). In addition to allowing comparisons with Fischer and Rowlett (14), triazolam and pregnanolone were chosen as pharmacological tool compounds with selectivity for BZ and neuroactive steroid sites of action on $GABA_A$ receptors, and for their relatively short durations of action, which allows for interpretation of findings with fewer complications due to drug accumulation across a self-administration session.

As mentioned previously, both BZs and neuroactive steroids engender sedative-motor side effects that can limit their usage. Based on the observation that, for abuse-related side effects, combinations of BZs and neuroactive steroids engender additive or infra-additive effects, there is the possibility that these combinations will similarly engender additive or infra-additive sedative-motor effects. To assess this possibility, the effects of triazolam, pregnanolone, and triazolam-pregnanolone combinations on species-typical and drug-induced behaviors also were determined in female rhesus monkeys using an observation procedure that provides reliable metrics for drug-induced behaviors, as well as alterations of species-typical behaviors by drugs (20–24).

2. Materials and methods

2.1. Subjects and surgery

Eight adult female rhesus macaques (*Macaca mulatta*) weighing between 8 and 10 kg at the start of the study were used in the self-administration ($N = 4$) and behavioral observation ($N = 4$) procedures. Monkeys were housed individually in a colony room under a 12-h light/dark cycle (lights on at 0600 h). Monkeys had free access to water and received sufficient monkey chow to maintain healthy weights as determined by veterinary staff. Monkeys were maintained in accordance with the *Guide for Care and Use of Laboratory Animals, Eighth Edition*. Research protocols were approved by the University of Mississippi Medical Center's Institutional Animal Care and Use Committee.

Monkeys were prepared with chronic indwelling venous catheters following the general surgical procedures described by Platt et al. (25). The external end of the catheter was fed through a fitted jacket and tether system and attached to a fluid swivel (Lomir Biomedical, Malone, NY, United States) mounted to custom-designed cage systems (Carter2 Systems, Hillsboro, OR, United States). The catheters were flushed daily with heparinized saline (100 IU/ml), and the exit site of the catheters was inspected routinely.

2.2. Drugs

Midazolam (Hospira Inc., Lake Forest, IL, United States) 5 mg/ml pharmaceutical stock was diluted with 0.9% saline solution. Triazolam (Sigma-Aldrich, St. Louis, MO, United States) was

dissolved in propylene glycol and diluted with sterile water to a 50% propylene glycol/50% sterile water solution. Pregnanolone (Tocris Bioscience, Bristol, United Kingdom) was dissolved in a 45% (w/v) 2-hydroxypropyl-β-cyclodextrin solution. Triazolam-pregnanolone combinations for the self-administration study were prepared by dissolving each drug separately at twice the concentration of the test dose. The separate solutions were then combined in a single syringe prior to test sessions to create the test combination. Triazolam-pregnanolone combinations for the observation study were prepared by dissolving each drug separately and then administered sequentially via the i.v. catheter. In these sessions, the test dose of triazolam was administered first, followed by the test dose of pregnanolone.

2.3. Self-administration procedure

Using the procedure described by Fischer and Rowlett (14), four female rhesus monkeys were trained to self-administer the BZ midazolam (0.056 mg/kg/injection) under a progressive-ratio (PR) schedule of i.v. drug injection. At the beginning of a daily session, a set of two white stimulus lights above a response lever was illuminated. Upon completion of a response requirement, the white lights were extinguished and a set of two red lights was illuminated for 1-s, coinciding with an injection. Each trial ended with either an injection or the expiration of a 30-min limited hold. Trials were separated by a 30-min timeout period, during which all lights were off and responding had no programmed consequences.

Daily experimental sessions consisted of five components made up of four trials each. The response requirement remained constant for each of the four trials within a component, but doubled during each subsequent component. The session ended when a monkey self-administered a maximum of 20 injections or when the response requirement was not completed for two consecutive trials. The PR schedule for three monkeys (identification numbers = 318-01, 143-03, and 388-06) consisted of a sequence of response requirements: 40, 80, 160, 320, and 640 responses per injection. The schedule for the fourth monkey (165-01) consisted of response requirements: 20, 40, 80, 160, and 320 responses per injection.

Once training was complete, midazolam or saline was made available on alternating baseline days and until responding was stable (i.e., ≥10 injections on midazolam sessions and ≤5 injections on saline sessions). Test (T) sessions with triazolam, pregnanolone, or triazolam-pregnanolone combinations were added to the alternating sequence of midazolam (M) and saline (S) sessions according to the following sequence: MTSMTSTMST, etc. The ratios of triazolam-pregnanolone used in test combinations were calculated from the ED_{50} values of triazolam and pregnanolone dose-response curves for each monkey (see Section 2.5.1 for description of ED_{50} determinations). From these values, triazolam-pregnanolone combinations of individualized relative potencies (26) of 1:0.3, 1:1, 1:3 were tested (see Supplementary Table S3 for actual dose combinations tested). Each dose/dose combination was evaluated at least twice. The individual triazolam:pregnanolone dose ratios varied considerably; therefore individual subject's data are shown for the self-administration studies. The individual ED_{50} values and dose ratios used to determine each combination are listed in Table 1.

2.4. Behavioral observation procedure

Behavioral observations were conducted using the focal animal sampling approach as described in Platt et al. (27) and modified for rhesus monkeys [cf., (20, 21, 28, 29)]. Observers (four total) met a 90% inter-observer reliability criterion prior to the experiments and were blind to the drug treatments. Twenty-six species-typical and characteristic drug-induced behaviors (see Supplementary Table S1 for all definitions of behaviors) were scored by recording each instance that a particular behavior occurred during 15-s intervals in a 5-min observation period.

For sedation measures, structured exposure to stimuli were included in the observation sessions (20). When a monkey was observed to have closed eyes, an assessment of the animal's responsiveness to the stimuli was determined. Specifically, the observer presented three stimuli: (1) walked at a normal pace towards the cage, (2) spoke the animal's name, and (3) tapped twice on the cage bars or moved the lock used to secure the door of the cage. If the monkey responded immediately (i.e., opened eyes and oriented to the observer), *rest/sleep posture* was scored. If the monkey attended more slowly (i.e., >3 s following stimuli) and was observed to be assuming an atypical posture that differed from the characteristic rest/sleep posture (e.g., unable to keep an upright posture), the observer scored *moderate sedation*. If the monkey did not open eyes across the 15-s interval after all three stimuli, the observer noted the loss of ability to respond to external stimuli and scored *deep sedation*. The assessment of sedation was initiated during the 5-min sampling period if the animal presented, at any time during that period, with its eyes closed. The result of this assessment was recorded for each remaining 15-s interval of the 60-s epoch unless eyes opened. Afterwards, eyes closing again reinitiated the assessment. If eyes remained closed, then the assessment was repeated at the beginning of the next 60-s epoch.

Monkeys were habituated to the observers' presence over several weeks prior to testing. Baseline data were collected following saline injections. The effects of triazolam and pregnanolone alone were determined first, followed by combinations of triazolam and pregnanolone. The ratios of triazolam-pregnanolone were based on 1:1, 1:3, and 1:9 proportions, in order to focus on primarily sedative-motor effects that occurred at the higher dose ranges of these drugs (see Supplementary Table S4 for actual dose combinations tested). Scoring occurred at 5, 10, 20, 40, 80, and 160 min after the i.v. injection. Different doses of each drug or drug combination were evaluated in a randomized order, with at least a 2-day drug-free period between tests. Unlike the self-administration data, in which variance

TABLE 1 Individual ED_{50} and triazolam:pregnanolone dose ratios used in self-administration.

	ED_{50} (mg/kg)		Triazolam: pregnanolone relative potency dose ratios		
Monkey	Triazolam	Pregnanolone	1:0.3	1:1	1:3
143-03	0.0040	0.064	1:5	1:16	1:48
318-01	0.0006	0.020	1:11	1:33	1:100
165-01	0.0011	0.040	1:12	1:36	1:109
388-06	0.0015	0.073	1:16	1:49	1:146

in potencies was relatively high among the monkeys, the low variability in observation allowed for use of group statistics.

2.5. Data analysis

All statistical tests were conducted using GraphPad Prism Version 8.4.3 for Windows (GraphPad Software, La Jolla, CA, United States). Parametric statistics were used unless noted otherwise, and for all analyses involving multiple conditions, the error rate (α) was constrained to $p \leq 0.05$.

2.5.1. Self-administration

Data for self-administration consisted of the number of injections/session as well as the last response requirement completed (break point, BP). For triazolam and pregnanolone alone, self-administration was analyzed initially by separate one-way repeated measures analysis of variance (ANOVA) and Bonferroni tests comparing each dose vs. vehicle tests. BP data were used to calculate BP_{max}, which is the maximum BP obtained for individual monkeys for a test drug, irrespective of dose. This measure was used to compare the relative reinforcing effectiveness of each test drug, as well as combinations vs. the drugs alone. Because of violations in homogeneity of variance, BP_{max} data were analyzed with non-parametric Friedman's ANOVA with Dunn's multiple comparison tests. To determine potencies, ED_{50} values for each test drug were determined by analyzing the data points encapsulated by the peak and trough on the ascending limb and conducting log-linear regression, or linear interpolation when only two data points were available. The drug combination data were analyzed primarily with isobolographic and dose addition methods, described in Section 2.5.3.

2.5.2. Behavioral observation

For each subject, scores for each behavior were calculated as the number of 15-s intervals in which the behavior occurred (max score = 20 in a 5-min observation period). These scores were averaged across subjects to obtain a group mean for each dose of each drug at each time point. Drug effects on each behavior were evaluated by conducting a two-way analysis of variance (ANOVA) with both time and dose as within-subject factors. Bonferroni's multiple-comparison tests were conducted to compare the effects of each dose of triazolam, pregnanolone, and triazolam-pregnanolone combinations to vehicle controls. Dose-response functions additionally were constructed by computing cumulative scores over the entire time period (20), which were used to calculate ED_{50} values using the same approach described for self-administration (log-linear regression or linear interpolation). The primary method for analyzing combined effects of the two drugs was isobolographic and dose-addition analyses, described in the next section.

2.5.3. Isobolographic and dose-addition analyses

Two methods of analyses were used to evaluate drug combination effects. First, the effects of triazolam-pregnanolone combinations were assessed graphically with the use of isobolograms (26, 30). Isobolograms were constructed by connecting the ED_{50} of triazolam alone plotted on the y axis with the ED_{50} of pregnanolone plotted on the x axis. The line of additivity connects these points and contains the loci of dose combinations that would produce an ED_{50} equal to the ED_{50} of pregnanolone or triazolam administered alone if the combination is additive. Dose combinations that fall below or to the left of the line of additivity indicate an ED_{50} was reached with lesser quantities of the drugs, suggestive of supra-additivity. In contrast, dose combinations that fall above or to the right of the line of additivity are suggestive of infra-additivity. Theoretical additive dose combinations (a, b) are described by the equation (30):

$$B = b + \frac{B_{50}}{\left[\frac{E_B}{E_c\left(1+\frac{A_{50}^q}{a^q}\right)} - 1\right]^{\frac{1}{p}}} \quad (1)$$

This equation adjusts for differences in maximum effect, which in the present study was manifest as pregnanolone having a lower maximal effect than triazolam for some monkeys. A given dose of pregnanolone was designated as A with B as a given dose of triazolam. The potencies (ED_{50} values) of pregnanolone and triazolam when administered alone were defined as A_{50} and B_{50}, respectively. The maximum effects of triazolam and pregnanolone were defined as E_B and E_c, respectively. The coefficients p and q refer to curve-fitting parameters (i.e., Hill coefficients).

Drug combination effects also were analyzed by comparing the experimentally determined ED_{50} values for each mixture (Z_{mix}) with predicted additive ED_{50} values (Z_{add}) as described by Tallarida (31). Z_{mix} was defined as the total drug dose (i.e., dose triazolam + dose pregnanolone) that produced an increase to 50% of the maximum effect in drug self-administration or observable behavioral effect. Across all endpoints, the mean experimentally determined ED_{50} values (Z_{mix}) and predicted additive ED_{50} values (Z_{add}) for each mixture were compared with a paired t-test. An interaction index (γ) also was calculated to quantify deviation from additivity for each drug combination (32). From this calculation, a γ value of 1 indicated additivity, γ values that approached 0 indicated a greater degree of supra-additivity, and γ values greater than 1 indicated a greater degree of infra-additivity. One-sample t-tests were calculated to determine if the mean of the interaction indexes for each combination were significantly different from the theoretical additive value of 1.

3. Results

3.1. Drug self-administration

3.1.1. Drugs alone

Figure 1 shows the reinforcing effects of triazolam and pregnanolone alone under the PR schedule of drug self-administration. Also shown are baseline values for midazolam and saline self-administration (Figure 1, top panel). On baseline days, the training dose of midazolam maintained 12.1 ± 0.6 injections/session and saline maintained 2.5 ± 0.6 injections/session. Vehicle levels (3.0 ± 0.5 injections/session, not shown on graph) were not different from saline levels. As can be seen in the top panel of Figure 1, triazolam availability resulted in an increase in the mean number of injections/session

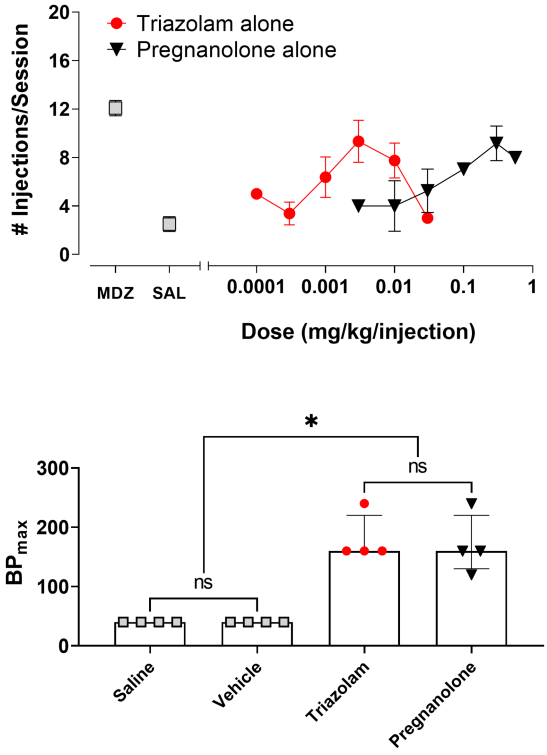

FIGURE 1
Self-administration of triazolam and pregnanolone under the progressive-ratio schedule of self-administration (N=4 female rhesus monkeys). Top panel: Mean number of injections/session±SEM for baseline self-administration of midazolam (point above "MDZ"), saline (point above "SAL"), and a range of doses of triazolam and pregnanolone alone. Bottom panel: Relative reinforcing effectiveness of saline, vehicle, triazolam and pregnanolone alone. Data are median BPmax (highest breakpoint achieved, irrespective of dose, with breakpoint defined as the last response requirement completed in a progressive-ratio response sequence) and inter-quartile ranges. Note that *p<0.05 for both saline and vehicle compared with triazolam and pregnanolone, and "ns" indicates lack of significance between saline and vehicle, as well as triazolam and pregnanolone (Dunn's multiple comparison tests).

relative to saline/vehicle levels up to 0.003 mg/kg/injection, followed by a decrease at higher doses, i.e., an inverted U-shaped function [repeated measures ANOVA: F (4, 12) = 7.40, p = 0.026 for vehicle and all triazolam doses]. No individual dose resulted in mean number of injections/session that differed from vehicle (Bonferroni t-tests, p's > 0.05). Similarly, pregnanolone availability resulted in an increase in the mean number of injections/session, although no appreciable decreases in self-administration were evident at doses up to 0.56 mg/kg/injection [repeated measures ANOVA: F (4, 12) = 11.73, p = 0.050 for vehicle and all pregnanolone doses]. Bonferroni tests showed that both 0.3 and 0.56 mg/kg/injection maintained higher injections/session than vehicle (p's < 0.05).

To compare relative reinforcing effectiveness among the two drugs, saline, and vehicle, the median BP_{max} values with interquartile ranges are shown in the bottom panel of Figure 1. Friedman's ANOVA showed a significant overall effect for all conditions: Q = 11.06, p = 0.0046. Dunn's multiple comparison's tests revealed that although both saline and vehicle values were significantly different from BP_{max} values for triazolam and pregnanolone, no statistically significant differences existed for either saline vs. vehicle or triazolam vs. pregnanolone.

3.1.2. Drug combinations

Due to the relatively large degree of variability among animals in the three drug combination conditions, individual subjects' data are presented in four separate panels for clarity (Figure 2). For two monkeys (143-03 and 388-06), combining triazolam and pregnanolone resulted in a proportion-dependent shift to the left in the triazolam dose-response function. For monkey 318-01, all ratios shifted the triazolam dose-response function to the left to essentially the same degree, whereas for monkey 165-01, no clear shift in the triazolam dose-response function was evident (the 1:1 ratio engendered a moderate rightward shift). Figure 3 shows the corresponding isobolograms based on the ED_{50} values of the drugs alone and combined, with dashed lines representing the theoretical lines of additivity derived from Equation (1). Note that two of the lines of additivity have slightly concave shapes, indicative of differences in the maximum number of injections/session obtained for triazolam vs. pregnanolone for these monkeys (318-01 and 165-01). Three of the four monkeys demonstrated 2–3 ratios that were below the line of additivity, suggesting supra-additive interactions for the reinforcing effects of triazolam and pregnanolone combined. Strikingly, monkey 165-01 showed additive effects (1:0.3) and infra-additive interactions (1:1 and 1:3), effects that clearly were in the opposite direction of the other monkeys. Examination of the dose-addition analysis for these combinations (Table 2) corroborated the overall results from the isobolographic analyses, with γ interaction indices showing values less than 1.0 for all ratios in three of the four monkeys, and γ values greater than 1.0 for the ratios in monkey 165-01. Statistical analyses of the γ interaction index data are shown in Figure 4, with data in the top panel including all subjects and data in the bottom panel excluding monkey 165-01. One sample t-tests conducted on the data in the top panel showed no significant differences from the theoretical value of 1.0; however, when 165-01 was excluded, the interaction indices for the 1:0.3 and 1:1 ratios, but not 1:3 ratio, were significantly below 1.0 (p's < 0.05). Therefore, combinations of triazolam and pregnanolone in ratios of 1:0.3 and 1:1 had supra-additive reinforcing effects. That is, lower combined doses resulted in reinforcing effects than would be expected if the reinforcing effects were simply additive when combined.

