MEDIZINISCHE UNIVERSITÄT WIEN

MEDICAL UNIVERSITY OF VIENNA

DIPLOMARBEIT / DIPLOMA THESIS

“The pharmacological properties of the new psychoactive substance (±)-cis-4,4'-dimethylaminorex (4,4'-DMAR)”

zur Erlangung des akademischen Grades

Doktor der gesamten Heilkunde
(Dr. med. univ.)

an der
Medizinischen Universität Wien

ausgeführt am
Institut für Pharmakologie

unter der Anleitung von

Univ. Prof. Dr. med. univ. Harald H. Sitte

eingereicht von

Mag. Julian Maier, BA BA

n00855111

Table of contents

Abstract

(±)-*cis*-4,4'-Dimethylaminorex (4,4'-DMAR) is a psychostimulant that has been associated with 31 fatalities and other adverse events in Europe between June 2013 and February 2014. However, the pharmacology of 4,4'-DMAR remains largely unexplored. In this thesis, its binding capabilities to monoamine receptors and transporters were assessed. *In vitro* uptake inhibition and transporter release assays were conducted to determine the effects of 4,4'-DMAR on human high-affinity transporters for dopamine (DAT), norepinephrine (NET) and serotonin (SERT). Furthermore, the interaction of 4,4'-DMAR with the vesicular monoamine transporter 2 (VMAT2) in rat phaeochromocytoma (PC12) cells and synaptic vesicles prepared from human striatum was explored. 4,4'-DMAR inhibited uptake mediated by human DAT, NET or SERT, respectively in the low micromolar range (IC_{50} values < 2 µM) and is therefore a more potent inhibitor than MDMA. Release assays identified 4,4'-DMAR as a substrate type releaser, capable of inducing transporter-mediated reverse transport via DAT, NET and SERT. In addition, 4,4'-DMAR inhibited both the rat and human isoforms of VMAT2 at a potency similar to 3,4-methylenedioxymethylamphetamine (MDMA). This thesis identified 4,4'-DMAR as a non-selective monoamine releasing agent. In contrast to the known effects of its parent substances aminorex and 4-methylaminorex, 4,4'-DMAR exerts profound effects on human SERT. The latter finding is consistent with the idea that fatalities associated with its abuse may be linked to monoaminergic toxicity including serotonin syndrome. The activity at VMAT2 suggests that chronic abuse of 4,4'-DMAR may result in long-term neurotoxicity.

Acknowledgements

All experimental work was performed by Julian Maier[i], Felix P Mayer[i], Marion Holy[i], Kathrin Jäntsch[i], Harald Reither[ii] and Sylvie Chaboz[iii].

Julian Maier[i], Felix P Mayer[i], Marion Holy[i], Kathrin Jäntsch[i], Christian Pifl[ii], Matthias E Liechti[iv], Dino Luethi[iv], Marius C Hoener[iii] and Harald H Sitte[i] designed the experiments.

Simon Brandt[v] and Lena Hirtler[vi] contributed materials.

Figures 1 to 4 were created by Julian Maier[i]. Experiments were conducted by Julian Maier[i] and Marion Holy[i].

Figure 5 was created by Julian Maier[i] and Felix P Mayer[i]. Experiments were conducted by Julian Maier[i], Felix P Mayer[i] and Kathrin Jäntsch[i].

Figure 6 was created by Christian Pifl[ii] and experiments were conducted by Harald Reither[ii].

Binding experiment data was provided by Matthias E Liechti[iv], Dino Luethi[iv] and Marius C Hoener[iii]. Experiments were conducted by Sylvie Chaboz[iii].

I have simultaneously worked on this diploma thesis and an article that will appear in a scientific journal in 2018. Therefore, parts of the Abstract, Introduction, Methods and Results sections will be, in a similar form, published in the journal *Neuropharmacology* in 2018.

---

i Medical University of Vienna, Center for Physiology and Pharmacology, Institute of Pharmacology, Währingerstraße 13A, 1090, Vienna, Austria

ii Medical University of Vienna, Center for Brain Research, Department of Molecular Neurosciences, Spitalgasse 4, 1090, Vienna, Austria

iii F. Hoffmann - La Roche Ltd., pRED, Roche Innovation Center Basel, Neuroscience Research, Department of Neurosymptomatic Domains, Grenzacherstraße 124, 4070, Basel, Switzerland

iv University Hospital Basel and University of Basel, Division of Clinical Pharmacology and Toxicology, Department of Biomedicine, Hebelstraße 20, 4031, Basel, Switzerland

v School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool, L3 3AF, UK

vi Medical University of Vienna, Center for Anatomy and Cell Biology, Währingerstraße 13, 1090, Vienna, Austria

List of Abbreviations

^{3}H-MPP^{+}	Tritiated 1-methyl-4-phenylpyridinium
^{3}H-5-HT	Tritiated Serotonin
4-MAR	4-Methylaminorex (Euphoria)
4,4’-DMAR	4,4'-Dimethylaminorex (Serotoni)
5-HT	5-hydroxytryptamine (Serotonin)
EC_{50}	Half maximal effective concentration
DAT	Dopamine transporter
IC_{50}	Half maximal inhibitory concentration
MAO	Monoamine oxidase
MAOI	Monoamine oxidase inhibitor
MDMA	3,4-Methylenedioxymethamphetamine (Ecstasy)
Na^{+}/K^{+}-ATPase	Sodium-potassium pump
NET	Norepinephrine transporter
SERT	Serotonin transporter
SLC	Solute carrier membrane transporter
SNRI	Serotonin–norepinephrine reuptake inhibitor
SSRI	Selective serotonin reuptake inhibitor
TAAR1	Trace amine associated receptor 1
TCA	Tricyclic antidepressant
VMAT2	Vesicular monoamine transporter2

Introduction

Between June 2013 and February 2014, 31 people in Europe died from the consumption of the new psychoactive substance (NPS) (±)-*cis*-4,4'-Dimethylaminorex [(±)-*cis*-4-methyl-5-(4-methylphenyl)-4,5-dihydro-1,3-oxazol-2-amine; 4,4'-DMAR] (EMCDDA, 2014). 21 of those 31 deaths occurred in the United Kingdom. To put this figure into context: Between 2003 and 2013, 76 people died from the consumption of NPS in the U.K. 21 of those deaths, approximately one quarter, were caused by 4,4'-DMAR, even though the substance had only been available for half a year (ONS, 2016).

Brandt *et al.* (2014) and McLaughlin *et al.* (2015) have analysed the chemical features of the substance and determined its monoamine transporter activity using rat brain synaptosomes. They concluded that 4,4'-DMAR acts as a potent releaser at DAT (SLC6A3), NET (SLC6A2) and SERT (SLC6A4), the monoamine transporters of dopamine, norepinephrine and 5-HT respectively. As will be examined in more detail, amphetamines and amphetamine-like substances, such as 4,4'-DMAR, increase the synaptic monoamine concentration via the induction of efflux through reverse transport and their interaction with the vesicular monoamine transporter VMAT2 (SLC18A2) (Sitte & Freissmuth 2015).

In comparison to its predecessors and structural analogues Aminorex and 4-MAR (4-Methylaminorex), 4-4'-DMAR has been shown to be a more potent releaser at SERT (Brandt *et al*, 2014). Aminorex and 4-MAR are amphetamine-like substances (Hofmaier *et al.* 2014; Kankaanpää *et al.* 2001) that are more potent DAT and NET than SERT releasing agents (Brandt *et al.* 2014). 4,4'-DMAR, on the other hand, is a potent non-selective monoamine releasing agent.

The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), hypothesises that serotonin syndrome and cardiovascular effects caused by norepinephrine as well as the combination of 4,4'-DMAR with various other drugs might have been possible causes of death (EMCDDA, 2014). Consequently, in 2015, the EU Council, advised by Brandt and the EMCDDA, decided to add the substance to the list of controlled substances, not readily available for the general public (EMCDDA, 2015).

This thesis is the first study to provide a human cell (HEK 293) based transporter binding and monoamine transporter interaction profile of the substance and therefore might shed light on 4,4'-

DMAR's short-term pharmacological effects and toxicity. Secondly, the thesis, utilising human striatal tissue and PC12 rat cells, both expressing VMAT2, offers an explanation of the possible long-term toxicity of 4,4'-DMAR, involving an inhibition of VMAT2, which has already been established for amphetamine and other amphetamine-related drugs before (Pifl *et al*. 2015). In addition, knowledge about deadly substances of abuse is not only relevant for the field of pharmacology and toxicology but also pertaining to the fields of public health, addiction, illicit drug use, drug regulation and policy.

Theoretical Framework

NET (SLC6A2), DAT (SLC6A3) and SERT (SLC6A4), as well as VMAT2 (SLC18A2), are part of the solute carrier (SLC) family of eukaryotic membrane transport proteins, which, via facilitative and secondary active transport, allow for the uptake or efflux of solutes (Schlessinger *et al*. 2010)[1]. While the exact number still remains enigmatic, current knowledge suggests that more than 400 genetically different SLCs are recognized (Perland & Fredriksson 2017; César-Razquin *et al*. 2015). If a transporter has a genetic sequence identity of at least 20% with another SLC, they belong to the same family (Hediger *et al*. 2013; Hediger *et al*. 2004). SLCs are currently grouped into 52 different families, with most (an exception is for example the SLC22 family – Colas *et al*. 2016) structurally similar SLCs having similar substrates (Perland & Fredriksson 2017; Schlessinger *et al*. 2010). It is established that most SLC families share very little sequence similarities amongst each other which distinguishes them from other membrane protein families, such as, e.g., G-Protein-coupled receptors (Colas *et al*. 2016; Höglund *et al*. 2011; Fredriksson *et al*. 2003). A search of SLC protein sequences against the Pfam (Protein families) database was conducted and the heterogenous results reveal that the known SLC families belong to 24 different Pfam superfamilies (Höglund *et al*. 2011; Perland *et al*. 2017). Only four clans (the major facilitator superfamily [MFS; approximately containing one third of all SLCs], the amino acid-polyamine-organocation (APC) superfamily, the cation:proton antiporter (CPA) superfamily and the drug/metabolite transporter (DMT) superfamily) encompass more than one SLC family (Höglund *et al*. 2011; Perland *et al*. 2017). Phylogenetic analyses unveil that 26 of the known 52 families can be further classified into four clusters, the α-, β-, γ- and δ-groups (Höglund *et al*. 2011; Fredriksson *et al*. 2008). Hidden Markov Models (HMMS) are being applied in order to identify novel SLCs, unique and repeated SLC sequences and orthologues (Perland *et al*. 2017; Perland and Fredriksson 2017; Höglund *et al*. 2011; Fredriksson *et al*. 2008; Sundberg *et al*. 2008). Still, even though 10% of the human genome contain sequences pertaining to transporters (Hediger *et al*. 2013) and SLCs are the largest transporter family, they are among the least (well-)researched gene groups (César-Razquin *et al*. 2015).

[1] Some special corner cases are for example the SLC51A and B, as well as the SLC3 family in conjunction with certain SLC7 members, in the sense that they are heterodimers that only demonstrate transporter function when interacting with each other (Colas *et al*. 2016).

All SLCs have in common that they are (trans-)membrane proteins that allow for specific substrates' lipid bilayer crossing (Perland *et al*. 2017). While a preponderance of cell membrane transporters is noteworthy, there nonetheless are intracellular exceptions such as the mitochondrial carrier family SLC25 (Pebay-Peyroula *et al*. 2003) or the vesicular glutamate and amine transporter families (SLC17 and 18 – Reimer 2013; Lawal & Krantz 2013). Another commonality is that, in general, SLCs contain ten to fourteen transmembrane α-helices (Colas *et al*. 2016; Schlessinger *et al*. 2010). While more and more crystal structures of SLCs have been solved over the last couple of years (Hediger *et al*. 2013), only four human SLC transporters' structures have been identified to date (Colas *et al*. 2016). A lot of knowledge could be garnered from working with eu- and prokaryotic homologues of human SLCs. An example are the insights gained from working with the bacterial, prokaryotic, leucine transporter (LeuT) (e.g. Rudnick *et al*. 2014; Shi *et al*. 2008; Singh *et al*. 2007; Zhou *et al*. 2007) and the homologous eukaryotic *Drosophilia melanogaster* structure (Penmatsa *et al*. 2013), which have contributed to the current understanding of how the human SLC6 family functions. X-ray crystallography has also led to a rise of molecular dynamics (MD) simulations on an atomic level trying to detail the exact mechanism of SLC action (Tamura & Hayashi 2017; Grouleff *et al*. 2015). Transport is illustrated with the alternating access model where SLC transporters alternate between inward and outward facing conformations via intermediate, i.e. occluded, states (Tamura & Hayashi 2017; Colas *et al*. 2016; Jardetzky 1966). Depending on the occupational state of the binding sites, the transporter alternates between conformational changes (Rudnick *et al*. 2014; Kristensen *et al*. 2011). Still, as Grouleff *et al*. (2015) highlight, the exact binding locations of many inhibitors, releasers and ions are currently heavily debated.

Approximately 190 SLCs have been linked to diseases but most have not yet been explored as drug targets (César-Razquin *et al*. 2015; Lin *et al*. 2015; Rask-Andersen *et al*. 2013). Most current drugs are inhibitors of SLC transporter activity (Lin *et al*. 2015). SLC families are for example targeted in the treatment of depression, epilepsy and addiction (SLC6), Parkinson and movement disorders (SLC18), gout (SLC22), diabetes (SLC5), utilized for diuresis (SLC12) and anti-neoplastic, as well as cardiovascular, applications are being investigated (Lin *et al*. 2015; Rask-Andersen *et al*. 2013). The, by far, most popular and well-researched SLC targeting drugs are inhibitors of SLC6A2, A3 and A4 (César-Razquin *et al*. 2015).

The SLC6 transporter family, containing more than 300 eu- and prokaryotic proteins, at least 20 of which can be found in humans, is, via the inclusion in the neurotransmitter:sodium symporter (NSS) family, part of the APC superfamily (Bala *et al*. 2013; Rudnick *et al*. 2014). Contrary to what the family name suggests, not all SLC6 members transporter neurotransmitters but rather also amino acids, taurine, creatine and betaine (Bröer & Gether 2012). It is possible to subdivide the family into four groups: First of all, a group labelled GABA transporters, containing three GABA transporters (SLC6A1, SLC6A11, SLC6A13), one taurine transporter (SLC6A6), one creatine transporter (SLC6A8) and one betaine transporter (SLC6A12); secondly, the subfamily of monoamine transporters comprising the aforementioned transporters of norepinephrine, dopamine and 5-HT (SLC6A2, SLC6A3 and SLC6A4); thirdly, neurotransmitter amino acid transporters for glycine (SLCA5, SLCA9), proline (SLC6A7) and neutral and cationic amino acids (SLC6A14) and lastly the nutrient amino acid transporters (SLC6A15-20), mostly transporting neutral amino acids (Rudnick *et al*. 2014; Bröer & Gether 2012). The four subfamilies share their mechanism of action. As their family name suggests, all SLC6 members are sodium symporters, transporting one to three sodium ions, with some additionally moving one chloride ion in conjunction with the substrate, while SLC6A4 further couples the efflux of potassium to substrate influx, as an antiport mechanism (Rudnick *et al*. 2014; Bröer & Gether 2012; Kristensen *et al*. 2011)[2]. Raising intracellular sodium levels, by blocking the Na^+/K^+-ATPase or dissipating the sodium gradient by administering the ionophore monensin, leads to an increase in neurotransmitter efflux (Sitte & Freissmuth 2015; Mollenhauer *et al*. 1990). Cellularly, SLC6 expression is regulated via steps of the secretory pathway (e.g. oligomerization and N- or C-terminus binding), post-translational modifications, such as glycosylation, ubiquination, phosphorylation (mostly via PKC [protein kinase C]) and dephosphorylation and various ways of transporter trafficking (internalization influenced by PKC, substrates and inhibitors) (Pramod *et al*. 2012; Kristensen *et al*. 2011; Ramamoorthy *et al*. 2011; Sager & Torres 2011; Melikian 2004). In addition, the presence of other molecules, such as membrane cholesterol, seems to be essential for transporter function (Vaughan & Foster 2013; Hong & Amara 2010; Scanlon *et al*. 2001; North & Fleischer 1983).

SERT (SLC6A4), located on chromosome 17q11.1-17q12 and expressed peripherally in the intestinal tract, the adrenal gland and platelets and centrally mostly in the median and dorsal raphe nuclei, is important for the regulation of emotions, mood, cognitive and motor functions and

[2] For the exact stoichiometry see Grouleff *et al*. (2015).

comprises 14 exons and polymorphic regions (Sara & Bouret 2012; Murphy *et al*. 2008; Canli & Lesch 2007; Torres *et al*. 2003; Kim *et al*. 1999; Heils *et al*. 1996; Lesch *et al*. 1994). Epigenetic DNA methylation is being discussed as a biomarker of adversities such as traumatic and stressful life events (Provenzi *et al*. 2016; van der Knaap *et al*. 2015; Dunam & Canli 2015). Certain SERT polymorphisms are associated with ulcerative colitis and IBS, as well as anxiety, affective disorders, substance abuse, attention deficit hyperactivity disorder, pulmonary hypertension and depression, seeming to influence individuals' reactions and responsiveness to antidepressants (Sikander *et al*. 2015; Acosta & Camilleri 2015; Murphy *et al*. 2008; Park *et al*. 2006; Kim *et al*. 1999; Gelernter *et al*. 1997). Genetically, depressive disorders seem to be highly heterogenous but SLC6A4 is being investigated as one possible locus of origin (Flint *et al*. 2014; Ripke *et al*. 2012; Risch *et al*. 2009). Medically, SERT is being targeted by antidepressant compounds, such as serotonin reuptake inhibitors (SSRIs, TCAs, SNRIs - Rask-Andersen *et al*. 2013). Drugs that exhibit a serotonergic profile of action, such as MDMA, a non-selective monoamine releasing agent, and cocaine or SSRIs and MAOIs can, when overdosed or combined with each other cause serotonin toxicity, serotonin syndrome (Greenier *et al*. 2014). The SLC6A4's crystallography structure reveals a central and allosteric binding site, as well as ion-binding sites and illuminates antidepressants' mechanism of action, with the drug locking the transporter in an outward-open confirmation by attaching to the central binding site (Coleman *et al*. 2016). The endogenous ligand of SERT, serotonin (5-Hydroxytryptamine), and its ways of synthesis and degradation, as well as the associated 5-HT receptors have been concisely described elsewhere (Spies *et al*. 2015; Bockaert *et al*. 2010; Eisenhofer *et al*. 2004; Barnes & Sharp 1999; Boadle-Biber 1993).

DAT (SLC6A3), located on chromosome 5p15.3, mostly expressed in the brain's mesolimbic, mesocortical and mesostriatal pathways and peripherally in the pancreas, stomach and kidney, is, like SERT, essential for the regulation of emotions, mood, cognitive and motor functions and contains 15 exons and a VNTR (variable number tandem repeat), responsible for polymorphisms (Costa *et al*. 2011; Torres *et al*. 2003; Vandenbergh *et al*. 2000; Ciliax *et al*. 1999; Vandenbergh *et al*. 1992). DAT polymorphisms correlate with addiction, attention deficit hyperactivity disorder, posttraumatic stress disorder, Parkinson's disease, bipolar disorder and depression (Li *et al*. 2016; Tong *et al*. 2015; Hansen *et al*. 2014; Zhai *et al*. 2014; Spencer *et al*. 2013; Vaughan & Foster 2013; Li *et al*. 2006; Erblich *et al*. 2005; Vandenbergh *et al*. 2000). Dopamine reuptake inhibitors are used to treat attention deficit hyperactivity disorder, narcolepsy, Parkinson's disease, as

antidepressants and consumed illicitly as well, e.g. cocaine (Huot *et al*. 2016; Kesselheim *et al*. 2012; Loland *et al*. 2012; Carroll *et al*. 2006; Adler & Chua 2002). In addition, partial uptake blockers that are allosteric DAT ligands are being identified as potential novel therapeutics (Rothman *et al*. 2015). Notable dopamine releasing agents, often consumed as illicit drugs, are amphetamine, cathinones, MDMA and tryptamine derivatives (Reith *et al*. 2015; Blough *et al*. 2014). DAT-SPECT (single-photon emission computed tomography) is applied in the diagnosis of Parkinsonism and early parkinsonian syndromes and increasingly used for research purposes (Suwijn *et al*. 2014; Scherfler *et al*. 2007). The fact that transport via SLC6A3 can be inhibited by extracellular zinc (Norregaard *et al*. 1998), was utilized to develop a human DAT model via MD simulation and zinc binding site-directed mutagenesis experiments (Stockner *et al*. 2013). In addition, *Drosophila melanogaster* DAT was crystallized to highlight the interaction between DAT and antidepressant drugs with the inhibitor occupying the substrate-binding site to the effect that the transported is stabilized in an outward-open confirmation (Penmatsa *et al*. 2015; Wang *et al*. 2015; Penmatsa *et al*. 2013). The endogenous ligand of the DAT, dopamine (3,4-dihydroxyphenylalanine), and its ways of synthesis and degradation, as well as the associated dopamine receptors have been concisely described elsewhere (Beaulieu *et al*. 2015; Broadley 2010; Eisenhofer *et al*. 2004). Interestingly, DAT and NET can substitute reciprocally in transporting dopamine and norepinephrine (Torres *et al*. 2003).

