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MDPI

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Special Issue Editor

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About the Special Issue Editor

Fabrizio Schifano (Prof.) is one of the few physicians in Europe with training and specialist qualifications in both psychiatry and clinical pharmacology. He has made an outstanding contribution to several areas, including the epidemiological, psychopathological and overdose issues relating to the misuse of new/novel psychoactive substances/NPS, the Internet and drugs. Professor Schifano has been the Principal Investigator of six consecutive EU Commission-funded, multi-centre (i.e., 12 EU countries), NPS-based research programmes since 2002.

Recent Changes in Drug Abuse Scenarios: The New/Novel Psychoactive Substances (NPS) Phenomenon

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Over the last decade, the emergence of a vast range of new/novel/emerging psychoactive substances (NPS) has progressively changed drug market scenarios, which have shifted from the 'street' to a 'virtual'/online environment.

Several definitions of NPS are in use, with the term 'new' not necessarily referring to new inventions but to substances that have recently been made available, possibly including failed pharmaceuticals or old patents which have been 'rediscovered' as 'recreational' molecules. Conversely, the term 'novel' can refer to something newly created, an old drug that has come back into fashion, or a known NPS molecule being used in an innovative or unusual way and hence presenting a 'novelty' appeal (Corkery et al., 2018) [1]. Though misleading, the terms 'legal highs' and 'research chemicals' have been used alternately to describe these molecules. NPS includes synthetic cannabinoids, cathinone derivatives, psychedelic phenethylamines, novel stimulants, synthetic opioids, tryptamine derivatives, phencyclidine-like dissociatives, piperazines, GABA-A/B receptor agonists, a range of prescribed medications, psychoactive plants/herbs, and a large series of image- and performance-enhancing drugs (IPED) (Schifano et al., 2015) [2]. Overall, users are typically attracted to NPS because of curiosity and the diffusion of social media users' experiences, easy availability or affordability from online drug shops, legality, intense psychoactive effects, and the likely lack of detection in routine drug screenings (Schifano et al., 2015) [2].

Between 2004 and 2017, some 700–800 examples of NPS were reported by related European and international drug agencies (UNODC, 2018 [3]; EMCDDA, 2018 [4]), with most molecules identified being synthetic cannabinoids, synthetic cathinones, phenethylamine derivatives, and synthetic opioids. However, it could be argued that the NPS scenario is much larger than that outlined by those molecules which have been seized or formally identified by EU and international agencies. Since the online NPS scenario typically predicts the real life NPS scenario (Schifano et al., 2015) [2], identifying what is being discussed online by web-based NPS enthusiasts, or 'e-psychonauts' (Orsolini et al., 2015) [5], may well be of interest. With this in mind, a crawling/navigating software (i.e., the 'NPS.Finder®') was recently designed by our group. In November 2017, it started to automatically scan, on a 24/7 basis, a vast range of psychonaut web forums for NPS. After a year of operation, it has been possible to estimate that the online/psychonaut web forum NPS scene may include some 4000 different molecules. The most popular examples of NPS mentioned in psychonaut forums have included synthetic cannabimimetics, synthetic opioids, phenethylamines, designer benzodiazepines, and prescribed drugs.

NPS use, especially for synthetic cannabinoids and novel psychedelics, has been associated with a range of untoward medical consequences, including vomiting, seizures, cardiovascular complications, and kidney failure (Schifano et al., 2017) [6]. By contrast, the main focus of this special issue is on the major psychopathological consequences of NPS use. Indeed, due to their complex pharmacodynamics, there are increasing levels of concern about the onset of acute or chronic psychopathological issues associated with NPS intake.

The occurrence of psychosis has been related to: (a) increased central dopamine levels, typically seen with novel psychedelic phenethylamines, novel stimulants and synthetic cathinones; (b) significant cannabinoid CB_1 receptor activation, which is associated with high potency synthetic cannabimimetics; (c) 5-HT_{2A} receptor activation, seen with latest generation phenethylamines, tryptamine derivatives and hallucinogenic plants; (d) antagonist activity at n-methyl-D-aspartate/NMDA receptors, observed with ketamine, methoxetamine/MXE, and their latest derivatives; and (e) k-opioid receptor activation, which is typically associated with both Salvia divinorum and Mitragyna speciosa/'Kratom' intake.

By considering the above, this special issue of Brain Sciences aims to provide an overview of a range of NPS-related issues. More precisely, Sahai et al. [7] present original *preclinical data* relating in silico and in vitro assessment of the psychoactive properties of a few dissociative diarylethylamines. Miolo et al. [8] focus on specific *analytical chemistry* issues relating to amphetamine-type stimulants and ketamine, while Parrott [9] argues that there are similarities between well-known recreational drugs and NPS in terms of *mood fluctuations/psychobiological instability issues*. Conversely, Cohen and Weinstein [10] present original *cognitive psychopharmacology* data relating to the use of organic and synthetic cannabinoids. From a *clinical point of view*, Bonaccorso et al. [11] introduce a case series of synthetic cannabinoid users presenting to acute psychiatric services with psychosis; Frisoni et al. [12] comment on the medical consequences of novel opioid intake; Martinotti et al. [13] provide a thorough overview of hallucinogen-persisting perceptual disorder, a clear issue of interest for NPS users; Schifano et al. [14] reflect on the misuse and abuse of prescribed medicines (e.g., benzodiazepine derivatives, methylphenidate look-alikes, and fentanyl analogues) in the NPS context; and Gittins et al. [15] provide empirical data relating NPS use by clients seeking treatment in the UK. Both Wadsworth et al. [16] and Miliano et al. [17] comment extensively on the *role of the open/deep web* in shaping and promoting changes in NPS scenarios. Finally, both Metastasio et al. [18] and Catalani et al. [19] offer original data which sheds further light on the expanding phenomenon of *IPED misuse/abuse*.

In conjunction with constant changes in basic structures from which emerging molecules can be derived, designed, and synthesized, the NPS market will continue to expand. This will pose a challenge, since NPS-related toxidromes are, per se, complex and unpredictable, and clinicians need to aim to be better educated in recognizing NPS-related toxicity issues. Drug control policies should be improved worldwide, and the list of examples of NPS should be constantly updated as improvements in analytical chemistry detection methods occur. Given the implications of NPS for mental health, psychiatric services should adapt to new drug scenarios while drafting new treatment strategies.

Conflicts of Interest: The author declares no conflict of interest.

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Octodrine: New Questions and Challenges in Sport Supplements

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Abstract: Background: Octodrine is the trade name for Dimethylhexylamine (DMHA), a central nervous stimulant that increases the uptake of dopamine and noradrenaline. Originally developed as a nasal decongestant in the 1950's, it has recently been re-introduced on the market as a pre-workout and 'fat-burner' product but its use remains unregulated. Our work provides the first observational cross-sectional analytic study on Octodrine as a new drug trend and its associated harms after a gap spanning seven decades. **Methods:** A comprehensive multilingual assessment of literature, websites, drug fora and other online resources was carried out with no time restriction in English, German, Russian and Arabic. Keywords included Octodrine's synonyms and chemical isomers. **Results:** Only five relevant publications emerged from the literature search, with most of the available data on body building websites and fora. Since 2015, Octodrine has been advertised online as "the next big thing" and "the god of stimulants," with captivating marketing strategies directed at athletes and a wider cohort of users. Reported side-effects include hypertension, dyspnoea and hyperthermia. **Conclusions:** The uncontrolled use of Octodrine, its physiological and psychoactive effects raise serious health implications with possible impact on athletes and doping practices. This new phenomenon needs to be thoroughly studied and monitored.

Keywords: octodrine; dimethylhexylamine; DMHA; ambredin; fitness; novel psychoactive substance; performance and image-enhancing drugs; anti-obesity agents; weight loss
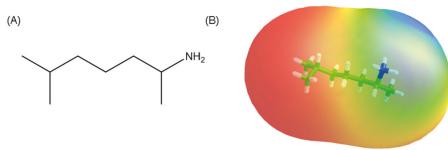
1. Introduction

The evolution of trends within drug use has recently been marked by a rapid expansion in the number of commercially-available psychoactive substances [1], with an increased number of young users [2] and relevant psychiatric consequences [3]. This includes both a proliferation of new drugs ('research chemicals' or 'RC's) with a distinct pharmacology and very little associated research evidence on their physiological or side effects, as well as an increase in the abuse of diverted prescription

medications [4,5] Octodrine sits somewhere between these two trends, being a traditionally-developed pharmaceutical but with no current, legitimate medical application.

The so-called "Performance and Image-Enhancing Drugs" (PIEDs) taken to enhance human abilities in a myriad of spheres, are one important emerging facet within this. These include substances with a perceived ability to enhance physical performance, psychological status, appearance, cognitive abilities and social relations and as such are sometimes referred to as 'lifestyle drugs' [6–10]. The concept of PIEDs is now well established and is acknowledged particularly in relation to the world of athletics [11,12]. The most well-known PIEDs are the anabolic steroids, peptides and hormones but their use is increasingly giving way to other types of substance to achieve specific goals. These can be physical in nature (e.g., tanning, weight loss, muscle gain, speed, strength, performance) or cognitive, such as the use of nootropics for professional or academic performance [13,14], or for social gain, where various categories of substance as a 'social lubricant' for social anxiety support). Over the past decade, more than 800 NPS were identified in over 102 countries by the EMCDDA and the UNODC Early Warning Systems [15,16] as well as our ongoing monitoring activities [1] and their number is constantly growing. Some of these compounds may represent a serious issue for public health and are changing the face of debates around doping by playing unfairly on the narrow line between legal and illegal [12]. The globalization of the online drugs market has made this a widespread phenomenon, reaching a new cohort of users, which includes not only the body builders and time-pressured professionals, who were initially associated with this trend but also students and others of all demographics [12,17,18].

In November 2016, Octodrine was found in an athlete engaged in a bodybuilding competition, later disqualified as he also tested positive for anabolic and stimulant drugs, included in the World Anti-doping Agency's (WADA) List of Prohibited Substances (Section S6 and S1) [19,20]. Octodrine is a psychoactive central nervous system stimulant. It is an amphipathic primary amine (Figure 1) [21] known under many names, including dimethyl hexylamine (DMHA) and 2-amino-6-methylheptane, 2-metil-5-amino-eptano. Its structure presents some similarities with that of other illegal stimulants like, AMP Citrate (DMBA), Ephedrine and 1.3-DMMA itself. With DMAA and AMP Citrate already phasing or phased out of current supplements, this drug was brought back on market as an alternative in pre-workout and 'fat-burner' products in 2016. Octodrine was originally developed in the United States as an aerosolized treatment for bronchitis, laryngitis and other conditions [22–24]. Its pharmacology was studied in the early 1950s, was investigated as an antitumor drug and used to be available as a nasal decongestant under the tradenames Vaporpac and Tickle Tackel Inhaler [25]. Sympathomimetic effects of DMHA were explained as alpha adrenergic agonist-mediated via G-protein-coupled receptors (GPCRs) [26]. Limited human data is available just from preliminary studies, while studies on activity and acute toxicity had been conducted on animals (cats, rabbits, dogs and pigs) [27–32]. Octodrine was found to increase the pain threshold, cardiac rate (positive chronotropic effect) and myocardial contractility (positive inotropic effect) [33–35]. The safety of Octodrine as an individual drug remains unknown due to the lack of any placebo-controlled trial but animal experiments suggest a potential for adverse cardiovascular effects. Structurally, there are two forms of DMHA: the naturally occurring 2-amino-5methylpetane and the synthetically derived 2-amino-6-methylheptane. The natural version can be found in extracts of Juglans Regia (Walnut Bark), Aconitum Kusnezoffii's and Kigelia Africana and it is often used for hunting purposes [36–43]. The synthetic version is the most widely used because less expensive and toxic to produce. It is therefore assumed that the DMHA used in supplements is synthetic. As of right now, this molecule is not on the 2016 WADA banned substances list but it fits perfectly in the category of the well-known Performance and Image Enhancing Drugs (PIEDs). Coveted by elite track and field athletes, DMHA is marketed to a broader demographic including beginners and non-professionals.
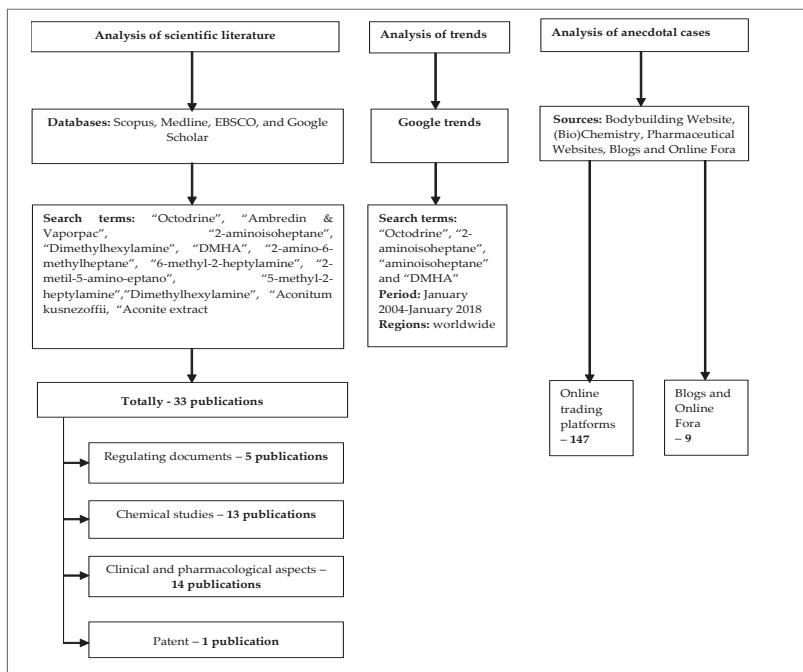
Figure 1. Chemical structure (**A**) and molecular lipophilicity potential (MLP) surface (**B**) of octodrine molecule (hydrophobic surfaces are depicted in red and polar surfaces are in blue) [21].

Considering the existing knowledge gap spanning seven decades and the re-emergence of Octodrine as a new drug trend, it was felt the need to further investigate the phenomenon in different communities, while exploring issues related to its e-commerce, consumption, motivations of use and potential negative impacts to health, among other features.

2. Materials and Methods

A literature review on Octodrine was carried out in the following databases: Scopus, Medline, EBSCO and Google Scholar (Figure 2). A list of keywords was compiled in accordance with a preliminary pilot study of literature and databases on the surface web and online e-commerce websites. Terms included: "Octodrine," "Ambredin & Vaporpac," "2-aminoisoheptane," "Dimethylhexylamine," "DMHA," "2-amino-6-methylheptane," "6-methyl-2-heptylamine," "2-metil-5-amino-eptano," "5-methyl-2-heptylamine,""Dimethylhexylamine," "Aconitum kusnezoffii, "Aconite extract," among others. The keywords also included synonyms of Octodrine in other languages and names of chemical isomers. Searches were carried out in English, Italian, German, Arabic and Russian.

Figure 2. Algorithm of the analysis.

No time restrictions were applied to the searches. Inclusion and exclusion criteria for literature data selection are defined in Table 1. Considering the lack of scientific investigations in the field and the absence of experimental and/or interventional studies in humans, additional qualitative systematic searches were carried out in the world-wide web to investigate the extent of diffusion of Octodrine, trading strategies for its distribution and the nature of the self-reported (subjective) experiences by users in English, German, Arabic and Russian. These included bodybuilding websites, chemistry and chemists' websites, pharmaceutical companies, online e-commerce stores as well as a range of fora posts/threads. The web snapshot was carried out on a regular basis (between November 2016 - January 2018) using a Google search. Only publicity available information was considered for the study and no posts/other contributions to fora discussions were made by the researchers. Additional data were also obtained by consulting Google Trends [44].

Table 1. Inclusion and exclusion criteria for selection of articles and the web analysed in this study.

Inclusion Criteria
1. Studies and publication related to Octodrine
2. Studies and publication of octodrine-related compounds and chemicals, in which Octodrine is an ingredient
3. Studies and publication in which octodrine is marginally included
4. English, German and Russian languages
5. All years of publication (no date restriction)
6. Surface web
7. Grey (unpublished) literature, including master's and doctorate theses
8. Fitness and body building websites
9. (Bio)chemistry, pharmacy and pharmaceutical websites
10. Online drug fora
11. Human and animal studies
12. Observational and experimental studies

Exclusion Criteria
1. Duplicate Articles
2. Initial screening for relevance (reading the title and abstract)
3. Articles found to be irrelevant by analysing the full article
4. Low scoring for an article on CASP critical appraisal tool (poor quality of appraised manuscript)

Ethical approval for this the study was granted by the School of Pharmacy Ethics Committee, University of Hertfordshire, Hatfield, United Kingdom (November 2013; PHAEC/10-42).

3. Results

3.1. Medical and Paramedical Database, Grey Literature

Various articles emerged from our literature searching but only eight of them [23,24,27–31,33] referred to Octodrine, Octodrine derivatives and Octodrine-related compounds in the entire scholarly-published literature (Table 2).

Table 2. Pharmacological and clinical properties of Octodrine (analysis of articles).

Reference	Author	Year of Publication	Name of Studied Substance or Medicament	Key Findings
Respiratory system				
[28]	Charlier, R.; Philippot, E.	1950	theophylline-diethylenediamine ethanoate	The aerosol with Octodrine demonstrated the property to increase respiratory volume
[29]	Charlier, R.	1951	2-amino-6-methyl-heptane	Animal experiment (dog) revealed bronchodilation, increased nasal and lung volume caused by 2-amino-6-methyl-heptane
[23]	Gode, J.	1958	Ambredin	Identification of bronchospasmolitic properties of Ambredin medicament consisting of Aceverine Hydrochloride, Octodrine Phosphate and Theophylline
[24]	Tschudin, M.L.	1960	Ambredin	
Cardiovascular system				
[30]	Fellows, E.J.	1947	2-amino-6-methylheptane	2-amino-6-methylheptane hydrochloride caused an increase in cardiac rate and amplitude of contraction in animal experiment (dog)
[27]	Marsh, D.F.; Herring, D.A.	1951	Methyl-2-heptylamine	Compared to others sympathomimetic amines, 6-Methyl-2-heptylamine focused the myocardial stimulant activity and increased force of myocardial contraction along with heart rate
[29]	Charlier, R.	1951	2-amino-6-methyl-heptane	Animal experiment (with dog) revealed growth in arterial blood pressure after the exposure of 2-amino-6-methyl-heptane
[34]	Oelkers, H.A.	1967	2-amino-6-methylheptane (+)-camphor-10-sulfonate	Inotropic properties of 2-amino-6-methylheptane (+)-camphor-10-sulfonate were identified
[31]	Trieb, G.; Nusser, E.	1974	Ordinal®retard	The medicament Ordinal® retard combining Octodrine, 3-octopamine and adenosine demonstrated pressure effects in treatment of patients with hypotension
Nervous system				
[30]	Fellows, E.J.	1947	2-amino-6-methylheptane	2-amino-6-methylheptane demonstrated local anaesthesia and elevation of local pain threshold in experiments with animals (rabbits, cats, dogs)
Antimicrobial activity				
[45]	Kim, K.; Zilbermintz, L.; Martchenko, M.	2015	Octodrine	Octodrine demonstrated antifungal activity in experiments with serum-grown *C. albicans*
[46]	Niu, H.; Cui, P.	2015	Octodrine	Octodrine demonstrated experimental activity against stationary phase *E. coli*

Reference to its multiple medicinal properties was found in five of these papers, which highlighted its sympathomimetic and broncho-spasmolytic effects, with possible further actions as a stimulant, anti-obesity and appetite suppressant agent. The molecule is cited also as an antimicrobial with specific antifungal activity [45], as a nasal decongestant [47] and as an ingredient of dietary supplements [48]. Other scholarly papers (a total of seven) made passing or limited reference to Octodrine, covering the chemical properties and analyses of several compounds including this one, or providing data on its antimicrobial effects only [45,46]. These are other scattered examples of relevant documentation, including an invention patent from 2012 [49]. However, the lack of experimental randomized controlled trials (RCTs) and other interventional studies on humans has led to a complete absence of systematic reviews and meta-analytic studies related to use of Octodrine as a medicinal agent or food supplement. Two of the three papers found on PubMed were published in the *Journal of Pharmacology and Experimental Therapeutics* in 1947 and 1951 respectively [27,30], while the third paper [29] was published at the *Archives internationales de pharmacodynamie et de thérapie*. Since the 1950s, there have been no other scholarly-published data specific for Octodrine in any peer-reviewed journal, neither observational nor experimental could be found on the entire web, including medical and paramedical databases, or unpublished literature. The substance remerged on the literature in 2017, when Cohen et al. published a study conducted on six different supplements: Game Day, Infrared, 2-Aminoisoheptane, Simply Skinny Pollen, Cannibal Ferox AMPed and Triple X. All these products disclosed on their label the words Octodrine, 2-amino-6-methylheptane and 6-methyl-2-heptanamine or listed the stimulant as if it were an extract of Aconitum kusnezoffii plant. Results showed that only one of them, Game Day, contained Octodrine, while the others contained different or banned stimulants [50].

3.1.1. Limited Data-Reporting in Scholarly Peer-Reviewed Papers and Invention Patents

There is limited mention of Octodrine in invention patents from 2012 in relation to a novel stable anaesthetic for reducing skin reactions [49].Two papers, pertinent to the disciplines of toxicology and chemical chromatography, examined Octodrine in terms of its physiochemical properties including relative retention time (RTT) and its identification in hair samples [51,52]. Furthermore, Niu et al. and Kim et al. [45,47] discussed the broad-spectrum antimicrobial effect, antifungal effect, anti-persister activity and application for the treatment of Candida albicans and uropathogenic strains of Escherichia coli. These two papers also discussed Octodrine microbial resistance. Additionally, Kuo et al. (2004) [26] and Schlessinger et al. (2011) [53], documented the sympathomimetic properties of Octodrine and effects related to norepinephrine transporter (NET) and G-protein-coupled receptors (GPCRs), which was in concordance with the results from 1947 and 1951 animal studies [27,30].

3.1.2. Google Trends

Google Trends provided valuable data in relation to the interest in Octodrine on the Web. Four keywords provided good insight on the trend as far back as the year 2004. These keywords are "Octodrine," "2-aminoisoheptane," "aminoisoheptane" and "DMHA." There was an obvious incremental increase of interest in Octodrine starting in the year 2012. This interest plateaued between 2013 and 2014 and was followed by a steep rise in 2014–2015, followed by a further escalation starting in 2015 before peaking by the January of 2018 [54]. Comparing to other three keywords, DMHA has demonstrated the greatest interest among Google users ranging between 9 searches in July 2008 and 100 searches in September 2017. The leading countries in terms of internet searches of Octodrine (DMHA) were the USA, Canada and Australia. On the Russian-language Internet, users showed no search activity for Octodrine, while intensively searching for DMAA. In June 2017, the quantity of DMAA searches in Russian-language zone was 100. Since 2004 the trend has demonstrated stable growth in this local online area [55]

3.1.3. Bodybuilding Website, (Bio)Chemistry, Pharmaceutical Websites, Blogs and Online Fora

Body building websites provided a major source of data, especially in relation to the analysis of online trading platforms and fora discussing Octodrine and its effects. A multilingual approach used in this part of the study facilitated the characterisation of regional and national features of sport-stimulant markets.

No specific inclusion criteria were imposed upon the body-building websites, beyond demonstrating a mention of DMHA or synonym. All such instances were included in the evaluation.

The English-language domain was investigated with relevant results. No results were produced from searches in Arabic. Thousands of websites can be located using the Google search terms "Octodrine" and its synonyms [56–59]. Popular brand names include: Olympus Labs CONQU3R Unleashed, Total War, Simply Skinny Pollen, AdrenaCLENV2, Game Day, Cannibal Ferox Amped, Giant Sports Giant Rush [57–66]. By January 2018, 68 English- and 6 German-language online shops selling Octodrine were identified. The product is often advertised as the "next big thing" in bodybuilding environments and described as the "new MDAA" whose effects are "just right" for dietary supplement users and/or stimulant-enthusiasts as it can allegedly enhance focus, experience and performance. Many of these sites also provide detailed information around usage and dosage, alongside with warnings on risks and severe side effects of this emerging molecule [56–59]. Professional scientific or pharmaceutical sites regarding the chemistry characterization of this compound can be found on the web, as well as "amateur" websites, displaying more generic scientific information on this potentially dangerous substance [67,68]. Online stores are predominantly American or Australian domains that ship their products all over the world. Octodrine is presented as a DMAA-like stimulant and predominantly sold as a fat-burner product or pre-workout formula. Some websites also recommended it for intensive study sessions, positioning this molecule among the Nootropics;

pharmaceuticals used to improve cognitive and executive function, memory and creativity in healthy individuals. Claims such as "It boosts dopamine and noradrenaline uptake, while slowing down reuptake just long enough for a solid workout or study session" are quite common [58].

Usually Octodrine is sold in powder (e.g., Cannibal Ferox AMPed, Olympus Labs CONQU3R Unleashed, Game Day, Total War) as pre-workout or in capsules (Infrared, Simply Skinny Pollen) as fat burner, with prices ranging from 1.75 to 3.75 dollars per serving.

German online trading platforms focus customers' attention on Octodrine with detailed feedback from the reviewers (estimation of taste, effects, "price-quality") [68] or vague offers of "fitness hardcore pre-workout booster for pumps and focus" [69]. The trader for amazon.de mentions Octodrine as additives "Oct" in "Arginine AKG, Beta-Alanine, Citrullin Malate complex" and omits a description of its side-effects. Standard marketing technologies widely used by German traders are also employed, such as discounts, world-wide express-delivery and even "halal" certification.

In terms of the Russian-language results, the majority consisted of bodybuilding resources linked to 73 online shops delivering Octodrine to the Russian Federation, the Ukraine and 4 Central Asian countries. In contrast, fora did not indicate significant popularity among local athletes and were predominantly arranged within Russian social nets [70,71]. All identified Russian-language websites run their activity legally on the surface web, a fact explained by the absence of any law enforcement restrictions referring to DMHA and DMAA in Russia and Ukraine. The relative lesser popularity of these particular stimulants is no doubt influenced by the availability of much cheaper locally produced analogues. An imported stimulant complex with DMHA and DMAA (from the USA) costs 1.27 ± 0.19 USD per unit compared to a local analogue for 0.49 ± 0.23 USD per unit. The local online platforms offer more than 20 brands of Octodrine. The immense variety of trading names of this substances is attributable to continuous rebranding as attempts to overcome counterfeit production [72]. By offering athletic stimulant complexes, Russian online shops strive to advertise DMHA and DMAA as the active components for the desirable results; less attention is paid to other substances such as vitamins, tyrosine, taurine and DMAE [73,74]. Trading and producing companies announced anti-inflammatory, anaesthetic, spasmolytic and anticonvulsant properties of Octodrine offering the "ideal" substance for "hard-core" training [72]. The use of Octodrine as a weight loss product is rarely advertised (e.g., only 9 Russian-language online shops were identified). Fat burners containing Octodrine are typically sold at a higher price point: 1.3 USD per unit in contrast to the 0.5 USD for the pre-training complex. Taking into account the legality of DMHA and DMAA in Russian-language territories and the absence of relevant trading regulations, local online shops did not notify their customers to possible side and toxic effects of the stimulants. Some shops explicitly claim that Octodrine has no side effects and that is potential is the same as caffeine [75]. For example, berserktakticalfarma.blogspot.com underlined the safety and high effectiveness of DMHA, emphasising that its potency is equal to 90% of DMAA [76]. Only one of 73 identified online websites warned that Octodrine and DMAA can be detected and could possibly mislead sport competition testing and thus advised users to cease Octodrine beforehand [74]. An additional online shop mentioned contraindications generally for stimulant complexes without specification on Octodrine [77].

Discussions on fora include suggested dosages, combinations, duration of action, among others. According to such anecdotal evidence, a "safe dose" is considered to be around 1mg/kg of bodyweight up to 160 mg per day, while others recommend 100 mg of the synthetic DMHA isomer and 75 mg of the natural one to reach the "sweet spot." In the Russian-language internet zone, the recommended dose of DMHA substantially differed between online shops (30 to 400 mg), as did the dosing schedule. For instance, the online retailer hulkfood.ru advised to take Octodrine for 45 days without stopping to gain significant desired stimulating effects before sporting competition [78].

Users suggest an intake approximately 15–30 or 30–60 min prior to working out [79,80]. Alternatively, if used for its appetite suppressant properties, DMHA consumption was advised between meals and never in the evening as it might affect sleeping. According to users, 25 mg

twice a day are enough to "keep one's mind off food" [81]. Experienced users shared their insights: "If used predominantly for its appetite suppressant properties, DMHA can be used during the day between meals. However, we recommend taking caution if using this ingredient late in the afternoon or early evening, as it has the potential to hinder your ability to sleep."

DMHA effects will occur ~15 to 60 min after consumption. The substance demonstrates potency to heighten level of mental focus, increase energy and reduce appetite, as well as raise feelings of wellbeing. Bloggers described a three-phase effect for pre-work-out complexes containing DMHA: (1) stimulation, (2) post-stimulation side effect symptoms and (3) sleep disturbances. Octodrine is frequently advised as a substance intensifying the first two phases with no impact on sleep [82]. Because of its stimulants effects, Octodrine has also been used outside fitness settings, including working environments as non-prescribed medication [83,84]. To boost athletic performance, it is sometimes ingested in combination with huperzine A, DMAE, n-acetyl tyrosine, alpha-gpc, noopept, phenotropil and picamilon to gain maximal focusing on training. Phenibut and ladasten strengthen the euphoric effects of Octodrine [82].

Side effects such as mood swings, tremor, concentration deficiency, over-stimulation, energy crashes, anxiety, high blood pressure, dyspnoea, rapid heartbeat and heartburn have been reported 6–8 h after the initial onset of effects [85,86]. Some users also reported eyes twitching (blepharospasm), pulsing sinus area (carotid sinus), mood fluctuation, absent-mindedness; a rise in blood pressure, piloroerection and hyperthermia following ingestion of Octodrine (e.g., Anabolicminds.com, 2016; Project Bodybuilding, 2016; kandeleria.ru). There were only a limited number of indications for professional athletes: some websites suggest avoiding consumption for ethical considerations, or because it could be considered a violation of the WADA restrictions [72,86,87].

Some fora provide information on abuse potential and possibility of dependency on Octodrine. Namely, users warned about withdrawal symptoms and growth of tolerance resulting from the short- and long-term use of the stimulant [71,82]. Experienced athletes recommend alternating 3–4 stimulant complexes each training day to overcome undesirable adaptation [77].

4. Discussion and Conclusions

To the best of the authors' knowledge, this study is the first to implement a systematic review of the literature or undertake cross-sectional analysis of the content of the web in relation to the diffusion and e-commerce of Octodrine. The authors also bring to the spotlight the noteworthiness of subsequent studies on Octodrine, specifically chemical analysis and receptor-ligand binding assays, with the aim is of reaching a full understanding of this widely used substance. The restrictions on amphetamine-type stimulants (e.g., DMAA, DMAE) exacerbate demands on new formulas tailored to the ambitions of bodybuilders and athletes. The sympathomimetic properties of Octodrine, inheriting the potential of DMAA, meet the expectations of "new-generation boosting" and stimulate the growth of global trading. Meanwhile discrepancies between practical experience and theoretical knowledge were observed in our study. Very little is known about its pharmacodynamics and pharmacokinetics profiles, or the chemical profile of the commercially-available Octodrine-related products.

On the Internet, Octodrine is misleadingly advertised as a "safe and legal" analogue of banned stimulants (e.g., DMAA and phenethylamine), making it potentially more attractive to new and experienced users. Intensive marketing campaigns with unlimited worldwide delivery, discounting programs and displays of "good" feedback from reviewers, have contributed to the re-emergence and current spread of Octodrine in the drug market. According to anecdotal for a, reports and trading information, motives of use go beyond athletics gyms: more often the drug is recommended as a day-life stimulator.

The psychoactive effects of Octodrine were neither previously described in literature nor studied despite its structural similarity to other drugs of abuse (DMAA, DMAE). Its metabolic pathway and adverse reactions have not been studied in humans, making its use in fitness settings extremely hazardous. A limited number of open claims were found on the potential risk, side effects and

complications of Octodrine use, while its properties as "hard-core" for advanced users are often emphasized. Sites exploited fragmented and sporadic "scientific news" describing only favourable effects of Octodrine, with research evidence being completely omitted. Only some English and German sites warned about ceasing Octodrine use before sport competitions. Meanwhile, on Russian-language trading platforms, DMHA was actively offered in combination with DMAA - attributable to the legal status of both in this geographical zone (Russia, Belarus, Kazakhstan, Kyrgyzstan). Amphetamine-type stimulants (such as DMAA) have showed more popularity, especially for the last two years.

The reported side effects of Octodrine suggest the strong need for further research. The desirable, stimulatory effects of Octodrine are accompanied with a range of mental and somatic symptoms, with frequent use of Octodrine being associated with tolerance, withdrawal symptoms and risks of dependence syndrome. We reiterate here the importance of focusing and asking direct questions on the pharmacological and toxicological properties of Octodrine. The addictive potential should be promptly identified and assessed. Hence, attention should be paid to further investigation in the field and consider the incorporation of Octodrine within WADA and FDA prohibited substances lists.

There are some limitations to our study. Only five peer-reviewed papers were directly pertinent to Octodrine and these were mainly published 6–7 decades ago. Other scholarly-published studies addressed Octodrine in a marginal way. The paucity of the available literature, absence of review articles, systematic reviews and meta-analytical studies clearly limit the extent of this present review. Taking into consideration lack of publications on the chemical analysis of Octodrine, a comprehensive chemical analysis is necessary for future research, with the aim of establishing the detection and identification of Octodrine, along with potential contaminants, excipients and other active ingredients in the currently promoted powder-products under the name Octodrine or DMHA. The sympathomimetic effect of Octodrine is well-understood. However, the central effects including the psychostimulant and anti-obesity effects are not adequately explored and/or reported, with no formally documented experimental studies nor case reports. Future studies should focus on the central effect of Octodrine and its correlation with patterns of cerebral dominance and the lateralization of brain function. Moreover, there is an inadequate body of data in relation to the geographic usage of Octodrine, particularly for contrasting the developing world (including the Middle East, North Africa and post-soviet regions) versus the developed world. In terms of multilingual analysis of websites and drug fora, only publicity available information was considered for the study to uphold observational status. Fora requesting registration were not included in the study. Self-reported experiences are only partially reliable and it may be inappropriate to trust such anecdotal evidence without independent verification.

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The Effects of Cannabinoids on Executive Functions: Evidence from Cannabis and Synthetic Cannabinoids—A Systematic Review

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Abstract: Background—Cannabis is the most popular illicit drug in the Western world. Repeated cannabis use has been associated with short and long-term range of adverse effects. Recently, new types of designer-drugs containing synthetic cannabinoids have been widespread. These synthetic cannabinoid drugs are associated with undesired adverse effects similar to those seen with cannabis use, yet, in more severe and long-lasting forms. Method—A literature search was conducted using electronic bibliographic databases up to 31 December 2017. Specific search strategies were employed using multiple keywords (e.g., "synthetic cannabinoids AND cognition," "cannabis AND cognition" and "cannabinoids AND cognition"). Results—The search has yielded 160 eligible studies including 37 preclinical studies (5 attention, 25 short-term memory, 7 cognitive flexibility) and 44 human studies (16 attention, 15 working memory, 13 cognitive flexibility). Both pre-clinical and clinical studies demonstrated an association between synthetic cannabinoids and executive-function impairment either after acute or repeated consumptions. These deficits differ in severity depending on several factors including the type of drug, dose of use, quantity, age of onset and duration of use. Conclusions—Understanding the nature of the impaired executive function following consumption of synthetic cannabinoids is crucial in view of the increasing use of these drugs.

Keywords: cannabis; synthetic cannabinoids; executive function

1. Introduction

The most popular illicit drug of the 21st century is cannabis, in its many forms and shapes [1–5]. According to the United Nation Office on Drugs and Crime (UNODC), approximately 181 million adults have used cannabis across the globe [2]. Moreover, in many countries more than 50% of young adults have used cannabis at least once in their lifetime [3]. Recently, new types of drugs that contain Synthetic Cannabinoids (SC) have become popular among drugs users worldwide [5–7]. SC drugs are associated with severe adverse effects (Table 1), have greater harm potential and they are more addictive than the traditional organic cannabis drugs [4,6–9]. Therefore, governments and health institutions across the Western world make major efforts in order to prevent the spread of SC and to improve the knowledge regarding SC and their potential risks [5,8]. One of the most notorious adverse effects that is associated with cannabinoids consumption is impairment of cognitive function [4]. Both pre-clinical and human studies drew a link between consumption of cannabinoids and long-term deficits of cognitive functions, especially high-order cognitive functions [4,5,10–13]. The purpose of the current review is to present and describe the acute and long-term effects of SC drugs in comparison with organic cannabis on executive function (EF) based on current literature from both human and animal research. A literature search was conducted using electronic bibliographic databases (PubMed®, ScienceDirect ®and Google Scholar platforms) up to 31 December 2017. Database-specific search strategies were employed using multiple keywords (e.g., "synthetic cannabinoids AND cognition,"

"cannabis AND cognition" and "cannabinoids AND cognition"). The search has yielded 160 eligible studies including 37 preclinical studies (5 attention, 25 short-term memory, 7 cognitive flexibility) (Table 2) and 44 human studies (16 attention, 15 working memory, 13 cognitive flexibility). Studies were included if they stated the following inclusion criteria: use of objective measurements of specific executive function (working memory, attention and cognitive flexibility) involving cannabinoid users (regular and recreational users) or cannabinoid treatments and a control group. Exclusion criteria were: studies that involved participants who had other neurological or psychiatric disorders or individuals who met criteria for alcohol dependence or other substance use disorders (abuse or dependence) different from cannabis and nicotine.

Table 1. Common clinical adverse effects induced after consuming synthetic cannabinoids.

Type of Effects	Symptoms
Psychosis	Recurrent psychosis episodes [9,14–16].
Agitation	Last for several hours after intoxication of SC [16–18].
Affect disturbance	Severe anxiety symptoms and panic attacks shortly after consuming SC [14,17–20].
Cognitive alterations	Impairment in memory and attention deficits [14,20–22].
Cardiovascular effects	Both tachycardia, tachyarrhythmia and cardiotoxicity were reported after exposure to SC [14,23].
Gastrointestinal effects	Nausea, vomiting and diarrhea after severe exposure to SC [14,24,25].

Table 2. Pre-clinical rodent studies of the effects of cannabinoid-agonists on executive function.

Animals	Cannabinoids Tested	Main Findings	Reference
Male Long–Evans rats	WIN55,212-2 and Δ9-THC	Dose-related attention impairments afteracute exposure to cannabinoid CB_1 receptor agonist. Impairments were reduced after treatment with CB_1 antagonist.	[26,27]
Male Sprague–Dawley rats	Δ9-THC	Decreased performance on a divided attention tasklasts for 2 weeks after chronic administration withcannabinoid CB_1 receptor agonist.	[28]
Male Sprague–Dawley rats	AM-4054	Decreased sustained attention after acute treatmentwith a cannabinoid CB_1 receptor agonist.Impairments were associated with task demands.	[14]
Male Sprague–Dawley rats	Δ9-THC	Impairments of visual attention on an operant signaldetection task after acute treatment with cannabinoid CB_1 receptor agonist.	[29]
Male Sprague–Dawley rats	WIN55,212-2	Deficits of working memory after chronic treatmentwith a cannabinoid CB_1 receptor agonist.	[30]
Female Long–Evans rats	Δ9-THC	Repeated administration with cannabinoid CB_1 receptoragonist in adolescence induced persistent impairment of working memory.	[31]
Male Sprague–Dawley rats	WIN55,212-2	Acute injection of cannabinoid CB_1 receptor agonist in late-adolescence period induced temporary impairment of short-term memory. Chronic treatment with cannabinoid CB_1 receptor agonist impair short-term memory for several weeks after the last administration.	[32]
Male Sprague–Dawley rats	Δ9-THC	Acute exposure to a cannabinoid CB_1 receptor agonistinduced working memory impairments	[33,34]
Male Sprague–Dawley rats, Lister rats and C57B16 mice	Δ9-THC	Working memory impairments were induced afterchronic treatment with a cannabinoid CB_1 receptor agonist.	[35,36]
Wild-type and CB_1 receptor knockout mice	JWH-081	Acute treatment with cannabinoid CB_1 receptor agonist induced short-term memory deficits in wild-type mice but not in knockout mice.	[37]
Male Long–Evans rats	HU-210	Acute treatment with a cannabinoid CB_1 receptor agonistinduced working memory deficits.	[38,39]
Male C57B1/6 mice	Δ9-THC	Acute injection of Δ9-THC disrupted performance of the working memory task, impairments were reversed by SR1417161A.	[40]
Male Wistar Rats	Δ9-THC	Acute administration induced set-shifting impairments24 h after treatment.	[41]

Table 2. *Cont.*

Animals	Cannabinoids Tested	Main Findings	Reference
Male albino Wistar rats	Δ9-THC	Acute treatment with a cannabinoid CB_1 receptor agonistinduced short-term memory deficits, impairments were attenuated after treatment with cannabinoid CB_1 antagonist.	[42]
Male ICR (CD-1) mice	JWH-018, JWH-018-Cl, JWH-018-Br and Δ9-THC	SCs dose-dependently impaired short- term memory. Their effects resulted more potent respect to that evoked by Δ9-THC.	[43]
Male Long–Evans rats	JWH-018	Chronic exposer to cannabinoid CB_1 receptor agonist induced spatial learning and short-term memory alterations well after the drugs exposure period.	[44]
Male Lister Hooded and Wistar rats	CP55,940	Acute administration of cannabinoid CB1 receptor agonist impaired short-term memory in both strains, yet, no long-term effects were observed.	[45]
Male Long–Evans rats	Δ9-THC	Acute treatment with a cannabinoid CB_1 receptor agonistinduced reversal learning deficits while set-shifting ability has maintained	[46]
Male Long–Evans rats	HU-210	Administration of the cannabinoid CB_1 receptor agonistelicited dose-dependent disruptive effects on set-shiftingperformance. Impairments were diminished afteradministration of the CB_1 antagonist AM251.	[47]
Male Albino Wistar rats	AB-PINACA or AB-FUBINACA compere with Δ9-THC	Two weeks after repeated administration of cannabinoid-agonist short-term memory impairments were observed, in SCs groups the impairments were greater and last for longer time.	[48]
Female and Male Sprague–Dawley rats	WIN55,212-2	Self-administration of SCs in low dosages during adolescence period improve or did not induce permanent memory impairments, while treatments of high dosages of SCs in adolescence period induced permanent short-term memory impairments.	[49,50]

2. Pharmacology of Organic Cannabis

Cannabis is the generic name of the psychoactive drug that is derived from the female plant *Cannabis sativa* [51]. There are more than 400 compounds including more than 60 cannabinoids, which are aryl-substituted meroterpenes unique to *Cannabis sativa* [52,53]. The main psychoactive ingredient in cannabis is Δ9-Tetrahydrocannabinol (Δ9-THC), which is the most potent cannabinoid that is present in the organic form of cannabis [53]. Besides Δ9-THC, organic cannabis products contain additional cannabinoids which do not induce psychoactive effects, such as Cannabidiol (CBD), Δ8-Tetrahydrocannabinol and Cannabinol [54–56]. Furthermore, CBD is considered a non-psychoactive cannabinoid that also moderates the psycho tropic effects of Δ9-THC [57–60].

The psychoactive effects of cannabis are dose-dependent [58,61,62] and there is evidence that as the content of Δ9-THC increases, the psychoactive effects of cannabis drugs increase [59,62]. Cannabinoid agonists in general and specifically Δ9-THC, exert their effects by acting on at least two types of endogenous cannabinoid receptors (CB1, CB2), which are widely distributed in numerous regions within mammals' brain [52,63,64]. Pacher and Kunos (2013) suggested that endocannabinoid receptors, the two endocannabinoid ligands and their related enzymes are the components of the Endo-Cannabinoid System (ECS), which is involved in a wide range of somatic and mental functions [65].

3. Synthetic Cannabinoids, from Therapeutic Agents to a Global Disease

3.1. Old Origins, New Trends

Since the discovery of Δ9-THC and the involvement of the ECS in a wide range of health conditions, cannabinoids have been synthesized for medical research purposes as promising research

and therapeutic tools [23,24]. In contrast to organic cannabinoids such as Δ9-THC, SCs selectively activate the endocannabinoid receptors [24,56,57].

In the beginning of the new millennium, a growing number of reports indicated that there were new psychoactive products which included mostly SC ingredients mixed with other herbal blends [6,66,67]. The production, distribution and use of SC drugs were initially neither controlled nor illegal, therefore they are presented as "legal-highs" [67], by various generic names such as; "Mr. Nice Guy," "Spice Gold," "Spice Diamond," "Yucatan Fire" and most commonly as "K2" or "Spice" [7,25]. These products were often sold without age restriction over multiple sources such as the internet and convenience stores [4,7–9,25,67]. As the popularity of SC drugs increased, their severe undesired adverse effects were observed; affective disorder, recurrent psychosis, tachycardia, seizures and prolonged hospitalization were not rare outcomes of SC intoxication [4,5,7–9]. Some of these adverse effects are related to the effect of additional psychoactive agents which these products contain [6,8,68].

Despite the fact that SCs are labeled as "not for human consumption" and "for aroma therapy use only," the popularity of these drugs appears to be growing [5]. SCs induce more intense effects than traditional cannabis, they are less expensive and they are undetectable in standardized drug tests. These unique features contribute to the growing numbers of recreational drug users who have used SCs [4,5].

3.2. The Psychoactive Ingredients of Synthetic Cannabinoid Products

Over than 140 products containing SC have been identified, although, the main psychoactive components of these products are different types of SCs which are categorized into four major groups including; (a) Aminoalkylindole or JWH series, (b) classical cannabinoids, (c) non-classical cannabinoids and (d) fatty acid amides (e.g., oleamide) [21,22,69,70].

The first generation of SC products mostly contain the series of 1-alkyl-3-(1-naphthoyl) indoles known as JWH compounds or aminoalkylinodels. This SC series is named after John W. Huffman who developed these ligands for medical research purposes [71] The JWH series advanced from computational melding of the chemical structural structures of Δ9-THC with previously developed aminoalkylinodels [71]. One of the first SC from this series to be abused is JWH-18 (1-penthyl-3-(1-napthoyl) indole), which features as easy synthesizable and high potency contribute to its popularity [9]. Compared to Δ9-THC, JWH-018 has 4 times the affinity for CB1 receptors and 10 time the affinity for the CB2 receptors [72]. JWH series represent the main psychoactive compounds detected in SC products across many countries [9].

Additional components detected in SC products include analogues of Δ9-THC, so-called classical cannabinoids such as HU-210 and HU-211. HU-210 developed in the middle of the 20th century at the Hebrew University (HU) [73] and is a hundred times more potent than Δ9-THC binds both CB_1 and CB_2 receptors [73,74] Similar to other SC, HU-210 acts as CB1 receptors full-agonists [73].

The cyclohexylphenol (CP) is a non-classical cannabinoids series synthesized by *Pfizer labs* in the early 1970s; examples include CP 59,540, CP 47,497 and their n-alkyl homologues [71]. Similar to JWH-018, CP-47,497 is included in large numbers within SC products e [67]. In addition, SCs from the CP series act as CB1 receptors full agonists [67]. However, within any given SC products, various types of SC are found in different concentrations [9,67] accompanied by additional psychoactive compounds from synthetic opioids such as O-desmethyltramadol, harmine and harmaline, which are inhibitors of the monoamine oxidase enzyme, to benzophenone (HM-40) and even caffeine [9,11,68]. There are several common features among different compounds of SC products which can highlight the risk potential which these drugs have and their related adverse effects. Firstly, SCs act as full agonists to CB1 receptors and some also bind to CB2 receptors [7]. Secondly, SCs are much more potent, easily cross the blood-brain barrier and have more affinity compared to organic psychoactive cannabinoids like Δ9-THC [68,69]. In addition, SC drugs do not contain CBD, which has high potency as an antagonist to CB1 and CB2 receptors and therefore it is able to revert the psychotic and anxiolytic

adverse effects of cannabinoid-agonists. It is suggested that the lack of CBD in SC products amplifies their psychotropic effects [4,6,75]. Moreover, SC products hold a unique characteristic, which is its ever-changing composition. The first generation of SC products commonly contain JWH-018, JWH-073 and CP-47,49, since these SCs became regulated, there has been an emergence of new types of SCs like JWH-081, JWH-210 and AM-2201, in an attempt to dodge regulations. Despite slight chemical structure modification, all of these SCs share the same main features and aim to mimic the psychoactive effects of Δ9-THC and even to transcend it [4,6,66,67,75].

4. Executive Function (EF) and the Long-Term Effects of Cannabinoids

4.1. The Three Core Factors Model of Executive Function

Although preclinical and human studies demonstrate that endocannabinoids involve and affect cognitive function in general and specifically high-order cognitive function [12,13,51,68], there is still a debate regarding the effects of chronic consumption of cannabinoid products such as cannabis or SCs on EF [12,13,46,70] (Table 1).

The term EF refers to "high-order" cognitive functions, which involve regulation, "lower-order" cognitive process and goal-directed behaviors [76,77]. EF generally clusters various cognitive abilities such as verbal reasoning, problem-solving, planning behaviors, sequencing, multi-tasking, cognitive flexibility, sustained attention, resistance to interferences and the ability to deal with novel information [77–80]. Due to the wide range of functions which are considered as executive or high-order, there is still an ongoing debate regarding the mechanisms which underlie executive function, performances and regarding which cognitive functions should be marked as executive [76].

Diamond (2013) suggested that EF should be divided into two subgroups: core EF and higher order EF [77]. Accordingly, the three cores EFs are (a) inhibition control or attention (b) Working Memory (WM) and (c) cognitive flexibility. The basic EFs are essential for the production of higher order cognitive functions such as verbal reasoning, problem-solving, planning behaviors, sequencing and multi-tasking. Accordingly, these functions do not involve much emotional arousal and they are logic based [77].

4.2. Cannabinoids and Attention-Evidence from Preclinical Studies

The ability to evaluate and allocate priority to external stimuli or internal habits and to optimize behavioral response requires attention [13,77]. These enable focus and selectively attend to desired stimuli and to inhibit response to irrelevant stimuli [77]. Studies have suggested that numerous brain regions facilitate attention performance, yet, it is mediated by the frontal lobes [81,82]. Additionally, the Anterior Cingulate Cortex (ACC) is a crucial factor in the execution of this function [82,83].

Preclinical studies provide strong evidence regarding the effects of repeated treatment with cannabinoid-agonists and impaired attention. The Lateralized Reaction Time task (LRT) of visuo-spatial attention that has been previously used in rats, is considered as a valid model for attention in rodents. In this paradigm, rodents need to attend to apparatus for the location of a visual stimulus over numbers of trails [83]. Arguello and Jentsch (2004) reported that acute treatments with the SC agonist WIN55212-2 (2.5 mg/kg) induced deficits in attention measured on the LRT task. In addition, treatment with SR141716A 1 mg/kg which is a CB1 antagonist reversed the WIN55212-2-induced attention impairment, although, when administered alone, this compound did not produce any effects on attention [26].