In addition to her data being strikingly different from the other monkeys, 165-01 was excluded for the analysis of interaction indices after a review of records of the subject's health revealed that over the course of ~6 months, this monkey showed no observable menses. This differed from the other subjects which all showed regular menses occurring approximately every month. Clinical hormonal assays using mass spectroscopy conducted with plasma samples from 165-01 revealed undetectable levels of progesterone, which combined with the lack of menses and the monkey's age (20 years at the time of the study) resulted in a clinical diagnosis of menopause, albeit at an age younger than is typical for rhesus monkeys.

Finally, BP_{max} values were analyzed for all combinations (and all monkeys) and no statistically significant effects were obtained when these values for the three ratios were included with the BP_{max} values for the drug alone. This included analyses that excluded monkey 165-01 (Friedman's ANOVA and Dunn's tests for all possible pairwise comparisons, p's > 0.05; data not shown).

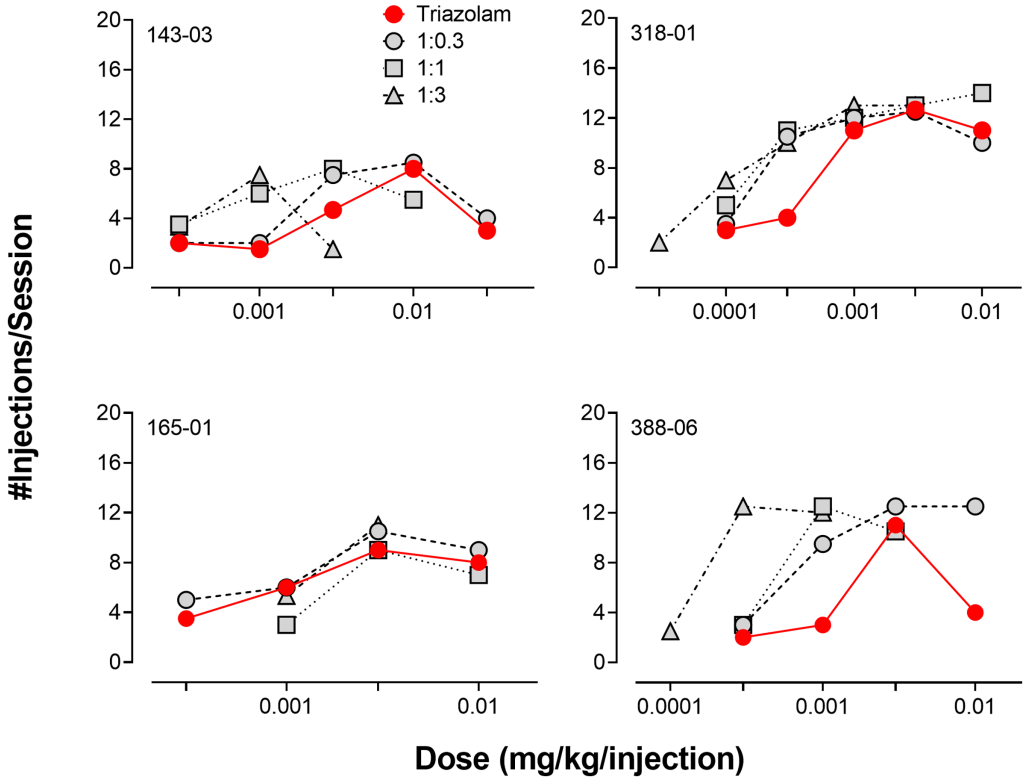

FIGURE 2
Comparison of self-administration of triazolam vs. triazolam-pregnanolone combinations in female rhesus monkeys responding under a progressive-ratio schedule (*N*=4 monkeys). Each individual panel depicts data for an individual monkey, identified by the 5-digit code in the upper left hand part of the panel. Ratios are fixed proportions of triazolam to pregnanolone, with three ratios tested: 1.0:0.3, 1.0:1.0, 1:0:3.0. Data are mean number of injections/session.

3.2. Behavioral observation

3.2.1. Summary of behavioral effects

Under baseline and vehicle conditions for triazolam and pregnanolone testing, monkeys displayed varying degrees of species-typical behavior, with self-groom, passive visual, tactile/oral exploration, forage, scratch, and locomotion being the most common behaviors (data not shown). Little to no behaviors indicative of sedative effects (e.g., rest/sleep posture or moderate/deep sedation) were observed during baseline conditions and vehicle sessions.

Of the 26 behaviors quantified during testing with triazolam, pregnanolone, and the three combinations (1:1, 1:3, 1:9), the majority of species-typical behaviors were not altered significantly (Supplementary Table S2). Exceptions were decreases in forage for triazolam alone, scratch and groom for the 1:3 combination, and groom for the 1:9 combination (Supplementary Table S2). In contrast, the two drugs alone and the combinations consistently increased measures of observable ataxia and deep sedation (note that rest/sleep posture and moderate sedation were not altered by any test condition). Because of the consistent and statistically significant effects on observable ataxia and deep sedation, these measures were chosen for isobolographic and dose-addition analyses, as described below.

3.2.2. Deep sedation

Significant increases in deep sedation were detected as a function of dose and time for both triazolam and pregnanolone alone [2-within repeated measures ANOVAs; triazolam dose × time interaction, $F(25, 75) = 1.70$, $p = 0.042$; pregnanolone dose × time interaction, $F(20, 60) = 272.8$, $p < 0.0001$]. For clarity, the top panel of Figure 5 shows the effects of the highest doses tested for triazolam (1.7 mg/kg, i.v.) and pregnanolone (3.0 mg/kg, i.v.). A striking difference between the two drugs is that triazolam engendered deep sedation from 5 to 80 min post-injection, whereas pregnanolone engendered deep sedation from 5 to 20 min post-injection only (Bonferroni multiple comparisons, p's < 0.05). Because of this difference in time course, analysis of the effects of the drug combinations were limited to the 5–10 min data only (i.e., scores were cumulated from 5 to 10 min). The middle panel of Figure 5 shows the mean cumulative scores for triazolam and the 1:1, 1:3, and 1:9 ratios of triazolam to pregnanolone as a function of dose. As can be seen in the Figure 5, all three ratios resulted in a leftward shift in the triazolam dose-response function, consistent with an overall enhanced effect on deep sedation by this drug combination.

Figure 5 (bottom panel) and Table 3 show the results of isobolographic and dose-addition analyses of the deep sedation data with triazolam and pregnanolone. As can be seen in the Figure 5, the 1:1 and 1:3 combinations were clearly below the theoretical line of additivity for this behavioral measure. The mean ED_{50} for the 1:9 combination also was below the line of additivity; however, the error bars associated with the pregnanolone effects clearly overlapped the additivity line. These observations were confirmed with the dose-addition analysis (Table 3), in which the experimentally determined

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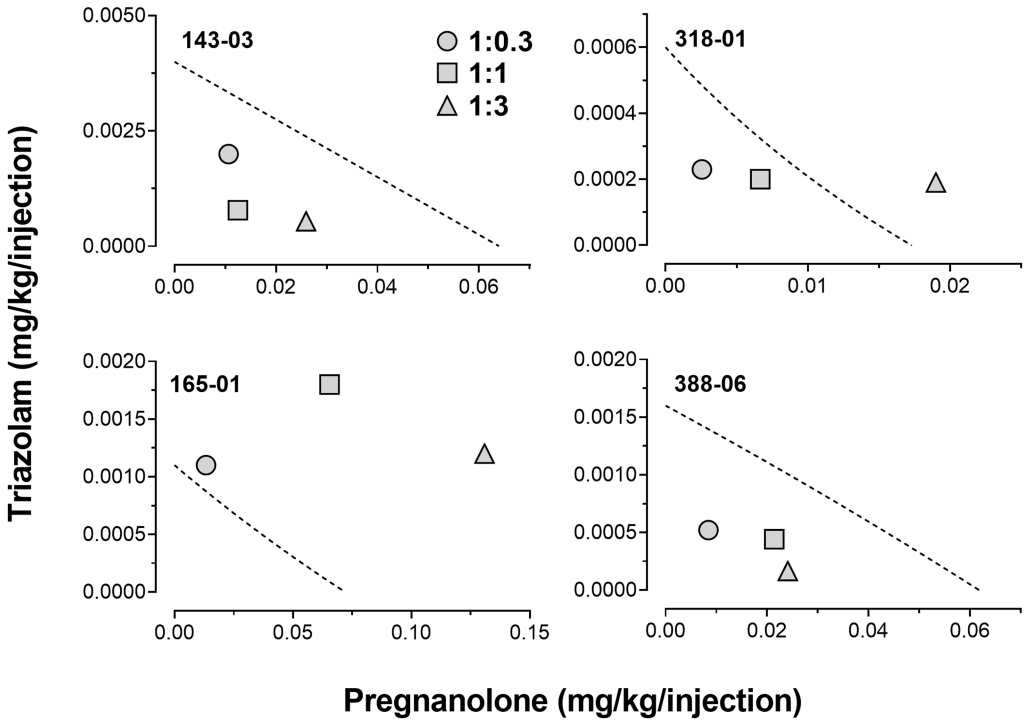

FIGURE 3
Isobolograms for triazolam-pregnanolone combinations in the progressive-ratio self-administration procedure. Each individual panel depicts data for an individual monkey, identified by the 5-digit code in the upper left hand part of the panel. Dashed lines represent theoretical lines of additive combinations for triazolam and pregnanolone (calculated via Equation 1, see text). The ED50 values for triazolam are plotted on the y-axes as a function of the corresponding ED50 values for pregnanolone on the x-axes. Individual data points represent ED50 values for combined effects of the two drugs, in proportions of 1:0.3, 1.0:1.0, and 1.0:3.0 triazolam to pregnanolone. Values below the line of additivity represent supra-additive interactions, whereas values above the line of additivity represent infra-additive interactions. Points close to or on the additivity line represent additive effects (i.e., no interaction).

TABLE 2 Predicted additive potency (Z_{add}) and experimentally determined potency (Z_{mix}) of triazolam-pregnanolone combinations on self-administration.

	Triazolam-pregnanolone combination								
	1:0.3			1:1			1:3		
Monkey	Z_{add}	Z_{mix}	γ*	Z_{add}	Z_{mix}	γ*	Z_{add}	Z_{mix}	γ*
143-03	0.019	0.013	0.67	0.034	0.013	0.39	0.049	0.027	0.56
318-01	0.0055	0.0028	0.51	0.010	0.0069	0.67	0.015	0.019	1.27
165-01	0.011	0.014	1.33	0.021	0.067	3.27	0.030	0.13	4.36
388-06	0.019	0.0090	0.46	0.037	0.022	0.59	0.055	0.024	0.56

*γ interaction index (i.e., Z_{mix}/Z_{add}).

ED_{50} values (Z_{mix}) for the 1:1 and 1:3 combinations were significantly less than the predicted additive ED_{50} values (Z_{add}; [1:1] $t(3) = 6.38$, $p < 0.01$; [1:3] $t(3) = 8.50$, $p < 0.01$), but no such significant difference was detected for the 1:9 combination. Similarly, one-sample *t*-tests showed that the γ interaction indices for the 1:1 and 1:3, but not 1:9 combinations, were significantly lower than the theoretical value of 1.0 (*p*'s < 0.05). In summary, these results indicate that combinations of triazolam and pregnanolone in ratios of 1:1 and 1:3 but not 1:9 had a supra-additive effect in inducing deep sedation. That is, combinations of lower doses of the component drugs resulted in significant levels of deep sedation that would not be expected if the drug effects were simply additive.

3.2.3. Observable ataxia

The pattern of effects observed for observable ataxia for the two drugs was somewhat more complex than that for deep sedation. In this regard, no dose × time interaction was evident for triazolam alone, although the main effect of dose was significant [$F(5, 15) = 5.048$, $p = 0.0065$]. Bonferroni *t*-tests revealed that the 0.3 mg/kg dose of triazolam showed an overall increase in the mean score for observable ataxia, irrespective of time ($p < 0.05$), and this dose is plotted in the top panel of Figure 6. Pregnanolone, in contrast, did show a significant dose × time interaction [$F(20, 60) = 33.97$, $p < 0.0001$]. The highest dose of pregnanolone is shown in Figure 6, and as with deep sedation, a relatively transient effect on observable ataxia was observed

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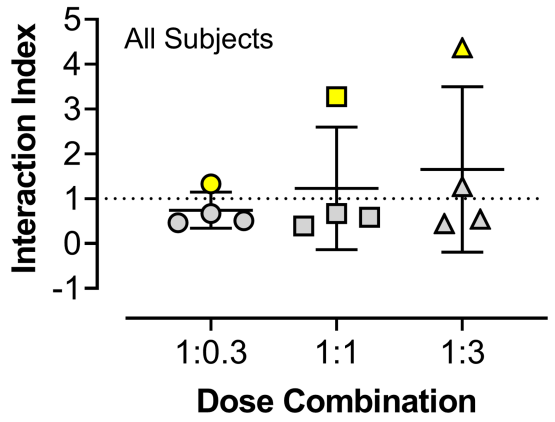


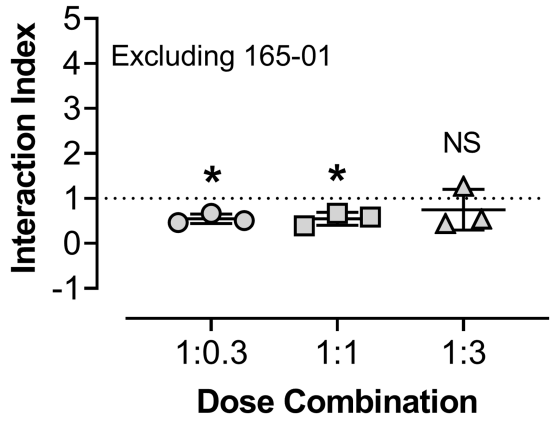
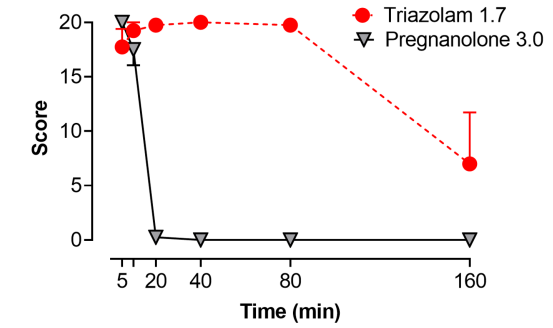
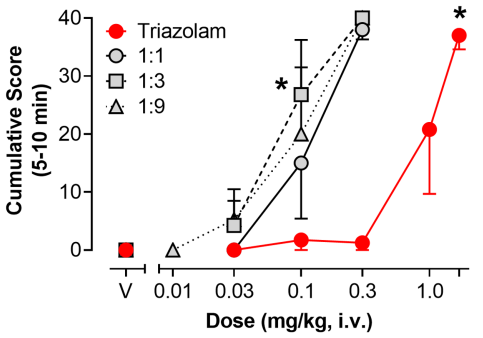


FIGURE 4
Statistical analyses of γ interaction index values obtained from dose-addition analyses of the combined reinforcing effects of triazolam and pregnanolone under the progressive-ratio procedure. The y-axis shows the interaction index values and the x-axis depicts the ratios of triazolam to pregnanolone. Symbols represent the four female rhesus monkeys. The top panel shows data for all subjects, with no significant differences observed vs. the theoretical value of 1.0 (additivity, one-sample *t*-tests, *p*'s>0.05). The bottom panel shows the analysis when subject 165-01 (yellow symbols in the top panel) was omitted from the statistical tests. For the 1:0.3 and 1:1 ratios of triazolam-pregnanolone, the interaction indices were significantly different from 1.0 (*$p<0.05$, one sample *t*-tests), whereas the 1:3 ratio did not differ from 1.0, i.e., additive effects were observed at this ratio combination.





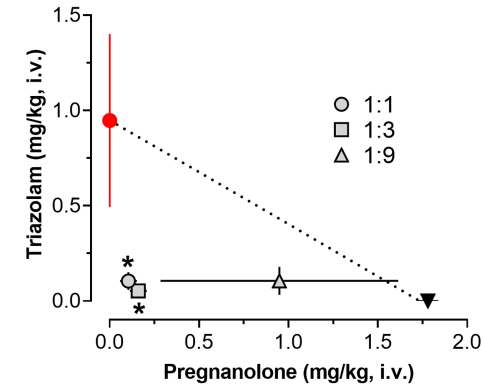


FIGURE 5
Deep sedation, scored *via* quantitative observation, following i.v. administration of triazolam and pregnanolone, alone and combined, in female rhesus monkeys (*N*=4). Top panel: Mean modified frequency scores±SEM as a function of time for deep sedation at doses significantly different from vehicle for triazolam and pregnanolone (1.7mg/kg and 3.0mg/kg, respectively). Middle panel: Dose-response functions for deep sedation after triazolam and triazolam-pregnanolone combinations (fixed ratio proportions of 1:1, 1:3, 1:9). Data are mean cumulative score±SEM for scores cumulated during the 5–10min observation sessions. Note that *$p<0.05$ vs. vehicle (V), Bonferroni *t*-tests. Bottom panel: Isobologram for the data depicted in the middle panel. Dashed lines represent theoretical lines of additive combinations for triazolam and pregnanolone (calculated via Equation 1, see text). The ED50 values for triazolam are plotted on the y-axes as a function of the corresponding ED50 values for pregnanolone on the x-axes. Individual data points represent ED50 values for combined effects of the two drugs, in proportions of 1:0.3, 1.0:1.0, and 1.0:3.0 triazolam to pregnanolone. Values below the line of additivity represent supra-additive interactions, whereas values above the line of additivity represent infra-additive interactions. Points close to or on the additivity line represent additive effects (i.e., no interaction). Error bars represent SEM values, and note that *$p<0.05$ based on dose addition analyses.