NET (SLC6A2) is located on chromosome 16q12.2, encoded by 14 exons and, similar to the aforementioned transporters, subject to genetic variation (Tellioglu & Robertson 2001; Brüss *et al*. 1993). NET is expressed in the plasma membranes of noradrenergic neurons in the central and peripheral nervous system, the lung and placenta and constitutive of the sympathetic nervous system (Zhou 2004; Schroeter *et al*. 2000). The locus coeruleus, located in the brainstem, is a noradrenergic nucleus, involved in the regulation of mood, sleep, behaviour, alertness and arousal (Sara & Bouret 2012; Zhou 2004). Polymorphisms and epigenetic DNA methylation have been associated with major depression, attention deficit hyperactivity disorder, heart failure, hypertension, tachycardia and orthostatic intolerance (Bayles *et al*. 2013; Zolk *et al*. 2012; Dong *et al*. 2009; Kim *et al*. 2008a; Kim *et al*. 2008b; Kim *et al*. 2006; Ono *et al*. 2003; Backs *et al*. 2001; Tellioglu & Robertson 2001; Shannon *et al*. 2000). Selective and non-selective norepinephrine reuptake inhibitors and releasing agents are utilised in the treatment of depression, anxiety, attention deficit hyperactivity disorder and narcolepsy (Schlessinger *et al*. 2011; Dell'Osso

et al. 2010; Billiard 2008; Hajos *et al*. 2004; Spencer *et al*. 2002; Gorman & Kent 1999; Luque & Rey 1999). The releasing agents amphetamine, MDMA and mephedrone and the reuptake inhibitor cocaine are seminal representatives of illicit substances targeting the SLC6A2 transporter (Rothman *et al*. 2001). In contrast to the previously described monoamine transporters, the crystallographic structure of NET remains, as of yet, unknown. The endogenous ligand of the norepinephrine transporter, norepinephrine, or noradrenaline, and its ways of synthesis and degradation, as well as the associated adrenergic α and β receptors have been concisely described elsewhere (Alexander *et al*. 2015; Broadley 2010; Eisenhofer *et al*. 2004).

The vesicular monoamine transporter 2, VMAT2 (SLC18A2), located on chromosome 10q25.3 and comprising 16 exons, can, in contrast to the exclusively in peripheral neuroendocrinal cells expressed VMAT1, be detected in enteric, peripheral and, predominately, central neurons (NCBI 2017; Wimalasena 2011; Fei *et al*. 2008; Eiden *et al*. 2004; Erickson *et al*. 1996; Peter *et al*. 1993; Surratt *et al*. 1993). SLC18A2 is part of the SLC18 family of vesicular neurotransmitter transporters, consisting of SLC18A1 (VMAT1), SLC18A2 (VMAT2), SLC18A3 (VAchT, the vesicular acetylcholine transporter), SLC18A4 (that is only expressed in insects) and SLC18B1, VPAT, the vesicular polyamine transporter, transporting spermine and spermidine (Takeuchi *et al*. 2017; Hiasa *et al*. 2014; Lawal & Krantz 2013). The first three transporters share between 40 and 60% sequence identity amongst each other (Eiden *et al*. 2004). Other vesicular transporters belonging to the SLC family are the vesicular glutamate transporters (SLC17) and the vesicular inhibitory amino acid transporter (SLC32) (Anne & Gasnier 2014). The SLC18 family's vesicular transporters, located in the membrane of secretory vesicles, operate via an antiport mechanism constituted by the exchange of one cytosolic cationic transmitter molecule for two luminal protons (Anne & Gasnier 2014; Lawal & Krantz 2013; Eiden *et al*. 2004; Parsons 2000). The necessary electrochemical gradient for transport is generated by a Mg^{2+}-dependent vacuolar (V-type) H^{+}-ATPase, residing on the membrane of cells involved in the secretory pathway (Chaudhry *et al*. 2008). Similar to the aforementioned monoamine transporters, (de-)phosphorylation and glycosylation are the most well-researched and -known mechanisms in the regulation and trafficking of the VMAT2 (Ramamoorthy *et al*. 2011; Fei *et al*. 2008). SLC18A2's exact structure has not been discovered yet but there exist 3D homology models built from known prokaryotic structures also belonging to the MFS superfamily (Liu *et al*. 2016; Anne & Gasnier 2014; Yaffe *et al*. 2013). In addition, it is pointed out that VMAT2 displays homology to bacterial resistance genes

and its mechanism of action bears resemblance to bacterial efflux pumps rather than other monoamine transporters, recognising many of the same cytotoxic compounds that bacterial drug-resistance pumps can identify (Eiden & Weihe 2011; Guillot & Miller 2009; Chaudhry *et al*. 2007). Current findings suggest that the multiple confirmations MFS models undergo during the transport cycle are consistent with the alternating access model (Yaffe *et al*. 2016). While many authors (Solovieff *et al*. 2014; Wimalasena 2011; Lin *et al*. 2010; Eiden *et al*. 2004) describe associations between impaired VMAT2 function and Parkinson's disease, schizophrenia, alcoholism and post-traumatic stress disorder, others emphasise the lack of unequivocal association of diseases and SLC18A2 polymorphisms (Lawal & Krantz 2013). One reason might be the extraordinary importance of a functioning SLC18A2 and that some mutations (and the full knockout of the SLC18A2 gene) might be incompatible with life (Lawal & Krantz 2013; Eiden & Weihe 2011; Lin *et al*. 2010; Takahashi *et al*. 1997). SLC18A2 prevents the inhibition of oxidative phosphorylation in mitochondria (and apoptosis), for example, caused by the active metabolite N-methyl-4-phenylpyridinium (MPP^+) of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Guillot & Miller 2009; Chaudhry *et al*. 2007; Staal & Sonsalla 2000). VMAT2 is therefore not only important for the controlled storage and release of monoamines but also for the prevention of cytosolic toxicity (Lohr *et al*. 2015). Interestingly, monoamine receptors can influence the expression of VMAT2 and *vice versa* (Guillot & Miller 2009; Fleckenstein *et al*. 2009). In addition, the function of VMAT2 is influenced by cytosolic neurotransmitter levels (Eiden & Weihe 2011). Pharmacological agents that increase the activity of the SLC18A2 (indirectly), such as pramipexole or apomorphine, are neuroprotective (Chaudhry *et al*. 2007). Analogously, inhibitors of VMAT2, such as the reserpine, a formerly used antihypertensive drug, and tetrabenazine, utilized in the treatment of hyperkinetic disorders (for example Huntington's disease), which bind to distinct sites, as well as many illicit drugs, cause, through the increase of cytosolic catecholamines, oxidative stress, leading to neurotoxic effects in catecholaminergic neurons (Wimalasena 2011; Guillot & Miller 2009; Zheng *et al*. 2006). Amphetamine-like drugs, such as methamphetamine or MDMA, inhibit VMAT2 and increase the concentration of catecholamines intra- and extracellularly (Guillot & Miller 2009). Still, other researchers argue that amphetamines, in fact, do not cause the inhibition of VMAT2 but rather act as releasing agents (Freyberg *et al*. 2016). A long-term effect of drug abuse is the reduction of VMAT2 expression (Narendran *et al*. 2012). This mechanism seems to be drug specific, with having been proven for cocaine users and also, while still debated, seeming likely for methamphetamine consumers (Narendran *et al*. 2012; Eiden & Weihe 2011; Fleckenstein

et al. 2009). Lohr *et al*. (2015) have demonstrated that mice overexpressing SLC18A2 are protected from methamphetamine caused neurotoxicity. The increase of VMAT2 availability might be a future therapeutic angle to consider in the development of a treatment for psychostimulant abuse and Parkinson's disease (Zheng *et al*. 2006). Reduced VMAT2 function is a consequence of Parkinson's disease and Alzheimer's disease and has been used as a marker to monitor the diseases' progression (Lawal & Krantz 2013; Wimalasena 2011; Chaudry *et al*. 2007; Chen *et al*. 2008; Miller *et al*. 1999)[3]. A hypermethylated SLC18A2 promoter region has been discussed as an indicator of prostate cancer (Haldrup *et al*. 2016). Various pesticides and environmental toxins inhibit VMAT2-mediated neurotransmitter uptake (Lawal & Krantz 2013). The endogenous ligands of the VMAT2 are 5-HT, dopamine, (nor-)epinephrine and histamine, listed by their binding affinities in descending order (Lawal & Krantz 2013).

Physiologically, after the synthesis, or (re)uptake, of monoamine neurotransmitters, VMAT2 (SLC18A2) aids the storage of monoamines into intracellular vesicles to avoid the neurotoxic effects of free cytosolic monoamines (Lin *et al*. 2010; Pifl *et al*. 2015). After an action potential reaches the presynaptic neuron and causes the influx of Ca^{2+}, VMAT2 mediates the process of releasing neurotransmitters from the intracellular vesicles into the synaptic cleft via exocytosis (Watson *et al*. 2006). The synaptic vesicles are retrieved and recycled via three distinct mechanisms: kiss and stay, kiss and run and clathrin-mediated endocytosis (Südhof & Rizo 2011; Egana *et al*. 2009). The monoamine release-caused chemical signal can be terminated via enzymatic degradation, diffusion (which is currently greatly debated – e.g. Matsson *et al*. 2015) or reuptake through neurotransmitter transporters (Watson *et al*. 2006). The subfamily of monoamine transporters (SLC6A2, SLC6A3 and SLC6A4) oversees the presynaptic reuptake of the monoamine neurotransmitters norepinephrine, dopamine and serotonin (Grouleff *et al*. 2015). The following VMAT2-mediated uptake into vesicles allows for the described transmitter cycle to start anew as soon as the next action potential is registered (Sitte & Freissmuth 2015). SLC18A2 mediates uptake not only into secretory synaptic vesicles but also large dense core vesicles where monoamines are stored until release (Grygoruk *et al*. 2014; Zhang *et al*. 2011; Fei *et al*. 2008; Bruns & Jahn 1997; Nirenberg *et al*. 1995). It has, additionally, been shown for DAT that the synaptic vesicle protein synaptogyrin-3, VMAT2 and the monoamine transporter form a spatially proximate complex that facilitates the ability to efficiently replenish synaptic vesicles (Egana *et al*.

[3] For a list of imaging agents and radioligands used for disease progression monitoring see Wimalasena (2011).

2009). Monoamine transporters are among the most common targets for illicit, new psychoactive, substances (NPS) (Kristensen *et al*. 2011). Amphetamines and amphetamine-type stimulants (ATS), such as 4,4'-DMAR, have to be substrates of monoamine transporters *and* the VMAT2 in order to exert their effects (Zheng *et al*. 2006; Chaudhry *et al*. 2007; Freyberg *et al*. 2016).

Amphetamine-type stimulants, a subfamily of the NPS, including methamphetamine, cathinones and MDMA (3,4-Methylenedioxymethamphetamine), have the second highest number of drug users worldwide, only preceded by the number of cannabis consumers, and are, after opioids, the second most frequent cause of diseases due to substance abuse (United Nations 2017). The historical, chemical, definition of amphetamines by Biel and Bopp (1978) is faced with the challenge that many compounds violate those initially defined criteria. Furthermore, the NPS market is innovative concerning the development of chemical substitutions or subtle changes of compounds to bypass legislative obstacles (McLaughlin *et al*. 2015; Sitte & Freissmuth 2015). The WHO (2017), basing their definition on the challenged chemical criteria, defines ATS as "amphetamine-type derivative[s] with hallucinogenic properties". Other definitions based on the mechanism of action of ATS face similar problems. While it is characteristic of ATS that they are releasing agents at NET, DAT and many also at SERT, with the most pronounced effects generally being caused at the first two transporters, some (e.g. methylphenidate) are inhibitors of reuptake and others (e.g. MDMA) show stronger effects at SERT than other ATS (Sitte & Freissmuth 2015; Rothman *et al*. 2001).[4] While ATS, in the form of plant-based products, have been around for millennia, the phase of synthetic amphetamines commenced around the beginning of the 20th century (Sulzer *et al*. 2005). Still, amphetamines are not only consumed as illicit substances but also used in the treatment of narcolepsy and as treatment for attention deficit hyperactivity disorder (Heal *et al*. 2013; Seiden *et al*. 1993).

Amphetamine-type stimulants compete with endogenous substrate intra- and extracellularly, i.e. they inhibit monoamine reuptake presynaptically and compete with substrate intracellularly for the transporter's primary binding site (Sitte & Freissmuth 2015). ATS and monoamines are similar in structure and molecular weight, which allows for the uptake of ATS via sodium (and chloride) symport into the cell (Heal *et al*. 2013; Rudnick & Clark 1993). This causes an increase in intracellular sodium levels and uncouples the physiological ion gradient, leading to augmented

[4] For a possible solution of the conundrum see Wittgenstein's concept of family resemblance (1986).

levels of neurotransmitter being transported outwardly (Sitte & Freissmuth 2015; Sitte & Freissmuth 2010). Transporter-mediated currents can cause the depolarization of the cell which has implications for the neuron's excitability (Baumann *et al*. 2014)[5]. ATS cause the inhibition of MAO, hindering the degradation of the released cytosolic monoamines (Sitte & Freissmuth 2015, Seiden *et al*. 1993). One recent study suggests that, contrary to the previous scientific consensus, ATS do not primarily inhibit VMAT2 but rather act as releasing agents that cause an accumulation of cytosolic monoamines (Freyberg *et al*. 2016). They, therefore, induce efflux of neurotransmitter via reverse transport and inhibit the reuptake of monoamines accumulated in the synaptic cleft, with both mechanisms, in conjunction, leading to an increase in synaptic monoamine concentrations and the activation of pre- and postsynaptic receptors (Sitte & Freissmuth 2015). The mechanism of reverse transport can be conceptualized building on the aforementioned alternating access model, that is facilitated through conformational changes caused, for example, by the phosphorylation of specific amino acids or oligomerization of the transporter (Bermingham & Blakely 2016; Sitte & Freissmuth 2015; Anderluh *et al*. 2014; Sager & Torres 2011; Sucic *et al*. 2010). With high intracellular substrate levels, the oligomeric transporters seesaw through inward-facing, occluded and outward-facing confirmations, releasing substrate in continuous and burst-like movements (Sitte & Freissmuth 2015; Kahlig *et al*. 2005).

Many amphetamine-type substances not only interact with SLC family members (e.g. in addition to the aforementioned: SLC22A3, SLC1A1, …) and other transporters (e.g. OCT3 [organic cation transporter 3] – Mayer *et al*. 2018) but also bind to receptors, such as, for example, various 5-HT, dopamine, histamine or adrenergic receptor subtypes (depending on the substance) and the, intracellularly located, $G_{s/q}$ coupled, trace amine associated receptor 1 (TAAR1) (Lam *et al*. 2015; Sitte & Freissmuth 2015; Underhill *et al*. 2014; Miller 2011; Zhu *et al*. 2010; Battaglia *et al*. 1988)[6]. Apart from trace amines, e.g. tryptamine, tyramine, octopamine and β- phenylethylamine, amphetamine and MDMA also bind to TAAR1 (Lam *et al*. 2015; Di Cara *et al*. 2011; Zucchi *et al*. 2006; Borowsky *et al*. 2001). While the exact mechanism of action of TAAR1 has not been completely illuminated yet, Revel *et al*. (2012) uncovered that TAAR1 partial agonists, in the presence of a full agonist (and therefore acting as a competitive antagonist), cause an increase in the firing frequency of serotonergic and dopaminergic neurons, while full agonists cause a decrease

[5] This ATS-caused depolarisation also seems to activate voltage-gated Ca^{2+} channels (Cameron *et al*. 2015).
[6] For a review of ATS' interaction with CART (cocaine- and amphetamine-regulated transcript) peptides see Vicentic & Jones (2007).

in firing frequency. Most investigators attribute inhibitory effects to the activation of the trace amine associated receptor 1 and report a TAAR1-caused decrease in dopamine and serotonin release (Revel *et al*. 2012; Di Cara *et al*. 2011; Lindemann *et al*. 2008)[7]. Activation of the receptor is therefore promoting neuroprotective effects and might also be a potential target in the treatment of ATS addiction (Miner *et al*. 2017; Sitte & Freissmuth 2015). Even though various antagonistic and agonistic therapies for ATS withdrawal have been investigated, none has, so far, been proven effective (Brensilver *et al*. 2013; Rothman & Baumann 2006). It is remarked that it might be necessary to combine anti-addictive drugs and behavioural therapy with neuroprotective agents (Yu *et al*. 2015; Rawson *et al*. 2010; Krasnova & Cadet 2009; Baldwin *et al*. 1993; Chuang 2004). In the short term, induced hypothermia might also be a possible neuroprotective measure (Garg *et al*. 2015; Yu *et al*. 2015; Krasnova & Cadet 2009). For the medical treatment of acute ATS overdoses see Rawson *et al*. (2010) and Schifano *et al*. (2015).

Drug users consider the euphoric, hallucinogenic and energising effects, as well as reduced fatigue, enhanced mental and physical abilities and increased self-confidence and sexual drive to be positive consequences of the consumption of ATS (Soussan & Kjellgren 2016; Rawson *et al*. 2010; Krasnova & Cadet 2009). Some researchers point out that MDMA, for example, also enhances prosocial behaviour and emotional empathy (Hysek *et al*. 2014; Schmid *et al*. 2014). Repeated consumption of the amphetamine-type stimulant methamphetamine increases the risk of Parkinson's disease and having a stroke, causes reduced memory and motor function, stereotypic movement, oral and skin diseases, as well as addiction, psychosis and depression (Callaghan *et al*. 2012; Rawson *et al*. 2010; Krasnova & Cadet 2009; Robinson & Becker 1985; Segal & Mandell 1974). Cardiopulmonary symptoms and diseases, hyperthermia, bruxism, insomnia, formication and agitation are among the acute effects of drug consumption (Rawson *et al*. 2010; Estler 1975). These side effects are not unique to methamphetamine but are characteristic of many ATS (Martin *et al.* 1971). MDMA, compared to other ATS, is a more efficacious releaser at SERT and can also cause serotonin syndrome (Greenier *et al*. 2014; Parrott 2002).

Considering long-term neurotoxic effects of ATS, it becomes evident that the substances subsumed under the umbrella term are quite heterogenous. Methamphetamine has been proven to be neurotoxic for humans by causing degenerative processes, decreasing the expression of monoamine

[7] On the other hand, Miller (2011) describes an increase in dopamine efflux due to TAAR1 activation.

transporters at dopaminergic and, to a lesser extent, serotonergic presynaptic terminals (Yu *et al*. 2015; Kitamura 2009; Krasnova & Cadet 2009; Nash & Yamamoto 1992)[8]. It is pointed out that inhibition of VMAT2 is highly likely to play a role in the development of ATS-related neurotoxic effects because it is followed by an increase of cytosolic monoamines causing oxidative stress (Lohr *et al*. 2015; Pifl *et al*. 2015; Wimalasena 2011; Lin *et al*. 2010; Guillot & Miller 2009; Krasnova & Cadet 2009). Various hypotheses concerning other possible mechanisms of toxicity have been described in great detail elsewhere (Yu *et al*. 2015; Krasnova & Cadet 2009). The neurotoxicity of MDMA is, on the other hand, more heavily debated. Although not undisputed, many animal studies (with mice, rats and monkeys) have proven neurotoxic effects at serotonergic axons, however, the results are more ambiguous concerning human MDMA usage (Halpin *et al*. 2016; Mueller *et al*. 2016; Garg *et al*. 2015; Carvalho *et al*. 2012; Baumann *et al*. 2007; Verrico *et al*. 2007; de la Torre *et al*. 2004; Lyles & Cadet 2003; Turner & Parrott 2000). Human data, mostly building on fMRI (functional magnetic resonance imaging) examinations, seems to suggest that moderate MDMA use (<50 lifetime consumption episodes or <100 tablets consumed) does not significantly correlate with neuronal changes, whereas heavy use does (Mueller *et al*. 2016; Garg *et al*. 2015). Still, comparisons across studies are not unproblematic due to differences in experiment design and statistical analyses, as well as their (lack of) accounting for poly-drug consumption (Miner *et al*. 2017; Mueller *et al*. 2016; Garg *et al*. 2015; Turner & Parrott 2000). There does not yet exist a clear-cut answer concerning MDMA's potential neurotoxicity because the collected human data is not completely conclusive and there are limitations concerning extrapolations from data gained from animal models to humans (Carvalho *et al*. 2012; de la Torre & Farré, 2004). The application of animal models and extrapolation of the obtained results to humans unveils one paramount problem, which is the difference in pharmacokinetic (ADME) properties that drugs exhibit between species. One uncertainty factor is, for example, the difference in metabolic degradation between species, e.g. monkeys seem to metabolise methamphetamine (but not MDMA), in contrast to rats, similarly to humans (Carvalho *et al*. 2012; Krasnova & Cadet 2009; Baumann *et al*. 2007; de la Torre & Farré 2004). Another difficulty related to the pharmacodynamic effects is the challenge of mimicking human, gradually escalating, drug use in the animal model with respect to the patterns of drug administration and their effects on the

[8] Dopamine receptors might play a modulating role in methamphetamine toxicity (Yu *et al*. 2015; Krasnova 2009). As Fei *et al*. (2008) point out, dopamine receptors might, with ATS present, cause a decrease in localization of VMAT2 to vesicles and therefore inhibit VMAT2 function.

organism (Krasnova & Cadet 2009). The chemically and pharmacologically related but heterogenous group of ATS displays similar effects that, in their severity, depend on the consumed substance and dosage, duration of consumption and combination with other substances. All ATS can cause various short-term complications and some exhibit neurotoxic effects in long-term usage.