A further study by Verrico et al (2004) examined the effect of repeated treatments with Δ9-THC on attention using the LRT task in rats. In their study, rats that were daily treated with Δ9-THC 20 mg/kg for 2 weeks, presented attention impairments which lasted 14 days after the last treatment with Δ9-THC [28]. Later-on, Miller et al. (2013) treated rats with small doses of novel SC agonists AM-4054 before performing a two-choice reaction time task, which measures sustained attention. They

reported that AM-4054 induced attention impairments which were positively correlated with task demands and harder trails were associated with poorer functions [14].

Some authors suggested that lesions of the medial prefrontal cortex or striatum can produce attention deficits similar to those presented after cannabinoid administration [84,85]. Chronic exposure to cannabinoid-agonists led to alterations within meso-limbic dopaminergic neurons [86], thus, cannabinoid-induced attention impairment might arise via continuous activation of CB1 receptors across the striatum or prefrontal cortex [83].

4.3. Cannabinoids and Attention-Evidence from Clinical Studies

The disruptive acute effect of cannabis on attention is widely described in clinical studies [87–90] and systematic reviews [11,70,87]. Yet, human studies failed to draw consistent evidence regarding the effect of chronic consumption of cannabinoids and impaired attention. While some studies described impairments of tasks which demand attention in chronic cannabis users [17,19,61,89,91], other studies demonstrated no differences in behavioral performance between cannabis users and non-users [22,87]. Since neuronal and functional alterations of the ACC region were consistency observed among chronic cannabis users [92] a recent review study suggested that the marginal effects that were observed in these studies are probably an outcome of a compensation mechanism that was developed among chronic users [87].

There are several tasks for measuring attention. In a paradigm such the Stroop task, a control of interference from of a pre-potent response is required [93]. Incongruent conditions of the classical Stroop color-word task contain color words written in another color. Subjects are required to ignore the semantic meaning of the word and instead attend to and report the color. Since humans are trained to read and to ignore other words' features such as font style or color, people are slower and prone to make more errors in the incongruent trials of the Stroop task [77].

On the Go/No-Go task, the participants do not inhibit natural response at the expense of another. On this task, participants are required to respond when target stimulus is presented and should not respond when a non-target stimulus appears [94]. Other tasks such as the Continuous Performance Task (CPT) are being used for measuring sustained attention. In this paradigm, participants are required to maintain attention over a continuous period in order to detect infrequent targets, thus ensuring that the goals of the behavior are kept over time [20].

Eldreth and colleagues (2004) have examined the performance on a modified Stroop task in which healthy individuals were compared with abstinent cannabis users. Although there were no behavioral differences between the groups, cannabis users had greater activation in prefrontal brain regions than non-users [95]. Similarly, Jager et al. (2006) observed moderate differences in brain activity between cannabis users and healthy individuals while performing attention and WM tasks. They reported that compared with healthy subjects, cannabis users presented hypo-activation in the left superior parietal cortex while performing the attention task [96].

Recently, Hatchard and colleagues (2014) observed a similar pattern among young cannabis users. Recreational cannabis users did not differ in performance on the modified Stroop task compared with non-users, however, differences in neuronal activity of several brain regions including the ACC and post-central gyrus were observed, suggesting that chronic consumption of cannabis affects neuronal process even in an absence of behavioral expressions [97]. In another study, Hester et al (2009) reported that alterations in attention correlated with neuronal hypo-activity of ACC in heavy cannabis users. The attention deficits expressed in performing more errors on the Go/No-go task, suggested that attention depended on cannabis consumption history, including doses, frequency and age of onset [98].

The studies described so far examined the complex association between chronic consumption of organic cannabis and impaired attention, yet, there is limited objective evidence for an association between chronic consumption of SCs and impaired attention in humans [9]. Cohen et al (2017) showed that SC users had more errors performing on the classic Stroop color-word task compared with regular cannabis users and healthy subjects [11]. Furthermore, several case reports described SC users

who experienced "thinking problems" which last from days to weeks following last consumption. However, attention deficits were less common and they were accompanied with additional symptoms such as affective disturbances and cognitive dysfunction including severe alterations in short-term memory [8,99,100].

4.4. Cannabinoids and Working Memory-Evidence from Preclinical Studies

Working Memory (WM) is defined as a cognitive mechanism for the temporary storage and manipulation of stored information [101], or simply, as a cognitive system which involves holding information in mind and mentally working with it [77].

The function of WM has been associated with integration of a wide range of neural networks. WM networks are associated with frontal-parietal regions including dorso-lateral pre-frontal cortex, ventro-lateral prefrontal cortex, pre-motor cortex, lateral parietal cortex and the frontal lobe [102]. An additional brain region which is considered a major component in WM is the hippocampus, which is essential for acquiring, encoding and consolidating new types of information. This information is represented and manipulated by the WM system in the prefrontal cortex [103]. In rodent models, changes in hippocampal morphology were observed following chronic treatments of various doses with cannabinoid agonists like Δ9-THC and WIN55,212-2, these neuronal alterations correlated with behavioral dysfunction [30–32].

Preclinical studies which used rodent as animal models, utilized both maze-based and instrumental tasks for investigating the effect of cannabinoids on WM [31]. Maze-based tasks require the rodent to use spatial cues correctly. These tasks are based on the navigational behaviors of rodent for foraging or in order to escape from predators [104]. Several works have suggested that chronic treatment of Δ9-THC induced WM impairments on in different types of maze-based tasks [33,34] and in water maze tasks [35,36,38]. These impairments are dose-related, thus greater impairments were observed after exposures to more potent cannabinoid-agonists [36,41]. Therefore, it is not surprising that SC agonists such as JWH-081 and HU-210 induce similar disruptive effect on WM performances in maze-based tasks [37,39]. In addition, similar impairment is induced with anti-cholinergic agents like physostigmine, suggesting that cannabinoid-agonists induce WM impairments due to interaction with acetylcholine system [105].

Instrumental WM tasks in rodents include the delayed matching to sample (DMTS) or delayed non-matching to sample (DNMS) tasks. During these tasks, the animal is initially presented with a stimulus and following delay period, both the original stimulus and a novel stimulus are presented. The animal must indicate either the sample stimulus or the novel stimulus follow the task's rule [31]. The effects of chronic treatment with cannabinoid agonists such as WIN55212-2 and Δ9-THC on WM in DMTS or DNMS paradigms are widely observed, both in rodents [27,41,42,106] and in primate models [107,108]. Again, most of the studies report that the disruptive effects of cannabinoid agonists are dose-dependent [31].

Recently, Barbieri et al. (2016) reported that administration of a CB1 receptors antagonist AM251 to mice as pre-treatment, fully prevented the disruptive effects of cannabinoid agonists including JWH-018 and Δ9-THC on WM, thus suggesting a CB1 receptor involvement in the effect of cannabinoids on WM [43]. Other studies reported that repeated treatment with SC agonists JWH-018 and CP55,940 in the puberty period induced severe WM impairments that remained in adulthood [44,45]. These findings are consistent with previous theories which suggested the involvement of ECS in brain development and that consumption of cannabinoid agonists in adolescence alter the function of the ECS [24,32,109,110].

Interestingly, some studies report contrary results where reduced impairments following repeated treatment with cannabinoid agonists were presented [38], although, this might be a result of tolerance [31]. In addition, further preclinical research is needed to examine the degree of persistence of deficiencies induced by chronic treatments with cannabinoid agonists [31]. Yet, a growing number of publications indicate that exposures to cannabinoids in early age are associated with greater and

persistent WM deficits, suggesting that the age of onset may be a mediating factor in the association between cannabinoids and WM performance [32,106,111–113].

4.5. Cannabinoids and Working Memory- Evidence from Clinical Studies

The disrupted effect of acute cannabis intoxication on WM performance in humans is widely documented [33,87]; however, there is a growing debate whether chronic cannabinoid consumption induces long-term impairments of WM [87,114,115].

The most common paradigm for measuring WM performance is the n-back task. During this task a sequence of constant stimuli in form of digits, shapes or numbers are presented to the subject, who need to decide if the presented stimulus is identical to a previous stimulus from n steps earlier. The load factor n reflects different WM loads; lower n represents an easier task [116]. Kanayama and colleagues (2004) investigated WM in chronic cannabis users and used functional Magnetic Resonance Imaging (fMRI) [117]. They reported that cannabis users did not show WM dysfunction; however, increased activation of several brain regions including prefrontal-cortex, ACC and basal-ganglia regions were observed. The authors suggested that chronic consumption of cannabis induced subtle neurophysiological deficits which are compensated by hyper-activation to meet the demands of the task [117,118].

In addition, an fMRI study which focused on hippocampus activity during performance on the n-back task, compared cannabis users with two control groups of healthy individuals and tobacco smokers [119]. Poorer performance was observed in cannabis users compared with both control groups on the task's overall score. Furthermore, cannabis users presented less activity in the right hippocampus across the task's conditions contrary to both control groups [119]. In a further neuroimaging study, Jager et al. (2007) examined the effects of cannabis use on neuronal activity in abstained cannabis users and healthy control participants during performance on the n-back task consisting of encoding and recall conditions [120]. Similar to previous studies [121], there were no differences between the groups in terms of behavioral performance. Interestingly, cannabis users exhibited hypo-activation in the right dorso-lateral pre-frontal cortex and in bilateral hippocampus regions. This reduced activity in WM responsible areas were limited to the encoding phase and were not presented in the rest of the task phases' [120].

Smith and colleagues (2010) used fMRI to examine the neuronal brain activity of heavy cannabis users and control non-users while performing different loads of the n-back task. The two groups presented similar WM performance, however, in contrast to other studies, cannabis users demonstrated hyper-activity in the right frontal gyrus, left middle inferior frontal gyrus and right superior temporal gyrus [122]. In a recent systematic review, Bossong and colleagues (2014) suggested that most functional neuroimaging studies present similar pattern of hyper neuronal brain activity in cannabis users compared with control participants that were accompanied with normal WM function [123]. They support the view that increased activity reflects greater neural effort in order to maintain good task performance [123]. On the other hand, a-3-year longitudinal neuroimaging study failed to find behavioral or functional differences between cannabis users and control participants, suggesting that a moderate use of cannabis may not have substantial effects on WM neural network and behavioral performance [124]. However, WM deficits in chronic cannabis users are more likely to be elicited in complex conditions [115]. Therefore, a lack of differences in WM performance between cannabis users and control participants does not necessarily indicate a lack of association between chronic consumption of cannabis and WM [123].

Convergent evidence from structural neuroimaging studies supports the last view indicating that chronic consumption of cannabis is associated with neuronal alterations in several brain regions which are involved in WM including reduction in size of the hippocampus and amygdala. In addition, these alterations correlated with the amount of cannabis use and dependence [125]. Recently, Battistella et al. (2014) reported similar data, where neuronal alterations in several brain regions including the parahippocampal gyrus were observed in chronic cannabis users compared with occasional users.

Furthermore, these alterations are associated with age of onset and frequency of cannabis use in the last 3 months [126].

To our knowledge, there is a limited number of available laboratory human studies investigating the association between persistence consumption of SC with WM performance. Yet, Castellanos and Thornton (2011) reported that young adults who used SC drugs experienced alterations in short-term memory; however, their main symptom was a severe psychotic episode [127]. Further reports described similar clinical manifestations where SC users experienced symptoms including alterations in short-term memory [128,129]. Cohen et al. (2017) demonstrated WM impairments observed among SC users compared with non-users and recreational cannabis users [11]. These reports are not surprising since CB1 receptors are highly distributed in the hippocampus and in prefrontal cortical regions [130,131], which are associated with WM [102]. In addition, SC products contain high-potency cannabinoid agonists, therefore it is reasonable that chronic consumption of SC induces impairments in WM function in more salient forms than those which are induced by organic cannabis [66,67].

4.6. Cannabinoids and Cognitive Flexibility- Evidence from Preclinical Studies

Cognitive flexibility has been described as the cognitive ability to think about multiple concepts simultaneously and to be able to switch between thinking about two different concepts [18]. Miyake et al. (2000) identified cognitive flexibility as the ability to shift one's thinking and attention between unrelated tasks, typically in response to a change in environmental demands [81]. Diamond (2013) expanded the view of the term and suggested that an additional feature of cognitive flexibility is being able to change perspectives spatially or inter-personally. Accordingly, for changing perspectives, an individual needs to inhibit the last perspective and to load a new perspective into WM [77]. In that sense, cognitive flexibility builds and depends on WM and inhibition control. Other aspects of cognitive flexibility involve changing the way of thinking in response to external demands and thinking "outside the box" [77].

In rodents, variations of attention set-shifting paradigms are being used to assess behavioral flexibility. During these tasks rats are required to change behavioral responses, by learning new stimulus-reward associations through earlier learned response inhibition tendencies [13]. These paradigms differentiate between two types of behavioral flexibility; (a) for successful extra-dimensional shifts the rats need to shift attention bias between different features of stimuli, (b) reversal-learning discriminations required the rats to update relations between stimuli and rewards presentation, in this inter-dimensional discrimination based on cue from a single modality [13,83]. This differentiation is important since these two aspects of behavioral flexibility are linked with different brain regions [13], while reversal learning is associated with orbito-frontal cortex [132], extra-dimensional shifts are mediated by the medial pre-frontal cortex [133,134].

Several preclinical studies investigated the effects of cannabinoid-agonists on cognitive flexibility and indicated inconsistent results. Egerton and colleagues (2005) reported that acute administration of 5 mg/kg Δ9-THC induced impairments in reversal learning, whilst attention set shifting ability was maintained [46]. Further primate research presented similar results using smaller doses and demonstrated that an acute administration of 0.5 mg/kg Δ9-THC induced more errors in reversal learning and it did not affect attention set shifting ability [135].

However, an additional rodent study has demonstrated different findings, whereby administration of 0.2 mg/kg of the SC agonists HU-210 2 days before measuring set-shifting, induced dose-dependent impairments in extra-dimensional set shifting ability [47]. These impairments were diminished after administration of a CB1 antagonist AM251. In addition, cannabinoids did not affect inter-dimensional reversal learning [47].

Further evidence regarding the effects of cannabinoids on cognitive flexibility was demonstrated by Varvel and Lichtman (2001). Knockout mice, which lack cannabinoid CB1 receptors presented impaired reversal learning in inter-dimensional water maze reversal learning task [40]. Their findings

support the view that the ECS are involved in execution of cognitive flexibility [13]. Consistent with earlier studies, Gomes and colleges (2015) recently indicated that rats which were repeatedly treated with 1.2 mg/kg of a CB1 agonist WIN55,212-2 for 2 weeks in adolescence, showed deficits in adulthood in performance of set-shifting tasks and alterations in dopamine levels in the ventral tegmental area. These alterations were present in adulthood and were similar to those which were shown in pre-clinical models of schizophrenia [136].

The conflicting results demonstrated by previous studies, reflect the need for further studies on the effect of cannabinoids on cognitive flexibility. The available evidence demonstrates that cannabinoids have indeed an effect on cognitive flexibility [13], possibly via modulation of dopamine and glutamate concentrations in several brain regions including the ACC and prefrontal cortex [13,83].

4.7. Cannabinoids and Cognitive Flexibility- Evidence from Clinical Studies

Recent studies using fMRI have found a variety of brain regions that were activated while performing cognitive processes that demand flexibility, including, the pre-frontal cortex, basal ganglia, ACC and posterior parietal cortex [137]. Some of the regions which underlie cognitive flexibility are involved in WM and inhibition control and thus, the findings support the hypothesis that cognitive flexibility depends both on WM and inhibition control [81]. In addition, levels of certain neurotransmitters such as monoamines in several brain regions are associated with cognitive flexibility [138].

Paradigms for investigating cognitive flexibility include a wide array of task-switching and set-shifting tasks. One of the oldest and most common task for measuring this performance is the Wisconsin Card Sorting Task (WCST) [139]. In this task, a number of stimulus cards are sorted by color, shape or number. The participant is required to conclude the correct sorting criterion on the basis of feedback. Set-shifting ability is required when sorting criterion has been changed and perseverative errors are the outcome of failure in set-shifting [77]. Additional tasks for measuring cognitive flexibility include verbal fluency and semantic fluency. In these tasks participants are required to demonstrate unusual patterns of thinking by answering a serial of verbal questions (What is common between a fly and a tree?) in order to be successful [77].

Acute intoxication of cannabis has disruptive effects on cognitive flexibility [62,105,140]. However, the evidence on non-acute effects of cannabinoids on cognitive flexibility have been inconsistent. Bolla et al. (2002) reported dose-related effects of cannabis use on cognitive function. They have examined several aspects of cognitive function including cognitive flexibility in heavy cannabis users compared with moderate and occasional users who abstained from cannabis for 28 days. Poorer performance was positively correlated with increased frequency of cannabis consumption [61].

Later on, Pope et al. (2003) has reported similar effects, except that deficits in performance on the WCST were observed in heavy cannabis users who had started smoking cannabis during adolescence [141]. In addition, there were no differences in flexibility performance between cannabis users who had started using cannabis in adulthood compared with non-users [141]. A further study demonstrated that heavy cannabis users' performance on the WCST resemble those of schizophrenic patients; however, there was no association between frequency of cannabis use and errors on the WCST [142]. Contrary to the last results, several studies indicated that while repeated consumption of cannabis has disruptive effects on some cognitive functions, impairments in cognitive flexibility were not presented in heavy cannabis users even after controlling for demographic variables [143,144].

In a systematic meta-analysis, Grant and colleagues (2003) examined the non-acute effects of cannabis on several aspects of cognitive function using strict inclusion criteria on a limited number of studies. The authors failed to find significant non-acute effects of cannabis consumption on cognitive flexibility. However, it should be noted that cognitive flexibility was referred as a component within the factor of abstraction reasoning [145]. This methodological issue is critical since abstract reasoning and cognitive flexibility are different components of EF [77].

The evidence so far points out to a lack of available evidence regarding the effects of SC on cognitive flexibility in humans. Altintas et al. (2016) examined several cognitive domains in SC users who experienced psychotic episodes and compared their performance with hospitalized schizophrenic patients. Interestingly, there were no differences between the groups in cognitive flexibility measurement [146]. Yet, their results cannot be interpreted as an outcome of SC use exclusively since it cannot be differentiated from psychotic symptoms that were observed among SC users as well. There are two additional aspects of the association between cannabinoid abuse and cognitive flexibility which should be noted. First, impairments in cognitive flexibility have been suggested to play a major role in continuous use of cannabinoids despite negative consequences [83]. Secondly, deficits in cognitive flexibility were associated with affective alterations [147]. Both greater mood alterations and greater rates of abuse are commonly observed among SC users and heavy cannabis users [9,148].

In summary, both pre-clinical and clinical findings suggest that the ECS are involved in cognitive flexibility [13]. Although, there are inconsistent findings in human studies, the non-acute disruptive effect of cannabinoids on cognitive flexibility is probably mediated by several factors including the age of onset and the frequency of cannabinoid consumption [58,141], yet, further exploration of the last relation is required.

5. Conclusions

Cannabinoid drugs, in both organic and synthetic forms became increasingly popular despite the potential harms associated with their use [6,10,87]. While the main psychoactive ingredient of cannabis is the CB1 receptor partial-agonist Δ9-THC [13,51–53], SC drugs contain varied types of cannabinoid-agonists which are more potent than organic cannabinoids [65,149]. Although SC and organic cannabinoids bind to the same CB receptors, the psychotropic effects of SC are more severe, more rigid and much more unpredictable than those induced by organic cannabinoids [4,5,65,75]. Taking into account the above evidence that SC drugs do not contain CBD, their harm potential is significant [5,75,114].

Taking together the recent finding of both animal and human studies, repeated consumption of cannabinoids is associated with EF impairments, yet, there is still a gap of knowledge regarding the last of these impairments [11,114]. The available data from both animal and human studies suggest that ECS involve and effect cognitive functions in general and EF specifically [9,10,12,13,83]. The ECS has a major role in neurodevelopmental and maturational process, which are especially prevalent during adolescence. Consumption of exogenous cannabinoids affect the functioning of the ECS, it is plausible that chronic consumption during early adolescence alters the neurodevelopmental maturational process during this period [5]. Consequently, it is not surprising that current evidence suggests that exposure to cannabinoids during the adolescent period may induce severe long-lasting cognitive impairments [5,78,96,108,147,148]. Furthermore, most of the current evidence indicates an association between the amount of cannabinoid consumption with the degree of impairment; more consumption, or consumption of drugs which contain more potent cannabinoids is associated with greater impairments [5,83,87]. Accordingly, although there is a limited number of human studies which examine both the acute and long-term effect of SCs on EF, it is reasonable to assume that SC which contain extremely potent cannabinoid-agonists may induce long-term EF-impairments [5–8]. Yet, further research is needed to expend to knowledge of the last phenomena.

It is important to note some of the limitations of the current review. Most of the available evidence regarding the effects of SCs on EF is based on pre-clinical studies. When interpreting these results, it is important to take into account that the methodological limitations which animal studies naturally hold. Firstly, while cannabis or SC users mostly use these drugs by smoking or inhaling [1,3,7], most of the pre-clinical studies mentioned in this review treated animals by intraperitoneal (I.P) injection which in contrast to inhaling induce greater effect in shorter time [9,32]. Furthermore, it is important to take into account that most of the mentioned pre-clinical studies have used specific SCs or pure Δ9-THC

for exploring their exclusive effect on a chosen factor [3,9,32]. In contrast to that, evidence from epidemiological data or human studies present information regarding the effects of SC or cannabis products which mostly contain a range of cannabinoids and in some cases additional psychoactive compounds [1,3,7,9,32].

Understanding the effects of cannabinoids on EF has considerable practical utility in the clinical setting. Executive function is essential to an individual's multiple abilities in daily life [77]. It has been suggested that due to impaired EF, patients may have difficulties in learning new coping behaviors and accordingly increases the likelihood of treatment dropout and poor treatment outcomes [12]. Therefore, the current review emphasizes the need of attention by the clinician regarding cognitive abilities of patients who suffer from cannabinoid abuse. In case of cognitive impairments, an alternative unique therapeutic method should be considered such as behavioral therapy [150] or introducing the patient with cognitive rehabilitation strategies [12]. This may be crucial, especially in cases of patients who are heavy cannabinoid users, or young patients who used cannabinoids in early age for persistent periods.

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Mood Fluctuation and Psychobiological Instability: The Same Core Functions Are Disrupted by Novel Psychoactive Substances and Established Recreational Drugs

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Abstract: Many novel psychoactive substances (NPS) have entered the recreational drug scene in recent years, yet the problems they cause are similar to those found with established drugs. This article will debate the psychobiological effects of these newer and more traditional substances. It will show how they disrupt the same core psychobiological functions, so damaging well-being in similar ways. Every psychoactive drug causes mood states to fluctuate. Users feel better on-drug, then feel worse off-drug. The strength of these mood fluctuations is closely related to their addiction potential. Cyclical changes can occur with many other core psychobiological functions, such as information processing and psychomotor speed. Hence the list of drug-related impairments can include: homeostatic imbalance, HPA axis disruption, increased stress, altered sleep patterns, neurohormonal changes, modified brain rhythms, neurocognitive impairments, and greater psychiatric vulnerability. Similar patterns of deficit are found with older drugs such as cocaine, nicotine and cannabis, and newer substances such as 3,4-methylenedioxymethamphetamine (MDMA), mephedrone and spice. All psychoactive drugs damage human well-being through similar basic neuropsychobiological mechanisms.

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1. Mood State Fluctuations

Psychoactive drugs, by definition, cause mood states to change and fluctuate. Hence an important factor for drug-induced distress is mood instability. This is found with sedative drugs such as alcohol or cannabis, and stimulant drugs such as cocaine or methamphetamine [1–6]. Indeed, the main reason that psychoactive drugs are used recreationally is for their "positive" mood effects, such as feelings of relaxation and pleasure [4]. Novel psychoactive substances (NPS) such as mephedrone and spice cannabinoids are very similar in this regard [6–9]. Although one of the paradoxes of drug taking is that many of the apparent mood gains represent the reversal of unpleasant abstinence feelings. Nicotine is a prime example of this pattern [10–12], although it also occurs with other substances [4].

Psychoactive drugs can also produce undesirable mood state changes. For instance, recreational cannabis, whether herbal or spice, can lead to feelings of tension and suspiciousness [2,13]. Stimulants such as cocaine, methamphetamine and mephedrone can also lead to feelings of anxiety and paranoia [1,14,15]. The acute mood effects of all psychoactive drugs can be highly variable and may differ considerably between individuals. On alcohol some users become happy and jovial, while others become moody and aggressive, especially after excess levels of consumption [5,16,17]. In a similar way, Le Strat et al. [18] documented a wide range of responses to an acute dose of cannabis. Furthermore, those with positive initial mood reactions were most likely to become regular cannabis

users, whereas those with negative or neutral mood reactions to cannabis tended not to persevere with its usage. Similar differences in mood response have also been empirically demonstrated with the methamphetamine derivative MDMA (3,4-methylenedioxymethamphetamine or "Ecstasy"). This drug is often described as the most euphoriant of all the recreational stimulants. Yet acute MDMA can release a wide range of feelings and cognitions-both positive and negative. Liechti et al. [19] found that an acute dose of MDMA in the laboratory led to significant increases in feelings of both introversion and extraversion, while feelings of happiness and depression were also significantly intensified. In a comprehensive review of the acute-dose MDMA mood literature, it was found that a wide range of positive and negative psychological material could be released/intensified [20]. For instance, psychotherapists who have incorporated MDMA into their therapy sessions have noted that the emergent psychological material can be difficult and stressful for the client to handle, although its release may also be important for potential therapeutic gains [20–24]. In positive environmental situations, the positive mood effects of MDMA predominate, whereas in neutral situations its mood effects can be more variable and less positive [14,15]. These adverse mood abreactions to MDMA can be more frequent than is commonly portrayed. In an article entitled: "Hug drug or thug drug", Reid et al. [25] noted that MDMA often generated feelings of anger and aggression. Rugani et al. [26] noted that acute psychotic patients who had used MDMA for recreational purposes demonstrated heightened levels of hostility, physical violence, and verbal aggression than non-users. The authors noted that this finding "surely runs counter to the expected entactogenic effects of Ecstasy" (for further debate, see [27]).

In a large Internet study of mephedrone and MDMA users, the acute mood effects of each drug were broadly similar, although some intriguing differences in pharmacodynamic tolerance were apparent [9]. A mixture of positive and negative mood changes is also found with established recreational stimulants such as cocaine [28], and laboratory doses of methamphetamine [14,15]. The effects of high doses of amphetamine and cocaine can be very strong, with reports of a physical rush or hit. These high doses can lead to intensely negative moods, with very severe feelings of suspiciousness, or clinical paranoia. Spice cannabinoids can also have far stronger effects that herbal cannabis, in both positive and negative ways [29]. The more extreme mood reactions of the more powerful drugs can lead to changeable and unpredictable patterns of behavior. However, these abreactions can occur with any psychoactive drug, irrespective of whether they are established or novel.

1.1. Drug Withdrawal and Repetitive Mood Vacillation

One of the main problems with every psychoactive drug is that the brief period of on-drug mood gains is followed by a period of neurochemical depletion afterwards, when the opposite mood states develop. These rebound moods are typically negative and aversive, and are readily reversed by taking the drug again. Hence every mood-altering drug has addiction potential. Indeed, the essence of addiction is these repetitive mood vacillations [4,30]. This pattern can be illustrated by legal stimulants such as nicotine, illicit drugs such as cocaine, or novel substances such as MDMA or mephedrone. The former two drugs have been extensively studied, with nicotine showing many pharmacokinetic and pharmacodynamic similarities to cocaine [31]. Both drugs are powerful Central Nervous System (CNS) stimulants, with the first cigarette of the day increasing heart rate substantially [32,33], which is one of the reasons tobacco smokers develop hypertension. In mood terms, tobacco smokers feel more alert after the first cigarette of the day, but soon this activation is lost, and after 20–60 min the regular smoker needs yet another cigarette. This mood fluctuation repeats over the rest of the day and recurs every day for the rest of their lives—until they quit or prematurely die [10,11,34].

Similar patterns of mood fluctuation occur with cocaine, since nasal snorting leads to a rapid hit, followed by a low-mood comedown, and the urgent need for another drug hit. Cocaine therefore displays high addiction potential, with crack cocaine being even more troublesome and addictive, due its extreme rapidity of action [4,35,36]. Khat leaf chewing occurs in many countries around the Horn of

Africa [37,38], with cathinone being slowly released into the systemic circulation. Cathinone displays weaker stimulant properties than cocaine. Yet Khat chewers also report acute mood gains, followed by negative moods on withdrawal, in a pattern identical to that found with other stimulants [37]. This is also evident with MDMA, although over a longer time period. Hence the acute moods peak after a couple of hours, while the recovery period can last for several days [28,39–42]. This long pharmacodynamic profile explains why Ecstasy/MDMA is only taken intermittently and displays lower addiction potential than most other stimulants [43,44].

Cannabis may be a sedative drug, but it shows a similar pattern of mood vacillation to the stimulant drug nicotine. Vandrey et al. [45] compared the mood changes found during withdrawal from cannabis and tobacco, and concluded that they were very similar. For instance, the unpleasant mood effects of cannabis withdrawal could include feelings of irritability, anger, and depression (i.e., very similar to tobacco), along with other problems such as impaired sleep, altered circadian rhythms, changed appetite, and drug cravings [45,46]. These symptoms of cannabis withdrawal have been formalized in standardized questionnaires such as the Cannabis Withdrawal Discomfort Scale [47], and the Marijuana Craving Questionnaire [48]. The high addictiveness of cannabis does make it difficult to understand current movements to make its usage legal [49].

1.2. Related Psychobiological Problems

Many other psychological skills and abilities may also fluctuate during drug stimulation and drug withdrawal. For instance, tobacco smokers display worse memories than non-smokers [50]. The reason for this is that new information is being laid down in memory and is being stored under a constantly changing background of nicotine levels. Hence memory storage and retrieval are both adversely affected by nicotine addiction [12]. Sleep is also adversely affected by nicotine addiction, while it improves to normal following smoking cessation [12]. The many psychobiological problems found in recreational stimulant users have been described in numerous comprehensive reviews [1,3,36,51,52].

Similarly, the addictiveness of herbal cannabis was noted earlier, while the stronger skunk varieties of herbal cannabis display greater potential [53]. Artificial spice cannabinoids are even stronger, and hence more damaging. Some of the artificial spices are full agonists for the cannabinoid receptor, whereas herbal cannabis is a weak partial agonist [54]. Hence spice displays far greater addictiveness, with some users committing suicide when they cannot access their normal drug supplies [6,29,55–58]. The practical consequences of cannabis dependency can be severe [49]. In the USA around 300,000 individuals approach professional drug addiction services for cannabis dependency every year [59]. Clinically disabling dependency occurs in around 10% of those who have ever tried the drug [60], while 65% of cannabis ever-users report some aspects of drug dependency [61], with young initiates the most vulnerable [62]. In summary, the core problems related to drug dependency are similar for stimulants and depressant drugs, and for older and newer psychoactive substances.

2. Homeostasis

One fundamental index of psychological balance and health is homeostasis. When the organism is well adapted to its environment, its daily rhythms of behavioral and physiological activity occur smoothly and efficiently. Selye [63] noted that disruption to homeostasis led to psychological imbalance, increased bio-physiological stress, and led to excessive energy expenditure. Furthermore, the repeated experience of acute stress led to cumulative chronic stress and this caused physical and psychological ill-health. Selye [63] showed that the Hypothalamic-Pituitary-Adrenal (HPA) axis was crucial for psychophysiological stability, with cortisol being the key neurohormone for maintaining homeostatic balance [64]. Healthy individuals showed regular circadian rhythms of cortisol secretion, and this master hormone helped to maintain the optimal secretion patterns for other important neurohormones [63,65,66].

Many psychoactive drugs affect neurohormonal secretions acutely, and when these drugs are taken repeatedly, they can lead to chronic stress. This may be illustrated with the recreational stimulant

MDMA [65,66]. In placebo-controlled laboratory trials, an acute dose of MDMA can generate a cortisol increase of around 150% [67]. Recreational Ecstasy/MDMA users show peak cortisol increases of around 800%, probably due to the combined effects of taking a stimulant drug in a stimulating environment [42,68]. The Cortisol Awakening Response can also be affected in recreational Ecstasy/MDMA users [69], with around 70% of recreational users complaining of disrupted sleep, even when drug-free [70]. Body temperatures can also change, with MDMA showing well-documented patterns of thermal change [71–73]. Synthetic cannabinoids such as AKB48 can induce hypothermia [74], while synthetic cathinones such as mephedrone can also affect thermal reactivity [75]. Returning to chronic stress, regular MDMA users show strong longer-term neurohormonal changes. When cortisol was measured in 3-month hair samples, the regular Ecstasy/MDMA users displayed 400% higher cortisol levels than non-user controls, while the light Ecstasy/MDMA users showed intermediate cortisol values [76]. MDMA is not the only recreational drug which can affect cortisol. Raganathan et al. [77] showed that acute tetrahydrocannabinol (THC) led to a significant increase in cortisol secretion. King et al. [78] found that chronic cannabis users had significantly higher salivary cortisol levels than non-user controls. Currently there is a paucity of empirical evidence on the neurohormonal effects of Novel Psychoactive Substances, and empirical studies in this area are therefore needed.

Psychiatric Aspects

All psychiatric disorders are dimensional, with symptoms ranging on a continuum from low to high. This core notion may seem rather obvious, but it needs to be stated since it can help explain how psychoactive drugs contribute to mental distress. The core processes described earlier, of mood state vacillation and homeostatic imbalance, can each contribute to mental instability, while those individuals with a predisposition to mental distress may be particularly vulnerable to the destabilizing effects of psychoactive drugs. For an example of this interactive psychiatric model applied to recreational MDMA, see Parrott [79].

The first written report of psychiatric problems being caused by any psychoactive drug were present in the world's oldest pharmacopoeia, attributed to Emperor Shen Nung in bronze-age China. This noted that when cannabis was taken in excess, it could produce "visions of devils" [80]. Modern research has confirmed that cannabis may cause a form of psychosis, with many similarities to classic schizophrenia [13]. Hence an acute dose of cannabis can induce bizarre thoughts and cognitions [81]. D'Souza and colleagues [82] administered THC and placebo to recreational cannabis users without any prior psychiatric history. The active cannabis condition led to significant increases in scores on the Positive and Negative Symptom Scale (PANSS), the standard rating scale for clinical symptoms of schizophrenia. Individual subjective experiences under the acute influence of THC included the following: "I thought I could see into the future" ... "I thought you were trying to program me" ... "I thought you could read my mind" ... "I thought I was god". These delusional thoughts as measured by raised PANSS positive symptom scores, can also correlate with changed patterns of brain activity [83].

When used regularly, cannabis can lead to both clinical psychosis, and other forms of psychiatric disorder [84]. The Swedish Conscript study was the first prospective investigation to demonstrate an association between cannabis and schizophrenia [85]. This finding has been replicated in further prospective studies, where regular cannabis use led to an increased risk of psychotic breakdown in later years. Le Bec et al. [86] undertook a comprehensive review and concluded that every published study showed a significant link between recreational cannabis and the later emergence of psychosis. One crucial factor is that the drug needs to be taken repeatedly and regularly. In one prospective study, Henquet et al. [87] found that occasional cannabis users showed no increase in risk, weekly-users showed a slightly increased risk, while daily-cannabis users showed a highly significant increase in later psychosis. Regular cannabis use is also associated with an increased risk for other mental health problems, such as depression and mania [88,89].

Recreational CNS stimulants can also cause greater psychiatric distress. Feyissa and Kelly [90] noted that Khat chewing could lead to a range of mental health problems including depression and hypomania; hence even weaker drugs such as cathinone can lead to psychiatric problems, while regular users of stronger stimulants such as amphetamine, cocaine or methamphetamine can experience a wide range of problems, including psychosis, depression, paranoia, psychomotor tics/tremors, eating disorders, and aggression [1,3,4,35,51,91]. Recreational MDMA is also associated with a wide range of adverse psychiatric consequences, such as clinical depression, aggression, problems with weight control, eating/food intake, and some of these issues may endure for years after drug cessation [26,76,92–99].

3. Neurocognitive Deficits

Cognitive skills are an important focus for most areas of applied psychology, and psychopharmacology is no exception. The extensive empirical literature demonstrates both acute and chronic drug influences. By definition, any drug which is psychoactive will affect not only mood states (see Section 1), but many other psychological functions including neurocognition. CNS stimulants such as cocaine or mephedrone will speed information processing, but also increase errors through increased carelessness and impulsivity. CNS depressants will generally lengthen reaction times but may increase errors through reduced alertness and vigilance/attention. When combined with drug-induced feelings of confidence, these changes can make any psychoactive drug dangerous for practical skills such as car driving [4,55,100]. Their chronic use can also be damaging. In an extensive review, Cruickshank and Dyer [1] noted that methamphetamine led to impairments in executive functioning, learning, memory, and motor skills. Other reviews have generated similar lists of neurocognitive impairments following other stimulants such as cocaine [52,101], or Ecstasy/MDMA [27,44,102–108]. Cannabis can lead to acute deficits in memory, learning, sustained attention, and higher cognitive skills, while its chronic use can lead to a wide range of cognitive deficits, even including a decline in general intelligence, with reduced IQ test scores [109–114]. Neuroimaging studies of regular cannabis users indicate deficits in various brain regions, such as the hippocampus and amygdala [114], with white matter degeneration and de-myelination [115].

4. Final Overview

Psychoactive drugs can damage human well-being simply by being psychoactive! In acute terms they may boost activity for a short period, but this is soon followed by a period of neurochemical recovery, when the opposite psychological states develop. These psychological fluctuations are readily seen in mood state changes of daily tobacco smokers (see Figure 1 in Parrott, [10]). However, moods and feelings provide just one index for other more general changes in psychological status. Many different psychological functions can be affected—in different ways by different drugs. They also affect many different neurotransmitter systems. Yet despite the multitude and variety of their neurotransmitter actions, in psychobiological terms these drugs all display the same underlying pattern of disrupted balance and equilibrium [4,11,30,38]. These core biological factors also explain why every psychoactive drug displays addiction potential. Regular users suffer from negative states off-drug, and feel better when on-drug, hence the "need" to take the drug repeatedly [10]. As novice users, the more they succumb to their new habit, the stronger their drug dependency becomes.

Psychoactive drugs also affect the HPA axis, causing hormonal dysregulation, and increasing the susceptibility for stress [4,66]. The healthy human organism displays a natural balance between sympathetic and parasympathetic nervous system activity. So, when humans take recreational drugs, they disturb this natural balance, and this leads to adverse consequences [4]. Proponents for drug use typically only talk about the short-term drug gains, and with this narrow focus, any psychoactive drug can be miss-described as beneficial. It is only by covering all aspects of their acute and chronic usage that a more complete picture of their damaging effects can emerge.

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The Dynamic Environment of Crypto Markets: The Lifespan of New Psychoactive Substances (NPS) and Vendors Selling NPS

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Abstract: The Internet has played a major role in the distribution of New Psychoactive Substances (NPS), and crypto markets are increasingly used for the anonymous sale of drugs, including NPS. This study explores the availability of individual NPS and vendors on the crypto markets and considers whether crypto markets are a reliable platform for the sale of NPS. Data was collected from 22 crypto markets that were accessed through the hidden web using the Onion Router (Tor). Data collection took place bimonthly from October 2015 to October 2016 as part of the CASSANDRA (**C**omputer **A**ssisted **S**olutions for **S**tudying the **A**vailability a**N**d **D**ist**R**ibution of novel psycho**A**ctive substances) project. In seven snapshots over 12 months, 808 unique vendors were found selling 256 unique NPS. The total number of individual NPS and vendors increased across the data collection period (increase of 93.6% and 71.6%, respectively). Only 24% ($n = 61$) of the total number of NPS and 4% ($n = 31$) of vendors appeared in every snapshot over the 12 months, whereas 21% ($n = 54$) of NPS and 45% ($n = 365$) of vendors only appeared once throughout the data collection. The individual NPS and vendors did not remain the same over the 12 months. However, the availability of NPS and vendors selling NPS grew. NPS consistently available on crypto markets could indicate popular substances.

Keywords: new psychoactive substances; legal highs; darknet; hidden web; crypto market

1. Introduction

New psychoactive substances (NPS) are an emerging phenomenon, and their representation on the hidden web (also known as the darknet) is under-researched. The Internet has played a major role in the distribution of NPS, and drug marketplaces (also known as crypto markets) on the hidden web are increasingly used for the anonymous sale of drugs, including NPS [1–3].

The European Union (EU) Early Warning System currently monitors over 650 substances [4]. However, not all substances are a cause of concern, and NPS rise and fall in popularity. Only a few have had sizable prevalence or media attention [5], and research has shown that the legal status of a substance is one of the factors determining why some NPS become popular and others do not [6]. Various legislations have been created to tackle NPS diffusion across Europe and the world. For example, the UK introduced the Psychoactive Substances Act in May 2016, which prohibited the import, export, and supply of psychoactive substances (with some exemptions) but permitted possession [7]. The Act achieved a reduction in UK-based online stores and offline retail stores [8]. As more countries seek to restrict access to NPS, the legal markets could be displaced by alternative routes such as street-level drug dealing or crypto markets [3]. However, movement to the illicit market

is dependent on its popularity, and therefore only those NPS with sufficient demand will transition into it [9].

On the hidden web, drugs can be sold and purchased anonymously through crypto markets that use an "eBay"-style system where buyers can publicly review the sellers and therefore build their reputation, attracting future transactions [10–14]. Conversely, negative reviews can discourage buyers and shun unworthy vendors from the crypto markets [11,15]. It is argued that this feedback mechanism and creation of a reputation are vital to the continuing function of the market and also represent an explanation for a vendor's lifespan [15,16]. To date, the majority of crypto market research has been conducted on the Silk Road [13,17–21], which was closed by law enforcement in 2013 [22]. Christin [20] found that most sellers disappeared before three months, and most items sold for less than three weeks. Christin [20] proposed the theory that short vendor lifespans could be due to lack of stock or to vendors using the "stealth mode". The stealth mode consists in vendors removing their listings or only selling their listings to a specific customer base [13]. Potentially, the turnover of vendors is a product of the instability of the crypto markets; individual crypto markets are said to be unpredictable and have a frequent turnover, causing instability in the community [23]. Law enforcements have succeeded in closing crypto markets in the past [14,22,24]. However, all successful operations have not stopped the online trade of drugs [25], and the number of vendors has increased [3]. Previous research on crypto markets have shown a small yet definite presence of NPS. Barratt, Ferris, and Winstock [18] examined the use of Silk Road in a sample from the UK, US, and Australia and found that out of the top 20 substances that were purchased, four of these were NPS. In addition, Van Buskirk et al. [3] explored crypto markets in 2015 and concluded that around one-fifth of vendors offered NPS.

Crypto markets are increasingly being used for the sale and purchase of drugs, regardless of the instability both the markets and the vendors show. To date, research has not focussed specifically on the lifespan of vendors selling NPS, substances whose popularity and availability are unpredictable. This paper, therefore, aims to explore the lifespan of individual NPS sold on the crypto markets, explore the lifespan of individual vendors selling NPS on the crypto markets, and consider whether crypto markets are a reliable platform for the sale of NPS.

2. Methods
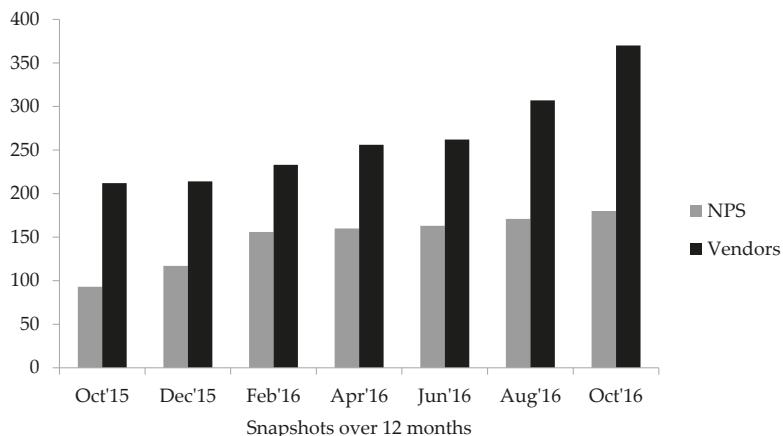
The study was part of the CASSANDRA (**C**omputer **A**ssisted **S**olutions for **S**tudying the **A**vailability a**N**d **D**ist**R**ibution of novel psycho**A**ctive substances) project [26]. The crypto markets on the hidden web were accessed through Tor (torproject.org, 501(c)(3), The Tor Project, Inc., Cambridge, MA, USA), and the data were collected bimonthly over 12 months in October, December (2015), February, April, June, August, and October (2016). The data were collected over two days in each of the seven snapshots. The crypto markets that were included sold NPS, were conducted in English, and had an open registration at the time of collection. The crypto markets present over the 12 months of data collection fluctuated because of crypto markets opening, adding NPS to their sales, or closing following exit scams or law enforcement. A table of the crypto markets included in this study can be found elsewhere [27].

Data were collected on each crypto market for NPS being sold that was visible to the researchers. The data collected were: name of the NPS (not including conventional illicit drugs, steroids, or prescription drugs), name of the vendor selling NPS, and name of the crypto market used by the vendor. Some NPS were sold under various aliases; these were categorised by their most common name. Furthermore, branded NPS that were commonly found on the visible Internet were categorised according to their contents. All analyses were performed using Microsoft Excel 2016 (Microsoft Office 2016, Microsoft, Redmond, WA, United States), unless otherwise specified.

All users on crypto markets were anonymous. The research was purely observational and did not involve interaction with either buyers or sellers. The study was approved by King's College London PNM Research Ethics reference number: LRS-15/16-3084 as part of the CASSANDRA project.
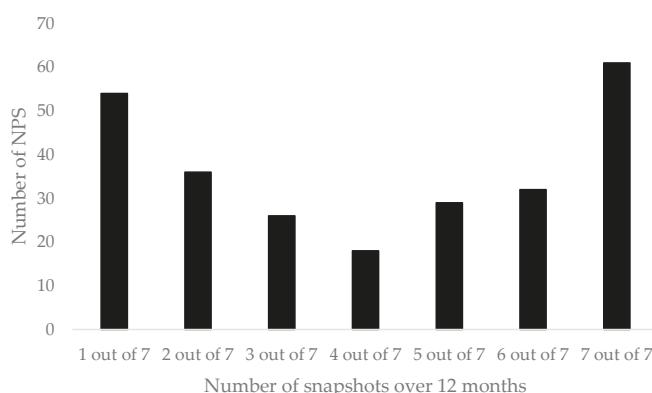
3. Results

The total number of individual NPS and vendors increased across the seven snapshots in the data collection period (increase of 93.6% and 71.6%, respectively) (Figure 1). Over 12 months, a total of 808 individual vendors were found selling 256 individual NPS.

Figure 1. Number of NPS and vendors in each month of data collection (seven snapshots in 12 months). NPS: New Psychoactive Substances.

A total corresponding to 21% ($n = 54$) of the NPS reported over the 12-month period only appeared in one snapshot (Figure 2). Fourteen percent ($n = 36$) of the total number of NPS appeared in two of the seven snapshots. Ten percent ($n = 26$) appeared in three of the seven snapshots, 7% ($n = 18$) appeared in four of the seven snapshots, 11% ($n = 29$) appeared in five of the seven snapshots, and 13% ($n = 32$) appeared in six of the seven snapshots. In addition, 24% ($n = 61$) of the total number of NPS appeared in each of the seven snapshots over the 12-month period (Table 1).

Figure 2. The number of NPS available across the seven snapshots (12 months) of data collection. NPS: New Psychoactive Substances.
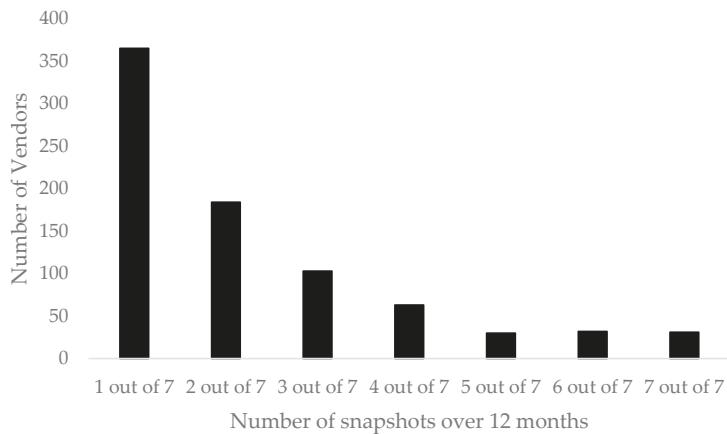
Table 1. New Psychoactive Substances that were available in every snapshot over 12 months of data collection ($n = 61$).

Benzodiazepine Analogue	Cathinone	Dissociative	Opioids	Phenethylamine	Synthetic Cannabinoid	Tryptamine	Other
Etizolam (Etilaam, Etizola, Eticalm)	3-CMC (3-chloromethcathinone)	3-MeO-PCP (3-methoxyphencyclidine)	Fu-F (furanylfentanyl)	25B-NBOMe (N-bombs, cimbi-36)	AB-CHMINACA (AB-C)	4-AcO-DMT (o-acetylpsilocin, psilacetin)	1P-LSD (NP-LAD, 1-Propionyl-d-lysergic acid diethylamide)
	3-MMC (3-methylmethcathinone, 3-mephedrone)	DXE (deschloroketamine, DCK, 2′oxo-PCM)	Kratom (ketum, kratum)	25C-NBOMe (N-bombs, cimbi-82)	MAM-2201 (4′-methyl-AM-2201, 5′-fluoro-JWH-122)	4-HO-MET (metocin, methylcybin)	AL-LAD (6-allyl-6-nor-LSD)
	4-CMC (4-chloromethcathinone, clephedrone)		W-18 (4-chloro-N-[1-[2-(4-nitrophenyl)ethyl]-2piperidinylidene] benzenesulfonamide)	25D-NBOMe (N-bombs)	NM-2201 (CBL-2201)	4-HO-MiPT (miprocin)	LSA (ergine, d-lyseramide, LAA, LA-111)
	4-EMC (4-ethylmethcathinone)			25i-NBOMe (N-bombs, cimbi-5)	"Spice"	5-MeO-DALT (N,N-Diallyl-5-methoxytryptamine, Foxtrot)	MPA (methylthienylpropamine, methiopropamine, methedrene, syndrax)
	4-FMC (4-fluoromethcathinone, flephedrone)			2CB (Tripstacy)		5-MeO-MiPT (5-Methoxy-N-methyl-N-isopropyltryptamine, Moxy)	MXP (methoxphenidine, 2-MeO-diphenidine)
	4F-PV8 (4-Fluoro-α-PHPP, para-fluoro-PV8)			2CE (Tripstacy)			Salvia
	4-MEC (4-methylethcathinone)			2CI (Tripstacy)			
	4-MMC (mephedrone, Mcat, meowmeow, bubble, drone)			2CP (Tripstacy)			
	5-ME (5-methyl-ethylone)			2FA (2-fluoroamphetamine, PAL-353)			
	a-PHP (alpha-PHP, PV7, a-pyrrolidinohexiophenone)			2FMA (2-fluoromethamphetamine)			
	a-PVP (a-pyrrolidinopentiophenone, flakka, k-prolintane, O-2387)			3-FPM (3-fluorophenmetrazine, PAL-593)			
	Dibutylone (bk-DMBDB, booty)			4FA (4-FMP, PAL-303, Flux, PFA)			
	Dimethocaine (DMC, larocaine)			5-EAPB (1-(benzofuran-5-yl)-N-ethylpropan-2-amine)			

Table 1. *Cont.*

Benzodiazepine Analogue	Cathinone	Dissociative	Opioids	Phenethylamine	Synthetic Cannabinoid	Tryptamine	Other
	Ethylone			5-MAPB (1-(benzofuran-5-yl)-N-methylpropan-2-amine)			
	MDPV (NRG-1, methylenedioxypyrovalerone)			6-APB (6-(2-aminopropyl)benzofuran)			
	Methylone (M1, MDMC, bk-MDMA)			DOC (4-Chloro-2,5-dimethoxyamphetamine)			
	PV9 (a-POP, alpha-POP)			DOM (2,5-Dimethoxy-4-methylamphetamine)			
	TH-PVP (3',4'-tetramethylene-α-Pyrrolidinovalerophenone)			ephedrine			
				Ethylphenidate (EPH)			
				MAL (methallylescaline)			
				MDA (3,4-Methylenedioxyamphetamine)			

Almost half of all vendors (45%, *n* = 365) appeared only once over the 12-month period (Figure 3). Twenty-three percent (*n* = 184) of the total number of vendors appeared in two snapshots over the 12-month period, 13% (*n* = 103) appeared in three snapshots, 8% (*n* = 63) appeared in four snapshots, 4% (*n* = 30) appeared in five snapshots, 4% (*n* = 32) appeared in six snapshots, and only 4% (*n* = 31) of the total number of vendors appeared in all seven snapshots.