(significant increase above vehicle levels at the 20-min time point only, Bonferroni *t*-test, $p<0.05$). Therefore, for observable ataxia, the dose combination analyses were conducted for the 5–20 min time points only.

Figure 6 (bottom panel) shows the dose-response functions for triazolam and the 1:1, 1:3, and 1:9 combinations of triazolam-pregnanolone as cumulative scores for the 5–20 min time period. Triazolam alone increased mean cumulative scores for observable ataxia up to 0.1 mg/kg; however, this effect dissipated as the dose of triazolam was increased, i.e., an inverted U-shaped function was observed (Bonferroni *t*-tests, *p*'s < 0.05). The lowest ratio, 1:1, appeared to result in a leftward shift of the descending limb of the triazolam dose-response function, but not the ascending limb. For the two higher ratios, the dose-response function appeared to be shifted primarily downward, with no significant effects on observable ataxia

TABLE 3 Predicted additive potency (Z_{add}) and experimentally determined potency (Z_{mix}) of triazolam-pregnanolone combinations on deep sedation.

Drug combination	Z_{add} (±95% CI)	Z_{mix} (±95% CI)	γ†
1:1	1.18 (0.39)	0.21 (0.09)*	0.18
1:3	1.39 (0.28)	0.21 (0.06)*	0.15
1:9	1.59 (0.15)	1.06 (0.74)	0.66

*An experimentally determined potency significantly different from the predicted additive potency ($p < 0.05$).

†γ interaction index (i.e., Z_{mix}/Z_{add}).

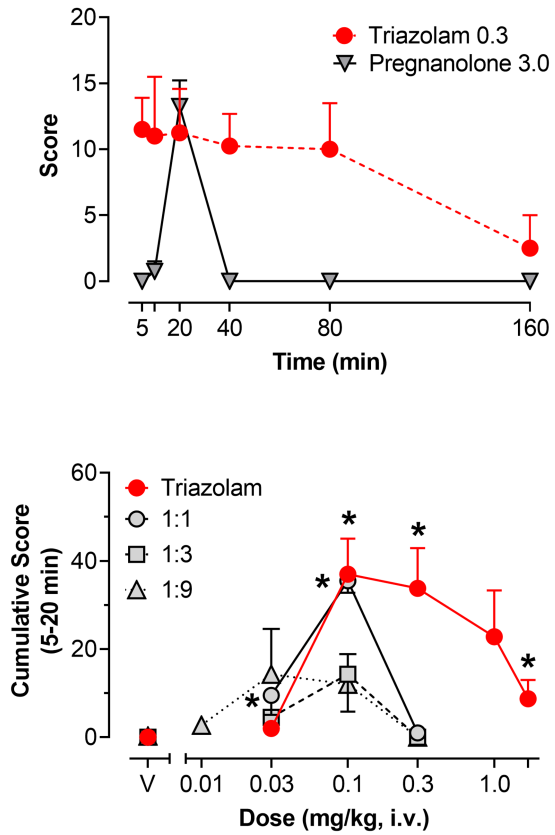


FIGURE 6
Observable ataxia, scored *via* quantitative observation, following i.v. administration of triazolam and pregnanolone, alone and combined, in female rhesus monkeys (N=4). Top panel: Mean modified frequency scores±SEM as a function of time for deep sedation at doses significantly different from vehicle for triazolam and pregnanolone (0.3mg/kg and 3.0mg/kg, respectively). Bottom panel: Dose-response functions for deep sedation after triazolam and triazolam-pregnanolone combinations (fixed ratio proportions of 1:1, 1:3, 1:9). Data are mean cumulative score±SEM for scores cumulated during the 5–20min observation sessions. Note that *p<0.05 vs. respective vehicle (V), Bonferroni t-tests.

obtained by either ANOVA or Bonferroni tests. Because these dose-response functions were essentially flat, no ED_{50} values could be computed and therefore isobolographic and dose-addition analyses were not conducted. Although these overall effects may be interpreted as an attenuation of the effects of triazolam on observable ataxia, it is important to note that the dose range of 0.01–0.3 mg/kg of triazolam was also where significant supra-additive effects on deep sedation occurred, raising the likely possibility that deep sedation masked the occurrence of observable ataxia.

4. Discussion

The present study evaluated the extent to which combinations of the BZ, triazolam, and the neuroactive steroid, pregnanolone, resulted in significant changes in reinforcing and sedative-motor effects in female rhesus monkeys. Regarding reinforcing effects, we previously found that self-administration of triazolam-pregnanolone combinations resulted in infra-additive interactions or additive effects in male monkeys (14). In the present study, we used the PR self-administration methods of Fischer and Rowlett (14) to repeat the combination studies in a group of female rhesus monkeys. Although the fact that these studies were not direct comparisons (i.e., females and males in a single experiment), there were apparent sex differences in self-administration, both for the drugs alone as well as combined. As shown in Table 4, triazolam and pregnanolone were 4- to 2-fold less potent in females compared with males, respectively, and showed 15–34% lower BP_{max} values, respectively, suggesting that both drugs were moderately less potent and effective as reinforcers in females vs. males. More strikingly, however, was the pattern of interactive effects between the two studies. In this regard, while the male monkeys in Fischer and Rowlett (14) demonstrated infra-additive to additive reinforcing effects when triazolam and pregnanolone were combined, three of four female monkeys in the present study showed robust supra-additive effects. The fourth monkey demonstrated infra-additive to additive effects; however, this monkey clinically presented with signs of menopause, including evidence of relatively low circulating progesterone levels. Based on these results, we propose that progesterone-derived neuroactive steroids (e.g., allopregnanolone) may act to enhance the reinforcing effects of triazolam and pregnanolone in female monkeys with normal cycles. Furthermore, these findings raise the possibility that reinforcing effects of BZ-neuroactive steroid combinations may synergistically enhance the abuse potential of the combinations, making development of low-dose BZ + neuroactive steroid combinations as therapeutic agents potentially untenable, at least in a subset of the patient population.

The finding that the nature of the interactions between a BZ and a neuroactive steroid may differ between females and males is not surprising, given that sex-specific behavioral and physiological effects of progesterone and neuroactive steroids are well documented (18, 19). In general, women consistently have been documented to be more likely to receive a BZ prescription than men, and to present with substance use disorders involving BZs at higher rates than men [(33); but see (34)], a finding that is largely international/cross-cultural in scope [e.g., (35)]. Preclinical evidence indicates that the effects of BZs can be altered by differences in circulating progesterone levels, e.g., a BZ engendered anxiolytic-like effects during early stages of the estrous cycle, but not the late diestrus phase, in rats (36). These results presumably are tied to differences in endogenous neuroactive steroid levels that are metabolized from progesterone. With respect to the $GABA_A$ subtype target site for BZs and neuroactive steroids, little is known about changes in subtype levels and/or constitution across either the menstrual or estrous cycle. However, (37) demonstrated a robust increase in neurons expressing α4, β1, and δ subunits during the late diestrus phase in rat midbrain, whereas levels of these subunits

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TABLE 4 Comparison of relative reinforcing potency and effectiveness values between male and female rhesus monkeys trained and maintained on a progressive-ratio schedule of midazolam reinforcement (*N*=4 per group).

	Triazolam	Pregnanolone
	ED_{50} Mean mg/kg/injection (SEM)	
Male[a]	0.00041 (0.00011)	0.023 (0.009)
Female	0.0018 (0.0007)	0.055 (0.011)
	BP_{max}[b] Mean responses (SEM)	
Male[a]	213 (53)	266 (53)
Female	180 (20)	171 (25)

[a]Data from male monkeys adapted from Fischer and Rowlett (14).
[b]BP_{max} is the highest breakpoint obtained, irrespective of dose, with breakpoint determined as last response requirement completed in a progressive-ratio sequence.

do not fluctuate across time in male rats. Electrophysiological evidence has accrued linking neuroactive steroid action to the extrasynaptically-located δ-subunit containing $GABA_A$ receptor (15). Therefore, it is feasible that enhanced action at δ-containing $GABA_A$ receptors by exogenous (and/or endogenous) neuroactive steroids may underly enhanced behavioral effects of BZ-neuroactive steroid combinations.

At present, we do not have direct evidence that the supra-additive effects of BZs and pregnanolone in self-administration was linked with progesterone metabolism. Although three of four monkeys exhibited normal cycles based on menstruation, the design of this study, in which drug vs. saline were available across days in a random order, did not allow for accurate tracking of progesterone (and estradiol) levels across the cycle. Specifically, the design of Fischer and Rowlett (14) involved randomized, single-day tests of drugs or drug combinations, counterbalanced in such a way as to reduce the influence of time factors. Nevertheless, systematic evaluation of the effects of neuroactive steroids on BZ self-administration over the course of the menstrual cycle is needed information, as well as conducting studies to more directly assess the role of progesterone (e.g., administration of 5α-reductase inhibitors to block neuroactive steroid formation).

Another goal of the present study was to expand the assessment of combinations of BZs and neuroactive steroids to sedative-motor effects, a prominent adverse effect of $GABA_A$ modulators in general. When tested alone, both triazolam and pregnanolone had marked effects on measures of deep sedation and observable ataxia, consistent with other findings using our quantitative observation methods (20, 21). Regarding deep sedation, combination of triazolam and pregnanolone resulted in leftward shifts in the dose-response function for triazolam that tended to not be proportion-dependent. Of the three ratios tested, two resulted in robust supra-additive interactions (1:1, 1:3) with the third trending to supra-additivity. Results with observable ataxia were more complex, with a tendency to decrease these effects rather than enhance them. However, this simply may reflect the enhancements in deep sedation "overriding" ataxic effects.

At present, we do not have sedative-motor data for BZ-neuroactive steroid combinations in male animals. Although not a direct measure of sedative-motor effects, we do have results from studies with male subjects involving operant lever-pressing maintained by food presentation. For example, in male rhesus monkeys, dose-dependent suppression of food-maintained responding by triazolam and pregnanolone demonstrated only additive effects when combined (14). However, in male rats trained under a schedule of food reinforcement, triazolam-pregnanolone combinations resulted in supra-additive interactions, a finding also seen with combinations of other BZs and neuroactive steroids (37). Similarly, food-maintained responding in the context of a triazolam discrimination procedure also showed supra-additive effects with triazolam-pregnanolone combinations in male rats (15), suggesting that at least in rats, the ability to respond/exert motor control may be impaired in an supra-additive fashion in males. However, these similarities with the present study should be interpreted with caution: It is important to note that although changes in schedule-controlled behavior may reflect sedative-motor effects, it is impossible to attribute decreases in operant behavior to any specific behavioral effect, i.e., the decreases could also reflect changes in appetite, motivation, associative processes, and so on.

Across the different behavioral procedures, the combinations of triazolam and pregnanolone were assessed using three different proportions, because changes from additivity often depends on the relative proportion of the drugs in the combinations (31). For both self-administration and deep sedation, there was a similar dependency on proportion: the ratios with the highest level of pregnanolone relative to triazolam (i.e., triazolam:pregnanolone = 1:3 and 1:9 for self-administration and deep sedation, respectively) were less likely to demonstrate supra-additivity. A cautionary note regarding this conclusion is that for self-administration, the 1:3 additive effect reflected results from a single monkey (318-01, see Figure 3), whereas for deep sedation, the average effect of the 1:9 proportion trended to supra-additivity, but did not achieve significance due to variability associated with the pregnanolone effect (see Figure 5). Nevertheless, these results suggest that nature of the interaction of, and perhaps the mechanism(s) underlying, triazolam and pregnanolone combinations may depend on the relative proportion of pregnanolone in the mixture, and further reinforce the utility of testing multiple proportions in drug combination studies.

There are several considerations worth noting for further evaluation of the results and conclusions of this study. Clearly, one monkey believed to be in menopause is an insufficient sample size for making strong conclusions about the role of circulating hormones in the combined effects of BZs and neuroactive steroids. More formal manipulations, such as ovariectomy, may provide a clearer test of this hypothesis. Because menopause is tied to aging, a study in younger monkeys with ovariectomies may help to disentangle whether there are simply age-related differences in responsiveness to BZ-neuroactive steroid combinations. Finally, a study designed to assess changes across the menstrual cycle, concomitant with measuring levels of reproductive hormones and/or endogenous neuroactive steroids, remains a high priority.

In Fischer and Rowlett (14), we raised the possibility that if combining BZs and neuroactive steroids resulted in supra-additive interactions with therapeutic endpoints but infra-additive interactions with endpoints related to adverse effects and/or toxicology, then combining these two drug classes at sub-therapeutic doses may be a viable strategy for developing improved pharmacotherapies (e.g., anxiolytics). The results from the present study present a significant challenge to this idea, due to the finding of pronounced supra-additive effects of the

combinations in both self-administration and sedative-motor effects. On the other hand, these data collectively point to a potentially significant sex difference in at least the reinforcing effects of $GABA_A$ positive modulators. This is especially noteworthy given the recent approvals of neuroactive steroids ganaxolone and brexanolone in the treatment of epilepsy and postpartum depression, respectively (39). Given the existence of pregnanolone and other neuroactive steroids in the CNS, along with their being metabolic products of reproductive hormones, information about these interactions may lead to a better understanding of sex differences underlying responses to these important psychiatric medications.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by University of Mississippi Medical Center's Institutional Animal Care and Use Committee.

Author contributions

JC, DP, DR-B, and JR were responsible for the study concept and design, assisted with data analysis and interpretation of findings. JC and DR-B contributed to data acquisition. JC and DP drafted the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

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EDITED BY
Yasser Khazaal,
Université de Lausanne, Switzerland

REVIEWED BY
Esa R. Korpi,
University of Helsinki, Finland
Hilary S. McCarren,
US Army Medical Research Institute of Chemical Defense, United States

*CORRESPONDENCE
Margot Ernst
✉ margot.ernst@meduniwien.ac.at

†These authors have contributed equally to this work and share first authorship

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Navigating the complex landscape of benzodiazepine- and Z-drug diversity: insights from comprehensive FDA adverse event reporting system analysis and beyond

Filip Koniuszewski[1†], Florian D. Vogel[1†], Irena Dajić[2], Thomas Seidel[3], Markus Kunze[1], Matthäus Willeit[2] and Margot Ernst[1*]

[1]Department of Pathobiology of the Nervous System, Center for Brain Research, Medical University Vienna, Vienna, Austria, [2]Department of Psychiatry and Psychotherapy, Medical University of Vienna, Vienna, Austria, [3]Department of Pharmaceutical Sciences, University of Vienna, Vienna, Austria

Introduction: Medications which target benzodiazepine (BZD) binding sites of GABAA receptors (GABAARs) have been in widespread use since the nineteen-sixties. They carry labels as anxiolytics, hypnotics or antiepileptics. All benzodiazepines and several nonbenzodiazepine Z-drugs share high affinity binding sites on certain subtypes of GABAA receptors, from which they can be displaced by the clinically used antagonist flumazenil. Additional binding sites exist and overlap in part with sites used by some general anaesthetics and barbiturates. Despite substantial preclinical efforts, it remains unclear which receptor subtypes and ligand features mediate individual drug effects. There is a paucity of literature comparing clinically observed adverse effect liabilities across substances in methodologically coherent ways.

Methods: In order to examine heterogeneity in clinical outcome, we screened the publicly available U.S. FDA adverse event reporting system (FAERS) database for reports of individual compounds and analyzed them for each sex individually with the use of disproportionality analysis. The complementary use of physico-chemical descriptors provides a molecular basis for the analysis of clinical observations of wanted and unwanted drug effects.

Results and Discussion: We found a multifaceted FAERS picture, and suggest that more thorough clinical and pharmacoepidemiologic investigations of the heterogenous side effect profiles for benzodiazepines and Z-drugs are needed. This may lead to more differentiated safety profiles and prescription practice for particular compounds, which in turn could potentially ease side effect burden in everyday clinical practice considerably. From both preclinical literature and pharmacovigilance data, there is converging evidence that this very large class of psychoactive molecules displays a broad range of distinctive unwanted effect profiles - too broad to be explained by the four canonical, so-called "diazepam-sensitive high-affinity interaction sites". The substance-specific signatures of compound effects may partly be mediated by phenomena such as occupancy of additional binding sites, and/or synergistic interactions with endogenous substances like steroids and endocannabinoids. These in turn drive the wanted and unwanted effects and sex differences of individual compounds.

KEYWORDS

Z-drugs, benzodiazepine binding sites, sex differences, adverse events, pharmacovigilance, side effects, FDA adverse event reporting system, benzodiazepine

1. Introduction

1.1. GABAA receptors

GABAA receptors are a heterogeneous protein family in the nervous system and in non-neuronal tissues. They assemble as transmembrane homo-or heteropentameric anion channels, which specifically conduct bicarbonate and chloride anions and are gated by the endogenous ligand GABA. In most instances, opening of neuronal channels facilitates chloride movement from the extracellular space into the cytoplasm, with a net inhibitory effect (1–3). GABAARs can be categorized into (1) postsynaptic receptors, which facilitate fast point to point communications between cells following action potentials, (2) extrasynaptic ones, which show high GABA affinity and a steady, non-desensitizing stream of ionic flow in order to provide tonic inhibition, as well as (3) perisynaptic receptors thought to chiefly gate synapses (4). Moreover, presynaptic GABAA receptors were described (5–7).

Due to the existence of 19 GABAA receptor genes encoding for α1-6, β1-3, γ1-3, δ, ε, π, ρ1-3, θ subunits in human and non-human mammals, and variants from splicing and RNA editing, the number of possible GABAAR pentamers is vast even considering the hitherto identified assembly rules (8–16). It is generally believed that most receptors contain two to three β- (or β-like subunits), one or two α-subunits, and one odd subunit which is most commonly γ2, oriented in a counter-clockwise manner, in α-β-α-γ-β-order. However, there is still an overwhelming number of receptor subtypes with unknown or divergent native receptor composition, assembly and stoichiometry (17, 18). Their physiological functions and pharmacological properties vary greatly, as known from heterologous expression systems as well as *in vitro* and *in vivo* studies in rodent systems (19–24).