Many ATS, such as 4-4'-DMAR, are labelled new psychoactive substance (NPS), the "new" standing for new on the market and not necessarily newly synthesised. The UNODC (2017), the United Nations Office on Drugs and Crime, and the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA 2017) define NPS as possibly hazardous, illicit, narcotic or psychotropic substances not listed as controlled substances by the United Nations drug conventions of 1961 and 1971[9]. The compound 4,4'-DMAR is, for example, deduced from a library of aminorex derivatives that were tested for their potential as anorectic medications in the early 1960s (Henderson *et al*. 1995). One major problem with NPS is the fact that they are often being sold without exact knowledge of their mode of action and their toxicological properties – indicated by many users referring to them as "research chemicals", in the sense that they embody the consumers, test subjects and researchers as one person (Mayer *et al*. 2016a)[10]. The lack of knowledge about new psychoactive substance's effects, potency and toxicity also contributed to the many deaths caused by 4,4'-Dimethylaminorex and led to the inception of this thesis, wanting to investigate 4,4'-DMAR's mechanism of action and explain its lethal effects. The main research question was: "How does 4,4'-Dimethylaminorex interact with the human monoamine transporters SLC6A2 (NET), SLC6A3 (DAT) and SLC6A4 (SERT), as well as the human vesicular monoamine transporter SLC18A2 (VMAT2)?". Therefore, the null hypothesis was that 4,4'-DMAR does not cause any effects at the aforementioned transporters, with the alternative hypothesis being that it does cause an effect. If one could reject the null hypothesis, it would then be interesting to examine the nature of the effect (inhibition or release) and then, as a final step, explain the substance's (side-)effects with its mode of action.

[9] These definitions are widely accepted but also quite often criticised due to their ambiguity. For a different taxonomy see Measham & Newcombe (2017).

[10] The website "reddit.com" contains a forum (open to the public), where drug users can discuss their insights, usage patterns, motivations, etc. (Reddit 2017). This allows for researchers to get an insider's perspective.

Methods

Cell culture

Human embryonic kidney (HEK293) cells, used for uptake inhibition and superfusion release assays, were maintained in a humidified atmosphere (37°C and 5% CO_2) at a subconfluential state in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat-inactivated Fetal Bovine Serum (FBS), penicillin (100 U x 100 mL^{-1}) and streptomycin (100 µg x 100 mL^{-1}). Geneticin (50 µg x mL^{-1}) was added to maintain the selection process. The generation of stable, monoclonal cell lines has been described elsewhere (Mayer *et al.* 2016a). Twenty-four hours prior to uptake inhibition experiments, HEK293 cells expressing the desired transporter, were seeded at a density of 40,000 cells per well onto poly- D-lysine (PDL) coated 96-well plates in a final volume of 200 µL per well. Analogously, 24 hours before release experiments, 40,000 cells per well were seeded onto poly- D-lysine coated glass coverslips (5 mm in diameter), that have been placed into a 96-well plate for a final volume of 200 µL per well (Mayer *et al.* 2016a).

Cells used for receptor and transporter binding assays and receptor activation assays, were cultured and prepared as recently described in detail (Luethi *et al*. 2017). For membrane preparations, the cells were harvested following application of trypsin/ethylenediaminetetraacetic acid (EDTA), washed with ice-cold PBS, pelleted via centrifugation (1000 rpm for 5 minutes at 4°C), frozen and stored at -80°C. The frozen pellets were then suspended in 20 mL (receptor binding) or 400 mL (transporter binding) HEPES-NaOH (20 mM, pH 7.4) containing 10 mM EDTA. After homogenization at 14,000 rpm for 20 seconds (receptor binding) or 10,000 rpm for 15 seconds (transporter binding), the homogenates were centrifuged at 48,000 g and 4°C for 30 minutes. For receptor binding assays, supernatants were discarded and the pellets resuspended in 20 ml HEPES-NaOH (20 mM, pH 7.4) containing 0.1 mM EDTA and homogenised at 14,000 rpm for 20 seconds. The centrifugation and removal of the supernatant was repeated, and the final pellet was resuspended in HEPES-NaOH that contained 0.1 mM EDTA and homogenized. The following transfected cell lines were used for the binding assays: HEK293 cells (5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, TAAR1, D_2, hDAT, hNET, and hSERT), Chinese hamster ovary (CHO) cells (α_{1A}), and Chinese hamster lung (CHL) cells (α_{2A}).

Rat pheochromocytoma cells (rPC12), used for VMAT2 uptake inhibition assays, were grown in PDL-coated cell culture dishes (10 cm diameter) in Opti-MEM (Gibco), supplemented with 5% Fetal Bovine Serum (FBS) and 10% horse serum, penicillin (100 U x 100 mL^{-1}) and streptomycin

(100 µg x 100 mL^{-1}). For VMAT2-assays, the cells were seeded at 40,000 cells per well onto PDL coated 96-well plates in a final volume of 200 µL per well 24 hours beforehand.

The human striatal tissue was derived from autopsied frozen half brains of subjects without evidence of any neurological or psychiatric disorder in their records as described earlier (Pifl *et al.* 2014). In brief, the brains were split into two hemispheres by a midsagittal cut. One half was utilized for neuropathological examination and the other half was frozen and later thawed and cut by hand into 3 to 5 mm thick slices at the beginning of the experiments (Pifl *et al.* 2014).

Experimental protocols and design

Uptake inhibition assays

For uptake inhibition experiments, DMEM was removed from the cell culture dishes and replaced with Krebs-HEPES-buffer (KHB; 25 mM HEPES, 120 mM NaCl, 5 mM KCl, 1.2 mM CaCl2, and 1.2 mM MgSO4 and 5 mM D-glucose, pH adjusted to 7.3) at a final volume of 200 µL per well. The cells were exposed to increasing concentrations of 4,4'-DMAR, diluted in KHB, and 3,4-methylendioxymethylamphetamine (MDMA), diluted in Milli-Q H2O, at a final volume of 200 µL per well for 10 minutes to ensure equilibrated conditions. This was the case for hSERT, hDAT, hNET transfected HEK cells as well as rat GAT1 (SLC6A1), expressed in HEK293 cells. For the 4,4'-DMAR experiments, tritiated substrate (0.2 µM [3H]5-HT for hSERT, 0.01 µM [3H]MPP+ for hDAT and hNET, 0.15 µM [3H]GABA for rGAT1) was added after ten minutes. Tritiated substrate utilized in the MDMA uptake inhibition assays was 0.2 µM [3H]5-HT for hSERT, 0,2µM [3H]DOP for hDAT and 0,015µM [3H]MPP+ for hNET. Uptake was terminated after 60 seconds for hSERT and 180 seconds for hDAT, hNET and rGAT1 by removing the tritiated substrate and washing the cells with 200 µL of ice-cold KHB. Afterwards, the cells were lysed in 100 µL 1% sodium dodecyl sulfate (SDS) per well. Uptake of tritiated substrate was determined with a beta-scintillation counter (Perkin Elmer, Waltham, MA, USA). In the 4,4'-DMAR assays, non-specific uptake was assessed in the presence of 10 µM paroxetine for SERT, 10 µM mazindole for hDAT, 10 µM nisoxetine for NET and 10 µM tiagabine for rGAT1. For the MDMA assays, non-specific uptake was assessed in presence of 100 µM paroxetine for hSERT, 30 µM cocaine for hDAT and 1000 µM cocaine/methylendioxypyrovaleron (MDPV) for hNET. Non-specific uptake was subtracted from the data to yield specific uptake values. Uptake in the absence of test drugs was defined as 100% and uptake in the presence of drugs was expressed as a percentage thereof. The half maximal inhibitory concentration was determined by non-linear regression fits according to

the equation: [Y=Bottom + (Top-Bottom)/(1+10^[(X-LogEC50])*HillSlope))] (Mayer *et al*. 2016a). rGAT1 expressing HEK293 cells were used as a negative control because amphetamines and amphetamine-type substances display no activity at GAT1 at pharmacologically relevant concentrations (Seidel *et al*. 2005).

Uptake into permeabilized PC12 cells attached to PDL-coated 96-well plates was measured as described earlier (Nakanishi *et al*. 1995), with minor modifications. PC12 cells were pre-incubated with 150 µM digitonin for 15 minutes at room temperature to permeabilize cell membranes. Subsequently, the cells were washed with HTMS buffer (50 mM Hepes-Tris buffer, 6 mM $MgCl_2$, 0.32 M sucrose, 2 mM ATP, pH 7.4 – adjusted with $MgOH_2$) (Nakanishi *et al*. 1995). Hundred nM nisoxetine and 100 nM mazindole were added to the buffer to inhibit DAT and NET. The cells were exposed to increasing concentrations of 4,4'-DMAR, MDMA or reserpine, diluted in HTMS buffer, at a final volume of 200 µL per well for 5 minutes. Subsequently, tritiated substrate (0.1 µM [^{3}H]5-HT) was added. Uptake was terminated after 15 minutes by aspirating the tritiated substrate and washing the cells with 200 µL ice-cold HTMS buffer. Afterwards, the cells were lysed with 100 µL 1% SDS per well. This solution was then transferred into counting vials, containing 2 mL scintillation cocktail. Uptake was determined with a beta-scintillation counter (Perkin Elmer, Waltham, MA, USA). Non-specific uptake was determined in presence of 1 µM reserpine and subtracted from the data to yield specific uptake values. Uptake in the absence of test drugs was defined as 100% and uptake in the presence of drugs was expressed as a percentage of control uptake. The half maximal inhibitory concentration was determined as mentioned above.

To evaluate VMAT2 uptake inhibition in human striatal tissue, seven samples were derived from autopsied frozen half brains. The specimens originated from voluntary body donations (6 females, 1 male, aged 87 ± 8 years) to the Center of Anatomy and Cell Biology, Medical University of Vienna, and were required to show no evidence of any neurological or psychiatric disorder in their records. The procedure has been described in more detail before (Pifl *et al*. 2014) but shall be briefly explained. Six-hundred mg of striatal tissue were homogenised in ice-cold 0.3 M sucrose containing 25 mM Tris (pH 7.4) and 10 µM pargyline at 4°C in a Teflon Potter-type homogenizer. Following homogenisation, the sample was centrifuged at 10,000 g for 15 minutes, resulting in a pellet (P2). The supernatant was centrifuged at 20,000 g for 30 minutes. Deionised H_2O was added to the P2 pallet, causing osmotic shock. The P2 sample was then centrifuged at 22,000 g for 15 minutes and 1.3 M potassium phosphate buffer in 1/10 of the volume (pH 7.4) was added. The

aforementioned supernatant was centrifuged at 100,000 g for 30 minutes with the resulting pellet being suspended in the osmotically shocked P2 pellet supernatant. Therefore, vesicles in the supernatants of P2-pellets and in H_2O-lysates of P2-pellets were combined again and stored at -80°C until uptake analysis (Pifl *et al.* 2014).

Transporter release assays

Dynamic transporter release assays allow for the assessment of monoamine-transporter-mediated reverse transport and avoid reuptake or retrograde diffusion of tritiated substrates by using a constant flow rate that causes the clearance of released substances (Pifl *et al.* 1995; Steinkellner *et al*. 2016; Mayer *et al*. 2016a). In brief, transporter-expressing cells are grown on glass-coverslips and pre-loaded with tritiated substrate by exposing the cells to 0.4 µM [^{3}H]5-HT (hSERT) 0.1 µM [^{3}H]MPP$^+$ (hDAT), 0.05 µM [^{3}H]MPP$^+$ (hNET) or 0.1 µM [^{3}H]GABA (rGAT1), respectively, for 20 minutes at 37°C. Subsequently, the cells are transferred into small chambers and superfused with KHB (0.7 mL min^{-1}). The superfusates are collected in counting vials (10 mL), containing 2 mL scintillation cocktail. After a cycle of two minutes, the next set of vials is automatically filled. The tubes delivering the drug-containing buffer are submerged in a water bath with a constant temperature of 25°C. To establish a stable basal release, the cells were superfused for 40 minutes before the collection of 2-minute fractions was initiated. At first, three basal fractions were collected before the cells were exposed to monensin (10 µM) or solvent for four fractions. The Na^+/H^+ ionophore monensin was chosen because it disrupts the pre-existing sodium gradient. The examined transporters all belong to the neurotransmitter-sodium-symporter SLC family (NSS). Thus, a dissipated sodium gradient selectively augments efflux triggered by substrates (Sitte & Freissmuth 2015). Afterwards, the cells were exposed to 10 µM 4,4'-DMAR or to a control substance, known to act as transportable substrate of the respective transporter, for five fractions. Three µM *para*-chloramphetamine (PCA) was used for hSERT-expressing cells, 10 µM amphetamine for hDAT and hNET and 100 µM GABA for rGAT1-expressing cells. Finally, the cells were lysed with 1% SDS. Afterwards the amount of tritiated substrate present in each vial was determined by a beta-scintillation counter (Perkin Elmer, Waltham, MA, USA). Efflux of tritium was expressed as a fractional rate, i.e. the radioactivity released during a fraction was expressed as the percentage of the total radioactivity present in the cells at the beginning of that fraction.

Receptor and transporter binding and activation assays

Receptor and transporter binding affinities were determined as described earlier in detail for each receptor and transporter (Luethi *et al*. 2017). In brief, membrane preparations overexpressing the respective receptors or transporters (human genes, with the exception of rat and mouse genes for TAAR1) were incubated with radiolabelled selective ligands at concentrations equal to K_d, and ligand displacement by the compounds was measured. Specific binding was determined as the difference between total binding (binding buffer alone) and nonspecific binding (in the presence of specific competitors). The radioligands and competitors utilised to determine nonspecific binding are summarised in Table 1.

Receptor	**rTAAR1**	**mTAAR1**	**5-HT$_{1A}$**	**5-HT$_{2A}$**	**5-HT$_{2C}$**
Radioligand	[^{3}H]RO5166017	[^{3}H]RO5166017	[^{3}H]-8-OH-DPAT	[^{3}H]Ketanserin	[^{3}H] Mesulergine
Concentration	3.5 nM	2.4 nM	0.9 nM	0.4 nM	1.4 nM
Non-specific binding	10 µM RO5166017	10 µM RO5166017	10 µM pindolol	10 µM spiperone	10 µM mianserin
K$_d$	2.8 nM	2.0 nM	1.39 nM	0.45 nM	1.6 nM
Receptor/ transporter	**α$_{1A}$**	**α$_{2A}$**	**D$_2$**	**hDAT**	**hNET**	**hSERT**
Radioligand	[^{3}H]Prazosin	[^{3}H]Rauwolscine	[^{3}H]Spiperone	[^{3}H]WIN3 5,428	*N*-methyl-[^{3}H]Nisoxetine	[^{3}H]Citalopram
Concentration	0.106 nM	2.0 nM	1.16 nM	3.3 nM	*2.9 nM*	1.5 nM
Non-specific binding	10 µM chlor-promazine	10 µM phentol-amine	10 µM spiperone	10 µM indatraline	10 µM indatraline	10 µM indatraline
K$_d$	0.044 nM	2.0 nM	0.26 nM	30 nM	37 nM	20 nM

Table 1: Receptors and transporters with their respective radioligands and non-specific binding determining substances, as used for radioligand binding assays.

The compounds were diluted in binding assay buffer (50 mM Tris/HCl, 10 mM MgCl2, 1 mM EGTA, pH 7.4). The concentrations tested ranged from 30 pM – 30 µM. Membrane suspension radioligand and test compounds were added to the microplates (Greiner, 96-well) at a final volume of 200 µL per well, incubated and shaken for 30 minutes at room temperature. The binding reaction was terminated by rapid filtration, using Unifilter-96 plates and pre-soaked GF/C glass filters

(incubated for 1 h in 0.3% polyethylenimine, washed with ice-cold washing buffer [50 mM Tris/HCl, pH 7.4]). Afterwards, scintillation cocktail (45 µL/well) was added and the plates were sealed. One hour later, radioactivity was determined by a Microplate Scintillation (Packard Instrument Company).

FLIPR assays were conducted as previously described (Luethi *et al*. 2017). In brief, HEK293 cells that expressed the human 5-HT_{2B} receptor were incubated in PDL-coated 96-well plates overnight. The growth medium was then removed by snap inversion, and 100 µl of calcium indicator Fluo-4 solution (Molecular Probes, Eugene, OR, USA) was added to each well. The plates were incubated for 45 minutes at 31 °C, the Fluo-4 solution was removed by snap inversion, and 100 µL of Fluo-4 solution was added a second time for 45 minutes at 31 °C. The cells were then washed and 100 µL assay buffer was added. Thereafter, the plates were placed in a fluorescence imaging plate reader (FLIPR), and 25 µL of the test substances diluted in assay buffer was added online. The increase in fluorescence was measured, and EC_{50} values were derived from the concentration-response curves using nonlinear regression. IC_{50} values were calculated by use of nonlinear regression curves for one-site models. Ki values were determined via the Cheng-Prusoff equation (Ki = IC_{50} / [1 + {radioligand concentration / Kd}]). The experiments were conducted as concentration-response curves covering 10 individual concentrations.

Data and statistical analysis

$IC_{50,}$ EC_{50} and AUC values were calculated and plotted with Microsoft Excel® 2010 (Microsoft Corporation, Redmond, WA, USA) and GraphPad Prism 7.03 (GraphPad Software Inc., San Diego, CA, U.S.A.), respectively. Transporter ratios were calculated as (1/numerator IC_{50}) divided by (1/denominator IC_{50}), e.g. the DAT/SERT ratio is expressed as (1/DAT IC_{50}) divided by (1/SERT IC_{50}) with higher values indicating greater selectivity for DAT. Release of preloaded tritiated substrate in the presence or absence of monensin was analysed by repeated measures two-way ANOVA (treatment x time) and Šidák's test. AUC data was also compared between groups by making use of the Mann-Whitney test. All results are expressed as mean ± SEM. *P* values less than 0.05 were considered significant. Parameters investigated are portrayed in Table 2 below. In these prospective, controlled laboratory experiments, the concentration of 4,4'-DMAR, and various control substances, was manipulated over time and the subsequently caused change in the amount of uptake or release of tritiated substrate was then measured.

Variable	Category	Type of	variable
Concentration of substances	log [conc] M	metric	independent
Monensin	Yes / No	nominal	independent
Time	in s/min	metric	independent
Uptake/release of tritiated substrate	µM	metric	dependent

Table 2: The investigated parameters.

Materials

(±)-*cis*-4,4'-DMAR hydrochloride was available from previous studies (Brandt *et al*. 2014). Reagents used in the experiments for uptake inhibition and release in HEK293 cells were used as described in Hofmaier *et al*. (2014). For uptake and release experiments [^{3}H]1-methyl-4-phenylpyridinium ([^{3}H]MPP$^+$; 80–85 µCi x mmol^{-1}) was purchased from American Radiolabeled Chemicals (St. Louis, MO, USA). [^{3}H]Dopamine (55 µCi x mmol^{-1}), [^{3}H]GABA (89,7 µCi x mmol^{-1}), [^{3}H]5-HT (28.3 µCi x mmol^{-1}), [^{3}H]8-OH-DPAT, [^{3}H]ketanserin, [^{3}H]mesulergine, [^{3}H]prazosin, [^{3}H]rauwolscine, [^{3}H]spiperone, N-methyl-[^{3}H]nisoxetine, [^{3}H]WIN35,428, and [^{3}H]citalopram were all from Perkin Elmer (Boston, MA, USA). [^{3}H]RO5166017 and RO5166017 were provided from F. Hoffmann-La Roche (Basel, Switzerland). All other chemicals and cell culture supplies were ordered from Sigma-Aldrich (St. Louis, MO, USA) with the exception of cell culture dishes, which were obtained from Sarstedt (Nümbrecht, Germany).