Figure 3. The number of vendors available across the seven snapshots (12 months) of data collection.

4. Discussion

The total number of individual NPS and vendors increased over the 12-month duration of the study. Almost half of the total number of vendors had a lifespan of only a few months, and the NPS advertised fluctuated over the study. One-fifth of NPS were available for the entirety of the study, whereas just under one-quarter were only available for a few months before disappearing. From the results of this study, the contents of the NPS market on the crypto markets varied, but a market was consistently available.

Research has shown that the use of crypto markets and the availability of substances including NPS have increased in recent years [3,20,24]. The results from this study complement these findings as the availability of NPS increased over the data collection period. The increased availability of NPS on the crypto markets suggests that NPS are being purchased via this method and that crypto markets are seen as a viable platform for the sale of NPS. This is necessary to observe because of the changing legislations surrounding these substances in countries around the globe, such as the ban in the UK that was implemented during this study [7]. Legal markets will look to migrate to other platforms.

This study found that 256 different NPS were available for purchase over the 12 months of the study, and nearly one-quarter of NPS remained available on the crypto markets, suggesting that these substances could be the most popular NPS. The majority of the NPS that were available in every snapshot were cathinones and phenethylamines (categorised according to [5]). However, there was frequent turnover throughout the study, whereby some NPS were available for purchase for a few months and then disappeared from availability. This pattern may have mirrored changes in popularity within the overall NPS market, fluctuating because of legality, ease of access, or similarities to traditional drugs [4,9]. It is possible that certain NPS were removed from the market because limited stocks were available to the vendors or because the demand of a specific NPS did not match up to its availability. Another possibility is that the feedback from the customers about the NPS was negative, and the stock was removed [15].

Almost half of the vendors were only captured in one snapshot over the course of the data collection, and the number of vendors who appeared in the data reduced as the snapshots increased (Figure 3). These findings show that the lifespan of the vendors was relatively short, and just under half of the vendors were available for under four months, mirroring what was found in the literature [20,28]. There are potential reasons for a short lifespan; research has suggested that vendors could only be selling their limited stock, or that vendors move into stealth mode after they have gathered a sufficient client base [13,20]. Furthermore, the crypto market system is built on trust and reputation; if vendors do not build an adequate reputation, they may remove themselves from the market either indefinitely or only apparently by setting up new accounts [15,28]. Only 4% of vendors remained available in every snapshot over the 12 months of this study. Perhaps, these consistent vendors were highly reputable vendors or vendors with a loyal customer base [28].

This study has several limitations. As this study was exploratory and lacked contact with the vendors, we were unable to verify whether the substances advertised were the same substances being purchased and received by the customers. Because of anonymity, another limitation is that studies like the present one are unable to capture how many vendor profiles are controlled by one person or if the number of vendors that are only available for a few months before disappearing are, in fact, opening and closing various accounts [29].

5. Conclusions

Online sales hold a predominant portion of the NPS market, and crypto markets are an emerging source of sale. NPS sold on crypto markets should be monitored to track the popularity and availability of specific substances. Our study found that vendors selling NPS had short lifespans, with half of the vendors only appearing once in the data collection. Individual NPS had longer lifespans, however not all NPS were available over the study period; only a quarter of the total NPS were available in all snapshots over the year. Regardless of the fluctuation of the vendors in the NPS market on the crypto markets, the number of NPS and vendors selling NPS increased over the year. The NPS that consistently appeared across the 12 months of data collection may suggest what substances are popular with the consumers. Crypto markets are therefore to be monitored for the sale of NPS, especially in light of the tightening regulations being applied in various countries.

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Review

Hallucinogen Persisting Perception Disorder: Etiology, Clinical Features, and Therapeutic Perspectives

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Abstract: Hallucinogen Persisting Perception Disorder (HPPD) is a rare, and therefore, poorly understood condition linked to hallucinogenic drugs consumption. The prevalence of this disorder is low; the condition is more often diagnosed in individuals with a history of previous psychological issues or substance misuse, but it can arise in anyone, even after a single exposure to triggering drugs. The aims of the present study are to review all the original studies about HPPD in order to evaluate the following: (1) the possible suggested etiologies; (2) the possible hallucinogens involved in HPPD induction; (3) the clinical features of both HPPD I and II; (4) the possible psychiatric comorbidities; and (5) the available and potential therapeutic strategies. We searched PubMed to identify original studies about psychedelics and Hallucinogen Persisting Perception Disorder (HPPD). Our research yielded a total of 45 papers, which have been analyzed and tabled to provide readers with the most updated and comprehensive literature review about the clinical features and treatment options for HPPD.

Keywords: Hallucinogen Persisting Perception Disorder; flashbacks; hallucinogenic substances; LSD; psychedelics; visual disturbances; perceptual disturbances

1. Introduction

Hallucinogens represent an enormous group of natural and synthetic agents [1,2]. The core features of hallucinogens include their being empathogenic and being able to induce alterations of consciousness, cognition, emotions, and perception. Their main characteristic is to profoundly affect a person's inner processes and the perception of the surrounding world. The perceptual distortions are mainly visual, as in the vast majority of induced psychoses [3–5]. The hallucinogenic properties of many natural products were known for thousands of years: popular healers, "brujos", and shamans used these substances in ancient times for medical, religious, spiritual, ritual, divination, and magical purposes. Nevertheless, the attention of western culture was drawn to psychedelics only at the beginning of the 20th century, but the turning point is considered to be 1938, the year in which the lysergic acid diethylamide, better known as LSD, was synthesized by Albert Hofmann. In the 1950s and 1960s, LSD was considered to have a therapeutic potential in the psychiatric field, allowing

patients to access unconscious material in therapeutic settings. This has been recently re-evaluated with uncertain results. After a mass diffusion of hallucinogens in the 1960s and 1970s, current prevalence data [6] from the United States highlight that more than 180,000 Americans report a recent use of LSD, and 32,000 a recent use of phencyclidine. Nowadays, the intake of hallucinogens is associated with shamanic ceremonies, workshops of underground therapy and self-experiences. In these frameworks, hallucinogenic substances are most commonly used alone, while in rave parties and social events they are often part of a heavy polyvalent use that frequently includes Novel Psychoactive Substances. These compounds, easily available on the Internet without any cultural barrier and sometimes without any advice from the group of peers, have profoundly changed the drug scenario [7–10]. Their use is becoming widespread, also due to their low cost and appealing market strategies [11,12]. However, significant medical and psychiatric problems have been reported for subjects using these drugs [13], regardless of previous psychiatric antecedents [14].

This paper will focus on a rare, and therefore, poorly understood aspect of hallucinogen consumption: the total or partial recurrence of perceptual disturbances that appeared during previous hallucinogenic "trips" or intoxications and re-emerged without recent use [4,5]. These returning syndromes are defined "benign flashbacks", or pervasive Hallucinogen Persisting Perception Disorder (HPPD). LSD is the model and prototype of the classical synthetic hallucinogen, and it is certainly the most explored and investigated substance associated with the etiology of this unique and captivating state [15]. HPPDs do not have a notable prevalence [16], and, therefore, they are frequently unrecognized [17,18].
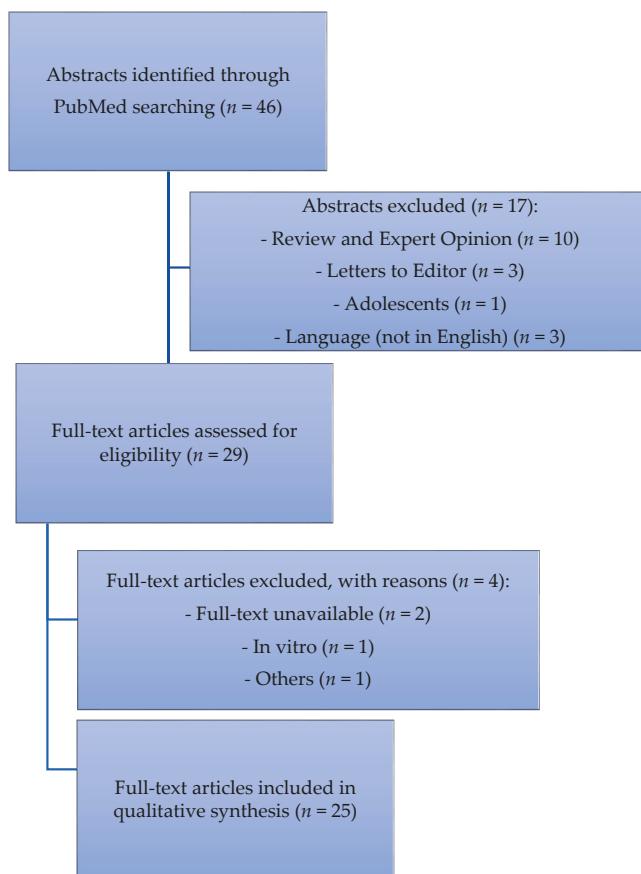
Classifications used to delineate and outline persisting perceptual disorders are now clearer than in the past [18]. Two major subtypes of hallucinogenic substance-use related recurring perceptual disturbances have been identified and reported [18]: (1) HPPD I, also described and named as benign Flashback and Flashback Type; and (2) HPPD II, also named HPPD Type II [17,18]. HPPD I has a short-term, reversible and benign course. Although visual images may provoke unpleasant feelings, re-experiencing the first hallucinogen intoxication may not lead to significant concern, distress, and impairment in individual, familial, social, occupational, or other important areas of functioning [17,18]. The impairment is mild and the prognosis is usually good. Some of the patients do not report being annoyed by these phenomena: they may indeed consider them as "free trips" resembling psychedelic experiences without consuming a psychoactive substance. Contrarily and conversely, HPPD II has a long-term, irreversible or slowly reversible and pervasive course [17,19]. The impairment of HPPD II is severe and the prognosis is worse. Some of the patients fail to adapt and live with these long-lasting recurrent "trips", and a consistent fraction needs to be constantly treated [19,20]. It has to be considered that the distinction between HPPD type I and HPPD type II has not yet been made in the Diagnostic and Statistical Manual of Mental Disorder, fifth edition (DSM-5) and it is still debated. HPPD type I is consistent with the diagnostic definition expressed by the International Classification of Disease, 10th (ICD-10), while HPPD type II better matches the DMS-5 criteria.

A vast list of psychoactive substances has been identified and linked with the development of this condition, including Magic Mushrooms (psilocybin) [21] and muscimol (*Amanita muscaria* (L.) Lam.) [22]; San Pedro cactus and Peyote (mescaline) [16,23]; ketamine [24]; dextromethorphan [25]; MDMA and MDA [26]; and cannabis and synthetic cannabinoids [27–33]. This condition has also been associated with the consumption of Ayahuasca, *Datura stramonium* L., *Salvia divinorum* Epling & Játiva, and *Tabernanthe iboga* (L.) Nutt., which contains ibogaine [17,18]. It is, therefore, clear that HPPD is not strictly associated with psychedelic consumption, but a number of hallucinogen-inducing substances may be correlated with its arising.

The aim of the present study is to review all the original studies about HPPD in order to evaluate (1) the possible suggested etiologies; (2) the possible hallucinogens involved in HPPD induction; (3) the clinical features of both HPPD I and II; (4) the possible psychiatric comorbidities; and (5) the available and potential therapeutic strategies.

2. Materials and Methods

We searched PubMed to identify original studies about psychedelics and Hallucinogen Persisting Perception Disorder (HPPD). The following search terms were used: "Hallucinogen Persisting Perception Disorder" OR "Hallucinogen Persisting Perceptual Disorder". The search was conducted on 15 September 2017 and yielded 46 records. We included all original articles (open-label or double-blind trials, prospective or retrospective observational studies, and case reports) written in English. We included all studies describing perceptual distortions in patients with a previous history of substance consumption. Reviews, commentaries, letters to the editor, and studies enrolling adolescents were excluded. All the authors agreed on the inclusion and exclusion criteria. We excluded 17 records by reading the titles and abstracts. By reading the full texts of the 29 remaining articles, we found 25 papers meeting our inclusion/exclusion criteria, and we, therefore, included them in the qualitative synthesis (Figure 1).

Figure 1. Flow-chart describing the data collection process.

3. Results

3.1. Suggested Etiologies

HPPDs are poorly understood due to the enormous range and variability of recurrent sensory disturbances, and the multiple distinct subtypes [17,18].

The main neurobiological hypothesis is that LSD consumers might develop chronic disinhibition of visual processors and dysfunction in the function of the central nervous system (CNS) [4,34–36]. This disinhibition may be linked to an LSD-generated intense current [37] that may determine the destruction or dysfunction [18] of cortical serotonergic inhibitory interneurons with *gamma*-Aminobutyric acid (GABAergic) outputs, implicated in sensory filtering mechanisms of unnecessary stimuli [34–36,38]. The efficacy of some treatment options in a subject with HPPD, such as pre-synaptic α_2 adrenergic agonists, selective serotonin reuptake inhibitor (SSRIs), benzodiazepines, and mood stabilizers would confirm this neurobiological hypothesis (see Section 3.2). Reverse tolerance or sensitization that emerges after LSD exposure may explain recurrent occurrences after the stimulus has been withdrawn [39]. Nonetheless, HPPD-like experiences, such as flashbacks, moments of derealization, and hyper-intense perceptions are reported in healthy populations and non-LSD exposed subjects [40]. Moving from biochemical receptor interactions towards macroscopic areas, a temporary or permanent impairment in the Lateral Geniculate Nucleus (LGN) has been hypothesized [4,41–43]. The LGN, which is located in the thalamus, is associated with visual perception pathways [41–43]. Recent research highlighted a brain dysfunction in patients with visual snow, located mainly in the right lingual gyrus [44], perhaps implying LSD involvement. Halpern et al. [40] suggested that HPPD can be due to a subtle over-activation of predominantly neural visual pathways that worsens anxiety in predisposed subjects after ingestion of arousal-altering drugs, including non-hallucinogenic substances. According to Holland and Passie, environmental triggering by specific situations or stimuli or other elements related to the original experience may be involved in flashback experiences [45].

3.2. Substances That Induce HPPD

Different substances have been associated with visual and perceptual disturbances (Table 1).

Table 1. Substances that induce Hallucinogen Persisting Perception Disorder (HPPD).

Authors	Cases (*n*)	Substances Inducing Perceptual Disturbances	Trigger Cues
Zobor, 2015 [29]	1	Cannabis	
Gaillard, 2003 [46]	2	Cannabis	
Lerner, 2014 [47]	2	Cannabis (Synthetic)	
Anderson, 2017 [48]	1	Cannabis and MDMA	Stress
Brodrick, 2016 [49]	1	Cannabis and LSD	
Coppola, 2017 [50]	1	Cannabis (Synthetic, JWH-122)	Cannabis consumption
Lerner, 2003 [51]	16	LSD	
Lerner, 2002 [20]	1	LSD	
Lerner, 2000 [52]	8	LSD	
Gaillard, 2003 [46]	1	LSD	Alcohol intake
Lev-Ran, 2017 [53]	40	LSD	Sexual intercourse or Intentional
Hermle, 2012 [54]	1	LSD	Stress
Lerner, 2014 [19]	2	LSD	
Abraham, 2001 [35]	38	LSD	Dark environment
Litjens, 2014 [26]	31	LSD	
Lerner, 2015 [55]	1	LSD	
Baggott, 2011 [56]	104	LSD	
Lev-Ran, 2015 [57]	37	LSD	
Lev-Ran, 2014 [58]	12	LSD	Situation and mental states
Lerner, 1997 [59]	2	LSD	
Abraham, 1996 [34]	3	LSD	
Espiard, 2005 [21]	1	PCP	Cannabis consumption
Lauterbach, 2000 [60]	1	Risperidone	

MDMA: 3,4-methylenedioxy-*N*-methylamphetamine; LSD: lysergic acid diethylamide; JWH-122: 4-methyl-1-(naphthalenyl)(1-pentyl-1H-indol-3-yl)-methanone.

According to the literature, we found that the majority of HPPD cases have been induced by LSD or phencyclidine (PCP) (14 studies, 294 patients) [17,19,21,26,35,46,51–53,55–59].

The use of cannabis has been associated with the development of perceptual distortions in seven patients [29,46,48,49,61]. In one case, it was associated with 3,4-Methylenedioxymethamphetamine (MDMA) and in another case with PCP [48,49]. In two patients, visual distortion followed the consumption of synthetic cannabinoids [61].

Lauterbach et al. reported the unique case of HPPD induced by the atypical antipsychotic Risperidone [60].

3.3. Clinical Features

According to DSM-5, Hallucinogen Persisting Perception Disorder is the recurrence of perceptive disturbances that firstly develop during intoxication. The contents of the perception and visual imagery range extensively [17,19]. DSM-5 and previous DSM editions report a list of the most common symptoms experienced by HPPD patients, but only a few symptoms have been described in the professional literature. The main group of symptoms reported by Criterion A of the DSM-5 are visual disturbances. In fact, as in the vast majority of induced psychoses, visual hallucinations are notably more common than auditory [3]. Regardless, every perceptual symptom that was experienced during intoxication may re-occur following hallucinogen withdrawal. We report a list of the main literature-reported visual disturbances in Table 2.

A latent period may antecede the onset of returning visual occurrences. This latent period may last from minutes, hours, or days up to years, and re-emerge as either HPPD I or II with or without any recognized or perceived precipitator [17,19]. Episodes of HPPD I and II may appear spontaneously or they may be triggered by identified and non-identified precipitators [18]. Episodes may be continuous, intermittent, or sudden. With regards to this point, neither HPPD I nor HPPD II can really be considered as persisting in a narrow sense of the word. Additionally, their differential diagnosis can only be proposed in terms of prognosis rather than clinical presentation.

However, HPPD I usually onsets with warning "auras", minor feelings of self-detachment, mild bewilderment, and mild depersonalization and derealization [17,18]. Conversely, the onset of HPPD II might be unexpected and abruptly detonate with bursting "auras", deep feelings of self-detachment, acute depersonalization-derealization [19].

The frequency of recurrence of perceptual distortions is lower for HPPD I than HPPD II [18]. Prior substance users can voluntarily elicit or produce visual disturbances with or without known triggers [4,17,18]. After HPPD II onset, hallucinogenic events tend to occur more frequently, and their duration and intensity increase. Subjects might perceive a partial or total loss of control.

Table 2. A representative, but not exhaustive, list of reported visual disturbances.

Symptom	Description
Symptom Reported by Diagnostic and Statistical Manual of Mental Disorder, fifth edition (DSM-5)	
Visual hallucinations	Perceptions in the absence of the objects. False perceived objects are often geometric figures.
Altered motion perception	False perceptions of movement in the peripheral visual fields
Flashes of color	
Color enhancement	Perception of intensified colors
Trails or tracers	Lines, stripes or bands that could be observed after animate and inanimate objects have already moved from their previous location. According to DSM-5, images left suspended in the path of a moving object as seen in stroboscopic photography
Palinopsia	Positive afterimages that continue to appear in one's vision after the exposure to the original image has ceased.
Halos	Colored light around a light source or an object

Table 2. *Cont.*

Symptom	Description
Micropsia	Misperception of images as too small
Macropsia	Misperception of images as too large
Common Symptoms Not Reported by DSM-5	
Floaters	Spots that seem to drift in front of the eye
Visualizations	Dots, points, particles, mottles or specks emerging in an obscure room
Fractals	Self-similarity perception or small parts that are seen having an equal and identical shape or form as the whole
Repetitions	Recurrence of inanimate or moving patterns or motives
Keenness	Undimmed color contrasts
Pareidolia	An image within an image like the imagery of objects or faces in a foggy arrangement
Superimpositions	Superimposed or overlapped geometric patterns
Distorted Perception of Distance	Objects were seen slightly closer or distant
Monochromatic Vision	The visual perception of distinct colors as one unique color with different tinges and tonalities
Intense fragmentation	The sense of disintegration of still or moving objects
Recurrent Synesthesia	Stimulation of one sensory pathway leads to automatic, involuntary reactions or experiences in a second sensory pathway
Geometric Phosphenes	Seeing light without light penetrating the eye.
Imagistic Phosphenes	Casual and unplanned formed images like non-humans (zoopsia) and human faces without geometric patterns or figures provoked by closing an eye and pressing it with a finger
Acquired Dyslexia	Difficulty with reading notwithstanding normal intelligence
Aeropsia or Visual Snow	Virtually seeing particles of air

3.4. Mental Illnesses Comorbid with HPPD

Recent observations reported a co-occurrence with depressive [20] and anxiety traits [51] and severe mental illnesses such as Major Depressive Disorder [23], Bipolar Disorder [23,62], and Schizophrenia Spectrum Disorders [17,58]. However, HPPD I and HPPD II onsets are not necessarily accompanied by any prominent additional psychiatric disorder, thus representing an independent condition [17,18]. In particular, the onset of HPPD II is often linked to a clear negative mood and affect. Anxiety and depressive features might aggravate new episodes. Anxiety might also evolve into a panic attack. Anticipatory anxiety may antecede future visual aberration events, and avoidant behavior may limit and restrict regular normal functioning [17,18]. Recently, in a study carried out by Halpern, a comprehensive survey of 20 subjects reporting Type-2 HPPD-like symptoms was presented and evaluated. The dissociative symptoms were consistently associated with HPPD, suggesting that HPPD is in most cases due to a subtle over-activation of predominantly neural visual pathways that worsens anxiety in predisposed subjects after the ingestion of arousal-altering drugs, including non-hallucinogenic substances. The authors report that many perceptual symptoms reported were not first experienced while intoxicated, and are partially associated with pre-existing psychiatric comorbidity, tempering the direct role of hallucinogens in the etiopathology of the disorder [40].

Only two observational studies and one case report evaluated psychotic patients with comorbid HPPD [57,58,60] (Table 3). Two observational, cross-sectional studies compared schizophrenic patients with prior use of LSD who developed HPPD (SCZ+HPPD, 49 patients) with those who did not (SCZ, 57 patients), for a total of 106 patients [57,58]. No differences between the two groups have been found with respect to demographic characteristics, age of psychotic onset, age of drug use onset, and type of substances abused [57,58]. As expected, SCZ+HPPD patients reported more distressing and horrific LSD experience ("bad trips") ($p < 0.05$) [57]. Interestingly, the positive subscale of the Positive and Negative Syndrome Scale (PANSS) did not differ between the two groups. On the

contrary, SCZ+HPPD patients showed lower scores in the PANSS negative subscale, the PANSS General Psychopathology Subscale, and the PANSS total scores ($p < 0.05$) [57]. Moreover, 67% of the schizophrenic patients comorbid with HPPD were able to distinguish between perceptual distortion and psychotic hallucinations [58], and 9 out of 12 patients could identify precursory cues for perceptual distortion (substance-induced cues, situational cues, and mental cues) [58]. Lauterbach et al. [60] reported a case of HPPD comorbid with psychosis, in which visual distortions were induced by antipsychotic treatment. Interestingly, the patient did not report any history of previous substance abuse [60]. The patient was treated with Risperidone, Clonazepam and Trazodone, and she reported visual disturbances resembling HPPD, in particular, illusions, after three subsequent Risperidone dosage increases [60].

Table 3. Observational studies and case reports comparing schizophrenic patients with HPPD (SCZ+HPPD) and schizophrenic patients without HPPD (SCZ) (* $p > 0.05$, ** $p < 0.05$).

Authors	Study	Number of Patients	Substances	Symptoms Description	Onset Perceptual Disorders	Recurrence of Perceptual Disorders	Treatment
Lev-Ran, 2015 [57]	Observational, cross-sectional, control study	80 hospitalized SCZ patient with past use of LSD 43 SCZ (DSM-IV-TR) 37 SCZ+HPPD (DSM-IV-TR) Onset of illness: 22.9 SCZ, 23.4 SCZ+HPPD *	Cannabis: 100% SCZ, 92% SCZ+HPPD * MDMA: 60% SCZ, 46% SCZ+HPPD * Opioids: 26% SCZ, 30% SCZ+HPPD 30% * Cocaine: 16% SCZ, 14% SCZ+HPPD * LSD initiation use: SCZ 17.9y, SCZ+HPPD 19.3y *	Adverse LSD experience (bad trip): 28% SCZ, 89% SCZ+HPPD ** PANSS: Positive symptoms: SCZ = SCZ+HPPD ** Negative symptoms: SCZ > SCZ+HPPD ** General psychopathology: SCZ > SCZ+HPPD ** Total score: SCZ > SCZ+HPPD **		Treatment ineffective in SCZ+HPPD	Antipsychotic medication
Lev-Ran, 2014 [58]	Observational	26 patients 14 SCZ (DSM-IV-TR)12 SCZ+HPPD (DSM-IV-TR) Demographic characteristic did not differ between the two groups	Past use of :LSD (100%) cannabis (100%) MDMA (7%) No differences between the two groups in age at onset of drug use and in number of incidences of hallucinogen use	67% SCZ+HPPD could distinguish HPPD symptoms from hallucination related to a psychotic state	9 SCZ+HPPD patients recognized precursory cues for perceptual distortion (7 substance-induced, 5 situational, and 2 mental cues)	12 patients experienced perceptual distortion (SCZ+HPPD)	Antipsychotic treatment. No significant differences in response to APS and adverse effects between the two groups
Lauterbach, 2000 [60]	Case report	1 psychotic patient	No reported substance abuse and hallucinogen exposure Risperidone Clonazepam Trazodone	HPPD-like symptoms: palinopsia, illusions, and visual disturbances	After risperidone treatment	Weekly recurrence. Remission in 48 h each time	

3.5. First-Line Medications

Pre-synaptic α$_2$ adrenergic agonists are a treatment with a low side-effect profile for patients with a previous history of substance-related disorders. Symptoms alleviation has been reported in some patients treated with these drugs [17,18,52,63]. The effectiveness may be based on the evidence that clonidine may elevate plasma GABA levels in humans, having a benzodiazepine-like effect. Clonidine may also decrease locus coeruleus activity, leading to a reduction of adrenergic activity [64], which can be effective in the management of PTSD [65]. Therefore, as in PTSD-related recurring flashbacks, some visual disturbances could be associated with excessive sympathetic nervous activity. Thus, these visual distortions could be ameliorated by Clonidine [52,63].

A dosage of 0.75 mg/die of Clonidine has been evaluated as a treatment option for nine HPPD patients [51,59] (Table 4). The total remission has been reported in a single patient with flashbacks and anxiety treated with 0.25 mg of Clonidine three times a day for two months [59]. In the 2 months open study on eight HPPD patients, the Clinical Global Impression (CGI) and Patient's Severity Perception significantly decreased between entry and endpoint scores [51], although two patients dropped out at week 3 and week 5, respectively [52]. Lofexidine (0.2–0.8 mg/day) is a sympatholytic centrally acting α$_2$ presynaptic adrenergic agonist that showed similar efficacy in some cases [23,65,66].

Benzodiazepines may be useful and effective in eliminating benign HPPD I and ameliorating, but not completely eradicating, pervasive HPPD II symptoms [18,67]. The effectiveness of Benzodiazepines may be related to their activity on the cortical serotonergic-inhibitory inter-neurons with GABAergic outputs [2,4]. Alprazolam (0.25–0.75 mg/day) has been prescribed with some success and Clonazepam (0.5–1.5 mg/day) appears to be the most reliable and effective benzodiazepine even at low doses [17,18,51,67]. Higher doses (4 mg/day) have also been used with good outcomes [68]. Clonazepam may act on serotonergic systems, improving, enhancing, and augmenting transmission [17,18,51,67], thus promoting alleviation and a marked improvement [51,67]. Clonazepam has been evaluated in three case reports and one open-label trial by Lerner [19,50,51]. In the clinical trial, 16 HPPD patients were treated with a Clonazepam dosage of 2 mg/day [51]. Their symptoms improved significantly after treatment initiation and the improvement persisted during a 6-month follow-up after treatment discontinuation [51]. The same author reported two cases of cannabis-induced visual disturbances and correlated anxiety features. In both cases, Clonazepam (2 mg/day) was effective in improving symptoms, but focal visual disturbances without anxiety (trailing phenomena in one case, and black moving spots in the second case) persisted during and after therapy [19]. More recently, Clonazepam (6 mg/day) has been proved to be effective in improving cannabis-induced HPPD symptoms [50]. On the other hand, the intrinsic abuse potential of benzodiazepines might be inconvenient in certain individuals with a past history of substance use [17,18]. Given the benign nature of HPPD I, the use of benzodiazepines should be proposed only for severe cases, in the acute phase, and for the short term.

HPPD patients appear to be sensitive to first-generation antipsychotics at low doses, requiring monitoring of extrapyramidal side effects. Haloperidol [69] and Trifluoperazine [70] were reported to be helpful. Perphenazine (4–8 mg/day) [17,23], Sulpiride (50–100 mg/day) [23], and Zuclopenthixol (2–10 mg/day) [17,23], at very low doses, are well tolerated and may be an effective treatment. Some of the long-acting first-generation antipsychotics may still be useful in co-occurring Psychotic Spectrum Disorders and HPPD II [58]. In one study, haloperidol was noted to reduce hallucinations, but an exacerbation of flashbacks in the early phases of treatment was highlighted as well [1,69].

The use of second-generation antipsychotics in HPPD patients without comorbid psychotic disorders is debated. Anderson recently reported the case of a young woman presenting prolonged and distressing multimodal pseudo-hallucinations, depressive symptoms, and anxiety, who was treated with Risperidone for three months without any significant improvement [48]. At the same time, conflicting evidence exists on the antipsychotics effects in psychotic HPPD patients. One study did not report differences in antipsychotic treatment response between SCZ and SCZ+HPPD patients [58]. On the other hand, a more recent study has shown the ineffectiveness of antipsychotic medications in an SCZ+HPPD population [57].

Table 4. Observational studies and case reports evaluating clinical presentation.

Authors	Study	Number of Patients	Substances	Symptoms Description	Onset Perceptual Disorders	Recurrence of Perceptual Disorders	Treatment
Lev-Ran, 2017 [53]	Observational cross-sectional study	40 (27 males); HPPD (DSM-IV-TR)	Previous use of LSD; Lifetime use of Cannabis	HPPD I: mean age 25.5 (3.7), times of LSD consumption: 7.1 (4.3), use of alcohol; perceptual disorders triggered by sexual intercourse, dark environment, and looking at still or moving objects; HPPD II: mean age 22.1 (2.8), times of LSD consumption 24.6 (1.4), use of SCS, stimulants and inhalants; intentionally triggering perceptual disturbances			None of the subjects included in the study received medications particularly targeted at treating HPPD
Zobor, 2015 [29]	Observational, cross-sectional, control study	Male, 23-year-old	Cannabis, previous 4-year history of heavy consumption (16–20 years)	Visual distortion: visual snow, sperm-like whizzing dot, jittering lights, floaters, photophobia, visual discomfort, positive and negative afterimages, impaired night vision, halos, starburst around lights; Ophthalmological examination: reduction of phosphene threshold, alteration in the EOG	During cannabis use period	Persistence despite cannabis withdrawal	No
		4 healthy subjects, mean age 25.5 years	Cannabis: Heavy consumption	Not reported		Not reported	No
Lerner, 2014 [19]	Case report	Male, 24-year-old	Cannabis: Three-year past history of social consumption; MDMA, LSD and cocaine (sporadically); Social Alcohol drinking	Visual disturbances (halos, color intensification, flashes of colors, distorted perception of distance)	During LSD intoxication	Recurrence one week after completely stopping all substance use: daily visual distortion; Disappearance after one year	Not accepted by the patients
		Female, 25-year-old	Cannabis: Three-year past history of social consumption; MDMA, LSD (sporadically); Social Alcohol drinking	Visual disturbances (positive afterimages, color intensification, flashes of colors, trailing phenomena)	During LSD intoxication	Recurrence four days after completely stopping all substance use: daily visual distortion; Improvement after one year; Trailing phenomena continued to appear intermittently	Not accepted by the patients
Gaillard, 2003 [46]	Case reports	Female, 18-year-old	Cannabis: Three-year past history of regular consumption	White dots when looking at a white wall or blue sky; "seeing shadows" on the left side, palinopsia, visual vibration upon awakening	During comatose episode following excessive use of cannabis	Recurrence after stopping all substance use: daily visual distortion	
		Male, 25-year-old	Cannabis: Two-year past history of regular and heavy consumption	Visual illusion and dyskinetopsia, difficult in depth perception	After two years of consumption	Symptoms persistence and increase after cannabis withdrawal + memory loss, and concentration deficits	

Table 4. *Cont.*

Authors	Study	Number of Patients	Substances	Symptoms Description	Onset Perceptual Disorders	Recurrence of Perceptual Disorders	Treatment
Abraham, 2001 [35]	Observational	38 HPPD cases	LSD: first mean use 18.1 (6.0) years; lifetime use 16 times (median)	7.11 (2.2) different types of visual hallucinations per subject	21 months after first use	Duration of visual hallucinations: 9.67 (7.68) years	
				The majority of subjects reported an intensification of visual hallucinations on emerging into a dark environment	13.5% subjects experienced symptoms within the first month of use, three subjects after a single use		
Litjens, 2014 [26]	Case series	31 HPPD cases; Web-questionnaire assessment	MDMA Cannabis	At least 2 different visual phenomena (visual snow, afterimages, flashes, illusory movement, and increased observation of floaters) with a minimum of one episode of disturbed perception every week (100%).	After a single drug exposure		
			LSD	Anxiety or panic in the weeks before or following the use of drugs (71%)			
				Depersonalization (32%)			
			80% serotonergic drugs	Derealization (39%)	After a period of extensive drug use		
Lerner, 2015 [55]	Case report	Male, 26-year-old	Cannabis: a Five-year history of occasional consumption; Alcohol: Social Consumption;	No distressing macropsia, micropsia, pelopsia and teleopsia, looking at still or moving objects and humans;	LSD intoxication	Recurrence two days after completely stopping all substance use: daily visual distortion	Not accepted by the patients
			LSD: Recreational use	Longer and distressing visual distortion experience with anxiety		Disappearance after one year	
Baggott, 2011 [56]	Observational Web-based questionnaire	2679 subjects	Median of 5 different drugs used by a single subject	224 subjects reported having at least one diagnosis associated with unusual visual experiences;	After exposure to LSD	The probability of experiencing constant or near-constant symptoms was predicted by greater past exposure to drugs and exposure to LSD	104 individuals considered symptoms impairing enough to seek treatment
		89.5% male, aged 21.6 (3.7) years		1487 individuals reported at least one abnormal visual experience;			
				587 endorsed at least one experience on a constant or near-constant basis			

Risperidone was usually prescribed due to its proven efficiency in the treatment of perceptual disturbances in Psychotic Spectrum Disorders, mainly in Schizophrenic Disorders. LSD seems to work as a partial agonist of postsynaptic serotonin receptors. Therefore, Risperidone, which is a strong antagonist of both postsynaptic 5-HT$_2$ and D$_2$ receptors, was expected to be convenient. In contrast with this supposition, Risperidone at recommended [71] and lower doses [72] worsens visual disturbances and accompanying anxiety, or does not show any effect [54]. This was presumably due to Risperidone's α_2 presynaptic antagonism and noradrenaline release [34]. In addition, Risperidone was associated to the re-experiencing of visual disturbances in some patients suffering from schizophrenia with a past history of LSD use [73]. One psychotic patient treated with Risperidone, Clonazepam, and Trazodone reported visual disturbances resembling HPPD after three subsequent Risperidone dosage increases [60]. At the same time, Risperidone has been shown to be effective in improving PCP-induced HPPD with anxiety in one patient, while in the same patient Olanzapine produced symptoms exacerbation [21].

Evidence not included in our systematic review suggested that low dosages of atypical antipsychotics may be useful, specifically Aripiprazole (5–10 mg/day) [23], also because of its efficacy in substance and alcohol use disorders [74].

Visual oddities and disturbances with sudden paroxysmal onset have been interpreted as visual seizures and prompted the use of antiepileptic drugs in HPPD. This consideration helped to explicate the efficacy of benzodiazepines and led to the prescription of Phenytoin [75,76]. Today, Phenytoin is not used for HPPD treatment due to its troubled side effect profile. Medications such as Valproic Acid (200–600 mg/day), Carbamazepine (200–600 mg/day), Oxcarbazepine (300 mg/day), Gabapentin (300–900 mg/day), Topiramate (25–100 mg/day), and Lamotrigine (50–100) may be useful [23], also because of their efficacy in substance and alcohol use disorders [77–79]. In a single case of HPPD symptoms and electroencephalographic (EEG) abnormalities, compatible with toxic encephalopathy, the visual hallucinations that recurred at any alcohol ingestion improved, but did not disappear with the use of Valproic Acid (1500 mg/day) [46]. Levetiracetam has shown to reduce some visual symptoms as well as HPPD related-depersonalization and derealization [80]. Lamotrigine has shown to be efficacious in a recent severe case of HPPD with some EEG abnormalities (Anderson et al., 2018). These medications may also be helpful when visual disturbances are accompanied by co-occurring mood swings and mood disorders.

Antidepressant medications could help in the management of co-occurring HPPD II with anxiety and depressive disorders [17,18,20,51,67]. HPPD II alone does not appear to be an appropriate target. There are questionable and controversial results regarding Sertraline, which has been reported to worsen [81] as well as to improve visual disturbances. Amelioration following long-term administration of SSRIs was attributed to the down-regulation of 5-HT$_2$ receptors, providing more evidence to corroborate the serotonergic mechanisms underlying this condition. Other prescribed SSRIs did not show any benefits. Norepinephrine reuptake inhibitors (NRIs) such as Reboxetine have been tried with some success in LSD-induced HPPD symptoms comorbid with Major Depressive Disorder [20]. Agomelatine, given its peculiar function on neurotrophic factors [74], could have some benefits on the syndrome, although no data are available until now.

3.6. Second Line Medications

Naltrexone has been usually used, alone or with other medications, in chronic patients with continuous visual imagery that previously did not respond to other medications [17,18].

Calcium Channel Blockers and Beta Blockers may be helpful in patients with co-occurring HPPD II and anxiety disorders [18]. Propanolol at low (20–60 mg/day) and high doses (240 mg/day), as well as Atenolol 25–50 mg/day, have been used to diminish accompanying anxiety of visual imagery [18,23]. Investigations of HPPD patients with EEG mapping showed that HPPD is represented by disinhibition [35] in the cerebral cortex [34]. The rationale behind this interesting and novel

approach is that improving sensory gating by dopaminergic enhancers may cause an inhibition of catechol-*O*-methyl transferase (COMT), that may improve HPPD symptomatology.

3.7. Brain Stimulation Treatments

Currently, brain stimulation treatments have been proposed as a possible therapeutic option to enhance the recovery of refractory symptoms in several disorders [82,83]. Repetitive Transcranial Magnetic Stimulation (rTMS) is a non-invasive brain stimulation approach that acts by modulating specific brain circuits. While high-frequency (>5 Hz) stimulation determines a depolarization of nerve cells, with long-term potentiation (LTP) effects, low-frequency stimulation protocols (1 Hz) determine the long-term depression (LTD) of the targeted area, with the possibility to induce the localized inhibition of specific disordered networks. According to the cortical hyperexcitability hypothesis about its pathogenesis, several case reports propose that rTMS could be a promising therapeutic method for refractory visual hallucinations in schizophrenia [84,85].

To date, no studies have investigated the potential use of rTMS in HPPD. Interestingly, Kilpatrick and Ermentrout (2012) [86] studied the spatiotemporal dynamics of neuronal networks in HPPD, with spike frequency adaptation. This study reported that altering parameters controlling the strength of synaptic connections in the network can lead to spatially structured activity suggestive of symptoms of HPPD. Future research is necessary to test the possible effectiveness of the rTMS neuromodulatory effect on HPPD. Putative targets of stimulation could be hypothesized to be located in the visual cortical areas, as well as in the occipitotemporal sulcus [87]. Functional neuroimaging may be beneficial in localizing a specific target for stimulation and may prevent wasting time and money on targets which are not as likely to be involved in the pathogenesis.

4. Discussion

It has to be highlighted that a limitation of the study might be represented by the search method: in fact, we decided to limit the literature search to the DSM terminology in order to exclude simple "flashback phenomena" that are commonly reported in psychopathology, and that may not follow the use of hallucinogens. This could have narrowed the results, preventing the inclusion of other studies using the ICD terminology, which is less "technical" about the issue.

The main consideration that has to be done with respect to HPPD is its rare and unpredictable nature [16]: current prevalence estimates are unknown, but DSM-5 suggests 4.2% [88]. The condition is more often diagnosed in individuals with a history of previous psychological issues or substance misuse [56], but it can arise in anyone, even after a single exposure (mostly to LSD, but it has also been reported after use of other psychedelics) [89]. In many cases, HPPD may also be explained in terms of a heightened awareness of and concern about ordinary visual phenomena, which is supported by the high rates of anxiety, obsessive-compulsive disorder, hypochondria, and paranoia seen in many patients [90].

The crucial movement towards a comprehensive clinical understanding of Hallucinogen Persisting Perception Spectrum Disorders (HPPSD) [23] is the establishment of an accepted operative nomenclature. This wide spectrum of disorders encompasses different subtypes, ranging from HPPD I to HPPD II, according to our hypothetical distinction. Among the innumerable triggers able to precipitate HPPD, prospectively, the use of natural and synthetic cannabinoids appears to be the most frequent. This is consistent with the rapid and vast diffusion of these novel psychoactive compounds, nowadays easily available without specific cultural filters and references [91,92]. Distinct substances, with completely different mechanisms of action, might lead or precipitate the genesis of HPPD, therefore suggesting a multifaceted etiology. Thus, it is accordingly conceivable that different medications could be useful and helpful in the treatment of different subtypes of HPPD. Tracers and trailing phenomena appear to be the most resistant symptoms. Concomitant coexisting psychiatric disorders can represent a further clinical challenge, with the clinical construct of the lysergic psychoma as a possible heuristic model. According to this theory, the presence of induced psychopathological

phenomena (the Psychoma) may trigger a specific reaction excepted by the not-affected part of the mind, trying to counteract the psychoma, which is perceived as a "foreign body in the mind". Of course, when the psychoma is strong and repeated in its nature, the possibility to determine a full-blown psychosis may become more concrete [93,94].

Regarding treatment options, a combination of medications may be needed according to the preceding or subsequent psychopathology. Given the limited literature about HPPD, a possible hypothesis about the pharmacotherapy of choice in relation to different etiologies has not been considered. However, the presence of psychiatric and neurological comorbidities could represent a valid criterion to address the choice. Clinical experience and an extensive and comprehensive knowledge of these phenomena are vital for successful treatment outcomes.

Controlled clinical investigations are mostly needed in order to better understand the etiology, mechanisms of action, clinical issues, and pharmacological treatment options for Hallucinogen Persisting Perception Spectrum Disorders (HPPSD).

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Exploration of the Use of New Psychoactive Substances by Individuals in Treatment for Substance Misuse in the UK

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Abstract: Substance misuse services need to meet the growing demand and needs of individuals using new psychoactive substances (NPS). A review of the literature identified a paucity of research regarding NPS use by these individuals and UK guidelines outline the need for locally tailored strategies. The purpose of this qualitative study was to identify and explore key themes in relation to the use of NPS by individuals receiving community treatment for their substance use. Electronic records identified demographics and semi-structured interviews were undertaken. A thematic analysis of transcripts identified a variety of substance use histories; 50% were prescribed opiate substitutes and 25% used NPS as a primary substance. All were males, age range 26–59 years (SD = 9), who predominantly smoked cannabinoids and snorted/injected stimulant NPS. The type of NPS used was determined by affordability, availability, side-effect profile and desired effects (physical and psychological: 25% reported weight loss as motivation for their use). Poly-pharmacy, supplementation and displacement of other drugs were prevalent. In conclusion, NPS use and associated experiences vary widely among people receiving substance use treatment. Development of effective recovery pathways should be tailored to individuals, and include harm reduction strategies, psychosocial interventions, and effective signposting. Services should be vigilant for NPS use, "on top" use and diversion of prescriptions.

Keywords: new psychoactive substances; substance use; cannabinoids; stimulants; substance misuse services; substance use treatment; psychosocial interventions; harm reduction

1. Introduction

"New Psychoactive Substances" (NPS) is "a generic term for . . . substances produced to mimic the effects of traditional illicit drugs" [1]. Formerly known as "legal highs", NPS have dramatically changed the UK drug scene and introduced a new challenge for healthcare professionals (HCPs) [2]. The internet and the media may have had a significant impact on the proliferation of this market. A study by Bright et al. (2013) showed that the media played a significant role in increasing the public's awareness of new NPS, which sparked curiosity and increased use. This, in turn, created a media "moral panic", resulting in legislative reactions, which led to the emergence of more harmful substances [3]. At a global level, NPS are unregulated products with unpredictable effects and clandestine chemists continuously and rapidly produce newly modified compounds [4]. The analysis of NPS products have found controlled substances, mixtures of active substances and different constituents even from the same supplier and using the same "brand" name [1,4,5]. People who use NPS present with unpredictable

adverse effects, creating a dilemma for HCPs [6]. Previously, NPS were often labelled as "not for human consumption", so that under the Medicines Act 1968, manufacturers of NPS were not legally required to list their ingredients or determine their safety [1,4,7,8]. The UK Government subsequently introduced the Psychoactive Substances Act (UK PSA) in May 2016, to prohibit NPS sale, supply, production, possession with intention to supply or possession in a custodial institution, with an aim to reduce use [9,10].

Owing to their continuous emergence and sheer numbers (increased from 166 substances in 2009 to over 740 in 2016) [11], NPS are categorized based on their chemical class [12], legal status [13] or chemical structure [14]. However, in clinical practice, NPS are more commonly considered in the context of their pharmacological effects and the substances they have been designed to replicate. Public Health England (PHE) [15] therefore categorizes NPS as: sedating, stimulating, hallucinogenic, cannabinoids, dissociative and "other" (not otherwise specified). Consequently, these PHE categories [15] are used by substance misuse services (SMSs) to record information on the National Drug Treatment Monitoring System (NDTMS) [16], which enables the monitoring of people receiving treatment and national comparisons.

There are a variety of guidelines available to support HCPs working in mental health and SMSs, such as those produced by the Department of Health [17] and the Novel Psychoactive Treatment UK Network (NEPTUNE) [4]; however, none cover specific details for all NPS categories. Due to their "novelty", there is a lack of clinical data to support an evidence-based approach to the management of individuals who use NPS [1]. Recent studies have shown that HCP baseline knowledge of NPS is poor and that they are less confident in managing acute toxicities related to the use of NPS compared with traditional illicit drugs [18,19]. Reports by both the Care Quality Commission and the HM Inspectorate of Probation showed that SMSs did not offer NPS-specific interventions and that people who use NPS poorly engaged [20]. Similarly, in Europe, HCPs in Italy within addiction, psychiatry, pediatrics and A&E services also reported a lack of knowledge and confidence with regards to NPS. They also affirmed that no questions are asked when taking drug histories during admission [2].

To understand the motivation for using NPS by individuals registered within SMSs, a detailed electronic search of NHS evidence was undertaken using the Allied and Complementary Medicine (AMED), Excerpta Medica Database (EMBASE), Health Management Information Consortium (HMIC), British Nursing Index (BNI), Cumulative Index to Nursing and Allied Health Literature (CINAHL), Medline, PsycInfo and Health Business Elite databases. This identified that the rapid proliferation of NPS use may be changing drug taking habits (by displacing or supplementing pre-existing drug repertoires), and may be affected by availability, price, purity and legal status [1,7,21,22]. In Ireland, in depth interviews found people switching from illicit substances to "legal" NPS, due to perceptions regarding improved effects, safety profile and overall higher quality for a lower price; however, the individuals were aged 18–33 years [23] and adult SMSs frequently treat people who are older than this. Another group of Irish interviewees were found to transition from nasal administration to injecting and binged excessively for long durations on stimulant NPS, but after they became controlled substances, their use reduced following headshop closures, increased prices and concerns about contamination [24]. Qualitative case reports of people using stimulant NPS in the UK, prior to the introduction of the UK PSA 2016, suggested their euphoric effects may lead to "more persistent patterns of drug use" due to their perceived legal status and poor quality of their illicit equivalents [21]. The analysis of cryptomarkets such as Alpha Bay, Valhalla, Agora and Evolution Market Place has also allowed the exploration of views and perspectives of vendors and customers on a large scale [25–28]. A study by Van Hout and Hearne (2017) has identified that cryptomarket customers prefer the sequential and concurrent use of psychedelics as well as NPSs, in particular, GABA (γ-aminobutyric acid)-activating NPSs [29]. The study showed that research of these market place forums can provide an insight on novel trends of NPS use. Similarly, in their study, Bright et al. (2013) utilized Google Trends as a data collection tool of NPS-specific news and employed it to generate media links related to "Kronic"—a Canadian brand known to contain cannabis. Interviewees stated that they are mainly motivated by the

poor detectability of this substance at workplaces, which outweigh the unknown harms associated with this substance [3].

