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CLASSICAL PSYCHEDELICS AND NBOMES AS SEROTONIN 2_B RECEPTOR AGONISTS: VALVULOPATHOGENIC SIGNALING PATHWAYS AND CARDIAC SAFETY CONCERNS

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Abstract

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ROIHUVUO; ELIAS: Classical psychedelics and NBOMes as serotonin 2_B receptor agonists: Valvulopathogenic signaling pathways and cardiac safety concerns

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The use of classical psychedelics such as psilocybin, lysergic acid diethylamide (LSD) and mescaline has continued despite prohibition. Classical psychedelics are becoming more approved as they are entering clinical trials. N-(2-methoxybenzyl) derivatives of phenethylamine (NBOMes) have appeared as designer drugs and caused fatal intoxications — such cases have been associated with classical compounds. Psychedelics are 5-HT2$_A$ agonists, but they are distinguished by biased agonism and additional serotonergic mechanisms.

Treatment with serotonin agonists for longer than three months has been associated with valvular heart disease (VHD). Mechanistic studies have shown that excess stimulation of the 5-HT2$_B$ receptor in valvular interstitial cells (VICs) initiates extracellular regulating kinase 2 (ERK2) signaling leading to a mitogenic response followed by valvular remodeling, compromised function and ultimately VHD. Therefore, classical psychedelics and possibly NBOMes could be valvulopathogens, depending on their 5-HT2$_B$ agonism and their intracellular pathways.

The aim of the study was to evaluate what are the mechanisms of action and their differences for classical psychedelics and NBOMes based on the current literature. Additionally, neurotoxicity related to 5-HT1$_A$ and 5-HT2$_A$ agonism was screened. The study question was answered through a systematic literature review, by using methods and orientation originating primarily from information sciences. Several NBOMes and all classical psychedelics except mescaline were 5-HT2$_B$ agonists. Psilocybin and NBOMes had nonspecific 5-HT2$_B$ activity. However, none of the compounds were associated with cases of VHD in the literature. LSD had low activity on a valvulopathogenic ERK2 readout. Clinical implications are unlikely due to infrequent dosing and low concentrations. The valvulopathogenic risks of classical psychedelics in known usage contexts are estimated to be low based on their mechanisms, but data on sensitive readouts is required. Ongoing clinical trials utilizing classical psychedelics are not likely to increase the risk of VHD.

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Klassisiin psykedeeleihin kuuluvat muun muassa psilosybiini, lysergihapon dietyyliamidi (LSD) sekä meskaliini, ja yhdisteitä käytetään edelleen huumausaineina. Niiden kliininen tutkimus on yleistymässä. Suoraan klassisten psykedeelien farmakodynamiikasta johtuneita kuolemantapauksia ei tunneta, kun taas fenetyyliamiinista johdettuihin N-2-metoksibentsyyli-muuntohuumeisiin (NBOMe) liittyy useita myrkytyskuolemia. Psykedeelien pääasiallinen vaikutusmekanismi on 5-HT2_A-agonismi. Vaikka klassiset psykedeelit vaikuttavat useisiin serotoniinireseptoreihin, ne ovat vähemmän toksisia kuin NBOMe-yhdisteet. Yhdisteitä on siksi arvioitava tapauskohtaisesti.

Yli kolme kuukautta kestävä serotoniiniagonistien käyttö on yhdistetty sydämen läppävikoihin. 5-HT2_B-agonismi lisää sydänläppien interstitiaalisolujen jakautumista ja fibroosia mm. ERK2-signalointiketjun välityksellä. Tavoitteena oli selvittää kirjallisuudesta, millaisia klassisten psykedeelien ja NBOMe-yhdisteiden vaikutusmekanismit ovat, ja onko näiden yhdisteiden raportoitu aiheuttaneen läppävikoja. Edelleen pyrittiin erittelemään 5-HT1_A-ja 5-HT2_A -mekanismeja, jotka selittäisivät eroja yhdisteiden neurotoksisuudessa. Tutkimus toteutettiin systemaattisena kirjallisuuskatsauksena, käyttäen pääosin informaatiotieteen menetelmiä.

Klassiset psykedeelit meskaliinia lukuun ottamatta olivat 5-HT2_B agonisteja. Läppävikatapauksia ei esiintynyt tutkimuksen aineistossa, eikä meskaliinilla teoriassa ole läppävikariskiä. LSD:llä havaittiin hyvin heikkoa aktiivisuutta ERK2-parametrissa. Tämän kliininen merkitys arvioitiin erittäin vähäiseksi, koska LSD:tä käytetään päihteenä tyypillisesti harvoin ja pitoisuudet elimistössä ovat erittäin matalia. Myös psilosybiinin sekä NBOMe-yhdisteiden läppävikariski päihdekäytössä lienee erittäin alhainen, mutta tarkempi mekanismiin pohjautuva ennuste edellyttäisi riskiä ennakoivan ERK2-aktiivisuuden määrittämistä. Tämän tutkimuksen perusteella pidetään epätodennäköisenä, että klassisten psykedeelien käyttö nykyisissä kliinisissä tutkimuksissa kohottaisi läppävikojen riskiä.

CONTENTS

ABBREVIATIONS

5-HT$\mathbf{x}_\mathbf{y}$: 5-hydroxytryptamine receptor, where numeric **x** denotes the family and character **y** marks the subtype within the family

DMT: dimethyltryptamine, a natural compound and a psychedelic found in various plants and animals with a long history of ceremonial use among indigenous people of South America

ERK2: extracellular regulating kinase 2, often used interchangeably with MAPK2

IP: inositol phosphate

LSD: lysergic acid diethylamide, a semisynthetic ergot derivative and a classical psychedelic used in neuropharmacological research and preliminary as an adjunct in psychotherapy

MAPK2: mitogen activated protein kinase 2

NBOMe: N-(2-methoxybenzyl) derivatives of psychedelic phenethylamine compounds, used in neuropharmacological research, as radioligands and occasionally as drugs despite high risk of toxicity

VHD: drug induced valvular heart disease

VIC: valvular interstitial cell

VEC: valvular endothelial cell

ECM: extracellular matrix

1. INTRODUCTION

Serotonergic functions have been an important target in pharmacology for decades. The substance serotonin was initially found in the gastrointestinal system and blood controlling motility and vascular tone, and eventually in the brain (Nichols and Nichols 2008). The compound was first called enteramine due to the intestinal origin, but it was found to be identical with the substance isolated from serum and eventually naming convention settled on serotonin. Additionally, the inadvertent interaction of human serotonin system and naturally occurring ergot alkaloids has endured since historical times. These compounds have occurred in crops and caused ergotism.

The more precise manipulation of the brain's serotonin system became available in western medicine with the discovery of compounds such as lysergic acid diethylamide (LSD), psilocybin, and mescaline. Psilocybin and mescaline are natural compounds and have been used for centuries or even millennia in traditional medicine and religious practices whereas LSD was first synthetized in 1938 (Hofmann 1979, Nichols 2016). These drugs form the group of classical psychedelics.

During the 1950's and early 60's legitimate research on psychedelics was conducted as LSD was distributed by Sandoz as Delysid and psilocybin as Indocybin (Nichols 2018, Geiger et al. 2018). The nature and rarity of severe adverse effects in clinical settings was recognized in 1960 (Cohen 1960), but only two years later the situation was aggravated by illicit trade and use (Cohen and Ditman 1962). The severity of adverse effects and toxicity in supervised use was subsequently evaluated with similar conclusions (Strassmann 1984, Halpern and Pope 1999), but psychedelics had already been prohibited in the late 60's mainly because of the cultural and political turmoil they were associated with (Nichols 2016).

After being largely a taboo for several decades, classical psychedelics are becoming increasingly popular in medical research (Liechti 2017, Bogenschutz and Ross 2018, Hynninen et al. 2020). This re-emerging interest has been so tremendous that "a psychedelic renaissance" (Sessa 2018) has been acknowledged and seen as a new era dawning in psychiatry (Nutt 2019). Clinical trials with modern protocols have yielded encouraging results in depression (Andersen et al. 2021). Other emerging indications include chronic pain (Castellanos et al. 2020) and cluster headache

(Schindler et al. 2015), which is reminiscent of the original usage context of ergot alkaloids. There is also ample evidence of a new usage pattern called microdosing, in which regular sub-perceptual doses are used (Kuypers et al. 2019). According to its proponents, microdosing has a positive impact on performance and pathological conditions (Passie 2019). Although microdoses are very small (e.g., 5-20 µg of LSD), they are taken far more frequently than substantially larger doses in traditional use. This more frequent usage pattern may have unpredictable effects on the risk profiles of these compounds.

Pharmacologically all classical psychedelics share an essential feature: they are 5-$HT2_A$ receptor agonists or partial agonists in the human body (Nichols 2016). Along with the 5-$HT2_A$ receptor, many psychedelics activate the 5-$HT2_B$ subtype which regulates both cardiovascular and neural functions such as development of the heart, valvulopathogenic responses (Hutcheson et al. 2011) and functioning of the raphe nuclei (Barnes et al. 2021). New research also indicates that 5-$HT2_B$ receptor may be relevant for profibrotic actions taking place in fibrosing interstitial lung diseases (Löfdahl et al. 2020). The desired psychological effects may come with the potential cardiovascular adverse effects, although the potential between different psychedelics may vary, and their risks and mechanisms are poorly characterized. This is the justification of the study.

The narrative literature review in chapter 2 summarizes the functions of serotonin and psychedelics, which are followed by a description of the anatomy and pathophysiology of heart valves. These themes are then conjoined to elucidate the role of serotonin signaling and the possible role of psychedelics in drug induced valvulopathies. In the experimental part, the literature is systematically reviewed to gain an understanding of the pharmacodynamics of the psychedelics, and its potential for toxic outcomes.

The purpose of the systematic review that follows is twofold. Firstly, to seek whether classical psychedelics and novel synthetic phenethylamine derivatives called NBOMes (N-(2-methoxybenzyl) share direct pharmacodynamic mechanisms, and intracellular pathways, or do these two groups of compounds differ. Secondly, to assess whether these compounds have a potential to cause drug induced valvular heart disease (VHD) via their possible 5-$HT2_B$ agonism. With this, it is also assessed if the use of psychedelics has already been associated with VHD. Additionally, neurotoxicological profiles are discussed.

2. LITERATURE REVIEW

Here I introduce the current cultural and scientific state of psychedelics, then proceed to describe the basics of serotonergic signaling and how it is related to the primary effects of these drugs in sections 2.2 and 2.3. Thereafter this review focuses on the functional anatomy and pathology of heart valves with the emphasis on conditions caused by serotonin agonists. The mechanism of drug induced valvular heart disease (VHD) is also discussed in section 2.6.

2.1 PSYCHEDELICS

Psychedelics were studied extensively during the 1950s and 60s (Nichols 2016). Research relied on their effects on the mind; hence the descriptive name psychedelics (mind-manifesting) which was coined by Humphrey Osmond in 1957 (Nichols 2016). They have also been labeled hallucinogens despite not consistently causing hallucinations as their primary effect, and earlier as psychotomimetics referring to behavioral alterations thought to resemble those seen in psychotic states. Each definition bears connotations to medical trends and as such they can be viewed as reflections of the cultural atmosphere surrounding this controversial topic. Psychedelics are viewed as psychopharmacologically unique rather than a composite of primary features of other drug classes (Jaffe 1990), and their subjection to culture dependent arbitrary and loaded nomenclature is questionable.

In the 1960s psychedelics were embedded into the western counterculture leading to a widespread unauthorized use. This was accompanied by an undertone of being a threat to the political and sociocultural status quo, consistent with the tendency of these drugs to loosen rigid patterns of thought and behavior (Nichols 2016). Eventually both clinical use and research of these compounds was halted nearly completely, and the views of acceptable, ethical, and justified psychopharmacological paradigms were fundamentally changed. In practice this manifested as a systematic rejection of 5-HT2_A receptor agonists in drug development regardless of whether a compound had or did not have psychedelic potency, while at the same time indirect serotonergic agonists, such as reuptake or monoamine-oxidase inhibitor antidepressants gained popularity and eventually became one of the cornerstones of psychopharmacology.

The use of classical psychedelics has prevailed despite prohibition. Krebs and Johansen (2013) have estimated that in the US population over 30 million people have used psychedelics whereas in Finland the lifetime prevalence of use in 2018 was 2,7% and 3,3% for LSD and psilocybin respectively (Terveyden ja hyvinvoinnin laitos 2018). At the same time, the development of unregulated alternatives as so-called designer drugs may have been accelerated.

Prohibition has not diminished the demands of drug markets, and these demands have then been fulfilled by substituting classical drugs with novel psychoactive compounds. When compared to classical drugs, their safety profiles are often poorly recognized by distributors and users alike. Highly potent and easily trafficked synthetic phenethylamine derivatives called NBOMes have been distributed as counterfeit LSD (Martins et al. 2017). As a result, NBOMes have directly caused multiple deaths in a relatively short history of use (Zawilska et al. 2020). In contrast, direct deaths related to classical psychedelics are virtually nonexistent even though they have been used without medical supervision for decades (van Amsterdam et al. 2011, Nichols 2016, Nichols and Grob 2018).

2.2 SEROTONIN AND RECEPTORS

Serotonin is widely present in nature. It has been found in various organisms including protozoans, plants (Erland et al. 2015), nervous systems and venoms of insects (Rillich and Stevenson 2018), and ultimately as a hormone and neurotransmitter in humans and other mammals. Interestingly, even certain species of fungi such as *Panaelous* (Nugent et al. 2004), *Psilocybe* (Lenz et al. 2021) and *Claviceps* (Gerhards et al. 2014) utilize tryptophan to produce compounds that closely resemble serotonin and mimic its actions in other organisms, thus eliciting behavioral responses. These compounds may have multiple biological roles, but their existence adds to the ubiquitous nature of serotonergic modulation. Thus, serotonin has an essential role in the complex interplay of biological systems from cellular level to interspecific interaction.

Chemically serotonin is an indole alkaloid catabolized from the amino acid tryptophan and consists of indole nucleus attached with an aminoalkyl (ethylamine) side chain (Fig 1), making it an indolalkylamine as well as a monoamine neurotransmitter along with for example the

phenethylamine derivative dopamine. Serotonin resembles especially psilocin (the active form of psilocybin) and bufotenin. The alkylamine side chain of indole psychedelics is typically methylated whereas it is often preserved in psychedelic phenethylamines (Fig 1).

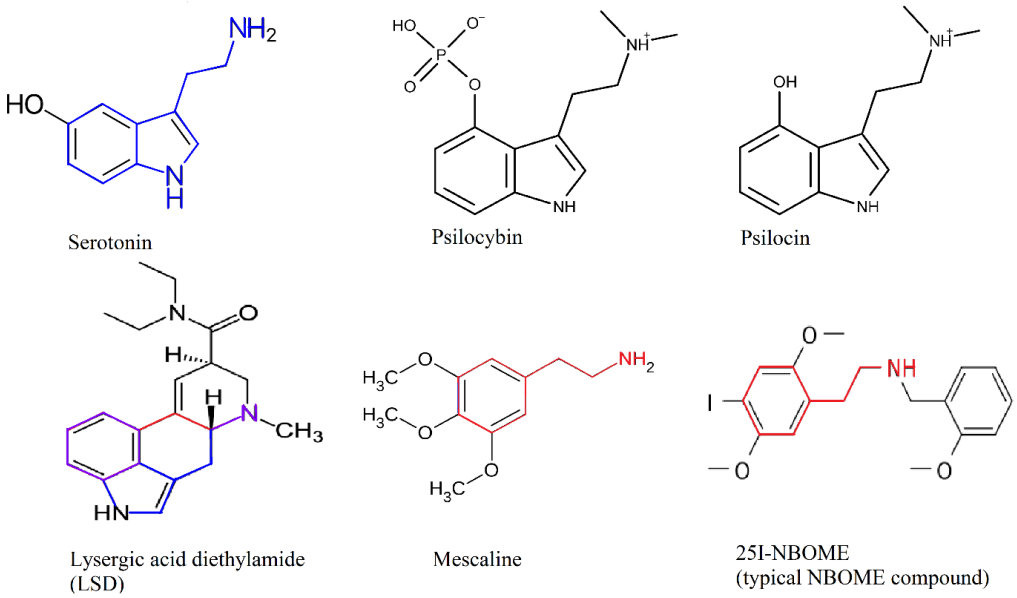

Figure 1. Structural formulas of serotonin, indolalkylamines psilocybin and psilocin, lysergic acid derivative LSD and phenethylamine derivatives mescaline and 25I-NBOMe. Tryptamine, phenethylamine, and their overlapping scaffolds are highlighted with blue, red, and magenta.

Serotonin regulates cortical and vascular development already during embryogenesis (Nebigil et al. 2001, Vitalis et al. 2007). The basis of the serotonergic system in the human brain is a set of raphe ("seam") nuclei in the brainstem, which synthesize serotonin and project extensively to both limbic and cortical areas (Jacobs and Azmitia 1992).

Serotonin is involved in several pathological states or symptoms, including nausea, vascular headaches, migraines, mood disorders and insomnia. Serotonin participates in controlling such functions as smooth muscle contraction, cardiovascular tone, mitogenesis, reflexes, perception, emotion responses, aggression, memory, and cognition. Manipulation of these functions can induce a variety of effects. These include therapeutic or adverse effects depending on the context. The development of triptans for migraine, selective serotonin reuptake inhibitors for depression and atypical antipsychotics for severe mental disorders seem both well-justified and inevitable.

Seven families of receptors form the basis of serotonergic signaling in humans, and several of these families consist of subtypes such as 5-HT2$_{A-C}$ (Barnes et al. 2021). Of these 15 serotonin receptor subtypes all but one are G-protein coupled and have modulatory actions on neurotransmission.

G-proteins are coupled to the intracellular loops of the receptor helices (Nichols and Nichols 2008). Binding of a ligand changes the receptor's conformation and initiates actions of secondary messengers such as G-proteins and β-arrestins. Therefore, in some cases activation of a single receptor may lead to multiple signaling pathways.

Cavero and Guillon (2014) describe agonists binding to a receptor but activating one or more pathways as perfectly and imperfectly biased, respectively (Fig 2). Biased agonism will be further examined in chapters 2.3.1 and 2.6 since it is relevant in neuronal effects of psychedelics and especially in the context of 5-HT2$_B$ agonism and valvulopathogenic signaling, respectively.

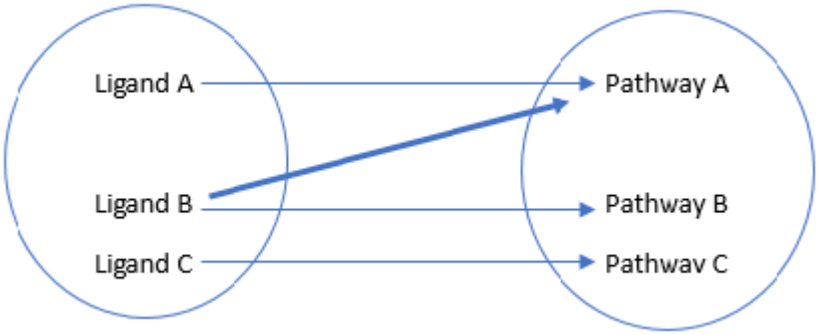

Figure 2. Functional selectivity as a relation. Ligand A and C are perfectly biased whereas B is imperfectly biased.

2.3 PSYCHEDELICS AS 5-HT2 AGONISTS

Psychedelics act as 5-HT2_A agonists which is believed to be the main mechanism of their psychedelic action. In this chapter I briefly review whether the agonism of psychedelics differs from the agonism of serotonin. This approach is later applied to 5-HT2_B signaling in section 2.6.

Marek and Aghajanian (1996) have identified LSD as having a maximal 5-HT2_A activity of around 40% in relation to serotonin, the activity of which LSD is able to block in high concentrations. LSD has a limited ability to induce neuronal firing in relation to serotonin. This is in accordance with the definition of LSD as a 5-HT2_A partial agonist, and this categorization applies to other classical psychedelics as well.

Partial agonistic activity could explain their ability to excite cortical pyramidal neurons (Aghajanian and Marek 2000) without causing evident epileptic activity (Lambe and Aghajanian 2006). On the other hand, NBOMes are associated with increased risk of seizures (Zawilska 2020). NBOMes are highly efficient 5-HT2_A agonists which is in accordance with more profound effects on neuronal firing.

In addition, psilocybin and very low concentrations of LSD can reduce serotonin turnover in the forebrain by suppressing raphe nuclei, which send serotonergic afferents to frontal areas and the rest of the brain (Aghajanian and Marek 1999a). This action is mediated by the 5-HT1_A autoreceptor.

5-HT1_A agonism has been thought to contribute to hyperthermia of serotonin syndrome based on animal models, but this is now attributed primarily to 5-HT2_A receptor stimulation instead (Francescangeli et al. 2019). It is conceivable that the risk of serotonin syndrome is attenuated by 5-HT2_A partial agonism and the ability to suppress raphe nuclei via 5-HT1_A agonism. 5-HT1_A agonism also inhibits synaptic secretion of serotonin (Cerrito and Raiteri 1979) and attenuates the excitability of pyramidal cells (Araneda and Andrade 1991, Tanaka and North 1993). These features are typical to indolamine psychedelics and possibly to the phenethylamine derivative mescaline which are not neurotoxic, whereas NBOMes seem to lack these features and are neurotoxic.

2.3.1 PSYCHEDELICS AND BIASED 5-HT2$_A$ AGONISM

Stimulation of the 5-HT2$_A$ receptor can activate at least two different and independent intracellular signaling pathways (Kurrasch-Orbaugh et al. 2003). These are the G-protein regulated phospholipase C and phospholipase A_2 pathways which exhibit different ratios between compounds. Different 5-HT2$_A$ agonists seem to activate these pathways in varying potencies and efficacies, with serotonin as a reference that stimulates both pathways maximally.

LSD and psilocybin are less efficient than serotonin at the 5-HT2$_A$ receptor (Kurrasch-Orbaugh et al. 2003). The half maximal effective concentration (EC_{50}) of LSD was 20 nM and the intrinsic activity was 56% for phospholipase A_2 activation whereas the EC_{50} of serotonin was 83 nM. LSD had a reverse profile for phospholipase C activation with an EC_{50} of 9.8 nM and intrinsic activity of 22% whereas the EC_{50} of serotonin was 120 nM. Psilocin — the active form of psilocybin — activated phospholipase A_2 with an EC_{50} of 86 nM and intrinsic activity of 42% whereas EC_{50} of serotonin was 83 nM. For phospholipase C activation the EC_{50} of psilocybin was 2300 nM and the EC_{50} of serotonin was 120 nM. Mescaline has been found to stimulate both pathways nearly equipotently with an intrinsic activity of around 50% (Moya et al. 2007). Based on these criteria all classical psychedelics act as partial agonists as suggested by neurophysiological studies.