Results

4,4'-DMAR binds to monoamine transporters

As shown in Table 3, 4,4'-DMAR binds to the monoamine transporters DAT, NET and SERT with significantly higher binding affinities when compared to monoamine receptors. One-way ANOVA (Tukey's multiple comparisons test) revealed that the K_i difference between $5HT_{2A}$ and $5HT_{2C}$ and the monoamine transporters is significant (P <0.0001) but the differences between the transporters' K_i values are only significant when NET and SERT are compared (P <0.05 [a comparison of DAT and SERT yields a P value of 0,053]). 4,4'-DMAR did not bind to rTAAR, mTAAR, $5HT_{1A}$, α_{1A}, α_{2A} and D_2 at the tested concentrations. 4,4'-DMAR activated $5HT_{2A}$ and $5HT_{2C}$ at higher concentrations with K_i values between 7.5 and 11.66 µM. I could therefore reject the null hypothesis and explore 4,4'-DMAR's interaction with monoamine transporters in more detail.

Receptor	**rTAAR1**	**mTAAR1**	**5-HT$_{1A}$**	**5-HT$_{2A}$**	**5-HT$_{2B}$**	**5-HT$_{2C}$**
	Receptor binding	Receptor binding	Receptor binding	Receptor binding	Activation potency	Receptor binding
	K_i ± SD [nM]	K_i ± SD [nM]	K_i ± SD [nM]	K_i ± SD [nM]	EC_{50} ± SD [nM]	K_i ± SD [nM]
	>5010	>4740	>17,400	8846 ± 862.6	>10,000	11,068 ± 561.1
Receptor/ Transporter	**α$_{1A}$**	**α$_{2A}$**	**D$_2$**	**hDAT**	**hNET**	**hSERT**
	Receptor binding	Receptor binding	Receptor binding	Transporter binding	Transporter binding	Transporter binding
	K_i ± SD [nM]	K_i ± SD [nM]	K_i ± SD [nM]	K_i ± SD [nM]	K_i ± SD [nM]	K_i ± SD [nM]
	>2120	>4970	>13,500	533.8 ± 44.2	266.8 ± 57	1881 ± 183.1

Table 3: Receptor and transporter binding affinities and 5-HT_{2B} activation potencies (as determined by FLIPR assay). K_i and EC_{50} values are given in nM (mean ± SD).

4,4'-DMAR inhibits transporter-mediated uptake in HEK293 cells

Uptake inhibition experiments were conducted to test how 4,4'-DMAR interacts with human monoamine transporters (i.e., hSERT, hDAT, hNET) and rGAT1, expressed in HEK293 cells. As shown in Figure 1, 4,4'-DMAR is a fully efficacious inhibitor of uptake mediated by hDAT, hNET and hSERT. No inhibitory effect could be observed in rGAT1-expressing cells. 4,4'-DMAR inhibited hSERT, hDAT and hNET with equal potency with IC_{50} values in the low micromolar range (hSERT = 1.75 µM – 95 % CI: 1.446 to 2.126; hDAT = 1.04 µM – 95 % CI: 0.848 to 1.282; hNET = 0.50 µM – 95 % CI: 0.447 to 0.553). Calculated ratios emphasize 4,4'-DMAR's low selectivity for one transporter over another (DAT/SERT ratio: 1.68; NET/SERT ratio: 3.50; DAT/NET ratio: 0.48). In contrast, even the highest concentration of 4,4'-DMAR tested (1000 µM) failed to achieve half-maximal inhibition of rGAT1-mediated uptake. As a reference comparator compound, I made use of MDMA because of its similar profile of action. MDMA inhibited hSERT (IC_{50} = 16.95 µM – 95 % CI: 13.41 to 21.43), hDAT (IC_{50} =17.62 µM – 95 % CI: 13.91 to 22.31) and hNET (IC_{50} = 4.57 µM – 95 % CI: 2.93 to 7.28). I did not conduct uptake inhibition experiments in rGAT1-expressing cells because our laboratory has, in the same cell line, shown before that MDMA does not interact with this transporter (Rosenauer *et al*. 2013).

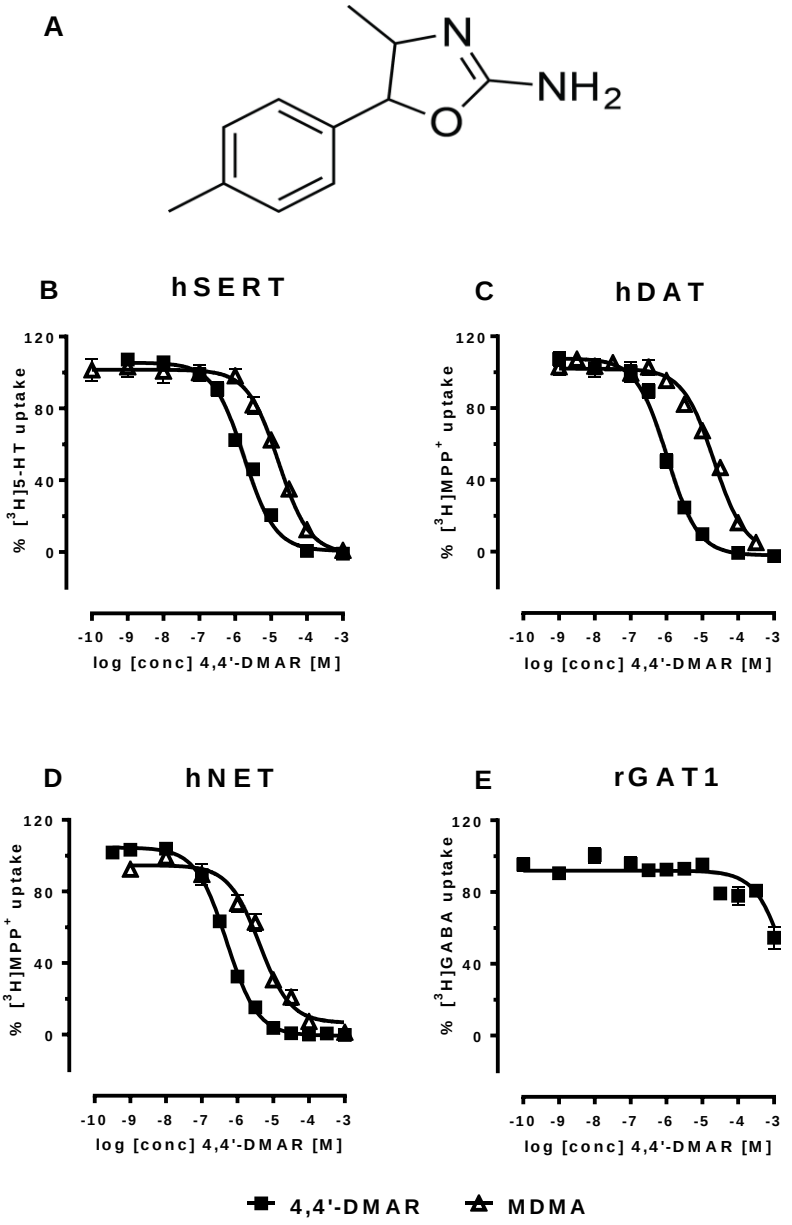

Figure 1:

Effects of (±)-cis-4,4'-dimethylaminorex (4,4'-DMAR) and 3,4-methylendioxymethylamphetamine (MDMA) on transporter-mediated uptake in HEK293 cells expressing hSERT, hDAT, hNET and rGAT1, respectively. (A) The chemical structure of 4,4'-DMAR. (B–E) Uptake of the indicated tritiated substrate into cells expressing the indicated transporters was determined in the presence of increasing concentrations of 4,4'-DMAR and MDMA. All symbols represent mean values ± SEM. The following numbers indicate the number of individual experiments with 4,4'-DMAR, performed in triplicate: hSERT: 5; hDAT: 5; hNET: 8; rGAT1: 8. The following numbers indicate the number of individual experiments with MDMA, performed in triplicate or duplicate: hSERT: 5; hDAT: 4; hNET: 6.

4,4'-DMAR induces transporter-mediated release in HEK293 cells

Data gained from uptake inhibition assays fail to conclusively reveal whether a drug acts as an inhibitor or as a substrate of transporters. Uptake inhibition assays measure the drug-caused change in intracellular substrate concentration and both, uptake inhibitors and releasing agents, cause less substrate to be located intracellularly (Scholze *et al*. 2000; Sitte *et al*. 2000; Mayer *et al*. 2016b). Hence, I performed release assays to investigate whether 4,4'-DMAR induces transporter mediated reverse transport. Dynamic superfusion experiments provide a decisive tool to monitor the effects of test drugs on plasmalemmal transporters (Raiteri *et al*. 1974; Sitte *et al*. 2000; Scholze *et al*. 2002). Cells expressing the transporter of interest were pre-loaded with radiolabelled substrate and exposed to 4,4'-DMAR (10 µM) in the absence or presence of monensin (10 µM). Monensin selectively augments substrate-induced release. In contrast, the effects of non-transported inhibitors remain unchanged (Mayer *et al*. 2016a; Scholze *et al*. 2000).

As shown in Figure 2, the presence of 4,4'-DMAR augmented the basal release of preloaded [^{3}H]substrate via hDAT, hNET and hSERT. On the contrary, 4,4'-DMAR had no effect on the release of [^{3}H]GABA from rGAT1-expressing cells. Two-way ANOVA (drug treatment x time) revealed that monensin + drug treatment significantly influenced the fractional release of [^{3}H]substrate, compared to buffer + drug. Šidák's post-hoc test was used to determine significant differences between the two treatment groups at each time point. The data revealed that monensin treatment significantly augmented 4,4'-DMAR-induced [^{3}H]substrate release. The effect of treatment at hDAT was $F_{1,26}$=4.25, $P < 0.05$, for hNET it was $F_{1,25}$=24.7, $P < 0.05$ and for hSERT it was $F_{1,28}$=373.22, $P < 0.05$. On the other hand, the results at rGAT revealed $F_{1,24}$=0.3, $P = 0.59$ and therefore no significant difference. This could be confirmed by the Mann-Whitney test ($P < 0.01$) for the human monoamine transporters but not for rGAT.

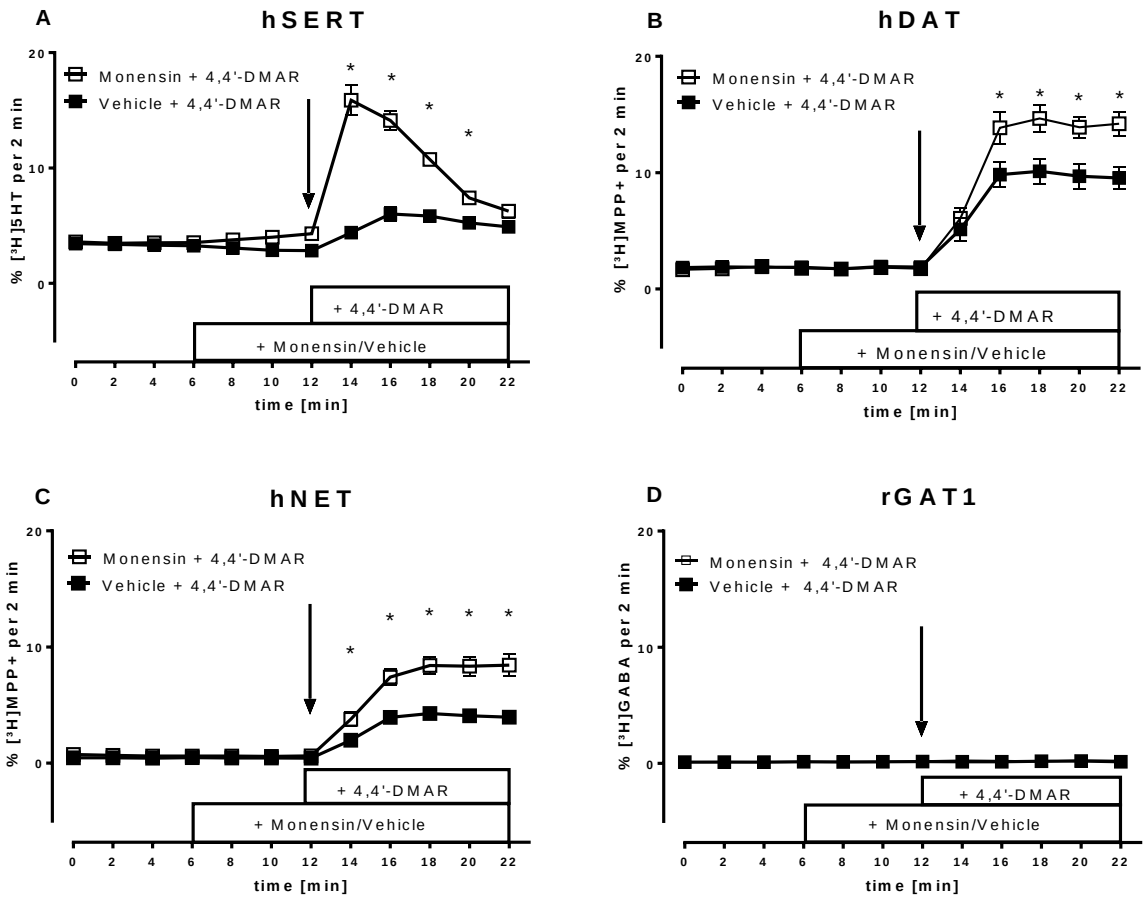

Figure 2:

Effects of 4,4'-DMAR on transporter-mediated release of preloaded radiolabelled substrate from HEK293 cells expressing hSERT, hDAT, hNET and rGAT, respectively. (A-D) Effects of 4,4'-DMAR on transporter-mediated efflux of tritiated substrates in HEK293 cells expressing the aforementioned monoamine transporters. After three basal fractions monensin or control buffer was added at t = 6 min (MON, 10 mM, indicated by black bar). Subsequently, the cells were exposed to 4,4'-DMAR at t = 12 min (indicated by arrow and bar). All data are represented as mean ± SEM. Data were analysed by repeated measures two-way ANOVA followed by Šidák's test. * denotes $p < 0.05$ when compared to the corresponding control buffer condition. The following numbers indicate the number of individual experiments performed in triplicate: hSERT: 5; hDAT: 5; hNET: 5; rGAT: 5.

To gauge the effect of 4,4'-DMAR on monoamine transporters in comparison to well-examined substances, I conducted control experiments with the substances *para*-chloramphetamine for hSERT, *d*-amphetamine for hDAT and hNET and GABA for rGAT1.

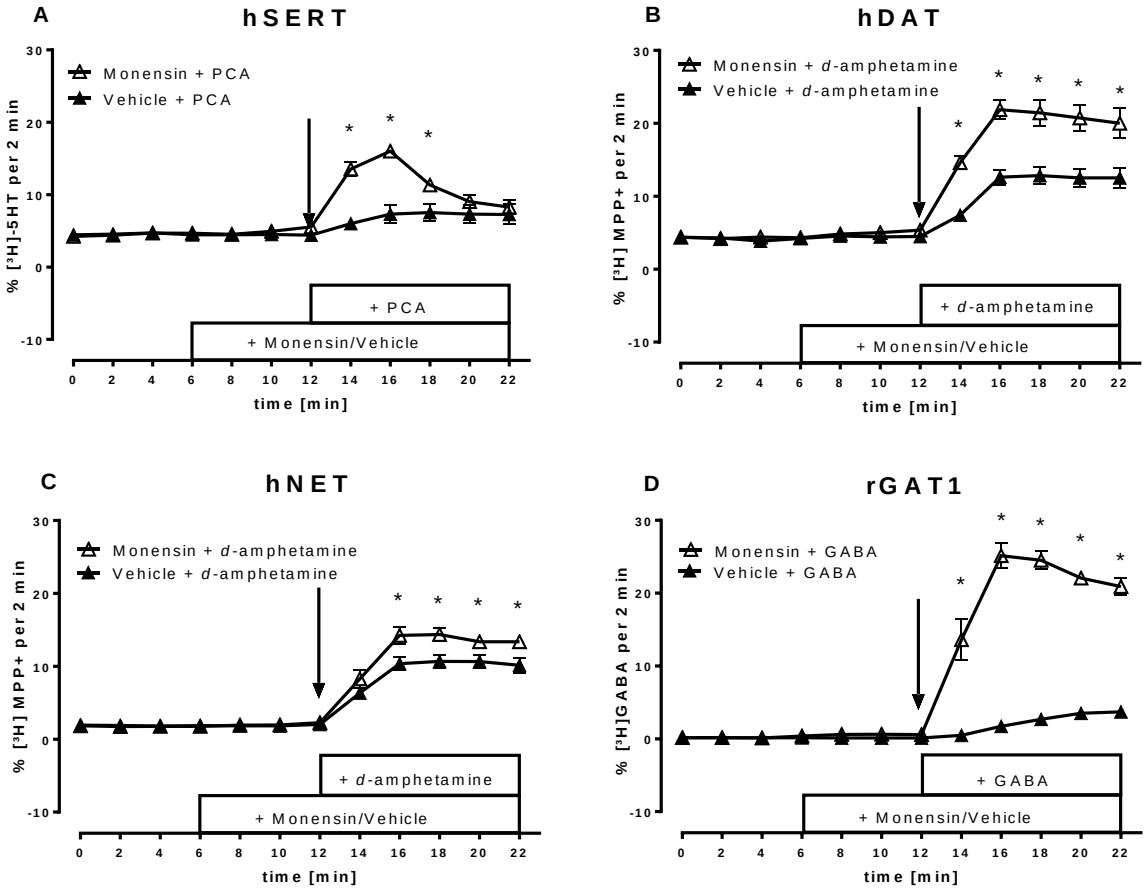

Figure 3:

Effects of 4,4'-DMAR on transporter-mediated release of preloaded radiolabelled substrate from HEK293 cells expressing hSERT, hDAT, hNET and rGAT, respectively. (A-D) Effects of the control substances (substances *para*-chloramphetamine for hSERT, *d*-amphetamine for hDAT and hNET and GABA for rGAT) on transporter-mediated efflux of tritiated substrates in HEK293 cells expressing the aforementioned monoamine transporters. After three basal fractions monensin or control buffer was added at t = 6 min (MON, 10 mM, indicated by black bar). Subsequently, the cells were exposed to 4,4'-DMAR at t = 12 min (indicated by arrow and bar). All data are represented as mean ± SEM. Data were analysed by repeated measures two-way ANOVA followed by Šidák's test. * denotes $p < 0.05$ when compared to the corresponding control buffer condition. The following numbers indicate the number of individual experiments performed in triplicate: hSERT: 5; hDAT: 5; hNET: 5; rGAT: 5.

Figure 3 reveals that the control substances augmented the basal release of preloaded [^{3}H]substrate at hDAT, hNET, hSERT and rGAT. Two-way ANOVA (drug treatment x time) revealed that monensin + drug treatment significantly influenced the fractional release of [^{3}H]substrate, compared to buffer + drug. Šidák's post-hoc test was used to determine significant differences between the two treatment groups at each time point. The data bring to light that monensin treatment significantly augmented control substance-induced [^{3}H]substrate release at hDAT, hSERT, rGAT but not hNET. The effect of treatment at hDAT was $F_{1,26}$=46.04, $P < 0.05$, $F_{1,27}$=192.05, $P < 0.05$ at rGAT, for hSERT it was $F_{1,27}$=7.91, $P < 0.05$ and for hNET $F_{1,27}$=3.2, $P < 0.1$ was the result.

To highlight the effect of 4,4'-DMAR and the control substances, with and without monensin added, on substrate release, Figure 4 portrays the area under the curve (AUC) for the nine fractions collected after drug treatment. 4,4'-DMAR has a strong effect on substrate release at hDAT and causes less release at hSERT and hNET and close to none via rGAT1. Interestingly, the AUC for hSERT is smaller than the AUC of hNET without monensin but larger with the addition of the ionophore. Data was analysed with the non-parametric Mann-Whitney test. $P < 0.05$ was considered significant and marked with an asterisk. Monensin augmentation, when compared to substance and vehicle groups, caused a significant increase in release at all transporter and all substances utilised with the exception of 4,4'-DMAR at the rGAT1.