In contrast, a field study of people attending "gay dance clubs" in London, who may be considered early adopters of new substances, found they were not deterred by changes in legislation and that stimulant NPS were more popular than any other substance [30]. The same survey found their addition to existing drug repertoires to supplement more established "club drugs" (e.g., ecstasy and cocaine), rather than replacing or displacing them [30]; however, use was affected by availability and purity [22]. A survey of experienced users in Holland also found that people who preferred stimulant NPS were undeterred by legal status and did not displace other illicit substances [31].

Perhaps the reasons for these conflicting findings is that individuals may not always be open or honest about their substance use behaviors when questioned, and NPS packaging may not always contain the expected substance [1,5,32]. Therefore, some novel approaches have been utilized, such as surveying websites and online forums such as Facebook and Twitter [29,33]. The former study found that NPS were used for pleasure, out of curiosity, alongside or as an alternative to other substances [33], whereas, the latter highlighted the diverted use of prescription medicines and poly-drug use due to "high level of social media engagement" [29]; however this methodology requires individuals to have internet access, which may not always be possible for or desired by people who have problems with substances, for example, if they are leading particularly chaotic lives or are homeless. Analyzing samples in isolation also does not allow for exploration of confounding factors or perhaps more importantly, discussion with individuals regarding their reasons for use, experiences with them or any treatment needs.

Historically, SMSs have focused on provision of services for people using crack-cocaine and opiates because these substances previously dominated the UK drug market [15]. Currently, people using NPS are increasingly presenting to SMSs with physical and psychological problems, including dependency [4,8,21]. Researchers working at the Camden and Islington NHS Foundation Trust studied 442 people who were engaging in substance use admitted to a mental health unit. The sample comprised 58 people who use NPS among which, 32 initially presented to A&E, 29 involved the police, 30 were sectioned under the Mental Health Act, and 46 presented with violence before and during admission; most of them were poly-substance users [34]. SMSs therefore need to adapt and change to meet the growing demand from people using NPS [8,35]. Once engaged, limited treatment data suggests that individuals often respond well, with high rates of successful treatment completion when compared to other substances [15]; however, concerns about poor engagement of high-risk people who engage in risky NPS use (e.g., MSM (men who have sex with men)) with SMSs remain [36]. They may present elsewhere such as sexual health services; therefore, this data may not be captured by NDTMS [37]. On general adult inpatient wards of a Scottish psychiatric hospital, NPS use was found to be prevalent among 22% of young male inpatients, in particular those with drug-induced psychosis and often used with other drugs including cannabis and prescribed opiate substitutes [38]. In Hungary, a study by Kapitány-Fövény et al. (2017) also outlined stimulant NPS prevalence among individuals receiving opiate substitution therapy. The main reasons for NPS use were curiosity and practical reasons (including availability) rather than psychopharmacological preferences [39].

Further research is required into the use of NPS by those attending SMSs as highlighted in the 2014 Home Office review [1]. For example, a large Hungarian needle exchange program study found that high risk drug users switched from injecting predominantly illicit amphetamine to NPS and the study recommended further exploration of purity, price and availability [40]. In depth interviews of eleven Irish high-risk stimulant NPS injectors recommended further investigation into their adverse health effects and displacement of other drugs [24]. Some people in SMSs use non-stimulant NPS and do not always inject, so further research is required, which should include all NPS types used via different routes of administration. UK research is particularly lacking, and more is required because SMSs, legislation and illicit drug supply chains can be very different to other countries.

As described above, published research, which definitively outlines the use of NPS by individuals, who engage with specialist adult SMSs in the UK was lacking. Due to the paucity of research, this study

will consequently have national and international resonance, contributing to existing knowledge of how NPS may be changing drug taking habits and consequently positively impact upon current practice.

The aim of the study was to explore NPS use by individuals receiving treatment for substance use by exploring their type and pattern of NPS use and associated positive and negative experiences.

2. Materials and Methods

In the South West Peninsula of England, the charities RISE (Recovery and Integration Service) in Devon (excluding Plymouth and Torbay) and Addaction in Cornwall have been commissioned to provide integrated specialist SMSs (where RISE is a subsidiary of Addaction, a national organization). RISE and Addaction work with people regardless of which substance(s) they have a problem with. They cover predominantly rural areas, where national data suggests that NPS use may be more common [1,15], although the reasons for this are poorly understood. The use of NPS by the population who engage with these services has not been investigated. This is required because, in accordance with PHE guidance [15], an improved, local understanding of NPS use is important to enable the development of an effective recovery pathway, which appropriately supports the needs of those requiring treatment. There is limited information on international, national or local recovery pathways, to which these SMSs can refer. Ethics approval was obtained from Aston University's Life and Health Sciences Ethics Committee on the 20 January 2015 (Project Identification Code No. 726). The study was reviewed and approved by Addaction/RISE internal governance processes on 13 February 2015. Time was then taken to undertake the study and to seek approval for its publication.

2.1. Study Design: Methodological Orientation and Theory

This was an explorative, qualitative study using thematic analysis [41–44]. Purposive sampling [41,42], where all eligible individuals (as outlined by the inclusion and exclusion criteria below) were identified by their Recovery Co-ordinators (RC) and invited to participate in face-to-face in depth semi-structured interviews [41,42], enabled a range of relevant views to be obtained. This methodology was chosen because it is suited to exploring knowledge in poorly understood areas such as an individual's use of NPS [41–44]. Such methodology has been shown to be successful in obtaining detailed information from individuals regarding their NPS use [42–44]. This study has been reviewed and approved by Addaction's internal governance processes and Aston University's Life and Health Sciences Research Ethics Committee.

Anonymizing results to protect confidentiality and the use of the same researcher, who has no prior knowledge of the interviewees (or ability to routinely impact on their care such as changes to their prescribed treatment) should have increased the likelihood of individuals sharing their personal views and experiences [42–44]. The interviews were conducted by a qualified pharmacist with many years of experience of working in mental health and substance misuse services.

2.2. Study Design: Participant Selection and Setting

Potential participants were contacted by their RC (who identified that the person had experience of NPS) and individuals were provided with an information sheet and consent form which provided a full explanation of the study. The person's ability to provide informed consent [45,46] and eligibility to participate was confirmed immediately prior to the interview. The interviews took place at one of the SMSs or established partner agency sites, where the individual usually attends for their treatment reviews. The presence of non-participants such as the person's RC was permitted if requested by the individual.

Inclusion criteria: Previous experience of using NPS and receiving community treatment (pharmacological/psychosocial) for substance use with the charities RISE in Devon or Addaction in Cornwall.

Exclusion criteria: In receipt of in-patient or prison services; known in a clinical capacity by the Researcher; presented with significant risk issues (after assessment by their RC); additional needs could not be met (for example if they required an interpreter because their first language was not English and the SMS was unable to facilitate) or lacked capacity to consent: particular care was taken

if the person was thought to have a disability, mental health problem or thought to be under the influence of substances [47,48].

2.3. Study Design: Data Collection

Halo is the electronic record system utilized by SMSs in the South-West Peninsula of England. Quantitative data was collated from the Halo system to identify key characteristics including the participants age, sex, employment, housing status and primary substances they use (such as opiates, cannabis, stimulants and alcohol), which the system classifies in accordance with PHE [15] for the NDTMS [16].

The interview guide (Supplementary Information 1 (SI1)) was piloted to ensure it was suitable for use, and open questions were used to avoid leading the person's responses. The piloting process involved local SMS managers and experienced HCPs reviewing the questions. The interviews were digitally recorded and fully transcribed verbatim [41]. The intention was to undertake approximately twenty interviews to achieve a degree of data saturation, where no new or relevant information is elicited; however, research suggests that as few as eleven or twelve may be required [49], and this was found to be the case in this study.

2.4. Data Analysis

During the transcription process any potentially identifying information was deleted and only Halo identification codes used to identify participants. Thematic analysis, which has been specifically designed for applied qualitative research that commences deductively from specified aims and objectives, was used to organize and make sense of the data using a framework approach [41–44]. Initially, a framework was developed from existing literature; this was then updated based on the new data obtained from the interviews using a stepwise approach: (a) interviews were audio-recorded and transcribed verbatim; (b) familiarization: initial recurrent themes were identified following immersion in the transcripts; (c) coding: researcher applied line-by-line codes to describe key issues, concepts and themes by which the data was examined and referenced; (d) developing an analytical framework: after coding the initial transcripts, the researcher in consultation with supervisor, developed a set of codes that could be applied to all subsequent transcripts. Codes were then grouped into categories; (e) applying the analytical framework: data was attached to the framework of codes and categories; (f) developing a framework matrix: to manage and summarize the data, it was put on to the framework to which the data related. At this stage all transcripts were actively searched for results, which contradicted the key conclusions; (g) data interpretation: the framework matrix was used to define concepts and discover associations between the themes to provide explanations for the results; and (h) the end result was a matrix of themes from each source.

3. Results

Twelve individuals participated in this study between July and September 2015 and their demographic particulars are summarized in Table 1. All participants were in receipt of psychological interventions, with an age range of 26–59 years (SD = 9; mean 37). Three people (all within Addaction Cornwall) were known to the Criminal Justice Team as they were subject to Court Treatment Orders (CTOs) for their substance use.

During the interviews, one participant (P_9) was accompanied by his RC following his request. Interviews had a mean duration of 11 min 23 s (with a range of 4 min 45 s to 24 min 57 s). No individuals had additional needs, which required support during the interviews. The data was collated and manually analyzed by the same researcher. When transcripts were independently reviewed for themes, the findings were discussed, and no differences were identified.

Table 1. Summary of information obtained from the electronic record system (Halo for all 12 participants.

Participant	P₁	P₂	P₃	P₄	P₅	P₆	P₇	P₈	P₉	P₁₀	P₁₁	P₁₂
Age (years)	28	33	34	31	40	37	36	59	45	44	26	32
Sex	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male
Service attended	RISE Devon	RISE Devon	Addaction Cornwall	Addaction Cornwall	RISE Devon	Addaction Cornwall	RISE Devon	RISE Devon	RISE Devon	Addaction Cornwall	Addaction Cornwall	RISE Devon
Under Criminal Justice	No	No	Yes	No	No	No	No	No	No	No	Yes	Yes
Housing problem	None	None	None	None	None	None	Acute problem	None	None	None	None	None
Employed	Yes	No	No	No	Yes	No	No	No	No	No	No	No
Substitute prescription	Methadone oral liquid 40 mg/day	None	Buprenorphine sublingual tablet 8 mg/day	Methadone oral liquid 50 mg/day	None	Methadone oral liquid 30 mg/day	None	None	Methadone oral liquid 100 mg/day	None	None	None
Primary Drug *	Morphine	Cocaine	Heroin	Heroin	NPS-stimulant	Heroin	Heroin	NPS-Cannabis	Heroin	Alcohol	Heroin	NPS-other
Secondary Drug *	Codeine	NPS-stimulant	Cannabis	None	NPS-Cannabis	None	Diazepam	None	None	None	Cannabis	None
Tertiary Drug *	None	None	None	None	None	None	NPS-other	None	None	None	None	None
Injecting status (number of days injected in last 28 days)	0	0	28	0	0	8	0	0	23	0	12	0

* In accordance with PHE classification [15] recorded for NDTMS purposes [16] on Halo.

Using thematic analysis [41–44], data from the interview transcripts were assigned to four core categories:

1. Substance use history;
2. Type and pattern of NPS use;
3. Positive experiences associated with NPS use;
4. Negative experiences associated with NPS use.

Thematic analysis [42–44] enabled further explorations of the data and identified sub-themes which are evidenced in Tables 1–4, including participants' quotations. A summary of the frequencies of the sub-themes is presented in supplementary information (SI2).

1. Substance use history

Individuals described a wide range of substance use histories and were at different stages in their recovery journeys; some described more complex histories and most used a variety of substances. There were varying perceptions of the severity of their substance use and some described ongoing entrenched behaviors:

> *"I'd been in accidents and the doctors had had me on codeine and dihydrocodeine and slow release morphine and stuff . . . I just gradually got used to it over the years and years and years and because I didn't have a regular doctor, I was seeing a different doctor each time who just kept giving me scripts . . . My body got used to it and I just started taking more and more . . . I'd just split up with my wife, and sort of going through a bit of a dodgy patch"* P1
>
> *"I'm a recovering alcoholic . . . Anything that would go up my nose was going"* P2
>
> *"Fertiliser–from off the farm . . . I inject heroin . . . tried every drug in the alphabet . . . got a crack addiction . . . started having counselling . . . gotta get my life sorted . . . going to rehab . . . Childhood–stepdad beat me up . . . "* P3
>
> *"I fell in with the wrong crowd . . . I lost both my parents in a car crash so sort of went downhill and then sort of started using heroin"* P4
>
> *"Started taking mind altering substances when I was 16 . . . these 20 years . . . changing my mental state on a daily basis . . . good help from a drug worker . . . got a job . . . more stable . . . My childhood trauma is what they think kind of led to my habitual use of drugs"* P5
>
> *"I can't put drugs away . . . just work my way through them to the bitter end"* P6
>
> *"I don't really know why I take them anymore. I think it's just a habit"* P7
>
> *"I used to do it [speed-balling] quite a lot . . . since I was a kid . . . once a month or so, I might have a drink but nothing to excess . . . never more than a bottle of whiskey"* P11

2. Type and pattern of NPS use

Table 2. Type and pattern of NPS use. A list of all the themes and sub-themes, with respective quotes by participants.

Participant	Quote	Theme	Sub-Theme
P1	*Plant food . . . Snorting it . . . Just a couple of times . . . it wasn't really something I got into heavily or used a lot . . . just a couple of times . . . A mate of mine had it at his house*	NPS type	"Other" (not otherwise specified) only
		Source	Friend only
		Frequency	Occasional
		Route of administration	Nasal only
P2	*Sparkle . . . exodus . . . I'm injecting [i/v] . . . back of my hands, my arms . . . I'm now snorting instead . . . I'm surprised they [veins] haven't collapsed yet to be fair . . . I was taught by the best . . . Sat down for an afternoon . . . talked process and cleanliness [injecting technique] . . . About three or four [times a day] it used to be five or six . . . I'm using another to come down on [synthetic cannabinoid] . . . a couple of tokes . . . at the end of the session, however many days that is-3 or 4 . . . I tend to get my money at the beginning of the fortnight and I'll plan out for it. I'm a typical user . . . [from a headshop] It's easier than buying, chasing a dealer*	NPS type	Stimulating and Cannabinoid
		Source	Headshop only
		Frequency	Daily (several times)
			Binging
		Route of administration	Nasal and Intra-venous injection
		Concomitant use	Cannabinoid NPS to end stimulant NPS use
		Preference	Accessibility
			Avoid dealers

Table 2. *Cont.*

Participant	Quote	Theme	Sub-Theme
		Harm reduction	Changed route of administration
			Safer injecting training
		Affordability	Budgeting
P₃	*Mephedrone ... Cherry Bomb [cannabis type] ... speedy ... Gocaine ... smoke ... snort powder ... banging up ... It was cheap ... shops and online ... you can buy it ... in a kilo and sell it off in ten bags ... made a lot of money ... dealt loads*	NPS type	Stimulating and Cannabinoid
		Source	Headshop and Online
		Route of administration	Nasal, Smoking and Intra-venous Injection
		Affordability	Cheap
			Dealing
P₄	*Pink Panthers and Eisenberg ... represent speed ... uppers ... Black Mamba ... I smoked them but I have been known to inject a Pink Panther ... I dibbled and dabbled ... it wasn't an everyday thing, only you know once every 3 or 4 weeks ... and then when I would come home, I would do a bit of heroin to go to sleep ... [head] shop*	NPS type	Stimulating & Cannabinoid
		Source	Headshop only
		Frequency	Occasional
		Route of administration	Smoking and Intra-venous Injection
		Concomitant use	Opiates to end stimulant NPS use
P₅	*[Speedy ones] the names they had for them were things like Gogaine, or Posh ... Stimulants were either taken orally or snorted ... if I was smoking synthetic cannabis, I would be using tobacco with it ... constantly ... either be becoming high on synthetic stimulants or coming down off that and using synthetic cannabis ... if I'd just been paid ... in one hit and then top up throughout the week ... I go in there for one thing, the temptation to buy something else ... My willpower was helped by a change of circumstances and that change of circumstances in that the shop's no longer selling it*	NPS type	Stimulating and Cannabinoid
		Source	Headshop only
		Frequency	Daily (several times)
		Route of administration	Oral, Nasal and Smoking
		Concomitant use	Cannabinoids with tobacco
			Cannabinoid NPS to end stimulant NPS use
		Preference	Accessibility
		Affordability	Budgeting
P₆	*The pot ones ... Spice ... Scorpion ... Clockwork Orange ... Pandora's Box ... Toxic Waste ... Smoking it neat in a pipe ... in a rolled cigarette ... I found that the dosage is important ... I'd do very small amounts ... I was writing it down, how much I was doing, taking tiny puffs ... I was on methadone at the time ... I substituted it [with NPS] ... At the newsagent, you were able to buy them pre-rolled and my friend who smokes pot ... headshop ... online from some dodgy retailer ... Couldn't get any marijuana*	NPS type	Stimulating and Cannabinoid
		Source	Headshop, Friend, Online and Other (newsagent)
		Route of administration	Smoking only
		Concomitant use	Displacement (prescribed opiates)
			Cannabinoids with tobacco
		Preference	Accessibility
		Harm reduction	Changed dose
P₇	*Diamond Dust, Lush, Sparkle [speedy types] ... Crystal ... IV ... In my arms ... Since I've been doing legal highs I haven't touched it [heroin] ... legal high shop*	NPS type	Stimulating only
		Source	Headshop only
		Route of administration	Intra-venous injection only
		Concomitant use	Displacement (illicit opiates)
P₈	*Low Rider, Cotton Candy, Strawberry Cough ... cannabis types ... Smoking them, putting them into joints or into a pipe ... just this and tobacco ... Every 20 min ... buy it in bulk of tens ... headshop*	NPS type	Cannabinoid only
		Source	Headshop only
		Frequency	Daily (several times)
		Route of administration	Smoking only
		Concomitant use	Cannabinoids with tobacco
		Affordability	Bulk-buying
P₉	*Mephedrone ... Devil's Dust ... an upper, a stimulant ... synthetic cannabinoids ... IV [groin] ... smoking [cannabinoids] ... No more often than once a day [cannabinoids] ... About every quarter of an hour [stimulants] ... obviously there were sleep breaks but it was about a 6-month period ... I didn't withdraw from opiates ... it took me away from heroin ... Group of friends and from a chap*	NPS type	Stimulating and Cannabinoid
		Source	"Street dealer" and Friend
		Frequency	Daily (several times)
			Binging
		Route of administration	Smoking and Intra-venous Injection
		Concomitant use	Displacement (illicit opiates)
P₁₀	*The powders and the puff ... Smoking it. Injecting it ... snorting everything ... Stopped me drinking ... no withdrawals ... Legal high shop ... Dealers as well*	NPS type	Stimulating and Cannabinoid
		Source	"Street dealer" and Headshop
		Route of administration	Nasal, Smoking & Intra-venous Injection
		Concomitant use	Displacement (alcohol)

Table 2. *Cont.*

Participant	Quote	Theme	Sub-Theme
P₁₁	*Mephedrone ... Methalone. A couple of hallucinogenic ones. And the pills that they did ... Hawaiian Woodrose ... They're a seed basically and they've got an LSA in them which is like a precursor to LSD ... smoking ... Usually I would just have a couple of Valium [to help come down from NPS] ... Internet ... It was just a sort of weekend thing ... Not very regularly.*	NPS type	Stimulating, Dissociative and Hallucinogenic
		Source	Online only
		Frequency	Occasional
		Route of administration	Smoking only
		Concomitant use	Benzodiazepines to end NPS use
P₁₂	*Crystal, Boom Dust ... Diamond Dust mephedrone ... smoke the cannabinoids ... Lotus ... The speedy ones ... Crystal Meth ones–the uppers ... opiates from Italy ... I snort them ... dabble them ... Put it in your mouth, let it dissolve ... smoking if they're the cannabinoid ones ... Putting it in with baccy, it makes it last longer ... If you mix them together, you hallucinate ... It's opportunist ... just down the road...just go to the shop and don't get ripped off by the drug dealers ... every day.*	NPS type	Stimulating, Cannabinoid and Sedating
		Source	Headshop only
		Frequency	Daily (several times)
		Route of administration	Oral, Nasal and Smoking
		Concomitant use	Potentiating effects
			Cannabinoids with tobacco
		Preference	Accessibility
			Avoid dealers
		Affordability	Cannabinoids with tobacco

3. Positive experiences associated with NPS use

Table 3. Positive experiences associated with NPS use. A list of all the themes and sub-themes, with respective quotes by participants.

Participant	Quote	Theme	Sub-Theme
P₁	*I wasn't thinking about the ex-wife ... The buzz ... like being drunk without the drink ... fuzzy, happy, chilled out sort of feeling*	Psychological effects	Escapism
			Relaxation
			Happiness
			Euphoria
P₂	*It allows me not to be me ... Without them, I'm a totally different person, I would never talk you right now without it ... allows me to be confident ... Part of it was to lose weight ... I was 17 stone when I started out on it ... just 10 times better than the drink ... It's stronger than street*	Psychological effects	Escapism
			Confidence
		Physical effects	Weight loss
		Preference	Strength
			Quality
P₃	*They keep me awake when I was out clubbing ... Was I allowed to sell them? I thought I was cos it was legal*	Psychological effects	Alertness/Energy
		Preference	Legal status
P₄	*It was about a fiver whereas you go out and buy a gram of coke and its 60 quid*	Preference	Affordability
P₅	*Wanting to escape from reality any way, or change that reality ... [stimulants] a heightened sense ... very energetic ... happy ... [cannabinoids] lethargic, very drowsy ... most of the time dozing on the sofa in front of the TV-that is what I wanted ... It's hundreds of times stronger ... would last for ages ... I got bored ... I was drawn to try them ... It's cheaper [than marijuana] ... 13.5 stone down to about 9.*	Psychological effects	Escapism
			Relaxation
			Happiness
			Alertness/Energy
		Physical effects	Weight loss
		Preference	Strength
			Affordability
			Curiosity
			Boredom
			Duration

<div align="center">**Table 3.** *Cont.*</div>

Participant	Quote	Theme	Sub-Theme
P$_6$	*More involved in the music, film ... my playing sounded great ... [it] wasn't very great ... they didn't have any downside at all ... they didn't relax you or send you to sleep–quite the opposite ... I went back because of the power it had over me. It scared me and I wanted it to ... I wanted to be able to do it without freaking out ... it would last for ages because it's so strong*	Psychological effects	Alertness/Energy
			Improved subjective experiences
			Overcome and control previous negative experiences
		Preference	Strength
			Duration
P$_7$	*You just get more for your money worth [than "street" amphetamine]*	Preference	Quality
			Affordability
P$_8$	*I like the relaxing, calming numb feeling ... The effect, it's quicker than marijuana. You get higher quicker ... faster*	Psychological effects	Relaxation
		Preference	Onset of action
P$_9$	*It was quite stimulating ... had an overriding desire to argue with Christians ... improved my darts. It deepened my appreciation of Johnny Cash ... You legalise [it] and I'll be back on it ...*	Psychological effects	Alertness/Energy
			Improved subjective experiences
		Preference	Legal status
P$_{10}$	*Make myself happy ... made me stronger ... gives me confidence ... I eat every day [resulting in weight gain when previously underweight]*	Psychological effects	Happiness
			Confidence
		Physical effects	Weight gain
P$_{11}$	*Totally a good feeling basically, made me feel quite happy and cheerful ... I tried most of them really to see what they were like I stuck to the illegal ones cos they work better ... I thought, yeah I'll try that–see what happens*	Psychological effects	Happiness
		Preference	Quality
			Curiosity
P$_{12}$	*[Stimulant NPS] gives me more energy ... [cannabinoid NPS] calms me down ... It enhances–changes your perspective on stuff ... Helps me listen to music ... I've lost a lot of weight doing them. I was 18 and a half stone before I started doing them ... I like to keep the weight off ... I've got a bad leg and it takes that pain away*	Psychological effects	Relaxation
			Alertness/Energy
			Improved subjective experiences
		Physical effects	Weight loss
			Analgesia

4. Negative experiences associated with NPS use

Table 4. Negative experiences associated with NPS use. A list of all the themes and sub-themes, with respective quotes by participants.

Participant	Quote	Theme	Sub-Theme
P$_1$	*My lip and my front teeth just went numb ... it makes your eyes water ... and your nose burnt sometimes. The nostrils would burn [when snorting NPS]*	Physical effects	Administration site
P$_2$	*A little bit dearer [than "street drugs"] ... It has turned me into a more devious person as well, although I've never stolen ... I am the nastiest person on it ... I don't like the shakes ... it makes me itch a bit ... eczema ... it's a really dangerous sweat, it's pretty embarrassing*	Psychological effects	Personality changes
		Physical effects	Tremor
			Eczema
			Sweating
			Itching
		Preference	Affordability
P$_3$	*I've had thoughts of ... killing myself ... not wanting to be here ... I didn't like the effects ... it'll kill you ... the heartbeat on the high ones ... you feel like, I've just injected here and my heart's going. It feels like I'm dying ... That's a vein there and it's just left a massive lump ... It's too powerful*	Psychological effects	Suicidal ideation
		Physical effects	Administration site
			Cardiac
		Preference	Strength
P$_4$	*I couldn't go out, struggled concentrating ... They don't make you feel good; all they do is make your heart feel like you're having a heart attack ... heart fluttering, palpitations ... Too strong, over-powering*	Psychological effects	Concentration
			Impaired activity of daily living
		Physical effects	Cardiac
		Preference	Strength

Table 4. *Cont.*

Participant	Quote	Theme	Sub-Theme
		Legal	Detained (Mental Health Act)
P5	*I stopped cleaning my flat . . . go for about 4 days without eating . . . lost interest in my home. I wasn't paying my bills . . . washing up not done for months . . . got sacked . . . all I wanted to do was get money and buy legal highs. That was my whole life . . . crawling around my carpet on my hands and knees . . . try and get enough for another rollie . . . that's how addictive they were . . . Eventually the police turned up and I basically got detained under the Mental Health Act for 24 h, until the effect of the drugs wore off . . . I started fitting in the street and I just couldn't control my body . . . it no longer had that kind of kick to it . . . I stopped buying it . . . you get a variation . . . the illegal high stuff is more consistent in its strength*	Psychological effects	Impaired activity of daily living
			Self-neglect
			Loss of control
			Dependency/Addiction
		Physical effects	Seizures
		Preference	Strength
			Quality
P6	*Too potent . . . I hate the fact that this is controlling me . . . I don't have control . . . Couldn't remember what I was doing . . . completely confused . . . Gave me panic attacks . . . Wouldn't have been in any fit state to go out . . . It seemed to speed my heart up to a frightening degree . . . I really thought I was gonna have a heart attack . . . my heart was thumping away madly . . . Hundreds of times stronger . . . it's easy to go over-board . . . the intensity of it was just on another level . . . I think they're more dangerous because they are just so intense . . . It could be a little bit hit and miss . . . it wouldn't have the same sort of effect*	Psychological effects	Impaired activity of daily living
			Confusion
			Impaired memory
			Panic attacks
			Loss of control
		Physical effects	Cardiac
		Preference	Strength
			Quality
P7	*It was pretty strong and I thought I was gonna collapse and die . . . It was the strength of it*	Preference	Strength
P8	*I want to keep to that high all the time . . . sleep [to stop] . . . It might make you cough . . . sweats*	Psychological effects	Dependency/Addiction
		Physical effects	Coughing
			Sweating
P9	*Abscesses*	Physical effects	Administration site
P10	*You get more addicted. You wanna another bag, another bag and another*	Psychological effects	Dependency/Addiction
			Cravings
P11	*I stuck to the illegal ones cos they work better*	Preference	Quality
P12	*I need them, cos I've been doing them for 6–7 years . . . I don't like having to wake up when I haven't got the money for them. Then I have to try to get the money for them. I don't like that part of it . . . they're quite addictive . . . done some quite reckless stuff to get them . . . trouble with the police . . . Makes me shake a lot . . . all the time*	Psychological effects	Dependency/Addiction
		Physical effects	Tremor
		Legal	Police involvement

4. Discussion

This study contributes to existing knowledge of how NPS are used by individuals, who engage with specialist adult SMSs. Seven (58%) reported opiates (illicit heroin/morphine) as their primary substance of use; this is in keeping with the finding that five (42%) of participants were in receipt of opiate substitution prescriptions. For a quarter of individuals, NPS was their primary substance of use in accordance with PHE classification [15], suggesting that while not as common as opiates (which SMSs are more familiar with treating [15]), NPS use is prevalent among individuals accessing treatment services, as previously suggested by both national and international findings [7,8,39].

The interviews allowed individuals to describe their experiences with all types and routes of administration of NPS; therefore, adding to the existing evidence base, particularly since previous studies [24,40] have largely focused on stimulant NPS, which were injected. Audio recordings allowed for more complete data and detailed transcriptions than relying on memory or notes alone and piloting should have increased the likelihood of individuals sharing their personal views and experiences [42–44].

Harm reduction measures aim to reduce the harm that someone may experience because of their ongoing substance use [45,46]. These include but are not limited to avoiding poly-pharmacy, administering by routes associated with less risk (such as smoking rather than injecting), not sharing drug paraphernalia and using smaller amounts. This study, as with other research, found that individuals did not usually know exactly which chemical they were taking [19,50]. Amount of experience with NPS and frequency of use varied greatly, from very occasional up to every 20 min. Frequent use was mainly observed with synthetic cannabinoid receptor agonists (SCRAs) and stimulant NPSs. Due the wide chemical diversity of SCRAs, little is known on their pharmacokinetics [51]. However, stimulants such as mephedrone may have a short duration of action warranting repeated dosing [52]. Overall substance use patterns fluctuated over time (with varying self-perceptions), which may be explained by individuals being at different stages in their recovery journeys. Participant ages

ranged from 26 to 59 (SD = 9); other studies have found and that most people using NPS are under 30 years old [1]. The difference may be because this study unlike national data reflects the age of those requiring treatment. Our results therefore show that SMSs need to manage individuals with a range of ages presenting with NPS use.

Previous studies and national reports have outlined a complex relationship between NPS and other substances, and the need for further investigation. Factors affecting use include substance availability, effects, safety profile, quality, price, purity and legal status [1,16,21–23,38]. This study equally found that NPS were favored over other substances for their perceived legal status, improved price, availability and higher quality, including quicker onset and duration of action, therefore adding to the existing knowledge base.

The large proportion (*n* = 9; 75%) of participants who stated they had experience with SCRAs is perhaps to be expected (SI2), given that cannabis, the traditional illicit substance, which these substances are attempting to mimic, has the highest prevalence rate of all illicit substances [53]. Preference for NPS type appeared to be dependent upon the required effect (usually stimulating or sedating). Some wanted to gain confidence, energy and alertness; others wanted to "escape reality", experience euphoria, feel happy, relaxed or were potentially self-medicating for underlying psychological trauma (and in one case (P$_{12}$) for leg pain). This has been found in other studies [30,54], where stimulating effects were particularly popular [21,31,54]. One study found that psychedelic effects were commonly favored [30]; although this could be due to the differing study environments (such as dance venues and festivals). Individuals also reported using NPS to alleviate feelings of boredom and out of curiosity, which has also been found previously [1,21,55] and similarly for traditional illicit substance use [1].

NPS were often mixed with other substances [34,50] and administered by a variety of routes, although snorting/injecting stimulant NPS and smoking SCRAs, the latter in combination with tobacco (*n* = 4; 33%) predominated (SI2). Stimulant and SCRA NPS were also used sequentially to balance the effects of each other. Excessive binging for long durations on stimulant NPS, followed by substances with sedating and relaxing effects to "come down" supports the results of another study [24]. Poly-pharmacy is associated with an increased risk of drug interactions and side-effects including overdose and death [7,45], so harm reduction advice, which SMSs offer, should include: overdose awareness, how to reduce frequency, avoid "binges", minimize polysubstance use, manage altered tolerance levels as well as the provision of take home naloxone [24,56].

The amount participants spent on their NPS varied widely and was affected by how much money they had access to, the source of NPS supply and pattern of use. To reduce costs NPS were bought in bulk or smoked with tobacco and one person (P$_3$) also described dealing NPS to fund his habit. NPS were described as comparatively cheap to traditional illicit drugs which has been similarly suggested by the Home Office [1]. A Dutch study has found them to be similar in price to the substances they are attempting to mimic [30] and this may be accounted for by differences between England and Holland drug markets.

Strength of NPS was perceived as both a positive and negative. Some found them to be too strong, leading to unwanted effects and overdoses; others preferred them for their strength, considering them to be more cost-effective and of higher quality. Overall, NPS use reduced if strength and quality was variable, and the NPS was viewed as inferior rather than superior to traditional illicit drugs; this may be highly dependent on the local drug markets and has been similarly identified by the Home Office [1].

Our findings support other research, which identified that the rapid proliferation of NPS use may be changing drug taking habits [22]. Several individuals reported NPS alleviating withdrawal symptoms including cravings, from other substances and in some cases completely substituted with NPS. SMSs should therefore provide individuals with advice on how to manage withdrawals (from all substances). Using NPS in addition to traditional illicit drugs or prescribed substitute medication to potentiate effects was also reported, particularly when then the person was presenting as more chaotic

as found in similar studies [24]. This is unsurprising since individuals in the early stages of recovery commonly present with more complex and riskier patterns of substance use.

Participants reported obtaining NPS from various sources; the majority had experience of using headshops (*n* = 9; 75%) (SI2) in contrast to a qualitative study that found that NPS were usually obtained from friends or online [30]. Headshops were often preferred for their convenience (particularly if they could avoid "dealers"), with usage reducing when they could no longer be legally sold. However, legal status did not always deter use, supporting Home Office findings, which suggest this may be due to the impact of legal status upon the quality of NPS [1,3]. Previous studies have equally conflicted in their findings on the effect of legal status on the decision to use NPS [24,30]. Purchasing them from high street vendors may add to the perception of this being "normal" behavior and the risk of them being considered as "safe" and "legal" and consequently increase NPS use. For example, one person (P$_3$) felt it was permissible to sell them because they did not consider them to be "drugs" in the same way as traditional illicit substances. Recent studies showed that there is an underlying competition between cryptomarkets and street networking, which may drive high quality of illicit substances as well as NPS [25–28]. However, an international drug testing service that was offered to cryptomarket users suggested that this may not be the case [25].

Participants described a wide range of physical and psychological problems including symptoms of NPS dependency reflecting national and international findings [4,8,57,58]. Similar to the findings of other studies [31,57,58], reported physical health effects included tremor, coughing, itching, seizures, eczema and sweating, and problems associated with specific routes of administration such as venous abscesses. Like other studies, cardiac effects were reported (*n* = 3; 25%) (SI2), usually following stimulant NPS use and sometimes lasting a few days [31,57,58]. While individuals described some of these effects as sometimes being prolonged, the long-term effects remain unknown. Individuals were not specifically asked about their experiences of acute compared to chronic effects or to distinguish between side-effects and withdrawal symptoms. This suggests further study development and provides additional support for the need for longitudinal research [1]. Unpleasant psychological effects, included feelings of loss of control, difficulties concentrating, impaired memory, confusion, personality changes, panic attacks and cravings; sometimes leading to crime, impaired activities of daily living and self-neglect. Suicidal ideation and in one case (P$_5$), being sectioned under the Mental Health Act following prolonged NPS use was reported. Individuals with pre-existing mental health conditions ("dual diagnosis") may be particularly at risk of psychological problems and local integrated pathways should outline how to obtain the required support quickly and effectively, especially in acute situations such as the person disclosing suicidal ideation.

High risk administration practices and side-effects such as vein damage as a direct result of the route of administration were described, although not as significant as identified by a similar Irish study, perhaps because it only included NPS mainly administered by injection [24]. To ameliorate this, individuals actively undertook harm reduction approaches such as switching from injecting to snorting and using less [45]. It is important that SMSs appropriately signpost to additional sources of help and support, for example tissue bioavailability services.

4.1. Limitations of the Study

Repeat interviews and member-checking was not implemented due to difficulties in re-establishing contact with the participants, time constraints and geographical problems. The purposive sampling [42–44] approach enabled suitable participants to be identified. Although small, tentative data saturation was achieved, as has been found to occur in other research [24,42–44,49]. A larger sample size may have enabled more generalizability; however, this smaller sample size allowed for more in-depth analysis.

Interviews require people to discuss their experiences in an artificial environment, which may lead to problems with reliability. However, this approach of using semi-structured interviews to

obtain detailed information from individuals regarding their NPS use has been successfully used previously [23,24,30].

Data was collected in a predominantly rural environment and further research should be conducted in more urban areas. The Devon RISE and Addaction Cornwall SMSs were almost equally represented (7:5 respectively). Men dominate SMSs and reflecting this, all our participants were men [1,7]. With a larger sample size, women may have been included, providing the opportunity for comparison.

Data was collected in 2015, when many NPS were sold openly in headshops and NPS markets have since changed. Currently, under the UK NPS Act 2016, headshops can no longer legally sell NPS and a clear shift from the surface to the dark net was observed [59] and motivation for NPS use may have consequently evolved. Implications of the new legislation include intentional poor disclosure of NPS use to HCPs and NPS emerging on the illicit market, often sold as the traditional illicit drug the NPS mimics, to increase profit margins [55]. The delayed reporting of this study is due to the time needed for the necessary approval for publication

4.2. Implications for HCPs and Policy-Makers

The way that the participants described NPS, easily made them identifiable in accordance with PHE categories used by NDTMS, therefore providing support for this approach in clinical practice [15]. However, there were significant discrepancies between NPS use recorded on Halo for the purposes of NDTMS [16] and disclosures made by individuals during the interviews. Therefore, all individuals presenting to SMSs should be asked about their use of NPS, the name of the substance, and reassessed regularly, because they may not routinely present or perceive this as an issue, especially if other substances dominate their pattern of substance use or if it changes over time. Additionally, this study highlights that individuals should be asked about NPS regardless of their age. With the increasing emergence of NPS (from approximately 478 in 2015 when this data was collected [14] to 740 in 2016 [11]), increasing chemical diversity, potency, and changing degree of purity [14,25], it is important that HCPs reassess regularly the use of novel or existing NPS and tailor harm reduction approaches to individuals. The importance of offering harm reduction interventions was demonstrated by the positive impact that they had on individuals; however, the reports of managing withdrawal symptoms by diverting prescribed medication or otherwise using polypharmacy must not be underestimated in clinical practice. HCPs must check that prescribed doses are optimized to reduce cravings and "on top" use. Additionally, adherence to medication regimens must be checked and community pharmacy teams must promptly notify prescribing services of missed doses.

Consequently, treatment needs may also fluctuate over time, so SMSs need to be able to accommodate this and regularly review the person's progress. These findings also provide support for using interviews to elicit more accurate data regarding people's NPS use and highlights that reviewing NDTMS data in isolation to assess the extent of NPS use is inadequate. This may be for a variety of reasons, including lack of staff vigilance when completing the required data set and suggests a potential training need.

Because of their overall experiences, some strongly felt that they would never use NPS again, while others continued to use despite their negative consequences. These findings corroborated with previous research on SCRAs, where users reported that these substances completely "hijacked their personalities" [60]. These negative effects may have a significant impact upon the person, including their employment, housing status and criminal record, and the wider community. SMSs are traditionally skilled in providing support with such problems but promoting awareness among staff about how best to identify individuals requiring more intensive support, or those whose needs may need to be prioritized, such as individuals with pre-existing conditions, those who are homeless or subject to CTOs, may enable this to be provided more promptly.

There was evidence that the SMSs were raising awareness of harm reduction by advising on alternative routes of administration, such as switching from injecting to snorting and offered safer

injecting technique sessions. Risks may be further reduced if the substance could be tested to confirm its content prior to administration and harm reduction advice [5,61] was provided at the time of purchase. Currently, neither is currently permissible in UK SMSs, therefore adding further weight to reviewing existing legislation.

SMSs need to understand the motivation for using NPS: when individuals disclosed their substance use histories, they were highly variable. In one case, iatrogenic dependency (P_1) was described, though reasons for use were frequently associated with traumatic life events, often from childhood. This has been found to occur in other NPS studies [21] and SMSs should therefore be sensitive to the needs of these individuals and tailor psychological interventions accordingly. When more significant disclosures are made, other services such as bereavement counselling and mental health teams should be signposted so that any unresolved issues can be managed effectively.

Some described improved abilities and experiences with NPS, often acknowledging this was perceived because of the mind-altering effects of the substance, such as having energy while on a night out and enjoyment of activities such as playing music. Such information may be of interest to policy-makers, where psychoactivity of newly emerging NPS has not yet been demonstrated. One individual (P_6), reported a desire to use NPS to overcome their previous negative experiences with these substances, alongside entrenched addictive behaviors and boredom due to a lack of meaningful daytime activities. This highlights the need for SMSs to promote a variety of psychosocial interventions to help occupy an individual's time in substance-free environments, which are tailored to the person's needs to enable them to progress with their recovery. Supporting people to develop skills which will enable them to find work is important because the number of participants in employment was comparatively low and boredom was cited as a reason for NPS use, which may be compounded by rural locations. Some may require more intensive RC support and there should be adequate provision for this.

The main positive physical health effects reported were upon weight: three (25%) stated that using predominantly stimulant NPS to keep their weight down was one of their main motivators for use. This may be a particular problem once the person stabilizes their NPS use and weight gain is observed, which may result in the occurrence of other eating disorder symptoms. In contrast, one individual (P_{10}) reported appetite stimulating effects which they found to be beneficial as they were otherwise losing weight due to self-neglect because of their chaotic drug use. However, it cannot be assumed that the food they were selecting to eat included "healthy options". In these situations, individuals should be offered supportive explanations that if their substance use stabilizes their weight will usually return without the need for stimulants. Malnutrition may be common in people in substance use treatment, which is one of many reasons why SMSs supporting activities such as "breakfast clubs" and the provision of "life-skills" courses which incorporate cooking techniques and general nutritional advice, should be actively encouraged. Everyone involved in the care of people with substance use problems (including GPs and community pharmacists in addition to specialist SMSs) should be vigilant for eating disorder behaviors and provide the required support or signpost as required: there may be training needs.

Three participants were subject to CTOs because of their substance use and would therefore require regular drug testing for SMSs to report on their progress. Since NPS may not always be detectable in routine drug screens and can produce false positives [5,55,61], SMSs could consider more specialist NPS-specific tests. These may be expensive (and not always possible due to the novelty of the substances), but if used sparingly and appropriately, they may be a useful tool. However, this is not without risk, since it may also perpetuate the demand for "new" NPS that are not detectable. Half of the participants were in receipt of a prescription for oral opiate substitution treatment and using NPS as a replacement for such medication was reported. The diversion of prescribed medication is highly likely in such situations. Therefore NPS-specific tests may also be useful for supporting decisions around continued prescribing in circumstances where there is evidence of "on top" use or diversion is suspected. Staff should receive appropriate training so that they are vigilant for "on-top"

use, diversion and substitution of the person's prescribed intervention with NPS, particularly if the individual is under a CTO.

NPS treatment systems are complex and require structured partnerships between multidisciplinary teams with a broad range of competencies. This includes: A&E departments, sexual, social care and mental health services, prison, probation staff and Young Offender teams, community adult and young people SMSs, youth services and organizations such as the police [6]. This is required to manage the myriad of health, social and criminal problems associated with NPS use and to address the diverse needs of individuals including young people, MSM, prisoners, and the homeless population. This approach should also involve people who use NPS and their carers. Drug detection is key to support diagnosis and treatment planning, adherence and outcomes [5,6,55,61]. Proactive, engaging and competent SMSs are needed to respond appropriately to meet the needs associated with NPS use. Regular education and training is of paramount importance to inform about types and degrees of harm, new patterns and trends of recreational drug use and changes to the drug scene [6]. NPS use should be considered in clinical assessments and management plans should be devised accordingly [20].

5. Conclusions

This study found that NPS are used by individuals across a range of ages, presenting to SMSs in Devon and Cornwall, but is highly variable. NPS may be favored over other substances for their perceived legal status, price, strength, availability and better quality, including quicker onset and duration of action and improved side-effect profile. Consequently, NPS use may be dependent on local drug markets and reduce if perceived to be variable in strength and quality in comparison to traditional illicit drugs. SCRAs and stimulant NPS are most frequently used and preference for NPS type is often dependent upon the required effect. Using poly-pharmacy (including NPS, traditional illicit substances and prescribed medication) to potentiate effects, manage side-effects and withdrawal symptoms occurs, sometimes resulting in displacement of substances. Therefore, SMS must remain vigilant and consider the use of NPS-specific tests, especially when substitute medication is prescribed. NPS are frequently administered by a variety of routes, including high risk injecting practices, though individuals can respond well to harm reduction interventions, which should be routinely provided and include the management of withdrawals. Results showed that a wide range of problematic physical and mental health effects may occur, including symptoms of dependency, which may lead to criminal activity, impaired activities of daily living and self-neglect. People's perception of the severity of their use, frequency and amount used varies widely and may change depending on their recovery journey, consequently fluctuating for individuals over time. People use NPS for a variety of reasons, including stimulant types taken for the intention of losing weight. As malnourishment is often an issue for individuals accessing SMS, the multidisciplinary team must be attentive, frequently revisit the current pattern of substance use and any associated issues, promptly refer to other services accordingly and may require further training. Local integrated pathways for individuals presenting with more complex issues and particular needs such as dual diagnosis and tissue bioavailability should be established to enable prompt access. SMSs should engage individuals with psychosocial interventions and meaningful daily activities, especially in more rural areas. It is important that current approaches to existing treatment strategies are adapted and recovery plans tailored to an individual's needs. Education and training is needed for HCPs working within various services where people who use NPS are encountered. Multidisciplinary and multiagency approaches should be nationally adopted to capture changing trends of NPS use.

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Mechanistic Insights into the Stimulant Properties of Novel Psychoactive Substances (NPS) and Their Discrimination by the Dopamine Transporter—In Silico and In Vitro Exploration of Dissociative Diarylethylamines

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Abstract: Novel psychoactive substances (NPS) may have unsuspected addiction potential through possessing stimulant properties. Stimulants normally act at the dopamine transporter (DAT) and thus increase dopamine (DA) availability in the brain, including nucleus accumbens, within the reward and addiction pathway. This paper aims to assess DAT responses to dissociative diarylethylamine NPS by means of in vitro and in silico approaches. We compared diphenidine (DPH) and 2-methoxydiphenidine (methoxphenidine, 2-MXP/MXP) for their binding to rat DAT, using autoradiography assessment of [^{125}I]RTI-121 displacement in rat striatal sections. The drugs' effects on electrically-evoked DA efflux were measured by means of fast cyclic voltammetry in rat accumbens slices. Computational modeling, molecular dynamics and alchemical free energy simulations were used to analyse the atomistic changes within DAT in response to each of the five dissociatives: DPH, 2-MXP, 3-MXP, 4-MXP and 2-Cl-DPH, and to calculate their relative binding free energy. DPH increased DA efflux as a result of its binding to DAT, whereas MXP had no significant effect on either DAT binding or evoked DA efflux. Our computational findings corroborate the above and explain the conformational responses and atomistic processes within DAT during its interactions with the dissociative NPS. We suggest DPH can have addictive liability, unlike MXP, despite the chemical similarities of these two NPS.

Keywords: dopamine; DAT; brain; addiction; molecular dynamics; free energy calculation; autoradiography; voltammetry; diphenidine

1. Introduction

A notable increase in the number of new psychoactive substances (NPS), formerly known as 'legal highs', 'bath salts' or 'designer drugs' ties in with the growing lines of evidence of their complex behavioural effects and health risks they may carry. NPS often resemble traditional drugs of abuse, although their pharmacological properties are unknown. Frequently, through minor chemical modification of the molecular structure of psychoactive drugs, different biological effects can be achieved, which includes the drug's addictive liability. The latter links with the stimulant effects of drugs and involves the neurotransmitter dopamine. Stimulants have been known to raise dopamine availability in the brain, including the brain's reward pathway that can be hijacked by stimulant
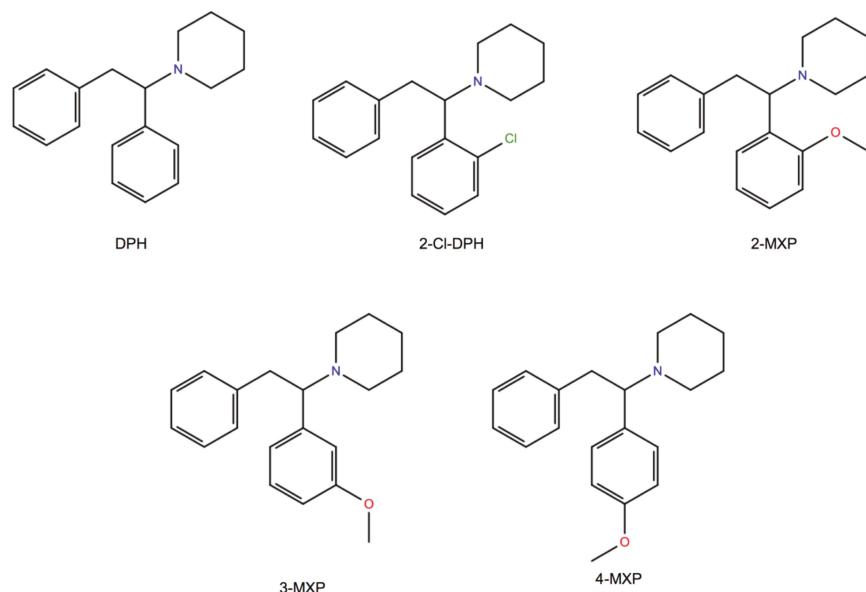
drugs [1]. Perceptions of pleasure and reward are associated with the release of dopamine in the nucleus accumbens as part of the reward pathway; a similar phenomenon is also implicated in responses to stimulants whose repeated use may lead to drug dependence [2].

The molecular target for stimulants is the dopamine transporter (DAT), which can be obstructed by the drug that binds to it. DAT belongs to the Solute Carrier 6 gene family (*SLC6A3*) that encodes several Na$^+$/Cl$^-$-dependent neurotransmitter transporters (the NSS or neurotransmitter:sodium symporter family), including transporters for norepinephrine, serotonin, GABA and glycine [3]. NSS proteins function to couple the transport of Na$^+$ down its concentration gradient with the uphill transport of the respective substrate. Additionally, several NSS proteins are characterized by co-transport of Cl$^-$ [4]. DAT, like the other members of this family of transporters, has both an intracellular amino- and carboxyl-termini and twelve transmembrane (TM) helical domains [5–7]. The substrate binding site is known to be deeply buried in the transporter structure. Referred to as the S1 site, it has been resolved by X-ray crystallography [6,8,9] and previous computational modeling [10–12] to describe a site that overlaps with that of dopamine and many of the popular psychostimulants. It is also clearly distinct from the site observed for antidepressant binding (S2 site) to the leucine transporter (LeuT) which is found facing the extracellular vestibule nearly 11 Å above the S1 site [13].