1.2. Benzodiazepines and Z-drugs

Benzodiazepines have dominated the pharmaceutical market of GABAA receptor targeting compounds since their introduction in the 1960s by Hoffmann La Roche (25). At the time, they replaced the previous generation of GABAA receptor targeting central nervous system (CNS) depressants, the barbiturates, due to a better pharmacological profile and safer use. They are a heterocyclic class of molecules chemically defined by an aromatic benzyl ring annulated to an unsaturated diazepine-ring (Figure 1). The compounds that incorporate a 1,4 – diazepine partial structure are the ones most frequently used clinically. To exert effects at low doses, BZDs require a high affinity binding site on GABAA receptors which is known to be localized at extracellular interfaces between an α1-3,5 "principal" subunit, together with a γ1-3 "complementary" subunit (27–33). Preclincial research and the low abundance of the γ3 subunit have led to the notion that the four sites formed by α1-3,5 together with γ2 account for the major share of drug effects that are mediated by the resulting four high affinity binding sites. Many receptors that lack the high affinity sites still can be modulated by BZDs in higher (micromolar) concentrations but lack low concentration BZD effects (34–36). However, it should be noted that the distinction is largely based on data originating from heterologous expression systems which do not account for endogenous GABAA receptor modulators and their allosteric interactions with BZD effects (37, 38).

As a class, BZDs have a broad variety of therapeutic effects, including anxiolysis, hypnosis, sedation, muscle relaxation and anticonvulsant effects (39–42). Dose-dependent euphorogenic and amnestic actions are described as well, which might contribute to their popularity in recreational and illicit use (43). In the Anatomical Therapeutic Chemical Classification (ATC) System, BZDs run under the codes N03 (antiepileptics), and N05 (psycholeptics) in which they are further divided into N05B (anxiolytics) and N05C (hypnotics and sedatives). They are useful therapeutics in many diseases and disorders, such as anxiety disorders, epilepsy or sleeping disorders. However, in most therapeutic regimens their broad pharmacological profile evokes unwanted or adverse effects in addition to the wanted effects. General side effects of BZDs include cognitive impairment (44), increased risk of fall and injury in the elderly (45), disturbance of sleep architecture (46), sedation, and muscle relaxation, among others (42, 47, 48). Sudden discontinuation after prolonged use may lead to withdrawal symptoms such as depressive mood, irritability, sleep disturbances, muscular tension, and tremor or even grand mal-like seizures. Treatment-emergent BZD use disorder is a rare, but sometimes serious adverse drug reaction. Additionally, in less than 1 % of patients or users, BZDs can induce paradoxical reactions ranging from talkativeness, restlessness, hyperactivity, excessive movement, to agitation and aggressive behavior in word and action, or even to seizures (46, 49, 50). Juveniles and elderly are especially susceptible to adverse effects (39).

Another group of molecules which target the high affinity benzodiazepine binding sites on GABAARs, are the Z-drugs: zaleplon, zolpidem, zopiclone and eszopiclone (see Figure 1). They were introduced in the 1990-ies and marketed as drugs of the millennium with claims for lower abuse potential and fewer side effects compared to BZDs. However, since their launch, the number of adverse event reports connected to the Z-drugs has been rising. They were shown to produce euphorogenic effects like prominently abused benzodiazepines such as lorazepam (Ativan), alprazolam (Xanax) and flunitrazepam (Rohypnol) (51). In addition, paradoxical reactions similar to those of BZDs have been described for Z-drugs as well (52). Overall, the side effects for Z-drugs are converging toward the ones observed for BZD administration, apart from a better performance on some cognitive measures in older populations (53). We will refer here to benzodiazepines and Z-drugs together as "BZ-site ligands" for brevity.

A variety of prescription drugs can affect benzodiazepine pharmacokinetics and effects by interfering with their liver

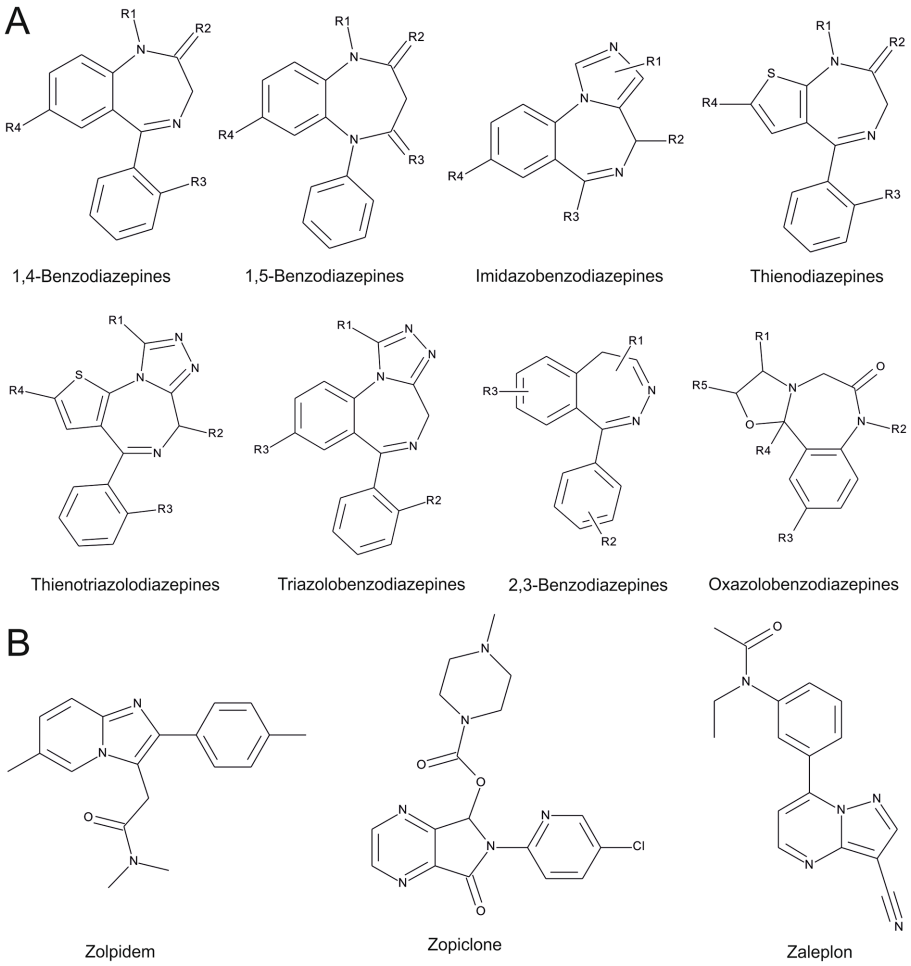


FIGURE 1
Chemical entities of benzodiazepines and Z-drugs, as in "New benzodiazepines in Europe review 2021" (26). **(A)** Benzodiazepine scaffolds are depicted. **(B)** Z-drug scaffolds, note that zopiclone comprises two entities (enantiomers), which are not reflected in this 2D- representation.

metabolism through the cytochrome P450 (CYP) system, especially isoenzyme 3A4 and 2C19 action (54–57). This can lead to accumulation of the compounds, with severe side effects, or to therapy failure due to accelerated clearance of BZ-site ligands. Combining diazepam (Valium) with drugs such as rifampicin or the antiepileptic drug carbamazepine can dramatically accelerate its clearance (58–60). Hormonal oral contraceptives, on the other hand, can reduce clearance and increase the half-lifetime for multiple benzodiazepines (61–65). Natural grapefruit juice can severely impair diazepam metabolism by inhibition of CYP3A4 (66, 67) leading to clinically relevant stronger diazepam effects and accumulation. It has been observed that additional benzodiazepines can interfere with other CYP isoenzyme activity and/or with glucuronidation (68–71).

1.3. Useful or problematic drugs: controversial issues concerning dose escalation, non-medical use, and unwanted effect severity

Between 1996 and 2014 the number of adults in the US that filled prescriptions for BZ-site ligands increased significantly (8.1 million, 4.1% to 13.5 million; 5.6%) (72). Accordingly, the total filled quantity tripled and overdose deaths involving BZ-site ligands quadrupled from 0.58 to 3.07 per 100.000 adults. There is an evident gap between prescription rates of BZ-site ligands between sexes reported in multiple sources, such that women receive prescriptions for these drugs about twice as often as men (73, 74). Remarkably, despite this fact and their widespread usage in the clinics, coherent systematic studies on sex differences in effects of BZ-site ligands are rare. The existing studies point toward a controversy in terms of substance misuse risk due to sex with some indicating male sex as a risk factor (75–77) and others vice versa (78–80). However, due to different study designs, comparison among them is difficult.

Owing to their widespread *in vivo* effects, BZ-site ligands are prominent among commonly misused drugs. Since they have mainly CNS depressant effects, they are categorized as "downers" (81). In the US, all benzodiazepines are controlled in schedule IV of the "Controlled Substances Act" meaning they are considered to have relatively low addictive properties while serving a medical need. Nonetheless, BZDs and to a similar extent Z-drugs can cause physical and psychological dependence after relatively short periods of time, which is why the rule for treatment regimen is "as short as possible, as

long as needed." An added concern is that BZ-site ligands may induce drug tolerance in many of their effects, meaning that a higher dose is required for achieving the same effects.

Therefore, although they are generally perceived as a safe class of compounds, BZDs and Z-drugs can be problematic in long term treatments and illicit drug use. If taken alone, the potential of benzodiazepine overdose to cause fatal adverse effects is comparatively low in contrast to other depressants, such as barbiturates, but existent (82–84). Between 2005 and 2011 the emergency department visits that involved BZ-site ligands almost doubled, according to the DAWN (Drug Abuse Warning Network) report (84). The risk for serious outcomes during an emergency department visit was higher for benzodiazepine users compared to non-users, and was escalated further by combining benzodiazepines with alcohol or opioids (84). In addition, BZDs have been shown to approximately double the risk for motor vehicle accidents (85), and similar effects have been described for zopiclone (86, 87).

BZ-site ligands are often not a primary drug of abuse, but are taken in combination with other drugs (43). In particular, BZDs with a rapid onset of action can create euphoric effects, usually observed at higher concentrations. Diazepam (Valium) and alprazolam (Xanax) are combined with methadone to potentiate its mood enhancing effect further (43). Cocaine and other stimulant users utilize BZDs to mitigate side effects (43) or for "coming down." The analysis of more than 1,200 oxycodone related drug abuse deaths from a postmortem database highlighted the prevalence of diazepam co-abuse in oxycodone users (84), as also described in other sources (88). The combination of alcohol and BZDs is particularly problematic given the low inhibition threshold of alcohol procurement by its social acceptance and easy accessibility (89). Furthermore, alcohol and BZ-site ligands both chiefly act as depressants, thus exerting a compounded effect when taken together. There is some evidence that for individuals with alcohol use disorder (AUD) a stronger psychoactive effect can be achieved after benzodiazepine administration. People with AUD in their familial history may also experience a different sensitivity and effects of alprazolam (90–93). Studies exist which describe drug – alcohol interactions and adverse outcomes that are associated with BZ-site ligands, but systematic comparisons between individual drugs are lacking. Thus, it remains unclear for most approved substances whether they are more or less problematic in different forms of medical and non-medical use, despite considerable anecdotal evidence that suggests that specific compounds are particularly well suited, e.g., as date rape drug, or have tendencies to elicit bad trips.

After all, Bz-site ligands have been an indispensable part of everyday clinical practice for decades and they remain so today (94). Attempts to restrict their use via tighter regulatory requirements for their prescription were followed by an increase in overdose emergencies involving drugs with a less favorable safety profile (95–97). Moreover, if due to excessive Bz-site ligand doses, acute sedation or respiratory depression are readily antagonized by intravenous flumazenil in clinical or emergency medicine settings. Thus, increased awareness and a more detailed understanding of the mechanisms mediating unwanted and at times dangerous BZD effects, in addition to supporting rational clinical decision making, could help to promote developing drugs with similar benefits but even more favorable risk profiles.

1.4. FAERS and use of pharmacovigilance data

At least partly because those substances are no longer protected by patents, comprehensive controlled studies and interindividual substance comparisons according to current scientific standards are lacking for the majority of BDZs & Z-drugs among indications in which they are currently used.

Pharmacovigilance is a rapidly growing scientific discipline that strives to detect, assess, understand and prevent drug-related issues and adverse events, thus in short includes every activity that is connected to better drug safety (98). To collect real world post marketing drug-adverse event observations, the U.S. government provides a federal database called FDA Adverse event reporting system (FAERS). The FAERS database includes adverse events, medication errors and product quality complaints that were submitted to the FDA by either health care professionals such as prescribers or pharmacists, but also by patients or other public members (99). Pharmacovigilance analysis typically utilizes these metrics to determine if a drug is associated with an adverse event. A greater value for these measures signifies a more substantial association between the medication and the unfavorable outcome. Thus, post-marketing pharmacovigilance data such as those in FAERS can provide highly useful signals for adverse reactions that were not observed in the early phases of the drug approval procedure. Due to the inherent limitations of the real-world data, such as a gap in provided dosages of the reported drug, co-usage of other substances, a lack of demographic data and others, specialized methods of data analysis have been developed (100–102). It is generally understood that a strong signal implies an association between a drug and an outcome, but cannot provide any evidence for causation. Thus, and due to other properties of real-life observations, pharmacovigilance data is not suitable for comparative pharmacology (101). However, it is the only data available to generate hypotheses on the basis of large numbers of real world observations and across a substantial number of drugs.

FAERS encourages use of "preferred terms" to report adverse events in MedDRA terms (See Figure 2). The MedDRA dictionary hierarchy is a categorization of medical terminology, which hence allows to analyze FAERS reports at the different MedDRA levels. The five levels of the dictionary are System Organ Class (SOC), High Level Group Term (HLGT), High Level Term (HLT), Preferred Term (PT), and Lowest Level Term (LLT) (103). For an overview of the MedDRA hierarchy and which levels were used in this study, see Figure 2A.

Since Bz-site ligands (comprising benzodiazepines and Z-drugs) are a broadly prescribed class of medications, the number of reports connected to their usage is vast. However, no comprehensive comparison between reports of individual compounds for Bz-site ligands has been performed to our knowledge yet. Here, we employ disproportionality analysis, which provides mathematically well-defined parameters for the strength of an association signal (104). Specifically, the commonly used information component (IC) value gives a measure of the strength of the quantitative dependency between the specific drug and the reported adverse event. Here we use the IC025, see Figure 2 and methods, which defines the endpoint of the 95% credibility interval (100). We analyzed a large FAERS dataset in order to generate individual drug profiles. We found and reported tendencies of drug-heterogeneity, some of which are confirmed by other sources containing clinical study data.

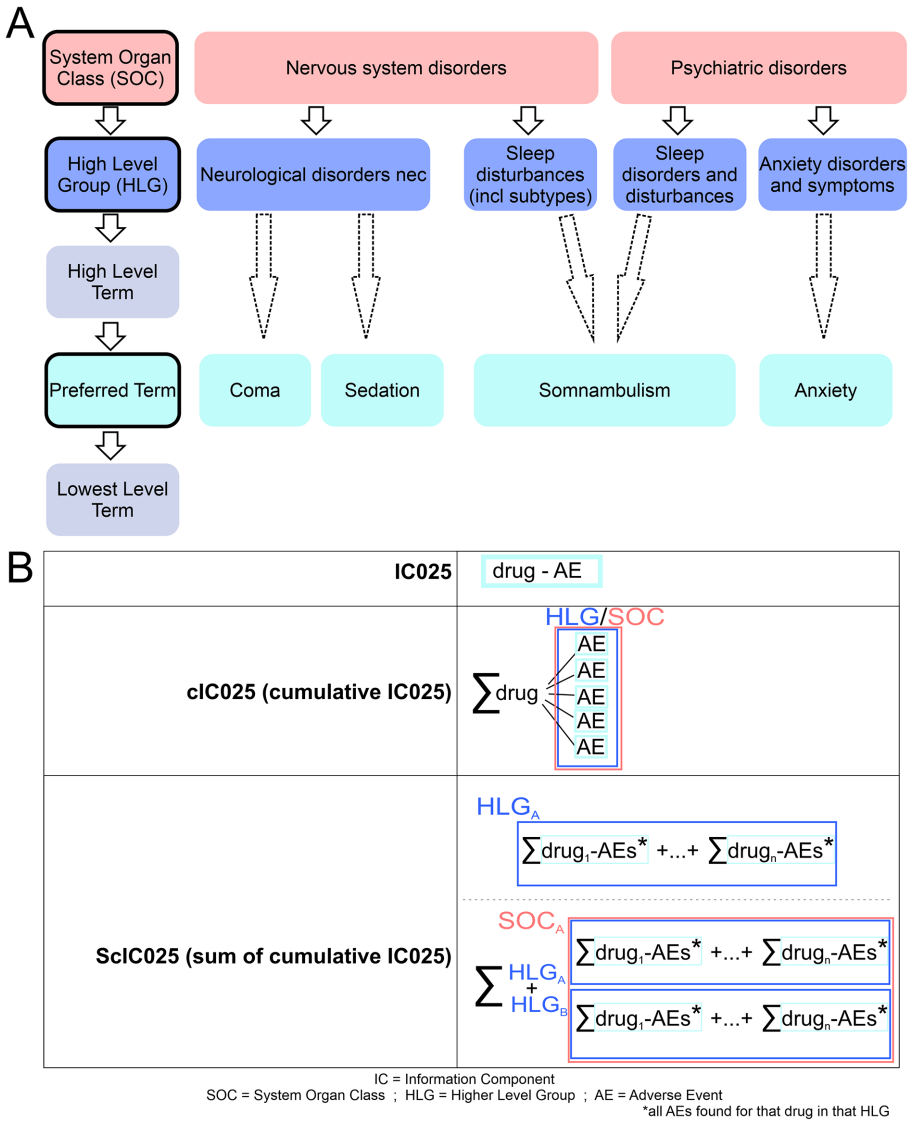


FIGURE 2
Overview of MedDra dictionary system and employed IC025 usage. **(A)** The five levels of MedDra hierarchy are displayed with specific examples to them; red: system organ class (SOC); blue: High level group (HLG), Cyan: Preferred term (PT). Arrows indicate the direction from higher levels to lower levels, dashed arrows indicate that we surpass the high level terms in the analysis shown in this work. **(B)** Different IC025 values and their respective calculations are shown. IC025 reflects on the drug-AE association; cIC025 is the sum of all IC025s for a drug within an HLG; ScIC025 gives the summation of cumulative IC025s for a HLG or SOC.