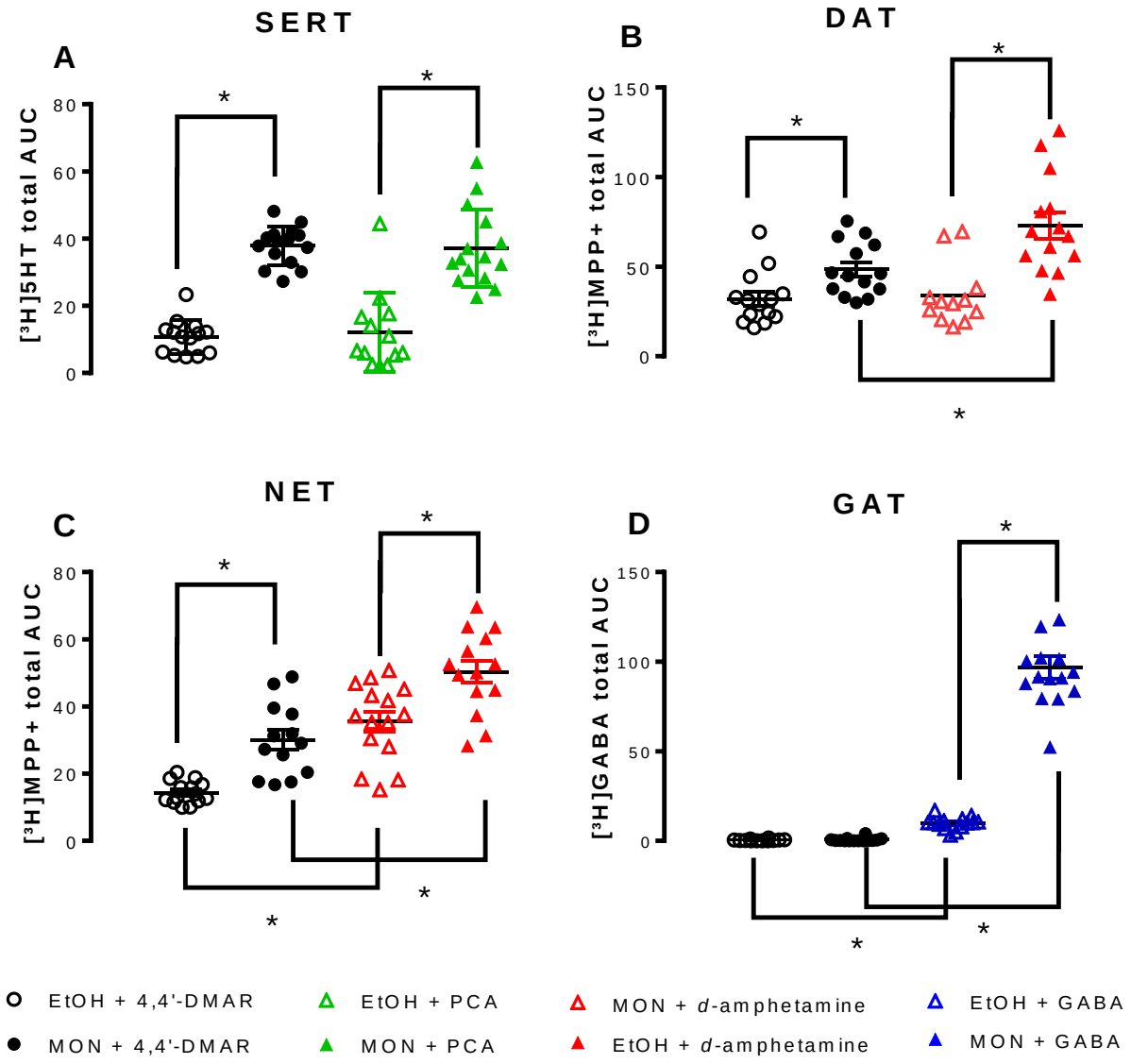

Figure 4:

Effects of 4,4'-DMAR on transporter-mediated release of preloaded radiolabelled substrate in HEK293 cells expressing hSERT, hDAT, hNET and rGAT1. [3H]-5-HT was used as the radiolabelled substrate for hSERT while release by hDAT- and hNET-expressing cells was performed using [3H]-MPP+. rGAT1 release experiments made use of [3H]-GABA. (A–D) For each transporter, AUC was calculated after drug treatment in the absence or presence of monensin (MON, 10 µM). Fully coloured symbols indicate vehicle + drug, whereas empty symbols indicate MON + drug. Bars represent mean values ± SEM. Data were analysed by the Mann-Whitney test. * denotes $p < 0.05$ and a significant difference between compared groups.

4,4'-DMAR inhibits VMAT2 uptake in rat PC12 cells

To investigate if 4,4'-DMAR inhibits VMAT2, I performed uptake inhibition assays in PC12 cells that exogenously express VMAT2 on monoaminergic vesicles. The major finding was that reserpine, MDMA and 4,4'-DMAR inhibited VMAT2 in a concentration-dependent manner (cf. Figure 5). Reserpine inhibited VMAT2 with an IC_{50} value of 0.04 µM (95% CI: 0.03 µM to 0.07 nM). MDMA and 4,4'-DMAR were weaker in that regard, with IC_{50} values of 26.47 µM (95% CI: 17.45 to 39.90) and 29.28 µM (95% CI: 16.29 to 52.61), respectively.

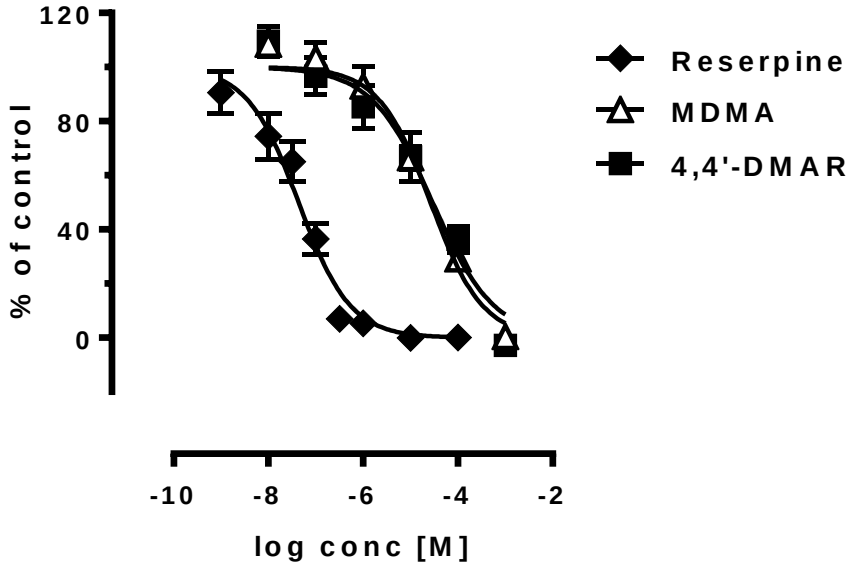

Figure 5:

Effects of 4,4'-DMAR, MDMA and reserpine on VMAT2-mediated uptake in PC12 cells. All symbols represent mean values ± SEM. The following numbers indicate the number of individual experiments performed in triplicate: DMAR: 8; MDMA: 8; reserpine: 5.

4,4'-DMAR inhibits VMAT2-mediated uptake in human striatal synaptic vesicles

Finally, uptake inhibition experiments in synaptic vesicles prepared from human striatum were conducted. Figure 6 shows that 4,4'-DMAR inhibits [^{3}H]dopamine uptake by human VMAT2 in the micromolar range (IC_{50} = 27.6 ± 7.7 µM), comparable to MDMA (IC_{50} = 28.3 ± 4.1 µM), but much less potently than reserpine (IC_{50} = 0.04 ± 0.006 µM).

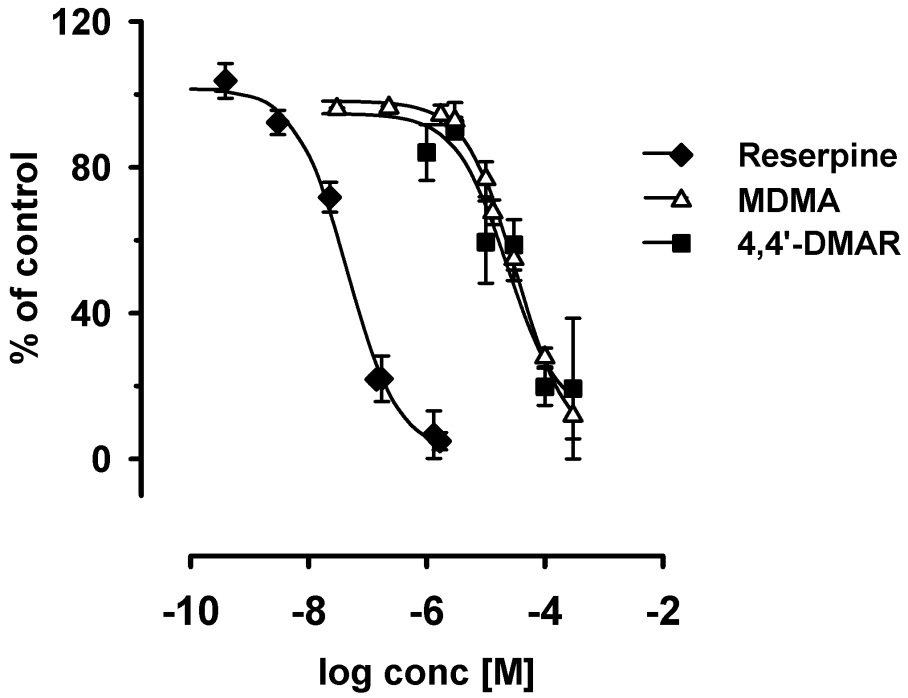

Figure 6:

Effects of 4,4'-DMAR, MDMA and reserpine on VMAT2-mediated uptake in human striatal synaptic vesicles. Symbols represent mean values ± SE. Control uptake was 18.6 ± 2.7 pmol x 4 min-1 x mg protein-1. The following numbers indicate the number of individual experiments performed in duplicate: 4,4'-DMAR: 6; MDMA: 7; reserpine: 4.

Discussion

Considering its potency and the high number of adverse effects caused, it is striking, as Loi *et al.* (2017) point out, that there is "only extremely little, if any, scientific knowledge" available about 4,4'-DMAR. Coppola and Mondola (2015) draw attention to two studies (Brandt *et al*. 2014; McLaughlin *et al*. 2015) making use of rat brain synaptosomes to determine the monoamine transporter activity of 4,4'-DMAR; however, no study has shed light onto the effects of 4,4'-DMAR on humans or human cells as of yet. The internet has been scoured for user reports, detailing the self-reported effects of the NPS on human beings, and internet snapshot surveys have been used to gain insights into the availability of 4,4'-DMAR (Nizar *et al*. 2014; Glanville *et al*. 2015; Loi *et al*. 2017). While, therefore, subjective reports of the drugs' effects on humans exist, this thesis' main goal was to establish the pharmacological properties of 4,4'-Dimethylaminorex, utilizing human cells, expressing human transporters, in order to further the understanding of the substance's effects and toxicity.

By conducting uptake inhibition experiments, I found out that 4,4'-DMAR is a fully efficacious inhibitor of uptake in HEK293 cells stably expressing hNET, hDAT and hSERT in the low micromolar range, with the order indicating rising IC_{50} values. I have also shown that it is a more potent inhibitor of uptake in monoamine transporters than MDMA. As a next step, to test whether the uptake inhibition assays' results were caused by 4,4'-DMAR being an inhibitor or a releaser, I determined transporter release data, which suggests that 4,4'-DMAR is a potent releaser at hDAT, hNET and hSERT. In accordance with McLaughlin *et al.* (2015), it has been shown that the NPS is a more potent releaser at hDAT than hNET, which is closely followed by hSERT, whereas Brandt *et al.* (2014) came to the conclusion that release at hDAT is followed by hSERT and then hNET. Lucchetti *et al*. (2017) conducted motivational-behavioural experiments with rats and concluded that 4,4'-DMAR is highly addictive, an effect that might be caused by its potency at inducing dopamine release. Still, the differences between the 4,4'-DMAR-caused release at the three monoamine transporters are relatively marginal; the observed differences between NET and SERT might be dependent on the individual cell line and their passage, and hence transporter expression-dependent. A possible consensus could be that 4,4'-DMAR is a more potent releaser at DAT, followed closely by similar effects at SERT and NET. The substance's unique feature would therefore be the, compared to other ATS, very high release of 5-HT. Brandt *et al.* (2014) have contrasted the release of 4,4'-DMAR with its parent substances' (4-MAR and Aminorex) release

and found out that their amount of release caused at DAT and NET was on par with or stronger than 4,4'-DMAR's. In contrast, the release at SERT is by far the most potent in 4,4'-DMAR. It is highly likely that the process of methylation has been a causal factor in the increase in potency between the substances (Schönherr & Cernak 2013). In addition, numerous publications show that ring-substitution in the *para*-position renders substrate-type releasers less selective for DAT over SERT (Mayer *et al*. 2017; Solis *et al*. 2017; Bonano *et al*. 2015; Sakloth *et al*. 2015; Cozzi *et al*. 2013). This more serotonergic profile of action is reflected in 4,4'-DMAR's street name "Serotoni".

The transporter binding experiments further substantiate that 4,4'-DMAR binds to hNET, hDAT and hSERT, the order indicating rising K_i values, with high affinities. In addition, the ATS also inhibits binding of other substances to 5-HT_{2A} and 5-HT_{2C} receptors, albeit with lower affinities. Interestingly, the 5-HT_2 subgroup of receptors (5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}), expressed not only in the CNS, but amongst other peripheral regions, also in the myocardium, endocardium and the heart valves, is implicated in cardiovascular complications (Baumann & Rothman 2009; Lauder *et al*. 2000). It has been proven for MDMA that binding to the 5-HT_{2B} receptor is associated with an increased occurrence of valvular heart disease (Baumann & Rothman 2009). Even though 4,4'-DMAR only binds to 5-HT_{2A} and 5-HT_{2C} receptors with lower affinities, this mechanism might be, in part, implicated in the causation of cardiac complications. In addition, it is pointed out that 5-HT_2 receptor binding might be responsible for hallucinogenic effects (Liechti & Vollenweider 2001). It is noteworthy that, in contrast to amphetamine and MDMA, 4,4'-DMAR does not bind to rat or mouse TAAR1 at the investigated concentrations (Lam *et al*. 2015; Zucchi *et al*. 2006). As mentioned before, TAAR1 agonists cause a decrease in firing frequency at serotonergic and dopaminergic axons. This means that, whereas MDMA and other ATS auto-inhibit the excitatory response they cause via the induction of efflux, 4,4'-DMAR does not (Di Cara *et al*. 2011). This lack of neuroprotective effects after 4,4'-DMAR consumption, might be one piece of the puzzle in the explanation of possible neurotoxic effects of the substance.

In order to further consider this angle, I examined 4,4'-DMAR's effects on VMAT2 in rat PC12 and human striatal cells in isolation and therefore conducted uptake inhibition experiments with blocked non-vesicular monoamine transporters. The results suggest that 4,4'-DMAR inhibits serotonin uptake via the rVMAT2 in PC12 cells, as well as dopamine uptake into synaptic vesicles prepared from human striatum with a potency similar to that of MDMA. Many investigators point out that the inhibition of VMAT2 and the following accumulation of substrate within the cytosol

might be responsible for long-term neurotoxicity (Lohr *et al*. 2015; Pifl *et al*. 2015; Wimalasena 2011; Lin *et al*. 2010; Guillot & Miller 2009). The neurotoxicity of 4-MAR has been the subject of controversy, with some results indicating long-term damage to serotonergic and dopaminergic axons (Bunker *et al*. 1990; Hanson *et al*. 1992) and others negating such an effect (Zheng *et al*. 1997). It has been pointed out that, compared to 4-MAR, 4,4'-DMAR can more freely pass the blood-brain barrier and has a brain-to-plasma ratio three times that of its parent substance (Lucchetti *et al*. 2017). VMAT2 and P-gp (P-glycoprotein, MDR1, multidrug resistance protein 1), a transporter located in the colon and jejunum, kidney, liver, pancreas and also the endothelial cells that make up the blood-brain barrier, share sequence homology and many inhibitors of one transporter have also proven effective at the other, e.g. reserpine (Staal *et al*. 2001). Therefore, inhibition of VMAT2 by 4,4'-DMAR hints at the possibility of the NPS also inhibiting P-gp and hindering its ability to pump substrate out of the cell (Staal *et al*. 2001; Thiebaut *et al*. 1987). Inhibition of P-GP allows drugs more easily to pass the blood-brain barrier, accumulating in neurons and increasing neurotoxic effects (Schinkel 1999). The increased passage of the blood-brain barrier might hint at a greater neurotoxic potential of 4,4'-DMAR in comparison to its parent substance 4-MAR

The findings of this thesis, in conjunction with the results reported by Brandt *et al*. (2014) and McLaughlin *et al*. (2015), suggest that 4,4'-DMAR's mechanism of action resembles the pharmacological profile of MDMA and, therefore, acts as a non-selective monoamine releasing agent that also affects VMAT2. When comparing inhibition data, it is noticeable that 4,4'-DMAR behaves very similarly to MDMA at hNET and hSERT and is a more potent inhibitor at hDAT (Simmler *et al*. 2013)[11]. McLaughlin *et al*. (2015) have compared the EC_{50} values of MDMA and 4,4'-DMAR in rat brain synaptosomes and found the latter to be the more potent releaser at SERT, DAT and NET (approximately between 5 and 10 times, depending on the transporter). The receptor and transporter binding profiles reveal that 4,4'-DMAR exhibits, depending on the monoamine transporter, ten to 100 times (NET > DAT > SERT) higher binding affinities than MDMA (Simmler *et al*. 2013; Simmler *et al*. 2014). In contrast to MDMA, 4,4'-DMAR does not bind to TAAR1 and 5-HT_{2B} at the tested concentrations, whereas only the latter binds to 5-HT_{2C} (Simmler *et al*. 2013; Baumann & Rothman 2009). The NPS' affinity towards 5-HT_{2A} (K_i = 8.8 µM) is comparable to

[11] MDMA IC_{50}: hSERT – 1.36 µM; hDAT – 17 µM; hNET – 0.45 µM (Simmler *et al*. 2013)
4,4'-DMAR IC_{50}: hSERT – 1.75 µM; hDAT 1.04µM; hNET – 0.5 µM

the affinity reported for MDMA (K_i = 5.9 µM) (Simmler *et al*. 2013). Even though the cardiotoxicity related to MDMA has been mostly linked to its interaction with the 5-HT_{2B} receptor, all 5-HT_2 receptors are expressed in cardiac cells (Baumann & Rothman 2009; Setola *et al*. 2003; Lauder *et al*. 2000). It might be possible that 5-HT_{2A} and 5-HT_{2C} binding could therefore be implicated in cardiac complications caused by 4,4'-DMAR. The VMAT2 assays suggest that the two substances are similar in their ability to inhibit VMAT2 (Pifl *et al*. 2015). The data allude to the possibility of 4,4'-DMAR-caused cardiotoxic and neurotoxic effects similar or, potentially, even stronger than those of MDMA. The lack of TAAR1 binding of 4,4'-DMAR, and therefore the loss of an auto-inhibitory pathway that is present for MDMA, might explain, in conjunction with the more potent release and the higher binding affinities compared to MDMA, 4,4'-DMAR's higher potency as a releasing agent.

According to the EMCDDA (2014), 4,4'-DMAR was often not consciously purchased but unwittingly consumed by victims who thought that they had procured MDMA or cocaine. It was therefore used by drug producers as an adulterant or masking agent to hide the lack of the expected active substance (Brunt *et al*. 2017; Giné *et al*. 2014; Hofmaier *et al*. 2014; Rosenauer *et al*. 2013). The same practice has been reported for 4-MAR, where the substance had also been misrepresented as cocaine or methamphetamine (Meririnne *et al*. 2004). This would offer an explanation of the many lethal drug-related events - non-lethal doses of what was thought to be MDMA turned out to be the more potent 4,4'-DMAR and caused deaths due to overdose. It is striking that only one death due to 4-MAR (Davis and Brewster, 1988) and several cases of pulmonary hypertension caused by Aminorex and 4-MAR (Fishman 1999; Gaine *et al*. 2000) have been reported so far. Rothman *et al*. (1999) attribute these to the effect of 5-HT. The admission notes and autopsy reports of 4,4'-DMAR victims, provided by the EMCDDA (2014), suggest symptoms typical of serotonin syndrome or serotonin toxicity and serotonin and norepinephrine caused cardiotoxicity (Greenier *et al*. 2014; Brandt *et al*. 2014). Investigators corroborate similar serotonergic and noradrenergic effects for MDMA (de la Torre *et al*. 2000a; Green *et al*. 2003; Steinkellner *et al*. 2011). With (i) the data gained from my release assays (and the relatively high hSERT release), (ii) the knowledge of the pharmacology and toxicology of 4,4'-DMAR's parent substances, (iii) its similarity to MDMA and (iv) the admission notes and autopsy reports provided by the EMCDDA (2014), taken together, the conclusion that the increase in hSERT release from Aminorex to 4,4'-DMAR has led to the increase in toxicity and frequency of lethal drug-related events seems plausible. By

identifying serotonin syndrome and noradrenergic sympathomimetic effects as, highly likely, causes of the lethality of 4,4'-DMAR, medical professionals are able to treat drug users appropriately (Schifano *et al*. 2015).

Taken one step further, concerning drug policy, it seems to be the case that harm reduction initiatives, such as the Trans European Drug Information (TEDI) project[12], where drug users can anonymously get their purchased substances chemically analysed and are alerted about possible adulterations and misrepresentations of drugs, allow for the twofold benefit of warning users individually and collectively and of gathering field data concerning drugs in circulation, supplementary to data gained from police and customs seizures (Brunt *et al*. 2017). This might aid in the prevention of further big-scope drug-related tragedies such as the 4,4'-DMAR caused series of deaths between June 2013 and February 2014. The alternative to harm reduction initiatives are blanket bans on psychoactive substances, as, for example, the one issued in the United Kingdom in 2016, their effectiveness being heavily debated in scientific literature (Negrei *et al*. 2017; Reuter & Pardo 2017; Baumann & Volkow 2016; Reuter & Pardo 2016; Gross 2015; Stevens *et al*. 2015; Fraser & Moore 2011).