A classical example of a drug that binds to DAT and obstructs the S1 site is cocaine [14]. Cocaine binding to the DAT reduces dopamine re-uptake from the extracellular compartment and leads to an increase in the synaptic concentrations of dopamine available for neurotransmission in the pathway affected (mesolimbic/mesocortical) [15]. Alternatively, drugs such as amphetamine, interact with the DAT when entering the presynaptic compartment where they displace newly synthesised dopamine [16], which also leads to an increase in basal dopamine concentrations in the synapse. The elevated concentration of extracellular (synaptic) DA underpins the behavioural response to stimulants, and with repeated use, may lead to drug dependence.

We have previously demonstrated a range of pro-dopaminergic effects among synthetic cathinones [17] and benzofurans [12,18] as studied by means of neurobiological methods in vitro, and also in silico, using molecular modeling of atomistic interactions between drugs and DAT [12]. By means of the in vitro fast cyclic voltammetry technique, we were able to estimate if the stimulant profile is more similar to that of amphetamine or cocaine. On the basis of radioligand binding studies we can visualise the drug's binding at the dopamine transporter in the mesolimbic and mesocortical pathways of the brain, and we could say that some of the synthetic cathinones, such as bupropion, ethcathinone or diethylpropion do not act as a substrate releaser at the DAT. That allowed us to conclude that the wide-ranging pro-dopaminergic effects of cathinones were consistent with their different behavioural effects and popularity as recreational drugs [17]. Additionally, the varied and complex atomistic mechanisms of interactions between stimulants and DAT, which result in an increase in dopaminergic tone typical of stimulant drugs, can be studied by means of virtual methods of molecular modeling [12], which we apply in the present study to understand how relatively minor structural differences translate into different pharmacological NPS profiles of relevance to their addictive potential.

Here, we choose to study in silico a class of compounds that rigorously share the core of the 1,2-diarylethylamine structure and an ethylamine nucleus with aromatic substitutions (Figure 1). We also study two of them, the dissociative NPS, diphenidine (DPH) and its methoxylated derivative 2-methoxydiphenidine (methoxphenidine, 2-MXP/MXP), in vitro using the above neurobiological methods. DPH and 2-MXP replaced a ketamine-like drug methoxetamine (MXE) which was banned the UK in 2013; MXE was branded as a bladder-friendly ketamine, while ketamine has been associated with cystitis and bladder fibrosis [19,20].

Figure 1. Molecular structures of diphenidine (DPH) and other aryl-substituted 1,2-diarylethylamines including 2-MeO-diphenidine (2-MXP), 3-MXP, 4-MXP and 2-Cl-DPH.

It is worth noting that DPH and 2-MXP have been described as relatively selective *N*-Methyl-D-aspartate receptor (NMDA receptor or NMDAR) antagonists [21–23]. NMDA antagonism has been accepted to underpin the dissociative state effects that include sensory distortions and hallucinations, and depersonalization [19,24,25], although monoamine binding sites can also play a role in the psychoactive profiles on these drugs [25]. The recent interest in the pharmacology of these compounds is justified by the fact that both DPH and 2-MXP have been associated with adverse health effects including deaths [21,26–29].

Interestingly, DPH and 2-MXP have different functional potencies (IC_{50}) at the dopamine transporter, 1.99 μM and 30 μM, respectively [22] and as confirmed in the recent study [30] where DPH was shown to be an inhibitor of the noradrenaline transporter (NET) (3.3 μM) and DAT (3.4 μM), while 2-MXP was mainly an inhibitor of the NET, with 7.8 μM inhibition compared to 65 μM at DAT. The monoamine transporter inhibition can contribute to their psychoactive properties and dictate addictive potential especially in the case of DPH which has a relatively high affinity (K_i = 0.23 μM) compared to 4.8 μM for 2-MXP at DAT [30].

Since this study relies heavily on rat in vitro data we endeavoured to be consistent in comparing the in silico data to the in vitro responses to psychostimulants in the same cellular background. As such, we utilised a previous homology model of the *Rattus norvegicus* dopamine transporter (rat DAT, rDAT) [12] to dock each of the five compounds (Figure 1). Their relative binding free energies were then calculated using alchemical free energy molecular dynamics simulations, particularly the free energy perturbation (FEP) method. The free energy predictions were subsequently compared with the experimental IC_{50} values that were reported earlier [22]. By using such in silico approaches we explored the possibility of predicting the DAT-binding properties, and thus addictive liability among this class of dissociative NPS. Awareness of addictive potential of NPS is important to both users and health services.

2. Methods

2.1. Animals

Eight week old male Wistar rats (Charles River, Harlow, UK) were kept on a 12/12 h light/dark cycle (lights on at 7 a.m.) with food and water *ad libitum*. Rats were treated in accordance with the U.K. Animals (Scientific Procedures) Act 1986 (related to the 1986 EU Directive 86/609/EEC) and sacrificed by cervical dislocation with no anaesthesia.

2.2. Reagents

All chemicals used were supplied by Sigma Chemicals (Poole, UK) except DPH and 2-MXP which were a gift from John Ramsey (TICTAC Communications Ltd., London, UK). The radioligand for the dopamine transporter, [^{125}I]RTI-121 (specific activity 81.4 TBq/mmol) was purchased from Perkin Elmer (Beaconsfield, UK).

Radioligand DAT binding study was conducted as previously described [8]. Briefly, brains were removed and frozen at −40 °C in a mixture of methanol and dry ice, then stored at −80 °C. Frozen brains were cut into 20 μm serial coronal sections to harvest the striatum at +1.7 mm to −0.3 mm versus bregma [31], collected onto polysine-coated slides and stored at −80 °C. The autoradiography procedure was conducted according to Strazielle et al., 1998 [32]: preincubation in 0.05 M NaPB pH 7.4, incubation with 20 pM [^{125}I]RTI-121 in NaPB pH 7.4 with increasing concentrations of the drugs tested (0–30 μM) for 60 min at room temperature; non-specific binding was assessed in the presence of 200 μM nomifensine. Slides were opposed to Kodak BioMax MR films for 4 days; autoradiograms were analysed using MCID™, Version 7.0, Imaging Research Inc. (St. Catharines, ON, Canada), *n* = 6 rats. Flat-field correction was applied. The striatal regions of interest were sampled in duplicates for relative optical density; left and right caudate values were averaged, and their means were calculated to assess the specific binding.

2.3. Fast Cyclic Voltammetry

Carbon Fibre Microelectrodes. Carbon fibre microelectrodes were constructed by inserting a single carbon fibre (Goodfellow Cambridge Ltd., Huntingdon, UK), 7 μm in diameter, into a 10 cm long borosilicate glass capillary tube. The capillary tube was then pulled using an electrode puller (P-30, Sutter Instruments Co., Novato, CA, USA) and the exposed carbon fibre was cut to approximately 70 μm under a microscope using a scalpel. Microelectrodes were backfilled with a saline solution before a length of copper wire was inserted into the end so it could be connected to the head-stage. A Ag/AgCl reference electrode and a steel wire auxiliary electrode were also connected to the head-stage and positioned within the recording chamber fluid, well away from the slice. Carbon fibre electrodes were calibrated using 5 or 10 μM dopamine in artificial cerebro-spinal fluid (aCSF).

Fast cyclic voltammetry. A triangular voltage waveform was applied to a carbon fibre microelectrode, which oxidises dopamine at ~600 mV. Calibrations of electrodes in a known concentration of dopamine allow the recorded Faradaic current to be converted into the relevant neurotransmitter concentration. Using a Millar Voltammetric Analyser (PD Systems, West Molesey, UK) we sampled dopamine at 8 Hz. Changes in the sampled signal were captured using a CED1401 micro3 analogue-to-digital converter (Cambridge Electronic Design (CED), Cambridge, UK), displayed using Spike2 v7.1 data capturing software.

Electrical stimulation protocol. Bipolar tungsten electrodes, with their tips 400 μm apart, were used to locally stimulate the core of the nucleus accumbens. Pseudo-one pulse stimulation was used to avoid the activation of autoreceptors [33] which occurs approximately 500 ms after striatal dopamine release [34,35]. A train of 10 × 1 ms 10 mA pulses at 100 Hz was applied every 5 min using a Neurolog NL800 stimulus isolator (Warner Instruments, Hamden, CT, USA) under computer control (Spike, CED, Cambridge, UK).

Experimental protocol. To begin an experiment, slices were transferred from the slice saver to a laminar flow recording chamber that was supplied with aCSF via gravity feed, at a rate of 100 mL/h. Slices were left to equilibrate in the recording chamber for 30 min before starting stimulation of the slice, and the tips of both stimulating and recording electrodes were placed in the accumbens to record monoamine release. Recording took place from the beginning of this 30 min period as large spontaneous release events of dopamine can occur, which is indicative of poor slice health [36], and on such occasions (5–10%) the experiments were terminated.

2.4. Statistics

DAT binding data were analysed with a 1-way ANOVA. Voltammetry data were analysed for peak effects of each drug concentration on each brain slice, which was typically found around 45–50 min after drug application. Statistical analysis was carried out using SigmaPlot (v. 11.0), a 1-way ANOVA (independent variable = concentration) was used with post-hoc Tukey's. In all graphs data are presented as means $\pm$ SEMs and significance is set at $p < 0.05$.

2.5. Computational System Setup

Modeling of the Rattus norvegicus dopamine transporter (rDAT). The construction and refinement of the homology model of the *Rattus norvegicus* dopamine (rDAT) transporter has been previously reported [12] using established protocols used in the construction of a human DAT (hDAT) model [37–40]. Briefly, we used Modeller 9v17 [41] and the previously published sequence alignment of the NSS family of proteins to first construct the transmembrane (TM) part of the rDAT (residues 57–589) based on the recent crystal structure of the *Drosophila melanogaster* dopamine transporter (dDAT) bound to dopamine (PDB ID: 4XP1) [9]. An adaptation of this sequence alignment, created by the Alignment-Annotator web server [42], is provided in Figure S1—in the Supplementary Material for convenience. The newly crystallized dDAT structure is well suited as a template for homology modeling of rDAT because the overall sequence identity is >50% [6], with the sequence identity between the TM segments of rDAT and dDAT being 61%, and having a Root Mean Square Deviation (RMSD) of <1 Å for the critical regions of the binding site and ion binding sites, TMs 1, 6 and 8 [12], which are key to the inferences we describe herein.

For completion, we also used the sequence alignment in Figure S1 and the N- and C-terminal regions modelled for hDAT from ab initio methods [40] to include Modeller 9v17 [41] homology models of the terminal domains for rDAT. Based on the align module of Modeller 9v17 [41], two functional Na^+ and one Cl^- ion in 4XP1 were also added to the S1 binding site. PROPKA [43] was used to determine the protonated state of the ionizable Glu490 residue of rDAT while a disulfide bond was introduced in EL2, between Cys180 and Cys189.

Compound preparation and docking. The chemical structures in the (S)-enantiomer of the five compounds (Figure 1) were built using the LigPrep module of Schrödinger Release 2017-1 [44] with the OPLS3 force field. The prepared compounds all carried a net positive charge as assigned by Epik [44]. The rDAT homology model was prepared using the Protein Preparation Wizard module in Maestro, following which the Induced fit docking (IFD) protocol, in the Schrödinger software suite was implemented to dock 2-MXP (Figure 1) to the homology model of rDAT. Since 2-MXP is one of the largest of the five compounds it was used to create a greater volume in the binding site, that was subsequently replaced by the other compounds. The residues Phe76, Asp79, Ser149, Val152, Tyr156, Asn157, Phe326, Val328 and Ser422, previously identified as important for binding psychostimulants of comparable size [10,11], were used to define the docking grid box. Dockings was then performed using standard precision (SP). Random initial positions and conformations of the ligand were screened for clashes with the protein and subsequently refined by allowing flexibility of the side-chains in the binding site.

The bound 2-MXP was then replaced with the other compounds to create the corresponding complexes (rDAT-DPH, rDAT-CLD, rDAT-3-MXP and rDAT-4-MXP) by aligning the backbone atoms

to overlap the binding positions, while maintaining the amino (NH) group interaction with Asp 79. In the absence of a crystal structure of DAT with these compounds we hypothesised that the best binding pose for these compounds would initially exploit the key interactions of residues Phe76, Asp79, Ser149, Val152, Tyr156, Asn157, Phe325, Val327 and Ser421, as seen in the crystal structures of dopamine, amphetamine, MDMA and cocaine [9] and which was also validated and exploited for docking 5-MAPB and 5-APB in a previous study [12]. The force-field parameters for the compounds were obtained from the Acellera small molecule parameterization tool implemented in the HTMD 1.11.2 suite [45].

2.6. Molecular Dynamics Simulations

The rDAT complexes (rDAT-DPH, rDAT-CLD, rDAT-2-MXP, rDAT-3-MXP and rDAT-4-MXP) were immersed in biophysically relevant membrane environments, a mixture of POPE/POPC/PIP_2/POPS/cholesterol, closely resembling the neuronal cell plasma membrane, with explicit water, internal ions and added salts as described previously. See the earlier work for details of the protocol [12].
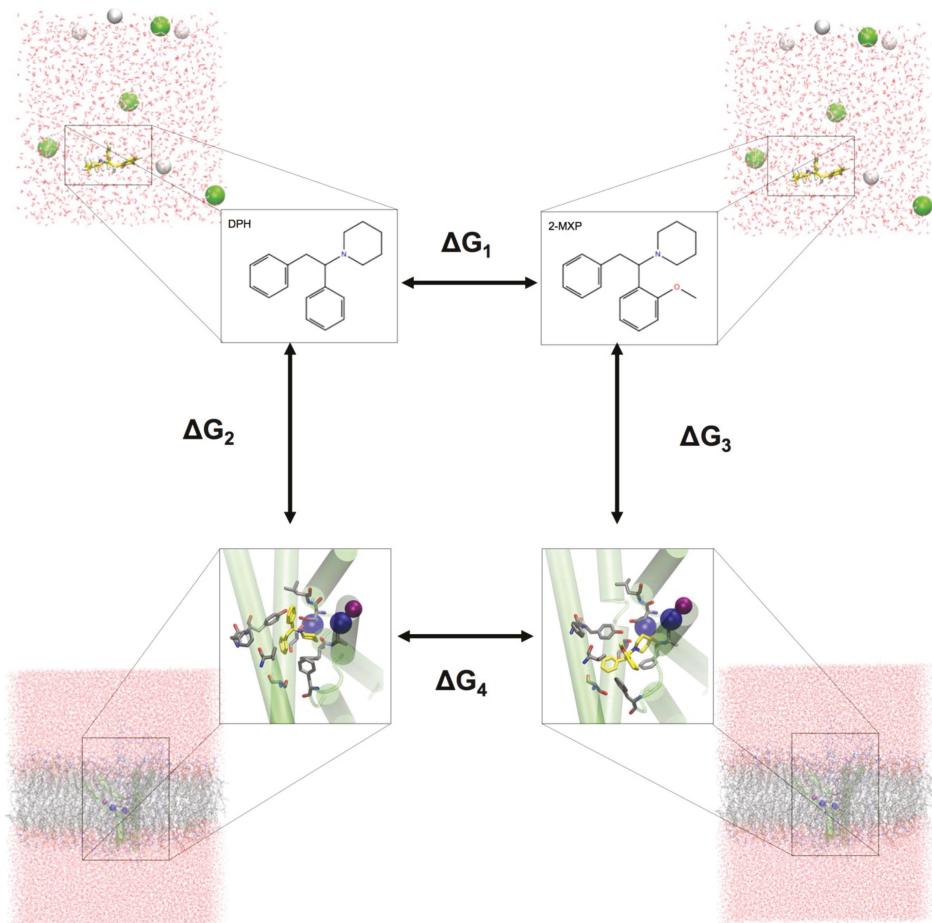
For all complexes a previously described multistep equilibration protocol [46] was performed with the NAMD software, version 2.9 [47] to remove the close contacts in the structure, the backbones were initially fixed and then harmonically constrained, and water was restrained by small forces from penetrating the protein-lipid interface. The constraints on the protein were released gradually in three steps of 300 ps each, changing the force constants from 1 to 0.5, and 0.1 kcal/(mol Å2), respectively, with a time step of 1 fs. This was then followed by a short (100 ns) unbiased MD simulation performed with a 2 fs integration time-step and under constant temperature (310 K) maintained with Langevin dynamics, and 1 atm constant pressure achieved by using the hybrid Nosé-Hoover Langevin piston method on a flexible periodic cell to capture long-range effects. The simulated system, including the transporter embedded in a membrane patch and water layers on each side containing Na^+ and Cl^- ions (corresponding to a concentration of 150 mM NaCl), was composed of approximately 266,590 atoms in a box with the final dimensions of 138 × 146 × 153 Å.

After this equilibration phase, unbiased production MD simulations were carried out using GPUS and the ACEMD software [48] with an established protocol [12,39] for a further 300 ns. Briefly, the simulations employed the all-atom CHARMM27 force field for proteins with CMAP corrections [49] as well as the CHARMM36 force field for lipids [50], the TIP3P water model, and the CHARMM-compatible force-field parameter set for PIP_2 lipids [51]. The PME method for electrostatic calculations was used, along with 4 fs integration time-step with standard mass repartitioning procedure for hydrogen atoms implemented in ACEMD. More details about the computational protocol can be found in (Khelashvili et al., 2015b). The trajectories were analysed with the R software [52] and VMD [53] for graphical representation.

In total, at least 3 μs of simulation time including the MD runs and the following free energy perturbation (FEP) calculations were accumulated.

2.7. Free Energy Perturbation Calculations

To evaluate the change in the binding free energy of the four compounds (2-Cl-DPH, 2-MXP, 3-MXP and 4-MXP) from DPH, we utilized a two-step free energy calculation approach. As an example, we show in Figure 2 the change in the binding free energy between compounds DPH and 2-MXP. This can be calculated by either $\Delta G_4 - \Delta G_1$ or $\Delta G_3 - \Delta G_2$. However, the change from $\Delta G_4 - \Delta G_1$ is computationally more practical. ΔG_1 represents the unbound solvent (ligand in water) state while ΔG_4 represents the bound (protein-ligand complex in water) state. We are calculating the relative binding affinity of two compounds rather than the absolute protein-ligand binding free energy calculation which is more challenging as the introduction of the protein adds significantly more degrees of freedom to the system [54].

Figure 2. The thermodynamic perturbation cycle between the compounds DPH and 2-MXP. A similar cycle would apply to the free energy change from DPH to the other three compounds (2-Cl-DPH, 3-MXP and 4-MXP). Here we highlight the complexity of the full systems in each step of the transformation as well as the components (compounds, protein, ions and membrane) that are boxed. ΔG_1 represents the unbound solvent (ligand in water) state while ΔG_4 represents the bound (protein-ligand complex in water) state.

Two independent transformations between each of the four compounds (2-Cl-DPH, 2-MXP, 3-MXP and 4-MXP) and DPH were calculated in both a water-solvent environment with both the protein (and membrane) present and absent, respectively. The free energy change ΔG of each perturbation was evaluated by the alchemical free-energy perturbation (FEP) approach [55,56] that has been applied previously to de novo mutations in the human dopamine transporter [37,38].
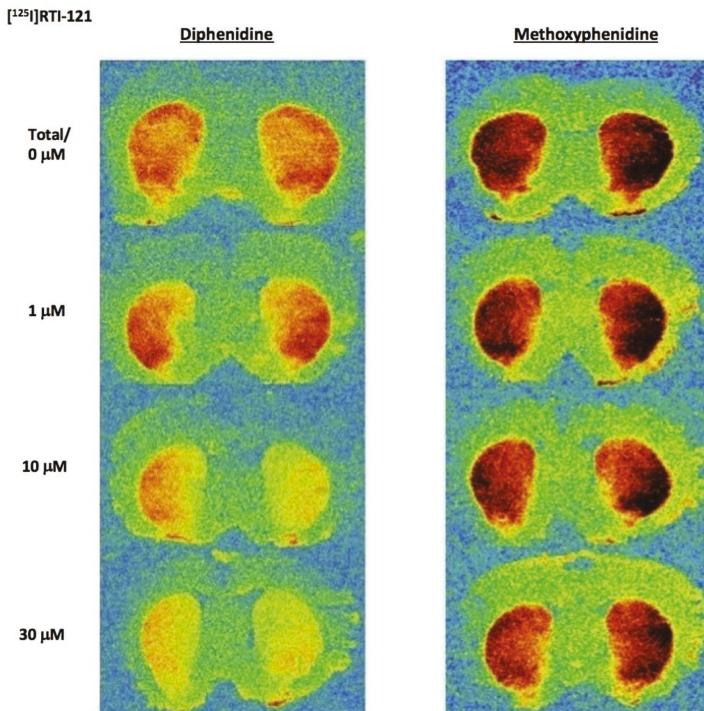
The free energy perturbation procedure with a softcore potential implementation was carried out with the NAMD software version 2.12 [47] and with the same simulation systems with explicit solvent as described above. For the FEP computation, the coupling parameter λ varied from 0 to 1 such that each window did not exceed 3 kcal/mol for a total of 400 ps for the full transformation. In the hysteresis tests the results differed from the annihilation in the same interval by ~1 kcal/mol. Each reported value is the average of at least three runs starting from different points (after at least 100 ns) of the MD

trajectories. Table S1 of the Supplemental Information gives the details of the bound (protein-ligand complex in water) state or ΔG_4. Using the restraining potential approach (Wang et al., 2006), a potential representing the interaction of the atoms being annihilated with the binding residues, including the crucial salt bridges between the N1 atom of each compound and the CG of Asp 79 and OH of Tyr 156 in the S1 site were applied [10,11]. The final solvation energies were calculated as the algebraic sum of the FEP and restraining energy values. A similar protocol has been described elsewhere [37,46,57].
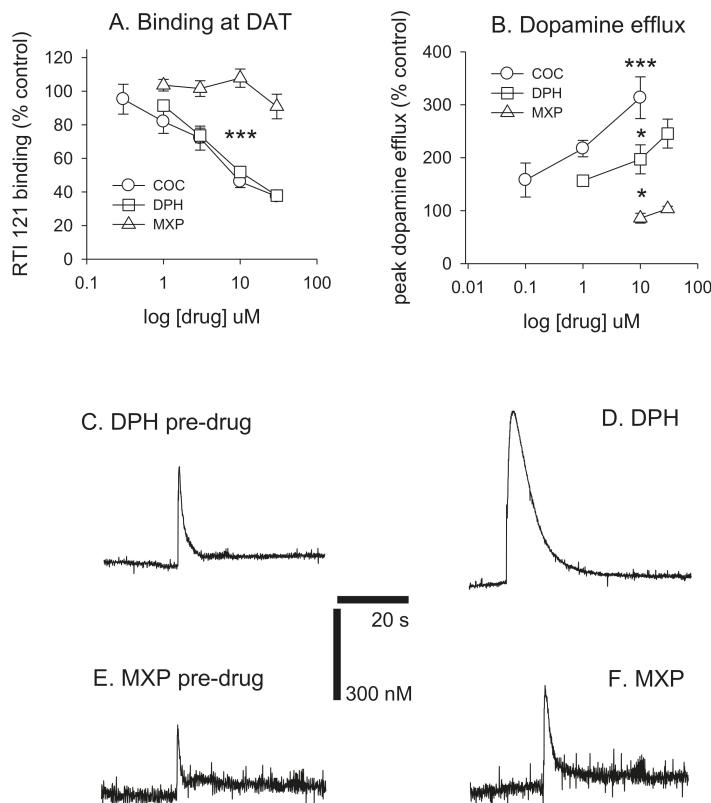
The aqueous solvation energy of the ligand in the simulation system was calculated for direct comparison in a $30 \times 30 \times 30$ Å^3 water box containing two Na$^+$ ions and one Cl$^-$ ion (equivalent to 120 mM NaCl) using exactly the same FEP/MD procedure as above but without restraints.

3. Results

Diphenidine displaced [^{125}I]RTI-121 (RTI) binding in a concentration-dependent manner ($F(4, 29) = 33.26$, $p < 0.001$) with 3, 10 and 30 µM causing a significant reduction in RTI binding ($p < 0.05$) (Figures 3 and 4A). There was no effect of methoxphenidine on RTI binding ($F(4, 29) = 1.47$, $p = 0.24$). Cocaine was also tested for comparison (autoradiograms not shown); cocaine displaced RTI binding in a concentration-dependent manner ($F(5, 35) = 18.701$, $p < 0.001$. Tukey's test revealed that 3, 10 and 30 µM cocaine significantly reduced RTI binding (all $p < 0.05$). A 1-way ANOVA was used to compare the 3 drugs at 10 µM (the only common concentration used in the dopamine efflux experiments) and there was a significant difference between the 3 drugs ($F(2, 17) = 72.683$, $p < 0.001$. Tukey's showed that both cocaine and diphenidine caused a significant reduction in RTI binding vs. methoxyphenidine (Figure 4A).

Figure 3. Representative autoradiograms showing the binding of the radioligand [^{125}I]RTI-121 in the brain striatal sections in the presence of increasing concentrations of DPH and 2-MXP (MXP).

Figure 4. Top panel (**A,B**): Comparative effects of DPH, 2-MXP (MXP) and cocaine-quantitative in vitro data: (**A**) Specific DAT binding expressed as % of [^{125}I]RTI-121 binding; *n* = 6 per drug concentration. (**B**) peak DA efflux as measured by means of fast cycling voltammetry *n* = 4–7. Bottom panels (**C**–**F**): Representative dopamine efflux events after brief electrical stimulation for DPH and 2-MXP (10 pulses at 100 Hz). (**C,E**) show control dopamine efflux prior to drug application. (**D,F**) show dopamine efflux 60 min after drug administration. 60 s of data is shown for each trace and peak dopamine efflux is ~250 nM under control conditions. DAT: dopamine transporter; COC: cocaine; DPH: diphenidine; MXP: methoxyphenidine. Values are means ± SEM. Panel A: *** *p* < 0.001 for both COC and DPH vs. MXP at 10 µM. Panel B: * *p* < 0.05 for COC vs. DPH and for DPH vs. MXP, *** *p* < 0.001 for COC vs. MXP, all for 10 µM drug concentrations. For drug concentrations vs. control statistics see text.

DPH also increased peak dopamine efflux after electrical stimulation ($F_{(3, 19)}$ = 14.405, *p* < 0.001, with 10 and 30 µM significantly increasing dopamine efflux vs. controls (both *p* < 0.05). 2-MXP had no significant effect on peak dopamine efflux $F_{(2, 15)}$ = 1.91, *p* = 0.187. MXP voltammetry was only tested at 10 and 30 µM. Cocaine also increased dopamine efflux ($F_{(3, 21)}$ = 14.907, *p* < 0.001). Tukey's revealed that both 1 and 10 µM significantly increased dopamine efflux (both *p* < 0.05). We also compared drugs at 10 µM using a 1-way ANOVA; $F_{(2, 14)}$ = 19.884, *p* < 0.001) with post-hoc Tukey's revealing that cocaine had a significantly greater effect on dopamine efflux vs. both diphenidine (*p* < 0.05) and methoxyphenidine (*p* < 0.001) and diphenidine had a significantly greater effect on dopamine efflux vs. methoxyphendine (*p* < 0.05). See Figure 4B. Dopamine efflux events after brief electrical stimulation (10 pulses at 100 Hz) are shown in Figure 4C–F, demonstrating the differences between DPH and 2-MHP in their respective abilities to increase dopamine levels at 30 µM.

*3.1. Relative Binding Free Energies*
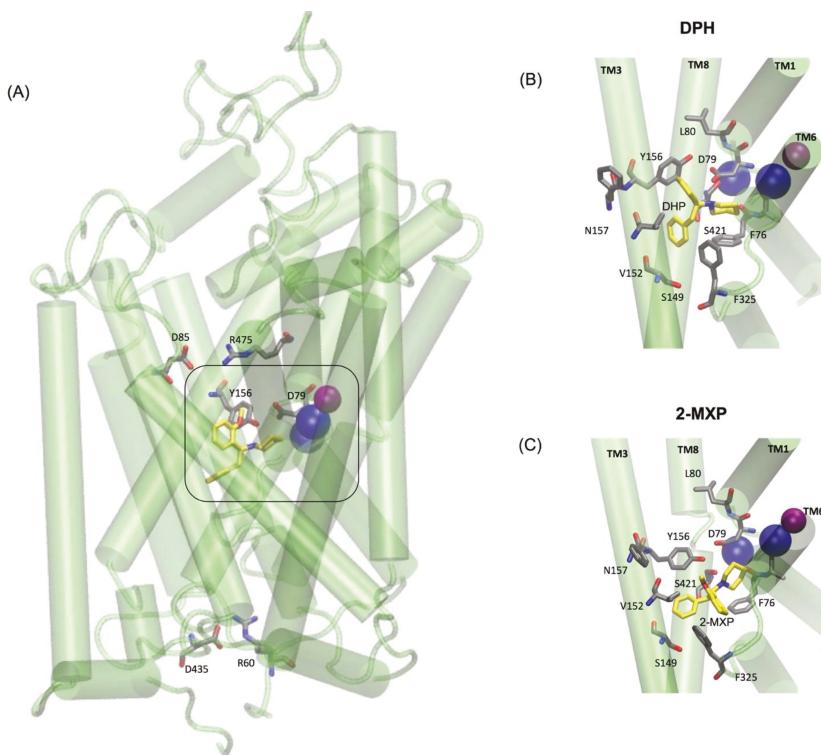
The binding free energy change from DPH to its aryl-substituted 1,2-diarylethylamines analogs (Figure 1) are calculated and compared with the previously determined experimental IC_{50} values from rDAT (Table 1) [22]. Free energy methods have been shown to have good chemical accuracy [56,58] in predicting the binding free energy change ($\Delta\Delta G$), which is the cost in free energy of substituting one compound with another compound. This is usually in combination with well-tuned force fields that have reasonable agreement with experimental results, where the RMSD in hydration free energy of small organic molecules between calculated and experiment are ~1.0 kcal/mol using the more common force fields like CHARMM. Therefore, the hypothetical binding poses for each compound as described above, which is shared by many other compounds in the binding site should give the best correlation between the predicted binding free energy and the experimentally measured binding affinity IC_{50} values. A positive value would suggest a cost in energy, thus the substituted compound should be less favourable. Conversely, a negative value means the substituent is more favourable.

Table 1. Calculated binding free energy change ($\Delta\Delta G$, kcal/mol) of the substituted analogs against DPH and their experimental IC_{50} values (μM)* from Wallach et al., 2016. ΔG_1 represents the unbound solvent (ligand in water) state while ΔG_4 represents the bound (protein-ligand complex in water) state.

Compound	ΔG_1 w.r.t. DPH (kcal/mol)	Average ΔG_4 w.r.t. DPH (kcal/mol)	$\Delta\Delta G_{4-1}$ w.r.t. DPH (kcal/mol)	IC_{50} (μM)*
DPH	0	0	0	1.99
3-MXP	1.90 ± 0.2	1.25 ± 0.5	-0.65 ± 0.3	0.587
4-MXP	2.64 ± 0.1	1.89 ± 0.5	-0.75 ± 0.4	2.23
2-Cl-DPH	-7.19 ± 0.2	-6.84 ± 0.7	0.35 ± 0.5	10.5
2-MXP	11.96 ± 0.2	15.59 ± 0.8	3.63 ± 0.6	30

Based on the initial rDAT complexes constructed by docking, MD simulations were performed to incorporate conformational flexibility into both rDAT and the corresponding ligands (2-MXP, 3-MXP, 4-MXP, DPH, CLD) to assess the persistence of the key interactions in the S1 site (Phe76, Asp79, Ser149, Val152, Tyr156, Asn157, Phe326, Val328 and Ser422). As such, the monitored Root Mean Square Deviation (RMSD) of the transmembrane regions of rDAT showed that the RMSDs reached equilibrium within 100 ns simulation for each complex (Figure S2A). Structural superimposition of the docking pose and a representative snapshot from the MD simulations for the five compounds in the rDAT binding site indicates some flexibility (e.g., the RMSDs of DPH and 2-MXP before and after MD simulation were 0.56 Å and 0.75 Å, respectively (Figure S2B)), but the key interactions such as the salt bridge between the protonated nitrogen of ligand and the Asp79 was preserved. As such, snapshots were taken at 100 ns and subsequently at 150 ns and 175 ns to further estimate the molecular basis of 2-MXP, 3-MXP, 4-MXP, DPH and CLD binding specificity to rDAT via the FEP method.

The two oppositely charged pairs of residues, Arg 85–Asp 475 and Arg 60–Asp 435, functioning as the extracellular (EC) and intracellular (IC) gates respectively, were maintained as well as the stabilizing hydrogen bond between Asp79 and Tyr156 shown to be important in stabilizing the S1 binding pocket [10,12] (Figure 5A). Additionally, as previously mentioned the binding pose for each compound was aligned so that they maintained the amine (NH) group interaction with Asp 79. For the purpose of the free-energy calculations this was aided by the use of distance restraints between the crucial salt bridge of the N1 atom of each compound and the CG of Asp 79 and OH of Tyr 156.

Figure 5. (**A**) Simplified representation of the molecular model of rDAT and the positions of the two oppositely charged pairs of residues, Arg 85–Asp 475 and Arg 60–Asp 435, functioning as the extracellular (EC) and intracellular (IC) gates respectively and the Asp79 and Tyr156 residues shown to be important in stabilizing the S1 binding pocket (boxed) via a hydrogen bond. rDAT in complex with (**B**) DPH (yellow) and (**C**) 2-MXP (yellow). Each of these distinct compounds occupies a binding pocket that is deeply buried in the transporter structure. Selected central binding site residues from each compound are shown in grey and labelled respectively. Additionally, blue and purple spheres represent the internal sodium and chloride ions, respectively.

Interestingly, the values for $\Delta\Delta G$ reveal ranked predictive binding free energies with respect to DPH as follows 4-MXP (-0.75 kcal/mol) > 3-MXP (-0.65 kcal/mol) > DPH (0 kcal/mol) > 2-Cl-DPH (0.35 kcal/mol) > 2-MXP (3.63 kcal/mol).
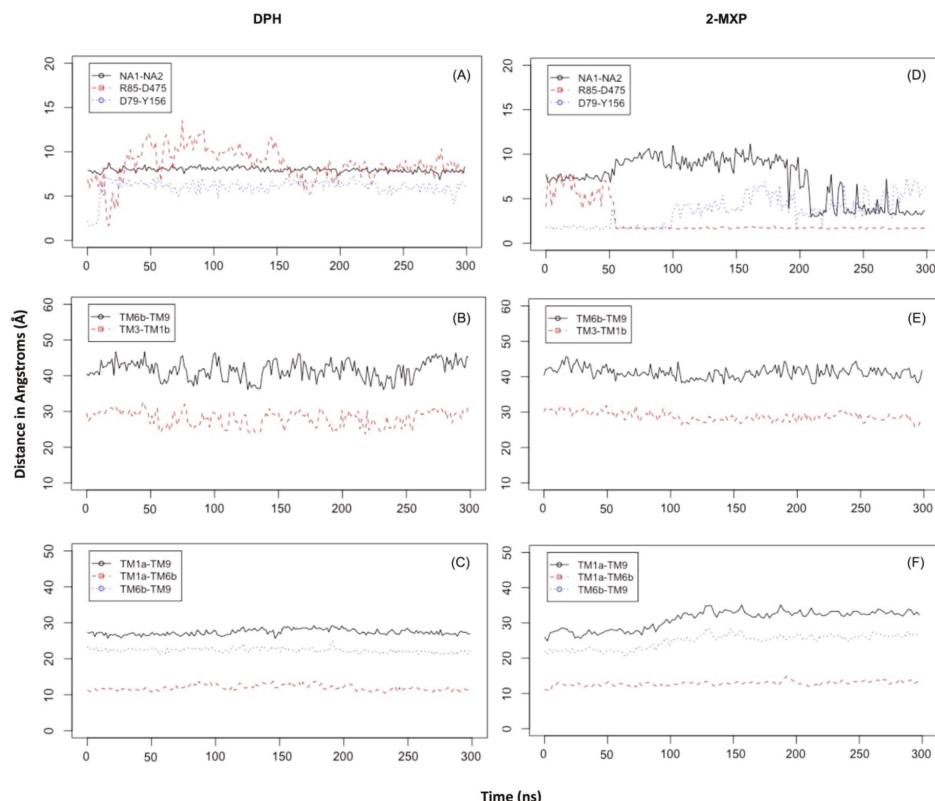
The $\Delta\Delta G$ values from DPH to 2-Cl-DPH (0.35 kcal/mol) and DPH to 2-MXP (3.63 kcal/mol) are positive indicating unfavourable substitutions, with the most unfavourable being 2-MXP. This is in agreement with the IC_{50} values whereby 2-MXP (30 μM) is less potent at rDAT compared to 2-Cl-DPH (10.5 μM).

The measured IC_{50} values for 3-MXP (0.587 μM) and 4-MXP (2.23 μM) while showing unfavourable changes are quite close to DPH (1.99 μM). While the change in binding free energies ($\Delta\Delta G$) are small, they suggest favourable substitutions for the methoxy group in 3-MXP (-0.65 kcal/mol) and 4-MXP (-0.75 kcal/mol). With 4-MXP ranking slightly more favourable than 3-MXP, which is in disagreement with experimental data as the IC_{50} values show that 4-MXP is more potent.
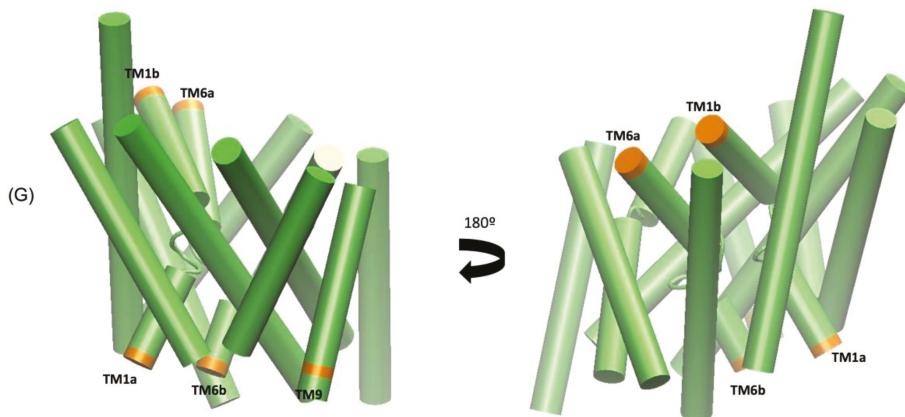
3.2. Molecular Dynamics Simulations

The extended molecular dynamics simulations also revealed some mechanistic insights into the different binding properties of each compound. The simulations reveal a number of concerted motions not only with the IC and EC gates but also distances between the Na1 and Na2 ions and the Asp 79 and Tyr 156 hydrogen bond. We also characterise the IC movements based on the concerted motions of the intracellular transmembrane TM1a, TM6b, and TM9 segments and additional movements on the extracellular side by the TM1b and TM6a segments, previously identified as markers for opening of the intracellular vestibule [39].

In DPH we see a disruption of the extracellular network with the ionic interaction between Arg 85 and Asp 475 for DPH (a feature not seen with the other compounds) (Figure 6A). This was complemented by the movements seen in the extracellular TM6b-TM9 and TM3-TM1b segments (Figure 6B). This suggests a mechanism involving a conformational change whereby DAT opens extracellularly. There is no evidence of Na2 ion destabilization or disruption of the hydrogen bond between Asp79 and Tyr156 during these timescales (Figure 6A) or any effect on the intracellular TM segments, TM1a-TM6b, TM1a-TM9, and TM6b-TM9 (Figure 6C).

Figure 6. *Cont.*

Figure 6. Time evolution in the simulations for rDAT when bound to DPH (**A**–**C**) and 2-MXP (**D**–**F**). (**A**,**D**) depict the distances between D79 and Y156, Na1 and Na2 and R85 and D475. (**B**,**C**,**E**,**F**) panels depict the Cβ-Cβ distances between residues in various extracellular and intracellular transmembrane (TM) segments respectively for I67 (in TM1a) and L446 (in TM9), I67 (in TM1a) and S332 (in TM6b) and S332 (in TM6b) and L446 (in TM9); E306 (in TM6a) and F171 (in TM3); and F171 (in TM3) and K92 (in TM1b). (G) The IC and EC segments in the protein TM regions from the middle and bottom panels are labelled and coloured in orange.

The simulations reveal that 2-MXP changes position in the rDAT binding site in such a way that its electron-donating, 2-methoxy (-CH$_3$O) group, that is also an ortho-substitution, is oriented away from the electron-donating phenolic-OH on Y156 (Figure 5C). Here we observe not only destabilization of the Na2 ion (Figure 6D) but also isomerization to the inward-facing state (as monitored by the movements in the intracellular TM segments—Figure 6F). There is no evidence of disruption of the extracellular network, instead the ionic interaction between Arg 85 and Asp 475 becomes more stable (Figure 6E).

At these timescales there is some evidence of isomerization with respect to 2-Cl-DPH at rDAT (Figure S1—in the Supplemental Material) as seen in Na1 and Na2 distance change (Figure S3A) and the increase in distances in the intracellular TM segments (Figure S3C). The extracellular network remains intact as well as the D79-Y156 hydrogen bond (Figures S3A and S3B). 2-Cl-DPH also changes position (tilts) in the binding site but it also contains an electron withdrawing chloride in the ortho-position, giving rise to an electron-poor ring substitution which could also explain its unfavourable IC$_{50}$ and ΔΔG values.

With respect to 3-MXP and 4-MXP, the -CH$_3$O group substitutions are classified as meta- and para-aromatic substitutions, respectively (Figures S4 and S5). Since we know that chemically the -CH3O group is described as an activator or electron donor to the ring at these positions this could potentially explain the more favourable ΔΔG values compared to 2-Cl-DPH and 2-MXP. Similarly, to 2-MXP, 4-MXP not only displays destabilization of the Na2 ion but also isomerization to the inward-facing state (Figure S5A,C). There is also no evidence of disruption of the extracellular network, instead the ionic interaction between Arg 85 and Asp 475 becomes much more stable (Figure S5B). At these timescales, 3-MXP does display evidence of isomerization to the inward-facing state (Figure S4C), with increased minimum distances changing between the intracellular TM segments, TM1a-TM6b, TM1a-TM9, and TM6b-TM9 but there is no change in the distance between Na1 and Na2 (Figure 4A) or the extracellular network (Figure 4B). Longer simulations are needed to fully explore these changes.

4. Discussion

In the present study, we analysed the mechanism of binding between rat DAT and aryl-substituted 1,2-diarylethylamines using docking and alchemical free energy approaches in conjunction with in vitro neurobiological experimental measurements, which we applied to 2 diarylethylamine NPS of that class, diphenidine and methoxphenidine. While DPH and 2-MXP exert their dissociative effects via *N*-methyl-D-aspartate receptor antagonism [22], their psychoactive effects can also be influenced by their binding to the monoamine transporters, with DPH and 2-MXP having their highest affinity for the DAT, followed by the noradrenaline transporter (NET) and serotonin transporter (SERT) [30]. DPH and 2-MXP have markedly different functional potencies at DAT, as represented by their IC_{50} values of 1.99 μM and 30 μM for DPH and 2-MXP, respectively, at human DAT stably expressed in HEK293 cells [22]. For the sake of comparison, the IC_{50} of cocaine as the most potent stimulant ranges from 0.4 μM to 1.3 μM at DAT expressed in transfected HEK293 cells, as published in the literature [59].

Our neurobiological findings suggest that DPH significantly increased dopamine efflux, as a result of binding to DAT, demonstrated in the competition with 125-iodine labelled RTI-121 in rat brain sections, whereas 2-MXP had no significant effect on either RTI-121 binding or evoked dopamine efflux in the nucleus accumbens in vitro. Interestingly, despite displacing RTI-121 binding with similar potency to cocaine, DPH had a significantly lesser effect on dopamine efflux as measured using fast cyclic voltammetry. These data, consistent with the IC_{50} differences reported by Wallach et al., 2016, suggest that DPH exhibits pro-dopaminergic stimulant-type effects in the brain tissue, and therefore this NPS can have some addictive liability. In addition to suggesting that DPH might have addictive liability due to its effects on dopamine efflux, we might also tentatively highlight its potential utility as a pharmacotherapy. Dopamine transporter ligands might be useful as substitution or maintenance treatments for psychostimulant abuse [60] or as treatments for attention deficit hyperactivity disorder (ADHD) or even as treatments for depression, for example bupropion. Given DPH is also an antagonist at NMDA receptors, as well as a DAT blocker, it may even have potential as a fast-acting antidepressant as seen with ketamine [61]. We have previously reviewed the potential use of novel psychoactive substances, including dissociatives, such as DPH, in CNS disorders [62].

On the contrary, 2-MXP, despite its apparent chemical similarity to DPH, does not exhibit pro-dopaminergic stimulant effects as assessed in the brain tissue in vitro. It is most interesting to understand why subtle structural differences result in such varied stimulant features of novel psychoactive substances (NPS) and what basic atomistic interactions of DAT as the biological target for stimulants decide about the discrimination of potential stimulants at DAT.

Therefore, we calculated relative binding free energy of the analogs 2-Cl-DPH and MXP. That exercise showed unfavourable substitutions when compared to DPH, in agreement with the experimental IC_{50} values, and consistent with our in vitro comparison of the pro-dopaminergic effects of DPH and MXP. While, the calculations using two other compounds 3-MXP and 4-MXP reveal favourable substitutions, although their IC_{50} values from Wallach et al. [12] show small unfavourable changes compared to DPH. Conformational changes emerging over long-scale simulations have also indicated different structural and dynamic elements of the mechanisms governing the interactions of these various compounds with DAT. Structural rearrangements in DAT when bound to DPH show evidence of an outward facing conformation, specifically the rearrangement of the extracellular gates, a well-known conformation adopted by inhibitors like cocaine [10,11,63,64]. This observation is supported by the present in vitro data where DPH acts as a DAT inhibitor but weaker than cocaine, which can cause a 4-fold increase in DA efflux, as previously observed under identical conditions [65]. In contrast when 2-MXP is bound to DAT, it shows evidence of adopting an inward facing conformation of DAT, such as the spontaneous release of Na2 and the rearrangement of the intracellular gates. Although this is a conformation adopted by many substrate releasers like amphetamine and 5-MAPB [12], 2-MXP does not appear to be a DAT inhibitor nor does it reveal reverse transport at the concentrations used in this study (up to 30 μM; data not shown) (Figure 4—bottom panel). Longer atomistic simulations are needed to resolve the rearrangements seen for 2-MXP as

well as 2-Cl-DPH, 3-MXP and 4-MXP that also show stabilisation of the inward facing conformation of DAT.

While the free energy pertubation method itself is a good measure of the binding affinity between two molecules, it does highlight some considerations for future studies, including the size and position of the substitution. Most importantly, the geometric and dynamic properties of a modelled protein-ligand complex contribute entropically to the binding mode of ligands. Thus, it is still challenging to rank with chemical accuracy a series of ligand analogues in a consistent way for systems where there are considerable fluctuations in the binding mode. In this study we face this issue calculating relative binding free energies of the aryl-substituted 1,2-diarylethylamines analogs because of the presence of multiple metastable ligand orientations which can cause convergence problems. Distance restraints were utilised but the use of orientational restraints may further accelerate the convergence of these calculations [66]. We aim to further investigate this with other classes of NPS with different scaffolds and substitutions. Calculating the free-energy of binding from different snapshots of long atomistic-simulations may also reveal the energy change associated with binding pose changes.

This study also demonstrates the ability of the alchemical free energy approach in combination with docking and homology modeling to be an effective means of investigating and characterising novel psychoactive substances. Additionally, these methods highlight that a structural analysis is important when creating a stimulant profile as it adds to the insights of binding and functional data derived from functional studies applied in animal models. To our best knowledge, this is the first application of relative binding free energy calculation in the studies on the biological effects of NPS, and similar approaches are plausible with some other biological targets, which would expand our understanding of stimulant mechanisms of NPS and other drugs of addiction, as psychoactive effects of drugs can be influenced by their binding to other monoamine transporters, namely NET and/or SERT. It is important to understand the molecular determinants of stimulant actions which may underlie their distinct pharmacological effects at DAT and also at other transporters like SERT and NET as well as species effects.

This study considered the responses of both in vitro data and in silico data to psychostimulants in the same cellular background, rDAT. A previous study by Dawn Han and Howard Gu show that when popular psychostimulant drugs like cocaine, methylphenidate, amphetamine, methamphetamine and MDMA are tested for their relative affinities, their sensitivities at the human and mouse transporters were similar (Ki values are within 4-fold) [67]. Therefore, there is translational significance to this study supporting the use of rodent models to represent pharmacological, functional and now structural changes of psychostimulants when acting in humans. This is not surprising because rDAT and mDAT share 99% sequence similarity and identity to hDAT. Nevertheless, there is evidence and reason to investigate in detail species effects that could be driving potential differences like abolished reward mechanisms through mutations in mDAT and hDAT [68,69] in further publications and from longer simulations.

To conclude, our in vitro study with the brain tissue indicates that diphenidine can increase dopamine efflux as a result of its binding to dopamine transporter, whereas methoxphenidine has no significant effect on either RTI-121 binding or evoked dopamine efflux. These data suggest that diphenidine can have some addictive liability, unlike methoxphenidine, despite the chemical similarities of these two NPS.

The present computational study supports the neurobiological findings of DPH when compared with 2-MXP, and provides novel insights into the mechanisms that underpin the pro-dopaminergic, hence stimulant, interactions between NPS and DAT. Longer atomistic simulations are needed to confidently resolve whether the DPH stimulant behaves in a cocaine-like manner in terms of its effects on the conformational rearrangements within the DAT. While this novel in silico approach informs about the potential addictive effects of a prevalent dissociative NPS, diphenidine, it also tackles the core physical mechanisms that decide about addictive properties of other NPS, of direct relevance to the health risks linked with their use.

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Review

Abuse of Prescription Drugs in the Context of Novel Psychoactive Substances (NPS): A Systematic Review

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Abstract: Recently, a range of prescription and over-the-counter drugs have been reportedly used as Novel Psychoactive Substances (NPS), due to their potential for abuse resulting from their high dosage/idiosyncratic methods of self-administration. This paper provides a systematic review of the topic, focusing on a range of medications which have emerged as being used recreationally, either on their own or in combination with NPS. Among gabapentinoids, pregabalin may present with higher addictive liability levels than gabapentin, with pregabalin being mostly identified in the context of opioid, polydrug intake. For antidepressants, their dopaminergic, stimulant-like, bupropion activities may explain their recreational value and diversion from the therapeutic intended use. In some vulnerable clients, a high dosage of venlafaxine ('baby ecstasy') is ingested for recreational purposes, whilst the occurrence of a clinically-relevant withdrawal syndrome may be a significant issue for all venlafaxine-treated patients. Considering second generation antipsychotics, olanzapine appears to be ingested at very large dosages as an 'ideal trip terminator', whilst the immediate-release quetiapine formulation may possess proper abuse liability levels. Within the image- and performance-enhancing drugs (IPEDs) group, the beta-2 agonist clenbuterol ('size zero pill') is reported to be self-administered for aggressive slimming purposes. Finally, high/very high dosage ingestion of the antidiarrhoeal loperamide has shown recent increasing levels of popularity due to its central recreational, anti-withdrawal, opiatergic effects. The emerging abuse of prescription drugs within the context of a rapidly modifying drug scenario represents a challenge for psychiatry, public health and drug-control policies.

