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i Ciències de l'Alimentació

FINAL DEGREE PROJECT

ROLE OF THE DOPAMINE AND SEROTONIN TRANSPORTERS IN ADDICTION: PREDICTION OF THE ABUSE POTENTIAL OF NEW PSYCHOACTIVE SUBSTANCES

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Main field: ***Pharmacology***

Secondary fields: ***Toxicology and Pharmaceutical Chemistry***

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ABBREVIATIONS LIST

- [^{3}H]MPP+: [^{3}H]1-methyl-4-phenylpyridinium
- 5-HT: serotonin
- α-PVP: α-pyrrolidinovalerophenone
- ADHD: attention deficit / hyperactivity disorder
- ATS: amphetamine-type stimulants
- DA: dopamine
- DALYs: disability-adjusted life years
- DAT: dopamine transporter
- DEA: Drug Enforcement Administration
- DMEM: Dulbecco's modified Eagle's medium
- EWA: Early Warning Advisory
- FBS: fetal bovine serum
- G418: geneticin
- HEK293: Human Embryonic Kidney
- ICSS: intracranial self-stimulation
- KHB: Krebs HEPES buffer
- MDMA: 3,4-methylenedioxymethamphetamine
- MDPV: 3,4-methylenedioxypyrovalerone
- Nacc: nucleus accumbens
- NE: norepinephrine
- NET: norepinephrine transporter
- NPS: new psychoactive substances
- NT: neurotransmitter
- PDL: Poly-D-lysine
- SDS: sodium dodecyl sulphate
- SERT: serotonin transporter
- UNODC: United Nations Office of Drug and Crime
- $VMAT_2$: vesicular monoamine transporter 2

1. ABSTRACT

Drugs of abuse are largely controlled and prohibited, however the emergence of New Psychoactive Substances (NPS) starts to be a threat to global health. NPS are created based on minor modifications on the structure of well-known drugs that can lead to new products avoiding current legislation, and have the same or even greater psychoactive and stimulant effects than classical drugs of abuse. Among NPS, synthetic cathinones are β-keto analogues of amphetamine, that mimic the effects of traditional psychostimulants such as cocaine or MDMA.
The aim of the present study was to review the role of the dopamine transporter and serotonin transporter in addiction, especially regarding NPS, and describe the pharmacological profile of 7 novel synthetic cathinones, as well as to compare them with standard psychoactive or prescribed compounds such as cocaine, MDMA, or paroxetine. To evidence the direct blockade of dopamine and serotonin reuptake by these novel synthetic cathinones, HEK293 cells were used to carry out monoamine uptake inhibition assays. Our study demonstrated that all 7 new compounds potently inhibit dopamine uptake and had very little effect on 5-HT uptake inhibition. Consequently, all these new substances possess high hDAT/hSERT ratios, even greater than cocaine. This selectivity may have a key role in their predictable abuse potential, since a higher ratio hDAT/hSERT may allow us to predict a higher addictive potential, and thus, a threat to public health.

Les drogues d'abús son substàncies il·legals, però l'aparició de Noves Substàncies Psicoactives (NPS) comença a suposar una amenaça per a la salut pública. Les NPS estan creades en base a petites modificacions de l'estructura de drogues conegudes que poden donar lloc a nous productes que eludeixen la legislació actual, i que tenen els mateixos o inclús més efectes psicoactius i estimulants que les drogues d'abús clàssiques. Entre les NPS, les catinones sintètiques son anàlegs β-cetònics de l'amfetamina que imiten els efectes dels psicoestimulants tradicionals com la cocaïna o el MDMA.
L'objectiu d'aquest estudi és revisar el paper del transportador de dopamina i de serotonina en l'addicció, especialment a NPS, així com descriure el perfil farmacològic de 7 noves catinones sintètiques, i comparar-les amb substàncies psicoactives de referència o substàncies prescrites, com la cocaïna, el MDMA, o la paroxetina.
Per evidenciar el blocatge directe de la recaptació de dopamina i serotonina per part d'aquestes 7 noves catinones sintètiques, es van utilitzar cèl·lules HEK293 per a realitzar un assaig d'inhibició de la recaptació de monoamines. Aquest experiments van demostrar que els 7 nous compostos inhibeixen potentment la recaptació de dopamina, però tenen molt poc efecte sobre la recaptació de serotonina. Conseqüentment, totes aquestes noves substàncies tenen un ratio hDAT/hSERT molt elevat, inclús major que el de la cocaïna. Aquesta selectivitat podria jugar un paper clau en el potencial d'abús, ja que un major ratio hDAT/hSERT podria predir un alt potencial d'abús, i per tant, una amenaça per a la salut pública.

2. INTEGRATION OF DIFFERENT FIELDS

Three different fields are integrated in this study: pharmacology, toxicology and pharmaceutical chemistry. Pharmacology is the main field studied in this work. It is defined as the science that studies how drugs interact with our body, as well as their actions and pharmacological properties. In this specific case, it will help us to understand the mechanism whereby drugs of abuse can cause addiction, as well as the pathways that lead to the effects that characterise them. This study focuses on new psychoactive substances, little known drugs of abuse, and pharmacology is essential to understand their mechanism of action.

The second integrated field is toxicology, the science that will help us to understand the undesired and harmful effects of drugs of abuse.

Finally, pharmaceutical chemistry will help us to understand the importance of the structure of the drugs and its impact on addiction and abuse liability.

These fields are highly related, and it is important to study all of them to fully understand the mechanism of drugs of abuse, as well as their consequences.

3. INTRODUCTION

The illegal drug situation in Europe and worldwide is particularly worrying. Around 29% of the adults aged between 15 and 64 years old living in Europe reported having used illicit drugs at least once in their lives. In this context, the most consumed illegal drug in Europe and worldwide is by far cannabis, followed by cocaine, MDMA and amphetamines (1). In 2018, nearly 1 in every 19 people worldwide reported having used illicit drugs at least once in the previous year, which represents 5,4% of the population worldwide (2). Moreover, the consumption of drugs of abuse starts at school periods, thereby facilitating the transition to more dangerous compounds, such as MDMA, due to a false sense of security when consuming drugs. (2)

Between 2009 and 2018, the number of drug users worldwide increased from 210 million to 269 million approximately, in other words, an increase of a 28%, partly as a result of the increase in the world population. (3)

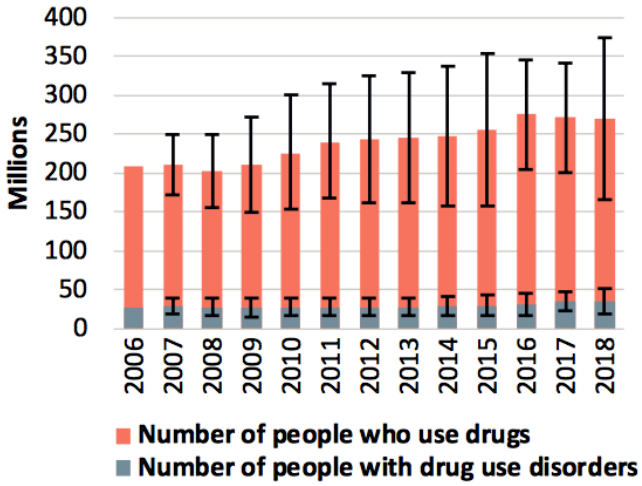


FIG.1 Global prevalence of drug use and drug use disorders, 2006-2018. (3)

Note: those estimations reflect the best available data.

Not only due to their addictive potential, but also because of their serious health consequences, authorities consider drugs of abuse a global issue and a threat to public health. Our objective as a society is to reduce the principal risk factors for drug abuse and to boost protection, in order to prevent the transition from sporadic consumption to regular consumption, and the transition from common drugs, such as alcohol or tobacco, to more dangerous ones.

The most important impact of drugs of abuse is their effect on well-being and health. The emergence of drug use disorders implies a transition from sporadic consumption to regular consumption, maintenance, and dependence or addiction. Moreover, the association between drug use disorders and mental health disorders has been demonstrated. This association is partly due to the negative effects of drug abuse on mental health disorders and to common risk factors between drug use disorders and mental health disorders. (2)

In fact, the use of drugs of abuse, especially regular consumption, is related to more than 80 well-known diseases, and it entails adverse health consequences including premature death, mental health disorders, fatal or non-fatal overdoses and other diseases such as HIV and Hepatitis C. (2)(4)

It is estimated that, in 2018, among the 269 million people who consumed drugs in the past year, 35,6 million people developed a drug use disorder. This corresponds to a prevalence among people aged 15-64 of 0,7% of drug use disorders worldwide. People suffering from drug use disorders have harmful patterns of drug use, drug dependence, and other health problems, such as mental health disorders, which stands out above the rest. It is unclear whether drug use disorders can cause mental health disorders or if there are common risk factors between drug use disorders and mental health disorders, but mental health disorders among people with drug use disorders has grown dramatically in the last years (2)(5)(6)(7). Moreover, drug abuse is also linked to social disorders. Drug consumption is associated with a low level of education, family disruption, lack of financial stability, poverty, and difficulties finding and keeping a job. These consequences can go beyond drug users and affect their families and community, just as communities can influence consumption behaviours of drug users (2)(8).