This thesis examined the pharmacological properties of 4,4'-DMAR by concentrating on its effects at human SLC transporters of the SLC6 and SLC18 families, as well as monoamine receptors and concluded that the NPS is an efficacious releaser at hDAT, hNET and hSERT, to which it also binds with high affinities. In addition, the substance was found out to be a potent inhibitor of VMAT2-mediated uptake. This, firstly, offers a possible and plausible explanation for the substance's short and long-term effects and toxicity, lending support to medical professionals to treat drug users appropriately. Secondly, because of the fact that ATS have to be substrates of monoamine transporters *and* the VMAT2 in order to exert their effects, it is hereafter recommended that future analyses of NPS include assays to determine the degree of inhibition at VMAT2 in order to provide a more holistic picture of the substances' mechanisms of action (Zheng *et al*. 2006; Chaudry *et al*. 2007; Freyberg *et al*. 2016). Even though 4,4'-DMAR has been banned in the EU, the results of this thesis remain highly relevant because the NPS is still available in online shops (Shenzhen Chemicals 2017; Pharmaceutical Chemistry Co. 2017; Online Research Chemicals Shop 2017). In addition, the NPS market is exceedingly dynamic and the information presented

[12] The project was a collaborative effort of mobile and stationary drug testing services in six European countries. From Vienna, Austria, the checkit! initiative participated in the project (Rosenauer *et al*. 2013).

herein might be relevant for our understanding of future Aminorex derivatives, which may be introduced into drug markets (McLaughlin *et al*. 2015).

While one recent study has identified four metabolites of 4,4'-DMAR in pharmacokinetic experiments with rats and metabolic experiments have also been conducted for 4-MAR, this thesis has generated a research gap concerning possible neurotoxic (via VMAT2) and cardiotoxic (via 5-HT_2 receptors and noradrenergic neurons) effects of 4,4'-DMAR in humans and animals that might be closed in a future animal model study (Lucchetti *et al*. 2017; Meririnne *et al*. 2004; Henderson *et al*. 1995). It would, in addition, be highly relevant to investigate whether 4,4'-DMAR is not only an inhibitor of VMAT2 but also of P-GP and MAO.

To conclude, the new psychoactive substance 4,4'-DMAR has been shown to be a potent non-selective monoamine transporter releasing agent that inhibits VMAT2-mediated uptake in human and rat cells. The latter result might explain its potential long-term neurotoxicity, caused by the accumulation of substrate in the cytosol. Compared to other amphetamine-type stimulants and its predecessors, 4,4'-DMAR has a very pronounced serotonergic profile of action similar to MDMA. However, 4,4'-DMAR was often mislabelled and sold as ecstasy even though it is a more potent releasing agent. The deaths can therefore be apprehended as overdoses, acutely causing serotonin and norepinephrine toxicity. This thesis aimed and succeeded at classifying the basic pharmacological properties of 4,4'-DMAR in human cells and provided plausible explanations for the substance’s short- and long-term effects.

References

Acosta A, Camilleri M (2015). Pharmacogenetics in irritable bowel syndrome. Expert Opinion on Drug Metabolism & Toxicology, 11(8): 1187-1191.

Adler LAA, Chua HC (2002). Management of ADHD in Adults. J Clin Psychiatry 63(12): 29-35.

Alexander SPH, Davenport AP, Kelly E, Marrion N, Peters JA, Benson HE *et al*. (2015). The concise guide to pharmacology 2015/16: G protein-coupled receptors. British Journal of Pharmacology 172: 5744-5869.

Anderluh A, Klotzsch E, Reismann A, Brameshuber M, Kudlacek O, Hauck Newman A *et al*. (2014). Single Molecule Analysis Reveals Coexistence of Stable Serotonin Transporter Monomers and Oligomers in the Live Cell Plasma Membrane. J Biol Chem 289(7): 4387-4394.

Anne C, Gasnier B (2014). Vesicular Neurotransmitter Transporters: Mechanistic Aspects. Current Topics in Membranes 73: 149-174.

Backs J, Haunstetter A, Gerber SH, Metz J, Borst MM, Strasser RH, Kübler W, Haass M (2001). The Neuronal Norepinephrine Transporter in Experimental Heart Failure: Evidence for a Posttranscriptional Downregulation. J Mol Cell Cardiol 33: 461-472.

Bala, PA, Foster J, Carvelli L, Henry LK (2013). SLC6 Transporters: Structure, Function, Regulation, Disease Association and Therapeutics. Mol Aspects Med 34(2-3): 197-219.

Baldwin HA, COlado MI, Murray TK, De Souza RJ, Green AR (1993). Striatal dopamine release in vivo following neurotoxic doses of methamphetamine and effect of the neuroprotective drugs,
chlormethiazole and dizocilpine. British Journal of Pharmacology 108: 590-596.

Barnes NM, Sharp T (1999). A review of central 5-HT receptors and their function. Neuropharmacology 38: 1038-1152.

Battaglia G, Brooks BP, Kulsakdinum C, De Souza EB (1988). Pharmacologic profile of MDMA (3,4-methylenedioxymethamphetamine) at various brain recognition sites. European Journal of Pharmacology 149: 159-163.

Baumann MH, Wang X, Rothman RB (2007). 3,4-Methylenedioxymethamphetamine (MDMA) neurotoxicity in rats: a reappraisal of past and present findings. Psychopharmacology 189: 407-424.

Baumann MH, Rothman RB (2009). Neural and cardiac toxicities associated with 3,4-methylendioxymethylamphetamine (MDMA). Int Rev Neurobiol 88: 257-296.

Baumann MH, Bulling S, Benaderet TS, Saha K, Ayestas MA, Partilla JS *et al*. (2014). Evidence for a Role of Transporter-Mediated Currents in the Depletion of Brain Serotonin Induced by Serotonin Transporter Substrates. Neuropsychopharmacology 39: 1355-1365.

Baumann MH, Volkow ND (2016). Abuse of New Psychoactive Substances: Threats and Solutions. Neuropsychopharmacology 41(3): 663-665.

Bayles R, Baker EK, Jowett JBM, Barton D, Esler M, El-Osta A, Lambert G (2013). Methylation of the SLC6a2 Gene Promoter in Major Depression and Panic Disorder. PLOS ONE 8(12); e83223.

Beaulieu J, Espinoza S, Gainetdinov RR (2015). Dopamine receptors – IUPHAR Review 13. British Journal of Pharmacology 172: 1-23.

Bermingham DP, Blakely RD (2016). Kinase-dependent Regulation of Monoamine Neurotransmitter Transporters. Pharmacol Rev 68: 888-953.

Biel JH, Bopp BA (1978). Amphetamines: structure – activity relationship. In: Iversen LL, Iversen SD, Snyder SH (eds). Stimulants. Handbook of Psychopharmacology, vol 11. Boston: Springer.

Billiard M (2008). Serotonin norepinephrine reuptake inhibitors (SNRIs) in anxiety disorders: a comprehensive review of their clinical efficacy. Neuropsychiatric Disease and Treatment 4(3): 557-566.

Blough BE, Landavazo A, Partilla JS, Decker AM, Page KM, Baumann MH, Rothman RB (2014). Alpha-ethyltryptamines as dual dopamine–serotonin releasers. Bioorganic and Medicinal Chemistry Letters 24(19): 4754-4758.

Boadle-Biber MC (1993). Regulation of Serotonin Synthesis. Prog. Biophys. Molec. Biol. 60: 1-15.

Bonano JS, Banks ML, Kolanos R, Sakloth F., Barnier ML, Glennon RA *et al*. (2015). Quantitative structure–activity relationship analysis of the pharmacology of para-substituted methcathinone analogues. Brit Pharmacol 172: 2433-2444.

Borowsky B, Adham N, Jones KA, Raddatz R, Artymyshyn R, Ogozalek *et al*. (2001). Trace amines: Identification of a family of mammalian G protein-coupled receptors. PNAS 98(16): 8966-8971.

Brandt S, Baumann M, Partilla J, Kavanagh P, Power J, Talbot B *et al*. (2014). Characterization of a novel and potentially lethal designer drug, (±)-cis-para-methyl-4-methylaminorex (4,4'-DMAR, or "Serotoni"). Drug Test Anal 6: 684–695.

Brensilver M, Heinzerling KG, Shoptaw S (2013). Pharmacotherapy of amphetamine-type stimulant dependence: An update. Drug and Alcohol Review 32: 449-460.

Bröer S, Gether U (2012). The solute carrier 6 family of transporters. British Journal of Pharmacology 167: 256-278.

Broadley KJ (2010). The vascular effects of trace amines and amphetamines. Pharmacology & Therapeutics 125: 363-375.

Bruns D, Jahn R (1997). Monoamine Transmitter Release from Small Synaptic and Large Dense-Core Vesicles. Advances in Pharmacology 42: 87-90.

Brunt TM, Nagy C, Bücheli A, Martins D, Ugarte M, Beduwe C *et al*. (2017). Drug testing in Europe : monitoring results of the Trans European Drug Information (TEDI) project. Drug Test Analysis 9(2): 188-198.

Brüss M, Kunz J, Lingen B, Bönisch H (1993). Chromosomal mapping of the human gene for the tricyclic antidepressant-sensitive noradrenaline transporter. Hum Genet 91: 278-280.

Bunker CF, Johnson M, Gibb JW, Bush LG, Hanson GR (1990). Neurochemical effects of an acute treatment with 4-methylaminorex: a new stimulant of abuse. Eur J Pharmacol 180: 103–11.

Callaghan RC, Cunningham JK, Sykes J, Kish SJ (2012). Increased risk of Parkinson's disease in individuals hospitalized with conditions related to the use of methamphetamine or other amphetamine-type drugs. Drug and Alcohol Dependence 120: 35-40.

Cameron KN, Solis EJ, Ruchala I, De Felice LJ, Eltit JM (2015). Amphetamine activates calcium channels through dopamine transporter-mediated depolarization. Cell Calcium 58: 457-466.

Canli T, Lesch KP (2007). Long story short: the serotonin transporter in emotion regulation and social cognition. Nature Neuroscience 10(9): 1103-1109.

Carroll FI, Howard JL, Howell LL, Fox BS, Kuhar MJ (2006). Development of the Dopamine Transporter Selective RTI-336 as a Pharmacotherapy for Cocaine Abuse. AAPS J 8(1): e196-e203.

Carvalho M, Carmo H, Costa VM, Capela JP, Pontes H, Remiao F *et al*. (2012). Toxicity of amphetamines: an update. Arch Toxicol 86: 1167-1231.

César-Razquin A, Snijder B, Frappier-Brinton T, Isserliin R, Gyimesi G, Bai X *et al*. (2015). A call for systematic research on solute carriers. Cell 162: 478-487.

Chaudhry FA, Edwards RH, Fonnum F (2007). Vesicular Neurotransmitter Transporters as Targets for Endogenous and Exogenous Toxic Substances. Annu Rev Pharmacol Toxicol 48: 277-301.

Chuang DM (2004). Neuroprotective and neurotrophic actions of the mood stabilizer lithium: can it be used to treat neurodegenerative diseases?. Crit Rev Neurobiol 16(1-2): 83-90.

Ciliax BJ, Drash GW, Staley JK, Haber S, Mobley CJ, Miller GW *et al*. (1999). Immunocytochemical Localization of the Dopamine Transporter in Human Brain. Journal of Comparative Neurology 409: 38-56.

Colas C, Ung PM, Schlessinger A (2016). SLC Transporters: Structure, function, and drug discovery. Medchemcom 7(6): 1069-1081.

Coleman JA, Green EM, Gouaux E (2016). X-ray structures and mechanism of the human serotonin transporter. Nature 532: 334-339.

Coppola M, Mondola R (2015). 4,4′-DMAR: Chemistry, Pharmacology and Toxicology of a New Synthetic Stimulant of Abuse. Basic & Clinical Pharmacology & Toxicology 117: 26–30.

Costa A, Riedel M, Müller U, Möller H, Ettinger U (2011). Relationship Between SLC6A3 Genotype and Striatal Dopamine Transporter Availability: A Meta-Analysis of Human Single Photon Emission Computed Tomography Studies. Synapse 65: 998-1005.

Cozzi NV, Brandt SD, Daley PF, Partilla JS, Rothman RB, Tulzer A *et al*. (2013). Pharmacological examination of trifluoromethyl ring-substituted methcathinone analogs. Eur J Pharmacol 699: 180-187.

Davis FT, Brewster ME (1988). A fatality involving U4EuH, a cyclic derivative of phenylpropanolamine. Journal of Forensic Sciences 33: 549-553.

De la Torre R, Farré M, Roset PN, Hernández López C, Mas M, Ortuno J *et al*. (2000a). Pharmacology of MDMA in Humans. Annals New York Academy of Science : 225-237.

De la Torre R, Farré M, Ortuno J, Mas M, Brenneisen R, Roset PN *et al*. (2000b). Non-linear pharmacokinetics of MDMA ('ecstasy') in humans. J Clin Pharmacol 49 : 104-109.

De la Torre R, Farré M (2004). Neurotoxicity of MDMA (ecstasy): the limitations of scaling from animals to humans. Trends in Pharmacological Sciences 25(10): 505-508.

De la Torre R, Farré M, Roset PN, Pizarro N, Abanades S, Segura M *et al*. (2004). Human Pharmacology of MDMA. Pharmacokinetics, Metabolism, and Disposition. Ther Drug Monit 26: 137-144.

Dell'Osso B, Buoli M, Baldwin DS, Altamura AC (2010). Serotonin norepinephrine reuptake inhibitors (SNRIs) in anxiety disorders: a comprehensive review of their clinical efficacy. Hum Psychopharmacol Clin Exp 25: 17-29.

DiCara B, Maggio R, Aloisi G, Rivet J, Lundius EG, Yoshitake T *et al*. (2011). Genetic Deletion of Trace Amine 1 Receptors Reveals Their Role in Auto-Inhibiting the Actions of Ecstasy (MDMA). Journal of Neuroscience 31(47): 16928-16940.

Dong C, Wong ML, Licinio J (2009). Sequence variations of ABCB1, SLC6A2, SLC6A3, SLC6A4, CREB1, CRHR1 and NTRK2: association with major depression and antidepressant response in Mexican-Americans. Molecular Psychiatry 14: 1105-1118.

Duman EA, Canli T (2015). Influence of life stress, 5-HTTLPR genotype, and SLC6A4 methylation on gene expression and stress response in healthy Caucasian males. Biology of Mood & Anxiety Disorders 5: 2.

Egana LA, Cuevas RA, Baust TB, Parra LA, Leak RK, Hochendoner S *et al*. (2009). Physical and Functional Interaction between the Dopamine Transporter and the Synaptic Vesicle Protein Synaptogyrin-3. Journal of Neuroscience 29(14): 4592-4604.

Eiden LE, Schäfer MK, Weihe E, Schütz B (2004). The vesicular amine transporter family (SLC18): amine/proton antiporters required for vesicular accumulation and regulated exocytotic secretion of monoamines and acetylcholine. Eur J Physiol 447: 636-640.

Eiden LE, Weihe E (2011). VMAT2: a dynamic regulator of brain monoaminergic neuronal function interacting with drugs of abuse. Ann NY Acad Sci 1216: 86-98.

Eisenhofer G, Kopin IJ, Goldstein DS (2004). Catecholamine Metabolism: A Contemporary View with Implications for Physiology and Medicine. Pharmacological Reviews 56(3): 331-349.

EMCDDA (2014). Risk Assessment Report of a new psychoactive substance: 4-methyl-5-(4-methylphenyl)-4,5-dihydrooxazol-2-amine: EU European Monitoring Centre for Drugs and Drug Addiction. [Online] Available from http://www.emcdda.europa.eu/system/files/publications/ 931/4%2C4'-DMAR%20Risk%20Assessment%20Report.pdf [Accessed: 23rd December 2017].

EMCDDA (2015). 4,4′-DMAR: EU European Monitoring Centre for Drugs and Drug Addiction. [Online] Available from http://www.emcdda.europa.eu/attachements.cfm/att_229825_EN_TDA S14006ENN.pdf [Accessed: 23rd December 2017].

EMCDDA (2017). Action on new drugs. [Online] Available from: http://www.emcdda.europa.eu/activities/action-on-new-drugs [Accessed: 23rd December 2017].

Erblich J, Lerman C, Self DW, Diaz GA, Bovbjerg DH (2005). Effects of dopamine D2 receptor (DRD2) and transporter (SLC6A3) polymorphisms on smoking cue-induced cigarette craving among African-American smokers. Molecular Psychiatry 10: 407-414.

Erickson JD, Schäfer MK, Bonner TI, Eiden LE, Weihe E (1996). Distinct pharmacological properties and distribution in neurons and endocrine cells of two isoforms of the human vesicular monoamine transporter. Proc Natl Acad Sci 93: 5166-5171.

Estler CJ (1975). Effect of Amphetamine-Type Psychostimulants on Brain Metabolism. Adv Pharmacol Chemother 13: 305-357.

Fei H, Grygoruk A, Brooks ES, Chen A, Krantz DE (2008). Trafficking of Vesicular Neurotransmitter Transporters. Traffic 9(9): 1425-1436.

Fishman A (1999). Aminorex to Fen/Phen. An Epidemic Foretold. Circulation 99: 156-161.

Fleckenstein AE, Volz TJ, Hanson GR (2009). Psychostimulant-induced Alterations in Vesicular Monoamine Transporter-2 Function: Neurotoxic and Therapeutic Implications. Neuropharmacology 56: 133-138.

Flint J, Kendler KS (2014). The Genetics of Major Depression. Neuron 81: 484-503.

Fraser S, Moore D (2011). Governing through problems: The formulation of policy on amphetamine-type stimulants (ATS) in Australia. International Journal of Drug Policy 22: 498-506.

Fredriksson R, Lagerström MC, Lundin LG, Schiöth HB (2003). The G-Protein-Coupled Receptors in the Human Genome Form Five Main Families. Phylogenetic Analysis, Paralogon Groups, and Fingerprints. Molecular Pharmacology 63(6): 1256-1272.

Fredriksson R, Nordström KJV, Stephansson O, Hägglund MGA, Schiöth HB (2008). The solute carrier (SLC) complement of the human genome: Phylogenetic classification reveals four major families. FEBS Letter 582: 3811-3816.

Freyberg Z, Sonders MS, Aguilar JI, Hiranita T, Karam CS, Flores J, *et al*. (2016). Mechanisms of amphetamine action illuminated through optical monitoring of dopamine synaptic vesicles in
Drosophila brain. Nat Commun 7: 10652.
Gaddum JH (1953). The Technique of Superfusion. Brit J Pharmacol 8: 321-326.

Gaine S, Rubin L, Kmetzo J, Palevsky H, Traill T (2000). Recreational Use of Aminorex and Pulmonary Hypertension. CHEST 118: 1496–1497.

Garg A, Kapoor S, Goel M, Chopra S, Chopra M, Kapoor A *et al*. (2015). Functional Magnetic Resonance Imaging in Abstinent MDMA Users: A Review. Current Drug Abuse Reviews 8 : 15-25.

Gelernter J, Kranzler H, Cubells JF (1997). Serotonin transporter protein (SLC6A4) allele
and haplotype frequencies and linkage disequilibria in African- and European-American and Japanese populations and in alcohol-dependent subjects. Human Genet 101: 243-246.

Giné CV, Espinosa IF, Vilamala MV (2014). New psychoactive substances as adulterants of controlled drugs. A worrying phenomenon?. Drug Test Analysis 6(7-8):819-24.

Glanville J, Dargan P, Wood DM (2015). 4-Methyl-5-(4-methylphenyl)-4,5-dihydrooxazol-2-amine (4,4′-DMAR, 4,4′-dimethylaminorex): availability, prevalence of use, desired effects
and acute toxicity. Hum Psychopharmacol Clin Exp 30: 193–198.

Gorman JM, Kent JM (1999). SSRIs and SNRIs: broad spectrum of efficacy beyond major depression. J Clin Psychiatry 60(4) : 33-38.

Green R, Mechan AO, Elliott JM, O'Shea E, Colado MI (2003). The Pharmacology and Clinical Pharmacology of 3,4-Methylenedioxymethamphetamine (MDMA, "Ecstasy"). Pharmacological Reviews 55(3): 463-508.

Greenier E, Lukyanova V, Reede L (2014). Serotonin Syndrome: Fentanyl and Selective Serotonin Reuptake Inhibitor Interactions. AANA Journal 82 (5): 340-345.

Gross M (2015). Drugs: blanket ban or harm reduction?. Current Biology 25: r523-r548.

Grouleff J, Ladefoged LK, Koldso H, Schlott B (2015). Monoamine transporters: insights from molecular dynamics simulations. Front Pharmacol 6: 235.