The novel psychoactive compounds differ from the classical ones regarding 5-HT2$_A$ biased agonism. As for NBOMes, 25I-NBOMe is found to have an intrinsic activity of 90% at phospholipase C making it nearly a full agonist in this regard (Hansen et al. 2014), which also applies to several related compounds (Jensen et al. 2017).

Since lisuride, the non-psychedelic N,N-diethylurea analog of LSD, has increased potency and intrinsic activity for phospholipase A_2, phospholipase C activation is suggested as the differentiating response between psychedelic and non-psychedelic 5-HT2$_A$ agonists (Kurrasch-Orbaugh et al. 2003). The low efficacy of LSD has been problematic as it does not seem to correlate with the perceived potency and capacity to induce subjective effects in humans. Clinically relevant doses of LSD produce plasma concentrations in the range of 3-12 nM (Dolder et al. 2017), which induce minor phospholipase C activation, i.e., less than 10% (Kurrasch-Orbaugh et al. 2003).

Serotonin, being a balanced agonist, should be capable of evoking psychedelic reactions during elevated concentrations if phospholipase C activation was a sufficient cause. Moreover, psilocin's low potency for phospholipase C activation suggests that effects elicited by physiologically relevant plasma concentrations do not require substantial activation of this pathway. Although serotonin is associated with mental perturbations, it is not considered a psychedelic per se, and serotonin syndrome is not considered to be a psychedelic state. Therefore, activation of phospholipase C likely is not the decisive feature of psychedelic 5-$HT2_A$ agonists.

2.3.2 PSYCHEDELICS AND β-ARRESTIN BIAS

Arrestins are molecules integral to G-protein coupled receptors, including the 5-HT2 receptor family. They are bound to intracellular regions of receptors (Schmid et al. 2008) and have distinct downstream effects (Liu et al. 2015). Signal transduction of activated G-protein receptor depends on the recruitment of intracellular mechanisms, which in turn depends on the receptor conformation. Differentially binding ligands may initiate different sets of responses via changes on the ratio of G-protein and arrestin recruitment. Therefore, the existence of G-protein and β-arrestin biased agonists has been suggested as an explanation for only certain 5-$HT2_A$ agonists being psychedelic.

Experimentally, 5-$HT2_B$ receptor has been used as a surrogate for 5-$HT2_A$ receptor. These experiments have shown that LSD has a higher ability to recruit β-arrestin2 when compared with other psychoactive ergolines (Wacker et al. 2017). By inference from the 5-$HT2_B$ receptor, it has been suggested that a high potency of β-arrestin2 recruitment is a surrogate for psychedelic activity at the 5-$HT2_A$ receptor. On the other hand, serotonin can recruit this pathway as well, whereas 5-methoxy-DMT cannot despite considered a psychedelic compound (Schmid and Bohn 2010). Nevertheless, Pottie et al. (2020) have evaluated psychedelic potencies of new psychoactive compounds based on β-arrestin2 recruitment. According to their results several NBOMes are more potent and efficient on β-arrestin2 recruitment than LSD or mescaline, which would be in accordance with their known pharmacological and toxicological profiles.

2.3.3 CONCLUSIONS ON PSYCHEDELICS AND BIASED 5-HT2$_A$ AGONISM

Here I briefly conceptualize three possible relations of the 5-HT2$_A$ agonism of serotonin and psychedelics (Fig 3). Firstly, psychedelics are not considered to damage the intracellular machinery or to be cytotoxic which could lead to nonspecific signaling. Secondly, psychedelic reaction does not seem to require the whole set of responses elicited by serotonin. Therefore, in terms of 5-HT2$_A$ signaling psychedelics can be understood as a subset (Fig 3A) or a proper subset (Fig 3B) which is necessarily only a part of the full set of serotonin.

If psychedelics are considered a subset, then equivalent intracellular signaling pathways are activated in different proportions which explains the differences between psychoactivity of serotonin and psychedelic 5-HT2$_A$ agonists. If psychedelics are a proper subset and serotonin is not considered to elicit a psychedelic response, an additional possibility is proposed: serotonin and psychedelics share the signaling necessary for a psychedelic reaction, but regarding serotonin this could be suppressed by a concomitant signaling (Fig 3C).

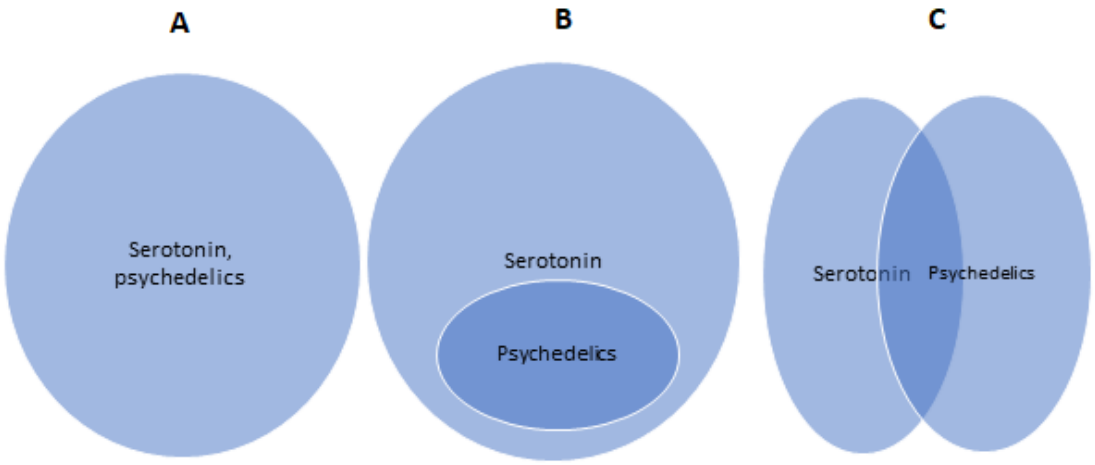

Figure 3. Conceptualization of the 5-HT2$_A$ signaling of serotonin and psychedelics. A: the signaling cascades of psychedelics are a subset of the cascades of serotonin and they initiate the same pathways but in different proportions. B: psychedelics are a proper subset of serotonin and initiate some but not all the signaling cascades of serotonin. C: signaling of serotonin and psychedelics intersect so that both have common and unique cascades.

In addition, there exists the possibility that signaling profiles elicited by serotonin and psychedelics intersect. In this case there would be shared and non-shared signaling. However, it is not yet certain whether there exists a single signaling feature which differentiates psychedelic 5-HT2_A agonists from serotonin and non-psychedelic 5-HT2_A agonists. It remains to be seen whether individual psychedelics can be classified more precisely in relation to serotonin based on differences in signaling profiles, and whether a single signaling cascade is enough to initiate a psychedelic reaction.

Overall, a vast body of literature exists about the detailed effects of psychedelic 5-HT2_A agonists. It is challenging to concisely present the most important information, and it is not in the scope of this thesis to perform a thorough literature review from this aspect. Thus, the reader is advised to rely on the work of Nichols (2016) and López-Giménez and González-Maeso (2018) if additional information is required.

2.4 HEART VALVES AND VALVULAR DISEASE

The properly functioning heart valve supports a unidirectional blood flow, and therefore pathological conditions may involve either obstructed outflow or regurgitation. Obstructed outflow is caused by stenosis whereas thickening of valve leaflets prevents them from closing and leads to regurgitation or leaky valve (Fig 4) which is prone to prolapsing (Hinton and Yutzey 2011). Other causes of regurgitation include e.g. myocardial infarction and aortic root dilation.

In both obstructed blood flow and regurgitation, the ventricles must compensate by contracting more forcibly, but this unsustainable demand leads to compromised ventricular function. This may result in congestive heart failure manifesting as angina, fainting and ultimately death. Since pharmacological treatments capable of reversing heart valve disease are not currently available (Hutcheson et al. 2011), surgical replacement of valves with artificial ones or grafts from animals remains as the standard treatment protocol (Hinton and Yutzey 2011).

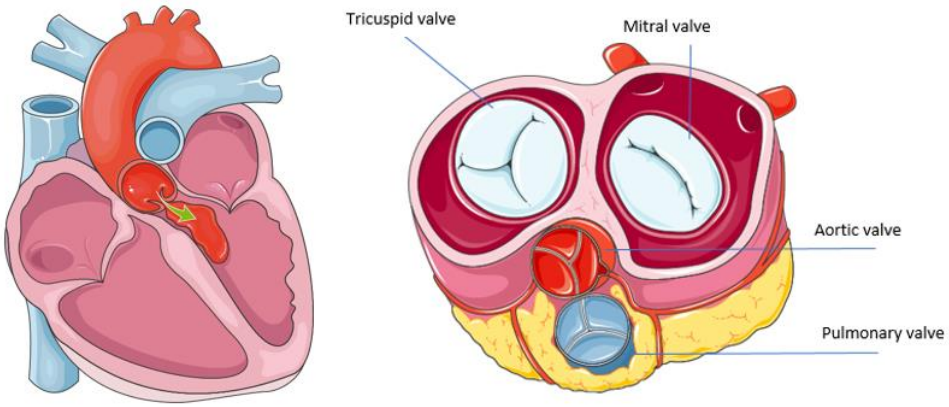

Figure 4. On the left: a schematic of improperly closing aortic valve causing regurgitation. On the right: cross section of a schematic heart exposing the valves (Servier Medical Art).

The prevalence of aortic valve disease is estimated between 1.4% (Hutcheson 2011) and 2.5% in the USA (Hinton and Yutzey 2011). Exact estimates are difficult to make since cases may be asymptomatic, but the risk increases with age so that aortic sclerosis is present in 29% of people at the age of 65 or older. Aortic sclerosis increases the risk of death from cardiovascular causes by approximately 50% and without signs of significantly compromised left ventricular outflow.

2.4.1 ANATOMY AND FUNCTION OF HEART VALVES

The structurally similar aortic and pulmonary valves are called semilunal since they consist of three leaflets resembling half-moon when closed (Hutcheson et al. 2011). When the left ventricle contracts during systole, the pressure becomes higher than in the aorta and causes the aortic valve to open, whereas higher pressure in the right ventricle than in the pulmonary artery opens the pulmonary valve. Both valves are closed when the ventricles relax during diastole and the ventricular pressures fall below those in the aortic and pulmonary arteries. As the blood flow along the aortic wall decelerates and ultimately reverses direction, it generates vortices behind the aortic valve leaflets to facilitate closing and minimizes reverse flow to the ventricle (Ayoub et al. 2016).

All surfaces of the valve leaflet are covered by valvular endothelial cells (VECs), but valvular interstitial cells (VICs) are the most prevalent cells in all valvular layers (Elangbam 2010). They maintain the integrity of heart valves and repair possible damages, and as such resemble both

fibroblasts and smooth muscle cells which express the 5-$HT2_B$ receptor (Hutcheson et al. 2011). Therefore, they have an essential role in ensuring valvular integrity, although there is the possibility of hypertrophy if this delicate balance were to become disturbed. According to Barnes et al. (2021), especially the mitogenic response of VIC are believed to be involved in 5-$HT2_B$ mediated valvular heart disease. The proposed cellular mechanisms of drug induced VHD are presented in more detail in chapter 2.6.

Since VICs produce the extracellular matrix responsible (ECM) for the durability of valves, alterations in the ECM reflect the viability of VICs (Elangbam 2010). ECM is composed of proteoglycans, collagens, and elastin. The structural units of proteoglycans are glycosaminoglycan chains. Maintaining the structural integrity of ECM is a process involving synthesis as well as degradation and reorganization, which are regulated by matrix metalloproteinases.

Histopathological processes of valves can be classified as myxomatous or fibrotic (Hinton and Yetzeu 2014). In myxomatous type collagen is degraded and elastic fibers are fragmented along with proteoglycan accumulation, which leads to a loss of valvular structure and leaking due to floppiness. Fibrotic type is associated with serotonin agonism and differentiated by collagen accumulation and degradation of proteoglycan which together lead to stiffening of the valve. This causes problems by restricting the opening of valve, eventually leading to narrowing of passages between cardiac compartments (stenosis). Cases of advanced stenosis are often accompanied by calcification which hardens the valves even further, thus increasing severity of the condition.

A thorough review of the anatomy of heart valves is provided by Misfeld and Sievers (2007), and their mechanobiology is examined in detail by Ayoub et al. (2016). In summary, complex interplay of mediators on multiple abstraction levels influences the integrity of heart valves.

2.5 KNOWN VALVULOPATHOGENIC DRUGS

In the 1960's overuse of serotonergic antimigraine drugs dihydroergotamine and methysergide was reported to induce cardiac and pulmonary fibrosis (Graham 1967). Elevated serotonin levels due to carcinoid syndrome were known to cause fibrosis during this time and use of ergot-based

drugs continued with additional safety precautions. Methysergide was also used to treat gastrointestinal symptoms of carcinoid syndrome since it acts as a partial serotonin agonist and blocks the effects of serotonin in high concentrations (Melmon et al. 1965).

Concerns of drug induced cardiac fibrosis became prominent in 1997 when VHD was observed in 24 women following the use of weight-loss combination drug of fenfluramine and phentermine (Cavero and Guillon 2014). The association of VHD with ergot-based drugs re-emerged when the antiparkinsonian drug pergolide (Pritchett et al. 2002) and later cabergoline were suspected to cause VHD, although the risk seems to be significantly dose-dependent with increasing cumulative dose corresponding to higher risk (Stiles et al. 2021).

The use of fenfluramine was widespread: the number of “Fen-Phen” prescriptions totaled over 18 million in 1996 (Connolly et al. 1997), and Hopkins and Polukoff (2003) estimate that during 1996 and 1998 2.5 % of US population had used appetite suppressing drugs, mostly fenfluramine and phentermine. Hypothetically, if each treatment lasted 3 months and regurgitation occurred in 1 of 8 patients as stated by Sachdev et al. (2002), there would have been over 2 million cases of “Fen-Phen” induced valve regurgitation based on the number of prescriptions.

It is difficult to estimate the actual exposure to fenfluramine based solely on prescriptions, and drug courses may have been shorter than 90 days. More importantly, Dahl et al. (2008) attributed 0.44 % of their study sample requiring heart valve surgery directly due to fenfluramine exposure, whereas Fournier and Zureik (2012) estimated that the fenfluramine prodrug benfluorex caused 1300 deaths due to valvular insufficiency in France during 1976 and 2009.

The histopathological examination of “Fen-Phen” induced VHD closely resembled that of carcinoid heart disease which is caused by a neoplasia of enterochromaffin cells producing abnormally high concentrations of serotonin in the blood (Cavero and Guillon 2014). According to Cosyns et al. (2013), drug induced VHD differs from rheumatic and primary calcific VHD by lack of calcification, commissural fusion, and stenosis. They state that this condition predominantly affects the left sided valves, while Cavero and Guillon (2014) consider both left and right sided valves to be affected.

Drug induced VHD is claimed to be exhibited in 6% to 25% percent of patients treated with either fenfluramine or methysergide for longer than 6 months, and about half of these have shown improvement with discontinuation of the drug so that the symptomatic pulmonary hypertension has subsided (Cavero and Guillon 2014). Conversely, according to Silberstein (2008) methysergide induces fibrotic changes in 1 of 5000 patients, and Cosyns et al. (2009) claimed that only sporadic case reports of ergotamine or methysergide causing VHD exist. Bhattacharyya et al. (2009) state the figures of 6 to 25% only for appetite suppressants with incidences increasing from 3 months onwards. The figures concerning fenfluramine seem to have fluctuated over the years, since Sachdev et al. (2002) found that fenfluramine treatment longer than 90 days increased the prevalence of VHD from 5.9% to 12% when compared with drug free obese patients (OR 2.2, CI 1.7-2.7).

The manufacture and use of both fenfluramine and methysergide have since ceased almost completely and the use of practically all ergot derivatives has been restricted (European Medicines Agency (EMA) 2013, 2014) as an additional safety precaution, although for example nicergoline has not been found to cause fibrosis (Fioravanti et al. 2014). Fenfluramine has been repurposed as an add-on treatment for Dravet's syndrome and is scarcely used (Ceulemans et al. 2012), serving as an example of how a drug's utility should not be determined solely based on its pharmacology but by contextual risk-benefit assessment as well.

2.5.1 MIXED MONOAMINERGIC ERGOT DERIVATIVES

As for the mixed monoamine agonist cabergoline used to treat Parkinson's disease, the incidence of valve regurgitation was estimated to increase 5 folds after 4.2 years of use with an excess risk of 21 per 10 000 per year, contrasted with a control incidence of 5.5 per 10 000 per year when no dopamine agonist was used (Schade et al. 2007). The incidence rate ratio was 4.9 for cabergoline treatment lasting longer than six months with a confidence interval as wide as 1.5 to 15.6, but in this study the total number of cases in cabergoline users was only six.

Treatment of prolactinomas with cabergoline requires significantly lower doses than Parkinson's disease (Stiles et al. 2021). A median weekly dose of 2.1 mg for 27 months (median cumulative dose 56 mg) was not associated with cardiac endpoints, whereas in the study population of Schade et al. (2007) the daily dose was over 3 mg in 80% of patients with cabergoline associated

valvulopathies. This discrepancy indicates that the valvulopathogenic effects may be related only to high dose therapy.

On the other hand, in their meta-analysis Simonis et al. (2007) claimed that moderate-to-severe valvular changes were evident in 26% of those using dopaminergic ergot derivatives along with prevalence of 10% in controls. The higher prevalence in controls when compared to 5% of Sachdev et al. (2002) is likely due to older patients. Simonis et al. (2007) describe this figure as convincing based on the prevalence in 70-year-old persons whereas the average age in studies included by Sachdev et al. (2002) was only 46 years.

The risk of certain mixed monoaminergic ergot derivatives might be overestimated since Cosyn et al. (2009) consider the association of bromocriptine with drug induced VHD "doubtful". This is in accordance with Jähnichen et al. (2005) proposing that the valvulopathogenic potential of bromocriptine differs from pergolide and cabergoline on the pharmacodynamical level. According to a systematic review by Tran et al. (2015) regurgitation was seen in 7.8% (13/167) of Parkinson's disease patients treated with bromocriptine, but 69% (9/13) of these were found in the same study. Frequency for cabergoline was 11.6%, 8.7% for pergolide and 6% for untreated healthy controls providing much lower frequencies than that of Simonis et al. (2007). When the apparent requirement of continuous treatment for three months is considered, it seems rather unlikely that the ergot derivative LSD or other psychedelics could substantially increase the risk of drug induced VHD unless used in nearly inconceivable amounts.

2.5.2 5-HT2_B AGONISM OF KNOWN VALVULOPATHOGENIC DRUGS

According to Cavero and Guillon (2014), a direct receptor mediated explanation for drug induced VHD is justified because neither methysergide, dopaminergic ergot derivatives nor fenfluramine routinely elevate plasma serotonin levels. Although fenfluramine has been shown to acutely elevate plasma serotonin levels, the increase is estimated insufficient to cause VHD and the 5-HT2_B agonist metabolite norfenfluramine is considered the causative agent (Zolkowska et al. 2008). Metabolic activation needs to be accounted also in the context of methysergide. It is rapidly metabolized into methylergometrine, which in turn has a longer elimination half-life and higher plasma concentrations (Bredberg et al. 1986).

Other drugs suspected or known to cause VHD were recognized as 5-HT2_B receptor agonists as well (Fitzgerald et al. 2000, Rothman et al. 2000). Setola et al. (2003) discovered that stimulation of the 5-HT2_B receptor in VICs could be the cause of mitogenic responses leading to valvular remodeling. It is found in aortic and mitral valves (Bhattacharyya et al. 2009), and the 5-HT2_A receptor has also been found in rat cardiac fibroblasts where it mediates their activation (Yabanoglu et al. 2009). Of these the 5-HT2_B receptor subtype is presumed necessary for the development of drug induced VHD. Additional evidence has accumulated from clinical use of lisuride (Hutcheson et al. 2009). Lisuride has been used for decades without a single case of drug induced or other VHD reported although it is a dopaminergic 5-HT2_A agonist and 5-HT2_B antagonist.

2.6 5-HT2_B RECEPTOR SIGNALING IN DRUG INDUCED VALVULAR HEART DISEASE

The 5-HT2_B receptor has a vital role in cardiac development (Nichols and Nichols 2008) despite initially presumed absent in the heart (Bonhaus et al. 1995). Structural and functional disturbances of the heart are seen in 5-HT2_B inactivated mice (Barnes et al. 2021), and developmental defects cause lethality in embryos. Cardiomyocytes exhibit structural deficits at the intercellular junctions and impaired contractility which cause partial lethality in neonates. Dilation of the left ventricle and impaired systolic function are seen in animals surviving to adulthood.

Chronic treatment with the sympathetic stimulant isoproterenol induces cardiac hypertrophy in mice, and 5-HT2_B antagonists can prevent this by preventing the generation of myocardial superoxide. The reader is advised to rely on the review of Barnes et al. (2021) if more information on the role of serotonin in cardiac development is required, since this part of the study concerns primarily the role of 5-HT2_B receptor in valvular remodeling in adulthood.

Gustafson et al. (2005) have shown that long-term administration of serotonin in high doses induces valvulopathies in rats. In addition, plasma serotonin levels are elevated in carcinoid heart disease which is characterized by pathological changes of the heart valves (Cavero and Guillon 2014). Moreover, Droogmans et al. (2009) detected valvular lesions after long-term treatment with a high dose of pergolide and treatment with 5-HT2_B antagonist cyproheptadine

prevented the formation of lesions. Therefore, 5-HT2$_B$ agonism is considered the key receptor mechanism of serotonergic drug induced valvulopathies.

The other known key mediators of heart valve disease may suggest which intracellular pathways are relevant in the development of 5-HT2$_B$ mediated valvulopathies. Cytokine transforming growth factor β1 (TGF-β1) is a well-characterized mediator (Hutcheson et al. 2011, Cavero and Guillon 2014), and 5-HT2$_B$ agonism is thought to crosstalk with the pathways of TGF-β1. According to Hutcheson et al. (2011), 5-HT2$_B$ agonism is directly responsible for the proliferation of VICs and accumulation of fibrotic ECM (Fig 5).