Molecular foundation for drug heterogeneity may be triggered by a variety of off-target and on-target effects. Conducting systematic investigations to explore every potential off-target effect of a drug may be impractical, given the vast number of molecules in the body. Thus, off target effects were not further considered in this study. For on-target heterogeneity, structural data provides hypotheses for mechanisms that can drive a multiplicity of overlapping and non-overlapping effects of the investigated drugs. These largely stem from multiple binding sites and their cooperativity at various receptor subtypes. The compounds for which informative FAERS records exist were thus also examined in terms of their chemical features that drive the pharmacodynamics with the hope to identify common drug properties that drive certain unwanted effects and a short overview of binding site heterogeneity within the family of GABAARs is also provided.

2. Results

We mined the publicly available FAERS (FDA Adverse Event Reporting System) data set from Khaleel et al. (105) to establish pharmacovigilance profiles per drug and sex, for all Bz-site ligands with sufficient data. For all steps of our analysis, the datasets from female and male reports were treated separately to obtain individual results per sex, in the same vein as done by Drug Central (106). The applied workflow is displayed in Figure 3 (see also the Methods

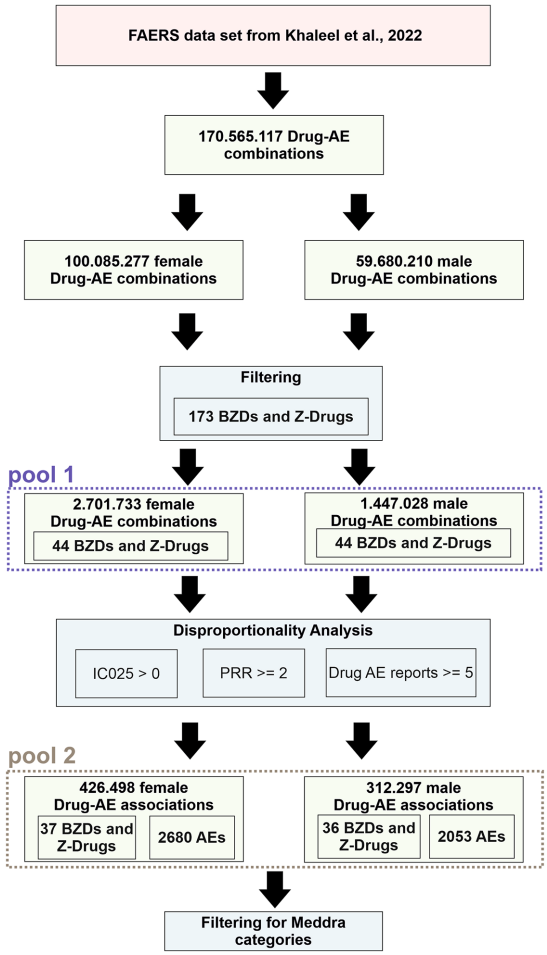


FIGURE 3
Pipeline that was performed on the FAERS dataset. Green boxes display the results obtained after the filtering steps, which are represented by blue boxes. Data that was used for analysis according to MedDRA categories is identified as "pool 1" and "pool 2" respectively.

section). The full data set comprised 170.565.117 drug – adverse event combinations, including 100.085.277 female drug-adverse event combinations and 59.680.210 male drug-adverse event combinations. These reports were filtered for 173 drugs composed of benzodiazepines and Z-drugs from our drug list (for detailed information, see Methods section and Supplementary Item 1). The filtering process left us with 2.701.733 female drug-adverse event combinations and 1.447.028 male drug-adverse event combinations, from the use of 44 benzodiazepines, for which a FAERS entry exists, and which are referred to as data pool 1 for brevity (see Figure 3; Supplementary Table S1). Disproportionality analysis was performed to identify drug-adverse event associations. Our primary criteria for inclusion of a record into data pool 2 were the commonly used thresholds of PRR > 2 and IC025 > 0 (107–110) as well as the existence of five or more records. Supplementary Item 2 provides the composition of pools 1 and 2 (see Figure 3) with respect to the total reports per drug that were analyzed. The main criterion used for subsequent data filtering was the IC025 value, as suggested by the UMC (Uppsala Monitoring Center) (111), since higher IC025 values reflect a stronger signal. Only 39 of the 44 drugs found in the dataset met the applied criteria and thus were used for further analysis (pool 2). The raw data is provided in Supplementary Items 3, 4 in Excel format.

The records from pool 2 (after the disproportionality analysis) were analyzed with the use of the MedDRA categories, and in some instances pool 1 data was utilized for comparison. To analyze pool 2 data, which contains only drug-AE associations, we employ the following nomenclature (see Figure 2B): IC025 denotes the value for a particular drug and an individual adverse effect combination where usually only positive values from pool 1 were used in the downstream calculations of aggregate values. Simple sums, cumulative IC025 (cIC025), are the aggregate of all positive IC025 values for a specific drug within a category (HLG or SOC). Summative cumulative IC025 (ScIC025) indicates the sum of cIC025-values for all drugs combined within the group (HLG or SOC), see Figure 2B.

2.1. Overview across all SOCs

At the highest MedDRA level of system organ classes (SOCs), associations were obtained for all 39 drugs, in 27 SOCs, Figure 3. To obtain an overview, the summed cumulative IC025 (ScIC025) values per SOC were computed and are displayed in Figure 4A. Not surprisingly, the largest summed cumulative signals were observed for "nervous system disorders" and "psychiatric disorders," together comprising the "neuropsychiatric" group. Owing to the widespread non-medical use of Bz-site ligands, it is not unexpected that the SOC "injury, poisoning and procedural complications" also displays a high ScIC025 as seen in Figure 4A, closely followed by "investigations." The top four SOCs were fully decomposed into the contributing HLGs, see Supplementary Figures S1–S3.

For each of the four top SOCs, we ranked the contributing drugs by ScIC025 over the whole SOC to investigate the gross contributions. The top 10+ drugs for each SOC are depicted in Figure 4B, where more than 10 drugs are shown because the top 10 differed between the sexes. It is noteworthy that each SOC features a unique drug ranking, and the top ranked drug is different for all four analyzed SOCs. Clobazam is top ranked in "nervous system disorders" for both sexes, and occurs in the top 10 for the other three SOCs as well. In the "psychiatric disorders," the top ranked drug is clonazepam, which is also found among the top 10 in all four datasets. Its relative contribution to each SOC differs in part considerably between sexes. Midazolam, as a procedural anesthetic, is the top ranked compound in the HLG "injury, poisoning and procedural complications," and is not among the top 10 in the "psychiatric disorders." The SOC "investigations" is very heterogeneous, as it does not reflect a single organ system but comprises parameter changes across all SOCs. There, striking differences in cIC025 between the male and female signals occur for several drugs, e.g., temazepam and nitrazepam. Closer inspection of this SOC and its constituent subgroups, see Supplementary Figure S3, reveals high ScIC025 value for females in cardiac investigations. This is also matched by the higher ScIC025 for females in the SOC "cardiac disorders," Figure 4A.

Results obtained for the four analyzed SOCs suggest a heterogeneous side effect pattern associated with individual compounds – while some drugs occur only in the top 10 of individual SOCs (e.g., brotizolam only occurs in "investigations"), others dominate several or all of the SOCs. In order to investigate drug

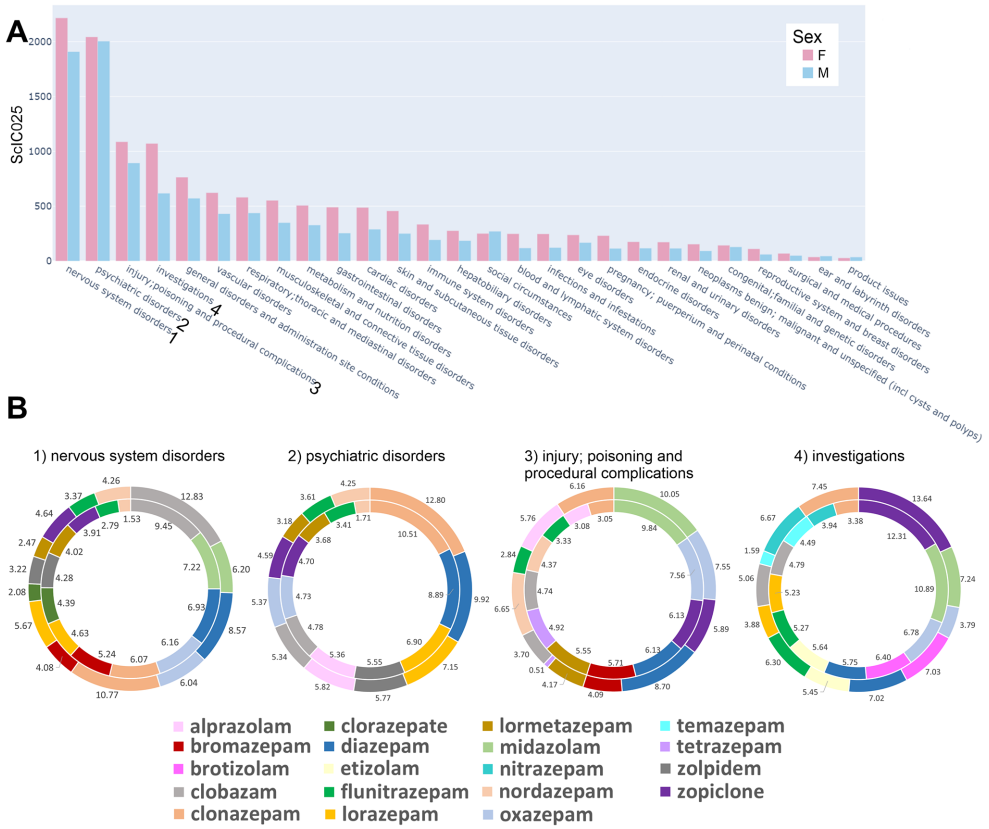


FIGURE 4
Distribution of AE associations across all organ system classes: **(A)** Summed cumulative IC025 (ScIC025) values per SOCs for all drugs which had a positive IC025 separated by sex. Bars reflecting female reports are light red, those reflecting male reports are light blue. **(B)** Pie charts are presented for the top 10 drugs for each sex (together 10 or more) with the highest cIC025 contribution to the four highest ranked system organ classes (SOCs) in terms of summed cIC025 (ScI025). The size of the displayed segments corresponds to the cIC025 contribution of each drug to the summed cumulative IC025 (ScIC025) and is shown as percentage. The outer circles reflect data for males, while the inner circles represent data for females, with the drugs sorted according to the female ScI025 rank values, starting at the top and descending in clockwise direction.

heterogeneity upon more detailed decomposition, we zoomed further into the top two SOCs – after merging them into "neuropsychiatric reports," see Figure 5. Supplementary Figures S1, S2 provide more details on the SOCs "nervous system disorders" and "psychiatric disorders" separately.

2.2. Analysis of neuropsychiatric AEs

Closeup analysis of the neuropsychiatric SOCs was performed in a next step. The largest contributing higher level groups in the neuropsychiatric SOCs are displayed in Figure 5. They comprise groups with "neurological/psychiatric disorders not elsewhere classifiable (nec)," two groups with disturbances in movement/motor systems, signs and symptoms related to sleep, anxiety signs, seizures, suicidal and self-injurious behaviors, and a group with disturbances in thinking and perception. The ScIC025 per HLG differs only to a small degree between sexes (Figure 5). As a next step we looked a drugs' contribution in terms of cIC025 to each HLG.

In line with the large contributions to the ScIC025 by clobazam, clonazepam and diazepam to the whole nervous system and psychiatric SOCs, these drugs are seen to have rather large cIC025 values in the individual neuropsychiatric HLGs as well. However, heterogeneity emerges at this level too: Clobazam is seen to contribute with a considerable association to the "seizures" HLG, as we have noted previously (112) but, e.g., with only a small signal to "anxiety disorders and symptoms." In the two groups concerned with movement and muscle symptoms, several drugs carry different association strength as can be seen in Figure 5B where clobazam has a stronger signal in males.

2.3. Neurological disorders not elsewhere classified

Interestingly, the HLG 1 ("neurological disorders nec," Figure 5A) accounts for almost half of the ScIC025 from the SOC "nervous system disorders" with a summed cumulative IC025 value of about 900 in females and 800 in males. This HLG was thus analyzed in detail at the level of the individual adverse event associations (= IC025 values), see Figure 6.

The major contributing AEs to this HLG are signs of sedation and over-sedation, including sedation, somnolence, sopor and coma, all indicative of CNS depression of various degrees (see Figure 6). The second largest group comprising agitation, restlessness and logorrhoea reflects paradoxical responses (see

nr	HLG	nr	HLG	nr	HLG
1	neurological disorders nec	2	psychiatric disorders nec	3	movement disorders (incl parkinsonism)
4	neuromuscular disorders	5	sleep disorders and disturbances	6	anxiety disorders and symptoms
7	seizures (incl subtypes)	8	suicidal and self-injurious behaviours nec	9	disturbances in thinking and perception

nervous system disorders psychiatric disorders

FIGURE 5
Distribution of AE associations across the organ system classes "nervous system disorders" and "psychiatric disorders": Higher level groups from the "nervous system -" and "psychiatric – disorders," in which a summed cumulative IC025 of both sexes adds up to 300 or higher, are displayed as polar bar charts. The individual cIC025 contribution per compound to the summed one in the neuro-psychiatric disorders is displayed as patterned segments as defined in the legend. Drugs in the polar bar chart are sorted by their total contribution of ScIC025 within the whole SOC per sex from high to low, with the higher ranked drugs at the outer rims. The table below identifies the nine largest contributions from the top two SOCs. green: nervous system disorders; blue: psychiatric disorders.

Figure 6). It is interesting to note that the cumulative signal for logorrhoea differs markedly between the sexes, and displays some drug specificity. For example, logorrhoea sticks out in the male dataset (IC025 = 5.3), and shows no association for females. This is due to the low number of reports (<5) and thus was not taken into pool 2. More detail can be found in the provided data in Supplementary Items 3, 4. In total, a large share of cIC025 in the neuropsychiatric groups thus reflects the known and expected signs of sedation and over-sedation on the one hand side, and paradoxical reactions on the other hand side.

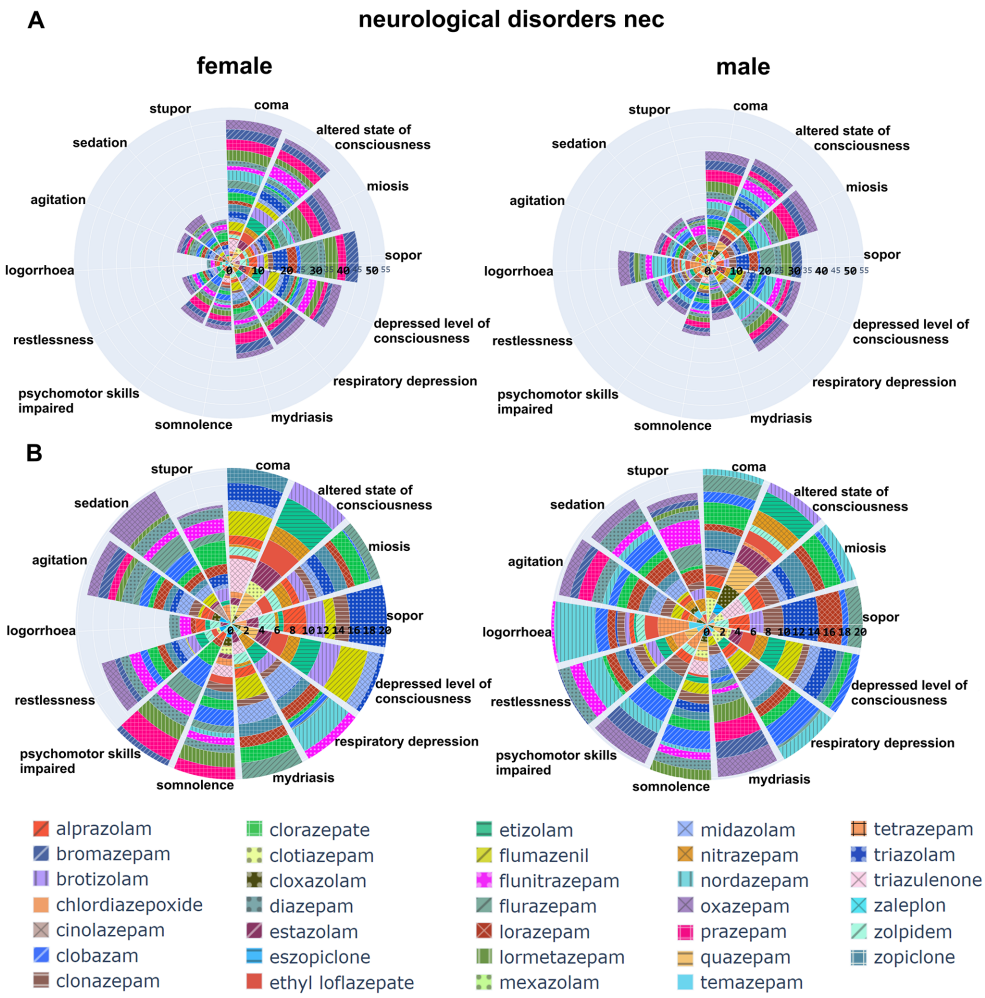


FIGURE 6
Distribution of AE associations across the HLG "neurological disorders nec": The top two polar bar charts display the individual adverse events that have a signal in this HLG as cumulative IC025 across all contributing drugs. For plotting, a cutoff was used: all AEs with a cumulative IC025>30 for both sexes added are plotted, the full dataset is in Supplementary Items 3, 4. The drugs are identified in the list on the bottom of the graph. Panel **B** is an enlarged view of panel **A**, note the cIC025 scale (0–20).