Grygoruk A, Chen A, Martin CA, Lawal HO, Fei H, Gutierrez G (2014). The Redistribution of Drosophila Vesicular Monoamine Transporter Mutants from Synaptic Vesicles to Large
Dense-Core Vesicles Impairs Amine-Dependent Behaviors. Journal of Neuroscience 34(20): 6924-6937.

Guillot TS, Miller GW (2009). Protective Actions of the Vesicular Monoamine Transporter
2 (VMAT2) in Monoaminergic Neurons. Mol Neurobiol 39: 149-170.

Hajós M, Fleishaker JC, Filipiak-Reisner JK, Brown MT, Wong EHF (2004). The Selective Norepinephrine Reuptake Inhibitor Antidepressant Reboxetine: Pharmacological and Clinical Profile. CNS Drug Reviews 10(1): 23-44.

Haldrup C, Lynnerup A, Storebjerg TM, Vang S, Wild P, Visakorpi T *et al*. (2016). Large-scale evaluation of SLC18A2 in prostate cancer reveals diagnostic and prognostic biomarker potential at three molecular levels. Molecular Oncology 10: 825-837.

Hansen FH, Skjorringe T, Yasmeen S, Arends NV, Sahai MA, Erreger K *et al*. (2014). Missense dopamine transporter mutations associate with adult parkinsonism and ADHD. Journal of Clinical Investigation 124(7): 3107-3120.

Hanson GR, Bunker CF, Johnson M, Bush L, Gibb JW (1992). Response of monoaminergic and neuropeptide systems to 4-methylaminorex: a new stimulant of abuse. Eur J Pharmaco. 218: 287–93.

Heal DJ, Smith SL, Gosden J, Nutt DJ (2013). Amphetamine, past and present – a pharmacological and clinical perspective. Journal of Psychopharmacology 27(6): 479-496.

Hediger MA, Romero MF, Peng J, Rolfs A, Takanaga H, Bruford EA (2004). The ABCs of solute carriers: physiological, pathological and therapeutic implications of human membrane transport proteins. Eur J Physiol 447: 465-468.

Hediger MA, Clémoncon B, Burrier RE, Bruford EA (2013). The ABCs of membrane transporters in health and disease (SLC series): Introduction. Molecular Aspects of Medicine 34: 95-107.

Heils A, Teufel A, Petri S, Stöber G, Riederer P, Bengel D, Lesch KP (1996). Allelic Variation of Human Serotonin Transporter Gene Expression. J Neurochem 66(6): 2621-2624.

Henderson GL, Harkey MR, Chueh Y (1995). Metabolism of 4-Methylaminorex ("EU4EA") in the Rat. Journal of Analytical Toxicology 19: 563-570.

Hiasa M, Miyaji T, Haruna Y, Takeuchi T, Harada Y, Mariyama S *et al*. (2014). Identification of a mammalian vesicular polyamine transporter. Sci Rep 4: 6836.

Hofmaier T, Luf A, Seddik A, Stockner T, Holy M, Freissmuth M *et al*. (2014). Aminorex, a metabolite of the cocaine adulterant levamisole, exerts amphetamine like actions at monoamine transporters. Neurochemistry International 73: 32–41.

Höglund PJ, Nordström KJV, Schiöth HB, Fredriksson R (2011). The Solute Carrier Families Have a Remarkably Long Evolutionary History with the Majority of the Human Families Present before Divergence of Bilaterian Species. Mol Biol Evol 28(4): 1531-1541.

Hong WC, Amara SG (2010). Membrane Cholesterol Modulates the Outward Facing Conformation of the Dopamine Transporter and Alters Cocaine Binding. Journal of Biological Chemistry 285(42): 32616-32626.

Huot P, Fox SH, Brotchie JM (2016). Dopamine Reuptake Inhibitors in Parkinson's Disease: A Review of Nonhuman Primate Studies and Clinical Trials. Journal of Pharmacology and Experimental Therapeutics 357: 562-569.

Hysek CM, Schmid Y, Simmler LD, Domes G, Heinrichs M, Eisenegger C *et al*. (2014). MDMA enhances emotional empathy and prosocial behavior. SCAN 9: 1645-1652.

Jardetzky O (1966). Simple allosteric model for membrane pumps. Nature 211: 969-970.

Kahlig KM, Binda F, Khoshbouei H, Blakely RD, McMahon DG, Javitch JA, Galli A (2005). Amphetamine induces dopamine efflux through a dopamine transporter channel. PNAS 102(9): 3495-3500.

Kankaanpää A, Meririnne E, Ellermaa S, Ariniemi K, Seppälä T (2001). Detection and assay of *cis*- and *trans*-isomers of 4-methylaminorex in urine, plasma and tissue samples. Forensic Science International 121: 57-64.

Kesselheim AS, Myers JA, Solomon DH, Winkelmayer WC, Levin R, Avorn J (2012). The Prevalence and Cost of Unapproved Uses of Top-Selling Orphan Drugs. PLoS ONE 7(2): e31894.

Kim DK, Lim S, Lee S, Sohn SE, Kim S, Hahn CG, Carroll BJ (1999). Serotonin transporter gene polymorphism and antidepressant response. Neuroreport 11(1): 215-219.

Kim C, Hahn MK, Joung Y, Anderson SL, Steele AH, Mazel-Robinson MS *et al*. (2006). A polymorphism in the norepinephrine transporter gene alters promoter activity and is associated with attention-deficit hyperactivity disorder. PNAS 103(50): 19164-19169.

Kim C, Waldman ID, Blakely RD, Kim K (2008a). Functional Gene Variation in the Human Norepinephrine Transporter Association with Attention Deficit Hyperactivity Disorder. Ann NY Acad Sci 1129: 256-260.

Kim JW, Biederman J, McGrath CL, Doyle AE, Mick E, Fagerness J *et al*. (2008b). Further evidence of association between two NET single-nucleotide polymorphisms with ADHD. Molecular Psychiatry 13: 624-630.

Kitamura O (2009). Detection of methamphetamine neurotoxicity in forensic autopsy cases. Legal Medicine 11: 63-65.

Krasnova IN, Cadet JL (2009). Methamphetamine toxicity and messengers of death. Brain Res Rev 60(2): 379-407.

Kristensen AS, Andersen J, Jorgensen TN, Sorensen L, Eriksen J, Loland CJ *et al*. (2011). SLC6 neurotransmitter transporters: structure, function, and regulation. Pharmacological Reviews 63(3): 585-640.

Lam VM, Espinoza S, Gersimov AS, Gainetdinov RR, Salahpour A (2015). In-vivo pharmacology of Trace-Amine Associated Receptor 1. European Journal of Pharmacology 763: 136-142.

Lauder JM, Wilkie MB, Wu C, Singh S (2000). Expression of 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors in the mouse embryo. Int J Devl Neuroscience 18: 653-662.

Lawal HO, Krantz DE (2013). SLC18: Vesicular neurotransmitter transporters for monoamines and acetylcholine. Molecular Aspects of Medicine 34: 360-372.

Lesch KP, Balling U, Gross J, Strauss K, Wolozin BL, Murphy DL, Riederer P (1994). Organization oft he human serotonin transporter gene. J Neural Transm 95: 157-162.

Li Y, Shao C, Zhang D, Zhao M, Lin L, Yan P *et al*. (2006). The Effect of Dopamine D2, D5 Receptor and Transporter (SLC6A3) Polymorphisms on the Cue-Elicited Heroin Craving in Chinese. American Journal of Medical Genetics Part B (Neuropsychiatric Genetics) 141B: 269-273.

Li L, Bao Y, He S, Wang G, Guan Y, Ma D (2016). The Association Between Genetic Variants in the Dopaminergic System and Posttraumatic Stress Disorder. A Meta-analysis. Medicine 95(11): e3074.

Liechti ME, Vollenweider FX (2001). Which neuroreceptors mediate the subjective effects of MDMA in humans? A summary of mechanistic studies. Human Psychopharmacology Clin Exp 16: 589-598.

Lin L, Yee SW, Kim RB, Giacomini KM (2015). SLC transporters as therapeutic targets: emerging opportunities. 14(8): 543-560.

Lin Z, Zhao Y, Chung C, Zhou Y, Xiong N, Glatt C, Isacson O (2010). High regulatability favors genetic selection in SLC18A2, a vesicular monoamine transporter essential for life. FASEB J. 24(7): 2191–2200.

Lindemann L, Meyer CA, Jeanneau K, Bradala A, Ozmen L, Bluethmann H *et al*. (2008). Trace Amine-Associated Receptor 1 Modulates Dopaminergic Activity. Journal of Pharmacology and Experimental Therapeutics 324(3): 948-956.

Liu M, Heng J, Gao Y, Wang X (2016). Crystal structures of MdfA complexed with acetylcholine and inhibitor reserpine. Biophys Rep 2(2-4): 78-85.

Lohr KM, Stout KA, Dunn AR, Wang M, Salahpou A, Guillot TS, Miller GW (2015). Increased Vesicular Monoamine Transporter 2 (VMAT2; Slc18a2) Protects against Methamphetamine Toxicity. ACS Chem Neurosci 6(5): 790-799.

Loi B, Zloh M, De Luca MA, Pintori N, Corkery J, Schifano F. (2017). 4,4′-Dimethylaminorex ("4,4′-DMAR";"Serotoni") misuse: A Web-based study. Hum Psychopharmacol Clin Exp 32: e2575.

Loland CJ, Mereu M, Okunola OM, Cao J, Prisinzano TE, Mazier S *et al*. (2012). R-Modafinil (Armodafinil): A unique dopamine uptake inhibitor and potential medication for psychostimulant abuse. Biol Psychiatry 72(5): 405-413.

Lucchetti J, Marzo CM, Passoni A, Di Clemente A, Moro F, Bagnati R *et al*. (2017). Brain Disposition of cis-para-Methyl-4-Methylaminorex (cis-4,49-DMAR) and Its Potential Metabolites after Acute and Chronic Treatment in Rats: Correlation with Central Behavioral Effects. Journal of Pharmacology and Experimental Therapeutics 361: 492-500.

Luethi D, Trachsel D, Hoener MC, Liechti ME (2017). Monoamine receptor interaction profiles of 4-thio-substituted phenethylamines (2C-T drugs). Neuropharmacology [Epub ahead of print].

Lyles J, Cadet JL (2003). Methylenedioxymethamphetamine (MDMA, Ecstasy) neurotoxicity: cellular and molecular mechanisms. Brain Research Reviews 42: 155-168.

Martin WR, Sloan JW, Sapira JD, Jasinski DR (1971). Physiologic, subjective, and behavioral effects of amphetamine, methamphetamine, ephedrine, phenmetrazine, and methylphenidate in man. Clin Pharmacol Ther 12(2): 245-258.

Matsson P, Fenu LA, Lundquist P, Wisniewski JR, Kansy M, Artursson P (2015). Quantifying the impact of transporters on cellular drug permeability. Trends in Pharmacological Sciences 36(5): 255-262.

Mayer FP, Schmid D, Owens WA, Gould GG, Apuschkin M, Kudlacek O *et al*. (2018). An unsuspected role for organic cation transporter 3 in the actions of amphetamine. Neuropsychopharmacology [epub ahead of print] doi:10.1038/s41386-018-0053-5.

Mayer FP, Burchardt NV, Decker AM, Partilla JS, Li Y, McLaughlin G *et al*. (2017). Fluorinated phenmetrazine "legal highs" act as substrates for high-affinity monoamine transporters of the SLC6 family. Neuropharmacology (Epub ahead of print). DOI: 10.1016/j.neuropharm.2017.10.006.

Mayer FP, Luf A, Nagy C, Holy M, Schmid R, Freissmuth M, Sitte HH (2016a). Application of a Combined Approach to Identify New Psychoactive Street Drugs and Decipher Their Mechanisms at Monoamine Transporters. In Neuropharmacology of New Psychoactive Substances (NPS). Eds Baumann M, Glennon R, Wiley J, Current Topics in Behavioral Neurosciences, vol 32. Springer: Cham, 333-350.

Mayer FP, Wimmer L, Dillon-Carter O, Partilla JS, Burchardt NV, Mihovilovic MD *et al*. (2016b). Phase I metabolites of mephedrone display biological activity as substrates at monoamine Transporters. Brit J Pharmacol 173: 2657-68.

McLaughlin G, Morris N, Kavanagh P, Power J, Twamley B, O'Brien J *et al*. (2015). Synthesis, characterization, and monoamine transporter activity of the new psychoactive substance 3′,4′-methylenedioxy-4-methylaminorex (MDMAR). Drug Test Anal 7: 555-64.

Measham F, Newcombe R (2016). What's so 'new' about new psychoactive substances? Definitions, prevalence, motivations, user groups and a proposed new taxonomy. In: The SAGE Handbook of Drug and Alcohol Studies. Kolind T, Hunt G, Thom B (eds.). Thousand Oaks: Sage.

Melikian HE (2004). Neurotransmitter transporter trafficking: endocytosis, recycling and regulation. Pharmacology & Therapeutics 104: 17-27.

Meririnne E, Ellermaa S, Kankaanpää A, Bardy A, Seppälä T (2004). Pharmacokinetics and Tissue Distribution of the Stereoisomers of 4-Methylaminorex in the Rat. The Journal Of Pharmacology And Experimental Therapeutics 309(3): 1198-1205.

Miller GW, Erickson JD, Perez JT, Penland SN, Mash DC, Rye DB, Levey AI (1999). Immunochemical Analysis of Vesicular Monoamine Transporter (VMAT2) Protein in Parkinson's Disease. Experimental Neurology 156: 138-148.

Miller GW (2011). The Emerging Role of Trace Amine Associated Receptor 1 in the Functional Regulation of Monoamine Transporters and Dopaminergic Activity. J Neurochem 116(2): 164-176.

Miner NB, Elmore JS, Baumann MH, Phillips TJ, Janowsky A (2017). Trace amine-associated receptor 1 regulation of methamphetamine-induced neurotoxicity. NeuroToxicology 63: 57-69.

Mollenhauer HH, Morre DJ, Rowe LD (1990). Alteration of intracellular traffic by monensin; mechanism, specificity and relationship to toxicity. Biochim Biophys Acta 1031: 225-246.

Mueller F, Lenz C, Steiner M, Dolder PC, Walter M, Lang UE *et al*. (2016). Neuroimaging in moderate MDMA use: A systematic review. Neuroscience and Biobehavioral Reviews 62: 21-34.

Murphy DL, Fox MA, Timpano KR, Moya PR, Ren-Patterson R, Andres AM *et al*. (2008). How the serotonin story is being rewritten by new gene-based discoveries principally related to SLC6A4, the serotonin transporter gene, which functions to influence all cellular serotonin systems. Neuropharmacology 55: 932-690.

Nakanishi N, Onozawa S, Matsumoto R, Hasegawa H, Minami N (1995). Vesicular Monoamine Uptake by Digitonin-Permeabilized PC12 Cells: Inhibitory Effect of Neuromodulators and Drugs on the Amine Transport. The Journal of Biochemistry 118: 291-296.

Narendran R, Lopresti BJ, Martinez D, Mason NS, Himes M, May MA *et al*. (2012). In vivo evidence for low striatal vesicular monoamine transporter 2 (VMAT2) availability in cocaine abusers. Am J Psychiatry 169: 55-63.

Nash JF, Yamamoto BK (1992). Methamphetamine neurotoxicity and striatal glutamate release: comparison to 3,4-methylendioxymethamphetamine. Brain Research 581: 237-243.

NCBI (2017). SLC18A2. [Online] Available from: https://www.ncbi.nlm.nih.gov/gene/6571 [Accessed: 23rd December 2017].

Negrei C, Galateanu B, Stan M, Balalau C, Dumitru MLB, Ozcagli E *et al*. (2017). Worldwide legislative challenges related to psychoactive drugs. Journal of Pharmaceutical Science 25:14.

Nirenberg MJ, Liu Y, Peter D, Edwards RH, Pickel VM (1995). The vesicular monoamine transporter 2 is present in small synaptic vesicles and preferentially localizes to large dense core vesicles in rat solitary tract nuclei. Proc Natl AcadSci 92: 8773-8777.

Nizar H, Dargan P, Wood D (2014). Using Internet Snapshot Surveys to Enhance Our Understanding of the Availability of the Novel Psychoactive Substance 4-Methylaminorex and 4,4′-Dimethylaminorex. J Med Toxicol 11(1): 80–84.

Norregaard L, Frederiksen D, Nielsen EO, Gether U (1998). Delineation of an endogenous zinc-binding site in the human dopamine transporter. EMBO J 17: 4266-4273.

North P, Fleischer S (1983). Alteration of Synaptic Membrane Cholesterol/Phospholipid Ratio Using a Lipid Transfer Protein. Journal of Biological Chemistry 258(2): 1242-1253.

Online Research Chemicals Shop (2017). 4,4'-DMAR. [Online] Available from http://onlinechemicalsshops.com/shop/buy-44dmar/ [Accessed: 4th August 2017].

Ono K, Iwanaga Y, Mannami T, Kokubo Y, Tomoike H, Komamura K *et al*. (2003). Epidemiological Evidence of an Association between SLC6A2 Gene Polymorphism and Hypertension. Hypertens Res 26: 685-689.

ONS (2016). Deaths involving legal highs in England and Wales: between 2004 and 2013: UK Office for National Statistics. [Online] Available from https://www.ons.gov.uk/peoplepopulationandcommunity/birthsdeathsandmarriages/deaths/articles/deathsinvolvinglegalhighsinenglandandwales/between2004and2013 [Accessed: 23rd December 2017].

Park JM, Choi MG, Park JA, Oh JH, Cho YK, Lee IS *et al*. (2006). Serotonin transporter gene polymorphism and irritable bowel syndrome. Neurogastroenterol Motil 18: 995-1000.

Parrott AC (2002). Recreational Ecstasy/MDMA, the serotonin syndrome, and serotonergic neurotoxicity. Pharmacology, Biochemistry and Behavior 71: 837-844.

Parsons SM (2000). Transport mechanisms in acetylcholine and monoamine storage. FASEB J 14: 2423-2434.

Pebay-Peyroula E, Dahout-Gonzalez C, Kahn R, Trèzéguet V, Lauquin GJ, Brandolin G (2003). Structure of mitochondrial ADP/ATP carrier in complex with carboxyatractyloside. Nature 426: 39-44.

Penmatsa A, Wang KH, Gouaux E (2013). X-ray structure of dopamine transporter elucidates antidepressant mechanism. Nature 503: 85-91.

Penmatsa A, Wang KH, Gouaux E (2015). X-ray structures of Drosophila dopamine transporter in complex with nisoxetine and reboxetine. Nature Struct Mol Biol 22(6): 506-508.

Perland E, Fredriksson R (2017). Classification systems of secondary active transporters. Trends Pharmacol 38(3): 305-315.

Perland E, Bagchi S. Klaesson A, Fredriksson R (2017). Characteristics of 29 novel atypical solute carriers of major facilitator superfamily type: evolutionary conservation, predicted structure and neuronal co-expression. Open Biol. 7: 170142.

Peter D, Finn P, Klisak I, Liu Y, Kojis T, Heinzmann C *et al*. (1993). Chromosomal Localization of the Human Vesicular Amine Transporter Genes. Genomics 18: 720-723.

Pharmaceutical Chemistry Co. (2017). 4,4'-DMAR. [Online] Available from http://pharma-chem.net/cathinones/4-4-dimethylaminorex-4-4-dmar.html [Accessed: 4th August 2017].

Pifl C, Drobny H, Reither H, Hornykiewicz O, Singer EA (1995). Mechanism of the Dopamine-Releasing Actions of Amphetamine and Cocaine: Plasmalemmal Dopamine Transporter versus Vesicular Monoamine Transporter. Molecular Pharmacology 47: 365-373.

Pifl C, Rajput A, Reither H, Blesa J, Cavada C, Obeso JA *et al*. (2014). Is Parkinson's Disease a Vesicular Dopamine Storage Disorder? Evidence from a Study in Isolated Synaptic Vesicles of Human and Nonhuman Primate Striatum. J Neurosci 24: 8210-8218.

Pifl C, Reither H, Hornykiewicz O (2015). The profile of mephedrone on human monoamine transporters differs from 3,4-methylenedioxymethamphetamine primarily by lower potency at the vesicular monoamine transporter. Eur J Pharmacol 755: 119-126.

Pramod AB, Foster J, Carvelli L, Henry LK (2013). SLC6 transporters: Structure, function, regulation, disease association and therapeutics. Molecular Aspects of Medicine 34: 197-219.

Provenzi L, giorda R, Beri S, Montirosso R (2016). SLC6A4 methylation as an epigenetic marker of life adversity exposures in humans: A systematic review of literature. Neurosci Biobehav Rev 71: 7-20.

PubChem (2017). 4,4'-DMAR. [Online] https://pubchem.ncbi.nlm.nih.gov/compound/20741615#section=Top [Accessed: 4th August 2017].

Raiteri M, Angelini F, Levi G (1974). A simple apparatus for studying the release of neurotransmitters from synaptosomes. Eur J Pharmacol 25: 411–414.