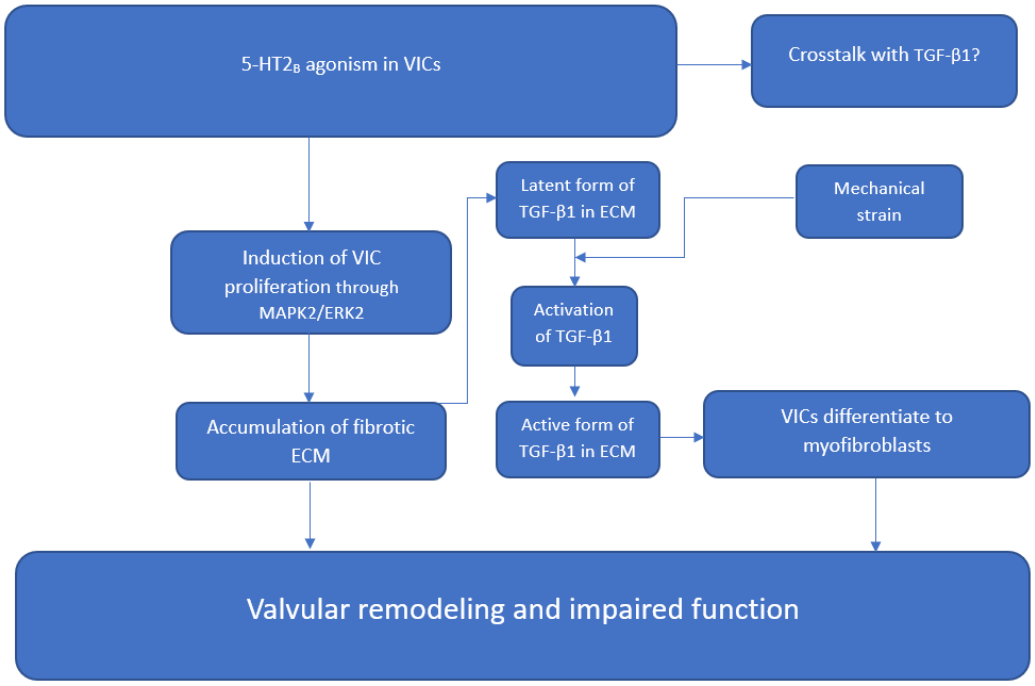

Figure 5. Stages of the valvulopathogenic process initiated by 5-HT2B receptor agonism in heart valves based on the description of Hutcheson et al. (2011).

The cytokine TGF-β1 induces transcription of mitogenic effector genes (Hutcheson et al. 2011). This increases synthesis of glycosaminoglycans and collagen which then cause valvular remodeling. TGF-β1 activates VICs into myofibroblasts and affects cellular processes through transcription factors known as Smads. TGF-β1 is stored in ECMs in latent form and can be activated by multiple cues, including myofibroblast contraction. Once activated, the subsequent

signaling may also lead to synthesis of the latent form. This can then be activated by VICs, which creates a self-sustained process.

When a compound's effect on valvulopathogenic signaling is evaluated, TGF-β1 activity is not used directly. Instead, other intracellular readouts are screened, which commonly include Ca^{2+}-release, phospholipase C mediated inositol phosphate (IP) accumulation and mitogen activated protein kinase 2 (MAPK2) phosphorylation, the nuclear factor of activated T-cells (NFAT) and β-arrestin recruitment (Cavero and Guillon 2014). The MAPK2, which is also known as extracellular signal regulating kinase 2 (ERK2), is activated by both β-arrestin and G-protein dependent signaling. The onset of β-arrestin dependent activation is longer but results in more lasting signaling (Eishingdrelo et al. 2015). Additionally, the β-arrestin pathway promotes receptor internalization and may have antagonistic activity on G-protein coupled signaling pathways.

Table 1. Intracellular readouts and their sensitivity to drug induced VHD for known valvulopathogens. MAPK2/ERK2 (Cavero and Guillon 2014) and NFAT (Papoian et al. 2017) are considered the most sensitive although they are not decisive.

Intracellular readout	**Sensitive to drug induced VHD**	**Insensitive to drug induced VHD**
MAPK2 / ERK2	**X**	
NFAT	**X**	
Ca^{2+} release		**X**
IP accumulation		**X**
β-arrestin recruitment		**X**

Signaling pathways can converge. For example, the activation of phospholipase C increases the amount of inositol triphosphate, causing the release of intracellular Ca^{2+} from endoplasmic reticulum to cytosol (Mognol et al. 2002). This leads to a signaling cascade activating the phosphatase calcineurin and starts the NFAT-activation. Therefore, states of the interconnected cellular signaling can be probed from discrete intracellular readouts.

Additional codependent signaling exists. For instance, calcineurin-NFAT and MEK1-ERK1/2 pathways are possible at least in cardiomyocytes (Sanna et al. 2004), but information about their role in cardiac fibroblasts is scarce. According to Papoian et al. (2017), definite patterns of functional selectivity categorically differentiating valvulopathogenic and non-valvulopathogenic 5-$HT2_B$ agonists have not been found.

Taken together, the 5-$HT2_B$ signaling is essential in cardiac development. In adulthood the integrity of cardiac functioning is partly regulated by the 5-$HT2_B$ receptor. Excess stimulation of the 5-$HT2_B$ receptor in VICs initiates MAPK2/ERK2 signaling leading to a mitogenic response followed by valvular remodeling, compromised function and ultimately VHD. Both 5-$HT2_A$ and 5-$HT2_B$ receptors may be involved in VHD as suggested by Kekewska et al. (2011), but evidence (Hutcheson et al. 2011) favors the 5-$HT2_B$ mediated rationale. G-protein and β-arrestin pathways

can have different effects on intracellular signaling, and biased agonism has been suggested as an explanation for certain 5-HT2$_B$ agonists being markedly valvulopathogenic.

2.6.1 CONCLUSIONS ON KNOWN VALVULOPATHOGENIC DRUGS AND THE ROLE OF 5-HT2$_B$ RECEPTOR

The association of 5-HT2$_B$ receptor and drug induced VHD has subsequently been studied further and it is well established in the literature. However, there seems to be some inconsistencies and possibly overestimations regarding the frequency of drug induced VHD and especially the role of various ergot derivatives (Table 2). The association with a low-dose therapy of e.g. cabergoline has not been established and sporadic cases resulting from migraine treatment with ergotamine have likely involved medication overuse. Ergot alkaloids have been implied as the only known cause besides serotonergic anorexigens. However, it seems that the use of ergot alkaloids has resulted in relatively few confirmed cases of serious drug induced VHD.

Table 2. Summary of drugs commonly associated with drug induced VHD.

Drug	Mechanism	5-HT2$_B$ agonism	Indication	VHD
Bromocriptine	Mixed monoamine agonist	Partial agonism? [j]	Parkinson's disease, hyperprolactinemia	Unclear [a, h]
Cabergoline	Mixed monoamine agonist	Yes	Parkinson's disease, hyperprolactinemia	Yes (Parkinson's disease) [a, b, h]
Ergotamine	Mixed monoamine agonist	Yes	Migraine	Case reports [a] Yes [c] Likely [a] Medication overuse?
Dihydroergotamine	Mixed monoamine agonist	Yes	Migraine	Yes [c]

Fenfluramine	Serotonin release and agonism	Yes	Weight loss	Yes [c, d] Strongest association (Prodrug benfluorex) [i]
Methysergide / methylergonovine	Serotonin antagonist / partial agonist	Yes (methylergonovine)	Migraine prophylaxis	Case reports [a] Yes [c] Likely [a] 0.02% [e]
Lisuride	Mixed monoamine agonist	Antagonist	Parkinson's disease	No [f]
Pergolide	Mixed monoamine agonist	Yes	Parkinson's disease	Yes [a, g, h]

a: (Cosyns et al. 2009), b: (Schade et al. 2007), c: (Cavero and Guillon 2014), d: (Sachdev et al. 2002), e: (Silberstein 2008), f: (Hutcheson et al. 2009), g: (Simonis et al. 2007), h: (Tran et al. 2015), i: (Fournier and Zureik 2012), j: (Jähnichen et al. 2005)

Prolonged use of various serotonergic drugs has been associated with valvulopathies which has led to withdrawal from markets or additional safety precautions. These include the serotonin releaser and agonist fenfluramine (Fitzgerald et al. 2000) and antiparkinsonian ergoline drugs sharing certain structural and pharmacological features with LSD (Rothman and Baumann 2009, Caputo et al. 2015).

Ultimately, the association of drug induced VHD has been strongest with fenfluramine. Fibrotic changes caused by fenfluramine have been restricted to heart valves, whereas methysergide has also been associated with retroperitoneal fibrosis (Rothman et al. 2000). Heart valves may be the most susceptible site, since in the year 2000 treatment duration in methysergide cases might have been significantly longer than in fenfluramine cases. Initially it seemed that fenfluramine affected primarily the aortic valve whereas methysergide and ergotamine had caused mitral

regurgitation (Rothman et al. 2000), but since then fenfluramine has been shown to disturb interstitial cell signaling in mitral valve as well (Connolly et al. 2009).

The risk has been dose-dependent and increases with treatment duration so that a minimum of three months has preceded symptoms. Most cases have been mild-to-moderate, but severe cases requiring valve replacement surgery have occurred. 5-$HT2_B$ antagonists have been found to attenuate fibrotic processes in myofibroblasts (Löfdal et al. 2016), but it is not known whether this might reverse structural changes caused by therapeutic use of 5-$HT2_B$ agonists.

2.7 ADDITIONAL SEROTONERGIC DRUGS WITH POSSIBLE RELEVANCE

Concerns about 5-$HT2_B$ agonism and cardiac safety have reached such an extent that the development of 5-$HT2_B$ agonists has been specifically banned by the Food and Drug Administration (FDA) regardless of the indication (Barnes et al. 2021). Many relatively common drugs such as the decongestant xylometazoline and antihypertensive ADHD medication guanfacine have been recognized as 5-$HT2_B$ agonists, raising concerns about their safety (Huang et al. 2009). Several triptans such as sumatriptan, almotriptan and zolmitriptan share the N,N-dimethylethylamine side chain and indole nucleus with psilocybin and psilocin (Vries et al. 1999, Telft-Hansen et al. 2000), and with eletriptan they have occasionally been reported to have 5-$HT2_B$ affinity (Soldin et al. 2013).

Definite information on the 5-$HT2_B$ affinities of these triptans is scarce. For example, it has been stated that sumatriptan does not bind to 5-$HT2_B$ (Schmuck et al. 1996), and the developers of almotriptan have reported an extensive binding profile except for 5-$HT2_B$ (Gras et al. 2002). This has not been considered problematic since all triptans are intended only for occasional use and none of them has been associated with VHD. Metabolic cleavage of certain triptans' ring-substitution would yield N,N-dimethyltryptamine (DMT) which in turn is a psychedelic compound with 5-$HT2_B$ affinity (Psychoactive drug screening program 2021), but this is not thought to occur in vivo.

Several related indolamine alkaloids like psilocybin, DMT and LSD have been reported effective in cluster headache (Schindler et al. 2015) and migraine (Schindler et al. 2020). The importance of 5-$HT2_B$ receptor partial agonism and subsequent receptor desensitization in prophylaxis is still

debated in the literature, but it has been presumed essential to dihydroergotamine (Schaerlinger et al. 2003) and generally supported in a review by Segelcke and Messlinger (2017). The risks of this mechanism are mitigated in most users of psychedelics by relatively infrequent dosing in preventative purposes, i.e., less than weekly.

Currently the most used serotonergic drugs are the selective serotonin reuptake inhibitors (SSRIs) which have been claimed to act as direct 5-$HT2_B$ agonists at therapeutic concentrations (Zhang 2010, Hertz 2015a). These claims have been criticized by Banas et al. (2015) with Hertz et al. (2015b) subsequently defending their original claims. Since then, research supporting a directly valvulopathogenic mechanism of SSRIs has been limited to a paper by Peng et al. (2018). Fluoxetine's Ki value is reported to be > 10 000 nM/L (Knight et al. 2004) and around 5000 nM/L together with norfluoxetine (Rothman et al. 2000). Although both are formerly described as 5-$HT2_B$ antagonists (Rothman et al. 2000), the combined concentration of fluoxetine and norfluoxetine could be close to 2000 nM/L (Ferguson and Hill 2006). This could have minor significance if these compounds acted as 5-$HT2_B$ agonists as claimed by Zhang (2010) and Hertz (2015a). In conclusion, association of drug induced VHD with serotonergic antidepressants has been suggested by De Backer et al. (2016) and Lin et al. (2016) with approximately 3 and 1.4-fold increases in risk, respectively. As of 2021, guidelines concerning the possibility of this adverse effect have not been announced by EMA or FDA.

3. AIMS OF THE STUDY

The classic and novel serotonergic psychedelics, for example psilocybin and NBOMes, respectively, may have both cardiovascular and neurotoxic side effects and harms. The aim of this study is to chart the individual harm potentials of these compounds, and their differences which are based on the compounds' distinct mechanisms of action. The first specific aim of the study is to present what are viewed as mechanisms of action of these compounds in the research literature, based on a systematic review. The motive for this approach is that the exact pharmacological mechanisms of the compound are necessary to understand their potential for peripheral side effects, with both currently known and unknown consequences.

Since serotonergic compounds have previously been suggested to have a risk of valvular heart disease, and especially 5-$HT2_B$ receptor has been associated with this risk, the second specific aim of the study is to find whether the use of classical psychedelics and NBOMes can be associated with valvular heart disease. This is done primarily by evaluating their 5-$HT2_B$ mechanisms and secondarily by searching explicit mentions of valvular heart disease in studies concerning the 5-$HT2_B$ mechanism of classical psychedelics and NBOMes.

In the narrative literature review the association of these compounds with VHD was not evident. Additionally, the mechanisms of action in existing reviews of psychedelics concern mainly 5-$HT1_A$ and 5-$HT2_A$ receptors. The mechanisms of action of these compounds are examined from a pharmacological and toxicological viewpoint.

LSD and psilocybin were selected as reviewed compounds as they are studied clinically and are indoles resembling serotonin, which is previously associated with VHD. Likewise, LSD is chemically related to ergot alkaloids, and ergot alkaloids have also been previously associated with drug induced VHD. On the other hand, mescaline and NBOMes were also selected as they are phenethylamine derivatives along with the known valvulopathogen fenfluramine. Therefore, the study includes compounds from all chemical groups known to contain valvulopathogens. NBOMes are abused despite being highly toxic and therefore it is relevant to include them in the review, as their mechanism of action differs from classical psychedelics and their harm potentials may be higher than those of classical psychedelics.

The main factors affecting the response of agonists are efficacy and the extent of bias. The partial and possibly the biased 5-HT2_A agonism contributes the safety of classical psychedelics. If these compounds are 5-HT2_B agonists, their agonism could be full or partial and biased (Fig 2). This would affect their 5-HT2_B related valvulopathogenic risk potentials. Therefore, it is examined whether serotonergic psychedelics are 5-HT2_B agonists and if so, can they be distinguished from valvulopathogenic 5-HT2_B agonists. It is anticipated that especially LSD and psilocybin would be 5-HT2_B agonists and therefore their mechanism of action could be associated with drug induced VHD.

The research questions are formulated as follows:

- What are the pharmacodynamic profiles of these compounds in the current literature?
    - This research question is answered by a systematic literature search. The search includes PubMed and Scopus databases.
- Based on their pharmacodynamic profiles, can serotonergic psychedelics be associated with drug induced VHD? Pharmacodynamic profiles are presented in chapter 5 section 5.4 and discussed in chapter 6 section 6.1.2. Conclusions are presented in chapter 6 section 6.8.
- Do mechanisms suggest patterns of 5-HT$1_{A,}$ 5-HT2_A and 5-HT2_B signaling relevant to neurotoxicological risks or the lack of neurotoxicity?
    - These research questions are answered by an additional literature search providing more detailed data on the pharmacology of classical psychedelics and NBOMes. The results are summarized in sections 5.4 and 5.5 and discussed in chapter 6 section 6.5.

4. MATERIALS AND METHODS

In order to answer the first research question "**what are the pharmacodynamic profiles of these compounds presented in the current literature?**", a systematic literature search was performed in PubMed. The search and the main results are presented in chapter 5. The steps of the review process are depicted as a flowchart (Fig 6) and the exact search queries are stated in the Appendix 1. Stating mechanisms of action in the abstract was selected as a preprocessing criterion to gain an overview about the most important direct mechanisms. The publications which included the 5-HT2_B mechanism in their abstracts were further examined in case they concerned cardiac complications as well.

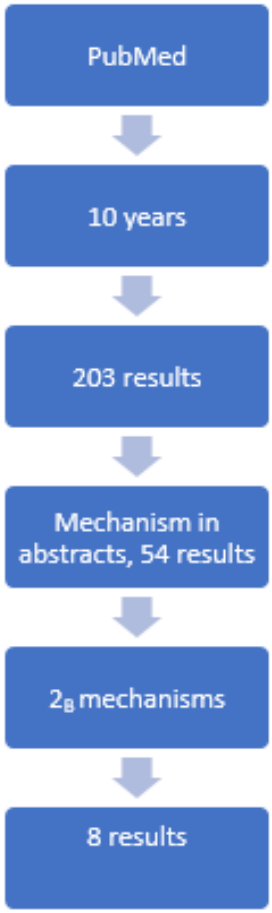

Figure 6. PubMed search process flowchart. Results were screened for mechanisms in abstracts and then for 5-HT2_B-mechanism.

The link between the 5-HT2$_B$ receptor and valvulopathies is established in the literature according to the narrative review. A complementing literature search (Appendix 1) was performed in Scopus to further evaluate the mechanism of drug induced VHD and classical psychedelics and NBOMes. This receptor may not be essential to the psychedelic activity of these compounds and the PubMed database search results depict the 5-HT2$_B$ receptor having a lesser, perhaps overlooked importance. Since the PubMed search yielded over 200 results with low incidences of 5-HT2$_B$ mechanism (5/14 for LSD, 1/17 for psilocybin, 2/18 for NBOMes and none for mescaline), two essential modifications were made to the search strategy to decrease the proportion of irrelevant mechanisms in the results: mechanism of action and related concepts were omitted and the 5-HT2$_B$ mechanism was added in the search query as a restrictive parameter.

This way the Scopus search was performed with a 5-HT2$_B$ selective and bottom-up approach as opposed to the PubMed search which started from mechanisms and proceeded to the 5-HT2$_B$ mechanism. The 5-HT2$_B$ related results were distributed comparably to the PubMed results when categorized by compounds. Finally, the PubMed results concerning cardiac sequelae were combined with the corresponding Scopus search results by producing a union (Fig 7). The results of these database searches overlapped so that the Scopus search yielded only one additional result. Together the first two methods answer to the first research question: **"What are the pharmacodynamic profiles of these compounds presented in the current literature? "**.

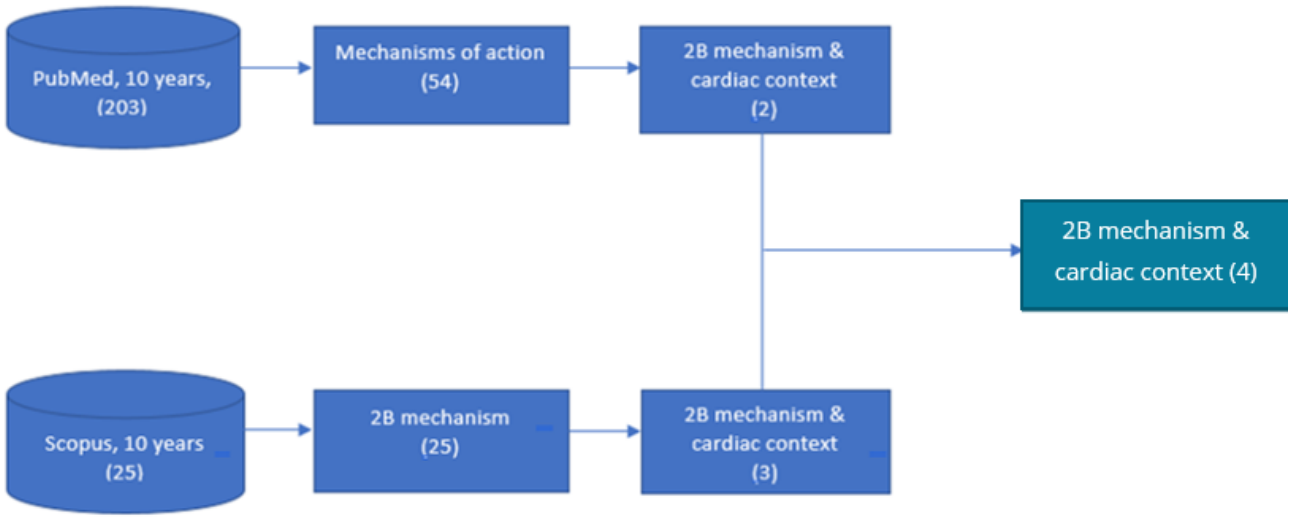

Figure 7. Flowchart of the process yielding results which concerned cardiac sequelae from both database searches. Duplicates were omitted during the process.

A comprehensive evaluation of the 5-HT2_B related mechanism and subsequent VHD risk requires additional pharmacodynamical data besides mechanisms in general. The same applies to the assessment of 5-HT1_A and 5-HT2_A related toxicology. Thus, a third literature search was conducted. The data (Appendices 2 and 3) include the 5-HT1_A, 5-HT2_A and 5-HT2_B receptor affinities and intracellular readouts of classical psychedelics and various NBOMes as well as known valvulopathogenic drugs. Potencies and efficacies were included if they were available in the literature.

The 5-HT2_B related data (Appendix 2) was evaluated with the method presented in Fig 8. This answered to the second research question: **"Based on their pharmacodynamic profiles, can serotonergic psychedelics be associated with drug induced VHD?".**

The 5-HT1_A and 5-HT2_A related pharmacodynamics (Appendix 3) were analyzed with the method depicted in Fig 9. This method answered to the third research question: **"Do mechanisms suggest patterns of 5-HT1A, 5-HT2A and 5-HT2B signaling relevant to neurotoxicological risks or the lack of neurotoxicity?"**.