More specifically, the Global Burden of Disease study indicates the negative effects of drug abuse in terms of years of “healthy” life lost, also called DALYs (Disability-Adjusted Life Years), and deaths. DALYs indicates the number of years of life lived with disability and the number of years lost due to premature death. The Study estimated that in 2017, 585 000 deaths worldwide were due to drug abuse, and that the number of DALYs attributed to drug abuse increased 17% over the past decade, being liver cancer and cirrhosis, caused by hepatitis C, and opioids use disorder the main causes. Furthermore, the European Drug Report raised an alarming problem: the increase in the number of deaths attributed to drug overdose among the 50-plus aged group, between 2012 and 2018. This fact stresses the relation between drug use disorders and age, and the relevance of rehabilitation and social integration, just as the importance of preventing drug use at school ages. (1)(2)(3)

3.1 TRADITIONAL DRUGS OF ABUSE (PSYCHOSTIMULANTS)

3.1.1 COCAINE

Among the most consumed drugs, with 18 million users in 2016 as defined in the UNODC (1), cocaine is considered the second illicit drug most widely used in Europe, only surpassed by cannabis. Cocaine is an alkaloid (see FIG. 2) extracted from the leaves of *Erythroxylon coca*, also known as the coca plant, originally from South America, from where it is illegally shipped to Europe. (9)

Cocaine is considered to be a highly addictive stimulant, with a powerful effect on the central nervous system, and nearly exclusively used for recreational purposes, although it had medical purposes as local anaesthetic. It is usually distributed as a crystalline white powder, and can be snorted, ingested or injected (10). On the other hand, among its main effects, cocaine produces euphoria, energy, wakefulness, hypersensitivity to touch,

sight and sound, agitation, sexual arousal, an intense feeling of happiness, loss of appetite and insomnia. Moreover, physiological effects of cocaine include vasoconstriction, dilated pupils, and increased heart rate, blood pressure and body temperature. (1)(3)(10)

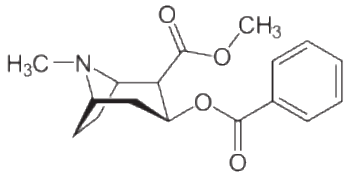
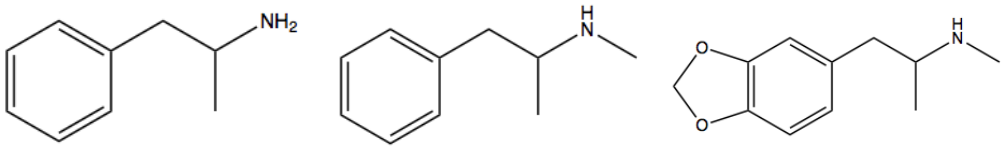


FIG. 2 Chemical structure of cocaine.

3.1.2 AMPHETAMINE-TYPE STIMULANTS (ATS)

Amphetamine-Type Stimulants (ATS) is a group of synthetic drugs that include amphetamine, methamphetamine and MDMA (3,4-Methylenedioxymethamphetamine), also known as “ecstasy” (see FIG. 3). Historically, this group of drugs has been used to treat attention deficit / hyperactivity disorder (ADHD), narcolepsy, depression, and obesity, due to their stimulant effect on the central nervous system. In fact, the use of ATS, such as MDMA, produce insomnia, loss of appetite, euphoria, energy, wakefulness, a feeling of well-being, increased alertness and concentration, and sexual arousal. A chronic use may cause hallucinations, depression, irritability, aggressive behaviours and paranoid psychosis and around 0,5% of the adult population worldwide is estimated to have consumed amphetamine-type stimulants in the past year, corresponding to 27 million people. Most notably, the seized quantity of “ecstasy” almost tripled from 2012 to 2016, fast becoming a grave danger to public health. (11)(12)(13)



AMPHETAMINE METHAMPHETAMINE MDMA

FIG.3 Chemical structure of amphetamine, methamphetamine and MDMA.

3.2 NEW PSYCHOACTIVE SUBSTANCES (SYNTHETIC CATHINONES)

Drugs of abuse are largely controlled and prohibited, but the emergence of New Psychoactive Substances (NPS) is threatening global health. In this regard, drug consume is growing exponentially, and this is partly due to the emergence of the so called NPS, that occupy a prominent place in the clandestine market.

NPS are created on the basis of minor modifications on the structure of well-known drugs, and its objective is to mimic the effects of drugs considered illegal, such as cocaine and MDMA. These minor modifications can lead to new products that avoid the legislation, and have the same or even greater psychoactive and stimulant effects. Thus,

these NPS are the legal alternative to traditional drugs of abuse, maintaining the effect that makes them so addictive and sought. More and more designed drugs are appearing in the black market, and this legal void allows the free marketing of these hazardous new substances. In addition, most of NPS are not controlled by the 1961 Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances. Since these new drugs are not controlled by any international convention, the legal situation of these drugs can vary from one country to another. (14) It is estimated that in 2019, approximately 60 countries had taken legal measures to control the free flow of these substances, but although many countries are rushing to legally control these NPS, once one is controlled, a new one takes its place in the clandestine market. For example, 3,4-methylenedioxypyrovalerone (MDPV) is considered a “first-generation cathinone”, classified by the Drug Enforcement Administration (DEA) as a Schedule I compound. The MDPV ban lead to the emergence of α-pyrrolidinovalerophenone (α-PVP), considered a “second-generation cathinone”, which only differs from MDPV by the lack of 3,4-methylendioxy group. (1)(14)(15)(16)(17)(18)(19)

For this reason, and due to the fact that globalisation and new drug trafficking routes are emerging, the NPS are spreading worldwide at a breakneck speed. It is estimated that 70% of the NPS reported in Europe have appeared in the last five years. Up to December 2020, 126 countries and territories from all over the world reported one or more NPS and 1047 NPS were reported to the UNODC Early Warning Advisory (EWA). (see FIG. 4)

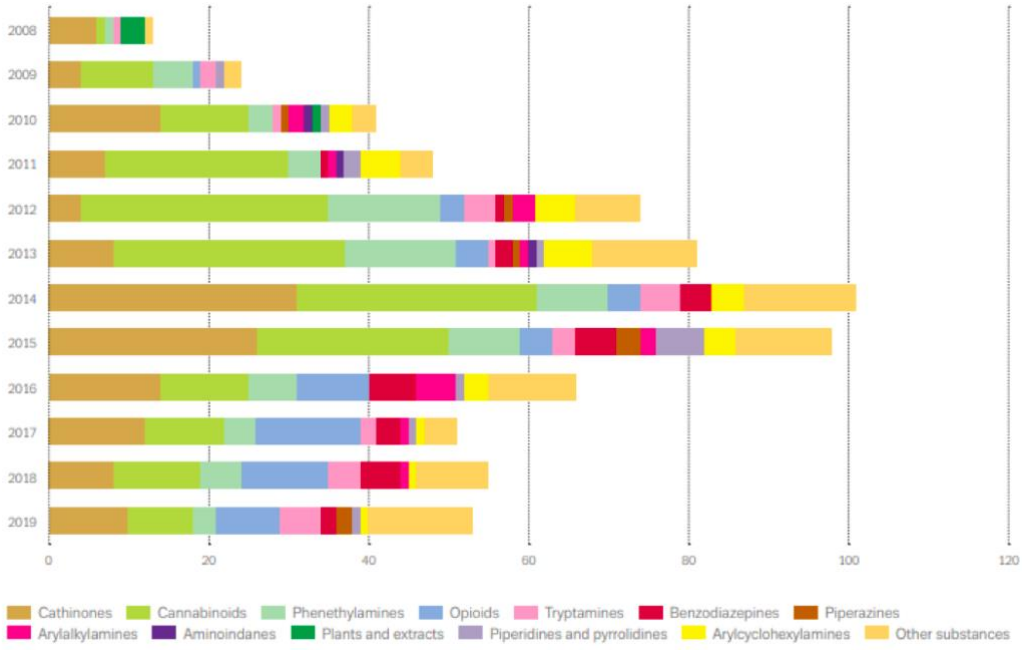


FIG. 4 NPS reported to the United Nations Office of Drug and Crime.
Source: UNODC World Drug Report 2020 (3)

There has been an exponential rise in the abuse of NPS sold as an alternative to illicit drugs such as cocaine or amphetamine, and the internet has facilitated this fact on a global scale (1)(20). Global authorities are particularly concerned with this situation, due to the fact that the appearance of this NPS entails a grave danger to public health, since

the consequences and the risks associated to the use and abuse of NPS are completely unknown. More specifically, it must be pointed out the appearance of a class of NPS known as synthetic cathinones, structurally related to amphetamines (see FIG. 5). In a chemical perspective, synthetic cathinones are β-keto analogues of amphetamine, and synthetic cathinones are derivatives of cathinone, a psychoactive product found on the plant *Catha edulis*, also known as Khat. Khat's stimulant effects are widely known and even today, chewing Khat leaves is still a popular practice in some countries in Africa and Arabia. However, it was not until 1970s that cathinone was not isolated and identified as the compound responsible for the psychoactive effects of Khat leaves. Since then, many derivatives have been created for therapeutic purposes. Nevertheless, the illegal use of synthetic cathinones first appeared in 2010 in the United States under the name of "bath salts" and since then, they constitute a large part of the NPS reported worldwide. (15)(21)(22)

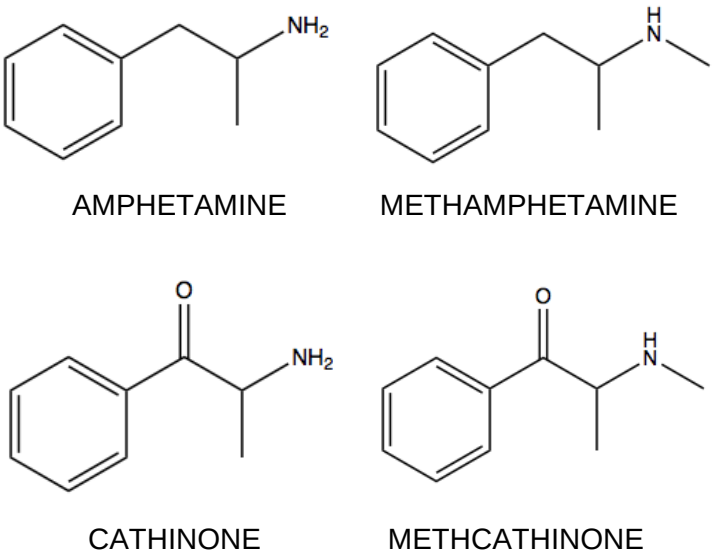


FIG. 5 Chemical structures of amphetamine, methamphetamine, cathinone and methcathinone.