Ramamoorthy S, Shippenberg TS, Jayanthi LD (2011). Regulation of monoamine transporters: role of transporter phosphorylation. Pharmacol Ther 129(2): 220-238.

Rask-Anderson M, Masuram S, Fredriksson R, Schiöth HB (2013). Solute carriers as drug targets: Current use, clinical trials and prospective. Molecular Aspects of Medicine 34: 702-710.

Rawson R, Gonzales R, Ling W (2010). Clinical Aspects of Methamphetamine. In: Addiction Medicine: Science and Practice. Johnson BA (ed.). New York: Springer.

Reddit (2007). Researchchemicals. [Online] Available from: https://www.reddit.com/r/researchchemicals/ [Accessed: 23rd December 2017].

Reimer RJ (2013). SLC17: a functionally diverse family of organic anion transporters. Mol Aspects Med 34(2-3): 350-359.

Reith MEA, Blough BE, Hong WC, Jones KT, Schmitt KC, Baumann MH *et al*. (2015). Behavioral, biological, and chemical perspectives on atypical agents targeting the dopamine transporter. Drug and Alcohol Dependence 147: 1-19.

Reuter P, Pardo B (2016). Can new psychoactive substances be regulated effectively? An assessment of the British Psychoactive Substances Bill. Addiction 112: 25-31.

Reuter P, Pardo B (2017). New psychoactive substances: Are there any good options for regulating new psychoactive substances?. International Journal of Drug Policy 40: 117-122.

Revel FG, Moreau J, Gainetdinov RR, Ferragud A, Velázquez-Sánchez C, Sotnikova TD *et al*. (2012). Trace Amine-Associated Receptor 1 Partial Agonism Reveals Novel Paradigm for Neuropsychiatric Therapeutics. Biol Psychiatry 72: 934-942.

Ripke, S, Wray NR, Lewis CM, Hamilton SP, Weissman MM, Breen G *et al*. (2013). A mega-analysis of genome-wide association studies for major depressive disorder. Mol Psychiatry 18(4): 497-511.

Risch N, Herrell R, Lehner T, Liang K, Eaves L, Hoh J *et al*. (2009). Interaction Between the Serotonin Transporter Gene (5-HTTLPR), Stressful Life Events, and Risk of Depression. A Meta-analysis. JAMA 301(23): 2462-2471.

Robinon TE, Becker JB (1986). Enduring Changes in Brain and Behavior Produced by Chronic Amphetamine Administration: A Review and Evaluation of Anima1 Models of Amphetamine Psychosis. Brain Research Reviews 11: 157-198.

Rosenauer R, Luf A, Holy M, Freissmuth M, Schmid R, Sitte HH (2013). A Combined Approach Using Transporter-Flux Assays and Mass Spectrometry to Examine Psychostimulant Street Drugs of Unknown Content. ACS Chem Neurosci (4): 182−190.

Rothman RB, Ayestas MA, Dersch CM, Baumann MH (1999). Aminorex, fenfluramine, and chlorphentermine are serotonin transporter substrates. Implications for primary pulmonary hypertension. Circulation 100: 869-75.

Rothman RB, Baumann MH, Dersch CM, Romero DV, Rice KC, Carroll I, Partilla JS (2001). Amphetamine-Type Central Nervous System Stimulants Release Norepinephrine More Potently Than They Release Dopamine and Serotonin. Synapse 39: 32-41.

Rothman RB, Baumann MH (2006). Balance between Dopamine and Serotonin Release Modulates Behavioral Effects of Amphetamine-Type Drugs. Ann NY Acad Sci 1074: 245-260.

Rothman RB, Ananthan S, Partilla JS, Saini SK, Moukha-Chafiq O, Pathak V, Baumann MH (2015). Studies of the Biogenic Amine Transporters 15. Identification of Novel Allosteric Dopamine Transporter Ligands with Nanomolar Potency. Journal of Pharmacology and Experimental Therapeutics 353: 529-538.

Rudnick G, Krämer R, Blakely RD, Murphy DL, Verrey F (2014). The SLC6 transporters: perspectives on structure, functions, regulation, and models for transporter dysfunction. Eur J Physiol 466: 25-42.

Rudnick G, Clark J (1993). From synapse to vesicle: the reuptake and storage of biogenic amine neurotransmitters. Biochim Biophys Acta 1144: 249-263.

Sager JJ, Torres GE (2011). Proteins Interacting with Monoamine Transporters: Current State

and Future Challenges. Biochemestry 50: 7295-7310.

Sakloth F, Kolanos R, Mosier PD, Bonano JS, Banks ML, Partilla JS *et al*. (2015). Steric parameters, molecular modelling and hydropathic interaction analysis of the pharmacology of para-substituted methcathinone analogues. Brit J Pharmacol 172: 2210-2218.

Sara SJ, Bouret S (2012). Orienting and Reorienting: The Locus Coeruleus Mediates Cognition through Arousal. Neuron 76: 130-141.

Scanlon SM, Williams DC, Schloss P (2001). Membrane Cholesterol Modulates Serotonin Transporter Activity. Biochemistry 40: 10507-10513.

Scherfler C, Schwarz J, Antonini A, Grosset D, Valldeoriola F, Marek K *et al*. (2007). Role of DAT-SPECT in the Diagnostic Work Up of Parkinsonism. Movement Disorders 22(9): 1229-1238.

Schifano F, Orsolini L, Duccio Papanti G, Corkery JM (2015). Novel psychoactive substances of interest for psychiatry. World Psychiatry 14: 15-26.

Schinkel AH (1999). P-Glycoprotein, a gatekeeper in the blood–brain barrier. Advanced Drug Delivery Reviews 36: 179-194.

Schlessinger A, Matsson P, Shima JE, Pieper U, Yee SW, Kelly L *et al*. (2010). Comparison of human solute carriers. Protein Science 19: 412-428.

Schlessinger A, Geier E, Fan H, Irwin JJ, Shoichet BK, Giacomini KM, Sali A (2011). Structure-based discovery of prescription drugs that interact with the norepinephrine transporter, NET. PNAS 108(38): 15810-15815.

Schmid Y, Hysek CM, Simmler LD, Crockett MJ, Quednow BB, Liechti ME (2014). Differential effects of MDMA and methylphenidate on social cognition. Journal of Psychopharmacology 28(9): 847-856.

Scholze P, Zwach J, Kattinger A, Pifl C, Singer EA, Sitte HH (2000). Transporter-mediated release: a superfusion study on human embryonic kidney cells stably expressing the human serotonin transporter. J Pharmacol Exp Ther 293: 870–878.

Scholze P, Norregaard L, Singer EA, Freissmuth M, Gether U, Sitte HH (2002). The role of zinc ions in reverse transport mediated by monoamine transporters. J Biol Chem 277: 21505–21513.

Schönherr H, Cernak T (2013). Profound Methyl Effects in Drug Discovery and a Call for New C-H Methylation Reactions. Angew Chem Int Ed 52: 12256-12267

Schroeter S, Apparsundaram S, Wiley RG, Miner LH, Sesack SR, Blakely RD (2000). Immunolocalization of the Cocaine- and Antidepressant-Sensitive l-Norepinephrine Transporter. Journal of Comparative Neurology 420: 211-232.

Segal DS, Mandell AJ (1974). Long-Term Administration of d-Amphetamine: Progressive Augmentation of Motor Activity and Stereotypy. Pharmacology, Biochemistry and Behavior 2: 249-255.

Seiden LS, Sabol KE (1993). Amphetamine: effects on catecholamine systems and behavior. Annu Rev Pharmacol Toxicol 32: 639-677.

Setola V, Hufeisen SJ, Grande-Allen KJ, Vesely I, Glannon RA, Blough B *et al*. (2003). 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy") induces fenfluramine-like proliferative actions on human cardiac valvular interstitial cells in vitro. Mol Pharmacol 63: 1223-1229.

Shannon JR, Flattem NL, Jordan J, Jacob G, Black BK, Biaggioni I *et al*. (2000). Orthostatic Intolerance and Tachycardia associated with Norephinephrine-transporter deficiency. New England Journal of Medicine 342(8): 541-549.

Shenzhen Chemicals (2017). 4,4'-DMAR. [Online] Available from http://shenzhenchemicals.com/product/44-dimethylaminorex-44-dmar-cas-1445569-01-6/ [Accessed: 4th August 2017].

Shi L, Quick M, Zhao Y, Weinstein H, Javitch JA (2008). The Mechanism of a Neurotransmitter:Sodium Symporter—Inward Release of Na+ and Substrate Is Triggered by Substrate in a Second Binding Site. Molecular Cell 30: 667-677.

Sikander A, Sinha SK, Prasad KK, Rana SV (2015). Association of Serotonin Transporter Promoter Polymorphism (5-HTTLPR) with Microscopic Colitis and Ulcerative Colitis. Dig Dis Sci 60: 887-894.

Simmler LD, Buser TA, Donzelli M, Schramm Y, Dieu LH, Huwyler J *et al*. (2013). Pharmacological characterization of designer cathinones in vitro. British Journal of Pharmacology 168: 458-470.

Simmler LD, Rickli A, Hoener MC, Liechti ME (2014). Monoamine transporter and receptor interaction profiles of a new series of designer cathinones. Neuropharmacology 79: 152-160.

Singh SK, Yamashita A, Gouaux E (2007). Antidepressant binding site in a bacterial homologue of neurotransmitter transporters. Nature 448: 952-956.

Sitte HH, Scholze P, Schloss P, Pifl C, Singer EA (2000). Characterization of carrier-mediated efflux in human embryonic kidney 293 cells stably expressing the rat serotonin transporter: a superfusion study. J Neurochem 74: 1317–1324.

Sitte HH, Freissmuth M (2015). Amphetamines, new psychoactive drugs and the monoamine transporter cycle. Trends in Pharmacological Sciences 36(1): 41-50.

Solis E, Partilla JS, Sakloth F, Ruchala I, Schwienteck KL, De Felice LJ *et al*. (2017). N-Alkylated analogs of 4-methylamphetamine (4-MA) differentially affect monoamine transporters and abuse liability. Neuropsychopharmacology 42: 1950-1961.

Solovieff N, Roberts AL, Ratanatharathorn A, Haloosim M, De Vivo I, King AP *et al*. (2014). Genetic Association Analysis of 300 Genes Identifies a Risk Haplotype in SLC18A2 for Post-traumatic Stress Disorder in Two Independent Samples. Neuropsychopharmacology 39: 1872-1879.

Soussan C, Kjellgren A (2016). The users of Novel Psychoactive Substances: Online survey about their characteristics, attitudes and motivations. International Journal of Drug Policy 32: 77-84.

Spencer T, Heiligenstein JH, Biederman J, Faries DE, Kratochvil CJ, Conners CK, Potter WZ (2002). Results from 2 proof-of-concept, placebo-controlled studies of atomoxetine in children with attention-deficit/hyperactivity disorder. J Clin Psychiatry 63(12): 1140-1147.

Spencer TJ, Biederman J, Faraone SV, Madras BK, Bonab AA, Dougherty DD *et al*. (2013). Functional Genomics of Attention-Deficit/ Hyperactivity Disorder (ADHD) Risk Alleles on Dopamine Transporter Binding in ADHD and Healthy Control Subjects. Biol Psychiatry 74(2): 84-89.

Spies M, Knudsen GM, Lanzenberger R, Kasper S (2015). The serotonin transporter in psychiatric disorders: insights from PET imaging. Lancet Psychiatry 2: 743-755.

Staal RGW, Sonsalla PK (2000). Inhibition of Brain Vesicular Monoamine Transporter (VMAT2) Enhances 1-Methyl-4-phenylpyridinium Neurotoxicity In Vivo in Rat Striata. Journal of Pharmacology and Experimental Therapeutics 293(2): 336-342.

Staal RGW, Yang J, Hait WN, Sonsalla PK (2001). Interactions of 1-methyl-4-phenylpyridinium and other compounds with P-glycoprotein: relevance to toxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Brain Research 910: 116-125.

Steinkellner T, Freissmuth M, Sitte HH, Montgomery T (2011). The ugly side of amphetamines: short- and long-term toxicity of 3,4-methylenedioxymethamphetamine (MDMA, 'Ecstasy'), methamphetamine and D-amphetamine. Biological Chemistry 392: 103-115.

Stevens A, Fortson R, Measham F, Sumnall H (2015). Legally flawed, scientifically problematic, potentially harmful: The UK Psychoactive Substance Bill. International Journal of Drug Policy 26: 1167-1170.

Stockner T, Montgomery TR, Kudlacek O, weissensteiner R, Ecker GF, Freissmuth M, Sitte HH (2013). Mutational Analysis of the High-Affinity Zinc Binding Site Validates a Refined Human Dopamine Transporter Homology Model. PLoS Comput Biol 9(2): e1002909.

Sucic S, Dallinger S, Zdrazil B, Weissensteiner R, Jorgensen TN, Holy M *et al*. (2010). The N Terminus of Monoamine Transporters Is a Lever Required for the Action of Amphetamines. Journal of Biological Chemistry 285(14): 10924-10938.

Südhof TC, Rizo J (2011). Synaptic vesicle exocytosis. Cold Spring Harb Perspect Biol 3: a005637.

Sulzer D, Sonders MS, Poulsen NW, Galli A (2005). Mechanisms of neurotransmitter release by amphetamines: A review. Progress in Neurobiology 75: 406-433.

Sundberg BE, Waag E, Jacobsson J, Stephansson O, Rumaks J, Svirskis S *et al*. (2008). The Evolutionary History and Tissue Mapping of Amino Acid Transporters Belonging to Solute Carrier Families SLC32, SLC36, and SLC38. J Mol Neurosci 35: 179-193.

Surrat CK, Persico AM, Yang X, Edgar SR, Bird GS, Hawkins AL *et al*. (1993). A human synaptic vesicle monoamine transporter cDNA predicts posttranslational modifications, reveals chromosome 10 gene localization and identifies TaqI RFLPs. FEBS J 3: 325-330.

Suwijn SR, de Bruin K, de Bie RMA, Booij J (2014). The role of SPECT imaging of the dopaminergic system in translational research on Parkinson's disease. Parkinsonism and Related Disorders 20S1: 184-186.

Takahashi N, Miner LL, Sora I, Ujike H, Revay RS, Kostic V *et al*. (1997). VMAT2 knockout mice: Heterozygotes display reduced amphetamine-conditioned reward, enhanced amphetamine
locomotion, and enhanced MPTP toxicity. Proc Natl Acad Sci 94: 9938-9943.

Takeuchi T, Harada Y, Moriyama S, Furuta K, Tanaka S, Miyaji T *et al*. (2017). Vesicular Polyamine Transporter Mediates Vesicular Storage and Release of Polyamine from Mast Cells. Journal of Biological Chemistry 292(9): 3909-3918.

Tamura K, Hayashi S (2017). Atomistic modeling of alternating access of a mitochondrial ADP/ATP membrane transporter with molecular simulations. PLOS ONE 12(7): e0181489.

Tellioglu T, Robertson D (2001). Genetic or acquired deficits in the norepinephrine transporter: current understanding of clinical implications. Expert Rev Mol Med: 1-10.

Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC (1987). Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. Proc Natl Acad Sci 84: 7735-7738.

Tong JHS, Cummins TDR, Johnson BP, McKinley L, Pickering HE, Fanning P *et al*. (2015). An Association Between a Dopamine Transporter Gene (SLC6A3) Haplotype and ADHD Symptom
Measures in Nonclinical Adults. Am J Med Genet Part B 168B: 89-96.

Turner JJD, Parrott AC (2000). 'Is MDMA a Human Neurotoxin?': Diverse Views from the Discussants. Neuropsychology 42: 42-48.

Underhill SM, Wheeler DS, Li M, Watts SD, Ingram SL, Amara SG (2014). Amphetamine Modulates Glutamatergic Neurotransmission through Endocytosis of the Excitatory Amino Acid Transporter EAAT3 in Dopamine Neurons. Neuron 83(2): 404-416.

United Nations (2017). World Drug Report. Booklet 1. [Online] Available from: https://www.unodc.org/wdr2017/index.html [Accessed: 23rd December 2017].

UNODC (2017). NPS. [Online] Available from: https://www.unodc.org/LSS/Page/NPS/ [Accessed: 23rd December 2017].

Vandenberg DJ, Persico AM, Hawkins AL, Griffin CA, Li X, Jabs EW *et al*. (1992). Human Dopamine Transporter Gene (DAT1) Maps to Chromosome 5pl 5.3 and Displays a VNTR. Genomics 14: 1104-1106.

Vandenberg DJ, Thompson MD, Cook EH, Bendahhou E, Nguyen T, Krasowski MD *et al*. (2000). Human dopamine transporter gene: coding region conservation among normal, Tourette's disorder, alcohol dependence and attention-deficit hyperactivity disorder populations. Molecular Psychiatry 5: 283-292.

Van der Knaap LJ, Riese H, Hudziak JJ, Verbiest M, Verhulst FC, Oldehinkel AJ, van Oort F (2015). Adverse Life Events and Allele-Specific Methylation of the Serotonin Transporter Gene (SLC6A4) in Adolescents: The TRAILS Study. Psychosomatic Medicine 77: 246-255.

Vaughan RA, Foster JD (2013). Mechanisms of dopamine transporter regulation in normal and disease states. Trend in Pharmacological Sciences 34(9): 489-496.

Verrico CD, Miller GM, Madras BK (2007). MDMA (Ecstasy) and human dopamine, norepinephrine, and serotonin transporters: implications for MDMA-induced neurotoxicity and treatment. Psychopharmacology 189: 489-503.

Vicentic A, Jones DC (2007). The CART (Cocaine- and Amphetamine-Regulated Transcript) System in Appetite and Drug Addiction. Journal of Pharmacology and Experimental Therapeutics 320(2): 499-506.

Wang KH, Penmatsa A, Gouaux E (2015). Neurotransmitter and psychostimulant recognition by the dopamine transporter. Nature 521: 322-327.

Watson CJ, Venton BJ, Kennedy RT (2006). In Vivo Measurements of Neurotransmitters by Microdialysis Sampling. Analytical Chemistry: 1391-1399.

WHO (2017). ATS. [Online] Available from: http://www.who.int/substance_abuse/facts/ATS/en/ [Accessed: 23rd December 2017].

Wimalasena K (2011). Vesicular Monoamine Transporters: Structure-Function, Pharmacology, and Medicinal Chemistry. Med ResRev 31(4): 483-519.

Wittgenstein L (1986). Philosophical Investigations. Translated by Anscombe GEM. Oxford: Blackwell.

Yaffe D, Radestock S, Shuster Y, Forrest LR, Schuldiner S (2013). Identification of molecular hinge points mediating alternating access in the vesicular monoamine transporter VMAT2. PNAS 110(15): e1332-4.

Yaffe D, Vergara-Jaque A, Forrest LR, Schuldiner S (2016). Emulating proton-induced conformational changes in the vesicular monoamine transporter VMAT2 by mutagenesis. PNAS 113(47): e7390-e7398.

Yu S, Zhu L, Shen Q, Bai X, DI X (2015). Recent Advances in Methamphetamine Neurotoxicity Mechanisms and Its Molecular Pathophysiology. Behavioural Neurology 2015: 103969.

Zhai D, Li S, Zhao Y, Lin Z (2014). SLC6A3 is a risk factor for Parkinson's disease: A meta-analysis of sixteen years' studies. Nerosci Lett 564: 99-104.

Zhang Z, Wu Y, Wang Z, Dunning FM, Rehfuss J, Ramanan D *et al*. (2011). Release mode of large and small dense-core vesicles specified by different synaptotagmin isoforms in PC12 cells. Mol Biol Cell 22(13): 2324-2336.

Zheng G, Dwoskin LP, Crooks PA (2006). Vesicular Monoamine Transporter 2: Role as a Novel Target for Drug Development. AAPS J 8(4): e682-e692.

Zheng Y, Russell B, Schmierer D, Laverty R (1997). The Effects of Aminorex and Related Compounds on Brain Monoamines and Metabolites in CBA Mice. J Pharm Pharmacol 49: 89-96.

Zhou J (2004). Norepinephrine transporter inhibitors and their therapeutic potential. Drugs Future 29(12): 1235-1244.

Zhou Z, Zhen J, Karpowich NK, Goetz RM, Law CJ, Reith MEA, Wang D (2007). LeuT-Desipramine structure reveals how antidepressants block neurotransmitter reuptake. Science 317: 1390-1393.

Zhu H, Appel DI, Gründemann D, Markowitz JS (2010). Interaction of organic cation transporter 3 (SLC22A3) and amphetamine. J Neurochem 114(1): 142-149.

Zolk O, Ott C, Fromm MF, Schmieder RE (2012). Effect of the rs168924 Single-Nucleotide Polymorphism in the SLC6A2 Catecholamine Transporter Gene on Blood Pressure in Caucasians. Journal of Clinical Hypertension 14(5): 293-298.

Zucchi R, Chiellini G, Scanlan TS, Grandy DK (2006). Trace amine-associated receptors and their ligands. British Journal of Pharmacology 149: 967-978.

List of tables and figures