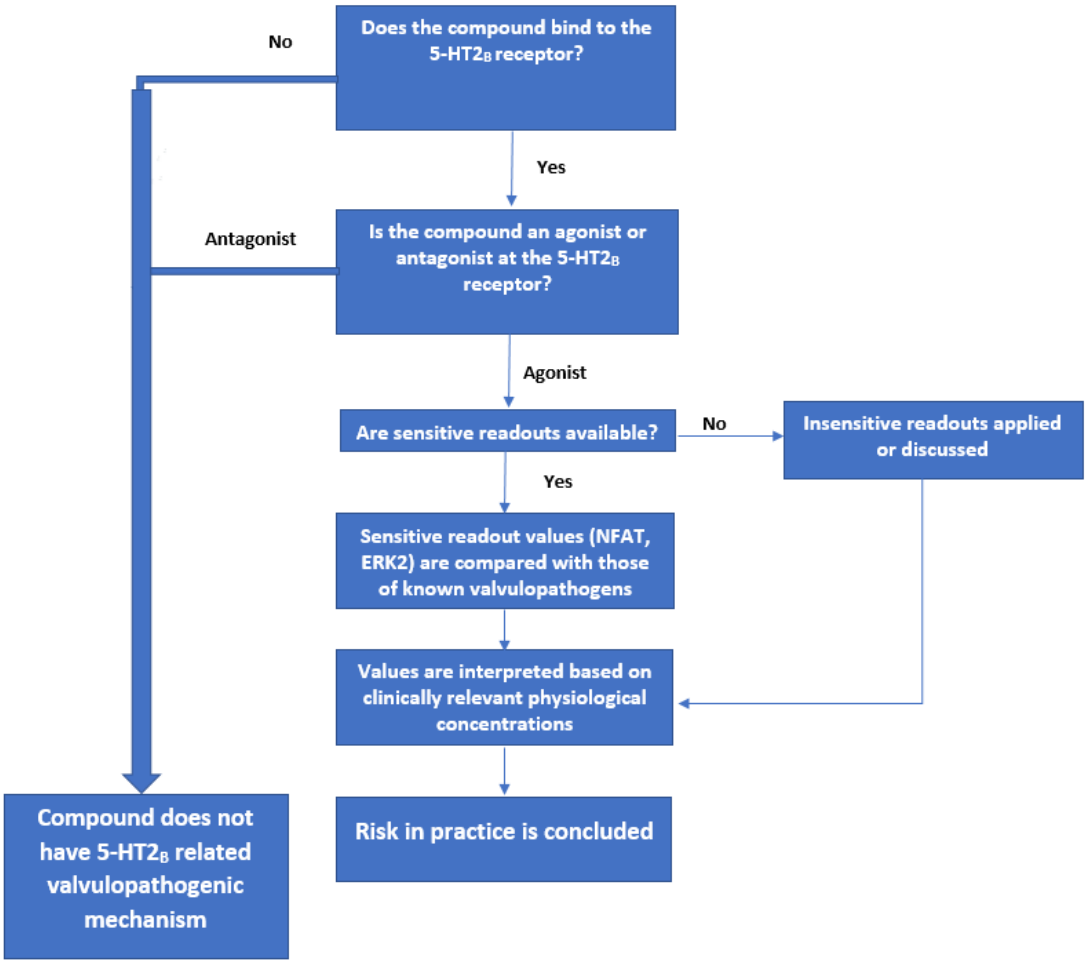

Figure 8. A flowchart demonstrating how a compound's approximated valvulopathogenic risk is deduced from the mechanistic data in principle. The aim is to evaluate the degree of mitogenic response induced by a 5-HT2_B receptor agonist and thus the hazard of valvular remodeling. This is accomplished by comparing primarily the sensitive ERK2 readout values with those of known valvulopathogenic drugs or non-valvulopathogenic 5-HT2_B agonists.

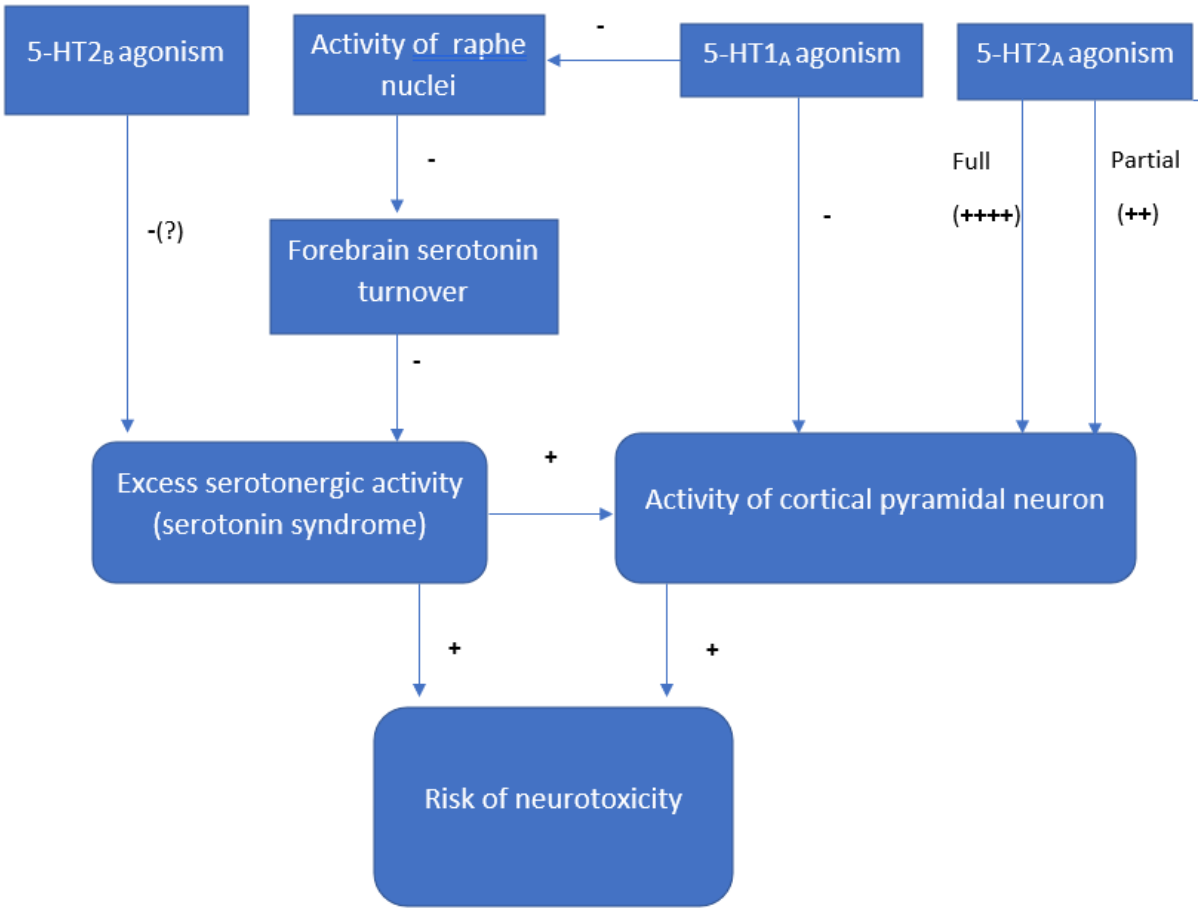

Figure 9. A simple model of serotonergic neurotoxicity focusing on serotonin levels and the activity of cortical pyramidal cells. Stimulatory and inhibitory activities are marked with + and – signs, respectively. 5-HT1$_A$ agonism produces hypothermia in animals (Francescangeli et al. 2019) and attenuates firing of pyramidal cells whereas partial and full 5-HT2$_A$ agonism increase their firing rate (Aghajanian and Marek 1999b). Full agonism is thought to have a greater effect than partial agonism. The excess activity of cortical pyramidal cells is believed to increase the risk of adverse outcomes such as seizures, as for example LSD is a partial agonist and typically does not provoke seizures (Lambe and Aghajanian 2006). 5-HT1$_A$ agonism also inhibits the dorsal raphe nucleus, which in turn decreases serotonin turnover (Aghajanian and Marek 1999a). Decreased serotonin turnover is then assumed to lower the amount of serotonin available to stimulate 5-HT2$_A$ receptors. 5-HT2$_B$ agonism was suggested to lower the risk of serotonin syndrome based on animal experiments (Diaz and Maroteaux 2011).

5. RESULTS

5.1. THE PHARMACODYNAMIC PROFILES OF CLASSICAL PSYCHEDELICS AND NBOMES

Based on the PubMed queries (Table 3), the direct activation of 5-HT2$_A$ receptor is mentioned in each article concerning the pharmacodynamics of classical psychedelics and NBOMes and is thus surrounded by a clear consensus. Therefore, 5-HT2$_A$ agonism or partial agonism is reported as necessary mechanism of classical psychedelics and NBOMes in the included literature. In addition, a pattern of 5-HT2$_A$ mechanism combined with another constant mechanism was not detected, and thus 5-HT2$_A$ mechanism is the necessary basis of the pharmacological profile of classical psychedelics and NBOMes. **This material answered to the first research question:** "What are the pharmacodynamic profiles of these compounds in the current literature?".

Table 3. Distribution of mechanisms of action based on Scopus and PubMed searches.

Mechanism	LSD	Psilocybin	Mescaline	NBOMe
Scopus search, 5-HT2$_B$				
5-HT2$_B$ (cardiac context)	7 (1)	3 (1)	1 (0)	4 (1)
PubMed search, 5-HT2B				
5-HT2$_B$ (cardiac context)	5 (0)	1 (0)	-	2 (2)
PubMed search, other mechanisms				
5-HT1$_A$	6	8	1	1
5-HT2$_A$	14	17	5	18
5-HT2$_C$	5	4	1	8
D$_1$	2	-	-	-

Mechanism	LSD	Psilocybin	Mescaline	NBOMe
D_2	3	-	-	-
D_3	1	-	-	-
5-HT6	1	1	-	-
5-HT7	1	1	-	-
α-adrenergic, unspecified	-	-	-	1
α1	-	-	-	1
α2	-	-	1	-
TAAR1	3	-	-	1
Total	**14**	**17**	**5**	**18**

5.2. ARE SEROTONERGIC PSYCHEDELICS EXPLICITLY ASSOCIATED WITH CARDIAC SAFETY CONCERNS IN THE CURRENT LITERATURE?

The Scopus search yielded 25 results within the past 10 years, 7 of which concerned LSD, 3 psilocybin, 1 mescaline and 4 NBOMes (Table 3). Explicit mentions of cardiac sequelae were distributed so that LSD (Family et al. 2020), psilocybin (Klein et al. 2020) and NBOMes (Eshleman et al. 2018) each gained one hit. The 5-$HT2_B$ and cardiac related studies from both databases (Fig 7) were combined and the duplicates were removed. This produced a set containing four studies: one phase 1 LSD study (Family et al. 2020), one structure-activity study for psilocybin and analogues (Klein et al. 2020), and two pharmacological studies for NBOMes (Rickli et al. 2015, Eshleman et al. 2018).

5.3 WHICH COMPOUNDS ARE USED IN CLINICAL TRIALS?

Since classical psychedelics are studied as potential medicines and the risk of drug induced VHD depends on the length of the treatment with 5-HT2$_B$ agonists, it is important to know how patients could be exposed to classical psychedelics in clinical contexts. Therefore, a supplementary search in ClinicalTrials.gov revealed that 7 studies using LSD are currently[1] about to launch: one early phase 1 study and three phase 1 and 2 studies, and only in one study the drug is administered repeatedly as a microdose of 13 µg (ClinicalTrials 2021). A total of 39 psilocybin studies were recruiting or not yet recruiting, and four of these were early phase 1 studies, 13 were phase 1 and 18 were phase 2 studies. The highest number of doses was four with an incidence of one, while most of the studies planned to use only one dose of psilocybin. Mescaline was about to be used in one study as a single dose. The acronym NBOMe yielded zero results, whereas the prefix ”Cimbi” yielded two studies using these compounds as PET-radioligands.

5.4 5-HT2$_B$ RELATED PHARMACODYNAMICS OF THE PSYCHEDELICS, AND THE DATA ON THE INTRACELLUAR SIGNALING

An extensive set of pharmacological data on the 5-HT2$_B$ agonism of classical psychedelics and NBOMes was searched from scientific publications. This included receptor affinities, potencies, and efficacies on various signaling pathways. These were used to profile the different psychedelics based on their mechanisms of action, and to elucidate if there are differences in the activation of for example ERK2 which is a sensitive readout predicting valvulopathogenic effect. Assessment and classification of compounds is presented in detail in sections 6.2.1, 6.2.2 and 6.2.3 where their valvulopathogenic risks are assessed based on the method presented in Fig 8. The whole set of rank orders of affinities, potencies and efficacies is presented in Appendix 2.

At the **5-HT2$_B$** receptor the affinities for suspected serotonergic valvulopathogens, classical psychedelics and NBOMes were both lower and higher than that of serotonin (1.54-8.71 nM). LSD (0.57-3.72 nM) and 25E-NBOMe (1.11 nM) had higher affinities than cabergoline (1.17 nM), 25I-NBOMe (1.4 nM) had a higher affinity than serotonin, and psilocin (4.6 nM) had a higher

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affinity than pergolide (7.08 nM). The affinity of mescaline (795 nM) was slightly lower than (-)-fenfluramine (680 nM) but higher than the affinity of (+)-fenfluramine (3920 nM). When the 5-$HT2_A$ is treated as the on-target receptor and 5-$HT2_B$ as the off-target, the safety margins increase with 5-$HT2_A$ selectivity so that NBOMes have higher margins than classical psychedelics.

The **ERK2** activation potency of LSD (110 nM) was lower than that of clinically used guanfacine (91 nM) and significantly lower than the potency of known valvulopathogen ergotamine (20 nM). The ERK2 efficacy of LSD (39%) was the lowest of included compounds with known ERK2 parameters and lower than for example those of guanfacine (59%), cabergoline (60%) and pergolide (79%).

On **PI hydrolysis** psilocin (58 nM) was slightly more potent than norfenfluramine (60 nM) but less potent than ergot alkaloids and related compounds. The efficacy of psilocin (45%) was lower than that of cabergoline (123%) and pergolide (112%) but higher than that of methylergonovine (40%) and (-)-norfenfluramine (24%).

On **IP accumulation** ergot derivatives were more potent than serotonin (9.33 nM) and (+)-norfenfluramine (25.9 nM) except for ergotamine, which was the least potent (43.7 nM). Methylergonovine and cabergoline were the most potent compounds with EC50 values of 0.063 and 0.76 nM, respectively. The efficacies of known valvulopathogenic compounds varied from that of pergolide (94%), cabergoline (77-89%) and (+)-norfenfluramine (88%) to that of methylergonovine (19%).

On **calcium flux** all compounds were less potent than serotonin (0.29-2.09 nM) which was followed by psilocin (2.37 nM), 25I-NBOMe (7.3 nM) and LSD (8.91-39 nM). Known valvulopathogens methylergonovine (21.4 nM), pergolide (66-88.5 nM) and cabergoline (398 nM) had lower potencies than serotonin or serotonergic psychedelics. Cabergoline (98.8%) and pergolide (74.1-94.2%) had the highest efficacies after serotonin and were followed by 25CN-NBOME (79%), 25I-NBOMe (65%), LSD (51%) and psilocin (39.2%).

5.5 5-$HT1_A$ & 5-$HT2_A$ RELATED PHARMACODYNAMICS

This material answered to the third research question: “Do mechanisms suggest patterns of 5-$HT1_A$, 5-$HT2_A$ and 5-$HT2_B$ signaling relevant to neurotoxicological risks or the lack of

neurotoxicity?". The material is further analyzed in section 6.5.1 where the neurotoxicological risks are assessed based on the method presented in Fig 9. The material is fully presented in Appendix 3.

At the **5-HT1$_A$** receptor LSD (1.1 nM) had higher affinity than serotonin (1.71 nM) followed by psilocin (49. nM), NBOMes (> 1000 nM) and mescaline (4600 nM). The binding of NBOMes, closely related compounds and mescaline averaged three and two orders of magnitude weaker than that of LSD and psilocybin, respectively. LSD (1.31 nM) was more potent than serotonin (2.88 nM) and the potencies of NBOMes were at least three orders of magnitude weaker (> 1000 nM). LSD had the highest efficacy (93%) followed by 25I-NBOH (74%) and 25D-NBOMe (55%).

At the **5-HT2$_A$** receptor classical psychedelics had generally lower affinites than NBOMes (0.044-4.9 nM). LSD (0.73-3.29 nM) had the highest affinity of classical psychedelics followed by psilocin (25-49 nM) and mescaline (551-6300 nM).

In **IP-1** assay 25E-NBOMe (0.5 nM) and 25N-NBOMe (0.51) had potencies greater than LSD (0.71 nM). The efficacies of NBOMes (85.9%-95.1%) were higher than that of LSD (64.5%). The assays of Rickli et al (2015, 2016) with slightly varying results are presented in Appendix 3.

In **Ca-assay** the 25CN-NBOMe, 25I-NBOMe and 25B-NBOMe had potencies around 1 nM and efficacies over 80%. The potency of LSD in Ca-mobilization was 33.8 nM, and the efficacy was 84.6%.

6. DISCUSSION

In this final chapter I discuss the results of the study, assess the risks of compounds according to the available data, and present the conclusions. Limitations of this study and the possibility of prospective studies are considered.

6.1 SYSTEMATIC LITERATURE REVIEW

Here I restate the most important findings of the systematic literature review. Readout sensitivity is also discussed since in the absence of ERK2/NFAT readouts the assessment of compounds was based mainly on insensitive readout data.

6.1.1 PHARMACODYNAMIC PROFILES IN THE LITERATURE

Based on the articles obtained through PubMed search, there were differences in the pharmacodynamics between the compounds. LSD had the most diverse target receptors (Table 3), and it could dose-dependently affect many of the same receptors as dopaminergic ergolines (Giacomelli et al. 1998, Appendices 2 and 3), such as lisuride (Newman-Tancredi et al. 2002). Psilocybin lacked the dopaminergic properties of LSD as well as the trace amine-associated receptor mechanism and had only serotonergic activity.

Mescaline (Aghajanian and Marek 1999a) and NBOMes (Appendix 3) were not found to significantly affect the 5-$HT1_A$ receptor. Although the alkylamine side chain of phenethylamine is preserved in mescaline, it was not discussed in the context of trace amine-associated receptor which is thought to bind phenethylamine. Mescaline was also the only compound without 5-$HT2_B$ hits in the PubMed search, but the possibility of this activity may not have been thoroughly examined and could be a false negative. This is because the awareness of 5-$HT2_B$ receptor has increased relatively recently and most studies on mescaline have been conducted priorly. Finally, the list of direct mechanisms of action of NBOMes resembled those of mescaline (Table 3) as they were not dopaminergic nor 5-$HT1_A$ or 5-$HT_{6\&7}$ agonists but mainly 5-$HT2_A$ and 5-$HT2_C$ agonists. This is in accordance with the intention of developing NBOMes as selective 5-$HT2_A$ agonists. The toxicity of different classical psychedelics and NBOMes does not increase with the number of mechanisms but rather with the differences in the intrinsic activity at the 5-$HT2_A$ receptor caused by the different ligands.

6.1.2 SEROTONERGIC PSYCHEDELICS EXCLUDING MESCALINE ARE ASSOCIATED WITH THE MECHANISM OF DRUG INDUCED VHD IN CURRENT LITERATURE

A systematic literature review was performed to find out whether classical psychedelics or NBOMes are associated with the mechanisms of drug induced VHD in current literature. Certain other serotonergic drugs (Sachdev et al. 2002, Simonis et al. 2007), and 5-HT2_B as a mechanistic mediator have been associated with VHD in the past. The systematic review revealed that this concern for psychedelics was discussed sporadically in scientific papers which focused on their mechanisms of action and were published during the last ten years, but without a single report of a confirmed case.

LSD was the most often mentioned compound in the context of 5-HT2_B mechanism, followed almost equally by NBOMes and psilocybin while mentions of mescaline have been sparse. This is soundly explained by the close association of valvular heart disease and ergolines, together with both LSD and psilocybin being studied clinically. Curiously, the association of the 5-HT2_B mechanism and valvulopathies was most often mentioned in the context of NBOMes, followed equally by LSD and psilocybin with no mentions of mescaline. NBOMes are used frequently in pharmacological studies and as PET-radioligands (Ettrup et al. 2010, Johansen et al. 2019). The dose in this context can be as low as 0.25 µg resulting in practically non-existent physiological activity (Johansen et al. 2019). Lastly, mescaline appears largely as a curiosity.

Since LSD, psilocybin and NBOMes were found to be 5-HT2_B agonists and are therefore recognized as possible valvulopathogens, the valvulopathogenic concerns of the compounds in the literature were in accordance with their pharmacological profiles. Mescaline was not associated with valvulopathogenic concerns in the literature, and it is not a 5-HT2_B agonist, indicating that the literature was coherent.

Coincidentally it was discovered that MDMA abuse has been linked to fibrotic heart disease. The causation is plausible since both fenfluramine and MDMA are serotonin releasing agents (Bhattacharyya et al. 2009). The search strategies focused on the overall mechanisms of action and the 5-HT2_B mechanism, and by combining them it was possible to obtain relevant information from different aspects with a relatively straightforward approach. This resulted in a pharmacological overview (Table 3), which clarified the proposed and most essential mechanisms of these compounds. The 5-HT2_B mechanism gained relatively little attention when

compared with other mechanisms, and upon further inspection the explicit connection between this mechanism and cardiac complications was even rarer.

6.1.3 INADEQUATE EVIDENCE OF DRUG INDUCED VHD CAUSED BY SEROTONERGIC PSYCHEDELICS

Reports of confirmed cases of serotonergic psychedelics as a cause of drug induced VHD were not found in the literature review of this thesis. Earlier publications could have contained relevant information, but on the other hand the scope of articles concerning drug induced VHD in the narrative review extended further than ten years and classical psychedelics were not associated with drug induced VHD. However, it could be that the authors did not consider classical psychedelics at all. Even so, LSD was not associated with valvulopathies in historical textbooks focusing on ergot alkaloids and related compounds (Berde and Schild 1978) and particularly on LSD (Sankar 1975). Kuypers et al. (2019) did not present evidence of valvulopathies associated with prolonged administration of LSD in studies performed in the 1960's. If such cases have occurred, they may have been undetected, or such findings may have been unpublished.

Most modern clinical trials use only a few psychoactive doses, which is not likely to carry the risk of drug induced VHD. On the other hand, prolonged microdosing with sub-perceptual doses could produce minor valvulopathogenic responses based on this study, but their significance is attenuated by typical dosing schedules which allow users to remain drug-free on most days. The risks of microdosing could be minimized further by a wash-out period for example every three or six months to avoid any cumulative effects on heart valves.

6.1.4 INTRACELLULAR SIGNALING READOUT SENSITIVITY

Several readouts were available for each class of compounds. However, they were generally insensitive to drug induced VHD. The sensitivity of readouts seemed to vary when predicting the risk of drug induced VHD. Thus, their sensitivities and the validity of their heuristic use is discussed.

Only few EC_{50} values concerning **Gq/11 signaling** were available for known or suspected valvulopathogens (Appendix 2). For serotonin, the EC_{50} was several times the plasma concentration, and for pergolide the difference was over 30-fold. On the other hand, the suspected valvulopathogen bromocriptine failed to activate Gq/11 signaling whereas lisuride had high potency but an efficacy of only 11.6%, indicating that this activity is not sufficient to cause VHD. The Gq/11 readout seems insensitive to valvulopathogens, but the cardiac safety of bromocriptine is supported by other readouts as well. Therefore, the ambiguous bromocriptine (Table 2) could be tentatively classified as a non-valvulopathogenic mixed monoaminergic ergot derivative as an example of applying readout data (Appendix 2).

Interestingly, of known or suspected valvulopathogens serotonin is the least potent at **IP-accumulation**. This indicates that this pathway could become relevant in hyperserotonergic conditions such as carcinoid syndrome. The EC_{50} for **calcium flux** is around the plasma concentration of serotonin in normal conditions, and it seems unlikely that this would reliably predict the valvulopathogenic potency of a compound. However, it could be that mitogenic responses were very sensitive to increases in this signaling above baseline activity.

An increase of intracellular Ca^{2+} level as well as activation of protein kinase C is required for ERK2 activity (Knauer et al. 2009). On the other hand, ERK2 activity was found to be independent of PI-3-kinase and tyrosine kinases. Therefore, stimulation of IP accumulation and intracellular calcium levels could predict ERK2 activation and might be used heuristically in the absence of an actual ERK2 readout. On the other hand, in case of LSD the relative efficacies for ERK2, PI turnover and Ca^{2+} mobilization were consistent whereas the potency for IP accumulation was nearly hundredfold, demonstrating the caveat in making assumptions based on potencies only. Thus, efficacy was the more uniform parameter.