Among these synthetic cathinones, popularly known as "bath salts", MDPV and α-PVP are the ones with highest demand on the clandestine market. However, some other synthetic cathinones are widely prescribed, for example bupropion, while others are considered a threat to public health.

Synthetic cathinones are psychomotor stimulants and the occasional use of these synthetic cathinones produces euphoria and other psychostimulant effects, such as increased alertness. Moreover, their consumption can cause major side effects such as delirium, hallucinations, tachycardia, hyperthermia, psychosis, and other neurological or cardiovascular adverse effects, although violent or combative behaviours are also frequent. The most dangerous adverse effect is "excited delirium", a stage in where the consumers show extreme agitation, hyperthermia and delirium, along with rhabdomyolysis and kidney failure (21). Nevertheless, the risks and consequences of the abuse of synthetic cathinones is still little known.

Regarding their route of administration, “bath salts” are mostly consumed orally or intranasally, although they can also be consumed intravenously and, of course, their consumption can lead to acute intoxications and even death, as well as drug dependence (15). However, there is no specific treatment, besides, the detection of these bath salts is very difficult and often impossible, and the rapid emergence and the huge number of NPS makes very difficult to develop early detection techniques (15)(20)(21). For these reasons, and due to the fact that there is still very little information on toxicity and long-term adverse effects, the appearance on the black market of NPS poses a health hazard for consumers and a challenge for prevention and treatment. (20)

3.3 PHARMACOLOGY OF DRUG ADDICTION (DOPAMINE REWARD PATHWAYS)

Addiction is defined as a chronic brain disease with genetic and sociocultural components. It has been shown that the brain has a mechanism to generate addiction and every action or behaviour has its consequences. There are positive reinforcing actions, essential to species survival, such as eat or drink, and negative reinforcing actions. Natural selection favours those who repeat positive reinforcing actions, and the capacity to repeat those actions can be genetic, but it can also be learnt. As previously mentioned, there is only one neurophysiological mechanism that promotes the repetition of positive reinforcing actions, and it is the dopaminergic rewarding pathway, composed of dopaminergic neurons specialised in interpretation of positive actions and learning, located in the mesocorticolimbic region of the brain. (see FIG. 6)

The mesocorticolimbic dopaminergic system is mainly composed of dopaminergic neurons that originates in the Ventral Tegmental Area and projects to Limbic and Cortical structures, among them we can find the amygdala, the nucleus accumbens (Nacc), and the prefrontal cortex. From a physiological perspective, these dopaminergic neurons are controlled by GABAergic neurons located in the Ventral Tegmental Area, that keep dopaminergic neurons inactive. When we eat or drink, the opioergic neurons, also located in the Ventral Tegmental Area, release β-endorphins, an endogenous neurotransmitter (NT) that binds to the Mu receptors of GABAergic neurons and block them. Consequently, there is a decrease in GABA, in which produces an increased activity of dopaminergic neurons that release dopamine (DA) in the Nacc, increasing DA concentration in this area. This exponential growth induces pleasure and eager to repeat, since it activates the DA reward pathway.

As it is a neurophysiological mechanism, it also needs self-regulation. This self-regulation mechanism is mediated by dynorphins, released together with DA when there is a stimulus. Dynorphins bind to Kappa receptors located in the dopaminergic neurons, and block the liberation of DA, thereby ending the dopaminergic reward pathway. The endorphine-dynorphine system constitutes a self-regulation mechanism of the reward pathway. (23)(24)(25)(26)

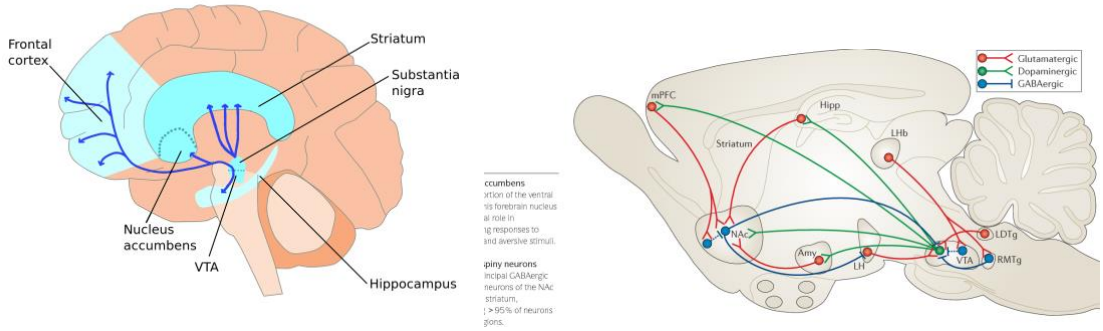


FIG. 6 Brain reward system. (27)(28)

Dopaminergic neurotransmissions take part in many physiological processes, such as movement, reward and addiction, and they are essential to the survival of the specie. Without this transmission, we would not have the necessary stimulus to eat, drink, or to reproduce, but improper dopaminergic transmission plays a key role in many diseases such as Parkinson's disease, schizophrenia, and of course, drug addiction. (29)

It has been demonstrated that several drugs of abuse, such as cocaine and amphetamines, have rewarding effects, since they raise extracellular DA concentrations in a specific part of the brain, the Nacc. Dopaminergic neurons in the Nacc, especially in the shell subregion, play a key role in the emergence of rewarding effects. Furthermore, the DA release in the Nacc also has an important role in the prefrontal cortex, the one responsible for regulating the rational control. Whit that in mind, it is foreseeable that drugs will produce changes in behaviour. Moreover, it has been demonstrated that the repeated use of drugs of abuse reduce the number of D2 receptors, located in the post-synaptic neuron and responsible for conveying the dopaminergic transmission and producing pleasure. This explains why in a chronic use of a drug, a same concentration does not achieve the same effect as the one achieved in the first use. (15)(23)(24)(30)

It is often asked if addiction is a lifestyle choice or if some people are more vulnerable due to their biology. Drugs of abuse produce their effect by activating rewarding pathways in the brain, therefore, although initial drug consume is a voluntary behaviour, repeated consumption involves brain pathways beyond our control, as they interfere with our self-control capacity over consumption behaviours. Nevertheless, it is true that there are factors that influence repeated consumption, such as biological vulnerabilities, or comorbid psychiatric problems, that increase the risk of becoming dependent to drugs. (24)

Drugs of abuse, such as traditional psychostimulants and synthetic cathinones, can activate the dopaminergic neurons in the mesolimbic area, since they disrupt the DA transporter (DAT), by acting as blockers or substrates. In both cases, the outcome is the same: an increase in extracellular DA concentrations, especially in the Nacc. This fact can alter the rewarding circuits and provoke addiction, since the raise in DA concentration produces great pleasure, positive emotions, learning and urge to repeat. Therefore, just by looking at the capacity of drugs to increase extracellular DA concentration, we can predict the abuse liability. (22)(31)

3.4 ROLE OF DOPAMINE AND SEROTONIN TRANSPORTERS ON DRUG ADDICTION

As mentioned before, several drugs of abuse, such as classical psychostimulants and synthetic cathinones, act on plasma membrane transporters for dopamine (DAT), serotonin (SERT) as well as norepinephrine (NET) (see FIG. 7). For example, the consumption of synthetic cathinones alters the normal function of these transporters, located in the neurons of central and peripheral nervous system, and thus, there is an increase of monoamines in the synaptic space, mechanism by which drugs exercise their function (32). Moreover, from a therapeutic perspective, the disruption of the DAT can involve many psychiatric disorders and neurological diseases, such as epilepsy, Parkinson's disease, Alzheimer's disease, autism, depression, obsessive-compulsive disorder and attention-deficit hyperactive disorder (ADHD), and DAT is a target for drugs of abuse, psychostimulants and therapeutic antidepressants. (33)(34)

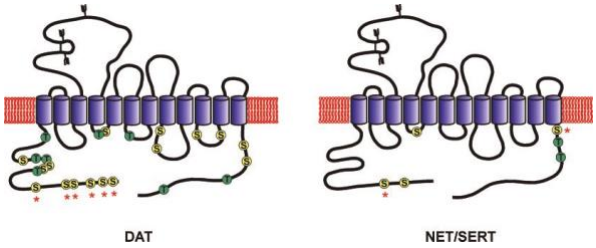


FIG. 7 Structure of the DAT, NET and SERT. (35)