2.4. Highest ranked psychiatric HLGs

To investigate contributions to the cumulative neuropsychiatric signal beyond sedation and paradoxical responses in more detail, we analyzed the four psychiatric HLGs with the highest summed cumulative IC025 (Figure 3A) individually as shown in Figure 7. These comprise "psychiatric disorders nec," "sleep disorders and disturbances," "anxiety disorders and symptoms," and "suicidal and self-injurious behavior." Since sleep related disturbances occur both in the psychiatric and nervous system disorder SOCs, we merged these prior to the analysis (see Figure 2; Supplementary Figure S4). The individual AEs that contribute to each of the groups are provided in Supplementary Figures S4–S7 and Supplementary Table S2.

The HLG"psychiatric disorders nec" displays a higher fraction of total reports for males compared to females, and relatively balanced values are seen for the remaining three HLGs (Figure 7A). Diazepam has the highest cumulative signals for both sexes in the groups"psychiatric disorders nec" and "suicidal and self-injurious behavior." Additionally, three of the five highest ranked drugs for both sexes are diazepam, alprazolam and clonazepam (Supplementary Table S2). Figure 7B provides for each drug the fractions of reports within each of the HLGs from panel A from the respective per drug 100% values. An interesting contributor to the male dataset "psychiatric disorders nec" is nordazepam, for which >13% of all associated AEs are from these four HLGs, Figure 7B. The group of "psychiatric disorders not elsewhere classifiable" comprises AE associations chiefly from abuse and withdrawal signs, see Supplementary Figure S5. We noted that nordazepam generally has a large signal, i.e., strong associations with "drug abuse," "substance abuse" and related AEs. To look into these AEs more closely, we extracted the IC025 for each abuse-, addiction-and withdrawal relevant term from the neuropsychiatric SOCs on a per drug basis, and observed considerable heterogeneity there as well, see Supplementary Figure S8. In addition to nordazepam, oxazepam and lormetazepam have rather high IC025 values for most AEs related to abuse/addiction compared to loprazolam and flunitrazepam (triazulenone), which have only weak signals for males and no association for females at all. Notably, alprazolam is the only drug that

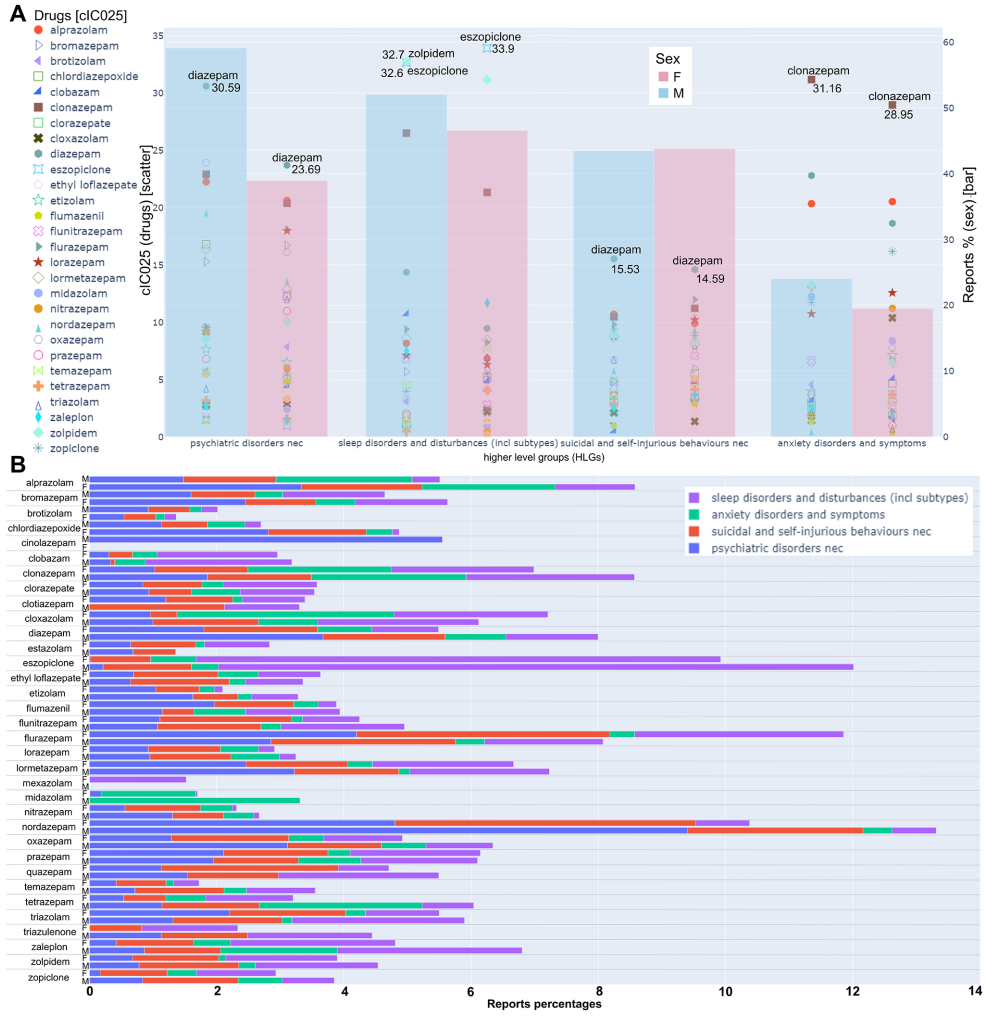


FIGURE 7
Detailed analysis of the top four psychiatric HLGs. **(A)** The left axis refers to the scatter plot. For each drug, the cumulative IC025 in the respective HLG is displayed. The drugs with the highest cIC025 value within each HLG are displayed on the graph; the respective cIC025 value is given next to the drug. The right axis refers to the bar graphs; blue: male, red: female; Bar height indicates the report percentage of all drugs within the HLG in relation to the reports of all drugs in all HLGs, as specified in the methods. Calculated as specified in the methods. **(B)** For each drug, the percentage of reports for a HLG in relation to the total reports from a drug are displayed as specified by the color legend. The raw data that is summarized in this Figure can be found in Supplementary Items 2–5 and Supplementary Table S2.

is associated with all investigated AEs in that bucket (Supplementary Figure S8).

Clonazepam not only features a strong cIC025 in the "psychiatric disorders nec," but is also among the top 5 ranked compounds in the HLGs "sleep disorders and disturbances," "suicidal and self-injurious behaviors nec" and "anxiety disorders and symptoms." In fact, for the male dataset, these four AE groups add up to nearly 10 % of all AEs this drug is associated with, Figure 7B.

In the merged HLG "sleep disorders and disturbances," the strongest signals are seen for eszopiclone and zolpidem. For the case of eszopiclone, the fraction of reports falling into this HLG is also exceptionally high, as seen in Figure 7B. At the level of the individual adverse events that add up to the drugs' cumulative signal in this group, eszopiclone is chiefly associated with signs of insomnia, while zolpidem is chiefly associated with various disturbances of sleep such as somnambulism and sleep related eating issues, see Supplementary Item 5. Interestingly, in sleep order and disturbances, two (male) and three (female) of the five highest ranked drugs are Z-drugs (Zolpidem, Eszopiclone, Zaleplon) as further described in Supplementary Table S2.

For the HLG "anxiety disorders and symptoms," clonazepam is the top ranked drug for both sexes. For some drugs, striking differences in the normalized report numbers between sexes are seen (Supplementary Figure S7). Specifically, eszopiclone has a high cumulative signal of this HLG for females, but not for males. Vice versa, and, e.g., midazolam, tetrazepam and zaleplon feature stronger associations for males. The top AE observed in this HLG, namely agitation, (Supplementary Figure S7) is also contained in the neurological disorders nec. Group and is not specific for anxiety related issues. For individual drug-AE combinations, agoraphobia and panic signs add up to considerable signal strength for the case of clonazepam, in both sexes.

The selected examples highlight the fact that individual drugs have different association strengths with adverse events that belong to

different groups of symptoms, and in part also between sexes. While the findings need to be interpreted with due care, the overall picture that emerges strongly suggests an unexpected degree of compound heterogeneity.

2.5. Sex differences

Given the occurrence of different signals for the two sexes in multiple datasets throughout our analysis, we next analyzed the data specifically with regard to sex differences in the neuropsychiatric SOCs on a per drug basis. In a first step, the merged neuropsychiatric data was visualized on a scatter plot where all drug/ AE pairs were plotted according to the respective sex-specific IC025 values, see Figure 8A. To capture those drug/ AE pairs, which have a stronger association in one sex, we filtered the data with an IC025 threshold of 2: 1 as displayed in Figure 8A. The further the ratio is from 1:1, the greater the distance between the data point and the diagonal. Thus, neuropsychiatric drug/AE events that occur in one sex only with a positive IC025, are reflected by points on the axes in Figure 8A. From the pools that have a signal ratio > 2:1 (and a signal in both sexes), we investigated the AEs with the highest cumulative IC025 from all the drugs per sex. The resulting top 20 for each sex are displayed in Figures 8B,C. The drug-AE pairs that have a signal only in one sex were also further analyzed by filtering for 20 drug-AE pairs with the highest IC025, see Supplementary Figures S9, S10. In addition, we identified the 10 drugs, which have the biggest contribution to adverse events in the same dataset, thus for all drug/AE pairs with a cIC025 ratio > 2:1 (Figure 8D).

Multiple psychiatric AEs are found in the data pool with stronger signals in one sex. Signs of paradoxical responses such as logorrhoea, mania, restlessness, and hypomania are seen for several drugs with a stronger association in males, but are absent from the top ranked AEs in the female >2:1 dataset. Nordazepam has an outstandingly strong signal for males not only for paradoxical responses, but a wide range of neuropsychiatric signs (see Supplementary Figure S11). Logorrhoea is associated with male reports exclusively for nordazepam, tetrazepam, and oxazepam see Supplementary Figure S9. For the data

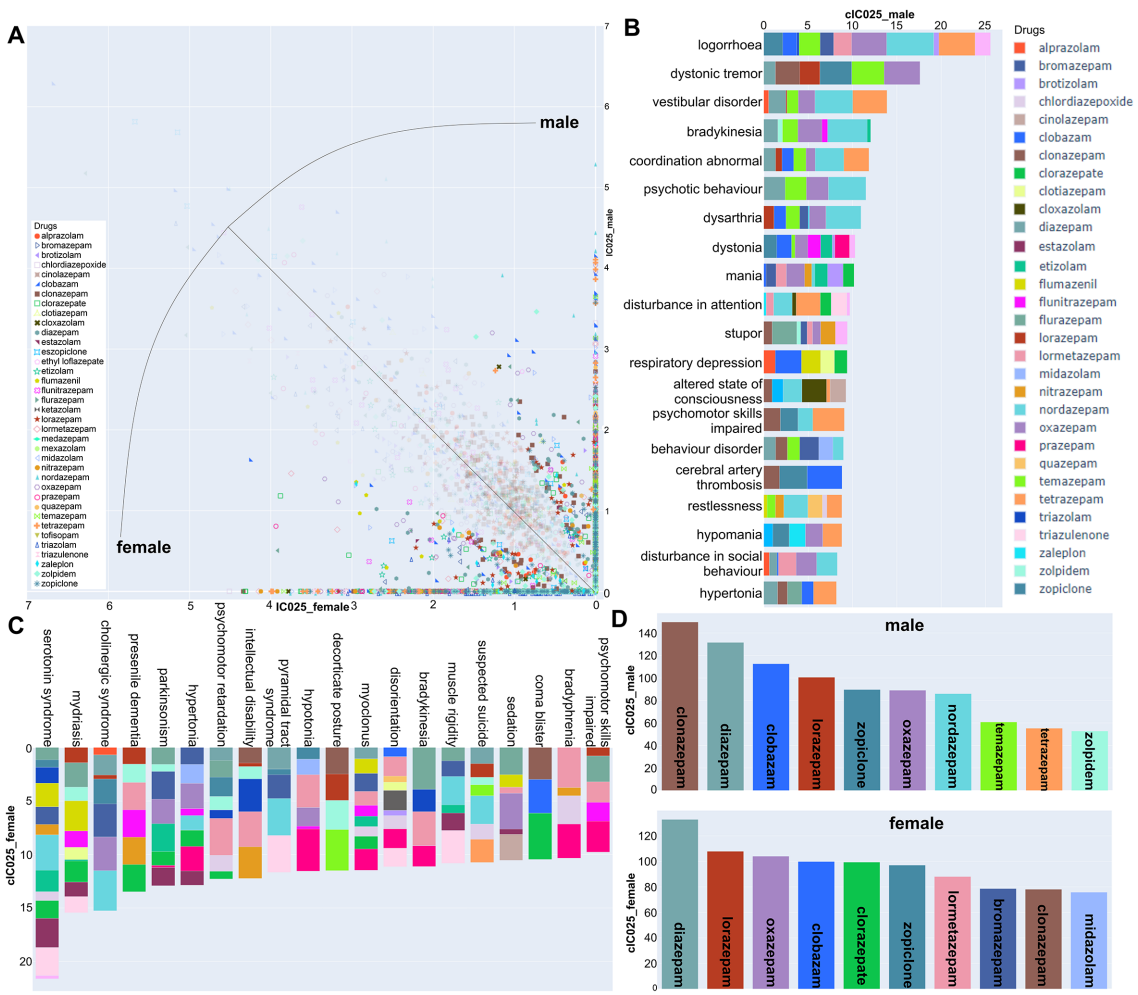


FIGURE 8
Sex differences in signal strength for neuropsychiatric AEs: **(A)** Scatter plot with all AE/drug pairs that exceed an IC025 ratio of 2:1 in either sex displayed in strong colors, pale colors for those <2. Points on the diagonal have equal IC025 for both sexes. **(B,C)** Top 20 neuropsychiatric AEs from the data of panel A above the 2:1 threshold toward one sex, are displayed (male: **B**; female: **C**) with the per drug contributions color coded. **(D)** The top 10 contributing drugs from the male and female data above threshold.

with a female: male ratio 2:1 or larger, the top ranked AEs are a mix of partly unspecific signs of neuropsychiatric changes. Among those AE-drug associations that are seen in females only, we note that anterograde amnesia is particularly strongly associated for clorazepate and lormetazepam, in the striking absence of an association for males (Supplementary Figure S10). Intrigued by this finding, and due to the relevance for illicit uses such as date rape of amnestic drugs, we mined the dataset for amnestic effects and confirmed the strikingly strong association for these two compounds, see Supplementary Figure S12. A minor point of interest here is that zolpidem is associated with a panel of amnestic AEs for both sexes, to a higher extent than the classical benzodiazepines.

Zolpidem is one of a few compounds with a stronger signal in the male:female >2:1 dataset, while the converse is true, e.g., for bromazepam and the already mentioned lormetazepam. Of the compounds which display stronger associations for males, the majority has more female reports in pool 2, thus, the difference in signal strength is likely a specific phenomenon. From the data that can be mined in this way from FAERS, findings concerning such sex-specific AE profiles of some drugs would be an interesting substrate for further pharmacoepidemiological and clinical follow up studies.

2.6. Physico-chemical descriptors

In total, pharmacovigilance data strongly suggests that the profiles of Bz-site ligands differ considerably in terms of the human *in vivo* effects that they can elicit, and that safety profiles for some of the drugs might be somewhat incomplete. The question of molecular drivers of such heterogeneity are manyfold, and deserve brief consideration to further align the pharmacovigilance derived effects with testable hypotheses for future research.

To get insight into molecular patterns of similarity and heterogeneity at the drug level, we evaluated all compounds based on physico-chemical and 3D properties. This was done as the integration of pharmacophore models and fingerprints in pharmacovigilance data analysis can reveal previously unknown safety concerns associated with a drug scaffold, and thus enable the implementation of measures to enhance drug safety. One such measure is to avoid certain moieties in drug development or to abstain from certain drugs to circumvent the occurrence of specific adverse events. Furthermore, this could allow for the exclusion of these derivatives in specific patient cohorts. A systematic view onto drug similarities and differences can also reveal unexpected cliffs in the structure–activity landscape and thus inform bed-to bench considerations for further drug development.

In the past, it has been difficult to establish structure–activity relationships for molecules targeting the GABAA receptors due to small changes in chemical scaffolds causing in part unexpected observed heterogeneity of structure–activity landscapes derived in heterologous expression systems, or even in preclinical and clinical *in vivo* outcomes (113, 114). The goal here was to approach the question of heterogeneity from the perspective of pharmacovigilance, and to relate the outcomes with ligand-based approaches. To accomplish this, we used two methods to describe the physico-chemical properties and 3D features of the molecules being studied. Firstly, we produced ligand fingerprints, which represent each molecule as a combination of recognized physico-chemical parameters, see Methods section. We then clustered the molecules, as shown in the upper panel of Figure 9. Secondly, we employed a pharmacophore model that incorporates both pharmacophore features (termed "color" by the used software) and molecular shape to group the substances based on their 3D orientation/size and functional groups, which are all critical factors in drug-protein target interactions. Their overlap in properties was used to group compounds, as shown Figure 9.

Our analysis revealed a more complex molecular landscape in the ligand fingerprint analysis than anticipated based on the 2D/3D-structure similarity of the compounds, which is consistent with the more traditional pharmacophore analysis. The pharmacophore demonstrated that all isomers of the "xazolam" compounds (cloxazolam, ketazolam, mexazolam, oxazolam) formed a distinct cluster due to their shared structural features. Similarly, the triazolo-compounds as well as the traditional 1,4-benzodiazepines such as diazepam and its metabolites also cluster together (Figure 9, lower panel). While ligand fingerprint analyses generally agree with the former, we resolved some unexpected clusters of compounds, such as zaleplon grouping together with lormetazepam (Figure 9, upper panel), which are structurally dissimilar based on 3D properties alone. Similarly, zolpidem formed a cluster closest to midazolam and clotiazepam, despite its distinct chemical 3D-body compared to the others. Thus, the ligand fingerprint analysis, based on various physicochemical properties, revealed a more nuanced picture, which is less intuitive but complements the 3D properties obtained by the pharmacophore results.