The insensitive readout activities shared by all known valvulopathogens were **PI hydrolysis and IP accumulation** (Appendix 2), since the EC_{50} values of pergolide for other readouts are significantly higher and for example ergotamine was not active in calcium assay (Unett et al. 2013). Additionally, suspected valvulopathogen bromocriptine and non-valvulopathogenic lisuride did not influence PI hydrolysis. The low IP accumulation efficacy of valvulopathogenic methylergonovine (28%) is likely compensated by high unbound plasma concentrations if IP

accumulation has any predictive value. The IP accumulation parameters were not available for psilocin.

Many ergolines are known to activate the **β-arrestin pathway**, which causes a prolonged activation of the ERK2 pathway (Eishingdrelo et al. 2015). Despite its close connection to the ERK2 pathway which predicts valvulopathogenic risk, the β-arrestin pathway activation is not considered a sensitive readout. Ergopeptines such as ergotamine and dihydroergotamine are the most heavily β-arrestin biased ergolines at the 5-$HT2_B$ receptor. LSD and cabergoline have a significantly lower but equal bias (Wacker et al. 2014). The influence of β-arrestin pathway on the valvulopathogenic risk of LSD would not be greater than that of cabergoline.

The β-arrestin bias could be interpreted in combination with the ERK2 parameters, although it is not clear whether ERK2 could be activated independently of β-arrestin. The influence of β-arrestin bias on ERK2 signaling should be studied further, as it is possible that the β-arrestin bias of LSD affects the kinetics of valvulopathogenic signaling. This could enhance the valvulopathogenic features of LSD when compared with the estimation based on ERK2 readouts alone.

Huang et al. (2009) have investigated different readouts based on exhaustive set of data and provide additional information on the heuristic use of various readouts. They conclude that calcium-flux based screening is suitable for the preliminary detection of 5-$HT2_B$ agonists, but more sophisticated analysis is required to distinguish compounds with actual valvulopathogenic potency.

The approach based on calcium-flux would further support the classification of bromocriptine as non-valvulopathogenic, since it was not found to be active in a calcium-flux assay. Surprisingly, ergotamine was not active in a calcium assay (Appendix 2) despite it is often considered a valvulopathogen (Table 2). The data of Huang et al. (2009) indicates that ergotamine has a very low potency for calcium flux, and they do not classify ergotamine as a valvulopathogen. Thus, single values in the literature cannot be fully trusted, and the interpretation of readouts demands both resources and expertise since a fully predictive theory has not been formulated yet.

6.2 ASSESSMENT AND CLASSIFICATION OF CLASSICAL PSYCHEDELICS AND NBOMEs

I propose a classification for classical psychedelics and NBOMes in relation to their approximated valvulopathogenic mechanism profiles. The premise for this classification is that serotonin agonists may be selective and biased. Accordingly, there are 5-HT2_A agonists which are not psychedelics, such as lisuride. Additionally, lisuride is not a 5-HT2_B agonist. Even though all 5-HT2_A agonists are not psychedelics, all 5-HT2_B agonists are generally considered to be valvulopathogens (Barnes et al. 2021). However, valvulopathogenic 5-HT2_B agonism is thought to require NFAT or MAPK2/ERK2 signaling, on which certain 5-HT2_B agonists have practically nonexistent efficacies and potencies. Thus, there are 5-HT2_B agonists which are not valvulopathogens in practice, such as guanfacine and ropinirole (Huang et al. 2009). Importantly, signaling pathways of valvulopathogenic 5-HT2_B agonists are not known to be related to the decisive signaling of psychedelic 5-HT2_A agonists. In conclusion, there could be psychedelic 5-HT2_A agonists, which are also 5-HT2_B agonists but not valvulopathogens. There could also be psychedelic 5-HT2_A agonists which are not 5-HT2_B agonists. According to this reasoning, classical psychedelics and NBOMes can be categorized to different sets (A, B and C) based on their properties (Fig 10).

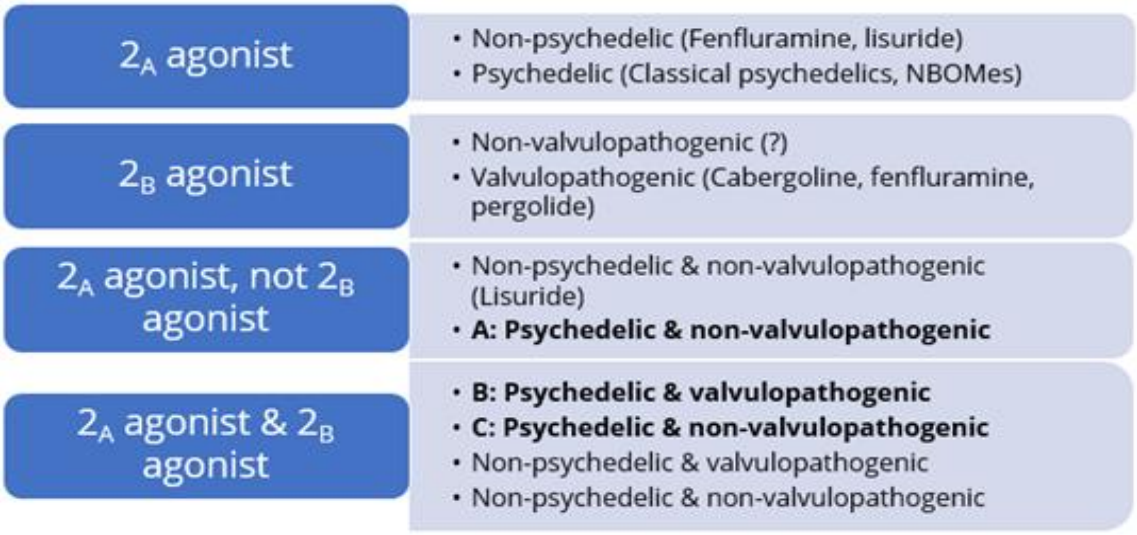

Figure 10. Diagram of relevant possible receptor profiles and corresponding effect sets. Both psychedelic and non-psychedelic 5-HT2_A agonists exist, and 5-HT2_B agonists can be valvulopathogenic. 5-HT2_B agonism is necessary for valvulopathogenic but not for psychedelic effects. The following sets are considered: **A:** psychedelic 5-HT2_A agonists which are not 5-HT2_B agonists and therefore not valvulopathogenic, **B:** psychedelic 5-HT2_A agonists and valvulopathogenic 5-HT2_B agonists, **C:** psychedelic 5-HT2_A agonists and non-valvulopathogenic 5-HT2_B agonists

6.2.1 ASSESSMENT AND CLASSIFICATION OF LSD

The Ki value of LSD for the 5-$HT2_B$ receptor was 0.57-3.72 which is similar or lower than that of serotonin (Appendix 2). This indicates that LSD could bind to the 5-$HT2_B$ receptor effectively and compete with the binding of serotonin. LSD acts as a partial agonist based on all available readouts (calcium flux, Gq/11, IP-1, ERK2).

LSD has a low potency for the sensitive valvulopathogenic ERK2 readout (110 nM) and the efficacy is 39%. Both the potency and efficacy of LSD on ERK2 are lower than those of all known valvulopathogens. For comparison, the possible valvulopathogen guanfacine has an ERK2 EC_{50} of 91 nM and efficacy of 59% (Huang et al. 2009). Guanfacine is not currently associated with drug induced VHD.

To my knowledge a weekly dose of 200 µg of LSD (Müller et al. 2020) is the highest exposure in current clinical practice. This dose of LSD produces peak plasma concentrations of around 10 nM/L, which are lower than the guanfacine concentrations of 40 nM/L seen with clinical doses (Boellner et al. 2007). Taken together, the ERK2 readout indicates that the risk of LSD is even lower than the risk of guanfacine.

Since microdosing produces plasma concentrations of around 1-2 nM/L, it is unlikely that clinical LSD concentrations could significantly activate the ERK2 pathway. Since LSD plasma concentrations during a regular microdosing protocol would be far below the ERK2 EC_{50} and the intrinsic activity is relatively low, valvulopathogenic responses are estimated to be practically nonexistent. According to the current literature, the risk would be lower than any of the known valvulopathogenic drugs.

Microdosing LSD could produce plasma concentrations resembling those of a 2 mg dose of cabergoline (Appendix 2), which has been safe when dosed weekly for endocrinological indications (Stiles et al. 2021). In terms of calcium flux LSD would be at least ten times more potent than cabergoline (Appendix 2), but cabergoline's EC_{50} for IP-accumulation would be exceeded during high dose therapy. This value was not available for LSD, but IP-accumulation has not been considered as a sensitive valvulopathogenic readout (Table 1).

LSD shares essential features with known valvulopathogens and therefore would be classified to set B (Fig 10) according to a strict interpretation. This was anticipated since LSD belongs to the

class of ergolines. In practice it would be justified to classify LSD to set C, since doses needed to actualize the risk would be far greater than those known to be required in currently recognized clinical indications.

6.2.2 ASSESSMENT AND CLASSIFICATION OF PSILOCYBIN

The Ki value of psilocin for the 5-$HT2_B$ receptor was stated in only one source citing the Psychoactive drug screening program database as the reference, but this particular value no longer existed in the database and its accuracy is therefore debatable. The Ki for psilocin (4.6) was lower than that of pergolide, a potent 5-$HT2_B$ agonist and a known valvulopathogen. The Ki for psilocin was also significantly lower than the value of the close analog DMT (Appendix 2), which further implies the possibility that psilocin's value is outdated. However, the Ki was in the same range as the value of serotonin. This indicates that psilocin could bind to the 5-$HT2_B$ receptor effectively and compete with the binding of serotonin.

Potencies and efficacies for sensitive readouts were not found in the literature. Therefore, the valvulopathogenic potency of psilocin cannot be ruled out or confirmed reliably. However, crude estimations can be made based on affinity and insensitive readouts.

A microdose of psilocybin containing mushrooms is reported to be 0.1-0.3 g (Johnstad 2018) which would correspond to about 1 to 3 milligrams of psilocybin. Since a dose of 0.3 mg/kg produces a peak plasma concentration of around 80 nM/L (Brown et al. 2017) and a 3 mg dose for a 50 kg person would equal 0.06 mg/kg, peak plasma concentrations following a microdose could theoretically be one fifth of those, that is 16 nM/L. This is clearly below the EC_{50} value of 58 nM for PI hydrolysis. The calcium flux EC_{50} (2.37 nM) by Klein et al. (2020) is very low and could be readily exceeded by a microdose, but the relevance of this is difficult to estimate. Moreover, the linear pharmacokinetics of very low psilocybin doses have yet to be established.

Definite classification of psilocybin was not possible as data on sensitive readouts was not available, and extrapolation from other readouts is complex and the results uncertain. Therefore, it is concluded that psilocybin could belong either to set B or C. Cases of VHD associated with psilocybin in humans or animals were not found. However, psilocin's high

potency (2.37 nM) in calcium flux assay warrants further analysis. On the other hand, the efficacy was only 39%, which could indicate a lower risk than suggested by the potency alone.

6.2.3 ASSESSMENT AND CLASSIFICATION OF MESCALINE AND NBOMES

The affinity of mescaline (795 nM) was the lowest of classical compounds and around that of (-)-fenfluramine (Appendix 2). However, low affinity does not mean lack of receptor activation. Mescaline's low affinity is compensated by relatively high doses which are approximately one and four orders of magnitude greater than those of psilocybin and LSD, respectively. Mescaline was not found to activate the 5-$HT2_B$ and therefore is not considered to have a mechanistic potential for being valvulopathogenic, justifying its classification to set A.

Many NBOMes such as 25I-NBOMe and 25E-NBOMe had a very high affinity to the 5-$HT2_B$ receptor. Generally, NBOMes seem to be partial agonists with efficacies less than 80%, while the plasma concentrations of hospitalized or deceased persons have exceeded the reported EC_{50} values for calcium flux. Since these compounds are known to have agonistic activity at the 5-$HT2_B$ receptor at low concentrations, they are considered to belong either to set B or C. The activity in calcium flux assays warrants further analysis according to the criteria of Huang et al. (2009). Definite classification of NBOMes was not possible due to the lack of sensitive readout data. However, NBOMes are currently used clinically only as radioligands in such a low dose that pharmacological effects are practically nonexistent.

6.3 SAFETY MARGINS

Safety margins for compounds can be calculated by using various parameters such as typical dose, binding affinity, potency on target and off-target receptors and functional assays. The predictive value of these remains debatable and as such they are best suited for early screening procedures for potential valvulopathogens. According to Papoian et al. (2017), the most accurately predictive safety margins are based on Ki values, and a Ki value of approximately one order of magnitude or less relative to 5-HT is considered to distinguish all known valvulopathogens. Based on this heuristic LSD, psilocybin, and 25D/E/I/N-NBOMes (Appendix 2) would be potential valvulopathogens and thus require additional testing or clinical monitoring.

Another method for determining safety margins would be calculating the ratio of off-target and on-target binding as demonstrated by Cavero and Guillon (2014). Psilocybin, mescaline, and LSD had the lowest safety margins, indicating that binding to the 5-$HT2_B$ receptor binding is inevitable when using these compounds. On the other hand, LSD and psilocin did not activate the receptor in the study of Rickli et al. (2016). Naturally, this approach is suitable only for agonists, as for example lisuride has negligible activity on functional assays despite a high binding affinity.

Safety margins based on binding do not consider the activation of signaling pathways and thus their utility as a stand-alone heuristic is debatable. Moreover, NBOMes bind with high affinity and possibly have a distinct profile of biased 5-$HT2_A$ agonism, hence causing highly toxic effects at plasma concentrations well within the 5-$HT2_B$/2_A safety margins. Thus, these safety margins may not be optimal in the context of psychedelic 5-$HT2_A$ agonists. Generally, Cavero and Guillon (2014) advocate the use of intracellular readouts, particularly IP accumulation and ERK2 for which the margins could also be calculated. However, especially the ERK2 values were lacking in the data of this study.

6.4 RISKS OF NOVEL COMPOUNDS

The safety issue is complicated in illicit use as more common drugs can be substituted with derivatives due to for example legal issues. Several lysergamides have appeared on the drug markets in recent years (Brandt et al. 2017), and while some are thought to act simply as prodrugs, others may have distinct pharmacological features. These include the other than N6-methyl-substituted lysergamides, such as the ethyl-, allyl- and propyl-substituted variants ETH-LAD, AL-LAD and PRO-LAD (Coney et al. 2017). Of these the PRO-LAD is particularly concerning, since bulky N6-substituents such as allyl and propyl have been linked to 5-$HT2_B$ agonism of cabergoline and pergolide, respectively (Kekewska et al. 2011).

Hypothetically, there could exist a lysergamide substitution pattern which would retain the desired features of LSD while excluding potentially harmful effects, although the toxicity of LSD is generally considered very low. On the other hand, a case-report has associated AL-LAD with a

fatal cardiac complication, suggesting the possibility of additional cardiotoxic mechanisms besides 5-HT2_B agonism (Blumenberg and Hendrickson 2020).

Novel psychoactive tryptamines (Rickli et al. 2016) and psilocybin analogs also exist (Klein et al. 2020), but the availability of psilocybin mushrooms likely limits the appeal of these designer drugs. On the other hand, the use of psilocybin could increase significantly regardless of its legality if it were to become a popular alternative to prescription drugs such as antidepressants. This raises the issue of harm reduction when substance use cannot be eradicated successfully but rather diverted to the safest possible alternatives.

6.5 NEUROTOXICOLOGICAL TRENDS

The final aim was formulated with mainly prospective research in mind. The objective was to detect patterns in the action mechanisms which would explain differences in neurotoxicity of classical psychedelics and NBOMes. It was found that 5-HT2_B agonism could reduce the risk of serotonin syndrome in mice (Diaz and Maroteaux 2011). It was also found that 5-HT1_A agonism could reduce the cortical excitability caused by 5-HT2_A agonism (Aghajanian and Marek 1999b). This opposing action could reduce the excitatory effects and thus the potential neurotoxicity of 5-HT2_A agonists.

It is proposed that 5-HT1_A agonism could attenuate neurotoxicity and especially the risk of serotonin syndrome via decreased serotonin turnover (Fig 9). The indoleamines LSD and psilocin can directly suppress the activity of dorsal raphe nucleus and reduce serotonin turnover in the forebrain (Aghajanian and Marek 1999a). 5-HT1_A agonism can suppress the release of serotonin through inhibitory autoreceptors and by inhibiting brainstem nuclei which contribute to volume transmission to the forebrain. This is in accordance with the known safety profile of LSD and psilocin.

6.5.1 ASSESSMENT OF 5-HT1_A AND 5-HT2_A RELATED NEUROTOXICITY

This analysis is based on the method described in Fig 9. Several NBOMes have even higher 5-HT2_A affinity than the very potent LSD, and both are active in doses less than one milligram. Several NBOMes are full 5-HT2_A agonist in IP-1 assay and Ca-assays (Jensen et al. 2017). In the

Ca-assay 25I-NBOMe is around 75-fold more potent than LSD while the efficacies are similar. Therefore, 25I-NBOMe could theoretically induce Ca-dependent signaling very potently when compared with LSD. This could correlate with excitotoxicity, which would be in accordance with known safety profiles of for example 25I-NBOMe and LSD. Additionally, NBOMes are more potent and efficient on β-arrestin2 recruitment than LSD or mescaline (Pottie et al. 2020), but the toxicological relevance of this action is not clear.

A pattern of very high ratios of 5-$HT1_A$/2_A Ki values was found for NBOMes, indicating their selective 5-$HT2_A$ agonism (Appendix 3). Doses required for relevant 5-$HT1_A$ receptor occupancy would likely far exceed those activating 5-$HT2_A$ receptors, which could contribute to the toxicity of NBOMes. The ratio of mescaline (< 1) suggested that 5-$HT1_A$ activation could contribute to its effects. On the other hand, mescaline has not been found to suppress the 5-$HT1_A$ autoreceptor regulated activity of the dorsal raphe nucleus, suggesting a very low efficacy and practical relevance (Aghajanian and Marek 1999a). The literature does not support that mescaline could act as a 5-$HT1_A$ agonist, although it may have indirect effects on the dorsal raphe nucleus and serotonin turnover. The possibility of NBOMes also having such an effect cannot be ruled out completely.

The ratio of LSD varies slightly depending on the selected values, which nevertheless indicate that LSD binds somewhat equally to the 5-$HT1_A$ and 5-$HT2_A$ receptors. In addition to acting as a full agonist at the 5-$HT1_A$ receptor, it has a lower Ki value and EC_{50} than serotonin itself and is a partial agonist of many additional serotonin receptors. This implies that it can both reduce the turnover of serotonin and substitute its activity, pervasively altering the state of serotonergic signaling in the brain. LSD had the most balanced profile of 5-$HT1_A$/2_A receptor activity followed by psilocin, and these compounds are known to be physiologically well-tolerated — no evidence of neurotoxicity was found. The ratio of psilocin was 2.5 which is still considered low, but neither the EC_{50} value nor efficacy at the 5-$HT1_A$ receptor were available.

6.5.2 CONCLUSIONS ON NEUROTOXICOLOGICAL TRENDS

The hypothetical anticonvulsant profile of serotonergic signaling (Meldrum and Naquet 1971) seems to require the activation of 5-$HT1_A$ receptors (Merlet et al. 2004). This assumption would be in accordance with the pharmacodynamics of fenfluramine, which stimulates both 5-$HT1_A$

and 5-HT2$_A$ receptors through serotonin release. Even so, this effect could be restricted to the visual cortex and be relevant mainly in photosensitive conditions (Meldrum and Naquet 1971) and in certain usage contexts such as dance festivals (Mohr et al. 2018, Salet et al. 2019), although serotonergic signaling has been stated to inhibit several overexcitable networks in animal models of epilepsies (Bagdy et al. 2007). I assume that selective and potent 5-HT2$_A$ agonists could have pronounced excitatory actions, and seizures are associated with NBOMes. Mescaline (Aghajanian and Marek 2000, Appendix 3) and NBOMes (Appendix 3) are thought to have negligible 5-HT1$_A$ activity. This would limit their safety when compared to the full 5-HT1$_A$ and partial 5-HT2$_A$ agonism of for example LSD.

Taken together, it is concluded that an ideal serotonergic psychedelic for clinical trials would be a somewhat balanced 5-HT1$_A$ and 5-HT2$_A$ agonist. Ideal serotonergic psychedelics could also be 5-HT2$_B$ agonists which would make the 5-HT2$_B$ mechanism toxicologically double-edged. Therefore, based on these criteria and the literature, the ideal compounds are LSD and psilocybin. The overall impact of these differential profiles and activity of the dorsal raphe nucleus on serotonergic neurotoxicity remains obscure, although it is presumed that a balanced profile contributes to safety. Finally, the results of Table 3 can be a beneficial starting point in prospective research concerning risks related to mechanisms other than 5-HT1$_A$, 5-HT2$_A$ and 5-HT2$_B$.

6.6 FUTURE DIRECTIONS

To predict the valvulopathogenic risks of the most clinically important classical psychedelics, prospective studies should focus on determining the ERK2 readout especially for psilocin but also for DMT. If the ERK2 data were available also for lysergamides and tryptamines distributed as designer drugs, their valvulopathogenic risks could be predicted preliminarily. This would have practical relevance since they can be used as substitutes to the original compounds. Although chronic use of NBOMes is thought to be rare, this data would make their precise toxicological assessment possible and add to scientific knowledge. Also, Finnish legislation requires that the court decisions for the penalties for narcotic crimes are based on the estimated dangerousness of use of the drug (Rikoslaki 50 luku 5.2§, Rikoslaki 50 luku 5.5§), even in cases in which no direct evidence is available.

DMT was not evaluated in this thesis. It is a natural compound with a long history of ritualized use in South America (Nichols 2016) and possesses 5-HT2_B activity (Appendix 2). DMT is orally active only when combined with monoamine oxidase inhibitors — such as β-carbolines in the form of ayahuasca — and can be administered more frequently than other psychedelic substances since it causes significantly less tolerance. Additionally, the pharmacodynamics of β-carbolines could contribute to the overall 5-HT2_B activity of DMT concoctions. Therefore, if DMT were a valvulopathogen, this usage pattern and combining of several substances might further affect the risk. Neither DMT nor β-carbolines used in ayahuasca are typically considered to be potential valvulopathogens, but this should be researched further.

Epidemiological studies of microdosing of classical psychedelics could provide valuable data about the association with VHD. On the other hand, classical psychedelics are largely illegal which could decrease the willingness to participate in such studies. Chronic administration to animals could also provide valuable toxicopathological data on heart valves, and the results could be used to assess safety margins in humans. Animal testing would provide means to calculate the “no observed adverse effect level” values, which in turn could be used to assess safety limits for these compounds in different usage contexts such as microdosing. Nonetheless, it seems that the valvulopathogenic effects of for example pergolide were not conclusive in animal testing until the study of Droogmans et al. (2009) when the compound had already been withdrawn from human use. Therefore, the sensitivity of animal tests in predicting valvulopathogenic risks in humans remains questionable.