Physiologically, the release of neurotransmitter occurs through the fusion of the synaptic vesicles with the cell membrane, a process called exocytosis. Thus, when the person is not under the influence of, for example, psychostimulants, the normal function of the monoamine transporter is to reuptake neurotransmitter molecules released into the synaptic space back to the neuronal cytoplasm thereby ending the action of monoamine signalling, as can be observed in FIG. 8. Moreover, the uptake of monoamine back to the cell reduces the stimulation of the post-synaptic receptor and the necessity of filling the synaptic vesicles with new neurotransmitter. (29)(31)

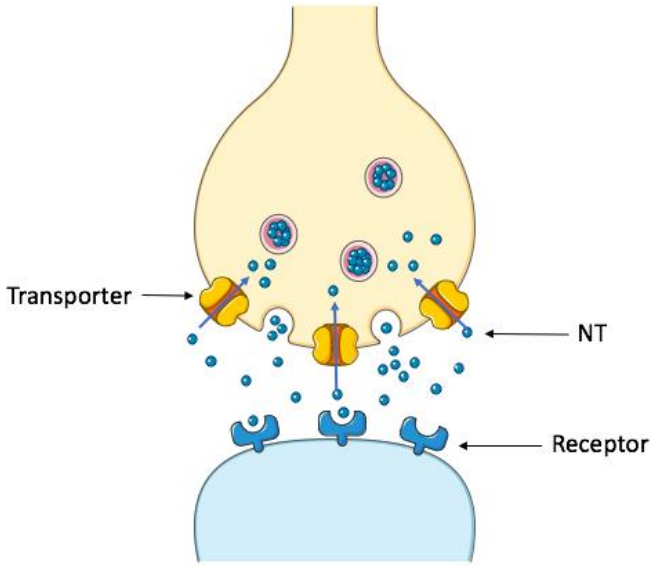


FIG. 8 Normal function of monoamine transporters

On the contrary, when consuming psychostimulants, the function of the monoamine transporter is altered. The effect of psychostimulants on the monoamine transporter can be mainly divided into two types: cocaine-like uptake blockers (FIG. 9) and amphetamine-like releasing agents (FIG. 10). The outcome in both cases is the same, an increase in extracellular concentrations of neurotransmitters, such as DA, serotonin (5-HT) or norepinephrine (NE). This is thought to be the mechanism of the addictive and rewarding properties of these kind of drugs. So, drugs that disrupt monoamine transporters will affect the monoamine transmission between neurons, leading to the characteristic effects of these kind of drugs. (15)(21)(22)(29)(32)(36)(37)(38)(39)

Cathinone and methcathinone are well-known monoamine transporter substrates, so it is not surprising that synthetic cathinones are also substrates of these transporters. However, synthetic cathinones such as α-PVP and its derivatives, belong to the first group, the cocaine-like uptake blockers. This group of drugs exert their effect by binding to the orthosteric site of monoamine transporter and blocking it, thus preventing DA reuptake. In general, cocaine-like uptake blockers act by blocking the DAT, causing a higher neurotransmitter concentration in the synaptic space, which results in an increased monoamine signalling that can lead to major psychostimulant effects and dependence. (15)(19)(21)(30)(42)

On the other side, amphetamine-like releasing agents act by entering to the intracellular space and releasing neurotransmitter from the intracellular space into the synaptic space by a non-exocytic mechanism (see FIG. 10). First of all, amphetamine-like releasing agents act like substrates of the monoamine transporter, thus they bind to the orthosteric site of the monoamine transporter and they are transported to the neuron cytoplasm. In this transport, sodium acts as a co-substrate, so when the amphetamine-like releasing agent is transported to the cytoplasm, sodium currents are also allowed into the cell. This mechanism raises intracellular sodium concentration and produces the depolarization of the cell, therefore contributing to a decrease in extracellular sodium concentration. This results in an increased affinity of the monoamine transporter for all the available intracellular substrates, to transport sodium outside the cell and balance intracellular and extracellular sodium concentrations. Therefore, monoamines are transported from the intracellular space to the synaptic space, with sodium as a co-substrate, by a non-exocytic mechanism. This process is called reverse transport. In summary, amphetamine-like releasing agents are capable to reverse the direction of the monoamine transporter, leading to a raise in monoamine concentrations in the synaptic space. (19)(32)(43)(44)

In addition to the reverse transport, amphetamine-like releasing agents have also indirect effects: they reverse the transporter $VMAT_2$ (vesicular monoamine transporter 2) to promote the release of neurotransmitter from the synaptic vesicles to the intracellular space. Amphetamine and its related compounds have weak base properties, whereas the cathecholaminergic vesicles are acid. It is believed that when amphetamine or its analogues enter the cell, they are diffused or transported by the $VMAT_2$ into the synaptic vesicle, where they accept protons and thus alkalinize the vesicle. This fact disrupts the electrochemical gradient necessary for vesicular DA sequestration and causes DA accumulation in the cytoplasm, since the DA accumulated in the vesicle is released into

the cytoplasm. The increase in cytoplasmic DA concentration also causes the reverse transport and the efflux of DA to the synaptic space. (32)(45)

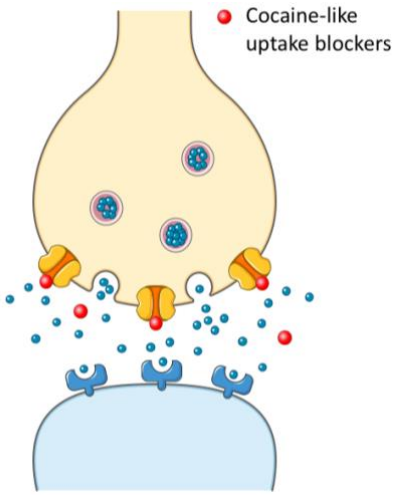


FIG. 9 Cocaine-like uptake blockers

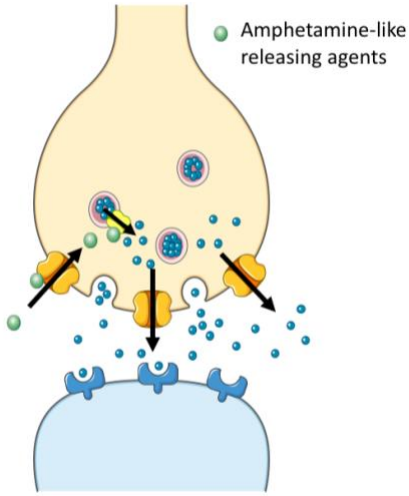


FIG. 10 Amphetamine-like releasing agents

Cocaine-like substances act as blockers, whereas amphetamine-like releasing agents are substrates of the monoamine transporter. In both cases, the result is an increase in extracellular concentrations of neurotransmitter, amplifying the chemical signalling in the central nervous system. Nevertheless, the acute and long-term effects of the two groups are very different (15)(19)(22)(32)(46)(47). Table 1 summarizes the main differences between these two groups of mechanisms.

It is important to note that, as amphetamine-like releasing agents enter into the cell, they can interact with proteins and supress neurotransmitter synthesis, hinder vesicular storage, which can lead to neurotransmitter deficits, and produce a loss of functional transporters. (22)(29)(32)

TABLE 1 Comparison between cocaine-like uptake blockers and amphetamine-like releasing agents

PARAMETER	COCAINE-LIKE UPTAKE BLOQUERS	AMPHETAMINE-LIKE RELEASING AGENTS
Inhibit neurotransmitter uptake	YES	YES
Enter into the neuron	NO	YES
Induce depolarization by inward sodium current	NO	YES
Induce reverse transport	NO	YES
Increase neurotransmitter concentrations in the synaptic space	YES	YES
Neurotransmitter deficits	NO	YES

As previously mentioned, most psychoactive substances can block or reverse the DAT, thereby increasing extracellular DA concentration in the Nacc. Besides their effect on DAT, many of these drugs can disrupt a related monoamine transporter, the SERT, and increase extracellular 5-HT concentrations. In fact, a growing evidence demonstrates that the abuse liability of a drug can be determined by its affinity to act at DAT vs. SERT. In fact, it has been reported that a higher affinity to DAT implies a higher addictiveness of the drug, since the mechanism of the drug is to disrupt this transporter, resulting in a higher DA concentration in some key brain reward structures, such as the Nacc. Some drugs are more akin to the DAT, resulting in higher addictiveness, and others are more akin to the SERT, resulting in less addictiveness. (15)(21)(48)(49)(50)(51)(52)

Paroxetine and cocaine are a good example to illustrate the range of effects that can have a drug depending on its selectivity for DAT vs. SERT. Both drugs share a similar mechanism of action: they block the monoamine transporters and consequently increase extracellular monoamine concentrations, but they differ in their capacity to block DAT and SERT. Paroxetine is SERT selective, whereas cocaine is more DAT selective. Paroxetine and other antidepressants are considered to be “pure” blockers of the SERT, since they block the SERT but hardly block the DAT. In this way, they are used to treat depression, since they increase 5-HT concentration in the synaptic space, without inducing remarkable addiction (40)(41). In contrast, cocaine shows a DAT > SERT selectivity that will determine its psychostimulant effects and its strong abuse potential. (48)(49)(50)(51)(52)

Another example is MDMA which disrupt the DAT, and to a large extent, it disrupts the SERT. Clinically, MDMA-like substances induce prosocial effects and well-being. Since they mainly act by inhibiting the SERT, their addictive potential is lower than amphetamine-like and cocaine-like substances. (53)(48)

Overall, drugs showing major selectivity for DAT over SERT have higher stimulant properties and a strong abuse-potential. Drugs that increase extracellular DA concentrations in the Nacc appear to promote rewarding effects, just like other psychostimulant drugs. Hence, the drug’s mechanism of action is less important than the selectivity for DAT or SERT, which may determine the psychostimulant effects and the abuse potential of the drug. For instance, to predict the abuse liability of a drug, it is important to calculate the ratio hDAT/hSERT. Consequently, a higher ratio hDAT/hSERT may allow us to predict a higher addictive potential (15), although it is impossible to ensure the addictive potential of a drug without performing other experiments, since the ratio hDAT/hSERT only measures the blockade of a monoamine transporter, and a correlation between the ratio hDAT/hSERT and the abuse liability may not exist.