2.7. Complexity of on target effects

The from FAERS signals suggested considerable compound heterogeneity is not too surprising in the light of the observed compound promiscuity at single GABAARs in the past combined with the existence of multiple homologous GABAAR subtypes (17, 115, 116). Recently structural data is accumulating that confirms and extends the existence of non-canonical binding sites and differential usage of binding modes, and thus provides structural correlates and hypotheses for effects specific to certain compounds. The current status of structural evidence is summarized in Figure 10.

The current structural evidence thus demonstrates several important points for the understanding of drug structure–activity relationships: (i) Compounds with a common chemical core can have different binding modes at the high affinity site, as demonstrated by the flumazenil binding mode that differs from the one observed for diazepam and alprazolam (118–120). (ii) The non-canonical sites that have been postulated on the basis of mutational studies are largely confirmed, and extended by the structural evidence (121, 122). The structural evidence again demonstrates distinct binding modes in these sites as well, as shown in Figure 9B (120). (iii) Biochemical evidence for further non-canonical binding sites, such as those observed at ECD β2+/γ2-interfaces (123) are supported by the observation of receptors that lack alpha subunits (17).

2.8. The complex relationship between chemical similarity and pharmacological trends

It is known that drug-protein interactions for ligands with chemical similarity form structure activity landscapes with "smooth"

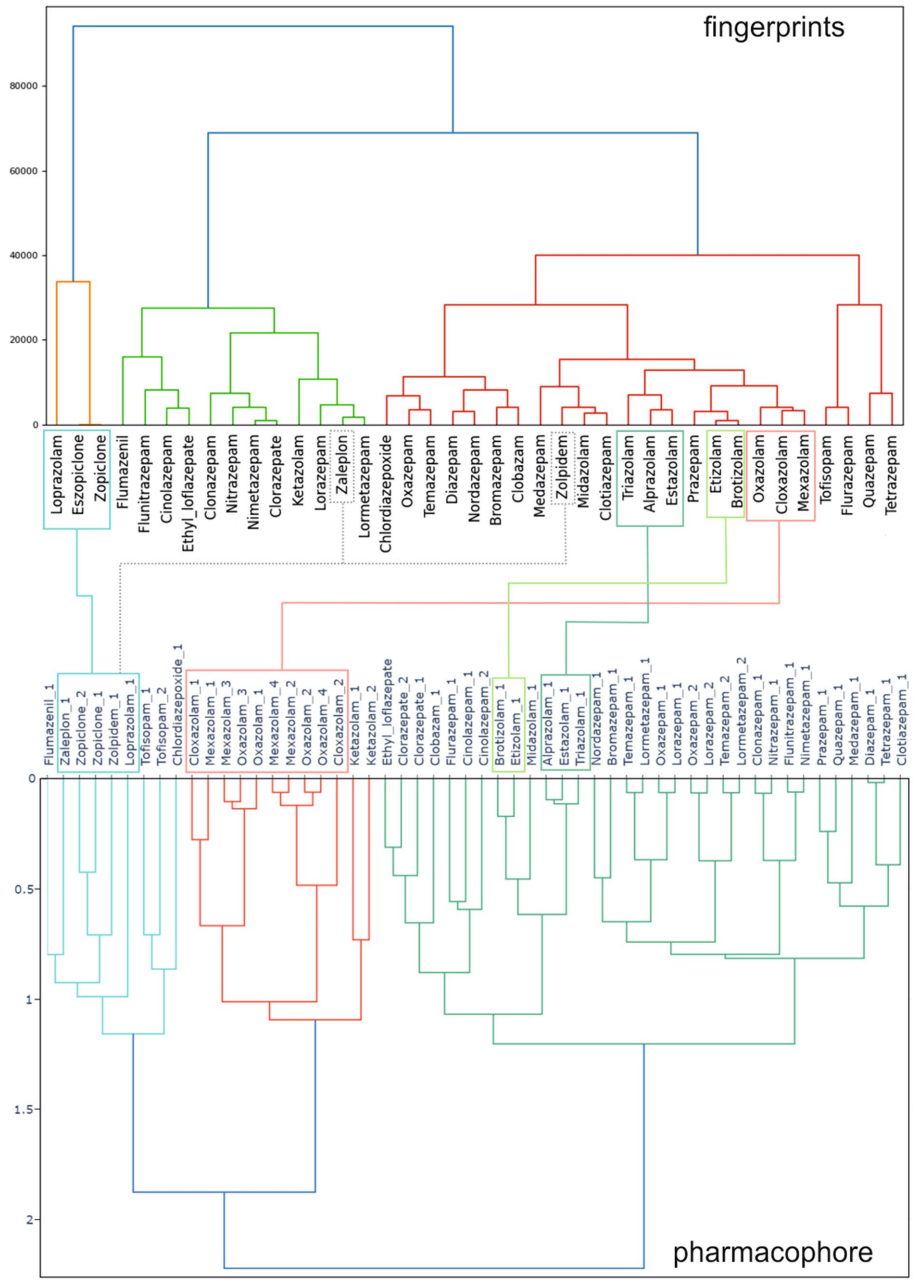
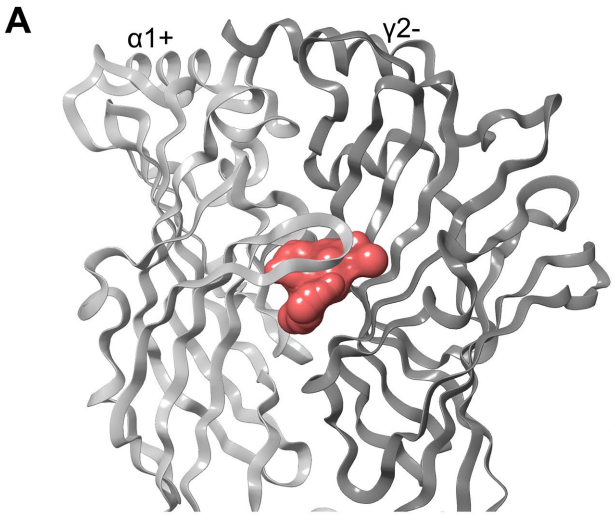
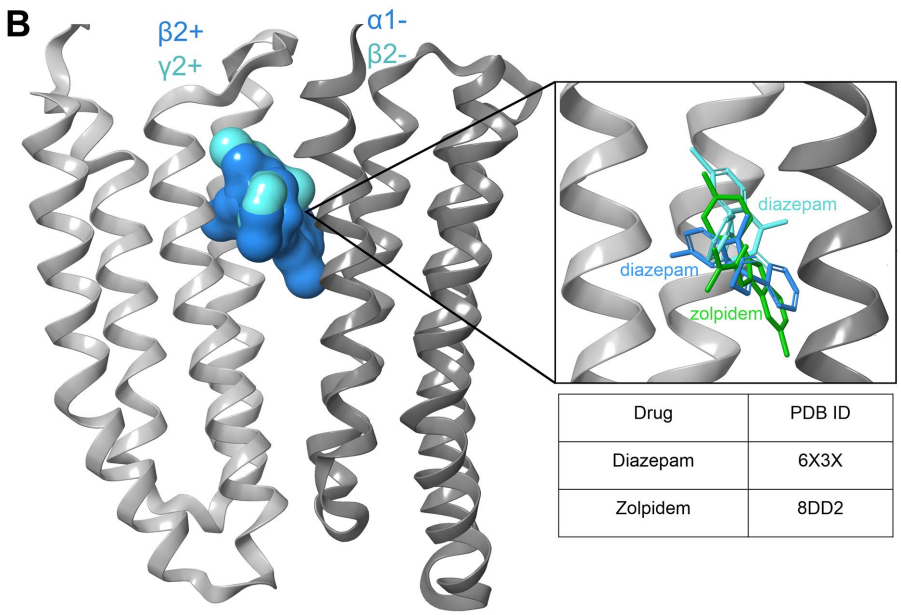


FIGURE 9
Clustering of 39 benzodiazepines and Z-drugs (from data pool 2 in Figure 2) based on their physico-chemical parameters and 3D properties. Upper panel: results of a ligand fingerprint analysis, which groups compounds based on their physico-chemical descriptors. Lower panel: results of a pharmacophore approach, which considers the size/shape and functional groups of compounds, thus is based chiefly on 3D properties of the molecules. Multiple stereoisomers of substances were considered for 3D- structure analysis and denoted by "_1" or "_2": For many compounds, only one structure exists, for enantiomers with a single chiral center two molecules exist as is the case for zopiclone, and for mexazolam, four molecules exist. The original values were obtained from vROCS® (for stereoisomer generation and shape/color calculations) and can be accessed in Supplementary Item 6. Connecting lines indicate some representative compounds that are grouped together by both, the ligand fingerprint analysis, and the pharmacophore approach. Dashed connecting lines indicate selected differences in clustering.

and "rugged" features, reflecting the interactions with binding sites that enable binding of similar molecules, and possess spatial features that lead to drops in activity due to small chemical changes. Thus, compounds that form clusters in chemical space are more likely to have overlapping pharmacological profiles – up to a degree. Intrigued by the seemingly dissimilar patterns of AEs found for zopiclone and eszopiclone, we analyzed these two compounds more closely along with another pair of chemically similar drugs, namely brotizolam and etizolam as displayed in Figure 11. For eszopiclone, the IC025 and fractional report share in the HLG "sleep disorders and disturbances" is outstandingly high. In contrast, zopiclone has no particularly strong association with any disturbance in sleep. This is intriguing as zopiclone is the racemic mix of eszopiclone and the presumed less affine/active R-enantiomer (124, 125).



Drug	PDB ID
Alprazolam	6HUO
Diazepam	6X3X
Flumazenil	6X3U
Zolpidem	8DD2



Drug	PDB ID
Diazepam	6X3X
Zolpidem	8DD2

FIGURE 10
Benzodiazepine and zolpidem binding sites. **(A)** Extracellular high affinity binding site (BS) shared by BZDs and Z-drugs, which requires an α1,2,3 or 5 subunit as a principal component (+) of the BS and a γ1-3 as a complementary part (–) of the interface. Light gray: α1+; dark gray: γ2-; red surface: ligands bound to the binding site in various structures as listed in the table. **(B)** Diazepam and zolpidem low affinity binding sites within the transmembrane domain (TMD) of the GABAA receptor. Diazepam density has been resolved in both the α2+/β2-, and the β2+/α1- pockets within the interface at the upper TMD between the two subunits. Zolpidem was found only in the latter, in 8DD2 (117). Light gray: β2+/γ2+ subunit, dark gray: α1–/β2- subunit; ligand surfaces are superposed in blue and cyan. The insert shows the three ligands in superposition, color coded to emphasize the different binding modes that diazepam displays in the two sites, respectively.

First, it is very interesting to note that the top 10 AEs for eszopiclone and zopiclone display no overlap at all. As expected from the data presented in Figure 5, the majority of strong AE associations for eszopiclone are disturbances in sleep and sleep-related phenomena. They affect both sexes to a comparable degree. For these sleep related AEs, zopiclone in stark contrast has a mixed pattern of positive and negative associations. The top 10 AEs for zopiclone cover a broad spectrum of neuropsychiatric phenomena, some of which lack corresponding reports for eszopiclone altogether. For dystonic tremor, only male reports exist, and for muscle spasticity, a negative association for males and a robust positive signal for females are seen. In the case of intentional self-injury, which bears a strong association for zopiclone, the negative IC025 for eszopiclone strongly implies that this adverse event is associated specifically with zopiclone. These data, taken together, suggest that R-zopiclone is not simply a molecule with lower affinity (124), but rather can exert highly specific and dominant side-effects and can potentially overcome the paradoxical responses to eszopiclone on the sleep-related side effects. We also compared another pair of drugs which cluster together very closely in both fingerprints and pharmacophore features, namely etizolam and brotizolam. For this case, the FAERS profiles are highly similar as would be anticipated.

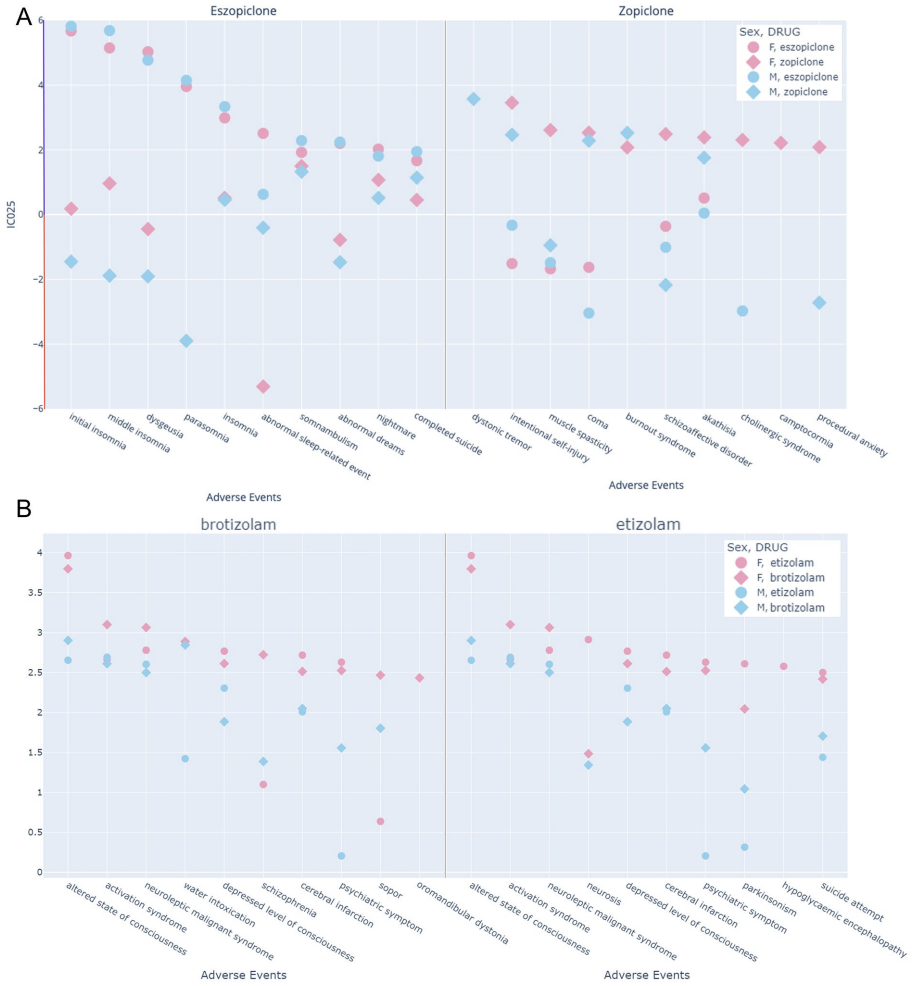


FIGURE 11
Top 10 neuropsychiatric AEs of four drugs to examine putative patterns of similarity induced by chemical similarity. **(A)** Eszopiclone and zopiclone: The left-hand side displays the top 10 adverse events of eszopiclone from the neuropsychiatric SOCs, as reflected by the IC025, and the corresponding values for zopiclone are displayed in addition. The right-hand side displays the top 10 adverse events of zopiclone from the neuropsychiatric SOCs, as reflected by the IC025, and the corresponding values for eszopiclone are displayed in addition. The y-axis reflects the IC025 value, which must be positive for an association between a drug and a reported adverse event. Data with negative values is taken from the data pool 1 prior to the disproportionality filter. **(B)** The left-hand side displays the top 10 adverse events of brotizolam from the neuropsychiatric SOCs, as reflected by the IC025, and the corresponding values for etizolam are displayed in addition. The right-hand side displays the top 10 adverse events of etizolam from the neuropsychiatric SOCs, as reflected by the IC025, and the corresponding values for brotizolam are displayed in addition. The y-axis reflects the IC025 value, which must be positive for an association between a drug and a reported adverse event. Data with negative values is taken from the data pool 1 prior to the disproportionality filter.

3. Discussion

As FAERS data has serious limitations that cannot be readily compensated for, pharmacoepidemiological studies would be needed to further substantiate or falsify the associations we identified. To address the question of robustness, we examined several selected drug-adverse event combinations that yield strong signals in our analysis with a semi-systematic search in the literature and in databases that rely in part on other means of evidence, such as the SIDER database (126) which chiefly utilizes product information, which in turn is derived from results obtained in appropriate clinical trials. The results of this analysis are provided in Table 1.

This compilation of converging pieces of evidence is far from comprehensive, but serves to demonstrate that strong associations derived from disproportionality analysis of large datasets often are confirmed in systematic studies.

With this study, we challenged the notion that benzodiazepines and Z-drugs are often considered to comprise a class of interchangeable drugs, apart from well accepted differences in pharmacokinetic properties. This is in striking contrast to anecdotal evidence and early preclinical literature (133). In order to examine data from human observations, we performed a comprehensive analysis of the pharmacovigilance data of BZ-site ligand associated AEs mined from the FDA adverse event reporting system. We included reports collected between 2004Q1 and 2021Q3. Those data suggest a diverse portfolio of AEs per compound, in part vastly different between individual compounds. This is partly reflected in product information and in the scientific literature, but systematic data and individual

TABLE 1 FAERS signals and other evidence for selected drug-AE event pairs: from the SIDER database, side effects reported as "frequent" or "common" are indicated with +, others as (+), and effects not mentioned there are indicated with -, n/a stands for absent drugs.

AE-Drug	IC025	PRR	SIDER	Studies* in agreement
Seizures- clobazam	M: 4.54\F: 4.71	M: 25.64\F: 28.77	–	(127, 128)
Aggression-Nordazepam	M: 3.81\F: 2.00	M: 18.89\F: 9.78	n/a	
Alanine-Aminotransferase level abnormal – Nitrazepam	M: 4.93\F: 4.66	M: 82.06\F: 65.95	–	(129)
Propofol Infusion Syndrome-Midazolam	M: 4.84\F: 5.26	M: 58.51\F: 104.06	–	(130, 131)
Dysgeusia – Eszopiclone	M: 4.77\F:5.03	M: 30.5\F: 35.27	Undefined frequency	(132)

*Studies include clinical trials and papers reporting or analyzing clinical studies.

safety profiles are scarce. Thus, pharmacovigilance data is a precious source of human observations and can play a pivotal role in the identification of individual compound profiles.