It would be relevant to further elucidate the possible contribution of 5-HT2_B agonism on the neuronal effects of psychedelics. Additionally, it might be tempting to produce compounds devoid of this effect to ensure their benign valvular safety profile, but this could have contradictory consequences on their acute hazards and neurological risks. Following this line of reasoning, the mechanisms of neurotoxicity could be examined further. Prospective studies could focus on the relationship of 5-HT2_B manipulation and the functioning of raphe nuclei (Boothman et al. 2003, Belmer et al. 2018), and whether this has any effect on neurotoxicity of certain serotonin agonists such as the NBOMes.

6.7 LIMITATIONS

Theoretical assessment of valvulopathogenic risk relies on the use and availability of relevant data. Despite the diversity of pharmacological data presented in Appendix 2, its utility for deduction of the risk potential was limited by the lack of sensitive readouts and a lack of more sophisticated data analysis. Therefore, prospective mechanistic studies should focus on determining the ERK1/2 potencies and efficacies at the 5-$HT2_B$ receptor. Results from this line of research could then ascertain how these compounds are to be classified according to the set-based approach (Fig 10) proposed in this thesis. Moreover, metabolites should also be considered when classifying compounds by these methods.

These literature reviews imply that neither VHD or the 5-$HT2_B$ mechanism are commonly discussed in the recent literature concerning classical psychedelics and NBOMes. Some instances may have been missed because the search query used in PubMed was focused on overall pharmacology and results concerning a more specific area could have been excluded.

Because the PubMed inclusion criteria was applied on the level of abstracts, instances of 5-$HT2_B$ mechanism only in the text body may have gone unnoticed. The probability and significance of these were considered very low, as the pharmacology would have been in the title and mechanisms mentioned only in passing later in the text are unlikely to be essential.

The literature review did not extend to publications older than ten years, but scientific interest in these compounds has re-emerged only recently and the 5-$HT2_B$ mechanism was unknown during the intense research in the 1960's. The ten-year time frame also corresponds to the period which NBOMes have existed.

Even if drug induced valvulopathies are unlikely to emerge, unexpected potentiating factors could be involved. These include the possibility of a particularly risky combination of biased agonism and increased risks due to receptor polymorphism, which could modify the affinity, potency and efficacy culminating in a novel variation of the standard agonistic profile. Individual pharmacokinetic variations arising from polymorphism and affecting metabolism should also be investigated thoroughly, as these could increase drug exposure in certain populations. The pharmacokinetics and subjective effects of LSD have already been shown to be affected by genetic polymorphisms of CYP2D6 enzyme (Vizeli et al. 2021). The pharmacokinetics of LSD in

older persons has been studied recently (Family et al. 2020), and the baseline risk of VHD is increased in this population.

Since this study is based purely on theoretical grounds, mainly by using informatics and deduction based on the mechanisms, it would be advisable to gather direct evidence for the psychedelics-VHD link by routinely inspecting the functioning of the heart valves in clinical studies if dosing of the substances lasts longer than three months. Such studies are anticipated if the recent finding of LSD potently increasing levels of brain-derived neurotrophic factor (BDNF) is confirmed (Hutten et al. 2020). This action could be beneficial for example in depression, as increased BDNF signaling is thought to be essential to the actions of all antidepressants (Casarotto et al. 2021). Moreover, the psychoactivity of such low doses of LSD are nonexistent or negligible.

Also, the theoretical predictions made of neurotoxicity and its mechanisms should be evaluated by a more practical approach utilizing methods of modern neuroscience, providing direct evidence on the possible risks. Thus, the model (Fig 9) of serotonergic neurotoxicity remains speculative.

The results of functional assays varied considerably since experimental conditions may influence the results and cannot be controlled absolutely. In addition, the significance of different readouts on a certain common endpoint is difficult to estimate, as the weight of effect varies along with the point of convergence. A more precise interpretation of readouts would have required extensive knowledge of cellular biochemistry, and resources were limited in this regard.

Currently it is not entirely clear how sensitive the recommended readouts are in predicting the risk of drug induced VHD. In this thesis I did not consider 5-$HT2_A$ or angiotensin receptor 1 related pharmacodynamics when assessing the risks, although their possible contribution in VHD has been proposed by Kekewska et al. (2011) and Hutcheson et al. (2009), respectively. In addition, Droogsman et al. (2009) evaluated the role of 5-$HT2_B$ receptor by using cyproheptadine, which is not a selective 5-$HT2_B$ antagonist and binds to 5-$HT2_A$ receptors with high affinity (Psychoactive drug screening program 2021). If 5-$HT2_A$ receptor agonism contributes to valvulopathies, it should be studied whether the signaling cascade responsible for psychedelic activity also relates to valvulopathogenic responses. Risk assessment guidelines focused

exclusively on the 5-HT2_B mechanism, and its validity was assumed as a premise. It was not possible to examine the validity of the 5-HT2_B mechanism thoroughly in the scope of this thesis.

It is suggested that the valvulopathogenic ERK2 process might be initiated by mechanisms other than 5-HT2_B activation, such as growth factors (Mutlak et al. 2015). Additionally, a specific pattern of ERK2 signaling could be markedly valvulopathogenic. Therefore, patterns initiated by different 5-HT2_B agonists should be studied in greater detail in the future.

This thesis discussed several areas besides the possible valvulopathogenic activities of the selected compounds, and some details may have been overlooked due to the relatively broad scope. Additionally, the methodology and orientation of this thesis originated primarily from information sciences rather than cell biology or biochemistry, which are central in a detailed analysis of the 5-HT2_B receptor signaling in drug induced VHD. Despite these limitations the findings of this study add to the existing literature and further clarify the uncertainty surrounding psychedelics and drug induced VHD (Kuypers et al. 2019). To my knowledge a systematic literature review focusing on this topic in this scale has not been done before.

6.8 CONCLUSIONS

Theoretically several NBOMes and all classical psychedelics except mescaline can be associated with drug induced VHD via their 5-HT2_B agonism. More data on the effects of psilocybin and NBOMes on valvulopathogenic signaling pathways is required for a thorough risk assessment. It is concluded that mescaline does not carry the risk of drug induced VHD and for LSD the risk is very low. Typical dosing regimens in clinical trials are unlikely to result in drug induced VHD and the risk of microdosing is predicted to be low for all studied compounds.

Serotonergic psychedelics are 5-HT2_A agonists. There are differences in efficacies and activated signaling pathways. Classical psychedelics are partial agonists whereas NBOMes can be full agonists. Of classical psychedelics psilocybin and LSD are also 5-HT1_A agonists which likely contributes to their physiological safety. NBOMes typically lack the 5-HT1_A agonism and are known to be neurotoxic. Several NBOMes and classical psychedelics except mescaline were 5-HT2_B agonists. 5-HT2_B agonism has a dual role in the safety of these compounds: it may protect

from serotonin syndrome in the central nervous system while simultaneously being necessary for drug induced VHD in the vascular system.

Classical psychedelics carry a lower risk of neurotoxicity than NBOMes. Due to their 5-$HT2_B$ agonism psilocin and LSD would be unlikely to pass current screening procedures in drug development despite their known safety profiles. This underlines their uniqueness as a treatment option, especially if central 5-$HT2_B$ agonism contributes to their psychoactive effects.

5-$HT2_B$ agonism of classical psychedelics is unlikely to compromise their clinical safety. However, caution is advised in prolonged dosing and among the elderly. If classical psychedelics are to be accepted as clinically viable treatment options, their physical risks need to be examined in detail. Otherwise, history might repeat itself and the optimism that surrounds this delicate matter might vanish abruptly if unforeseen adverse effects were to arise. In addition, legislative bodies and courts require evidence of drug harms and theories which can accurately predict the risks of drugs. Taken together, the results of this thesis have both medical and legislative value.

REFERENCES

Adam K. Klein, Muhammad Chatha, Lauren J. Laskowski, Emilie I. Anderson, Simon D. Brandt, Stephen J. Chapman, John D. McCorvy, and Adam L. (2020). Investigation of the Structure–Activity Relationships of Psilocybin Analogues Halberstadt ACS Pharmacology & Translational Science Article ASAP DOI: 10.1021/acsptsci.0c00176

Aghajanian, G. K., & Marek, G. J. (1999a). Serotonin and hallucinogens. Neuropsychopharmacology: official publication of the American College of *Neuropsychopharmacology*, 21(2 Suppl), 16S–23S. https://doi.org/10.1016/S0893-133X(98)00135-3

Aghajanian, G. K., & Marek, G. J. (1999b). Serotonin, via 5-HT2A receptors, increases EPSCs in layer V pyramidal cells of prefrontal cortex by an asynchronous mode of glutamate release. *Brain research*, *825*(1-2), 161–171. https://doi.org/10.1016/s0006-8993(99)01224-x

Aghajanian, G. K., & Marek, G. J. (2000). Serotonin model of schizophrenia: emerging role of glutamate mechanisms. Brain research. *Brain research reviews*, 31(2-3), 302–312. https://doi.org/10.1016/s0165-0173(99)00046-6

van Amsterdam, J., Opperhuizen, A., & van den Brink, W. (2011). Harm potential of magic mushroom use: a review. *Regulatory toxicology and pharmacology: RTP*, *59*(3), 423–429. https://doi.org/10.1016/j.yrtph.2011.01.006

Andersen, K., Carhart-Harris, R., Nutt, D. J., & Erritzoe, D. (2021). Therapeutic effects of classic serotonergic psychedelics: A systematic review of modern-era clinical studies. *Acta psychiatrica Scandinavica*, *143*(2), 101–118. https://doi.org/10.1111/acps.13249

Araneda, R., & Andrade, R. (1991). 5-Hydroxytryptamine2 and 5-hydroxytryptamine 1A receptors mediate opposing responses on membrane excitability in rat association cortex. *Neuroscience*, 40(2), 399–412. https://doi.org/10.1016/0306-4522(91)90128-b

Ayoub, S., Ferrari, G., Gorman, R. C., Gorman, J. H., Schoen, F. J., & Sacks, M. S. (2016). Heart Valve Biomechanics and Underlying Mechanobiology. *Comprehensive Physiology*, 6(4), 1743–1780. https://doi.org/10.1002/cphy.c150048

Bagdy, G., Kecskemeti, V., Riba, P., & Jakus, R. (2007). Serotonin and epilepsy. *Journal of neurochemistry*, 100(4), 857–873. https://doi.org/10.1111/j.1471-4159.2006.04277.x

Banas, S. M., Diaz, S. L., Doly, S., Belmer, A., & Maroteaux, L. (2015). Commentary: Chronic SSRI stimulation of astrocytic 5-HT2B receptors change multiple gene expressions/editings and metabolism of glutamate, glucose and glycogen: a potential paradigm shift. *Frontiers in behavioral neuroscience*, *9*, 207. https://doi.org/10.3389/fnbeh.2015.00207

Barnes, N. M., Ahern, G. P., Becamel, C., Bockaert, J., Camilleri, M., Chaumont-Dubel, S., Claeysen, S., Cunningham, K. A., Fone, K. C., Gershon, M., Di Giovanni, G., Goodfellow, N. M., Halberstadt, A. L., Hartley, R. M., Hassaine, G., Herrick-Davis, K., Hovius, R., Lacivita, E., Lambe, E. K., Leopoldo, M., … Hoyer, D. (2021). International Union of Basic and Clinical Pharmacology. CX. Classification of Receptors for 5-hydroxytryptamine; Pharmacology and Function. *Pharmacological reviews*, *73*(1), 310–520. https://doi.org/10.1124/pr.118.015552

Belmer, A., Quentin, E., Diaz, S. L., Guiard, B. P., Fernandez, S. P., Doly, S., Banas, S. M., Pitychoutis, P. M., Moutkine, I., Muzerelle, A., Tchenio, A., Roumier, A., Mameli, M., & Maroteaux, L. (2018). Positive regulation of raphe serotonin neurons by serotonin 2B receptors. *Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology*, *43*(7), 1623–1632. https://doi.org/10.1038/s41386-018-0013-0

Berde, B., & Schild, H. O. (1978). *Ergot alkaloids and related compounds*. Berlin; New York: Springer-Verlag.

Bhattacharyya, S., Schapira, A. H., Mikhailidis, D. P., & Davar, J. (2009). Drug-induced fibrotic valvular heart disease. *Lancet* (London, England), 374(9689), 577–585. https://doi.org/10.1016/S0140-6736(09)60252-X

Blumenberg, A., & Hendrickson, R. G. (2020). A letter reporting a case of fatal ventricular dysrhythmia associated with the LSD analog AL-LAD. *Clinical toxicology (Philadelphia, Pa.)*, 58(2), 143–145. https://doi.org/10.1080/15563650.2019.1609685

Bogenschutz, M. P., & Ross, S. (2018). Therapeutic Applications of Classic Hallucinogens. *Current topics in behavioral neurosciences*, 36, 361–391. https://doi.org/10.1007/7854_2016_464

Bonhaus, D. W., Bach, C., DeSouza, A., Salazar, F. H., Matsuoka, B. D., Zuppan, P., Chan, H. W., & Eglen, R. M. (1995). The pharmacology and distribution of human 5-hydroxytryptamine2B (5-

HT2B) receptor gene products: comparison with 5-HT2A and 5-HT2C receptors. *British journal of pharmacology*, *115*(4), 622–628. https://doi.org/10.1111/j.1476-5381.1995.tb14977.x

Boothman, L. J., Allers, K. A., Rasmussen, K., & Sharp, T. (2003). Evidence that central 5-HT2A and 5-HT2B/C receptors regulate 5-HT cell firing in the dorsal raphe nucleus of the anaesthetised rat. *British journal of pharmacology*, 139(5), 998–1004. https://doi.org/10.1111/j.1476-5381.1995.tb14977.x

Brand, T., & Anderson, G. M. (2011). The measurement of platelet-poor plasma serotonin: a systematic review of prior reports and recommendations for improved analysis. *Clinical chemistry*, 57(10), 1376–1386. https://doi.org/10.1373/clinchem.2011.163824

Brandt, S. D., Kavanagh, P. V., Westphal, F., Elliott, S. P., Wallach, J., Stratford, A., Nichols, D. E., & Halberstadt, A. L. (2017). Return of the lysergamides. Part III: Analytical characterization of N6 -ethyl-6-norlysergic acid diethylamide (ETH-LAD) and 1-propionyl ETH-LAD (1P-ETH-LAD). *Drug testing and analysis*, 9(10), 1641–1649. https://doi.org/10.1002/dta.2196

Bredberg, U., Eyjolfsdottir, G. S., Paalzow, L., Tfelt-Hansen, P., & Tfelt-Hansen, V. (1986). Pharmacokinetics of methysergide and its metabolite methylergometrine in man. *European journal of clinical pharmacology*, *30*(1), 75–77. https://doi.org/10.1007/BF00614199

Brown, R. T., Nicholas, C. R., Cozzi, N. V., Gassman, M. C., Cooper, K. M., Muller, D., Thomas, C. D., Hetzel, S. J., Henriquez, K. M., Ribaudo, A. S., & Hutson, P. R. (2017). Pharmacokinetics of Escalating Doses of Oral Psilocybin in Healthy Adults. *Clinical pharmacokinetics*, 56(12), 1543–1554. https://doi.org/10.1007/s40262-017-0540-6

Caputo, C., Prior, D., & Inder, W. J. (2015). The need for annual echocardiography to detect cabergoline-associated valvulopathy in patients with prolactinoma: a systematic review and additional clinical data. *The lancet. Diabetes & endocrinology*, *3*(11), 906–913. https://doi.org/10.1016/S2213-8587(14)70212-8

Casarotto, P. C., Girych, M., Fred, S. M., Kovaleva, V., Moliner, R., Enkavi, G., Biojone, C., Cannarozzo, C., Sahu, M. P., Kaurinkoski, K., Brunello, C. A., Steinzeig, A., Winkel, F., Patil, S., Vestring, S., Serchov, T., Diniz, C., Laukkanen, L., Cardon, I., Antila, H., … Castrén, E. (2021).

Antidepressant drugs act by directly binding to TRKB neurotrophin receptors. *Cell*, 184(5), 1299–1313.e19. https://doi.org/10.1016/j.cell.2021.01.034

Castellanos, J. P., Woolley, C., Bruno, K. A., Zeidan, F., Halberstadt, A., & Furnish, T. (2020). Chronic pain and psychedelics: a review and proposed mechanism of action. Regional anesthesia and pain medicine, 45(7), 486–494. https://doi.org/10.1136/rapm-2020-101273

Cavero, I., & Guillon, J. M. (2014). Safety Pharmacology assessment of drugs with biased 5-HT(2B) receptor agonism mediating cardiac valvulopathy. *Journal of pharmacological and toxicological methods*, *69*(2), 150–161. https://doi.org/10.1016/j.vascn.2013.12.004

Cerrito, F., & Raiteri, M. (1979). Serotonin release is modulated by presynaptic autoreceptors. *European journal of pharmacology*, 57(4), 427–430. https://doi.org/10.1016/0014-2999(79)90506-5

Ceulemans, B., Boel, M., Leyssens, K., Van Rossem, C., Neels, P., Jorens, P. G., & Lagae, L. (2012). Successful use of fenfluramine as an add-on treatment for Dravet syndrome. *Epilepsia*, *53*(7), 1131–1139. https://doi.org/10.1111/j.1528-1167.2012.03495.x

Cohen S. (1960). Lysergic acid diethylamide: side effects and complications. *The Journal of nervous and mental disease*, *130*, 30–40. https://doi.org/10.1097/00005053-196001000-00005

Cohen, S., & Ditman, K. S. (1962). Complications associated with lysergic acid diethylamide (LSD-25). *JAMA*, 181, 161–162. https://doi.org/10.1001/jama.1962.03050280091013b

Coney, L. D., Maier, L. J., Ferris, J. A., Winstock, A. R., & Barratt, M. J. (2017). Genie in a blotter: A comparative study of LSD and LSD analogues' effects and user profile. *Human psychopharmacology*, 32(3), 10.1002/hup.2599. https://doi.org/10.1002/hup.2599

Connolly, H. M., Crary, J. L., McGoon, M. D., Hensrud, D. D., Edwards, B. S., Edwards, W. D., & Schaff, H. V. (1997). Valvular heart disease associated with fenfluramine-phentermine. *The New England journal of medicine*, 337(9), 581–588. https://doi.org/10.1056/NEJM199708283370901

Connolly, J. M., Bakay, M. A., Fulmer, J. T., Gorman, R. C., Gorman, J. H., 3rd, Oyama, M. A., & Levy, R. J. (2009). Fenfluramine disrupts the mitral valve interstitial cell response to serotonin. *The American journal of pathology*, 175(3), 988–997. https://doi.org/10.2353/ajpath.2009.081101

Cussac, D., Boutet-Robinet, E., Ailhaud, M. C., Newman-Tancredi, A., Martel, J. C., Danty, N., & Rauly-Lestienne, I. (2008). Agonist-directed trafficking of signalling at serotonin 5-HT2A, 5-HT2B and 5-HT2C-VSV receptors mediated Gq/11 activation and calcium mobilisation in CHO cells. *European journal of pharmacology*, 594(1-3), 32–38. https://doi.org/10.1016/j.ejphar.2008.07.040

Dahl, C. F., Allen, M. R., Urie, P. M., & Hopkins, P. N. (2008). Valvular regurgitation and surgery associated with fenfluramine use: an analysis of 5743 individuals. *BMC medicine*, 6, 34. https://doi.org/10.1186/1741-7015-6-34

De Backer, T., Petrovic, M., Audenaert, K., Coeman, M., & De Bacquer, D. (2016). A happy valve in a happy patient? Serotonergic antidepressants and the risk of valvular heart disease (SERVAL). A case-control study. *Acta clinica Belgica*, *71*(1), 57–62. https://doi.org/10.1080/17843286.2015.1125563

De Gregorio, D., Enns, J. P., Nuñez, N. A., Posa, L., & Gobbi, G. (2018). d-Lysergic acid diethylamide, psilocybin, and other classic hallucinogens: Mechanism of action and potential therapeutic applications in mood disorders. *Progress in brain research*, 242, 69–96. https://doi.org/10.1016/bs.pbr.2018.07.00

Deleu, D., Northway, M. G., & Hanssens, Y. (2002). Clinical pharmacokinetic and pharmacodynamic properties of drugs used in the treatment of Parkinson's disease. *Clinical pharmacokinetics*, 41(4), 261–309. https://doi.org/10.2165/00003088-200241040-00003

Dolder, P. C., Schmid, Y., Haschke, M., Rentsch, K. M., & Liechti, M. E. (2015). Pharmacokinetics and Concentration-Effect Relationship of Oral LSD in Humans. *The international journal of neuropsychopharmacology*, *19*(1), pyv072. https://doi.org/10.1093/ijnp/pyv072

Dolder, P. C., Schmid, Y., Steuer, A. E., Kraemer, T., Rentsch, K. M., Hammann, F., & Liechti, M. E. (2017). Pharmacokinetics and Pharmacodynamics of Lysergic Acid Diethylamide in Healthy Subjects. *Clinical pharmacokinetics*, 56(10), 1219–1230. https://doi.org/10.1007/s40262-017-0513-9

Droogmans, S., Roosens, B., Cosyns, B., Degaillier, C., Hernot, S., Weytjens, C., Garbar, C., Caveliers, V., Pipeleers-Marichal, M., Franken, P. R., Lahoutte, T., Schoors, D., & Van Camp, G. (2009). Cyproheptadine prevents pergolide-induced valvulopathy in rats: an echocardiographic

and histopathological study. American journal of physiology. *Heart and circulatory physiology*, 296(6), H1940–H1948. https://doi.org/10.1152/ajpheart.01177.2008

Eishingdrelo, H., Sun, W., Li, H., Wang, L., Eishingdrelo, A., Dai, S., McKew, J. C., & Zheng, W. (2015). ERK and β-arrestin interaction: a converging point of signaling pathways for multiple types of cell surface receptors. *Journal of biomolecular screening*, *20*(3), 341–349. https://doi.org/10.1177/1087057114557233

European Medicines Agency, (EMA). (2013). New restrictions on use of medicines containing ergot derivatives. Retrieved March 30th 2021, from https://www.ema.europa.eu/en/news/new-restrictions-use-medicines-containing-ergot-derivatives

European Medicines Agency, (EMA). (2014). Restrictions on use of medicines containing ergot derivatives. Retrieved March 30th 2021, from https://www.ema.europa.eu/en/medicines/human/referrals/ergot-derivatives

Erland, L. A., Murch, S. J., Reiter, R. J., & Saxena, P. K. (2015). A new balancing act: The many roles of melatonin and serotonin in plant growth and development. *Plant signaling & behavior*, *10*(11), e1096469. https://doi.org/10.1080/15592324.2015.1096469

Eshleman, A. J., Wolfrum, K. M., Reed, J. F., Kim, S. O., Johnson, R. A., & Janowsky, A. (2018). Neurochemical pharmacology of psychoactive substituted N-benzylphenethylamines: High potency agonists at 5-HT2A receptors. *Biochemical pharmacology*, 158, 27–34. https://doi.org/10.1016/j.bcp.2018.09.024

Ettrup, A., Palner, M., Gillings, N., Santini, M. A., Hansen, M., Kornum, B. R., Rasmussen, L. K., Någren, K., Madsen, J., Begtrup, M., & Knudsen, G. M. (2010). Radiosynthesis and evaluation of 11C-CIMBI-5 as a 5-HT2A receptor agonist radioligand for PET. *Journal of nuclear medicine: official publication, Society of Nuclear Medicine*, *51*(11), 1763–1770. https://doi.org/10.2967/jnumed.109.074021

Family, N., Maillet, E. L., Williams, L., Krediet, E., Carhart-Harris, R. L., Williams, T. M., Nichols, C. D., Goble, D. J., & Raz, S. (2020). Safety, tolerability, pharmacokinetics, and pharmacodynamics of low dose lysergic acid diethylamide (LSD) in healthy older volunteers. *Psychopharmacology*, *237*(3), 841–853. https://doi.org/10.1007/s00213-019-05417-7

Ferguson, J. M., & Hill, H. (2006). Pharmacokinetics of fluoxetine in elderly men and women. Gerontology, 52(1), 45–50. https://doi.org/10.1159/000089825

Fioravanti, M., Nakashima, T., Xu, J., & Garg, A. (2014). A systematic review and meta-analysis assessing adverse event profile and tolerability of nicergoline. *BMJ open*, *4*(7), e005090. https://doi.org/10.1136/bmjopen-2014-005090

Fitzgerald, L. W., Burn, T. C., Brown, B. S., Patterson, J. P., Corjay, M. H., Valentine, P. A., Sun, J. H., Link, J. R., Abbaszade, I., Hollis, J. M., Largent, B. L., Hartig, P. R., Hollis, G. F., Meunier, P. C., Robichaud, A. J., & Robertson, D. W. (2000). Possible role of valvular serotonin 5-HT(2B) receptors in the cardiopathy associated with fenfluramine. *Molecular pharmacology*, *57*(1), 75–81.