Drug self-administration models are the most widely used *in vivo* experiments to prove abuse potential. Drug-taking behaviours seen in animals during drug self-administration procedures are equivalent to drug-taking behaviours shown by human drug abusers. Substances like cocaine or amphetamine act as “reinforcers” since the delivery rates are higher with the drug than with the vehicle, whereas substances like Paroxetine do not act as “reinforcers”. This, as well as other drug comparisons, allow us to hypothesises that substances with higher affinity to DAT produce reinforcing effects while substances with higher affinity to SERT are less reinforcing. (15)(48)

Intracranial self-stimulation (ICSS) is a behavioural procedure that can also provide helpful information of preclinical abuse potential testing. The results of this test allows to rank the abuse potential of drugs and correlate well with drug self-administration data. In this procedure, intracranial electrodes are implanted in experimental animals to target key regions of the brain. Negus and Miller (54) suggested a correlation between DAT selectivity versus SERT and a behavioural efficacy to facilitate ICSS. Hence, they demonstrated a positive correlation between a high ratio hDAT/hSERT and a drug's high abuse potential (15)(54).

Moreover, it has been demonstrated that the structure of the molecule plays an important role in the abuse potential of the drug, since a small variance in the structure can change its affinity to disrupt DAT or SERT. (17)(48)

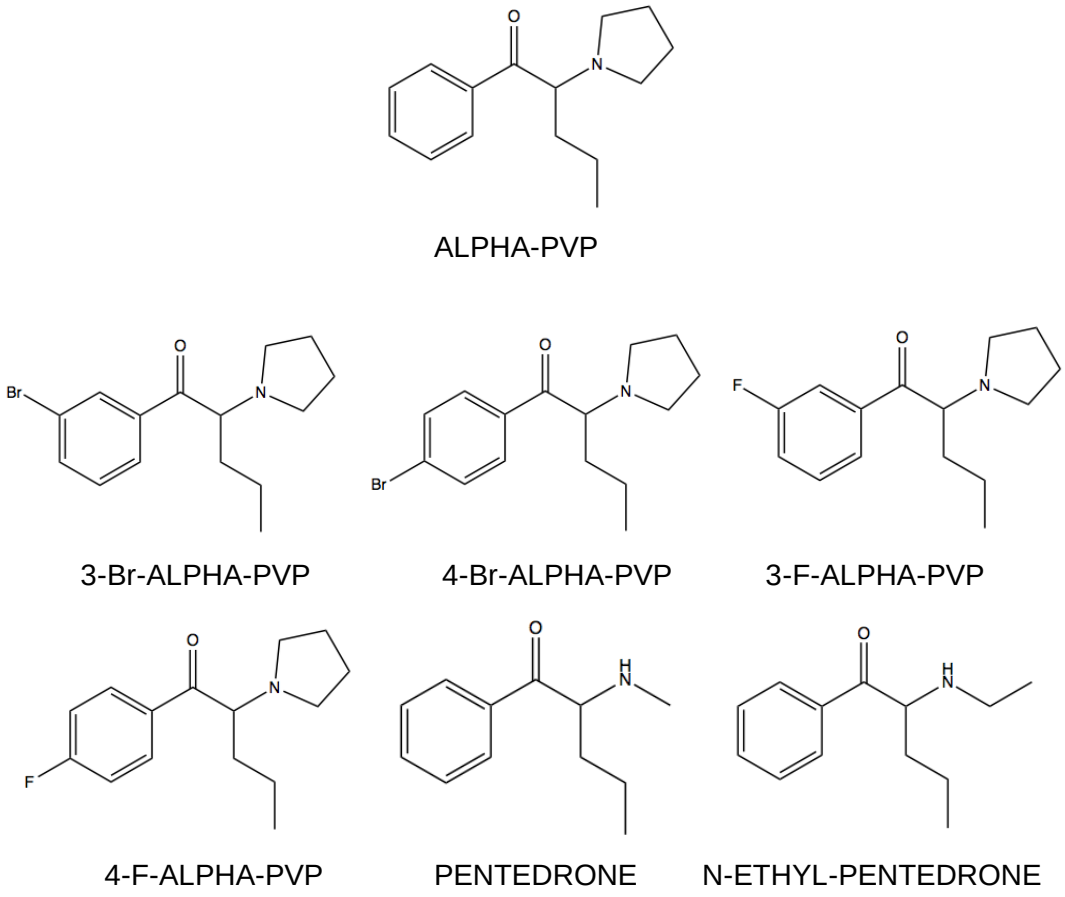


FIG. 11 Chemical structures of α-PVP and derivatives, Pentedrone and N-Ethyl-Pentedrone

Thus, the aim of this study is to determine the abuse potential of NPS such as novel synthetic cathinones by measuring their ratio DAT/SERT. This study demonstrates that some NPS may be even more powerful and addictive than the already well-known drugs of abuse, since small changes in the structure can lead to a higher ratio hDAT/hSERT.

4. OBJECTIVES

The illicit drug market has drastically changed over the last decade. The appearance of NPS represents a threat to public health. The rapid emergence of unknown substances, the lack of data and the changing nature of those substances represent a challenge for prevention and treatment. The main objective of this project is to evaluate the interaction of NPS with monoamine transporters involved in drug addiction.

Briefly, the specific objectives are:

- To evaluate the potential of some novel synthetic cathinones to inhibit DA and 5-HT reuptake *in vitro*.
- To determine structural modifications that lead to DAT or SERT selectivity.
- To compare the predicted in vitro abuse liability of these novel synthetic cathinones with well-known drugs of abuse.
- To predict the abuse potential of these novel synthetic cathinones through their DAT/SERT ratio.

5. MATERIALS AND METHODS

5.1 DRUGS, MATERIALS AND REAGENTS

The novel synthetic cathinones used in this study were synthetized as hydrochloride (HCl) salts and were generously provided by Institut Químic de Sarrià. Paroxetine was purchased from Sigma. Cocaine-HCl and MDMA-HCl were generously provided by Institut de Toxicologia de Catalunya, and Parc Cientific de Barcelona, respectively. [^{3}H] 5-HT was purchased from Perkin Elmer Inc. (Boston, MA, USA) and [^{3}H]1-Methyl-4-phenylpyridinium ([^{3}H]MPP+) was supplied by American Radiolabeled Chemicals (St. Louis, MO, USA). Cell culture dishes and 96-well plates were obtained from Sarstedt (Nuembrecht, Germany). Dulbecco's modified Eagle's medium (DMEM), antibiotics and culture serums were purchased from GIBCO (Invitrogen Corp., Paisley, UK), and all the reagents were from analytical grade.

5.2 CELL CULTURE

Uptake experiments were carried out using Human Embryonic Kidney (HEK293) cells with stable expression of the yellow-fluorescent protein (YFP)-tagged version of the human hDAT and hSERT (see FIG. 12). Transfected HEK293 cells expressing different monoamine transporters were generously provided by Dr. Harald's lab (Medical University of Vienna, Austria). This cell line has already been used in monoamine uptake inhibition assays with satisfactory results (17).

Cells were preserved in culture in a humidified atmosphere (5% CO_2, 37^{o}C) in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/100 mL penicillin and 100 U/100 mL streptomycin and cultured to a subconfluential state. 50 µg/mL of geneticin (G418) were used to maintain the selection process and the fluorescent protein was used to monitor the transporter expression in transfected cells. To ensure a successful growth, the cells were constantly transferred to new lab plates, in order to prevent agglomerations and to guarantee that they have the sufficient nutrients and space to grow correctly. The FBS is used as a supplement to the medium, and provides nutrients and hormonal and growth factors. It also prevents disruptions in the cell growth and other toxic effects, such as pH changes, proteolytic activity or the presence of heavy metals.

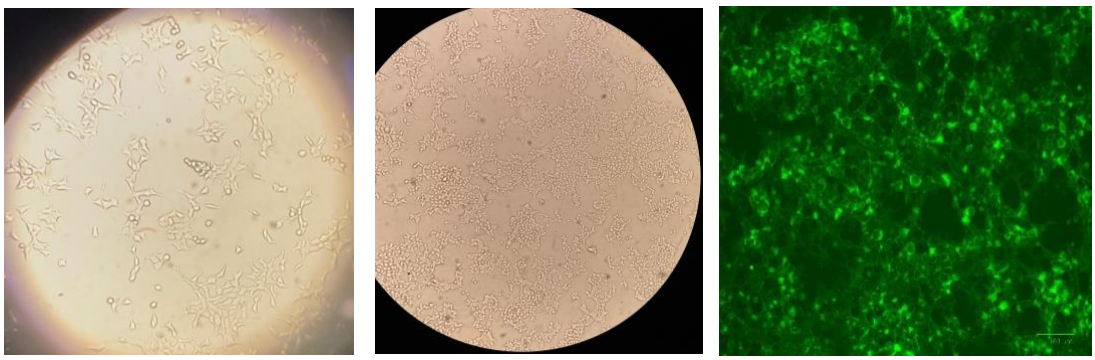


FIG. 12 Microscopic image of HEK293 cells stably expressing hSERT and hDAT on the membrane surface.