Keywords: drug abuse; novel psychoactive substances; NPS; pharmacovigilance; prescribing drugs' abuse

1. Introduction

Novel Psychoactive Substances (NPS; 'legal highs' or 'research chemicals') are molecules designed to mimic the effects of legal traditional recreational drugs with intense psychoactive effects and virtual non-detectability in routine drug screenings. NPS include synthetic cannabinoids, cathinone derivatives, psychedelic phenethylamines, novel stimulants, synthetic opioids, tryptamine derivatives, phencyclidine-like dissociatives, piperazines, psychoactive plants/herbs and a range of prescribed medications [1]. The term NPS was first used by United Nations Office on Drugs and Crime (UNODC) to refer to "substances of abuse, either in a pure form or a preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1961 Convention on Psychotropic Substances, but which may pose a public health threat" [2]. At present, the emergence of NPS, typically from outside Western countries [3], represents a considerable public health challenge. Moreover, in order to circumvent the present controls and regulations, NPS are constantly diversifying and being replaced [4]. This is being facilitated by the growing number of anonymous online marketplaces, called 'cryptomarkets',

which host many anonymous sellers whilst using untraceable cryptocurrencies [5]. NPS users report a range of reasons behind their preference for NPS as opposed to traditional drugs such as cannabis, cocaine and heroin, including typical lack of detectability, greater affordability, lack of stigma, and relative ease of online acquisition [6]. Recently, however, the phenomenon of using prescription drugs in an idiosyncratic way to resemble, or counteract, the effects of NPS, has increasingly been described. This phenomenon refers not only to high potency opioids (e.g., fentanyl) and 'exotic'/designer benzodiazepines—molecules already having been reported to be addictive [1]—but also: gabapentinoids [7], a range of stimulants [1], antipsychotics [8], antidepressants [9] and image- and performance-enhancing drugs (IPEDS, e.g., anabolic steroids, vitamins, clenbuterol and salbutamol) [10]. Among over-the-counter drugs, the two most common agents reportedly ingested in intentional abuse cases are the antitussive, dextromethorphan [11], and loperamide, a common antidiarrhoeal drug [12].
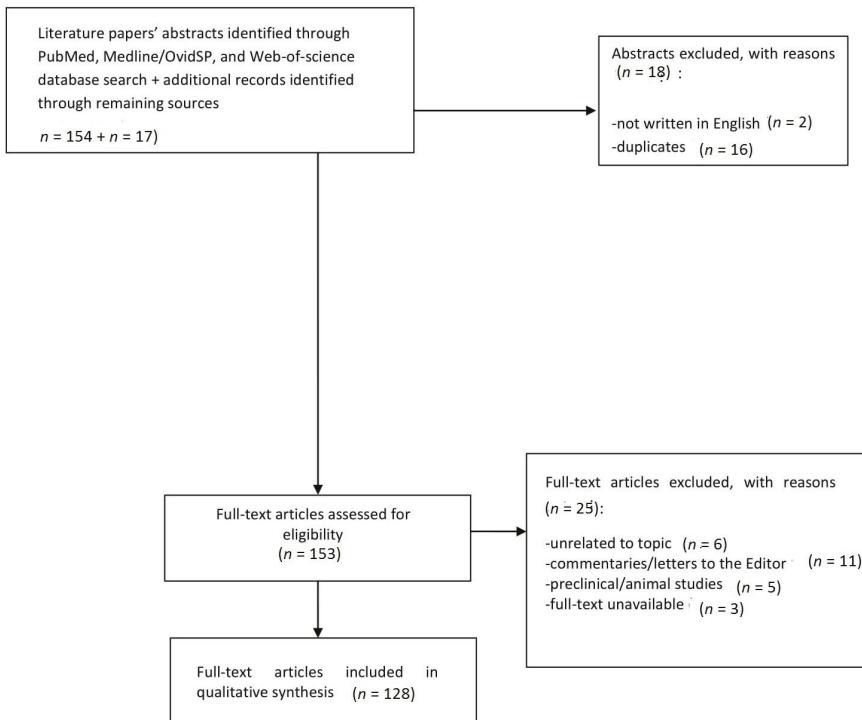
Any pharmacovigilance approach aims to detect, assess, understand and hopefully prevent adverse effects or any other medicine-related problems. From this point of view, there is a growing attention on prescription drugs and their addictive liability levels/diversion potential [7,8,10,12]. As the intended and the actual use of medicines differ between clinical trials and real-world use, pharmacovigilance activities are well placed to focus on the post-marketing phase. In Europe, those activities are coordinated by the European Medicines Agency (EMA) [13] through EudraVigilance (EV), which is the system for collecting, managing and analyzing information on suspected adverse reactions to medicines which have been authorized in the European Economic Area (EEA) [14].

This paper aims to provide a systematic review of the available literature relating to a preselected range of prescription medicines (pregabalin, gabapentin, quetiapine, olanzapine, venlafaxine, bupropion, loperamide, clenbuterol and salbutamol) previously reported as possibly being misused as NPS. For each molecule, a range of preclinical, epidemiological, and clinical pharmacological data will be provided.

2. Materials and Methods

A systematic review was carried out, consistent with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [15]. A literature search was performed on PubMed, Medline/OvidSP (includes Embase), and Web-of-Science; the current search was completed in February 2018 and was not associated with any time restrictions. We focused on pregabalin, gabapentin, quetiapine, olanzapine, venlafaxine, bupropion, clenbuterol, salbutamol and loperamide [Title/Abstract]. For each molecule, a number of search terms [Title/Abstract] were considered as follows: 'misuse', 'abuse', 'dependence', 'withdrawal', 'off-label use' and 'non-medical use'. In addition, the authors performed further secondary searches by using the reference listing of all eligible papers. All titles/abstracts were examined, and full texts of potentially relevant papers obtained. Relevant works were selected in order to obtain a full representation of the available literature data on the selected topic. Eligible studies were identified if they possessed a range of characteristics, including (1) peer-reviewed clinical/human studies; (2) at least an abstract with estimates and/or full availability of results; and (3) focusing on the misuse/abuse/dependence/withdrawal of pregabalin; gabapentin; quetiapine; olanzapine, venlafaxine, bupropion, loperamide, clenbuterol and salbutamol. The entire range of literature papers were included, e.g., experimental and observational studies; case reports; case series; and fatalities' reports. Although letters to the editor, conference proceedings, and book chapters were excluded from the systematic review, they were still considered in the retrieval of further secondary searches. SC independently extracted and collected relevant data; FS contributed to the analysis of the results and discussed possible issues and disagreements during the revision of the paper with SC.

From an initial list of 171 studies, 151 were identified as relevant and appropriate in terms of quality according to PRISMA checklists. Following this, duplicates, papers lacking an English abstract, letters to the editor, animal studies and papers unrelated to the topic were excluded, and 128 papers were finally considered for the current study. A flow diagram (Figure 1) describes the reasons for study inclusion/exclusion at each stage, is here provided.

Figure 1. Selection of retrieved studies.

3. Results

3.1. Gabapentinoids

Recently, the gabapentinoids, pregabalin and gabapentin, have increasingly been reported to be abused at the EU-wide level, in parallel with increasing levels of prescriptions, related fatalities and a growing black market [16–19]. Gabapentinoids are anticonvulsants but are also prescribed for a range of clinical conditions in neurology, psychiatry and rheumatology, whilst being used off-label for the treatment of benzodiazepine and alcohol dependence. Their effects are the result of calcium channel binding, resulting in decreased central excitability levels. Compared to gabapentin, pregabalin's binding affinity and potency are six times higher; pregabalin's more significant misuse potential may also be due to its more rapid absorption, faster onset of action, much faster attainment of maximum plasma concentration and higher bioavailability (>90%, irrespective of the dosage). Furthermore, gabapentinoids are thought to possess GABA-mimetic properties, whilst possibly having direct/indirect effects on the dopaminergic 'reward' system [7]. Gabapentionoid web enthusiasts report the ingestion of this compound alone or in combination with other drugs (e.g., cannabis, alcohol, opioids and other prescribed drugs), at a dosage range of 1000–4800 mg for gabapentin [20], and 750–12,000 mg for pregabalin [7]. Typical psychoactive effects include a sense of well-being/relaxation, euphoria, and even hallucinations [1]. In 2005, the Drug Enforcement Administration (DEA) placed pregabalin into Schedule V of the Controlled Substances Act (CSA) because of its potential for abuse [21] and a similar scheduling approach has recently been approved in the UK. Chiappini and Schifano [7] recently assessed the EMA EV database of pregabalin and gabapentin misuse-related Adverse Drug Reactions (ADRs) over the last decade. According to the Proportional Reporting Ratio (PRR) computation, abuse/dependence issues were more frequently reported for pregabalin compared

with gabapentin, hence confirming its higher addictive liability levels [7,22]. Furthermore, Emergency Department presentations involving intentional drug overdoses recorded by the National Self-Harm Registry (Ireland; 2007–2015), showed that gabapentinoids have been increasingly identified over time, with high dosages and polydrug abuse being reported [23]. Indeed, gabapentinoid fatalities are typically observed when these molecules are associated with other psychoactive drugs, especially opioids and other sedatives whose effects are potentiated by gabapentinoids [24,25].

3.2. Antidepressants

Consistent with a worldwide rise in antidepressant consumption [26,27], bupropion and venlafaxine have anecdotally emerged as increasingly being abused [1,28,29]. In examining a range of online communities and specialized web services, several antidepressant misusers' experiences may be identified [20]. These reports emphasise both bupropion's stimulant effects and venlafaxine's dissociative properties. Indeed, bupropion described as being ingested in very large quantities (up to 4050 mg/day, roughly 14 times higher than the maximal therapeutic dosage) in order to achieve an 'amphetamine-like high' [30]. In most abuse cases, its recreational use is associated with oral or nasal administration, but intravenous use has also been reported [28,30–34]. Bupropion pharmacology relies on its action both as a selective inhibitor of catecholamines (noradrenaline and DA) reuptake [35,36], and as a non-competitive antagonist of nicotinic acetylcholine receptors, hence being prescribed as well as an aid in smoking cessation [36]. Bupropion is known to be a cathinone derivative, that is, a beta-ketone amphetamine analogue with dopaminergic and noradrenergic effects, which may explain its misuse potential [37,38]. This is a reason for concern since bupropion is also used 'off-label' in a range of conditions, including attention-deficit/hyperactivity disorder, chronic fatigue, sexual dysfunction, and obesity. The adverse effects of bupropion misuse range from nasal pain to irritability, agitation, cardiac toxicity, hallucinations and seizures [39,40]. A retrospective review [41] on bupropion cases of intentional abuse reported to the US National Poison Data System highlighted an increase of 75% from 2000 to 2012, with the typical effects reported including tachycardia, seizures, agitation/irritability, hallucinations/delusions, and tremor; similar data were identified by the Toxicology Data Network of the US National Institute of Health (Toxnet) [42]. Typical bupropion abusers may present with a history of drug addiction [38,43,44] and/or are inmates, with bupropion having been removed from some US prison formularies [45–47]. Conversely, venlafaxine is a selective serotonin-norepinephrine reuptake inhibitor (SNRI) antidepressant, indicated [48] for the treatment of major depressive episodes, generalised anxiety disorder and social phobia, with off-label use including obsessive-compulsive disorder and chronic pain syndromes. Its reuptake effects are dose-dependent, with action progressively including serotonin (5-HT), norepinephrine (NE) and dopamine (DA). Venlafaxine's main active metabolite, desvenlafaxine, is highly inhibitive of NE transporter activities, further increasing the rate of DA turnover in the prefrontal cortex [49]. Both venlafaxine and its metabolite are not associated with monoamine oxidase inhibitory activity, which is responsible for the degradation of DA. Hence, venlafaxine abuse may be associated with DA increase in the prefrontal cortex [50], high affinity for D2 receptors adaptive changes in D3 receptors following its chronic administration and, finally, with the desensitisation of both 5-HT1A and beta-adrenergic receptors [51]. Dependence and withdrawal symptoms associated with both SSRIs and SNRIs have already been described, specifically with abrupt discontinuation of venlafaxine (including Extended Release (XR) formulation) after long-term use [9,52–54]. Symptoms range from mild to severe and include nausea, depression, suicidal thoughts, disorientation, stomach cramps, panic attacks, sexual dysfunction, headaches and occasional psychotic symptoms [55–59]; a newborn discontinuation syndrome has been described as well, at times associated with encephalopathy or paroxysmal episodes [60]. The management of venlafaxine withdrawal includes the use of other antidepressants (Ads) or venlafaxine tapering doses [61,62]. Furthermore, venlafaxine/'baby ecstasy' abuse has been reported, typically being the result of the intake of very large doses [63–65]. Consistent with this, studies have assessed drug and pharmaceutical consumption in England through wastewater analysis and

comparing it to NHS prescription statistics. Discrepancies have been observed in the case of venlafaxine, suggesting sales of non-prescribed venlafaxine, which are, therefore, not included within NHS data [66]. Furthermore, in a retrospective review of the records of the New Zealand National Poisons Centre over the period 2003-2012, rapidly increasing levels of enquiries were identified for a range of prescription medicines, including venlafaxine [67]. According to the EMA EV database from the last decade [68], the misuse-/abuse-/dependence- and withdrawal-related ADRs reported respectively for bupropion and venlafaxine show that bupropion may possess a higher recreational value due to its dopaminergic and stimulant-like activity, whilst the occurrence of a venlafaxine-withdrawal syndrome may be a significant issue for venlafaxine-treated patients; these data were confirmed by analysis of the UK-based Yellow Card Scheme [68].

3.3. Antipsychotics

Consistent with their increased prescription and availability [69], second-generation antipsychotic (SGA) (e.g., quetiapine and olanzapine) abuse has recently been reported [1,70–72]. Quetiapine appears to be the most documented SGA being abused; it is commonly administered in the 400–800 mg/day range for the treatment of schizophrenia; bipolar disorder; and as an add-on in major depression and anxiety [73–76]. Quetiapine is anecdotally known as 'Susie Q'; 'Quell'; and 'baby heroin' [75–79], with 'Q ball' and 'Maq ball', respectively, being combinations with cocaine, and marijuana. Crushed quetiapine tablets can be self-administered through nasal insufflation [79–81], although both oral [81–84], and intravenous [85–87] routes of administration have been reported. Consistent with these anecdotal clinical observations, post-marketing surveillance reports indicate an increase in quetiapine availability on the black-market [75,79,88–90]. Furthermore, quetiapine, either on its own or in combination with heroin and/or alcohol [91], is consistently associated with high rates of ambulance attendances, indicating greater community-level harm relative to other atypical antipsychotics [92]. Indeed, between 2005 and 2011, quetiapine-related Emergency Department visits increased in the USA by 90%, from 35,581 to 67,497 attendances [93]. A recent US National Poison Data retrospective analysis identified all cases of single-substance SGA exposures coded as 'intentional abuse' [94] during a 10-year period (2003–2013), quetiapine being the most represented molecule, followed by risperidone and olanzapine. Prison inmates and opioid addicts seem to represent the most at-risk populations [24,75,76,95–97]. Quetiapine psychotropic effects [86,87] are associated with both increased levels of DA in the nucleus accumbens (NAc) area [89,98–100] and D2 receptor blockage. As some pharmacodynamic mechanisms are shared by other non-misused SGAs [101–104], other factors [105,106] or pharmacological effects explaining the molecule misuse potential may include norquetiapine-related norepinephrine reuptake blockade [75], 5-HT7 antagonist properties and sigma receptor activation [107,108]. Quetiapine pharmacokinetics, mediated by the cytochrome CYP3A4, may play a part, as well, in facilitating its misuse [109]. Its XR formulation may be less frequently abused, due to the delayed (by approximately 3 h) and blunted (by approximately 67%) serum peak [88]; the tablet coating may also make snorting of the crushed tablets quite problematic [89].

Another SGA, olanzapine, is normally prescribed at a dosage of 5–20 mg oral daily in order to treat schizophrenia, bipolar disorder and resistant depression. Whilst being widely prescribed, it has been anecdotally reported, at dosages up to 50 mg, as the 'ideal trip terminator/modulator' after a psychedelic drug binge [110]. According to discussion forums/specialised websites [111], olanzapine is also being used to treat unwanted 'comedown' symptoms from drug/alcohol intake [112,113]. Consistent with this, clients on methadone maintenance treatment attending the National Drug Treatment Centre (NDTC) in Dublin reported levels of non-medical use of olanzapine, with dosages of up to 100 mg/day, in order to manage anxiety and improve sleep, and in a minority of cases, to 'get stoned' [114]. Olanzapine activity involves GABA-A receptors [115], hence the associated sedation, the rewarding glutamatergic stimulation of the ventral tegmental area DAergic neurons [116], the 5HT2C and histamine/H1 antagonist properties and the potent inhibiting action on the muscarinic M1 receptors [115,116]. In comparing quetiapine with olanzapine through the UK

Prescription Cost Analysis and the Drug Analysis Profiles of the Yellow Card Scheme, quetiapine was shown to be slightly less frequently prescribed but associated with a smaller total number of general reports, and hence, a comparatively higher number of abuse/dependence/withdrawal ADR reports [117,118]. In line with this, the OPPIDUM French addictovigilance network highlighted the emerging misuse of prescription molecules, and this included quetiapine as well [119]. Information from the previous 10 years from the EMA EV database relating to quetiapine and olanzapine misuse/abuse/dependence/withdrawal-related ADR reports [8] shows a higher misuse risk for quetiapine in comparison with olanzapine for the selected ADR reports. Indeed, quetiapine XR formulation was represented in only a smalll proportion of misuse cases, with both nasal and parenteral administration having been identified. Of particular interest was, in comparison with olanzapine, a higher risk of discontinuation/withdrawal syndrome following the abrupt cessation of quetiapine [75,113,120]. Finally, consistent with previous data [75,82–85,90,121–123], the quetiapine- and olanzapine-related fatalities reported on the EMA EV database were typically the result of a polydrug intake, which included opiates/opioids, antidepressants, and over-the-counter drugs [124,125].

3.4. Image-And Performance-Enhancing Drugs (IPEDs)

Over the last few decades, a range of prescribed and non-prescribed enhancement drugs have increasingly been self-administered [72] in order to improve the ageing process, and sexual performances, and to reduce hair loss, fatigue and other physiological conditions which are, at times, considered pathological in a society that strongly emphasises the importance of physical appearance [126]. Prescribed image- and performance-enhancing drugs (IPEDs) include anabolic-androgenic steroids (AAS), human growth hormone (hGH), steroid hormones (e.g., androstenedione), insulin, erythropoietin, diuretics, but also, β-2 agonists (e.g., clenbuterol and salbutamol) [127]. Their misuse is typically carried out within a polypharmacy context [128] with alcohol, cannabis/cannabinoids, cocaine, amphetamines/methamphetamines being ingested as ancillary drugs. Moreover, the recent reporting of IPED injecting practices are a reason for concern [129]; these mostly involve anabolic androgenic steroids, non-steroidal anabolic hormones (e.g., hGH and insulin), tanning peptides, cosmetic injectables such as botox and dermal fillers, etc. [130–132]. Among non-steroidal anabolic hormones, insulin seems to be misused for performance-enhancement purposes through several administration routes (intravenous, intramuscular and subcutaneous); indeed, insulin may help in achieving a decrease in fat deposition, an increase in muscle mass and positive mood changes, although serious hypoglycaemic episodes and other medical sequelae can occur as well [133,134]. Within the IPED group, anti-asthmatic beta-2 agonists have recently emerged as having potential for misuse, e.g., salbutamol for its performance-enhancing effects and clenbuterol for its hypertrophic and lipolytic effects. They are both included in the list of prohibited substances released by the World Anti-Doping Agency (WADA) [135], with salbutamol being allowed only as a component in the treatment regimen for athletes with asthma. Clenbuterol, even if different from anabolic steroids, has been also prohibited as an anabolic agent since 2006. In parallel with this, the Food and Drug Administration (FDA) banned the use of clenbuterol in food animals in 1991 and the European Union (EU) followed suit in 1996 [136]. Beta-2 agonists are synthetic molecules with sympathomimetic activity, prescribed as bronchodilators for the treatment of asthma. Clenbuterol is licensed for human use only in a few countries (Austria, Germany, Italy, Spain and Mexico), but not in the UK or the USA [137]. Clenbuterol, as a 'size zero pill', is popular and widely available on the web, being considered an ergo/thermogenic drug and hence, an anabolic burner [138], similar to caffeine, ephedrine, and thyroid hormones. Clenbuterol-associated lypolisis can occur via both β-2 adrenergic agonism and its specific action on the adipocytes' β-3 adrenergic receptors, which further facilitates lipolysis and weight loss [139–141]. While anti-asthmatic clenbuterol dosage ranges between 20 and 40mcg daily, the typical 'fat burning' dose is in the 120–160 mcg daily range; dosage starts at 40 mcg daily, gradually increases, and then remains at the highest dosage for a duration of 2–4 weeks [142]. In parallel with this, recent years have seen an increase in clenbuterol

exposure reported to poison control centres [143], with the molecule being used either as a dietary supplement [144] or as an adulterant in illicit drugs, such as cocaine [145]. Its adverse effects are dose-dependent and may include dysrhythmias and myocardial injury, headache, abdominal pain, nausea, and rhabdomyolysis [136,146–148]. Reports relating to salbutamol misuse have been less frequently mentioned [149–151]. Similar to clenbuterol, salbutamol's adverse effects are dose-related and may include tremor, restlessness, anxiety/agitation, tachycardia, atrial fibrillation, and myocardial ischaemia, especially in cases of overdosage, chronic use, or intravenous injection [152]. With respect to salbutamol, clenbuterol's higher levels of abuse potential could be associated with its pharmacological characteristics [143], such as its prolonged elimination half-life (35 h) and its higher lipophilicity, which can be associated with a fast transition through the blood–brain barrier. Consistent with this, salbutamol has been described as significantly less potent on a reinforcement schedule than clenbuterol [149,152–154]. Clenbuterol abuse-related fatalities have consistently been reported in the literature [136,140,147,155], whilst salbutamol is considered safer [156]. In this regard, Milano et al. [10] studied the 2006–2016 EMA clenbuterol- and salbutamol-related, misuse/abuse/dependence/withdrawal/overdose/off-label spontaneous reports. They found that clenbuterol, in comparison with salbutamol, had higher levels of misuse/abuse. These clenbuterol-related data were most typically from males and were associated with the intake of steroids [10], hence confirming previous reports [157,158].

3.5. Over-The-Counter (OTC) Medicines—Loperamide

Currently, over-the-counter (OTC) abuse ('pharming') is an internationally recognised problem, and the recent emergence of new forms, including online, of medicine supply, is alarming clinicians and health authorities nationwide. The EU introduced a strong legal framework for the licensing, manufacturing and distribution of medicines [159], but no measures have been taken so far for the distribution of OTC drugs, and it is hence, difficult to quantify their actual misuse and abuse [159–168]. Over previous years, the OTC antidiarrhoeal medicine loperamide has increasingly been reported as being diverted and used to achieve recreational effects [159–162]. Loperamide acts as a potent mu-opioid receptor agonist, albeit with predominantly peripheral activity on the myenteric plexus, hence primarily increasing the intestinal transit time by decreasing propulsive activity. Secondary peripheral effects are seen at κ-opioid and δ-opioid receptors as well [169,170]. Loperamide was initially placed by the US FDA in Schedule V of the Controlled Substance Act but then, after having assessed its safety profile with the conclusion of low levels of physical dependence risk, in 1988, it was made available for OTC use. In the 2–16 mg daily dosage, loperamide is considered safe and devoid of misuse abuse potential because of its rapid metabolism and poor blood–brain barrier (BBB) penetration. In doses of 50–300 mg, however, loperamide ingestion has been associated with euphoria, central nervous system depression [171–174] and even death [175]. Its diversion potential may be associated with its use as a relief from opioid withdrawal [176]. Anecdotally described as the 'poor's' methadone' [177], detailed loperamide dosage titration regimens are being reported online [20]. Related misuse case series [178] have reported both extremely high daily intakes (up to 1200 mg), and associated cardiotoxicity issues, such as QTc prolongation and torsades de pointes, QRS prolongation, ventricular dysrhythmias [179–182], syncope, and cardiac arrest [12,179,183,184]. The cardiotoxicity mechanism of loperamide is not clearly understood, although it may be due to potent inhibition of cardiac ion channels which is, in turn, associated with delayed repolarisation and QT prolongation [185–187]. Consequently, the FDA [175] has recently warned clinicians and users about the combination of loperamide with other drugs or herbal products that are known to prolong the QT interval, including Class 1A (e.g., quinidine, procainamide) or Class III drugs (e.g., amiodarone, sotalol) antiarrhythmics, antipsychotics (e.g., chlorpromazine, haloperidol, thioridazine, ziprasidone), antibiotics (e.g., moxifloxacin), and methadone. Loperamide ingestion has also been reported in association with P-glycoprotein (P-gp) substrates (e.g., quetiapine, cetirizine, oxycodone) or inhibitors (e.g., fluoxetine, citalopram, sertraline, omeprazole, quinine, quinidine, propranolol, ritonavir). These associations are associated with an increase in the low bioavailability of loperamide,

normally being <2%; plasmatic concentration; levels of euphoric effects; the capacity of possible contrasting opioid withdrawal symptoms [186–189]; and toxicity effects [175]. Concurrent use of loperamide with CYP3A4 inhibitors (e.g., itraconazole, grapefruit juice, omeprazole, tonic water and cimetidine) or CYP2C8 inhibitors (e.g., gemfibrozil) can increase its plasma levels as well, with recurrent ventricular tachycardia having been reported in a patient who was taking large recreational doses of both loperamide and the CYP3A4 inhibitor, famotidine [190]. Treatment of loperamide intoxication involves the use of naloxone, which may not be able to directly reverse loperamide cardiotoxic effects [191,192].

4. Discussion

The ever-increasing number of NPS emerging worldwide and the parallel changes in drug scenarios represent a challenge for psychiatric, public health and drug-control policies [193]. In line with this, the current systematic review has focused on a different range of prescribed medications which are indeed being used as NPS [1]. Within both online drug forum communities and social networks, there are some educated/informed users (the 'psychonauts') [194] who typically 'test' a range of psychotropics, including prescribed drugs, to achieve specific mindsets and eventually, share this information with peers [193]. However, in parallel with recently increased levels of access to the web, a large number of vulnerable subjects, including both children/adolescents and psychiatric patients, have been exposed to a range of 'pro-drug' information, and this is a reason for concern [193]. Although a number of online 'rogue' pharmacies have been shut down, this typically prompts the sellers to move to servers in overseas countries, leading to a growing black market [195].

It is intriguing that, for the range of prescription molecules discussed, including the fairly recently introduced gabapentinoids, pre-marketing processes were not been able to appropriately identify their abuse/misuse potential. However, similar to what happened with benzodiazepines and *z*-hypnotics, this potential has finally emerged over time. Present data seem to suggest that abuse liability-focused, pre-marketing laboratory testing may need to consider interaction studies with alcohol and/or other drugs [194,196]. Furthermore, post-marketing surveillance for substance abuse [197] should routinely be carried out to assess the abuse potential of newly released drugs, especially those with activity on the central nervous system (CNS) [198]. Indeed, lack of information on the abuse/misuse potential of a new medicine's interaction with the CNS does not mean that a specific medicine does not actually produce these effects. Furthermore, in order to look at how medicines are actually used in real life, modern pharmacovigilance should identify a range of technical tools and approaches to go beyond spontaneous reporting systems. Physicians should be vigilant when prescribing drugs with an abuse/misuse/diversion potential and carefully evaluate the possibility for some clients (inmates; people with a personal history of misuse or abuse) to be more vulnerable to these misuse activities. Finally, while a continuum of related professional training is needed, it may be important to consider a strategy to increase clients' access to treatment services, possibly through enhanced links between community pharmacists, who are the first professionals to identify a repeat supply issue, and prescribers/clinicians [198].

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Transitioning Bodies. The Case of Self-Prescribing Sexual Hormones in Gender Affirmation in Individuals Attending Psychiatric Services

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Abstract: Self-prescribing of sexual hormones for gender affirmation is a potentially widespread
and poorly studied phenomenon that many clinicians are unaware of. The uncontrolled use
of hormones poses significant health hazards, which have not been previously reported in the
literature. We have collected seven clinical cases in general adult psychiatry settings (both inpatient
and outpatients), describing transgender and gender non-conforming individuals' (TGNC)
self-prescribing and self-administering hormones bought from the Internet without any medical
consultation. Among these cases, two were taking androgens, and the rest were taking oestrogens.
The main reason for self-administration of hormones seems to be the lack of access to specialised
care due to discrimination and long waiting lists. We advocate for clinicians to be aware of
the phenomenon and proactively help TGNC individuals by enquiring about self-prescribing of
hormones, providing information and referring to the most appropriate treatment centre as well
as encourage a public debate on the discrimination and the stigma that TGNC population suffer
from. Overall, there is an urgent need for the implementation of different and innovative health care
services for TGNC individuals as well as more targeted prevention strategies on such underreported
and highly risky behaviours. Furthermore, it is necessary for every clinician involved in the care for
TGNC people to be aware of their special needs and be able to be an allied and an advocate to help in
reducing stigma and discrimination that affect the access to care for this often underserved population.

Keywords: transgender; gender reassignment; gender affirmation; self-medication; hormonal
replacement therapy (HRT); LGTBQ health; gender dysphoria; do it yoursfelf (DIY); identity; barriers
to care; discrimination

1. Introduction

A significant proportion of the population defines themselves as transgender, intersex non-binary
or gender non-conforming (in this paper, we will use "TGNC" for "transgender and gender
nonconforming" people as recommended by the American Psychological Association (APA)
guidelines [1]). A recent demographic study estimates that in the USA 0.39% (about one million
people) of the population define themselves as TGNC [2]. The exact number, however, might be bigger,
and it is very difficult to quantify the precise number due to the complex methodology in estimating the
numbers when gender non-conforming individuals are also considered in the statistics [3]. To estimate
the same data in the UK, it is more difficult because the Office for National Statistics (ONS) does

not produce estimates of the number of TGNC people living in the United Kingdom (Office for National Statistics [4]). The Gender Identity Research and Education Society (GIRES) estimates a prevalence of 1% TGNC individuals in the UK adult population [5]. TGNC people are individuals that do not identify or exclusively identify with the sex assigned to them at birth. Intersex individuals are people with a less common combination of hormones, chromosomes, and anatomy that are used to assign sex at birth. There are many examples such as Klinefelter Syndrome, Androgen Insensitivity Syndrome, and Congenital Adrenal Hyperplasia. Non Binary people are individuals that do not identify themselves completely as female/male or woman/man. Gender non-conforming people are, according to the American Psychological Association "those who have a gender identity that is not fully aligned with their sex assigned at birth" [1].

A significant number of these individuals often decide to undergo a gender affirmation process. This process consists of using sexual hormones and often undergoing surgery to affirm to the gender that they belong to. In the UK, the prevalence of the population that has sought medical care is estimated to be 0.025%, and about 0.015% are likely to have undergone a transition [5].

A recent survey made by the University of California, Los Angeles (UCLA) Centre for Health Policy and Research showed that 27% of youth between 12 and 17 in California are gender non-conforming [6]. According to another document from the same institution, such a population typically presents a "*conflict between a person's physical or assigned gender and the gender with which he/she/they identify. People with gender dysphoria may be very uncomfortable with the gender they were assigned, sometimes described as being uncomfortable with their body (particularly developments during puberty) or being uncomfortable with the expected roles of their assigned gender*" [7].

It could be, therefore, be argued that gender affirmation is a very important procedure to improve the quality of life and mental wellbeing of TGNC individuals. However, very little attention has been paid to this phenomenon.

A recent prospective study [8] assessing the psychopathology during the gender affirmation process has shown that the psychoneurotic distress measured with the Symptom Checklist-90 Revised SCL-90-R, improves after the start of the hormonal treatment, and anxiety, depression, interpersonal sensitivity, and hostility also tend to improve. This progress is so important that after the completion of the gender affirmation procedure via hormonal treatment and surgery, the psychopathology (assessed with a specific scale) is comparable to the one of the general population [8]. Another prospective study has also demonstrated that people with gender dysphoria treated with hormones presented a significant improvement at the Body Uneasiness Test (BUT) compared to the non-treated condition [9].

The use of hormones, however, might have significant side-effects or may lead to severe medical complications [10]. In particular, Cross-sex Hormone Therapy (CHT) female to male has been associated with a potential risk of cardiovascular disease, cancer (breast, ovarian and endometrial), osteoporosis; in the case of male to female therapy, there is a risk of venous thromboembolism, and potentially cardiovascular disease and cancer [10].

In clinical settings, gender affirmation is a complex and long-lasting procedure, involving many different healthcare professionals including psychiatrists, psychologists, endocrinologists, plastic surgeons, speech and language therapists, and counsellors. This procedure also needs a multidisciplinary approach with a schedule that allows time for physical and social transition [11–13]. The World Professional Association for Transgender Health (WPATH) published the most commonly accepted clinical guidelines and 'Standards of Care' for TGNC [14], including established general eligibility criteria for feminising or masculinising hormone therapy. These include (a) persistent, well-documented gender dysphoria; (b) capacity to make a fully informed decision and to consent for treatment; (c) age of majority in a given country; (d) if significant medical or mental concerns are present, they must be reasonably well-controlled.

The aim of this article is to raise awareness among mental health professionals about a phenomenon that is already known in specialist settings (e.g., gender affirmation clinics and substance misuse services) but less well known in different settings. Central to this paper is a collection of different

clinical cases collected in inpatients and outpatients National Health Service (NHS) clinics in Suffolk (a rural county north east of London) and London (Camden and Islington Boroughs). It is also desirable that by raising awareness, TGNC patients will find that mental health clinicians are not only therapists but also advocates and allied in their gender affirmation journey. In response to the existing lack of knowledge among the health professionals, NHS England recently released [15] a document assessing the individual suitability for endocrine and other pharmacological treatments. Suggested arrangements for medical practitioners include: (a) prescription of endocrine and other pharmacological interventions for the purpose of harm reduction and acting in the best interest for reducing gender dysphoria; (b) the assessment of risks, benefits and limitations of such a pharmacological intervention and the assurance that the individual meets the relevant eligibility criteria set out by the World Professional Association for Transgender Health Standards of Care (2011); (c) the provision of patient-specific prescribing guidance to the General Practitioner (GP), including adequately-detailed information about the necessary pre-treatment assessments, and advice on dosages, administration, initiation, duration of treatment among others; (d) the preparation of written advice to the GP when the individual is discharged. Further details on specific treatments have been outlined in Table 1. An additional statement by the General Medical Council (GMC) clarifies that GPs can prescribe hormones to TGNC individuals [16].

Table 1. Types of treatment for masculinisation and feminisation [15]

Aim	Type of Preparation	Notes	Recommendations
Medications for masculinisation	Testosterone preparations	Include testosterone injections and transdermal gels	Avoid smoking (risk of thrombosis)
	Medications to suppress hyptolamic-pituitary-gonadal activity		Avoid smoking (risk of thrombosis)
Medications for feminisation	Estradiol preparations	Doses necessary to achieve serum estradiol levels typical of pre-menopausal woman. Include oral estradiol and transdermal estradiol as patches and gels (for people over 40 years old). Ethinylestradiol will not be recommended	Avoid if Body Mass Index > 40; avoid smoking (risk of thrombosis)
	Medications to suppress hypothalamic-pituitary-gonadal activity and endogenous testosterone release	Include gonadotropin releasing hormone analogues and 5-alpha reductase inhibitors	Avoid if Body Mass Index > 40; avoid smoking (risk of thrombosis)
	Ornithine decarboxylase inhibitors	May be recommended as an adjunct to facial hair reduction interventions	Avoid smoking (risk of thrombosis)

Despite the availability of such clinical guidance and advice, TGNC individuals still do not receive the care they often need because of stigma, discrimination, and lack of awareness in health care settings [17,18]. A large population study from the 2014-5 Behavioural Risk Factor Surveillance System by Gonzales et al. found that "TGNC adults were more likely to be uninsured and have unmet health care needs and were less likely to have routine care, compared to cisgender (non-transgender) women". Reasons for such barriers to health care included discrimination in health care, health insurance policies, employment and inadequate health policy and regulations [19]. Although very few population surveys of this kind have been carried out, another study in Ontario confirmed that 43.9% of TGNC individuals experienced inequalities in the access to healthcare and remained medically unsupervised [20].

An additional element of concern to this phenomenon is the self-administration of CHT without clinical supervision. Evidence of such hazardous behaviour emerged from studies among TGNC population in Canada [21] and from patients attending gender reassignment clinics in the United Kingdom. In the latter case, it has been estimated that 23% of individuals referred to gender

reassignment clinic were self-administering hormones, mainly bought online (70%). Such behaviour appeared to be more common among trans women, as 32% of the female sample was using hormones at the moment of referral. Alarmingly, individuals that were purchasing hormones online appeared to be less informed about the risks and the side effects of hormonal therapy [22].

The growing number of illicit online pharmacies selling counterfeited products, including "Performance and Image-Enhancing Drugs" (PIEDs) and sexual enhancers [23] taken to enhance human abilities in a myriad of spheres, is another important emerging faucet within this [24–27]. PIEDs include substances with a perceived ability to enhance physical performance, psychological status and appearance, cognitive abilities and social relations, and as such, are sometimes referred to as 'lifestyle drugs'. It has been estimated that approximately 97% of websites selling pharmaceutical products are of illicit nature [26]. Individuals can purchase a wide range of unregulated and untested medicines in these websites which are freely sold without a prescription, and at discounted prices [27,28].

2. Clinical Cases

A number of TGNC patients cases that have started the gender affirmation process without any medical/specialist support were collected in two general adult psychiatry assessment clinics (outpatients) in London and Suffolk between 2014 and 2018. Information was obtained as part of the routine history taking during a psychiatric assessment and no specific questionnaire was designed for the psychiatry interview. The patients were informed at the moment of the assessment that some of the information given in an anonymised version would be used for a case presentation and a case report article. The patients that were included in the article were requested to give informed consent. The assessment clinics are the first point of contact with mental health where new referrals from GPs and other health professionals are seen. Suffolk is a rural county, predominantly a white English population, with chronic lack of access to mental health and other specialist health services that are conversely available in London, where most of the specialist and national health services are located. Consulted patients were either attending the clinics, or inpatient in psychiatric wards for the assessment and treatment of mental health conditions unrelated to their gender definition. The clinics and the wards were for general adult patients only. During the psychiatric interview, it emerged, worryingly that a certain number of TGNC individuals were purchasing hormones through illegal on-line pharmacies and were using them without any medical advice or monitoring (not even at General Practitioner level) using CHT protocols that were available online or receiving advice from online forums and blogs.

The length of the current clinical procedure, which involves long waiting lists, various passages of assessment and treatment, was criticized and perceived as a barrier for receiving the standard treatment. Patients preferred to purchase the hormones online and advocated a quick and easy CHT while trusting unsolicited online protocols from non-medical professionals for a faster result. Mistrust of medical professionals has also been previously reported as a potential cause [29]. Resilience on the Internet for medical advice concerning injecting practices and dosages among other features also indicates an underlying lack of engagement with medical professionals and limited practitioner knowledge regarding these patterns of use [30].

Hormone therapy in gender affirmation may affect different organs and systems [31]. For this reason, any hormonal treatment should be prescribed and supervised by a specialist and should also be discussed in depth with the patient to prepare him/her for the treatment. In this way, it is possible to monitor and manage the treatment effectively as well as to address any of the side effects described above. Hormonal treatment with oestrogens also requires diuretics to counteract the water retention associated with their use. Diuretics should also be used under medical supervision, and the renal function and electrolytes of the patients should be checked regularly to prevent, especially in summer, potentially dangerous dehydration and electrolytes imbalances.

In this paper, seven cases of TGNC individuals are described. They were assessed in a psychiatric clinic or admitted onto a psychiatric ward for different reasons, and they were using hormones

and other drugs for gender affirmation without any medical supervision and purchasing all these medications through unlicensed online dealers.

2.1. Oestrogens

Case 1 is a 24-year-old trans woman, working as a plumber, and single. She was referred for a psychiatric screening as the first step for the referral pathway to the Gender Identity Clinic after she disclosed to her GP that she was using hormones purchased online. The patient has no previous history of mental or physical illness, she described herself as TGNC since age 14 and started the transition, without medical supervision two years before the assessment. The patient joined on line forums where she received the information regarding the hormonal protocols and the websites selling hormones. She started the treatment on her own and subsequently asked the GP to be referred for gender reassignment/gender affirmation. At the time of the assessment, the patient did not present any comorbid psychiatric or physical conditions.

Case 2 is a 22-year-old trans woman with a previous history of depression at the age of 14 that was successfully treated with a Serotonin Reuptake Inhibitor (SSRI). The patient stated that she felt like a person "trapped in the wrong body" ever since she could remember. She complained of being bullied at school for this reason, and she thinks that the bullying and the non-acceptance from her friends caused the depressive episodes in her teens. She started the protocol online and asked the GP to continue prescribing, and the GP asked for a psychiatric opinion before proceeding. She had been using oestrogens, finasteride, and spironolactone intermittently in the last two years. The patient also has type I diabetes, treated with insulin. According to the GPs notes, the compliance with the insulin treatment and the control of his diabetes is not optimal. The mental state examination was unremarkable; the patient presented, however, traits of emotionally unstable personality disorder.

Case 3 is a 19-year-old trans woman, a college student, and single. She was referred by the GP for a psychiatric assessment following numerous suicidal attempts and self-harm episodes. She had a provisional psychiatric diagnosis of Emotionally Unstable Personality Disorder—Borderline type. During the psychiatric assessment, she disclosed using oestrogens and finasteride purchased online in order to proceed with the transition. She also reported that since she started using these hormones the emotional instability became more severe and was partly responsible for the deterioration of her clinical presentation.

Case 4 is a 26-year-old trans woman, living with a partner and working as an administrator. She was referred by her GP for psychiatric assessment as part of the procedure for a referral to the Gender Identity Clinic. The patient had no previous history of mental illness, no medical comorbidity. During the interview she disclosed using oestrogen cream and finasteride tablets purchased online although she did not disclose it before (even with the GP). The mental state examination was unremarkable.

Case 5 is a 36-year-old TGNC woman, single and unemployed. She was referred by the GP for a psychiatric assessment due to low mood and polysubstance misuse (cocaine, cannabis, amphetamine and gamma-Hidroxybutyric acid (GHB). During the interview, the patient stated that she has been taking oestrogen, spironolactone and finasteride for 15 years. A year ago, she was referred to the gender reassignment clinic where she was diagnosed with moderate depression and generalised anxiety disorder, but she was asked to see a general adult psychiatrist for the treatment. The patient complained that the main contributing factors to her depression were the stigmatisation and the lack of acceptance, in which she felt that she was victimised mainly by members of her family and her community. She also was frustrated with the length of the waiting list.

2.2. Androgens

Case 6 is a 25-year-old TGNC man, unemployed, living with his partner. The patient was referred for a psychiatric assessment after he disclosed to the GP about using androgens without medical advice and supervision. The patient complained of The Gender Identity Research and Education Society

feeling "uneasy with his body" since the age of 12. He started to purchase androgens online, 2 years before the psychiatric assessment, following a protocol available online. The patient also has stage 4 renal failure, and he was under the care of the renal team.

Case 7 is a 27-years-old TGNC man and a university student. He was admitted to an inpatient psychiatric unit after a suicidal attempt. The patient was presenting with moderate depression, on admission, he reported of using androgens purchased online while on the waiting list for an assessment by the Gender Reassignment Clinic. The patient was using the hormones without any medical advice or supervision. The low mood and the suicidal attempt were linked to his stressful situation at the university (where he struggled to cope with academic pressure) rather than to the hormonal treatment, that he also stated that he found the hormones to be beneficial. He was also frustrated by the long waiting list before he was able to start his gender affirmation process.

3. Discussion and Conclusions

In our paper, we discussed the clinical cases collected by the same clinician in his clinical practice in different settings, over the span of 4 years. We are aware that it is a very limited picture and we do not think that it is necessarily representative of the entire United Kingdom. We think, however, that this raises the question of access to gender affirmation treatment and the role of every clinician as an advocate for our patients. We are aware that this phenomenon has been described before, but we believe that many clinicians are not aware of it.

Self-prescribing of sexual hormones is a widespread, but poorly studied phenomenon. As highlighted in our work, the lack of access to specialised centres, stigmatisation and marginalisation of the TGNC population as well as the motivations underlying DIY hormonal treatment, deserve further consideration.

To the best of our knowledge, this is the first report of DIY hormonal treatment in general adult psychiatric settings. Previous articles that have described the trend of self-prescribing and administrations of hormones came from sexual health clinics and gender affirmation clinics [19,20]. Psychiatric assessment clinics and psychiatric inpatient wards are often the first port of call of individuals in distress. It is very important, therefore, that the staff working in these services are aware of the particular needs of the TGNC individuals. In particular, the need to establish an environment of respect and non-stigmatisation is very important in developing an effective therapeutic relationship. It is important that the terminology used is appropriate and respectful. This applies particularly to the pronouns and it is always important to check with the patients which pronouns they prefer. It is also important to tactfully ask if they have started the gender affirmation process on their own without any clinical supervision. Psychiatrists, GPs, Sexually Transmitted Diseases' (STD) specialists and all other clinicians should be informed about this under reported trend while encouraging the safe prescribing practice of sexual hormones.

As suggested by NHS England [15], the current assessment needs to be improved by proactively asking TGNC patients whether they are taking hormones and where they are sourcing them from.

The role of mental health services is particularly important because, before the gender affirmation process, TGNC individuals suffer from a higher rate of mental illnesses and mental discomfort (often due to stigma, discrimination and non-acceptance by family and society). For this reason, mental health professionals are more likely to encounter TGNC individuals in need of support but also have a crucial role to play as an advocate. It is, therefore, important when assessing TGNC individuals to respectfully enquire whether they have started the transition and if this is happening under medical care or not. If not, it is necessary to ask if they are sourcing hormones and other medications online or from other unlicensed sources. If this is the case, clinicians have the duty not only to inform the patients of the risk but also to suggest safer alternatives and support if necessary, the individuals in this process.

A common theme that emerged from our case studies was the use of hormones purchased online without any clinical guidance or supervision before and during the treatment. As previously

argued [28,32], the Internet often provides a channel for accessing peer-group experiences and disseminating such risky behaviours. The underlying interpersonal trust embedded in such sub-cultural groups, as seen for instance on discussion fora or social networking, can reinforce the establishment of risk taking norms, especially among early adopters. In addition, the intake of previously untested and unregulated medicinal products can expose users to a series of unwanted side-effects, especially in potentially risky, if unsupervised, medical practices such as intramuscular (IM) injections [33,34]. The shipping process is also questionable with the risk that even where the product is genuine, it may arrive in a condition that renders it unsafe for use. Buyers may also receive counterfeited products and, therefore, using compounds that may be toxic or even lethal. It is also concerning that substances like hormones which have a significant effect on the body and the mind, are used without guidance and monitoring of the side effects. Furthermore, the route of administration (e.g., IM) can lead to additional health risks, both chronic and acute [33].

Further studies need to be carried out to evaluate the motivation underlying such a poorly researched trend. The main reason for such behaviours seems to be due to the difficulty in accessing gender reassignment/gender affirmation treatments, leaving a significant part of the TGNC population in a condition of discomfort that make them more vulnerable to the onset of psychiatric illnesses (depression, anxiety) as well as other unhealthy conditions or practices (e.g., smoking, drug intake) [18,35,36]. Furthermore, as their need for medical assistance may grow [36], trans individuals often experience problematic accesses to healthcare in terms of professionals' education and discriminatory practices [37]. This is also due to the length of the waiting lists (up to three years) that pushes TGNC individuals into obtaining hormones online [38]. Despite the various efforts made by NHS England, the GMC and the Royal Colleges to improve the situation, a significant group of individuals still do not receive the care that they need and deserve in the United Kingdom. This may also be due to the chronic lack of funds for these services despite recent years' additional funding [39]. Gender affirmation is not the treatment of an illness but is a procedure that improves the wellbeing of TGNC and non-binary individuals and, therefore, should be appropriately funded and supported by every clinician [40].

It is necessary, therefore, to address such gaps in public health policy and clinical practice knowledge regarding gender affirmation and establish alternative services. Local clinic services, complementary to the NHS provided treatment, may be crucial to the wellbeing of individuals who are feeling disenfranchised and are not attending the gender reassignment/gender affirmation clinics while considering the treatment [41]. Examples of such services can be found in the United States with the Transgender Health Services program (STRIDE) based in San Francisco, which is structured as a peer-based model providing hormone therapy and support for the general and the psychological health of TGNC individuals [42]. Different gender reassignment/gender affirmation programs are also available in Canada. In British Columbia and Ontario projects like Trans Care BC (British Columbia) and Trans Health Connection (Ontario) aim to facilitate the access to care for TGNC by providing information and support for the TGNC community [43,44].

In summary, we believe that increasing the access to gender affirmation services alone, however, is not enough and it is necessary to raise awareness among every clinician about the special needs that TGNC individuals have when accessing healthcare. It would be necessary, to achieve this goal, to design and disseminate among all clinicians, a questionnaire regarding their attitude towards TGNC individuals, their knowledge about the gender affirmation process and the specific clinical needs that the TGNC population has. In this way, it would be possible to establish the educational needs that clinicians have and, therefore, consider specific training. This would enable improvement of access to care for TGNC individuals by fighting stigma and creating a more inclusive service. This approach should be across all the services that might care for TGNC individuals. Furthermore, disseminating knowledge and raising awareness might have another beneficial effect by transforming conscious clinicians in health to advocate for the TGNC population. In this way, it may be possible to address not only the problem highlighted in this article (the difficulty of accessing gender affirmation clinics) but

also the more widespread discrimination that TGNC individuals face when accessing various health care services.

As we have seen in our study, the implementation of such different and innovative health care services for TGNC individuals as well as more targeted prevention strategies on such underreported and highly risky behaviours have become a necessity in the United Kingdom and elsewhere. Closer attention should also be paid to the online market of DIY hormones, and an open dialogue with LGBTQ organisations should be established to support TGNC individuals and better understand their unmet needs.