Here we focused chiefly on neuropsychiatric AEs, as the BZ-site ligands are mostly classified as psychotropic substances, apart from a few antiepileptics. In the data extracted for neuropsychiatric MedDRA terms, only 11 of the 39 investigated drugs are responsible for more than 58% of the total neuropsychiatric ScIC025 (see Supplementary Figure S13) – this suggests that the currently available drugs show different dispositions to induce adverse neuropsychiatric signs. Our data suggests that this is not chiefly due to factors such as prescription bias, because we find drugs with small numbers of records (e.g., nordazepam) as well as compounds with high report numbers (e.g., diazepam) in the group with strong neuropsychiatric AE signals, but also highly prescribed substances such as triazolam or eszopiclone with a relatively low cumulative neuropsychiatric signal, see Figure 5 and Supplementary Figures S1, S2.

Outside of the neuropsychiatric SOCs, it was interesting to note though that in the MedDRA higher level group "investigations" we also found a high cIC025 signal. Interestingly, in records from females, changes in electrocardiogram parameters and in blood pressure are particularly strong compared to males (see Supplementary Figures S3, S14, S15).

Not surprisingly, multiple signs of over-sedation and related effects dominate the total neuropsychiatric ScIC025, closely followed by a very strong cumulative signal for signs indicative of paradoxical responses as seen in the group "neurological disorders nec," see Figure 6. The observation that only few of the compounds strongly associate with signs of paradoxical responses might suggest that they are specific to certain drugs, and thus, their incidence should be quantified per drug and not for the class as a whole. At least at the level of pharmacovigilance we find clear signs for a high degree of drug specificity, which is in good agreement with preclinical work. The most startling observation in this category is probably the very strong association of eszopiclone, classified as a hypnotic drug, with different forms of insomnia and other sleep disturbance signs which is nearly absent in the zopiclone data (Figures 7, 11).

In the psychiatric higher-level groups, apart from expected effects such as signs of confusional states and impaired psychomotor responses due to over-sedation, we observe strong signals also for anxiety symptoms, and for self-injurious behaviors. In this group, we note another difference between zopiclone and eszopiclone: Self-injurious behaviors are strongly associated with zopiclone only (see Figure 11). In total, the FAERS data suggests considerable drug heterogeneity. This applies not only to unwanted effects of medically used BZ-site ligands, but also issues related to non-medical drug use. We extracted the IC025 values per drug for signs and symptoms of dependence, drug abuse, and for withdrawal symptoms (Supplementary Figure S8). While this dataset needs to be interpreted with due care and may be biased by many confounding factors, it does feature considerable drug heterogeneity that is worthy of further investigation.

Non-medical drug use can be recreational due to desired drug effects, e.g., as downers or to enhance effects of other psychoactive substances, or for illicit purposes such as "date rape" drug administration. In this context, amnestic effects are of particular interest. We noted that anterograde amnesia is associated with female records exclusively for the case of lormetazepam and clorazepate with an IC025 > 3 (Supplementary Figures S10, S12). Only 17 (of 39 drugs in our pool of disproportionately strong associations) drugs were found to be associated with any amnestic effects, and for example among the Z-drugs, zaleplon has none. These findings suggest that the individual drugs show also considerable heterogeneity with respect to properties that are compatible with abuse as date rape drugs. While the limitations of pharmacovigilance data fully apply, further follow up of such hints toward drug heterogeneity should stimulate systematic investigations.

In this study we specifically identified pronounced drug differences in AE event profiles of a substantial number of compounds. Taking into consideration that benzodiazepines are prescribed approximately twice as often to women as to men in the US, a similar ratio of adverse event reports in the FAERS database would be anticipated in absence of sex specific factors involved. However, additional layers of complexity have to be taken into consideration that limit data interpretation: (1) the possibility that adverse reports are more often reported for a specific sex, even if they occur in the other sex as well (2) that some benzodiazepines are prescribed more often than others for women (such as for anxiety disorders) and might bias the reports therefore for certain indications toward one sex, (3) the dark figure of individuals abusing benzodiazepines without prescription, which is reported to be higher in men, (4) the missing total number of prescriptions for a specific drug and sex in the FAERS database from which the reports result, and (5) the reports resulting from prescription for different indications, and thus different dosages which information is mostly lacking (6) the reports based on illicit use that is lacking prescription and is combined with other substances such as opioids and alcohol often and (7) others.

However, preclinical and *in vitro* research provides some hints though why some compounds may display sex differences that are not due to data bias: Supra- additive effects with endogenous cannabinoids

(37) and (neuro-) steroids (134) have been observed for benzodiazepines *in vitro*. Recent studies propose native GABAA receptors to possibly harbor endogenous allopregnanolone before the addition of benzodiazepines (38).

We attempted to correlate molecular properties with FAERS derived drug profiles which we identified. Overall, we found very little evidence for any correlations between the chemical compound properties and their pharmacovigilance fingerprints. The limitations of a ligand-based approach to structure–activity relationships is impressively demonstrated by the vastly different FAERS profiles of eszopiclone and zopiclone. Our data implies the coexistence of two phenomena: As evidenced by the highly overlapping chemical and FAERS profiles of etizolam and brotizolam (see Figure 11), highly similar compounds can share most key properties as drugs – and in contrast, steep cliffs in structure activity landscapes can occur as well as appears to be the case for S- and R- zopiclone. The latter phenomenon has its structural correlation in the multitude of binding sites with which each molecule can interact with individual affinity and efficacy. It has long been hypothesized that a multitude of distinct receptor subtypes with unique binding sites are and mediate the broad range of *in vivo* effects that are observed for benzodiazepine site ligands as reviewed in (135). In line with this, research from rodent models on subtype specific pharmacology has had limited translation success (113), which is at least in part owed to different transcriptomes of neuronal cell types, e.g., in the limbic system and discrepancies in regio-specific subunit expression between animals and humans (136). Hence, there is accumulating evidence that not only the so-called "high affinity" binding sites of these drugs contribute to pharmacologically relevant effects, but that additional binding sites that are shared in part with general anesthetics also contribute to the observed *in vivo* spectrum of effects. Differences in compounds' ability to utilize these interaction sites will lead to specific pharmacodynamic profiles. It is already clear that the tendency for individual BZ-site ligands to occupy additional sites apart from the canonical high affinity sites is different among compounds (120, 122), which can be expected to impact massively on the spectrum of *in vivo* effects due to the near complete lack of isoform- differences in the low affinity sites (137). A recent surge in structural findings allows an updated view of known and putative allosteric sites by which wanted and unwanted pharmacological effects are potentially mediated, as summarized in Figure 10.

Moreover, even the heterogeneity of compound binding and effects at the canonical sites is vastly understudied: The pharmacology for γ1 and γ3 is very incomplete, and the high expression level of the γ1 subunit in human limbic system structures might account for highly specific drug effects for substances that act on γ1- containing receptors (133). An added layer of complexity comes from the modulatory efficacy, which can range per compound, substance concentration and high affinity site from strong GABA enhancing (PAM) effects to strong GABA diminishing (NAM) effects (133, 138–140). For most approved benzodiazepines and Z-drugs, data of their modulatory effect in the major receptor subtypes is completely lacking and PAM effects are assumed, with the exception of the "antagonistic" chiefly silent modulator flumazenil. Beyond the vast diversity of allosteric sites used by "Bz-site-ligands" on GABAARs, off-target effects certainly may be drivers of individual drug effects as well, even though broad panel CNS- target assays indicate that most of these compounds have fewer off-targets compared to many other CNS therapeutics.

In summary, this study provides insights into the pharmacological properties of BZD compounds and Z-drugs and helps to inform clinical decision-making and drug development in this area. The analysis of the FAERS dataset and the application of ligand fingerprint and pharmacophore analyses reveal a more nuanced picture of the heterogeneity of BZ-site ligands, which can help to identify potential therapeutic uses and adverse effects as well as shape clinical studies on this topic in the future. The FAERS profiles of many compounds suggest sex-specific side effects.

Our analysis produced strong drug-AE associations. While pharmacovigilance data cannot confirm alerts nor offer mechanistic interpretations, we hope our findings stimulate follow up research, and potentially adaptations of prescription practice to meet modern standards of sex-specific care. If appropriate clinical studies can confirm some of the associations derived from the FAERS dataset, product information and subsequently also the legal classification of individual compounds could conceivably be adjusted to account for increased risks of unwanted effects by the addition of specific warnings to product information.

4. Materials and methods

4.1. Data mining

Four publicly available sources were used to generate a list of benzodiazepines and Z-drugs: Drugbank (141), Wikipedia (142, 143), and Wikidata (144). To accomplish this, different data extraction techniques were utilized for each source. To collect pharmaceuticals from Drugbank, for example, a Python script was used to filter compounds associated with each GABAAR subunit, and this list was then manually screened for benzodiazepines and Z-drugs. We used SPARQL to search Wikidata for drugs related to any of the 19 components. We also obtained benzodiazepines from two Wikipedia pages (142, 143). The final list included 173 benzodiazepines and Z-drugs.

4.2. FAERS analysis

To conduct the pharmacovigilance analysis of benzodiazepines and Z-drugs, a FAERS (FDA Adverse Event Reporting System) dataset was utilized, which was taken from Khaleel et al. (105). This dataset covers adverse event reports from Q1 2004 to Q3 2021. Initially, the dataset was divided into male and female subsets. Records with unknown sex were removed. Records from female reports that deal with occurrences of the offspring were removed by manual curation if the need arose. Afterwards, a disproportionality analysis was performed for each drug-adverse event pair in both datasets.

Disproportionality analysis was used to assess the association strength between drug use and reported unfavorable outcome (or adverse event, AE) (100–102). For each drug-adverse event pair, the information component (IC), 95% confidence interval of IC (IC025) (100, 102), proportional reporting ratio (PRR), and reporting odds ratio (ROR) was calculated (101, 105). The IC was employed to evaluate the likelihood of genuine values falling within an assigned

range. The Uppsala Monitoring Centre developed and validated this approach using Bayesian neural networks to create the information component IC (111, 145), which represents the logarithmic base 2 of observed/expected ratios and is commonly used for analyzing WHO databases (100, 102, 146). In numerous studies conducted by both UMC and other researcher groups, IC025 has been utilized as a benchmark for identifying positive drug-adverse event connections (102, 104, 147–151). Further investigations employ the PRR measure with the IC025 and necessitate a minimum of 5 observations to ensure an affirmative signal (107–110), which we followed in this work. All relevant records were extracted for the 173 drugs on our drug list belonging to the benzodiazepines and Z-drugs categories from the dataset and examined the system organ classes (SOCs) and higher level groups (HLGs) within the MedDRA. For this MedDra Version 22.1 was used and only adverse events have been considered which could be identified in this MedDra version. The level of individual AEs was analyzed where appropriate by application of filters. Cumulative IC025 values, as well as relative and absolute report numbers were obtained as appropriate sums from the filtered records.

	Drug	All other drugs	Total
Adverse event	a	b	a + b
All other adverse events	c	d	c + d
Total	a + c	b + d	n = a + b + c + d

a = Reports of the drug of interest with the adverse event of interest. b = Reports of all other drugs with the adverse event of interest. c = Total drug reports of all other adverse events. d = Total reports of all other drugs with all other adverse events.

$$Proportional\ Reporting\ Ratio\ (PRR) = \frac{\frac{a}{(a+c)}}{\frac{b}{(b+d)}}$$

$$Information\ component\ (IC) = \log_2 \frac{a+0.5}{a_{\exp}+0.5}$$

$$a_{\exp} = \frac{(a+b)*(a+c)}{(a+b+c+d)}$$

$$IC025 = IC - 3.3*(a+0.5)^{-\frac{1}{2}} - 2*(a+0.5)^{-\frac{3}{2}}$$

$$Reporting\ Odds\ Ratio\ (ROR) = \frac{\frac{a}{c}}{\frac{b}{d}}$$

For all calculations, the python libraries pandas 2.8.2 and numpy 1.22.4 have been used with python 3.10. All the figures with the FAERS results have been generated with plotly 5.13 with python 3.10.

Calculation which has been used in Figure 4B:
Calculation:

$$\text{Drug percentage} = \frac{cumulative\ IC025\ of\ a\ specific\ drug\ in\ SOC}{sum\ of\ \ cumulative\ IC025\ of\ all\ drugs\ in\ SOC} * 100.$$

Calculation which has been used in Figure 7B:
Calculation:

$$\text{Reports percentage} = \frac{total\ reports\ of\ \ drug\ in\ HLG}{total\ reports\ of\ \ drug} * 100$$

4.3. Ligand based methods and creation of plots

4.3.1. Fingerprints

To calculate multiple molecular fingerprints from the sdf files of 39 drugs, Python 3.10 and PyBioMed 1.0 library were utilized which included moe, ghosecrippenfingerprint, cats2d, connectivity and topology. Further analysis was carried out through principal component analysis (PCA) using scikit-learn 1.0.2 library while plotly version 5.13 was used to create the dendrogram for graphical representation of the results.

4.3.2. 3D-structure similarity analysis

3D structures of the investigated drugs were retrieved from PubChem (152) as individual SD-files which were then merged to obtain a single file for further processing. The drug data set SD-file was then processed by the software Flipper (153) [version 3.1.1.2, executed with the following settings: (enumEZ true-enumNitrogen false-enumRS true-enumSpecifiedStereo true-warts true)], to enumerate all possible stereoisomers of drugs having one or more stereocenters. In the output file (SD format) the stereoisomers of the drugs are distinguished by a name suffix that consists of an underscore followed by the number of the stereoisomer. The stereoisomer enriched drug data set was then subjected to conformer ensemble generation using the software OMEGA (153, 154). Default settings were used for all parameters except for the energy-window (−ewindow) and the RMSD-threshold (−rms) setting for which values of 20.0 and 0.25, respectively, were chosen to obtain a more detailed representation of the conformational space of the compounds. The generated conformers were stored as a single SD-file which then served as input for molecular shape-based drug similarity calculations using the program ROCS (154). In order to calculate shape similarity values for all possible drug stereoisomer pairs, the obtained multi-conformer SD-file was specified both as an input file for the query (= reference) structures (−query) as well as for the evaluated database molecules (−dbase). For other ROCS parameters the preset defaults were used with the exception of the multi-conformer query flag, which was set to false (−mcquery false), the single-conformer database flag, that was set to true (−scdbase true) and the "per query structure generated ROCS reports" were merged into a single report output file (−report one). By means of a Python script ('report_to_dist_matrix.py), the obtained ROCS report file was then converted to 'shape distance' matrices based on the listed ColorTanimoto, ShapeTanimoto and TanimotoCombo scores of all drug stereoisomer pairs. Only the TanimotoCombo was used for further processing. For a drug

stereoisomer pair *ij*, the shape distance D_{ij}, which can adopt values in the range [0, 1], is calculated from the maximum similarity score S_{ij} that was encountered among all evaluated conformer pairings as follows:

$$D_{ij} = 1 - S_{ij}/S_{max}$$

S_{max} denotes the maximum value the particular ROCS similarity score can reach (1.0 for Color- and ShapeTanimoto, 2.0 for TanimotoCombo) and is used to scale the similarity score S_{ij} to the range [0, 1].

The combo scores of ROCS have been taken for further analysis. The dendograms have been created using python 3.10 with the library plotly 5.13.0.

4.4. Analysis of structural data

The PDB was mined for all structures of GABAA receptors with any of the analyzed BZ-site ligands in the complex. The resulting structures [8DD2 (subunit rendering), 6X3X (118), 6HUO (119), 6X3U (120)] were superposed and rendered with Schrödinger/Maestro Version 13.1.141.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found at: https://github.com/FilipKon/Drug_Diversity_FAERS.

Author contributions

FK and TS performed experiments. FV, FK, ID, MW, MK, TS, and ME contributed to writing. FV, ME, MW, ID, and FK contributed to shape the scope of the manuscript and literature research. ID and MW were involved in the choice of drugs. FV and ME designed experiments. ME provided funding and supervised the work. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2023.1188101/full#supplementary-material

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Glossary

ATC	Anatomical Therapeutic Chemical Classification System
AE	Adverse Event
AUD	Alcohol Use Disorder
BS	Binding site
BZD	Benzodiazepine
cIC025	Cumulative IC025
CNS	Central Nervous System
CYP	Cytochrome P450 System
DAWN	Drug Abuse Warning Network
FAERS	FDA Adverse Event Reporting System
GABAAR	GABAA Receptor
HLG	Higher Level Group
IC	Information component
OC	Oral Contraceptives
PRR	Proportional Reporting Ratios
PT	Preferred Term
ROR	Reporting Odds Ratio
ScIC025	Summed cumulative IC025
SOC	System Organ Class
+	Principal component of the binding site
-	Complementary component of the binding site

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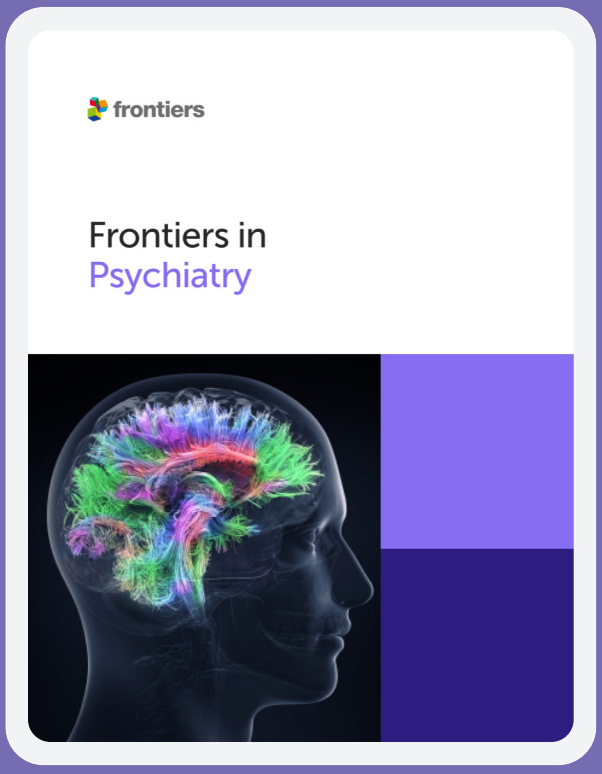

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