5.3 UPTAKE INHIBITION ASSAYS IN HEK293 CELLS

HEK293 cells expressing different monoamine transporters at a density of 0,36 million cells per well were seed in a Poly-D-lysine (PDL)-coated 96 well culture plate, 24 hours before the experiment. The PDL coat allows the cells to adhere to the well culture plate so they are not removed with the medium the day of the experiment.

First, on the day of experiment, the medium was removed from the cell culture 96-well plates and replaced with 200 µl/well of Krebs HEPES buffer (KHB: 10 mM HEPES, 120 mM NaCl, 3 mM KCl, 2 mM $CaCl_2 \cdot 2\ H2O$, 2 mM $MgCl_2 \cdot 6\ H_2O$, and supplemented with 20 mM D-glucose, pH adjusted to 7.3). Second, 3,6 µl of the different concentrated drugs were diluted in KHB at a final volume of 360 µl/eppendorf. Two types of eppendorfs were used: the preincubation eppendorfs, with only KHB and drug, and the incubation eppendorfs, with KHB, drug, and [^{3}H]MPP+ for hDAT or [^{3}H]5-HT for hSERT.
The drug concentrations used for DAT uptake differed from a drug to another. For α-PVP and derivatives, pentedrone and n-ethyl-pentedrone the concentrations used were: 0.001, 0.01, 0.03, 0.1, 0.3, 1, 10 and 100 µM, for cocaine: 0.001, 0.01, 0.1, 0.3, 1, 3, 10 and 100 µM, for MDMA: 0.001,0.01, 0.1, 1, 3, 10, 100 and 1000 µM and for paroxetine: 0.01, 0.1, 1, 3, 10, 30 and 100 µM. The drug concentrations used for SERT uptake also differed from a drug to another. For α-PVP and derivatives the concentrations used were: 0.01, 0.1, 1, 10, 30, 100, 300 and 1000 µM, for cocaine and MDMA: 0.01, 0.1, 1, 3, 10, 30, 100 and 1000 µM, for paroxetine: 0.0001,0.001, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, and 100 µM and for Pentedrone and N-Ethyl-Pentedrone: 0.01, 1, 3, 10, 30, 100 and 1000 µM.

To start the uptake, the different concentrations of the drugs diluted in KHB were used to incubate cells for 5 minutes, to ensure equilibrated conditions, in a process called preincubation. After, the addition of the radiolabelled compounds was performed during the incubation period: 0,02 µM of [^{3}H]MPP+ for hDAT, and 0,1 µM of [^{3}H]5-HT for hSERT. The incubation period lasts 1 minute for SERT and 3 minutes for DAT. During the preincubation time, the drug starts blocking the monoamine transporter and during the incubation time, the radiolabelled compound competes with the drug to bind to the monoamine transporter in order to enter the cell.
After incubation, the cells were washed with ice-cold KHB to end the uptake, and then, cell lysis was induced through the addition of 200 µL of 1% sodium dodecyl sulphate (SDS). The lysate was mixed with scintillation liquid and a beta-scintillation counter (Perkin Elmer, Waltham, MA, USA) quantified the radioactivity, in order to calculate the concentration of drug that entered the cell. Radioactivity was measured for all the samples with triple determination, and counted in counts per minute (cpm) via liquid scintillation spectrophotometry.

Parallel samples of non-specific uptake were determined in the presence of 100 µM of cocaine for hDAT and 30 µM of fluoxetine for hSERT. The value of the non-specific uptake was <10% of total uptake, and to obtain specific uptake, the non-specific values were subtracted from the total uptake. In the absence of drugs, the uptake was normalized to 100% and the uptake with the different concentrations of a drug was expressed as a percentage thereof. The data was normalised and calculated in relation to control samples. All determinations were performed in triplicate.

5.4 DATA ANALYSIS

Competition curves were plotted and fitted by nonlinear regression. To measure the potency of the substituted cathinones and standard compounds at monoamine transporters, the IC_{50} was determined by creating a constrained curve using GraphPad Prism Software and by plotting the percent values against the logarithms of the respective drug concentration. The IC_{50} represents the half maximal inhibitory concentration, in other words, it indicates the concentration of an inhibitory substance needed to inhibit *in vitro* a biological process by 50% (50). To obtain IC_{50} value, data was adjusted to a sigmoidal dose-response curve. The IC_{50} was then used to calculated the hDAT/hSERT ratio: hDAT/hSERT ratio = (1/DAT IC_{50}) / (1/SERT IC_{50}).

Data analysis of uptake inhibition assays were obtained using GraphPad Prism 8.0 (GraphPad software, San Diego, CA, USA). Statistical analysis was needed to compare the potency of the studied drugs of abuse at inhibiting DAT and SERT. One-way ANOVA followed by Tukey's test was used to analyse the data obtained in the uptake inhibition assays. No matching or pairing data was applied and Tukey test was used to compare between drugs, using multiple comparison. The α error probability was set at 0,05 ($p<0,05$). For statistical analysis see Annex II.

6. RESULTS

[^{3}H]MPP$^+$ and [^{3}H]5-HT uptake inhibition assays where carried out for cocaine, paroxetine, MDMA, pentedrone, N-ethyl-pentedrone and α-PVP and its halogenated derivatives to obtain information about the DAT and SERT blockade in HEK293 cells. For a better comparison and clarity, two different analysis were performed. The first one includes cocaine, MDMA, paroxetine, α-PVP, pentedrone and n-ethyl-pentedrone. In the second one, we included cocaine, MDMA, paroxetine, α-PVP and its halogenated derivatives.

All tested drugs showed a different profile in the uptake inhibition assays as showed in FIG. 13, 14, 15 and 16. In summary, α-PVP and its halogenated derivatives demonstrated high uptake inhibition potency at hDAT and very low at hSERT, whereas paroxetine showed high uptake inhibition potency at hSERT and very low at hDAT. MDMA and cocaine exhibited similar potency at hSERT, but cocaine showed higher uptake inhibition potency at hDAT than MDMA.

Particularly, in the first analysis, the order of potency at inhibiting DAT is represented as a concentration-response curve in FIG. 13. According to the statistical analysis (see Annex II), the order of potency of the substances tested is: α-PVP = pentedrone = N-ethyl-pentedrone = cocaine > MDMA > paroxetine. Similarly, for hSERT, the order of potency of the substances in FIG. 14 is: paroxetine = cocaine = MDMA = N-ethyl-pentedrone = pentedrone > α-PVP.

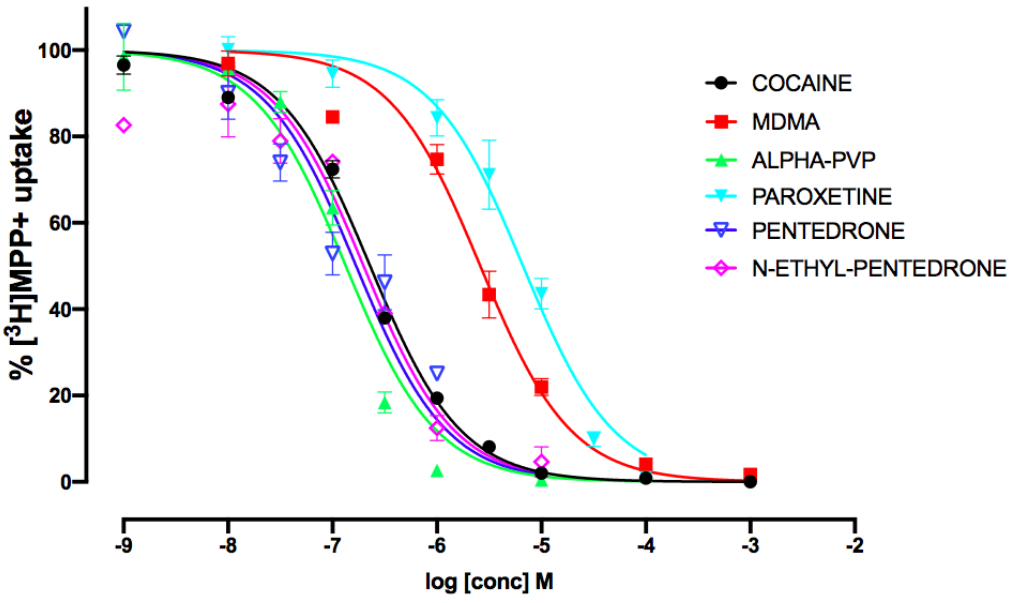


FIG. 13 Uptake inhibition assay at hDAT in transfected HEK293 cells. Effect of cocaine, MDMA, paroxetine, α-PVP, pentedrone and N-ethyl-pentedrone on [^{3}H]MPP$^+$. *Data are expressed as a percentage of control uptake (mean ± SEM) of 6 independent experiments performed in triplicate.*