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A Study on Photostability of Amphetamines and Ketamine in Hair Irradiated under Artificial Sunlight

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Abstract: Drugs incorporated into hair are exposed to the environment, and cosmetic and chemical treatments, with possible decreases in their content. Knowledge concerning the effect of sunlight on drug content in hair can be helpful to forensic toxicologists, in particular, when investigating drug concentrations above or below pre-determined cut-offs. Twenty authentic positive hair samples were selected which had previously tested positive for amphetamines and/or ketamine. Washed hair were divided into two identical strands, with the former exposed at 765 W/m^2 (300–800 nm spectrum of irradiance) for 48 h in a solar simulator, and the latter kept in the dark. Hair samples were extracted and analyzed by liquid chromatography high-resolution mass spectrometry detection. The percentage of photodegradation was calculated for each analyte (i.e., amphetamine, methamphetamine, methylendioxyamphetamine, ketamine, and norketamine). In parallel, photodegradation processes of standard molecules dissolved in aqueous and organic solutions were studied. In 20 hair samples positive for the targeted analytes, exposure to artificial sunlight induced an appreciable decrease in drug concentrations. The concentration ranges in the non-irradiated hair samples were 0.01–24 ng/mg, and 65% of samples exhibited a decrease in post-irradiation samples, with reduction from 3% to 100%. When more drugs were present in the same hair sample (i.e., MDMA and ketamine) the degradation yields were compound dependent. A degradation product induced by irradiation of ketamine in aqueous and methanol solutions was identified; it was also found to be present in a true positive hair sample after irradiation. Ketamine, amphetamines, and their metabolites incorporated in the hair of drug users undergo degradation when irradiated by artificial sunlight. Only for ketamine was a photoproduct identified in irradiated standard solutions and in true positive irradiated hair. When decisional cut-offs are applied to hair analysis, photodegradation must be taken into account since sunlight may produce false negative results. Moreover, new markers could be investigated as evidence of illicit drug use.

Keywords: hair; solar light; photodegradation; amphetamines; MDMA; ketamine

1. Introduction

The main advantage of hair as a testing matrix is the ability to provide information relating to historical drug exposure. Hair analysis has many applications within forensic (e.g., drug-related

deaths, drug-facilitated crimes (DFCs), child protection) and clinical toxicology (e.g., drug rehabilitation programs, workplace drug testing) [1–4].

The stability of drugs in hair, however, is affected by exposure to sunlight and weathering, cosmetic chemical treatments (i.e., oxidative dyeing, bleaching, or permanent wave), and physical damage [5–7]. In particular, exposure to sunlight and/or artificial light for many hours per day can induce photodegradation (i.e., photolysis) of licit/illicit drugs through the formation of free radicals (produced by the drug itself or formed by eumelanin and pheomelanin, and their oxidative products oxyeumelanin, and oxypheomelanin) or photosensitization reactions by intermolecular energy transfer. [8]

Previous studies on this matter have been published by Skopp et al. [9] in which cannabinoids detected in hair and affected by solar radiation were shown to reduce in concentration. More recently, Favretto et al. [10] evaluated the effect of light exposure on methadone, cocaine, and heroin metabolites in hair.

In order to better understand the role and underlying mechanisms of solar light exposure on decreasing concentrations of drugs in hair, and following our previous [10] photodegradation studies on UVA- and UVB-induced changes, the aim of the present work was to evaluate the photodegradation of several common stimulant drugs (i.e., amphetamines and ketamine (KET)) in true positive hair samples exposed to the whole spectrum of sunlight in a solar simulator. The use of a solar simulator, including visible light (400–800 nm) and part of UV radiation from 400 nm to 3 μnm (UVA = 400–315 nm, UVB = 315–280 nm), mimics exposure to environmental sunlight.

Amphetamine-based drugs are illegal synthetic stimulants that share a common structural backbone. The four amphetamines considered in the present work are the most used in Europe: amphetamine (AMF), methamphetamine (MA), 3,4-methylenedioxymethamphetamine (MDMA), and 3,4-methylenedioxyamphetamine (MDA).

Amphetamine can be metabolized along two pathways: either by hydroxylation of the aromatic ring to 4-hydroxyamphetamine or by deamination of the side chain to benzyl methyl ketone, which can then be degraded to benzoic acid. Methamphetamine is metabolized by cytochrome P450 (CYP), mainly by the CYP2D and CYP3A subfamilies, leading to the production of 4-hydroxyamphetamine and AMF. The half-life of MA is about 10 h, and 35–45% of a dose is excreted unchanged in the urine over a period of several days. Other metabolites, such as 4-hydroxymethamphetamine, norephedrine, and 4-hydroxynorephedrine, can also be found in urine in substantial quantities [11–13].

In addition, MDMA (i.e., 3,4-methylenedioxymethamphetamine, ecstasy) is a widely abused psychostimulant drug that acts as a powerful releaser and/or reuptake inhibitor of serotonin (5-HT), dopamine (DA), and norepinephrine (NE). Its metabolism depends on the following main metabolic pathways: (1) *O*-demethylenation followed by catechol-*O*-methyltransferase (COMT) methylation and/or glucuronide/sulfate conjugation; and (2) *N*-dealkylation, deamination, and oxidation. MDMA *N*-demethylation gives rise to 3,4-methylenedioxyamphetamine (MDA). The elimination half-life of MDMA is about 8–9 h, lower than those reported for MA (10–12 h) or AMF (12–15 h) [14,15].

Ketamine is a dissociative anesthetic drug that functions as an antagonist of the *N*-methyl-D-aspartate receptor and enhances the antinociceptive effects of conventional opioid analgesia, binding to μ opioid and σ receptors. Ketamine is increasingly misused as a recreational and "club drug" because of its hallucinogenic and stimulant effects, and also as a "date-rape" drug (to facilitate sexual assault). Ketamine is metabolized in the liver by the P450 system, and CYP3A4 is the main enzyme responsible for transforming *N*-demethylation into norketamine (NKET), 4-hydroxy-ketamine, and 6-hydroxy-ketamine. The elimination half-life is about 2 h, and the predominant metabolite of urinary excretion over a 72 h period is dehydronorketamine (DHNK) (16%), along with conjugates of hydroxylated ketamine metabolites [16,17].

The consumption of alcohol and/or drugs is associated with an increased risk of being the victim of a sexual assault. A retrospective case series in London on 1014 cases of claimed drug-facilitated sexual assaults (DFSAs) showed that in 34% of samples (blood and/or urine), an illicit drug (with or

without alcohol) was found, of which 10.8% contained cocaine, 4.6% "ecstasy" (MDMA), and 2.3% AMF [18,19]. In DFC cases, alleged victims are often unconscious leading up to the assault due to the amnesiac effects of the drug(s) administered, thus a considerable amount of time may be spent before the victim reports the incident. Consequently, hair analysis is of primary importance compared to blood and urine analysis, particularly when the occasional consumption is sufficiently high; however, exposure to the environment may affect the stability of drugs in the keratin matrix.

For these reasons, reliable interpretation of the analytical results is fundamental for a correct interpretation of a positive or negative result, and general knowledge of the photostability of drug analytes in the biological matrix must be considered. However, no data have been published until now on the effect of light on KET and amphetamines, and their respective metabolites in the keratin matrix; only morphine and 6-monoacetylmorphine (6-MAM) photodegradation have been studied by UVA and UVB irradiation [20].

In the present paper, levels of AMF, MA, MDMA, MDA, KET, and NKET were determined by means of liquid chromatography high-resolution mass spectrometry (HPLC-HRMS) [21,22] in authentic hair samples from drugs users before and after irradiation under the whole spectrum of sunlight in a solar simulator.

Although the photodegradation of a molecule not only depends on the wavelength used, but also on its physical state (solid or liquid) and the environment in which it is irradiated (i.e., solvent, polarity, pH, and the presence of salts, oxygen, and other compounds in the sample), we tested the same analytes in a pure methanol and water solution irradiated under the whole spectrum of sunlight to gather general information on their behavior and to study their kinetics of photodegradation.

2. Experiments

2.1. Chemicals

Amphetamine, MA, MDMA, MDA, KET, and NKET were purchased from LGC Promochem Cerilliant (Teddington, Middlesex, UK) as pure solutions in methanol at 1.0 mg/mL. The internal standards (IS) MA-D5, AMF-D5, MDMA-D5, MDA-D5, and KET-D3 were methanol solutions also from LGC Promochem at 1.0 mg/mL.

Methanol, dichloromethane, and acetonitrile (Merck, Darmstadt, Germany) were high-performance liquid chromatography (HPLC) grade. Trifluoroacetic acid (TFA) was from Sigma (Sigma-Aldrich, Milan, Italy). High-performance liquid chromatography water was prepared using a Milli-Q Plus (Millipore, Molsheim, France) system. All other reagents were from Sigma-Aldrich (St Louis, MO, USA).

2.2. Hair Samples

Authentic positive hairs were selected from samples at the laboratory that had previously tested positive for amphetamines and/or KET. Hair samples were collected with scissors from the posterior vertex and cut as close to the scalp as possible, wrapped in aluminum foil, and kept at room temperature until analysis. As recorded in donor interviews, all samples had not been submitted to previous cosmetic or chemical treatments.

2.3. Irradiation Procedure

Irradiation was performed in a Suntest CPS+ (Atlas, Linsengericht, Germany) equipped with a 1.8 kW xenon lamp and a glass filter (cut-off 310 nm) according to Option 1 of ICH Guideline Q1B (European Medicines Agency, London, UK, ICH Topic Q1B–Photostability testing of new active substances and medical products, 1998). The dark samples were maintained at solar box temperature during irradiation.

2.4. Photolysis Experiments in Solution (In Vitro Study)

Solutions of the compounds at concentrations ranging from 10^{-5} to 10^{-4} M in methanol and water were irradiated in the Suntest CPS+ with times increasing from 1 h up to 3 h.

Photolysis was evaluated using Cary 50 UV-Vis spectrophotometer (Varian, Milan, Italy) analyzing the change in the original spectrum upon irradiation, as already described [20], and by high-resolution mass spectrometry (HRMS). At selected UV doses, the solutions were diluted to 10^{-6} M in methanol and water, and analyzed by direct injection HRMS to measure the photodegradation of the analytes by recording the decrease of the ion signal of protonated molecules obtained by electrospray ionization (ESI) of solutions kept in the dark. The results are the mean of at least three experiments.

2.5. Photolysis Experiments in Hair Samples (In Vivo Study)

Hairs 5–7 cm long were divided into two approximately identical strands: the former was put between two 5×5 cm optical glasses and exposed at 765 W/m^2 (spectrum of irradiance: 310–800 nm) for 48 h in the solar simulator to an endpoint corresponding to two months exposure under the sunlight, and the latter was kept as a dark control in the same chamber of irradiation covered with an aluminum foil.

2.6. Hair Sample Preparation and Extraction

Hair samples were decontaminated with 3 mL of water and 3 mL of methanol. Pulverization was applied by the automatic homogenizer Precellys® Evolution (Bertin Technologies, Genoa, Italy) at a speed of 6000 RPM, cycle 9×30 s, pause 30 s. Solid phase extraction (SPE) was performed by Oasis MCX (Waters, Milford, MA, USA) cartridges. Sample preparation consisted of the addition of 2 ng/mg of IS and 3 mL of a methanol/TFA (90:10, *v/v*) solution to 25 mg powdered hair samples, ultrasonication for 1 h, and incubation overnight at 45 °C. After centrifugation, the methanolic solutions were dried under a stream of nitrogen at 40 °C. The residues were reconstituted in 3 mL of 0.1 M phosphate buffer pH 6 and subjected to SPE. After, cartridges of the sample conditioned with 3 mL methanol and 3 mL 0.1 M phosphate buffer pH 6 were loaded. Cleanup was accomplished by sequential washes with 3 mL of water, 3 mL of 0.1 N hydrochloric acid, and 3 mL of methanol. Cartridges were dried for 10 min under vacuum before elution with 2 mL of dichloromethane: 2-propanol (80:20, *v/v*) 2% ammonium hydroxide. Eluates were evaporated to dryness with nitrogen at 40 °C, reconstituted in 200 μL of water (0.1% formic acid)/acetonitrile 9:1 mixture, and 25 μL were injected into the LC-HRMS system.

2.7. High-Resolution Mass Spectrometry (HRMS)

All measurements were performed on an LTQ-Orbitrap (Thermo Fisher Scientific, Bremen, Germany) high-accuracy, high-resolution mass spectrometer operating in positive ESI mode and equipped with a Surveyor MS Pump.

2.8. Electrospray Ionization HRMS

For the analysis of pure standard solutions, direct injection analysis was performed with a syringe pump delivering solutions at 10 μL/min directly into the ESI source. The positive ion ESI parameters were as follows: capillary voltage 10 V, sheath gas flow rate 20 (arbitrary units, a.u.), auxiliary gas (N$_2$) flow rate 5 (a.u.), sweep gas flow rate 5 (a.u.), and capillary temperature 275 °C. Profile full-scan mass spectra were acquired in the Orbitrap in the *m/z* range 120–700 with a target mass resolution of 100,000 (FWHM as defined at 400 *m/z*) and a scan time of 0.65 s.

2.9. High-Performance Liquid Chromatography HRMS

For the determination of drug concentrations in hair and in standard solutions, 25 μL of solutions or extracts were injected into an Atlantis T3 (150 $\times$ 1.0 mm, 3 μm) column (Waters Corporation, Milford, MA, USA). High-performance liquid chromatography separation was achieved by gradient

elution at a constant flow rate of 300 µL/min. High-performance liquid chromatography conditions: A (water, 0.1% formic acid) and B (methanol, 0.1% HCOOH); initial conditions 10% B, linear gradient to 25% B in 4 min, 25% B hold from 4 min to 7 min, then ramped to 40% B in 5 min, to 60% B in 4 min and to 90% B in 2 min; 90% B hold from 18 min to 26 min. The column temperature was 40 °C. Mass spectrometry conditions were: positive ion ESI; capillary voltage 10 V, sheath gas flow rate 50 (arbitrary units, a.u.), auxiliary gas (N_2) flow rate 5 (a.u.), sweep gas flow rate 5 (a.u.), and capillary temperature 275 °C. Profile full-scan mass spectra were acquired in the Orbitrap in the m/z range 120–700 with a target mass resolution of 60,000 (FWHM as defined at 400 m/z) and a scan time of 0.45 s. Detection of the analytes and the IS was based on retention time, accurate mass measurements of MH[+] ions, and correspondence of the observed isotopic pattern to the calculated one. Drug concentrations were determined from peak area ratios of analyte to its IS compared to calibrator curves of peak area ratios to concentrations. The method was fully validated exhibiting a linear range from 0.1 ng/mg to 50 ng/mg (determined from regression with $1/x^2$ weighting utilizing six calibration points), lower limits of quantification of 0.01 ng/mg and limits of detection of 0.005 ng/mg for all the target analytes, intra-day imprecision, inaccuracy always lower than 19% and 20%, and inter-day imprecision and inaccuracy always lower than 21% and 22%, respectively. Extraction efficiency was determined in the range of 85–100% for the different substances.

3. Results

3.1. Photodegradation in Methanol and Water Solutions Exposed to Suntest CPS+

Preliminary, stock methanol, and water solutions for all the analytes diluted to 10^{-4} M were irradiated inside the photostability test chamber from 1 h to 3 h (each hour corresponding to about 70 J/cm^2), and their absorption spectra before and after irradiation were recorded (Supplementary Figures S1A1–S1F2).

Amphetamine dissolved in methanol was characterized by the only band at around 205–210 nm, mainly corresponding to the solvent, thus remaining unmodified in all the irradiated solutions (see Supplementary Figure S1A1). However, a shift to 205 nm and a small increase in spectral intensity were detected by increasing irradiation. When irradiated in water, AMF was characterized by two bands (around 220 nm and 240 nm) decreasing as irradiation dose increased (see Supplementary Figure S1A2).

Methamphetamine, both in methanol and water (Figures S1B1 and B2, respectively), presented curves similar to AMF, with the unique difference being the absorption at 205 nm in water which increased under irradiation.
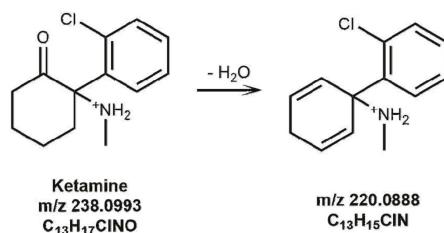
In addition, MDA in methanol (Figure S1C1) was characterized by three bands at 208 nm, 235 nm, and 285 nm. The irradiated solutions presented similar curves with increasing absorption and a small blue shift at 230 nm and 280 nm. Similar spectra were obtained for MDA in water (Figure S1C2), with two bands at 230 nm and 285 nm increasing in relation to irradiation time.

Furthermore, MDMA in methanol showed two bands centered at 235 nm and at 285 nm, indicating a small absorption increase and a small blue shift (Figure S1D1). A new band of absorption (300–340 nm) appeared which increased upon irradiation. In water, MDMA presented the same absorption band with a higher increase at 235 nm than at 285 nm, and the appearance of a band at 320 nm (Figure S1D2).
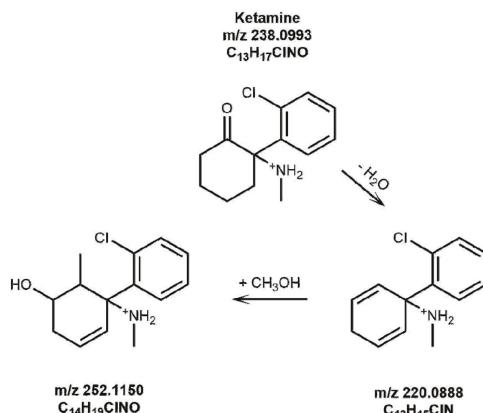
Ketamine in methanol was characterized by a single absorption band (205–230 nm). When irradiated, this band slightly increased and a shoulder (230–270 nm) appeared, as evidenced in Supplementary Figure S1E. On the contrary, in water, KET did not change its absorption spectrum upon irradiation (Figure S1E2). Absorption spectrum of NKET in methanol increased upon irradiation at 210–220 nm and in the range of 240–250 nm (Figure S1F1). In water, NKET showed a similar behavior upon irradiation for the band 240–250 nm, with slight changes (Figure S1F2).

3.2. Identification of New Photoproducts

All the irradiated solutions in the solar simulator, including the control solutions kept in the dark, were diluted to 10^{-5} M either in methanol or water, and analyzed by direct infusion HRMS, with the aim of identifying the compounds eventually formed upon irradiation. Interestingly, for KET and NKET only, some new species were observed in irradiated solutions. In HRMS spectrum of KET irradiated in water solution, its [M+H]$^+$ ion at 238.0993 m/z ($C_{13}H_{17}ClNO$) was accompanied by a new ionic species at 220.0888 m/z ($C_{13}H_{15}ClN$), corresponding to the loss of H_2O. In methanol, two species were observed at 220.0888 m/z and at 252.0786 m/z ($C_{13}H_{15}ClNO_2$), with the latter corresponding to the loss of H_2 and to the photo-addition of one oxygen atom. The proposed structures of KET photoproducts are shown in Figures 1 and 2. It must be highlighted that the ionic species identified were new protonated molecules present in solution, and they are not fragment ions produced by collisional experiments on precursor ions.

Figure 1. Proposed structures of ketamine (KET) photoproducts formed in a water solution upon irradiation for 3 h.
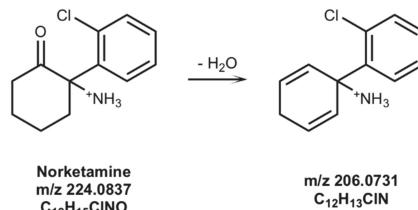

Figure 2. Proposed structures of KET photoproducts formed in a methanol solution upon irradiation for 3 h.

In HRMS spectrum of the methanol solution of NKET irradiated in the solar simulator, [M+H]$^+$ ions at 224.0837 m/z ($C_{12}H_{15}ClNO$) were accompanied by a new species at 206.0731 m/z ($C_{12}H_{13}ClN$), corresponding to the loss of H_2O. The proposed structure of NKET photoproducts formed in methanol is shown in Figure 3 analogously to KET. However, differently from KET, no photoproduct was detected in HRMS spectrum of NKET irradiated in water solution.
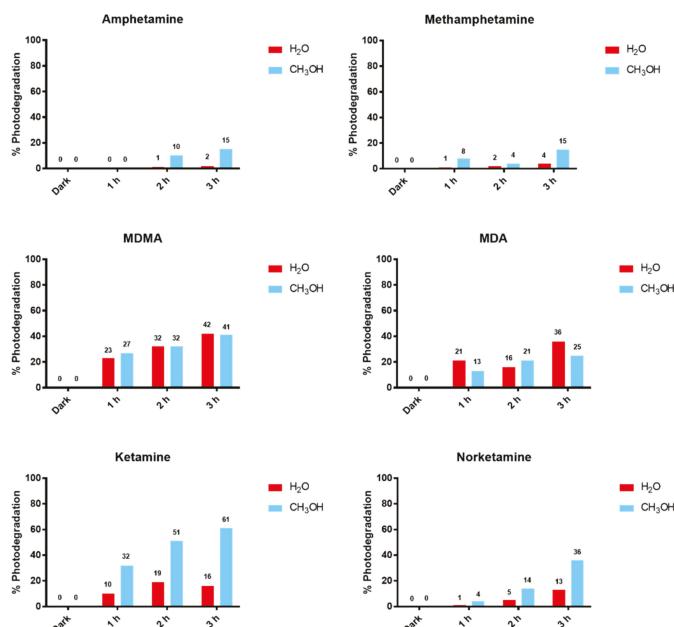
Vice versa, for all the other analytes (i.e., AMF, MA, and MDMA), both in water and methanol solutions, no photoproducts were evidenced by direct HRMS analysis. To avoid ionization suppression

phenomena that could occur in a mixture and shield the presence of less abundant species, irradiated solutions were also analyzed by HPLC-HRMS. The ratios of peak areas observed for samples upon irradiation vs those analogous samples kept in the dark were calculated as "percent degradation". In Figure 4, the yields of photodegradation obtained for all the analytes after 1 h, 2 h, and 3 h of irradiation are reported.

Figure 3. Proposed structures of norketamine (NKET) photoproducts formed in a methanol solution upon irradiation for 3 h.

As may be observed, AMF and MA in water show the highest photostability: the photodegradation was 1% and 4%, respectively, while in methanol it increased up to 15%. In addition, MDMA and MDA in methanol and water both presented similar photodegradation with a linear relationship with irradiation time: MDMA from 23% to 42% and MDA from 13% to 36%. For KET and NKET, the photodegradation yield was significantly higher in methanol (KET 61%; NKET 36%) than in water (KET 16%; NKET 13%) after 3 h.

Figure 4. Relative degradation of target drugs in methanol and water solutions with increasing time of irradiation in the solar simulator (% photodegradation calculated using the peak area of protonated molecules in solutions kept in the dark as controls for 0% degradation).

3.3. Photodegradation in Hair Exposed to Solar Box

The main goal of the study was to observe the photo-induced degradation of drugs in hair. In Figure 5, the concentrations of each drug for all seventeen hair samples irradiated in the solar simulator or kept in the dark (control) are presented.

Physical characteristics				Dark						Irradiated					
Color	Thickness	Straight	Curly	MET (ng/mg)	AMP (ng/mg)	MDMA (ng/mg)	MDA (ng/mg)	KET (ng/mg)	NKET (ng/mg)	MET (ng/mg)	AMP (ng/mg)	MDMA (ng/mg)	MDA (ng/mg)	KET (ng/mg)	NKET (ng/mg)
Brown 1	Thin	●		-*	-	-	-	0.02	-	-*	-	-	-	0.02	-
Brown 2	Thin	●		-	-	-	-	0.04	0.02	-	-	-	-	0.03	0.01
Brown 3	Thin	●		-	-	2.34	0.34	23.8	3.17	-	-	0.34	0.34	4.2	0.85
Brown 4	Thick		●	-	-	-	-	0.12	-	-	-	-	-	0.05	-
Brown 5	Thin	●		-	0.06	-	-	0.10	0.03	-	0.04	-	-	0.07	0.01
Brown 6	Thick	●		-	-	0.20	-	-	-	-	-	0.118	-	-	-
Brown 7	Thin	●		-	-	0.69	0.05	-	-	-	-	0.76	0.04	-	-
Brown 8	Thick	●		-	-	0.09	-	-	-	-	-	0.07	-	-	-
Brown 9	Thick		●	-	-	-	-	0.06	0.06	-	-	-	-	0.04	0.04
Brown 10	Thin	●		-	-	-	-	0.15	0.02	-	-	-	-	0.09	0.01
Brown 11	Thick			-	-	-	-	0.28	0.02	-	-	-	-	0.22	0.03
Brown 12	Thick		●	-	-	-	-	0.05	0.01	-	-	-	-	0.02	0.01
Dark Brown 1	Thick	●		-	-	-	-	0.34	0.02	-	-	-	-	0.09	0.02
Black 1	Thin	●		-	0.99	1.40	0.07	14.3	2.43	-	0.64	1.63	0.06	8.85	1.46
Black 2	Thin	●		0.97	-	-	-	-	-	0.84	-	-	-	-	-
Black 3	Thin	●		24.1	0.93	-	-	-	-	12.7	0.7	-	-	-	-
Black 4	Thin	●		-	-	0.45	-	-	-	-	-	0.32	-	-	-

Figure 5. Drug concentrations in hair samples with their physical characteristics calculated by high-performance liquid chromatography high-resolution mass spectrometry (HPLC-HRMS) in both aliquots kept in the dark or irradiated in the solar simulator. -: absent at limit of detection (LOD, 0.005 ng/mg); (−) observed % increase.
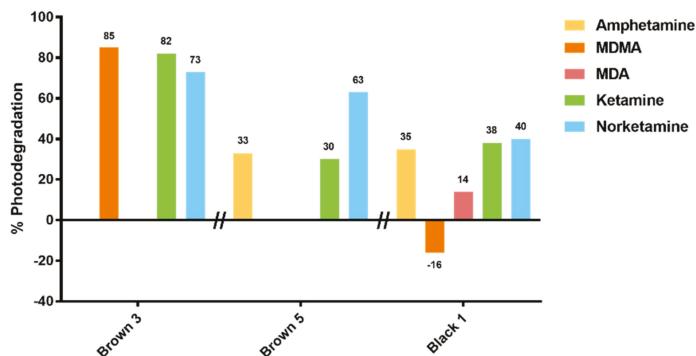
In Figure 6, the percentages of photodegradation are reported. Calculations were made using the concentrations of samples kept in the dark as control:

$$\% \text{ photodegradation} = (\text{drug conc.}_{\text{dark}} - \text{drug conc.}_{\text{irradiatied}})/\text{drug conc.}_{\text{dark}} \times 100.$$

Figure 6. Percentage of photodegradation, average, standard deviation, and range of drugs in hair samples irradiated at 765 W/m^2 in the solar simulator.

When comparing results from three poly-drug abusers, photodegradation of all the analytes was generally obtained, with the highest comparable photodegradation yields observed in sample Brown 3 (see Figure 7).

Figure 7. Percentage of photodegradation of amphetamine (AMF), KET, and NKET in three different hair samples. In these three samples, photodegradation seems to depend on the hair owner.

4. Discussion

From our results, a clearly different behavior of KET and NKET was evidenced when compared to all the other drugs and metabolites under study. Indeed, these drugs seem photo-unstable, both in solution and in hair matrix. In particular, for the experiments in solution, KET and NKET photo-induced products were identified; in hair samples their degradation was higher (average 42% and 29%) than all the other compounds (average 8–30%).

No color effect seems to be present, although no fair hair samples were present in this study, and the number of samples was limited. It is well known that the color of hair depends on the relative amount of pheomelanin (red) and eumelanin (black), the first defense against UV in human hair and skin [23–26]. Generally, a part of the light is absorbed by the hair matrix itself without any photochemical effect. In dark hair, the eumelanin can protect the drugs/metabolites with a higher degree than in fair hair. However, melanin may also react with oxygen under irradiation, producing reactive species, such as superoxide anion, that can induce photolysis of melanin itself [27], thus weakening the photoprotective effect of the pigment. In this context, no clear-cut interpretation of the role of hair color can be made.
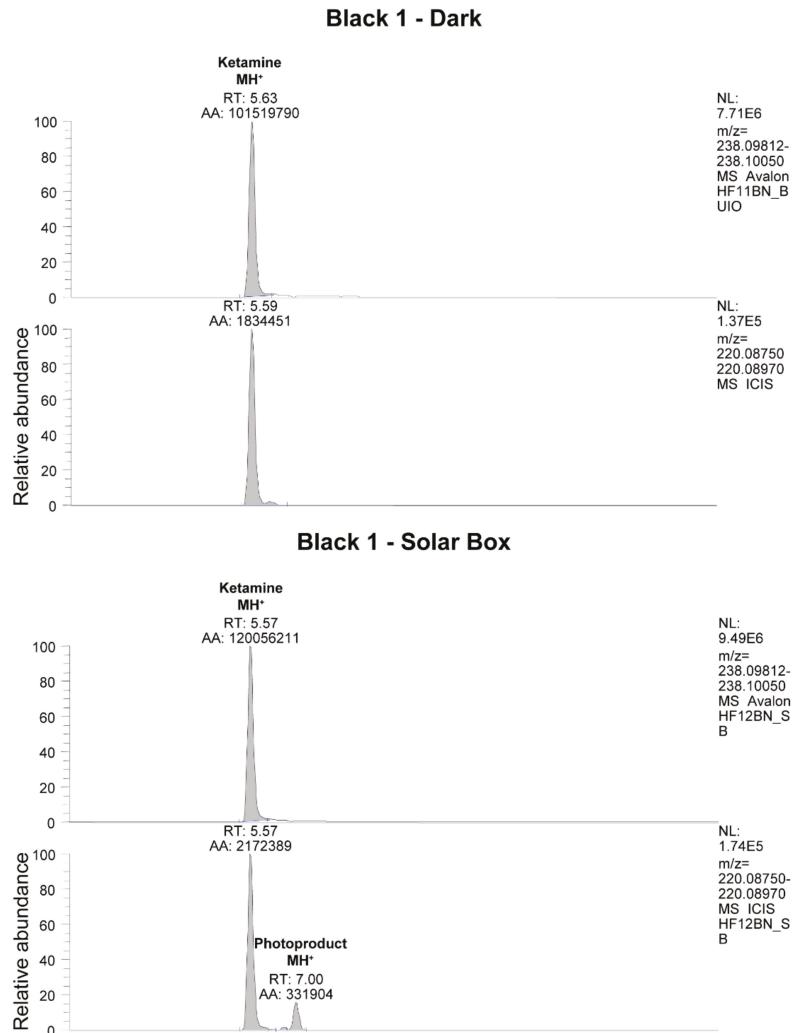
Regarding MA and AMF, on the basis of the in vitro experiments, they were expected to photodegrade less readily than KET and NKET. Unexpectedly, the experiments in hair revealed an average degradation of 30% and 31% for MA and AMF, respectively, with a range of 13–47% and 25–35%.

The presence of photoproducts was also investigated in irradiated hair samples. In one sample, the species at 220.08750 m/z, corresponding to the photoproduct of KET shown in Figure 1, could be identified by LC-HRMS as evidenced in Figure 8.

The importance of this finding must be highlighted, since no previous study has identified stable photoproducts in hair for KET, nor for any other substance previously investigated (i.e., cannabinoids, cocaine, opiates, methadone [9,10,20,21]).

The "apparent production" of NKET upon irradiation of sample Brown 11 was at first sight surprising, but could be reasonably related to the physical decomposition of the matrix, leaving KET more labile during the irradiation experiment and favoring its transformation to the metabolite. Furthermore, the increase of MDMA in sample Black 1 and Brown 7 upon irradiation can be rationalized (as already demonstrated in previous studies) by a greater lability of the keratin matrix

with consequent greater yield during the extraction of the analytes in the liquid acid phase with access to deeper layers of the hair structure.

Figure 8. LC-HRMS analysis of hair sample Black 1 taken in the dark and after irradiation in the solar simulator. In the irradiated sample, the presence of a KET photoproduct with 220.08750 *m/z* is evident.

5. Conclusions

Amphetamine, MA MDA, MDMA, KET, and NKET incorporated in hair undergo degradation when irradiated by artificial sunlight, suggesting that they can suffer photodegradation under natural sunlight. With this work, for the first time, the presence of a photoproduct of KET was evidenced in one true positive sample.

Since the detection of drugs in hair is often used as evidence of illegal acts with consequences on the freedom of persons, the UV-Vis effects on the integrity of the drugs and their metabolites should be considered when single administration needs to be evidenced. When decisional cut-offs are applied

to hair analysis (e.g., for granting a driving license, a job, or a child custody), it must be taken into account that hair exposed to sunlight may produce false negative results and lead to misjudgment. When possible, the detection of photoproducts of a drug under investigation can be a key factor in a case management.

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Sales and Advertising Channels of New Psychoactive Substances (NPS): Internet, Social Networks, and Smartphone Apps

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Abstract: In the last decade, the trend of drug consumption has completely changed, and several new psychoactive substances (NPS) have appeared on the drug market as legal alternatives to common drugs of abuse. Designed to reproduce the effects of illegal substances like cannabis, ecstasy, cocaine, or ketamine, NPS are only in part controlled by UN conventions and represent an emerging threat to global public health. The effects of NPS greatly differ from drug to drug and relatively scarce information is available at present about their pharmacology and potential toxic effects. Yet, compared to more traditional drugs, more dangerous short- and long-term effects have been associated with their use, and hospitalizations and fatal intoxications have also been reported after NPS use. In the era of cyberculture, the Internet acts as an ideal platform to promote and market these compounds, leading to a global phenomenon. Hidden by several aliases, these substances are sold across the web, and information about consumption is shared by online communities through drug fora, YouTube channels, social networks, and smartphone applications (apps). This review intends to provide an overview and analysis of social media that contribute to the popularity of NPS especially among young people. The possibility of using the same channels responsible for their growing diffusion to make users aware of the risks associated with NPS use is proposed.

1. Introduction

In the last 10 years, an increasing number of new psychoactive substances (NPS) has flooded the drug market. NPS are drugs of misuse not included in the International United Nations Conventions, which can easily bypass the supply reduction strategies of law enforcement agencies and sanctions related to the use and sale of illicit substances. The advent of NPS has contributed to the appearance and growth of a new "drug scenario" characterized by an increased number of drug users among young people and the consumption of drugs with unknown effects or safety profiles. At the initial stage of the phenomenon, NPS are typically used by a small group of people. After the use of these substances becomes well-known, their widespread marketing through media and Internet sales begins. This sequence of events causes the beginning of an epidemic diffusion that is eventually prevented by law enforcement agencies that perform important actions and fight against the trafficking and sale of NPS. Unfortunately, the subsequent legal control of these substances only initiates the reformulation of NPS, which induces a typical loop that is highly dangerous to public health. In order to understand

the full spectrum of the complex issue of NPS, we provide here an updated overview of the specific field of sales and advertising channels of NPS that represent an important ring in this chain of events.

2. The Complex Issue of the New Psychoactive Substances (NPS)

NPS are synthetic compounds that are very popular worldwide, as shown by the alarming number of NPS (779) reported between 2008 and 2017 by 111 countries and territories [1–4]. Designed in order to substitute classical drugs of abuse with legal surrogates, their expansion leads to an endless effort made by governments and law enforcement agencies to try to contain this phenomenon. A mix of features makes them very attractive, including the difficulty to detect them in human fluid samples by standard drug screening test, their ambiguous legal status, and, as in the case of synthetic cannabinoids, the perceived low risk despite their toxic effects and abuse liability [5,6]. Noteworthy, the exponential increase in the market size of these compounds has been facilitated by the World Wide Web (WWW), where information about their purchase and use are shared, advertised, and spread to everyone. In light of the changing scenario for drug marketing and advertising, the aim of this review is to analyze the role of the web in this emerging trend, focusing on social networks and smartphone applications (apps).

In the current world where communication is based on the Internet and social networks, online sites operate on both the surface and deep web [7–10] to supply NPS labeled as "not for human consumption" and sold as plant fertilizers, incense, bath salts, or with other aliases in order to avoid legislative controls [11]. The dark net plays a key role in this "super safe drug dealing", which buyers and sellers can access anonymously to provide drugs and pay for them with a virtual wallet [2]. Essentially, a few clicks are enough to supply highly psychoactive substances, cheaply and in a low-risk way [12,13], even through smartphone apps [14,15]. Therefore, NPS can be sold to everyone, including very young people, in complete anonymity and easily avoiding law enforcement [10,16,17]. Along with the emergence of new psychoactive drugs in the world drug market, new concepts are emerging to better describe this new, global phenomenon and its associated health consequences. That is, the term "spiceophrenia" has been proposed by Papanti and collaborators [18] to describe the psychotic symptoms (e.g., hallucinations, delusions) that likely occur in chronic users of synthetic cannabinoids. It has been reported that the use of Spice/K2 drugs may exacerbate psychotic symptoms in vulnerable individuals or trigger psychosis in individuals with no previous history of psychosis [19]. The synthetic cannabinoids present in these products may also induce important adverse neuropsychiatric consequences, including acute and lasting psychosis, since their pro-psychotic effects are likely related to the activity of the CB_1-receptor on dopaminergic, serotoninergic, and glutamatergic pathways [20].
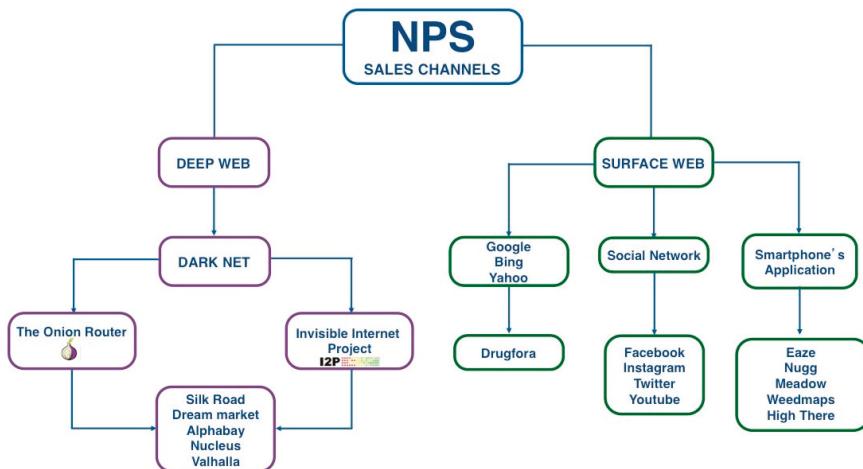
Similarly, within the e-health context, the term "e-addictology" has been recently used to indicate new technologies for assessing pathological dependencies and intervening on addictive behaviors, including computerized adaptive testing, e-health programs, web-based interventions, and digital phenotyping [21]. Importantly, new technologies can profoundly change not only the way illegal drugs are supplied, but they can also improve our understanding of drug addiction and favor the development of new interventions for addictive disorders [21].

Because not everyone has the finances or the technical skills to create or manage an Internet site, Facebook is often used as an alternative channel for sales and for advertising the use of these kinds of products [8]. On several drug fora, such as www.drugs-forum.com or www.erowid.org, these compounds are promoted and their subjective effects are discussed [9], but drug-related contents also exist on virtually all social networking sites, picture- and video-sharing services, and drug-dedicated apps. Drug selling through social media has also been reported, often using drug slang and jargon [22]. The changing policy on marijuana use in some states in North America, i.e., legalization of medical cannabis, led to an increased rate of cannabis use both in young people and adults [23,24] even though the causal effect of legalization has not been firmly assessed [25]. On the other hand, for young people,

is it difficult to recognize the risk of marijuana consumption if the law allows its use for medical purposes, and this might represents a "gateway of curiosity" [26].

3. The Deep Web and the Surface Web: Market Resilience

By typing a keyword in a search engine query such as Google, Bing, Yahoo, or others, web surfers can obtain a list of results belonging to the "surface web", while other, nonsearchable contents are referred to as the "deep web" or "invisible web" (see Figure 1). The deep web is often confused with the "dark net", but the two terms are not synonymous and overlap only partially. Basically, the deep web contains all the information stored online which is not indexed by search engines, with most information hidden simply because it is irrelevant for most users. Access to the deep web does not require special tools and a visitor can use specialized search engines or directories to locate the data for which he or she is looking. The dark web, instead, represents a small part of the deep web containing information hidden on purpose, and it typically requires special tools to enter. Like the surface web, the dark web is scattered among servers around the world and represents the portion of the Internet most frequently known for illicit activities. The most common way to access it is through The Onion Router (TOR) and the Invisible Internet Project (I2P).


Figure 1. NPS marketing, advertising, and communication network.

Developed in 2010 by the U.S. Army, the TOR browser is able to encrypt a user's IP address [27], thus making all the operations untraceable. In this way, the anonymous identities of administrators, sellers, and customers are protected [28–31] and the safe payment of any illicit good is guaranteed by of the use of cryptocurrencies, mainly bitcoins and litecoins, i.e., virtual money not controlled by government [32]. The peer-to-peer software I2P, instead, was created in 2003 purposely for illegal activities [31] in order to provide anonymous access and to bypass police and law enforcement surveillance. I2P uses a ".i2p" domain, different from TOR's classic Internet domain (WWW), to allow users to host services by I2P's homepage. The anonymous status in the web is also maintained by encryption of e-mails, files, and messages using different cryptosystems such as Pretty Good Privacy (PGP), the Amnesiac Incognito Live System, and the Tails [33].

Recently, online drug dealing has started replacing the old way of supplying drugs of abuse. Surfing in both the surface and deep web, it is possible to buy traditional illicit drugs but also temporarily legal NPS [1].

In this hidden world, the most famous platform is the Silk Road hub. Born but shutdown by the Federal Bureau of Investigation (FBI) in October 2013, it impressively reappeared after a month under the name Silk Road 2.0 in order to supply to demanding customers [34]. Although Silk Road 2.0 was closed in November 2014, it got back on track in May 2016 and is now available as Silk Road 3.0. Moreover, in recent years, many cryptomarkets became available for buying and selling NPS, including Dream Market [33] and others such as Alphabay, Nucleus, and Valhalla, which were shut down in 2016 and 2017, respectively. In addition, a collection of data from drug fora and blogs on the surface web shows that people who possess the knowledge for using the deep web are also able to access drug marketplaces and buy drugs, including NPS [31]. Since the late 2000s, a number of studies have investigated the online supply of NPS through online shops, among which was the two-year, European Commission-funded "Psychonaut 2002 project", coordinated by Fabrizio Schifano, that provided a quantitative and qualitative assessment of the online supply of NPS in a time-specific context, i.e.,"snapshot" [35]. More recently, another European project, the I-TREND (Internet Tools for Research in Europe on New Drugs) project, cofinanced by the Drug Prevention and Information Programme of the European Union, monitored the evolution of online shops and online user fora, conducted an online survey focused on NPS users, and, based on the analysis of samples and the exchange of reference standards among laboratories, ultimately produced a "top list" of NPS at the national level [22].

However, since the deep web remains inaccessible to everyone, research of NPS occurs also on the surface web, where several websites are currently selling them, advertising the products as incense, bath salts, fresheners, plant fertilizers, etc. Notably, when inserting into classical searching engines, such as Google, keywords like "legal highs" or "herbal highs", many websites are listed that offer drugs which are still legal thanks to the time that typically elapses from the appearance of a new substance into the market and its introduction in the list of regulated substances [13]. Few of these websites explicitly sold NPS; gaudy pictures, reduced price for first purchase, proposals for use of new equipment (e.g., vaporizers or smoking pipes), gift ideas, and holidays sales are only some examples of the tricks they use to capture the attention of young consumers. Everyone who is looking for a new sensorial experience and willing to try a psychoactive substance is encouraged to make the purchase with guaranteed secure payment and fast shipment.

4. Sharing Information: Drug Fora and YouTube

A large proportion of the world's population uses social networking websites, especially young adults. Therefore, it is not unexpected that conversations about drug use have transferred onto Internet drug fora and message boards [36]. The nature of conversations on drug fora (e.g., www.drugs-forum.com, www.erowid.org) varies significantly. Indeed, drug fora are used for many purposes, including sharing methods of using drugs and learning about new drugs but also for harm reduction purposes [37]. Notably, many users declare to access drug fora primarily to learn how to handle drugs more safely. As a matter of fact, some users claim to be experts and provide detailed guidelines on doses and routes of administration for each drugs class, advising against dangerous drugs interactions as well [38]. The types of visitors and/or participants to drug fora are diverse, but many of them can be considered recreational users who do not consider themselves drug addicts, do not look for treatment, and are not planning to discontinue drug use [39,40].

Drug fora are also very popular among NPS consumers. They are used to report their experiences of the positive and negative effects of substances and to provide advice on doses, routes of administration, and on how to obtain them easily [39], frequently sharing their favorite substances using pharmacological language.

The impact of conversations on drug fora on drug-use behaviors is not known, but it is reasonable to argue that monitoring such discussions could help policy and law enforcement agencies to identify emerging trends in drug use and markets [41]. In addition to drug fora, it is very common to find trip reports on YouTube, the most popular video-sharing site used by teenagers (among other users),

and also on the picture-sharing sites Flickr and Instagram. Previously used to report marijuana-, tobacco-, and alcohol-related experiences [42,43], a number of videos of various NPS are now available on YouTube, in which consumers describe in first person all proven effects including negative aspects of their experiences. Sometimes live streams after ingestion of the drug are posted. Many videos can be classified as "cautionary videos" (better known as vernacular prevention videos), others as "hedonistic/celebratory videos" (but not for crystal meth or heroin), and some are "do-it-yourself" (DIY) videos where, for example, detailed instructions on how to grow your own cannabis are provided [44]. Considering the novelty-seeking propensity of young people, this easily available online information might promote the use of these substances. Concern has been expressed for the potential negative impact of social media content depicting drug use and related behaviors [45].

5. Social Networks and Smartphone Apps

In these times, the way to surf the Internet has radically changed and social networks are the new leaders of this trend, with a large percentage of use by teenagers [17]. According to a recent survey from a leading global information and measurement company [46], Internet users engage longer in social media sites and apps than in activity on any other type of website. The same survey estimated that the social networking giant Facebook has currently more than 1.6 billion registered users, that the video-sharing site YouTube has more than 1 billion active users, while the social streaming site Twitter has more than 500 million registered users worldwide. Given these numbers, all these platforms have inevitably attracted the interest of drug suppliers, which strictly follow their evolution and diffusion among young people over time. In the last few years, several social networks have acquired important roles in market places for both NPS and illicit drugs [33] (see Figure 2). Simply looking on Facebook, it is now possible to find information and direct links to proceed with the purchase of several NPS or to simply share your experiences in groups created for drug-users only. Even the picture- and video-sharing service Instagram, despite its different use compared to the more famous Facebook, is used to look for new possible customers [47]. Several ambiguous profiles are used to post pictures of their products with hashtags such as #cannabiseeds, #headshop, #herbalicense, and #over18sonly. In 2014, drugabuse.com, for example, published on Instagram an infographic documenting drug-dealer activity [48].

Facebook	Instagram	Twitter
legal highs	#cannabiseeds	#legalhights
pharmaceuticals	#headshop	#ResearchChemical
chemicals	#herbalicense	#syntheticdrug
researchchemicals	#over18sonly	

Figure 2. Keywords and hashtags in social networks with explicit content on NPS.

On Twitter, by simply typing #legalhighs, it is possible to buy "the blue stuff", otherwise known as methamphetamine, of the famous *Breaking Bad* television series as well as other #ResearchChemicals, with free shipping offers and credit/debit card payments accepted. All the users, indeed, can be part of a #highsociety, allowing them to share their #proudstoners daily states of mind. Very recently, epidemiologists and linguistic scientists used Twitter to test the feasibility of producing

a fully-automated "drug term discovery" system capable of tracking emerging NPS terms in real time [49], which confirms that data collected on Twitter may be used to explore trends in NPS selling and use [50]. Along with other cyber drug communities (e.g., blogs, drug fora, Facebook), Twitter allowed the identification and characterization of a new generation of NPS users, the so-called "e-psychonauts", that considered themselves as psychedelic researchers, mind navigators, or chemicals experimenters [51].

In a technological world where about 2.4 billion people use a smartphone and an increasing number of people use apps, drug dealers adapt their activity accordingly and create simple apps that make buying any kind of psychoactive substance easier, including NPS. Although some apps are designed to prevent drug use, such as "Your Face on Meth" (where you can upload your picture and see its physical degradation over time potentially resulting from using methamphetamine), many apps are created specifically to promote drug use [52].

In North America, the number of cannabis-related smartphone apps is very impressive. In 2014, the number of apps returned from searches using terms like "cannabis" and "marijuana" were 124 and 218, respectively, in Apple's Store, and 250 for both on Google Play [14]. These apps have several content codes, contain information on different cannabis strains and synthetic cannabinoids mixture (e.g., K2, Spice), advice for growing cannabis, and recipes for cooking "special meals". Several apps create a connection with medical marijuana doctors to obtain a prescription, while other apps, such as Eaze, Nugg, Meadow, and Weed Maps, offer to trace medical dispensaries of marijuana, indicating to users the closest spot based on their location in addition to finding the closest doctor that will recommend medical marijuana [14,15]. Additionally, using the app High There, for instance, it is possible to match people to smoke together, while other apps, similar to Instagram and used mostly in Europe and the United States, are utilized for posting photos, videos, or texts related to marijuana or psychoactive substances. Noteworthy, apps useful for making untraceable calls to drug dealers are becoming very popular.

6. Conclusions

New psychoactive substances are very popular among young people and online communities, but very little information about toxicological and side effects are available on the Internet. The web-based open sale of unregulated NPS has shown a steady increase in recent years; the easy availability of NPS and the fluctuating dynamics of this new drug market represent a public health concern and an intricate regulatory issue. Research in the field is increasing, and several groups of clinical and preclinical researchers worldwide are investigating the central and peripheral effects of synthetic cannabinoids and synthetic cathinones [1,53–58], synthetic opioids and ketamine-like compounds [59,60], and many others [61,62]. Yet, it is fundamental to share scientific evidence on risks related to the consumption of these compounds using the same channels that promote them. Analyses of social media may represent a new approach to uncover and track changes in drug terms and markets in near real time. In conclusion, the NPS phenomenon is intricate and still very difficult to control. Using the same channels responsible for their growing diffusion to disseminate information and scientific knowledge about the risks associated with their use could represent a potential new approach to limit the diffusion of these dangerous substances.

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Synthetic Cannabinoid use in a Case Series of Patients with Psychosis Presenting to Acute Psychiatric Settings: Clinical Presentation and Management Issues

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Abstract: Background: Novel Psychoactive Substances (NPS) are a heterogeneous class of synthetic molecules including synthetic cannabinoid receptor agonists (SCRAs). Psychosis is associated with SCRAs use. There is limited knowledge regarding the structured assessment and psychometric evaluation of clinical presentations, analytical toxicology and clinical management plans of patients presenting with psychosis and SCRAs misuse. Methods: We gathered information regarding the clinical presentations, toxicology and care plans of patients with psychosis and SCRAs misuse admitted to inpatients services. Clinical presentations were assessed using the PANSS scale. Vital signs data were collected using the National Early Warning Signs tool. Analytic chemistry data were collected using urine drug screening tests for traditional psychoactive substances and NPS. Results: We described the clinical presentation and management plan of four patients with psychosis and misuse of SCRAs. Conclusion: The formulation of an informed clinical management plan requires a structured assessment, identification of the index NPS, pharmacological interventions, increases in nursing observations, changes to leave status and monitoring of the vital signs. The objective from using these interventions is to maintain stable physical health whilst rapidly improving the altered mental state.

1. Introduction

Recent statistics in England have reported an increased number of hospital admissions with a primary diagnosis of drug-related mental health/behavioural disorders and poisoning by illicit drugs, respectively of 12 and 40% compared to statistics released in 2006/07 [1]. Over the last five years deaths involving Novel Psychoactive Substances (NPS) have sadly increased, with a further 8% increase in 2016 [2]. NPS represent an emerging and concerning global phenomenon [3–5] mainly due to: (1) the difficulties posed in the clinical management, of both mental and physical health; and (2) the absence of a clear and formal/structured description of the clinical presentation of patients using NPS, with obvious repercussions on clinical management.