Fournier, A., & Zureik, M. (2012). Estimate of deaths due to valvular insufficiency attributable to the use of benfluorex in France. *Pharmacoepidemiology and drug safety*, 21(4), 343–351. https://doi.org/10.1002/pds.3213

Francescangeli, J., Karamchandani, K., Powell, M., & Bonavia, A. (2019). The Serotonin Syndrome: From Molecular Mechanisms to Clinical Practice. *International journal of molecular sciences*, 20(9), 2288. https://doi.org/10.3390/ijms20092288

Gee, P., Schep, L. J., Jensen, B. P., Moore, G., & Barrington, S. (2016). Case series: toxicity from 25B-NBOMe--a cluster of N-bomb cases. *Clinical toxicology (Philadelphia, Pa.)*, 54(2), 141–146. https://doi.org/10.3109/15563650.2015.1115056

Geiger, H. A., Wurst, M. G., & Daniels, R. N. (2018). DARK Classics in Chemical Neuroscience: Psilocybin. *ACS chemical neuroscience*, *9*(10), 2438–2447. https://doi.org/10.1021/acschemneuro.8b00186

Gerhards, N., Neubauer, L., Tudzynski, P., & Li, S. M. (2014). Biosynthetic pathways of ergot alkaloids. *Toxins*, *6*(12), 3281–3295. https://doi.org/10.3390/toxins6123281

Giacomelli, S., Palmery, M., Romanelli, L., Cheng, C. Y., & Silvestrini, B. (1998). Lysergic acid diethylamide (LSD) is a partial agonist of D2 dopaminergic receptors and it potentiates dopamine-mediated prolactin secretion in lactotrophs in vitro. Life sciences, 63(3), 215–222. https://doi.org/10.1016/s0024-3205(98)00262-8

Graham J. R. (1967). Cardiac and pulmonary fibrosis during methysergide therapy for headache. *The American journal of the medical sciences*, *254*(1), 1–12. https://doi.org/10.1097/00000441-196707000-00001

Gras, J., Llenas, J., Jansat, J. M., Jáuregui, J., Cabarrocas, X., & Palacios, J. M. (2002). Almotriptan, a new anti-migraine agent: a review. *CNS drug reviews*, *8*(3), 217–234. https://doi.org/10.1111/j.1527-3458.2002.tb00226.x

Gustafsson, B. I., Tømmerås, K., Nordrum, I., Loennechen, J. P., Brunsvik, A., Solligård, E., Fossmark, R., Bakke, I., Syversen, U., & Waldum, H. (2005). Long-term serotonin administration induces heart valve disease in rats. *Circulation*, 111(12), 1517–1522. https://doi.org/10.1161/01.CIR.0000159356.42064.48

Halberstadt, A. L., & Geyer, M. A. (2011). Multiple receptors contribute to the behavioral effects of indoleamine hallucinogens. *Neuropharmacology*, 61(3), 364–381. https://doi.org/10.1016/j.neuropharm.2011.01.017

Halpern, J. H., & Pope, H. G., Jr (1999). Do hallucinogens cause residual neuropsychological toxicity?. *Drug and alcohol dependence*, *53*(3), 247–256. https://doi.org/10.1016/s0376-8716(98)00129-x

Hansen, M., Phonekeo, K., Paine, J. S., Leth-Petersen, S., Begtrup, M., Bräuner-Osborne, H., & Kristensen, J. L. (2014). Synthesis and structure-activity relationships of N-benzyl phenethylamines as 5-HT2A/2C agonists. *ACS chemical neuroscience*, 5(3), 243–249. https://doi.org/10.1021/cn400216u

Henry, J. L., Epley, J., & Rohrig, T. P. (2003). The analysis and distribution of mescaline in postmortem tissues. *Journal of analytical toxicology*, 27(6), 381–382. https://doi-org.ezproxy.uef.fi:2443/10.1093/jat/27.6.381

Hertz, L., Rothman, D. L., Li, B., & Peng, L. (2015). Response: Commentary: Chronic SSRI Stimulation of Astrocytic 5-HT2B Receptors Change Multiple Gene Expressions/Editings and Metabolism of Glutamate, Glucose and Glycogen: A Potential Paradigm Shift. *Frontiers in behavioral neuroscience*, *9*, 308. https://doi.org/10.3389/fnbeh.2015.00308

Hinton, R. B., & Yutzey, K. E. (2011). Heart valve structure and function in development and disease. *Annual review of physiology*, 73, 29–46. https://doi.org/10.1146/annurev-physiol-012110-142145

Hofmann A. (1979). How LSD originated. *Journal of psychedelic drugs*, 11(1-2), 53–60. https://doi.org/10.1080/02791072.1979.10472092

Hopkins, P. N., & Polukoff, G. I. (2003). Risk of valvular heart disease associated with use of fenfluramine. *BMC cardiovascular disorders*, 3, 5. https://doi.org/10.1186/1471-2261-3-5

Huang, X. P., Setola, V., Yadav, P. N., Allen, J. A., Rogan, S. C., Hanson, B. J., Revankar, C., Robers, M., Doucette, C., & Roth, B. L. (2009). Parallel functional activity profiling reveals valvulopathogens are potent 5-hydroxytryptamine(2B) receptor agonists: implications for drug safety assessment. *Molecular pharmacology*, *76*(4), 710–722. https://doi.org/10.1124/mol.109.058057

Hutcheson, J. D., Setola, V., Roth, B. L., & Merryman, W. D. (2011). Serotonin receptors and heart valve disease--it was meant 2B. *Pharmacology & therapeutics*, 132(2), 146–157. https://doi.org/10.1016/j.pharmthera.2011.03.008

Hutten, Nadia R. P. W, Mason, N. L., Dolder, P. C., Theunissen, E. L., Holze, F., Liechti, M. E., et al. (2020). Low doses of LSD acutely increase BDNF blood plasma levels in healthy volunteers. *ACS Pharmacology & Translational Science*, doi:10.1021/acsptsci.0c00099

Hynninen E., Moliner R., Ekelund J., Korpi E.R., Elsilä L. (2020). Psykedeelien kliiniset mahdollisuudet ja biologiset mekanismit. *Lääketieteellinen aikakauskirja Duodecim*, 136(13), 1531–9.

Jacobs, B. L., & Azmitia, E. C. (1992). Structure and function of the brain serotonin system. *Physiological reviews*, 72(1), 165–229. https://doi.org/10.1152/physrev.1992.72.1.165

Jaffe JH. (1990) Drug addiction and drug abuse, in Goodman and Gilman's the Pharmacological Basis of Therapeutics (Goodman AG, Rall TW, Nies AS, Taylor P. eds) 8th ed, pp 522–573, McGraw Hill, New York.

Jensen, A. A., McCorvy, J. D., Leth-Petersen, S., Bundgaard, C., Liebscher, G., Kenakin, T. P., Bräuner-Osborne, H., Kehler, J., & Kristensen, J. L. (2017). Detailed Characterization of the In Vitro

Pharmacological and Pharmacokinetic Properties of N-(2-Hydroxybenzyl)-2,5-Dimethoxy-4-Cyanophenylethylamine (25CN-NBOH), a Highly Selective and Brain-Penetrant 5-HT2A Receptor Agonist. *The Journal of pharmacology and experimental therapeutics*, 361(3), 441–453. https://doi.org/10.1124/jpet.117.239905

Johansen, A., Holm, S., Dall, B., Keller, S., Kristensen, J. L., Knudsen, G. M., & Hansen, H. D. (2019). Human biodistribution and radiation dosimetry of the 5-HT_{2A} receptor agonist Cimbi-36 labeled with carbon-11 in two positions. *EJNMMI research*, *9*(1), 71. https://doi.org/10.1186/s13550-019-0527-4

Johnstad P. G. (2018). Powerful substances in tiny amounts: An interview study of psychedelic microdosing. *Nordisk alkohol- & narkotikatidskrift: NAT*, 35(1), 39–51. https://doi.org/10.1177/1455072517753339

Jähnichen, S., Horowski, R., & Pertz, H. H. (2005). Agonism at 5-HT2B receptors is not a class effect of the ergolines. *European journal of pharmacology*, 513(3), 225–228. https://doi.org/10.1016/j.ejphar.2005.03.010

Kamińska, K., Świt, P., & Malek, K. (2020). 2-(4-iodo-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl]ethanamine (25I-NBOME): A harmful hallucinogen review. *Journal of Analytical Toxicology*, 44(9), 947-956. doi:10.1093/jat/bkaa022

Kekewska, A., Hübner, H., Gmeiner, P., & Pertz, H. H. (2011). The bulky N6 substituent of cabergoline is responsible for agonism of this drug at 5-hydroxytryptamine 5-HT2A and 5-HT2B receptors and thus is a determinant of valvular heart disease. *The Journal of pharmacology and experimental therapeutics*, 338(1), 381–391. https://doi.org/10.1124/jpet.111.181255

Klein, A. K., Chatha, M., Laskowski, L. J., Anderson, E. I., Brandt, S. D., Chapman, S. J., et al. (2020). Investigation of the Structure–Activity relationships of psilocybin analogues. *ACS Pharmacology & Translational Science*, doi:10.1021/acsptsci.0c00176

Knauer, C. S., Campbell, J. E., Chio, C. L., & Fitzgerald, L. W. (2009). Pharmacological characterization of mitogen-activated protein kinase activation by recombinant human 5-HT2C, 5-HT2A, and 5-HT2B receptors. *Naunyn-Schmiedeberg's archives of pharmacology*, 379(5), 461–471. https://doi.org/10.1007/s00210-008-0378-4

Knight, A. R., Misra, A., Quirk, K., Benwell, K., Revell, D., Kennett, G., & Bickerdike, M. (2004). Pharmacological characterisation of the agonist radioligand binding site of 5-HT(2A), 5-HT(2B) and 5-HT(2C) receptors. *Naunyn-Schmiedeberg's archives of pharmacology*, *370*(2), 114–123. https://doi.org/10.1007/s00210-004-0951-4

Krebs, T. S., & Johansen, P. Ø. (2013). Over 30 million psychedelic users in the United States. *F1000Research*, *2*, 98. https://doi.org/10.12688/f1000research.2-98.v1

Kristofic, J. J., Chmiel, J. D., Jackson, G. F., Vorce, S. P., Holler, J. M., Robinson, S. L., & Bosy, T. Z. (2016). Detection of 25C-NBOMe in Three Related Cases. *Journal of analytical toxicology*, 40(6), 466–472. https://doi.org/10.1093/jat/bkw035

Kurrasch-Orbaugh, D. M., Watts, V. J., Barker, E. L., & Nichols, D. E. (2003). Serotonin 5-hydroxytryptamine 2A receptor-coupled phospholipase C and phospholipase A2 signaling pathways have different receptor reserves. *The Journal of pharmacology and experimental therapeutics*, 304(1), 229–237. https://doi.org/10.1124/jpet.102.042184

Kuypers, K. P., Ng, L., Erritzoe, D., Knudsen, G. M., Nichols, C. D., Nichols, D. E., Pani, L., Soula, A., & Nutt, D. (2019). Microdosing psychedelics: More questions than answers? An overview and suggestions for future research. *Journal of psychopharmacology (Oxford, England)*, *33*(9), 1039–1057. https://doi.org/10.1177/0269881119857204

Lambe, E. K., & Aghajanian, G. K. (2006). Hallucinogen-induced UP states in the brain slice of rat prefrontal cortex: role of glutamate spillover and NR2B-NMDA receptors. *Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology*, *31*(8), 1682–1689. https://doi.org/10.1038/sj.npp.1300944

Lenz, C., Sherwood, A., Kargbo, R., & Hoffmeister, D. (2021). Taking Different Roads: l-Tryptophan as the Origin of Psilocybe Natural Products. *ChemPlusChem*, *86*(1), 28–35. https://doi.org/10.1002/cplu.202000581

Liechti M. E. (2017). Modern Clinical Research on LSD. *Neuropsychopharmacology*: official publication of the American College of Neuropsychopharmacology, 42(11), 2114–2127. https://doi.org/10.1038/npp.2017.86

Lin, C. H., Hsiao, F. Y., Liu, Y. B., Gau, S. S., Wang, C. C., & Shen, L. J. (2016). Antidepressants and Valvular Heart Disease: A Nested Case-Control Study in Taiwan. *Medicine*, 95(14), e3172. https://doi.org/10.1097/MD.0000000000003172

Liu, Y., Yang, Y., Ward, R., An, S., Guo, X. X., Li, W., & Xu, T. R. (2015). Biased signalling: the instinctive skill of the cell in the selection of appropriate signalling pathways. *The Biochemical journal*, 470(2), 155–167. https://doi.org/10.1042/BJ20150358

López-Giménez, J. F., & González-Maeso, J. (2018). Hallucinogens and Serotonin 5-HT2A Receptor-Mediated Signaling Pathways. *Current topics in behavioral neurosciences*, 36, 45–73. https://doi.org/10.1007/7854_2017_478

Löfdahl, A., Tornling, G., Wigén, J., Larsson-Callerfelt, A. K., Wenglén, C., & Westergren-Thorsson, G. (2020). Pathological Insight into 5-HT2B Receptor Activation in Fibrosing Interstitial Lung Diseases. International journal of molecular sciences, 22(1), 225. https://doi.org/10.3390/ijms22010225

Marek, G. J., & Aghajanian, G. K. (1996). LSD and the phenethylamine hallucinogen DOI are potent partial agonists at 5-HT2A receptors on interneurons in rat piriform cortex. *The Journal of pharmacology and experimental therapeutics*, 278(3), 1373–1382.

Martins, D., Barratt, M. J., Pires, C. V., Carvalho, H., Vilamala, M. V., Espinosa, I. F., & Valente, H. (2017). The detection and prevention of unintentional consumption of DOx and 25x-NBOMe at Portugal's Boom Festival. *Human psychopharmacology*, *32*(3), 10.1002/hup.2608. https://doi.org/10.1002/hup.2608

Meldrum, B. S., & Naquet, R. (1971). Effects of psilocybin, dimethyltryptamine, mescaline and various lysergic acid derivatives on the EEG and on photically induced epilepsy in the baboon (Papio papio). *Electroencephalography and clinical neurophysiology*, 31(6), 563–572. https://doi.org/10.1016/0013-4694(71)90072-1

Melmon, K. L., Sjoerdsma, A., Oates, J. A., & Laster, L. (1965). Treatment of malabsorption and diarrhea of the carcinoid syndrome with methysergide. *Gastroenterology*, *48*, 18–24.

Merlet, I., Ostrowsky, K., Costes, N., Ryvlin, P., Isnard, J., Faillenot, I., Lavenne, F., Dufournel, D., Le Bars, D., & Mauguière, F. (2004). 5-HT1A receptor binding and intracerebral activity in temporal

lobe epilepsy: an [18F]MPPF-PET study. *Brain: a journal of neurology*, 127(Pt 4), 900–913. https://doi.org/10.1093/brain/awh109

Millan, M. J., Maiofiss, L., Cussac, D., Audinot, V., Boutin, J. A., & Newman-Tancredi, A. (2002). Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. I. A multivariate analysis of the binding profiles of 14 drugs at 21 native and cloned human receptor subtypes. *The Journal of pharmacology and experimental therapeutics*, 303(2), 791–804. https://doi.org/10.1124/jpet.102.039867

Mognol, G. P., Carneiro, F. R., Robbs, B. K., Faget, D. V., & Viola, J. P. (2016). Cell cycle and apoptosis regulation by NFAT transcription factors: new roles for an old player. *Cell death & disease*, *7*(4), e2199. https://doi.org/10.1038/cddis.2016.97

Mohr, A., Friscia, M., Yeakel, J. K., & Logan, B. K. (2018). Use of synthetic stimulants and hallucinogens in a cohort of electronic dance music festival attendees. Forensic science international, 282, 168–178. https://doi.org/10.1016/j.forsciint.2017.11.017

Monte, A. P., Waldman, S. R., Marona-Lewicka, D., Wainscott, D. B., Nelson, D. L., Sanders-Bush, E., & Nichols, D. E. (1997). Dihydrobenzofuran analogues of hallucinogens. 4. Mescaline derivatives. *Journal of medicinal chemistry*, 40(19), 2997–3008. https://doi.org/10.1021/jm970219x

Moya, P. R., Berg, K. A., Gutiérrez-Hernandez, M. A., Sáez-Briones, P., Reyes-Parada, M., Cassels, B. K., & Clarke, W. P. (2007). Functional selectivity of hallucinogenic phenethylamine and phenylisopropylamine derivatives at human 5-hydroxytryptamine (5-HT)2A and 5-HT2C receptors. *The Journal of pharmacology and experimental therapeutics*, 321(3), 1054–1061. https://doi.org/10.1124/jpet.106.117507

Mutlak, M., & Kehat, I. (2015). Extracellular signal-regulated kinases 1/2 as regulators of cardiac hypertrophy. *Frontiers in pharmacology*, 6, 149. https://doi.org/10.3389/fphar.2015.00149

Müller, F., Mühlhauser, M., Holze, F., Lang, U. E., Walter, M., Liechti, M. E., & Borgwardt, S. (2020). Treatment of a Complex Personality Disorder Using Repeated Doses of LSD-A Case Report on Significant Improvements in the Absence of Acute Drug Effects. *Frontiers in psychiatry*, *11*, 573953. https://doi.org/10.3389/fpsyt.2020.573953

Nebigil, C. G., Etienne, N., Schaerlinger, B., Hickel, P., Launay, J. M., & Maroteaux, L. (2001). Developmentally regulated serotonin 5-HT2B receptors. *International journal of developmental neuroscience: the official journal of the International Society for Developmental Neuroscience*, *19*(4), 365–372. https://doi.org/10.1016/s0736-5748(01)00022-3

Newman-Tancredi, A., Cussac, D., Quentric, Y., Touzard, M., Verrièle, L., Carpentier, N., & Millan, M. J. (2002). Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. III. Agonist and antagonist properties at serotonin, 5-HT(1) and 5-HT(2), receptor subtypes. *The Journal of pharmacology and experimental therapeutics*, 303(2), 815–822. https://doi.org/10.1124/jpet.102.039883

Nichols, D. E., & Nichols, C. D. (2008). Serotonin receptors. *Chemical reviews*, *108*(5), 1614–1641. https://doi.org/10.1021/cr078224o

Nichols, D. E., Sassano, M. F., Halberstadt, A. L., Klein, L. M., Brandt, S. D., Elliott, S. P., & Fiedler, W. J. (2015). N-Benzyl-5-methoxytryptamines as Potent Serotonin 5-HT2 Receptor Family Agonists and Comparison with a Series of Phenethylamine Analogues. *ACS chemical neuroscience*, 6(7), 1165–1175. https://doi.org/10.1021/cn500292d

Nichols D. E. (2016). Psychedelics. *Pharmacological reviews*, 68(2), 264–355. https://doi.org/10.1124/pr.115.011478

Nichols D. E. (2018). Dark Classics in Chemical Neuroscience: Lysergic Acid Diethylamide (LSD). *ACS chemical neuroscience*, *9*(10), 2331–2343. https://doi.org/10.1021/acschemneuro.8b00043

Nichols, D. E., & Grob, C. S. (2018). Is LSD toxic?. *Forensic science international*, *284*, 141–145. https://doi.org/10.1016/j.forsciint.2018.01.006

Nugent, K. G., & Saville, B. J. (2004). Forensic analysis of hallucinogenic fungi: a DNA-based approach. *Forensic science international*, *140*(2-3), 147–157. https://doi.org/10.1016/j.forsciint.2003.11.022

Nutt D. (2019). Psychedelic drugs-a new era in psychiatry? . *Dialogues in clinical neuroscience*, 21(2), 139–147. https://doi.org/10.31887/DCNS.2019.21.2/dnutt

Papoian, T., Jagadeesh, G., Saulnier, M., Simpson, N., Ravindran, A., Yang, B., Laniyonu, A. A., Khan, I., & Szarfman, A. (2017). Regulatory Forum Review*: Utility of in Vitro Secondary

Pharmacology Data to Assess Risk of Drug-induced Valvular Heart Disease in Humans: Regulatory Considerations. *Toxicologic pathology*, 45(3), 381–388. https://doi.org/10.1177/0192623317690609

Peng, L., Song, D., Li, B., & Verkhratsky, A. (2018). Astroglial 5-HT2B receptor in mood disorders. *Expert review of neurotherapeutics*, 18(5), 435–442. https://doi.org/10.1080/14737175.2018.1458612