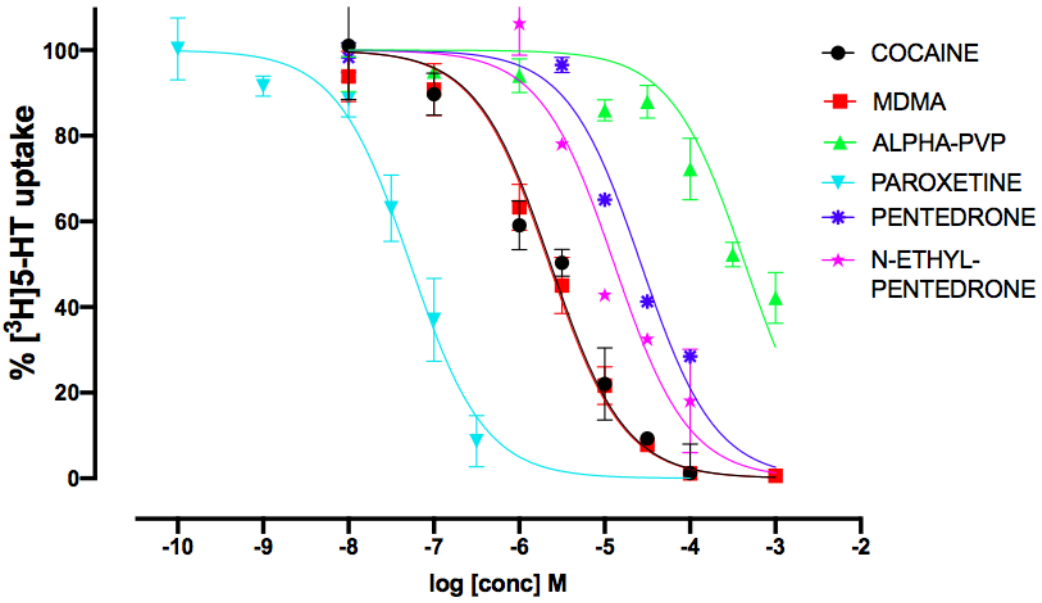


FIG. 14 Uptake inhibition assay at hSERT in transfected HEK293 cells. Effect of cocaine, MDMA, paroxetine, α-PVP, pentedrone and N-ethyl-pentedrone on [^{3}H]5-HT. *Data are expressed as a percentage of control uptake (mean ± SEM) of 6 independent experiments performed in triplicate.*

To fully understand these results, it is important to have in mind that uptake inhibition assays measure the concentration of [^{3}H]5-HT and [^{3}H]MPP$^+$ (a compound that mimics the effect of [^{3}H]DA) inside the cell once the experiment is over. The studied drugs are supposed to block the monoamine transporters, so a drug that potently inhibit DA or 5-HT uptake will lead to a lower concentration of monoamines inside the cell.

In the second analysis, the order of potency of the halogenated analogues of α-PVP for hDAT is showed in FIG. 15. All the NPS studied showed high uptake inhibition at hDAT, while weak potency at hSERT, although the inhibition potency differs depending on the NPS studied. For hDAT, α-PVP and its halogenated derivatives showed a high uptake inhibition potency, similar to the uptake inhibition potency of cocaine, a widely known psychostimulant. Briefly, the order of potency of the substances in FIG. 15 is: α-PVP = 3-Br-α-PVP = 3-F-α-PVP = 4-F-α-PVP = 4-Br-α-PVP = cocaine > MDMA > paroxetine. On the other hand, the order of potency of the halogenated analogues of α-PVP for hSERT is showed in FIG. 16. α-PVP, 3-F-α-PVP and 4-F-α-PVP showed weaker potency for hSERT than cocaine. Thus, the order of potency of the substances according to the statistical analysis (see Annex II) is: paroxetine = MDMA = cocaine = 4-Br-α-PVP = 3-Br-α-PVP > 3-F-α-PVP = 4-F-α-PVP = α-PVP. Finally, Table 2 showed the IC_{50} values of all tested compounds at inhibiting hDAT and hSERT.

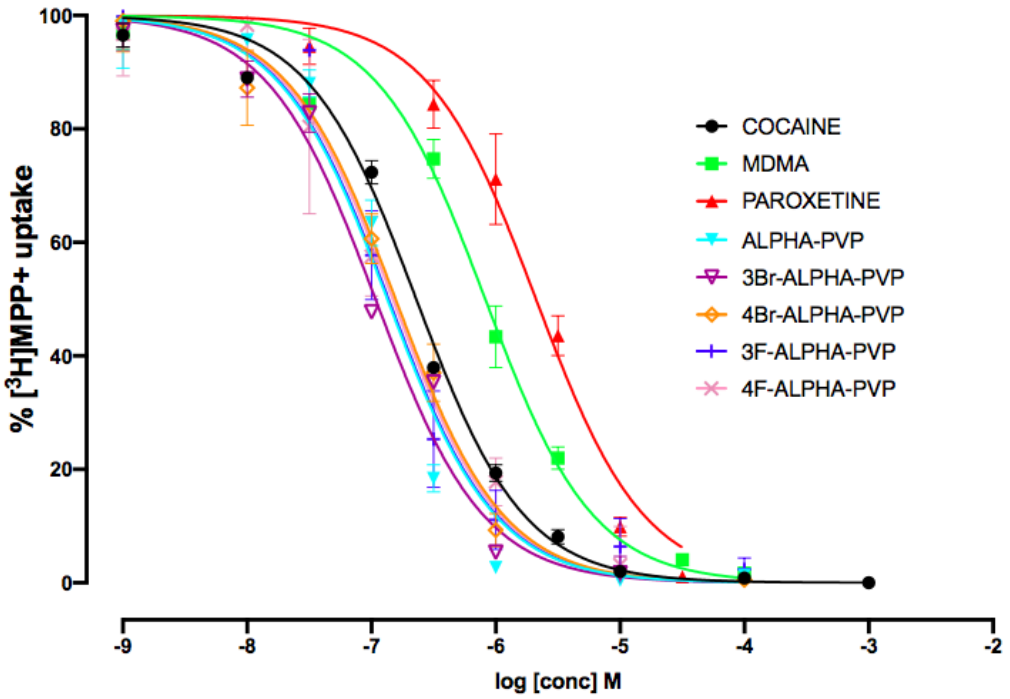


FIG. 15 Uptake inhibition assay at hDAT in transfected HEK293 cells. Effect of synthetic cathinones, cocaine, MDMA and paroxetine on [^{3}H]MPP$^+$. *Data are expressed as a percentage of control uptake (mean ± SEM) of 8 independent experiments performed in triplicate.*

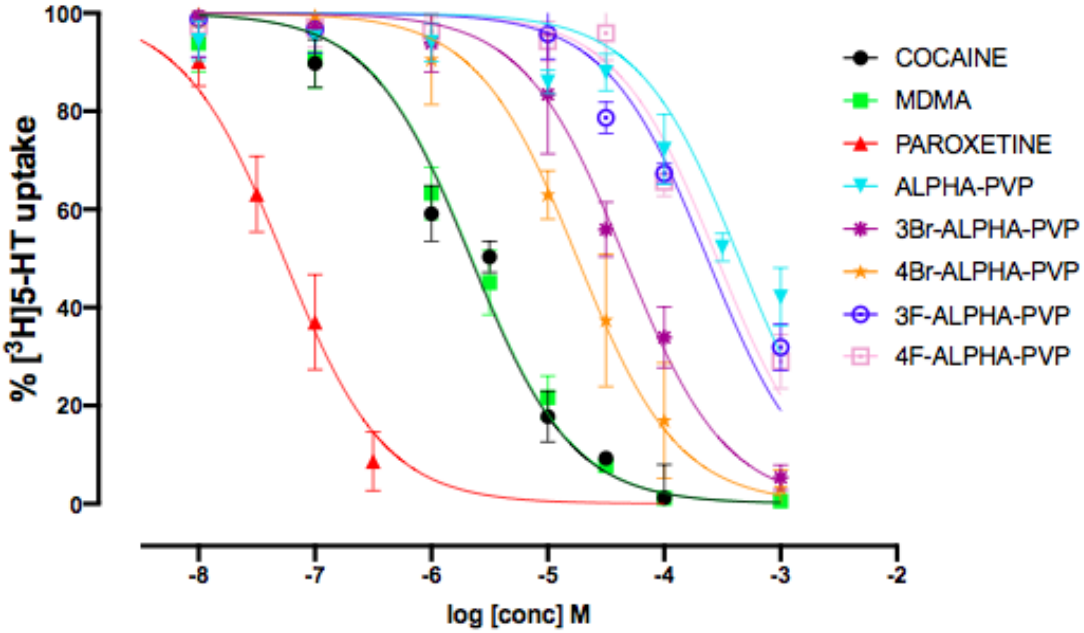


FIG. 16 Uptake inhibition assay at hSERT in transfected HEK293 cells. Effect of synthetic cathinones, cocaine, MDMA and paroxetine on [^{3}H]5-HT. *Data are expressed as a percentage of control uptake (mean ± SEM) of 8 independent experiments performed in triplicate.*

TABLE 2. Potency of substituted cathinones and standard compounds at monoamine transporters. Values of IC_{50} are given as µM (mean ± SEM).