NPS are a heterogeneous class of typically synthetic molecules including: synthetic cannabinoid receptor agonists (SCRAs), synthetic cathinones, amphetamine-derivatives, psychedelic phenethylamines, ketamine derivatives, novel tryptamines, synthetic opioids and sedatives (GABA-A/B agonists) [3,6,7]. Trends in illicit drug use over the last decade clearly show that adolescents and young adults give preference to NPS instead of traditional psychoactive substances (TPS) (e.g., cannabis, cocaine, heroin, amphetamines, LSD, ecstasy, ketamine, etc), because NPS are cheap and easily available either on the street or from websites [6–8]. They are difficult to identify in blood or urine drug screening (UDS) [9]. Lifetime NPS consumption was reported by 8% of young individuals in 2015 [10] up from 5% in 2011 [6]. Young adults (aged 16 to 24) are around twice as likely to have used an NPS in their lifetime compared to older adults (aged 16 to 59) [11]. It has recently been reported that the three psychiatric diagnoses most frequently associated with NPS use are bipolar disorder (23.1%), personality disorders (11.8%), and schizophrenia and related disorders (11.6%) [12].

In comparison to TPS users, poly NPS users are more likely to be young males [11], with daily use of traditional cannabis, weekly or more use of ecstasy, recent LSD use, higher levels of poly drug use, and a history of overdose on any drug in the past year [13]. Young adults attending nightlife events in pubs and discos are also more prone to poly-substance use, mainly combining NPS with alcohol and cocaine [14]. NPS users also tend to have a forensic history and a history of promiscuous sexual activity (e.g., chem-sex) [13].

Few studies have attempted to provide a psychopathological description of the clinical presentation of NPS users in acute settings. For example, in an observational cohort study enrolling consecutive adult patients presenting to an Emergency Department (ED) in London, the most common clinical features identified were seizures and agitation [15]. In a recent study looking into the impact of NPS misuse on admissions to an acute psychiatric facility in London, increased levels of violence in the group of NPS users were identified ([16]. Data collected in the Accident and Emergency departments (A&Es) of ten European countries have shown that the association between NPS use and the occurrence of psychosis varied considerably, depending on the type of drug used [17]. In particular, psychotic symptoms were noted in 6.3% of a large sample (5529 consecutive cases), with psychosis being more common amongst NPS users that had used tryptamines, methylenedioxypyrovalerone (MDPV), methylphenidate, SCRAs and amphetamine-type compounds [17]. A mounting range of evidence suggests that SCRAs can trigger the onset of acute psychosis in vulnerable individuals and/or exacerbate psychotic episodes in those patients with a previous psychiatric history. The literature reports a wide range of psychopathological issues such as paranoid thoughts, increases in aggressive and combative behavior, together with confusion, agitation and suicidal thoughts [18]. It has been suggested that SCRAs may have a higher psychosis-inducing potential compared to natural cannabis [19] because of the lack of cannabidiol—a substance associated with the medicinal effects of cannabis. For a review on existing studies and models of cannabis induced psychosis see a review from Murray et al [20].

Professionals usually report feeling less confident about managing NPS compared to TPS users, specifically because of the lack of clear guidance regarding the clinical management and the increased risk of toxicity [10–18,21]. For example, with the ingestion of NPS with high serotonergic activity (e.g.: psychedelic phenethylamines), misusers may present with hyperthermia, seizures, and hyponatraemia.

Conversely, NPS with high dopaminergic activity (e.g., methylphenidate-like drugs such as some synthetic cathinones) are highly addictive and associated with prolonged stimulation, insomnia, agitation, and psychosis [22,23]. Furthermore, most SCRAs are at times associated with medical emergencies such as hypertension, myocardial infarction, renal failure [24], elevated heart rate, hyperglycaemia, nausea, vomiting, hypokalaemia, and seizures [18]. Moreover, in view of the increased use of latest generation of sedatives or ultra-high potency fentanyl derivatives, the assessment of vital parameters is of paramount importance when NPS users are presenting to an emergency or acute facility [3–6].

The implementation of an appropriate and safe clinical management plan is commonly based on patients' accounts on which kind of NPS have been used. Acute mental health and emergency services are not routinely equipped with urine drug screening tests (UDS) for NPS in order to identify and provide an appropriate toxicological confirmation [15]. This causes considerable limitations when offering a targeted treatment strategy that can address patients' presentations and potentially life-threatening intoxications [9,13,25].

At present, there is a dearth of detailed information relating to the clinical presentation of NPS users in acute mental health settings, especially in terms of: (1) descriptions of behavioural and psychopathological features using structured assessment and psychometric scales; (2) analytical toxicology and identification of the index NPS used; and (3) appropriate and evidence-based clinical management plans. This results in a range of difficulties in formulating targeted/individually tailored treatment plans.

The aims of this case series are: (1) to offer a standardized description of NPS users' clinical presentations to provide clinicians with objective and measurable clinical pictures aimed at shaping protocols and standard operating procedures when NPS use is suspected and/or detected; (2) to provide best practice advice in the management of mental state alterations and medical complications, with the goal of reducing the number of serious adverse outcomes associated with NPS use.

2. Materials and Methods

2.1. Study Design and Recruitment

Data on presentation and clinical management of 4 cases, selected amongst patients consecutively admitted to two acute psychiatric wards from June 2017 to June 2018 at Highgate Mental Health Centre—Camden & Islington NHS Foundation Trust—were retrospectively collected using a database. Patients were aged 18 and 65 years and admitted because of presenting with psychotic illnesses and with a history of NPS use before or during admission. Data were collected using a standardized database to capture a range of information at baseline i.e., the time of the admission, and then during and after NPS intake. The information collected revolved around: (1) the clinical presentation, with a formal description of the psychotic symptoms; (2) the type of recreational drug(s) used; (3) the physical health outcomes; (4) the levels of psychiatric inpatient observation and leave status.

The clinical presentation was identified with the help of clinical notes and corroborated by the retrospective scoring of the Positive and Negative Syndrome Scale (PANSS), a multi-item questionnaire widely used to quantify disease severity in schizophrenia and psychosis [26]. Monitoring data of vital signs were collected using the National Early Warning Signs tool (NEWS) [27]. Data on the type of substance used were collected using routine drug screening tests for traditional psychoactive substances whilst a more thorough analysis (urine and/or oral fluids tests; Alere Laboratories technologies) was carried out to identify the index drug(s) used by both NPS and TPS patients. All subjects identified received medications included in the British National Formulary (BNF), such as benzodiazepines (BZO) and antipsychotics (first and second generation), with the choice guided by the clinical presentation and the drug testing results. Levels of nursing care, such as close or general observations, were also recorded together with the possibility of leave in the hospital grounds , either accompanied by staff (escorted) or alone (unescorted).

2.2. Participants

2.2.1. Inclusion Criteria

Cases were selected amongst patients with recent or current histories of NPS use, aged 18–65 years, presenting to acute services with a psychotic illness classified by the International Classification of Diseases, Mental and Behavioral Disorders [28] as schizophrenia, schizotypal, delusional, and other non-mood psychotic disorders (ICD-10 codes: F20-29), mood (affective) disorders (ICD-10 codes: F30-39), and mental and behavioral disorders due to psychoactive substances (ICD-10 codes: F10-19).

2.2.2. Exclusion Criteria

Patients were excluded from the study if: (1) the psychotic symptoms were precipitated by an organic cause; (2) they had moderate or severe learning disability; (3) they suffered from a medical or neurological illness or (4) had an insufficient command of the English language.

2.3. Measures

2.3.1. Psychometric Measures

The Positive and Negative Symptoms Scale (PANSS) is a multi-item questionnaire widely used to quantify disease severity in schizophrenia and to assess the severity of positive (or productive) and negative (or deficit) symptoms of psychosis [26]. PANSS is easy to administer and is based on the clinician's interview with the patient; data are gathered looking at the patient's mental state over the previous week, with the patient's family and/or his/her acquaintances being able to provide further information. PANSS consists of 30 items and takes 45–50 min to be completed by the clinician.

The National Early Warning Score (NEWS; Royal College of Physicians, London, UK) [27] is a standardized system for the assessment of acute illness in adults. It is based on six vital signs such as respiratory rate, oxygen saturation, temperature, blood pressure, pulse/heart rate, and alertness. Each parameter yields a score of between 0 and 3 so that when the scores for all the parameters are summed a total NEWS score of between 0 and 18 is achieved. A score of 7 or greater indicates that a patient is likely to be critically medically unwell.

2.3.2. Laboratory Measures

Standard urine drug screening tests were used to detect TPS such as heroin, cocaine, amphetamine, THC, and methadone. For NPS, the Alere drug screening urine and oral fluids tests were used. The urine tests provided a rapid screening of 30 synthetic cannabinoids at once whilst the oral screening test was able to detect mephedrone.

3. Clinical Vignettes

3.1. Case 1—Mr A

28 years old Caucasian male, single and unemployed, living alone, with a positive forensic history and a diagnosis of Paranoid Schizophrenia. The patient had a 4 years' history of psychosis with frequent relapses (5 admissions in 4 years). He was transferred to an acute treatment ward from a psychiatric intensive care unit (PICU). At the time of the transfer the patient was stable and on treatment with Risperdal Consta 37.5 mg fortnightly + Olanzapine 10 mg daily + Pregabalin 100 mg daily. The PANSS score was 73/210 and his psychopathology was mainly characterized by positive symptoms: delusional mood, persecutory and grandiose delusions and second and third person auditory hallucinations. The UDS was initially negative but, one week after the transfer, Mr A's mental state deteriorated suddenly and he became very agitated and verbally and physically aggressive. He presented with a bizarre and repetitive behaviour consisting of stopping and remaining immobile for a few minutes and then running fast along the ward corridor. He also had second and third person

auditory hallucinations, persecutory delusions and thought disorganization. He started to fear the hospital ward's electronic fire alarms. He believed that the fire alarms were cameras that were spying on him and he was very preoccupied with specific members of the staff whom he believed were there to kill him. The hallucinations also became very severe and he was responding to internal stimuli constantly throughout the day. The total PANSS score was 109/210 and the UDS was positive for SCRAs. We decided to increase Olanzapine to 20 mg, daily and to add Clonazepam 8 mg, daily to manage the agitated behaviour and the psychotic symptoms. We also increased the level of monitoring of his vital measures by completing the NEWS scores twice a day. NEWS scored 2 with increased heart rate and fluctuating blood pressure. We considered a transfer to PICU but since the patient was starting to respond well to the new treatment plan and the reason for the relapse was evident (NPS intake), we decided to continue to treat the patient on the acute ward. Mr. A responded well to the change/increase of medications, his symptoms improved in 24 h and within 7 days from the acute intoxication the PANSS scores reduced to 74/210.

3.2. Case 2—Ms T

32 years old Caucasian woman, single and unemployed, living alone, with no forensic history and a diagnosis of Schizoaffective Disorder and poly-substance misuse (mainly crack cocaine and heroin). Ms T was stabilized on a combination of Aripiprazole 30 mg, daily + Lithium carbonate 800 mg, at night. The PANSS score was 95/210 and the UDS was negative for all illicit substances. Four weeks later, the patient's mental state deteriorated suddenly. She became physically and verbally very aggressive with severe features of sexual disinhibition. The patient presented with delusional mood and with complex grandiose and persecutory delusions such that she believed she was part of a secret army and she had powers to kill people with her thoughts. She also believed she was being chased by the Albanian mafia and had to fight for her life. The patient also became very aggressive with members of staff and on four occasions it was necessary to call the emergency team to provide extra sedation. The PANSS score was consistent with the deterioration of her mental state, scoring 115/210 and the UDS tested positive for both SCRAs and THC. The clinical team felt that the patient needed a more robust pharmacological treatment plan and therefore Haloperidol 10 mg daily + Clonazepam 8 mg daily were added. The patient remained acutely unwell for more than 72 h. Ten days after the intoxication Ms T remained still irritable and agitated. The PANSS score was 115/210, 10 points higher than the baseline, and the UDS continued to test positive for SCRAs. NEWS were increased to twice a day but the score was always within range (0 or 1) with tachycardia being the only altered parameter. Meanwhile, other patients on the ward tested positive for SCRAs and it was suspected that Ms T was bringing SCRAs to the ward. At that stage, leave was suspended and a stricter search policy was enforced on the ward. The patient's mental state improved further and ten days later her urine tests were negative for SCRAs.

3.3. Case 3—Mr Y

20 years old Black-Caribbean male, single and unemployed, living with friends and with no forensic history, was quickly re-admitted to a treatment ward following the sudden onset of bizarre behaviour after an earlier discharge from another ward. The diagnosis was First Psychotic Episode in the context of poly-substance misuse. On admission, Mr Y was on Haloperidol Decanoate 50 mg, monthly + Haloperidol 10 mg, at night (on reducing regime). He appeared severely thought-disordered, sexually disinhibited and aroused, approaching other patients for sex or suddenly becoming physically aggressive by spitting on others. The PANSS score was 116/210 with prominent positive symptoms (positive symptoms subscale 40/49). He presented as being severely disruptive, chaotic, and intrusive into other patients' care, attacking staff and other patients, urinating on the floor and spitting at other people's faces. Mr Y was therefore treated with Aripiprazole 9.75 mg three times a day + Clonazepam 6 mg daily in divided doses. Observation levels were increased to 2:1 arms' length to reduce risks of retaliation from others due to sexually inappropriate and aggressive behaviour. NEWS monitoring

was increased to hourly to monitor any possible deterioration in physical health. UDS were positive for benzodiazepines and SCRAs. The patient remained unwell. Observation levels were maintained at 2:1 arms' length and NEWS monitoring decreased to TDS once physical outcomes remained stable for 12 h. After 72 h the clinical condition improved with a reduction of PANSS score to 98/210. Eventually, because of the continued high risk of retaliation from others Mr. Y was transferred to a Psychiatric Intensive Care Unit (PICU).

3.4. Case 4—Mr G

39 year old Asian British man, married and unemployed, living with his family and with a long forensic history. Mr G had a long-standing history of Bipolar Disorder since the age of 28. He had a history of numerous admissions, was non-compliant with his medications, and engaged poorly with his community team. He presented with a long-term history of poly-substance misuse (e.g., alcohol, cocaine, MDMA, cannabis, "legal highs"). He had previously been treated with a mood stabilizer (Sodium Valproate); Zuclopenthixol and Risperidone Depot (both stopped due to sexual dysfunction); Olanzapine and Quetiapine (both stopped due to poor response). At the time of his admission to Highgate Mental Health Centre, he was administered Abilify Depot 400 mg, monthly with no or little efficacy. He was transferred from another ward on Section 3 due to a manic relapse, with no leave and a diagnosis of Bipolar Affective Disorder (BPAD, current episode manic). Mr. G had a long history of violence towards staff and patients (he broke a nurse's nose and stabbed another patient with a pen). At the time of the admission, he was very agitated, aggressive and intimidating, banging his fist on the table and threatening staff with a glass bottle. He also showed bizarre behavior, e.g., wearing sunglasses whilst indoors, holding pieces of paper with some incomprehensible notes on Hitler, quantum physics and aliens. He was thought disordered with grandiose delusional beliefs regarding him being the King of Egypt and able to cause a nuclear war. It proved very difficult to verbally de-escalate him and he did not agree to change his medication regime as he believed that he should be treated "only with love". The PANSS score was 108/210. Abilify was withdrawn and Zuclopenthixol started whilst he continued the rest of his medications. On admission, UDS was negative for both NPS and TPS. A week later, UDS was positive for both benzodiazepines and SCRAs, and NEWS was increased to TDS. Two weeks later, UDS continued to be positive to SCRAs, no changes to his leave status were made, with garden leave being maintained. Three weeks after admission, UDS was positive for benzodiazepines and THC and four weeks later the admission UDS was positive for THC and SCRAs. After admission, his mental state remained unsettled with refractory manic positive symptoms and a poor response to medication. The PANSS score was 123/210. Hence, his leave was stopped and, a week later, his UDS became negative for all substances, SCRAs included. His positive symptoms started to improve with a reduction of PANSS to 66/210. Over the following four weeks Mr. G appeared well kempt and settled on the ward, with no grandiose delusions and no further episodes of aggression. He showed a satisfactory response to Zuclopenthixol 300 mg, weekly + Sodium Valproate 1200 mg. UDS was negative for all substances and, therefore, Mr. G was safely discharged to the community team.

4. Discussion

NPS misuse is a recent phenomenon and knowledge of its effects, either in the short or the long term, on the population is relatively poor [3]. There is an increasing amount of knowledge regarding the effect that NPS have on individuals with severe mental illness [12]. However, well documented evidence of the negative impact are limited and most cases have not been corroborated by analytic chemistry evidence of the NPS used [15]. Furthermore, there is a paucity of data to guide the monitoring and management of patients with severe mental illness who take NPS, and then suffer from acute psychopathology and physical ill-health [3].

To the best of our knowledge, our case series is the first and only attempt made at describing, with the use of a specific psychometric scale (PANSS), the effects of acute intoxication with NPS (mainly

SCRAs) identified through UDS in an acute hospital setting in patients suffering from severe psychotic disorders. All four cases show how clinically significant the impact of SCRAs use was on their mental states. The cases showed marked and sudden clinical deteriorations, with intense exacerbations of positive symptoms, psychomotor agitation, sexual disinhibition, verbal/physical aggression, and poor responses to medications. The latter phenomenon makes the clinical and risk management of these patients more difficult, and it is therefore necessary to develop appropriate management plans to minimize such risks.

In order to set up an appropriate and safe Informed Clinical Management Plan (ICMP) (Table 1), the first step advised here is to establish, whenever possible, which NPS is responsible for the intoxication due to their wide range of effects. We advocate, therefore, the use and further development of reliable drug tests to identify the specific NPS types associated with particular clinical presentations. Accurate testing for NPS would assist in establishing clear diagnoses, formulating ICMPs and identifying the most effective treatments for intoxications with particular NPS.

Table 1. Description of the Camden & Islington—Informed Clinical Management Plan (ICMP).

Camden & Islington—Informed Clinical Management Plan (ICMP)	
MENTAL STATE assessment	
Using a psychometric scale (PANSS)	Monitoring mental state
NPS detection	
Using specific analytic toxicology to detect NPS (UDS and/or oral swabs)	Monitoring access to substances and leaves (reduced/suspended—escorted/unescorted)
MEDICATION & PHYSICAL HEALTH monitoring	
Using benzodiazepines (BDZ) and/or second generation antipsychotics (SGA) when possible	Monitoring physical health (NEWS) Levels of nursing observations

The cases described here were all characterized by acute SCRA intoxications. The clinical presentations were characterized by an acute onset of agitation and aggressive behavior; the symptoms decreased in intensity and frequency in no less than 72 h. The management of acute intoxications was by identifying the substances responsible for the sudden deteriorations, and by treating the symptoms with benzodiazepines and antipsychotic medications. In addition vital parameters were monitored, nursing observations were increased, and leave statuses were changed. These measures led to rapid and successful resolutions of symptoms and reduced the need for transfer to more intensive care settings. Furthermore, they promoted more rapid step-down and recovery in the community.

In general terms, pharmacological treatment remains the mainstay of treatment. However, the novelty of the use of medications according to our protocol is that pharmacological interventions are guided by NEWS and toxicology results. For example, haloperidol should be avoided in patients that have used cathinones for the toxic effect on cardiac rhythm; and benzodiazepines should be avoided in patient with a NEWS score of 3 because of low oxygen saturations levels.

In terms of pharmacological treatment, the use of BDZ has been recommended with or without a second-generation antipsychotic (SGA) to reduce the risk of cardiac side effects [22,23]. Benzodiazepines remain the first line treatment, although their use needs to be weighed against the risk of respiratory depression when given to subjects who have ingested alcohol and/or unknown substances [6–18]. Amongst the SGAs, whilst aripiprazole is probably the safest antipsychotic to be used in such scenarios because of its negligible effect on QTc, olanzapine has proven to be effective in treating psychotic symptoms caused by NPS [29].

5. Limitations

We are aware that four cases are not representative of the multi-faceted spectrum of presentations with NPS use/intoxication and observation of a wider sample size is necessary. However, we believe

that this study is important in generating hypotheses that may lead to more comprehensive projects such as case control studies.

Furthermore, although the four cases presented were objectively described by using the PANSS to provide an objective measurement of the clinical observation, this was made retrospectively. We are also aware that the patients described were intoxicated with SCRAs. No other NPS such as cathinones or mephedrone were detected in our sample population. Therefore, our clinical description-albeit exhaustive and comprehensive-is limited to a subgroup of NPS users using only SCRAs. Moreover unfortunately, the UDS screening tests that were available were not able to identify with higher specificity the type of SCRAs used.

It is worth noting how in terms of diagnostic categories our patients sample was not homogenous as one of the four patients was presenting with bipolar disorder. This may be an additional confounding factor; and, in a large enough sample, patients should be divided according to diagnoses.

Finally, a randomized controlled trial (RCT) to establish which ICMP is necessary to establish which treatment plan is most effective in the management of individuals with a severe mental illness intoxicated with NPS would be helpful.

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Novel Synthetic Opioids: The Pathologist's Point of View

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Abstract: Background: New Psychoactive Substances (NPS) constitute a broad range of hundreds of natural and synthetic drugs, including synthetic opioids, synthetic cannabinoids, synthetic cathinones, and other NPS classes, which were not controlled from 1961 to 1971 by the United Nations drug control conventions. Among these, synthetic opioids represent a major threat to public health. Methods: A literature search was carried out using public databases (such as PubMed, Google Scholar, and Scopus) to survey fentanyl-, fentanyl analogs-, and other synthetic opioid-related deaths. Keywords including "fentanyl", "fentanyl analogs", "death", "overdose", "intoxication", "synthetic opioids", "Novel Psychoactive Substances", "MT-45", "AH-7921", and "U-47700" were used for the inquiry. Results: From our literature examination, we inferred the frequent implication of fentanyls and synthetic opioids in side effects, which primarily affected the central nervous system and the cardiovascular and pulmonary systems. The data showed a great variety of substances and lethal concentrations. Multidrug-related deaths appeared very common, in most reported cases. Conclusions: The investigation of the contribution of novel synthetic opioid intoxication to death should be based on a multidisciplinary approach aimed at framing each case and directing the investigation towards targeted toxicological analyses.

Keywords: fentanyl; NPS; synthetic opioids; MT-45; AH-7921; U-47700; forensic pathology

1. Introduction

New Psychoactive Substances (NPS) are a heterogeneous class of non-controlled substances available on the global illicit drug market (e.g., smart shops, internet, "darknet"). The use of NPS, often consumed along with other drugs of abuse and alcohol, has resulted in a significantly growing number of emergency admissions due to overdoses and a high number of deaths. By July 2017, 739 different NPS were reported to United Nations Office on Drugs and Crime (UNODC) [1]. According to the The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)report, with an overall total of 38 substances, synthetic opioids have become the fourth largest group of substances monitored in 2017, after synthetic cannabinoids (179 substances), cathinones (130), and phenethylamines (94) [2].

The class of synthetic opioids include fentanyl, its analogs used in medical therapy (e.g., sufentanil, alfentanil, and remifentanil) [3], and novel non-pharmaceutical fentanyls not approved for human

medical use (e.g., acetylfentanyl, acryloylfentanyl, carfentanil, α-methylfentanyl, 3-methylfentanyl, furanylfentanyl, 4-fluorobutyrylfentanyl, 4-methoxybutyrylfentanyl, 4-chloroisobutyrylfentanyl, 4-fluoroisobutyrylfentanyl, tetrahydrofuranylfentanyl, valerylfentanyl, cyclopentylfentanyl, and ocfentanil), and compounds with different chemical structures, such as MT-45 (1-cyclohexyl-4-(1,2-diphenylethyl)piperazine), AH-7921 (3,4-dichloro-*N*-{[1(dimethylamino) cyclohexyl]methyl} benzamide) and U-47700 (3,4-dichloro- *N*-[(1R,2R)-2-(dimethylamino)cyclohexyl]-*N*-methylbenzamide) [4,5]. They are used on their own or more often in combination with heroin or other opioids [6,7]. This paper critically examines the literature on deaths related to fentanyls and synthetic opioid overdose, alone or in combination with other psychoactive drugs (i.e., cocaine, benzodiazepine, alcohol, and other opioids) and investigates the characteristics and complexity of such deaths, analyzing all data useful for the forensic pathologist.

2. Materials and Methods

A literature search was carried out using public databases (such as PubMed, Google Scholar, and Scopus) to revise fentanyl-, fentanyl analogs- and others synthetic opioids-related deaths. Keywords including "fentanyl", "fentanyl analogs", "death", "overdose", "intoxication", "synthetic opioids", "Novel Psychoactive Substances", "MT-45", "AH-7921", and "U-47700" were used for the inquiry. The data were collected from 1990 to June 2018. Only deaths were considered. There were no language restrictions. All types of papers were included. We also reviewed the reference lists of the identified publications and PubMed suggestions. The full texts of all the eligible papers were obtained. Finally, 128 articles were included in this review. The examined data included the circumstances of death (e.g., trauma, external injuries) and drug exposure (pharmaceutical versus illicit drug use). With regard to the concentrations of the compounds, blood and, when stated, liver, urine, stomach content, kidney, brain, vitreous humor, and nasal swab concentrations were reported.

3. Results

3.1. Synthetic Opioid Overview

Novel synthetic opioids, similar to the classical opioids morphine and heroin, selectively bind to the μ-, δ-, and κ-opioid receptors in the peripheral and central nervous system (CNS), thereby simulating the effects of endogenous opiates. However, they generally show greater selectivity towards the μ-opioid receptor subtype than morphine [8]. Stimulation of the μ-opioid receptor promotes the exchange of GTP (Guanosine Triphosphate) for GDP (Guanosine Diphosphate) in the G-protein complex and subsequently inhibits adenylate cyclase in cells causing a decrease in intracellular cAMP (Cyclic Adenosine Monophosphate). In addition, the activation of the μ-opioid receptors inhibits calcium and potassium ion channel conductance [8]. All these molecular events cause cellular membrane hyperpolarization and inhibit tonic neural activity with a consequent reduction in the release of several neurotransmitters, such as substance P, GABA, dopamine, acetylcholine, and noradrenaline [9].

These neurochemical changes are mainly responsible for the pharmaco-toxicological effects induced by synthetic opioids. Typically, acute intoxication induced by "classical" and novel synthetic opioids is characterized by miosis (but later the pupils may become dilated), a reduced level of consciousness (CNS (central nervous system) depression), respiratory depression, hypoxia, acidosis, hypotension, bradycardia, shock, gastric hypomotility, paralytic ileus, pulmonary edema, lethargy, coma, and even death.

In the last few years, the novel fentanyls have become a serious concern. These substances currently dominate the synthetic opioid group, with a total of 28 reports since they first surfaced in 2012. Fentanyl, which is the prototypical compound of this class, is a synthetic, lipophilic phenylpiperidine opioid agonist. It was developed in the 1960s by Paul Janssen in Belgium, and it is now available therapeutically as an intravenous, transbucal, or transdermal preparation, commonly used for surgical

anesthesia and to treat severe chronic pain [10]. Because of its highly potent opioid euphoric effects, fentanyl leads addicts to rapidly abuse this drug through a variety of different methods, including the oral abuse of transdermal fentanyl patches [4]. The clinical effects are dose dependent, ranging from the induction of analgesia alone by serum concentrations of 0.3–0.7 ng/mL to the loss of protective airway reflexes and CNS depression by serum concentrations >3 ng/mL [11]. In addition, fentanyl and its analogs produce drowsiness and euphoria [12]. The most common side effects may include nausea, dizziness, vomiting, fatigue, headache, and constipation. The repeated use of fentanyls leads to the development of tolerance and dependence [13]. Typical withdrawal symptoms involve sweating, anxiety, diarrhea, bone pain, abdominal cramps, and shivers or goose "flesh" [12,14].

From a pharmacological point of view, fentanyl and its analogs are significantly more potent than morphine, with effects' magnitudes ranging from 1.5–7 times (butyrylfentanyl) to 10,000 times (carfentanil) those of morphine [14], and are characterized by high lipid solubility, rapid onset of action, and short duration of action. Like other types of opioid analgesics, such as morphine, methadone, and heroin, fentanyls produce their main effects by stimulating at nanomolar affinity the μ-opioid receptor [15–18]. In particular, they induce acute analgesia, relaxation, euphoria, sedation, bradycardia, hypothermia, depression of the central nervous system and respiratory function [16,19–21]. The last of the listed side effects poses the greatest danger to users and it is responsible for fentanyls-related significant morbidity and mortality [22]. The timely administration of the antidote naloxone [23] can rapidly reverse the severe respiratory depression caused by fentanyls [24], although multiple naloxone doses may be required [25]. The rapid administration of naloxone following fentanyl's overuse is essential, because of the rapid onset of action of the drug that can cause respiratory depression within two minutes [26]. The optimal dose range and methods of administration of naloxone are still not clear. The UK Department of Health recommends the following naloxone dose regimen: an initial dose of 0.4 mg intravenously, followed by up to two doses of 0.8 mg. If the latter two doses are ineffective, a further 2 mg dose should be provided [27]. In fact, despite the fact that fentanyl shows an affinity for μ-opioid receptor (Ki ~ 1.346 nM) similar to that of morphine (Ki ~ 1.168 nM) [28], it displays a greater potency (EC_{50} ~ 0.15 nM) than morphine (EC_{50} ~ 2.4 nM) in functional biological assays [29]. On this basis, higher naloxone doses may be necessary to reverse the adverse effects of fentanyls (i.e., lofentanil) with greater μ-opioid receptor affinity (Ki ~ 0.023 nM) [30] and potency (EC50 ~ 0.03 nM) [29].

Because of the narrow therapeutic index of fentanyl (and, presumably, of its analogs) its recreational use is highly dangerous, especially in opioid-intolerant users. High doses might hasten death due to respiratory arrest and pulmonary edema. Fentanyl analogs are clandestinely developed for recreational use [15,30–34]. These compounds have been synthesized by modification or replacement of the fentanyl's propionyl chain or by replacement of its ethylphenyl moiety. The obtained analogs have been further modified by substitution with fluoro, chloro, or methoxy groups at the *N*-phenyl ring. Among fentanyl analogs, carfentanil [methyl 1-(2-phenylethyl)-4-(*N*-propanoylanilino) piperidine-4-carboxylate] is considered one of the most lethal opioids, showing an extremely high clinical potency. It is used in research and, in some countries, as a veterinary medicine to immobilize large animals. Between November 2016 and April 2017, carfentanil was involved in at least 61 deaths in eight European countries. The vast majority of those deaths are related to heroin consumption [2].

Fentanyls have been found in a range of physical and dosage forms in Europe. The most common form is powder, but they have also been detected in liquids and tablets. E-liquids containing fentanyls that can be vaped using electronic cigarettes have also been reported [2].

These forms are easily absorbed through more convenient administration routes than injection, yet provide the consumers with psychoactive effects similar to those obtained with injectable forms. However, their use may pose a high risk of accidental overdose. In fact, nasal sprays and e-liquids could make fentanyls use more attractive and socially acceptable, promoting their spread and usage.

In addition to fentanyls, other novel synthetic opioids with chemical structures different from fentanyl, i.e., MT-45, AH-7921, and U-47700, have appeared on the recreational drug

market and are causing intoxication and potentially fatal outcomes in consumers. MT-45 (1-cyclohexyl-4-(1,2-diphenylethyl) piperazine)), also known as IC-6, CDEP, AC1L8SAC, and NSC 299236, has shown particular effects, such as paresthesia in limbs, hand weakness, balance disturbances, vision impairments, and hearing impairment or loss [35]. In three cases, unusual side effects have been reported, such as loss and depigmentation of hair, folliculitis and dermatitis, painful intertriginous dermatitis, and elevated liver enzymes [36]. MT-45 has been associated with many reports of fatal intoxications in Europe; in particular, Sweden reported 28 analytically confirmed deaths between November 2013 and July 2014 [37,38]. In vitro and in vivo metabolism studies have shown that MT-45 is biotransformed into active hydroxylated compounds [39] that may contribute to the overall pharmaco-toxicological profile of MT-45 in vivo [40]. AH-7921 (3,4-dichloro-*N*-[1(dimethylamino) cyclohexyl] methyl benzamide) belongs to a series of compounds known as cyclohexylamines [41]. The drug exhibited similar potency to morphine in preclinical studies [42]. The compound is taken orally, nasally, by smoking, and, less commonly, by intravenous injection [43]. The main clinical effects included hypertension, tachycardia, and seizures. The first death associated with AH-7921 use was reported by Norway in December 2012 [43]. There has been one confirmed fatality from AH-7921 in the United States, but a number of deaths have been associated with this drug in Europe [44,45].

U-47700 (3,4-dichloro-*N*-[(1R,2R)-2-(dimethylamino)yclohexyl]-*N*-methylbenzamide) is a structural isomer of AH-7921. It is also known as "fake morphine" or "U4" in the recreational drug market and it is sometimes also referred to as "pink", because impurities in its synthesis cause the drug powder to be slightly pink in color. In preclinical studies, U-47700 is about 1/10 as potent as fentanyl, but 7.5 times more potent than morphine [46,47]. During 2016, a significant number of U-47700 acute intoxication cases were reported in the USA. The clinical symptoms are consistent with those of traditional opioids [5]. U-47700 has caused at least 46 deaths from overdose in the United States [48–51].

3.2. Circumstantial Data and External Examination

It is very important to sample any potential drug-containing material in the area around a dead body, taking into account the numerous opioid administration routes (drug paraphernalia, powders, syringes, vials, pills, patches etc.). It is also important to look for signs of administration on the body, considering, however, that about 20% [52] of subjects take fentanyl or analogs by inhalation, ingestion, or, rarely, transdermal route, therefore puncture marks are not always evident.

Even in the absence of external signs specific for opioid intoxication, it is possible to observe non-specific signs of asphyxia, such as petechiae [53].

3.3. Autopsy-Pathological Findings

The data from the literature review showed that the new synthetic opioids produce similar clinical effects as the traditional ones [54]; therefore, we sought the typical findings of heroin intoxication trying to capture the differences and identify additional typical findings of fentanyl- and synthetic opioid-related deaths. The autopsy findings collected from the case reports treated in the literature were homogeneous with respect to the detected findings. The routine histological data were not very specific and did not reveal indicative signs of intoxication [13,55].

3.3.1. Central Nervous System

The major autoptic relief found in the CNS was cerebral oedema. This finding was reported for several drugs (fentanyl [56,57], acetylfentanyl [58], butyrylfentany [59], furanylfentanyl [60,61], ocfentanil [62], AH-7921 [63], U-47700 [64], MT-45 [65]). A case of fatal cerebral hemorrhage induced by acetylfentanyl was reported [66], and another case of a 19-month-old girl poisoned by a transdermal administration of fentanyl who developed leukoencephalopathy was described [67]; in this case, the girl survived, however, investigators declared that this is not always the outcome [55].

3.3.2. Cardiovascular System

A reported uncommon intoxication symptom is chest pain mimicking acute coronary syndrome with non-specific T-wave changes on the electrocardiogram [68]. It is necessary to distinguish the alterations induced acutely by the drug from those due to pre-existing pathologies. Most of the observed cardiovascular pathological findings, such as hypertrophy [69], cardiomegaly [56,70,71], cardiac fibrosis [72,73], atherosclerosis [69,74], are not attributable to an acute intoxication but, in some cases, they may be compatible with chronic drug intake. The presence of pericardial petechiae [53] can be interpreted as a generic sign of asphyxia, due to opioid-related respiratory failure.

3.3.3. Pulmonary

The main effect of fentanyl and its analogues on the respiratory system is respiratory depression. Furthermore, fentanyl can cause chest wall rigidity and apnea, particularly with rapid intravenous administration [75], a factor that can contribute to respiratory failure. Rare adverse effects after fentanyl usage include diffuse alveolar hemorrhage immediately after insufflating fentanyl powder [72]. The major pathological findings are pulmonary congestion [53,54,69,70,73,74,76–82] and pulmonary oedema, which are common to all the investigated drugs [57,58,70,73,74,78–81,83]. Signs found occasionally are petechiae on the pleura [57,82] and aspiration of gastric contents inside the trachea and bronchi [57,76,81].

A few cases of fentanyl patch aspiration have been reported, where the patch was found in the airways [71,84]. Microscopically, small amounts of foreign material have been reported in the lungs, consistent with prior intravenous drug abuse [80].

3.3.4. Others

Another common sign is generalized visceral congestion [51,60,65]. Hepatic parenchyma alterations, such as liver cirrhosis [74], chronic active hepatitis [82], fatty degeneration [62,70,84], hepatomegaly [62,70], are common but due to pre-existing conditions or chronic abuse of narcotics.

3.4. Sampling

The samples commonly taken for toxicological analysis consisted of peripheral blood [84], central blood [85], urine, and liver. Less commonly collected samples were vitreous humour [78], brain, kidney, bile, and gastric content.

Among these, the least susceptible site to post-mortem redistribution is the liver (in relation to fentanyl) [86]. However, there is currently no consensus on the ideal sampling site [87].

3.5. Lethal Concentrations

The lethal concentrations found in the literature are reported in Table 1. For each drug, the routes of administration and relative potency compared to morphine are shown, in addition to the dose (and the corresponding tissue). Table 2 shows the concentration data reported in multidrug-associated deaths.

Table 1. Lethal concentrations data reported in the literature.

	Potency Ratio to Morphine [14]	Administration Route Associated with Overdose	Blood Concentration (ng/mL)	Other Concentrations (Site, ng/mL)
Acetylfentanyl [53,58,80,88]	15.7	Nasal, intravenous	153–260 247.5–285 (heart)	Liver 100–2400 ng/g; urine 2.6–2720 ng/mL; stomach content 880 ng/mL; vitreous humor 131–240 ng/mL.
Alpha-Methylfentanyl [89]	56.9	Intravenous	3.1	liver 78 ng/g; bile 6.4 ng/mL.
Butyrylfentanyl [59,71]	1.5–7.0	Nasal, rectal, intravenous, sublingual	66–99 ng/mL; 39–220 ng/mL (heart)	liver 41–57 ng/g; kidney 160 ng/g, muscle 100 ng/g; vitreous humor 32 ng/mL; bile 260 ng/mL; urine 64 ng/mL; gastric contents 590 ng/mL; brain 93 ng/g.
Carfentanil [90]	10,000		0.11–0.88	
4-Fluorobutyrfentanyl [91]	Unknown	By smoking	91–112	urine, 200–414 ng/mL; liver, 411–902 ng/g; kidney 136–197 ng/g.
Furanylfentanyl [49,60]	Unknown	Nasal, intravenous	0.43–26	
3-Methylfentanyl [83,92]	48.5–7000	Intravenous	0.3–1.9	
Ocfentanil [62,93]	90	Nasal, by smoking	9.1–15.3; 23.3–27.9 (heart)	vitreous humor 12.5 ng/mL; urine 6.0 ng/mL; bile 13.7 ng/mL; liver 31.2 ng/g; kidney 51.2 ng/g, brain 37.9 ng/g; nasal swabs 2999 ng/swab.
AH-7921 [5,44,64,66]	Unknown	Oral, nasal, by smoking, intravenous	330–6600 480–3900 (heart)	urine 760–6000 ng/mL; bile 17,000 ng/mL; liver 530–26,000 ng/g; kidney 7200 ng/g; brain, 7700 ng/g; vitreous humor 190 ng/mL; stomach content, 40 µg/mL.
U-47700 [5,49,94,95]	7.5	Oral, nasal, intrarectal, smoking, intravenous	59–525 1347 (heart)	Urine 360–1393 ng/mL; liver 430–1700 ng/g; kidney 270 ng/g; lung 320 ng/g; brain 97 ng/g.
MT-45 [5,66,96]	Unknown	Oral, nasal, intrarectal, intravenous	520–660 1300 (heart)	Urine 370 ng/mL; vitreous humor 260 ng/mL; gastric content 49 µg/mL; liver 24 µg/g.
Fentanyl [70,82,86,87,97,98]	100	Oral, transdermal, nasal, intravenous	0.5–383	Urine 2.9–895 ng/mL; gastric content 31.6–745 µg/mL; liver 5.8–613 µg/g.

Table 2. Concentrations data reported in multidrug-associated deaths.

	Associated Drugs	Blood Concentration (ng/mL)	Other Concentrations (Site, ng/mL)
Acetylfentanyl	Butyrylfentanyl [69,81]	Acetylfentanyl: 21–38; 32–95 (heart) Butyrylfentanyl: 3.7–58; 9.2–97 (heart)	Acetylfentanyl: vitreous humor 38–68 ng/mL; bile 330 ng/mL; urine 8–690 ng/mL; gastric contents 170–28,000 ng/mL; brain 200 ng/g; liver 110–160 ng/g. Butyrylfentanyl: vitreous humor 9.8–40 ng/mL; bile 49 ng/mL; urine 2–670 ng/mL; gastric contents 170–4000 ng/mL; brain 63 ng/g; liver 39–320 ng/g.
	Fentanyl [85,99]	Acetylfentanyl: 0.13–12 Fentanyl: 0.24–21	
	Fentanyl, heroin [100]	Acetylfentanyl: 12 Morphine (free): negative Morphine (total): 20 Fentanyl: 15	
	Fentanyl, heroin, cocaine [100]	Acetylfentanyl: 9 Morphine (free): 30 Morphine (total): 60 Fentanyl: 20 Cocaine: 70 Benzoylecgonine: 970	
	Fentanyl, heroin, alprazolam [100]	Acetylfentanyl: 2 Morphine (free): 20 Morphine (total) <20 Alprazolam 30 Fentanyl: 19	
	Furanylfentanyl, diphenhydramine [49]	Acetylfentanyl: 0.65 Furanylfentanyl: 12.9 Diphenhydramine: 140	
	Morphine [100]	Acetylfentanyl: 400 Morphine (free): 30 Morphine (total): 70	
	Alprazolam [100]	Acetyl Fentanyl 560–600 Alprazolam 20–230	
	4-MethoxyPV8 and others [53]	Acetylfentanyl: 153 4-MethoxyPV8: 389 7-Aminonitrazepam: 200 Phenobarbital: 7700 Methylphenidate: 30	Acetylfentanyl: urine 240 ng/mL; gastric contents 880 ng/mL. 4-MethoxyPV8: urine 245 ng/mL; gastric contents 500 ng/mL.

Table 2. *Cont.*

	Associated Drugs	Blood Concentration (ng/mL)	Other Concentrations (Site, ng/mL)
Butyrrylfentanyl	U-47700 [49]	Butyrrylfentanyl: 26 / U-47700: 17	
Furanylfentanyl	U-47700 [49]	Furanylfentanyl: 2.5–26 / U-47700: 105–490	
	U-47700, Heroin [49]	Furanylfentanyl: 56 / U-47700: 107 / Morphine: 48	
	Fentanyl [60]	Furanylfentanyl: 0.4 / Fentanyl: 1.27	
	Carfentanil [101]	Furanylfentanyl: 0.34 / Carfentanil: 1.3	
U-47700	Fentanyl [102]	U-47700: 13.8 / Fentanyl: 10.9	U-47700: urine 71 ng/mL
	Diphenhydramine [49]	U-47700: 103 / Diphenhydramine: 694	
	Diphenhydramine, alprazolam, doxylamine [96]	U-4770:190 / Diphenhydramine: 140 / Alprazolam: 120 / Doxylamine: 300	
Fentanyl	Heroin [82,98,100]	Fentanyl: 2.7–16 / Morphine (free): <20–100 / Morphine (total): 30–240	
	Heroin, hydromorphone [100]	Fentanyl: 15 / Morphine (free): 20 / Morphine (total): 60 / Hydromorphone (free): <20 / Hydromorphone (total): 40	
	Heroin, methamphetamine [100]	Fentanyl: 0.004 / Morphine (free): 100 / Morphine (total): 90 / Methamphetamine: 270	
	Heroin, methadone, alprazolam [100]	Fentanyl 7–38 / Morphine (free): 20–50 / Morphine (total): 40–80 / Methadone: 320–400 / Alprazolam: 30	

Table 2. *Cont.*

Associated Drugs	Blood Concentration (ng/mL)	Other Concentrations (Site, ng/mL)
Oxycodone [97]	Fentanyl: 14 Oxycodone: 420	
Oxycodone, citalopram [97]	Fentanyl: 6.7 Oxycodone: 500 Citalopram: 200	
Oxycodone, codeine [97]	Fentanyl: 10 Oxycodone: 270 Codeine: 280	
Methadone [103]	Fentanyl: 5 Methadone: 540 Oxycodone: 70 Trazodone: 246	
Hydrocodone [103]	Fentanyl: 90 Hydrocodone: 240	
Morphine [103]	Fentanyl: 10 Morphine (total): 3230	
Cocaine [97,103]	Fentanyl: 12–34 Cocaine: 50–780 Benzoylecgonine: 31–4100	
Methamphetamine [71]	Fentanyl: 8.6 Methanphetamine: 1456	
Phenobarbital, nordiazepam, diazepam	Fentanyl: 20 Phenobarbital: 7000 Nordiazepam: 72 Diazepam: 58	
Bromazepam [55]	Fentanyl: 60.6; 94.1 (heart) Norfentanyl: 19.8; 50.1 (heart) Bromazepam: 874	Fentanyl: urine 152.2 ng/mL; brain 70.4 ng/g; kidney 161.3 ng/g; stomach content 536.8 ng/mL; liver 203.2 ng/g; bile 274.2 ng/mL Norfentanyl: urine 172.2 ng/mL; brain 5.6 ng/g; kidney 172.9 ng/g; stomach content 54.4 ng/mL; liver 164.6 ng/g; bile 436.4 ng/mL
Alprazolam, tramadol [103]	Fentanyl: 12 Alprazolam: 13 Tramadol: 1500	
7-Aminoclonazepam, Sertraline [82]	Fentanyl: 13.8 7-Aminoclonazepam: 57.1 Sertraline: 91.9	
U-49900, Methoxy-Phencyclidine [104]	Tetrahydrofuranylfentanyl: 339 U-49900: 1.5 Methoxy-Phencyclidine: 1.0	Tetrahydrofuranylfentanyl: urine >5000 ng/mL U-49900: urine 2.2 ng/mL Methoxy-Phencyclidine: 31.8 ng/mL

Fentanyl (row label spanning the upper rows)

Tetrahydrofuranylfentanyl (row label for last row)

4. Discussion

This review deals with human pathological findings that are directly attributable to the known toxic actions of fentanyls and other synthetic opioids. In the past few years, it has become more and more evident that fentanyls and other synthetic opioids are potentially extremely harmful. Fentanyl-related deaths have increased over the years [47,52], so it is necessary to review the data available on these analog NPS. The results from our literature analysis revealed the lethal potential of fentanyls and other synthetic opioids; a large number of different routes of substance administration have also surfaced, through which all of these compounds are potentially lethal.

In addition, a broad range of side effects associated with fentanyls and other synthetic opioids have emerged, posing serious health issues, which primarily concern the central nervous system, cardiovascular and pulmonary systems, and liver. Macroscopic examinations, autopsy data, and histopathological elements were collected from the literature, leaving evidence that mainly refers to opioid intoxication. The investigation of the cause of death provoked by fentanyl or other synthetic opioid abuse was based on a multidisciplinary approach aimed at framing each case and directing the investigations towards targeted toxicological analyses. This approach should be adopted in all cases of death from uncertain or questionable causes [66,70]. Past medical history and ante-mortem distribution, crime scene investigation, post-mortem toxicology examination, and toxicology findings should be carefully analyzed and considered on a case-by-case basis in light of all other data [11,98,105–107].

The examination of the literature showed that a large number of deaths associated with fentanyl and other synthetic opioids involved the abuse of other psychoactive substances. In a previous review [22] of various case studies of fentanyl-related deaths, it was speculated that the deaths were associated with drugs of abuse such other opiates (up to 64%), cocaine (up to 65%), cannabinoids (up to 50%), amphetamines (up to 40%), but also ethanol (up to 22.9%) and medicines like barbiturates (up to 27%), benzodiazepines (up to 52.2%), antidepressants (up to 48%). These data can lead to a series of considerations. The first is inherent in the contribution to the deaths of the substances detected together with synthetic opioids. Animal studies have shown that some classes of substances, such as benzodiazepines, have a synergistic effect with opioids.

Although the pharmacokinetic and pharmacodynamic interactions between benzodiazepines and opioids are not yet fully understood [108], pre-clinical studies suggest a synergistic effect on opioid-induced respiratory depression (measured as % increase in pCO_2) [109]. Forensic data show the risks of this drug combination: the concomitant use of benzodiazepines and "traditional" opioids is associated with the occurrence of opioid overdoses [110,111]. This occurrence is very common, and the co-use of opioid and benzodiazepine could be aimed at amplifying the subjective effects of the opioid [112,113]. However, the current knowledge does not permit to establish the exact contribution of benzodiazepines in opioid-related deaths, considering that many opioid addicts are also chronic benzodiazepine users [114,115].

Regarding the co-administration of ethanol and opioids, it can be dangerous because it enhances the positive subjective effects that contribute to the abuse and affects physical and cognitive functions. It is no coincidence that alcohol and opioid abuses often coexist [116]. Fatal intoxications involving opioids are frequently associated with alcohol use and are likely due to combined CNS- and respiratory-depressant effects [117,118].

Concerning the co-administration of cocaine and synthetic opioids, animal studies have shown that combinations of cocaine and remifentanil can lead to a strong additivity [119], maybe for their synergistic action on the mesolimbic dopaminergic system [120]. Unfortunately, data on humans are not yet reported in the literature. Recently, there has been an increase in cocaine-related overdoses; however, a large part of this increase is due to the simultaneous intake of opioids, especially synthetic ones [121]. Often, the use of a synthetic opioid is accidental, due to an unknown contamination of a cocaine stock [122,123].

The same assumption can be formulated for the deaths from the co-administration of heroin and fentanyl. A recent study showed a strong association between the number of tested samples of seized

drugs where fentanyl was detected and unintentional overdose deaths in which fentanyl was also identified [124]. This data are consistent with previous studies that have shown that a significant proportion of drug users unintentionally consume fentanyl, which is present in the substances they are taking [7,122,125]. However, the real diffusion of illicit fentanyl use in the general population is difficult to assess, because routine toxicology screens will not detect synthetic opioids that have little structural homology to morphine and other commonly tested opioids [14].

5. Conclusions

In Conclusion, the confirmation or exclusion of opioid overdoses is one of the major challenges for forensic pathologists, considering what has been said and that autopsy findings are not specific. It is therefore necessary that the forensic pathologist have a broader approach and, on the basis of the data collected, request a chemical-analytical analysis to point out NPS [87].

The role of the forensic pathologist in close collaboration with the forensic toxicologist is very important: together, they can identify new cases of fentanyl and synthetic opioid intoxication. The identification of NPS is essential to stop the social problems related to the spread of these new dangerous and highly addictive substances among our population.

Conflicts of Interest: The authors declare no conflict of interest.

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