MONOAMINE UPTAKE INHIBITION TRANSFECTED HEK293 CELLS			
COMPOUND	**[^{3}H] MPP$^+$UPTAKE AT hDAT**	**[^{3}H] 5-HT UPTAKE AT hSERT**	**hDAT / hSERT ratio**
PAROXETINE	6.43 ± 0.77	0.058 ± 0.007	0.009
MDMA	2.60 ± 0.23	2.04 ± 0.28	0.8
COCAINE	0.23 ± 0.013	1.85 ± 0.29	8
α -PVP	0.09 ± 0.026	443.4 ± 60.51	>2000
3Br-α -PVP	0.12 ± 0.016	40.91 ± 5.30	349
4Br-α -PVP	0.16 ± 0.013	23.15 ± 3.22	149
3F-α -PVP	0.13 ± 0.017	423.78 ± 116.60	>2000
4F-α -PVP	0.15 ± 0.019	279.43 ± 24.10	1810
PENTEDRONE	0.15 ± 0.066	49.45 ± 23.16	335.151
N-ETHYL-PENTEDRONE	0.19 ± 0.001	19.37 ± 3.14	98.45

hDAT/hSERT ratio = 1/DAT IC_{50} : 1/SERT IC_{50}

7. DISCUSSION

The emergence of NPS poses a major challenge for global authorities, therefore, several studies have been conducted to investigate their abuse potential and their toxicological and pharmacological properties *in vitro* and *in vivo* (17)(18). Thus, the main objective of this study was to describe the pharmacological profile of 7 synthetic cathinones, and compare them to standard psychoactive compounds such as cocaine and MDMA, and to a standard SERT inhibitor such as paroxetine.

To evidence the direct blockade of DA reuptake by these novel synthetic cathinones, HEK293 cells were used to carry out uptake inhibition assays. This experiments proved that all 7 novel synthetic cathinones potently inhibit DA uptake and had very little effect on 5-HT uptake inhibition. This selectivity may have a key role in their predictable abuse potential, as reported by many other studies (14)(17)(22)(55). In fact, in the present study, all 7 compounds demonstrated a high hDAT/hSERT ratio, which may predict their high abuse liability. (17)
In comparison with cocaine, a well-known psychoactive compound with high addictive potential, all 7 synthetic cathinones showed a higher hDAT/hSERT ratio than cocaine. Therefore, their predictable abuse liability may be higher than this well-known substance, increasing the necessity to study the effects of these NPS in order to expand the knowledge about these new drugs and predict possible negative effects of their consumption.

Our results are in concordance with other studies, which concluded that most of the synthetic cathinones that have appeared in the last years have higher hDAT/hSERT ratios than the well-known drugs of abuse. Synthetic cathinones, also known as “bath salts”, share a phenethylamine pharmacophore with amphetamine which is thought the responsible of the effects of synthetic cathinones, as reported by Riley et al. (55). This fact is thought to be involved in the rewarding and stimulant effects of a large number of drugs of abuse (22)(56). Furthermore, it is important to highlight the role of structural modifications in the selectivity of synthetic cathinones for DAT or SERT. The addictive potential of drugs arises from the reward strength they produce; hence, higher selectivity for DAT implies a predictable greater rewarding effect. Moreover, minor modifications in the structure of a drug may lead to changes in its ratio hDAT/hSERT (57)(58)(17). In fact, the 7 compounds tested in this study differed in their amino group or the halogenated aromatic substitution. Firstly, regarding the substitutions in the amino group, the *in vitro* experiments reveal that the ratio hDAT/hSERT increases from a methyl to a pyrrolidine ring. Pentedrone and α-PVP only differ in the amino group, but their ratio hDAT/hSERT vary greatly. This fact may involve the pyrrolidine ring as the principal responsible of the high ratio hDAT/hSERT of some novel synthetic cathinones, and thus the responsible of the predictable high abuse liability of these drugs. Riley et al. (55), Kolanos et al. (59) and Duart-Castells et al. (17), demonstrated that the ratio hDAT/hSERT increases from a methyl to a pyrrolidine ring, but decreases when the pyrrolidine ring is expanded to a piperidine ring, supporting our theory that involves the pyrrolidine ring as one of the principal responsible of the predictable high abuse liability of these novel synthetic cathinones. However, the length of the α-carbon side chain of MDPV and α-PVP also plays an important role in the inhibition of dopamine reuptake, as demonstrated by Kolanos et al. (59). They synthetized several analogues of α-PVP with structural

modifications on the α-carbon side chain and reported that when decreasing the length of the α-carbon side chain from a propyl to a methyl or even eliminating the α-carbon side chain, the analogues displayed reduced potency at inhibiting hDAT uptake. This is the reason why in this study we have focused on 7 NPS with an α-carbon side chain similar to α-PVP.

Regarding the halogenated aromatic substitution, the potency to block hDAT and hSERT varies among the different substituents used in this study, bromine and fluoride. Aromatic bromination demonstrated higher potency at blocking hSERT reuptake than aromatic fluorination, while similar potency at blocking hDAT reuptake. This fact may be due to the similarities regarding the volume of fluor and hydrogen, since they have a similar size, and thus, their capacity to bind to DAT and SERT may be similar to α-PVP. Thus, it is predictable that aromatic bromination may decrease the abuse liability of the drug, since its hDAT/hSERT ratio is lower than aromatic fluorination.
Sáez-Briones et al. (60) and Slack et al. (61) supported this hypothesis, and demonstrated that adding an aromatic bromine substituent increase the SERT affinity, obtaining substituted compounds that are potent inhibitors of 5-HT uptake and therefore have predictable lower abuse liability than fluorinated or non-substituted compounds, correlating with the results obtained in this study.

On the other hand, the position of the substituent seems also to influences the hDAT/hSERT ratio of the drug. Drugs with substituents in position 3 have a higher ratio hDAT/hSERT than their counterparts with substituents in position 4. This may be due to the significance of their spatial structure when binding to DAT. However, the results when determining the IC_{50} showed that an aromatic substitution decreases the selectivity for DAT and increases the selectivity for SERT. Thus, the ratio hDAT/hSERT decreases when adding an aromatic substitution. As reported in other studies (62)(63)(64), the position of the substituents plays a very important role in the hDAT/hSERT ratio of the drugs. Wee et al. (62) studied 4 new compounds structurally related to amphetamine, with aromatic substitutions in either position 3 and 4. They concluded that aromatic substitutions in position 4 increases the potency of the drug as a 5-HT release and therefore, decreases its hDAT/hSERT ratio, in correlation with the results observed in the present study. Moreover, they observed that the aromatic fluorination in position 4 does not increase the potency of the drug as a 5-HT releaser with the same potency as a methyl substitution in position 4. Wellman et al. (63) and Negus et al. (64) also demonstrated that substitutions in position 4 (for example PAL-313) increase the SERT activity, and thus decrease the hDAT/hSERT ratio.

Focusing on well-known drugs of abuse, the hDAT/hSERT ratio of MDMA is ten times lower than cocaine, which may influence the fact that cocaine is more addictive than MDMA, and the difference in their psychoactive effect (53). However, their potency for SERT is similar. Although it has been reported that MDMA inhibits preferably 5-HT uptake rather than DA uptake (53), the results obtained in this experiments reported a similar potency for SERT. This may be due to the fact that MDMA and cocaine have different mechanisms of action. Cocaine act by blocking the transporter, whereas MDMA belongs to the amphetamine-like releasing agents group and act by inducing reverse transport. This mechanism implies the entrance of the drug into the intracellular space, what leads to a reverse transport of monoamines. In fact, our uptake inhibition

experiments are not able to measure monoamine releasing properties, which may influence in our results. Thus, more experiments are needed in order to investigate feasible releasing properties of novel synthetic cathinones and other compounds.

Paroxetine belongs to the group of selective 5-HT uptake inhibitors, hence, it mainly inhibits 5-HT reuptake, but has very little effect on DA reuptake. This selectivity is responsible of its main effect: antidepressant and its low abuse liability. For this reason, paroxetine is used in this study to compare among substances. Thus, having in mind the results of this study, MDMA is the more similar substance in terms of SERT potency, which may lead us to think that its reinforcing effects will be lower than the reinforcing effects of α-PVP, and the well-being effect will also be higher, in accordance with previous findings. (53)

Finally, our results are consistent with the results observed in previous studies (14)(17) about the abuse potential and rewarding effects of synthetic cathinones. All 7 compounds evidenced a higher ratio hDAT/hSERT than cocaine, which involves a prediction of higher rewarding properties and addictive potential. Moreover, the observed structure-activity relation also correlates with the results reported in previous studies (14)(17)(59)(60)(61)(62)(63)(64) and demonstrates that there is a close relation between the structure of the drug and the capacity of each drug to block monoamine transporters.

8. CONCLUSION

In summary,

- The 7 novel synthetic cathinones tested have proven to be potent DA uptake inhibitors, but weak 5-HT uptake inhibitors.

- The IC_{50} values for hDAT decreases when increasing the length of the amino group from a methyl to a pyrrolidine ring, and increases when the aromatic substituent is a bromine in position 4 instead of a fluoride in position 3. This correlation enables us to establish an association between the structure of the molecule and its selectivity for DAT or SERT.

- The 7 synthetic cathinones tested have proven to have high ratios hDAT/hSERT, even higher than cocaine, a drug with known abuse liability, which suggests they may have a serious negative impact on public health.

Consequently, and although more research needs to be done, the abuse potential of novel synthetic cathinones must be taken as a serious and dangerous health threat.

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