Porter, R. H., Benwell, K. R., Lamb, H., Malcolm, C. S., Allen, N. H., Revell, D. F., Adams, D. R., & Sheardown, M. J. (1999). Functional characterization of agonists at recombinant human 5-HT2A, 5-HT2B and 5-HT2C receptors in CHO-K1 cells. *British journal of pharmacology*, 128(1), 13–20. https://doi.org/10.1038/sj.bjp.0702751

Pottie, E., Dedecker, P., & Stove, C. P. (2020). Identification of psychedelic new psychoactive substances (NPS) showing biased agonism at the 5-HT2AR through simultaneous use of β-arrestin 2 and miniGαq bioassays. *Biochemical pharmacology*, 182, 114251. https://doi.org/10.1016/j.bcp.2020.114251

Pritchett, A. M., Morrison, J. F., Edwards, W. D., Schaff, H. V., Connolly, H. M., & Espinosa, R. E. (2002). Valvular heart disease in patients taking pergolide. *Mayo Clinic proceedings*, *77*(12), 1280–1286. https://doi.org/10.4065/77.12.1280

Psychoactive drug screening program (PDSP). (2021). Retrieved March 2021, from https://pdsp.unc.edu/databases/pdsp.php

Rickli, A., Luethi, D., Reinisch, J., Buchy, D., Hoener, M. C., & Liechti, M. E. (2015). Receptor interaction profiles of novel N-2-methoxybenzyl (NBOMe) derivatives of 2,5-dimethoxy-substituted phenethylamines (2C drugs). *Neuropharmacology*, *99*, 546–553. https://doi.org/10.1016/j.neuropharm.2015.08.034

Rickli, A., Moning, O. D., Hoener, M. C., & Liechti, M. E. (2016). Receptor interaction profiles of novel psychoactive tryptamines compared with classic hallucinogens. *European neuropsychopharmacology: the journal of the European College of Neuropsychopharmacology*, 26(8), 1327–1337. https://doi.org/10.1016/j.euroneuro.2016.05.001

Rikoslaki, 50 luku — Huumausainelaki . Retrieved 4th November 2021, from https://www.finlex.fi/fi/laki/ajantasa/2008/20080373

Rillich, J., & Stevenson, P. A. (2018). Serotonin Mediates Depression of Aggression After Acute and Chronic Social Defeat Stress in a Model Insect. *Frontiers in behavioral neuroscience*, 12, 233. https://doi.org/10.3389/fnbeh.2018.00233

Rothman, R. B., Baumann, M. H., Savage, J. E., Rauser, L., McBride, A., Hufeisen, S. J., & Roth, B. L. (2000). Evidence for possible involvement of 5-HT(2B) receptors in the cardiac valvulopathy associated with fenfluramine and other serotonergic medications. *Circulation*, *102*(23), 2836–2841. https://doi.org/10.1161/01.cir.102.23.2836

Rothman, R. B., & Baumann, M. H. (2009). Serotonergic drugs and valvular heart disease. *Expert opinion on drug safety*, *8*(3), 317–329. https://doi.org/10.1517/14740330902931524

Sachdev, M., Miller, W. C., Ryan, T., & Jollis, J. G. (2002). Effect of fenfluramine-derivative diet pills on cardiac valves: a meta-analysis of observational studies. *American heart journal*, *144*(6), 1065–1073. https://doi.org/10.1067/mhj.2002.126733

Salet, N., Visser, M., Stam, C., & Smulders, Y. M. (2019). Stroboscopic light effects during electronic dance music festivals and photosensitive epilepsy: a cohort study and case report. BMJ open, 9(6), e023442. https://doi.org/10.1136/bmjopen-2018-023442

Sankar, D. V. Siva. (1975). LSD: A Total Study. Westbury, N.Y: PJD Publications

Saper, J. R., & Silberstein, S. (2006). Pharmacology of dihydroergotamine and evidence for efficacy and safety in migraine. *Headache*, 46 Suppl 4, S171–S181. https://doi.org/10.1111/j.1526-4610.2006.00601.x

Sard, H., Kumaran, G., Morency, C., Roth, B. L., Toth, B. A., He, P., & Shuster, L. (2005). SAR of psilocybin analogs: discovery of a selective 5-HT 2C agonist. *Bioorganic & medicinal chemistry letters*, 15(20), 4555–4559. https://doi.org/10.1016/j.bmcl.2005.06.104

Schade, R., Andersohn, F., Suissa, S., Haverkamp, W., & Garbe, E. (2007). Dopamine agonists and the risk of cardiac-valve regurgitation. *The New England journal of medicine*, *356*(1), 29–38. https://doi.org/10.1056/NEJMoa062222

Schaerlinger, B., Hickel, P., Etienne, N., Guesnier, L., & Maroteaux, L. (2003). Agonist actions of dihydroergotamine at 5-HT2B and 5-HT2C receptors and their possible relevance to antimigraine efficacy. *British journal of pharmacology*, *140*(2), 277–284. https://doi.org/10.1038/sj.bjp.0705437

Schindler, E. A., Gottschalk, C. H., Weil, M. J., Shapiro, R. E., Wright, D. A., & Sewell, R. A. (2015). Indoleamine Hallucinogens in Cluster Headache: Results of the Clusterbusters Medication Use Survey. *Journal of psychoactive drugs*, *47*(5), 372–381. https://doi.org/10.1080/02791072.2015.1107664

Schindler, E., Sewell, R. A., Gottschalk, C. H., Luddy, C., Flynn, L. T., Lindsey, H., Pittman, B. P., Cozzi, N. V., & D'Souza, D. C. (2020). Exploratory Controlled Study of the Migraine-Suppressing Effects of Psilocybin. *Neurotherapeutics: the journal of the American Society for Experimental NeuroTherapeutics*, 10.1007/s13311-020-00962-y. Advance online publication. https://doi.org/10.1007/s13311-020-00962-y

Schmid, C. L., Raehal, K. M., & Bohn, L. M. (2008). Agonist-directed signaling of the serotonin 2A receptor depends on beta-arrestin-2 interactions in vivo. *Proceedings of the National Academy of Sciences of the United States of America*, 105(3), 1079–1084. https://doi.org/10.1073/pnas.0708862105

Schmid, C. L., & Bohn, L. M. (2010). Serotonin, but not N-methyltryptamines, activates the serotonin 2A receptor via a βarrestin2/src/akt signaling complex in vivo Society for *Neuroscience*. doi:10.1523/JNEUROSCI.1665-10.2010

Schmuck, K., Ullmer, C., Kalkman, H. O., Probst, A., & Lubbert, H. (1996). Activation of meningeal 5-HT2B receptors: an early step in the generation of migraine headache?. *The European journal of neuroscience*, 8(5), 959–967. https://doi.org/10.1111/j.1460-9568.1996.tb01583.x

Segelcke, D., & Messlinger, K. (2017). Putative role of 5-HT_{2B} receptors in migraine pathophysiology. *Cephalalgia: an international journal of headache*, *37*(4), 365–371. https://doi.org/10.1177/0333102416646760

Sessa B. (2018). The 21st century psychedelic renaissance: heroic steps forward on the back of an elephant. *Psychopharmacology*, 235(2), 551–560. https://doi.org/10.1007/s00213-017-4713-7

Servier Medical Art. (2021). Retrieved 28th April 2021, from https://smart.servier.com/.

Setola, V., Hufeisen, S. J., Grande-Allen, K. J., Vesely, I., Glennon, R. A., Blough, B., Rothman, R. B., & Roth, B. L. (2003). 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy") induces fenfluramine-like proliferative actions on human cardiac valvular interstitial cells in vitro. *Molecular pharmacology*, 63(6), 1223–1229. https://doi.org/10.1124/mol.63.6.1223

Silberstein SD. (2008). Methysergide. *Cephalalgia*, 18(7), 421-435. https://doi.org/10.1111/j.1468-2982.1998.1807421.x

Simonis, G., Fuhrmann, J. T., & Strasser, R. H. (2007). Meta-analysis of heart valve abnormalities in Parkinson's disease patients treated with dopamine agonists. *Movement disorders: official journal of the Movement Disorder Society*, 22(13), 1936–1942. https://doi.org/10.1002/mds.21639

Soldin, O. P., Dahlin, J., & O'Mara, D. M. (2008). Triptans in pregnancy. *Therapeutic drug monitoring*, 30(1), 5–9. https://doi.org/10.1097/FTD.0b013e318162c89b

Stiles, C. E., Lloyd, G., Bhattacharyya, S., Steeds, R. P., Boomla, K., Bestwick, J. P., & Drake, W. M. (2021). Incidence of Cabergoline-Associated Valvulopathy in Primary Care Patients With Prolactinoma Using Hard Cardiac Endpoints. *The Journal of clinical endocrinology and metabolism*, *106*(2), e711–e720. https://doi.org/10.1210/clinem/dgaa882

Strassman R. J. (1984). Adverse reactions to psychedelic drugs. A review of the literature. *The Journal of nervous and mental disease*, *172*(10), 577–595. https://doi.org/10.1097/00005053-198410000-00001

Tanaka, E., & North, R. A. (1993). Actions of 5-hydroxytryptamine on neurons of the rat cingulate cortex. *Journal of neurophysiology*, 69(5), 1749–1757. https://doi.org/10.1152/jn.1993.69.5.1749

Terveyden ja hyvinvoinnin laitos, (THL). (2018). Retrieved 15th February 2021, from https://www.julkari.fi/bitstream/handle/10024/137660/Suomalaisten_huumeiden_k%c3%a4ytt%c3%b6_ja_huumeasenteet_2018_tilastoraportti.pdf?sequence=3&isAllowed=y

Tran, T., Brophy, J. M., Suissa, S., & Renoux, C. (2015). Risks of Cardiac Valve Regurgitation and Heart Failure Associated with Ergot- and Non-Ergot-Derived Dopamine Agonist Use in Patients with Parkinson's Disease: A Systematic Review of Observational Studies. *CNS drugs*, 29(12), 985–998. https://doi.org/10.1007/s40263-015-0293-4

Unett, D. J., Gatlin, J., Anthony, T. L., Buzard, D. J., Chang, S., Chen, C., Chen, X., Dang, H. T., Frazer, J., Le, M. K., Sadeque, A. J., Xing, C., & Gaidarov, I. (2013). Kinetics of 5-HT2B receptor signaling: profound agonist-dependent effects on signaling onset and duration. *The Journal of pharmacology and experimental therapeutics*, 347(3), 645–659. https://doi.org/10.1124/jpet.113.207670

Wacker, D., Wang, C., Katritch, V., Han, G. W., Huang, X. P., Vardy, E., McCorvy, J. D., Jiang, Y., Chu, M., Siu, F. Y., Liu, W., Xu, H. E., Cherezov, V., Roth, B. L., & Stevens, R. C. (2013). Structural features for functional selectivity at serotonin receptors. *Science* (New York, N.Y.), 340(6132), 615–619. https://doi.org/10.1126/science.1232808

Vitalis, T., Cases, O., Passemard, S., Callebert, J., & Parnavelas, J. G. (2007). Embryonic depletion of serotonin affects cortical development. *The European journal of neuroscience*, *26*(2), 331–344. https://doi.org/10.1111/j.1460-9568.2007.05661.x

Vizeli, P., Straumann, I., Holze, F., Schmid, Y., Dolder, P. C., & Liechti, M. E. (2021). Genetic influence of CYP2D6 on pharmacokinetics and acute subjective effects of LSD in a pooled analysis. Scientific reports, 11(1), 10851. https://doi.org/10.1038/s41598-021-90343-y

Vries, P. D., Villalón, C. M., & Saxena, P. R. (1999). Pharmacology of triptans. *Emerging Drugs, 4(1), 107–125.* doi:10.1517/14728214.4.1.107

Zawilska, J. B., Kacela, M., & Adamowicz, P. (2020). NBOMes-Highly Potent and Toxic Alternatives of LSD. *Frontiers in neuroscience*, *14*, 78. https://doi.org/10.3389/fnins.2020.00078

Zhang, S., Li, B., Lovatt, D., Xu, J., Song, D., Goldman, S. A., Nedergaard, M., Hertz, L., & Peng, L. (2010). 5-HT2B receptors are expressed on astrocytes from brain and in culture and are a chronic target for all five conventional 'serotonin-specific reuptake inhibitors'. *Neuron glia biology*, *6*(2), 113–125. https://doi.org/10.1017/S1740925X10000141

Zolkowska, D., Baumann, M. H., & Rothman, R. B. (2008). Chronic fenfluramine administration increases plasma serotonin (5-hydroxytryptamine) to nontoxic levels. *The Journal of pharmacology and experimental therapeutics*, *324*(2), 791–797. https://doi.org/10.1124/jpet.107.132654

APPENDIX 1

PubMed search:

#1 (nbome[tw] OR nbomes[tw] OR "phenethylamine derivative*"[tw] OR "phenylethylamine derivative*"[tw] OR "n-benzylphenethylamine*"[tw] OR "n-methoxybenzyl*"[tw] OR "n-benzylmethoxy*"[tw]) AND ("mechanism of action"[tw] OR "mechanisms of action"[tw] OR "mode of action"[tw] OR "modes of action"[tw] OR "action mechanism*"[tw] OR pharmacolog*[tw] OR neuropharmacolog*[tw] OR pharmacodynamic*[tw])

#2 "psilocybin/pharmacology"[majr]

#3 "lysergic acid diethylamide/pharmacology"[majr] AND review

#4 ("lysergic acid diethylamide"[tw] OR lsd[tw]) AND ("mechanism of action"[tw] OR "mechanisms of action"[tw] OR "mode of action"[tw] OR "modes of action"[tw] OR "action mechanism*"[tw] OR "pharmacologic action*"[tw] OR pharmacodynamic*[tw])

#5 "mescaline/pharmacology"[majr]

#6 (psilocybin[tw] OR mescaline[tw]) AND ("mechanism of action"[tw] OR "mechanisms of action"[tw] OR "mode of action"[tw] OR "modes of action"[tw] OR "action mechanism*"[tw] OR "pharmacologic action*"[tw] OR pharmacodynamic*[tw])

#7 #1 OR #2 OR #3 OR #4 OR #5 OR #6

Scopus search:

TITLE-ABS-KEY ((*psilocybin* OR *"lysergic acid diethylamide"* OR *lsd* OR *mescaline* OR *nbome* OR *nbomes* OR *"phenethylamine derivative*"* OR *"phenylethylamine derivative*"* OR *"n-benzylphenethylamine*"* OR *"n-methoxybenzyl*"* OR *"n-benzylmethoxy*"*) AND *{5-HT2B} OR {5-hydroxytryptamine receptor 2B}*)

APPENDIX 2

		5-HT2$_B$		
Compound	Affinity (Ki nM/L)	Potency (EC_{50} nM/L)	Efficacy (% of 5-HT)	Plasma concentration
Serotonin	1.54-8.71 (^{3}H5-HT) [a]	2.09 (Calcium flux) [b] 6.91 (Gq/11) [m] 0.32 (Calcium flux) [m] 1.78 (Calcium flux) [o] 0.21 (Calcium flux) [q] 9.33 (IP-acc.) [s] 1 (PI) [e] 0.4-6.2 nM (ERK2) [10, 11]	106 (Calcium flux) [b] 100.3 (Gq/11) [m] 109.9 (Calcium flux) [m] 100 (Calcium flux) [o] 100 (Calcium flux) [x] 99 (IP-accumulation) [s] 100 [e] 100 (ERK2) [10, 11]	~1 nM/L [9]
LSD	0.97 (^{3}H5-HT) [p] 3.72 ([^{3}H]LSD) [a] 0.60 ([^{3}H]Mesulergine) [m] 0.57 (^{3}H5-HT) [k]	8.91 (Calcium flux) [b] 12000 [c] 2.09 (Gq/11) [m] 39 (Calcium flux) [m] 3.07 (IP-1) [k] 110 nM (ERK2)[10]	51 (Calcium flux) [b] 71 [c] 43.4 (Gq/11) [m] 86.6 (Calcium flux) [m] 23.3 (IP-1) [k] 39 (ERK2)[10]	1.3 - 3.1 ng/ml 4 – 9.6 nM/L (100-200 µg) [1] 0.96-1.56 nM/L (10-20 µg) [2]
Bromocriptine	56.2 ([^{3}H]Mesulergine) [g]	1.29 (PI) [f] 2630 (Gq/11) [m] NA (Calcium flux) [m]	NA (PI) [f] NA (Gq/11) [m] NA (Calcium flux) [m]	1.3-6.5 ng/ml [4] (12.5-100 mg/d) Unbound: 0.2-1 nM/L [4]

Cabergoline	1.17 ([^{3}H]Mesulergine) [g]	2.57 (PI) [f] 398 (Calcium flux) [o] 0.76 (IP-acc.) [s] 4 (ERK2) [11]	123 (PI) [f] 98.8 (Calcium flux) [o] 89 (IP-acc.) [s] 60 (ERK2) [11]	Unbound: 0.044-1.59 nM/L (0.5-7 mg/d) [s]
Dihydro-ergotamine	24.2 ([^{3}H]LSD) [a]	1000 (Calcium assay) [s] 7.6 (IP-acc.) [s] 30 (PI) [e] 5 (ERK2) [11]	73 (Calcium assay) [s] 64 (IP-acc.) [s] 73 (PI) [e] 71 (ERK2) [11]	1 - < 10 ng/ml [z] Unbound: 0.35 (1 mg/im.), 0.4-0.96 (1.5 mg/sc.) 3-4 (1 mg/sc.) nM/L [s]
Ergotamine	2.61 (^{3}H5-HT) [a]	NA (Calcium assay) [s] 43.7 (IP-acc.) [s] 20 (ERK2) [11]	NA (Calcium assay) [s] 45 (IP-acc.) [s] 74 (ERK2) [11]	0.1 - < 0.5 ng/ml [z] Unbound: 0.2 nM/L (1 mg/im.) [s]
Lisuride	1.31 ([^{3}H]Mesulergine) [g]	NA [b] NA (PI) [f] 2.45 (Gq/11) [m] 537(Calcium flux) [m]	NA [b] NA [f] 11.6 (Gq/11) [m] 27.9 (Calcium flux) [m]	0.2-3.3 ng/ml Unbound: 0.41-6.83 nM/L [4]
Methylergonovine	0.45 (^{3}H5-HT) [p]	0.8 (PI) [e] 21.4 (Calcium flux) [o] 0.063 (IP-acc.) [s] NA (Calcium assay) [s]	40 (PI) [e] 49.5 (Calcium flux) [o] 28 (IP-acc.) [s] NA (Calcium assay) [s]	Unbound: 27.7 nM/L [s]

		2 (ERK2) [11]	53 (ERK2) [11]	
Pergolide	7.08 ([^{3}H]Mesulergine) [g - 14] [e]	53 (PI) [e] 6.03 (PI) [f] 33.1 (Gq/11) [m] 66 (Calcium flux) [m] 88.5 (Calcium flux) [o] 6.3 (IP-accumulation) [s] 1 (ERK2) [11]	112 (PI) [e] 113 (PI) [f] 88.9 (Gq/11) [m] 94.2 (Calcium flux) [m] 74.1 (Calcium flux) [o] 94 (IP-accumulation) [s] 79 (ERK2) [11]	Unbound: 0.27 – 0.87 nM/L (0.75-3 mg/day) [s]
Psilocin	4.6 [t]	2.37 (Calcium flux) [q] 58 (PI) [l] > 20 000 [d]	39.2 (Calcium flux) [q] 45 (PI) [l] NA [d]	16 ng/ml (0.3 mg/kg) 26 ng/ml (0.45 mg/kg) 37.6 ng/ml (0.6 mg/kg) 78.33-127-184 nM/L [5]
DMT	106.7 ([^{3}H]LSD) [a]	3400 [d]	19 [d]	-
4-OH-DIPT	-	5.12 [q] 460 [d]	97.0 [q] 55 [d]	-
4-OH-DPT	-	2.23 [q]	94.1 [q]	-
4-OH-EPT	-	4.34 [q]	89.2 [q]	
Mescaline	795 (^{3}H5-HT) [v]	> 20 000 [c]	NA [c]	3800 ng/ml (500 mg HCl)

				2950 ng/ml (blood), 2200 (brain) [3] 14000-18000 nM/L
25B-NBOMe	-	10 [c]	19 [c]	0.7-10.1 ng/ml 1.84-26.56 nM/L [7]
25C-NBOMe	-	100 [c]	16 [c]	0.48-1.04-2.07 ng/ml 1.4-3.1-6.2 nM/L [8]
25D-NBOMe	2.05 (^{3}H5-HT) [k]	100 [c] 32.3 (IP-1) [k]	22 [c] 47.7 (IP-1) [k]	-
25E-NBOMe	1.11 (^{3}H5-HT) [k]	6 [c] 23.5 (IP-1) [k]	26 [c] 49.4 (IP-1) [k]	-
25H-NBOMe	62.9 (^{3}H5-HT) [k]	340 [c] 463 [k]	11 [c] 37.7 (IP-1) [k]	-
25I-NBOMe	1.4 (^{125}I-DOI) [j]	7.3 (Calcium flux) [h] 15 (Calcium flux) [j] 130 [c]	79 (Calcium flux) [h] 65 (Calcium flux) [j] 32 [c]	0.45-7.5-28 ng/ml 1 – 65.53 nM/L [6]
25I-NBOH	1.91 (^{3}H5-HT) [k]	111 (IP-1) [k]	21.3 (IP-1) [k]	-
25N-NBOMe	8.7 (^{3}H5-HT) [k]	70 [c] 47 (IP-1) [k]	26 [c] 57.6 (IP-1) [k]	-
25P-NBOMe	-	170 [c]	23 [c]	-

25T2-NBOMe	-	40 [c]	31 [c]	-
25T4-NBOMe	-	200 [c]	27 [c]	-
25T7-NBOMe	-	310 [c]	14 [c]	-
25CN-NBOH	30 ([^{3}H]LSD) [h]	59 (Calcium flux) [h]	60 (Calcium flux) [h]	-
25CN-NBOMe	47 ([^{3}H]LSD) [h]	12 (Calcium flux) [h]	79 (Calcium flux) [h]	-
25CN-NBF	380 ([^{3}H]LSD) [h]	940 (Calcium flux) [h]	24 (Calcium flux) [h]	-
25CN-NBMD	65 ([^{3}H]LSD) [h]	220 (Calcium flux) [h]	31 (Calcium flux) [h]	-
Mescaline-NBOMe	-	> 20 000 [c]	NA [c]	-
(+)-Fenfluramine	3920 ([^{3}H]LSD) [y]	379 (IP-acc) [n]	38 (IP-acc.) [n]	-
(-)-Fenfluramine	680 ([^{3}H]LSD) [y]	1284 (IP-acc) [n]	47 (IP-acc) [n]	
(+)-Norfenfluramine	27 ([^{3}H]LSD) [y] 11.2 [n]	24 (PI-hydrolysis) [y] 23 (Ca-mobilization) [y] 25.9 (IP)[s]	 88 (IP) [s]	
(-)-Norfenfluramine	65 ([^{3}H]LSD) [y] 47.8 [n]	292 (PI) [y] 357 (IP-acc.) [n] 239 (Ca-mobilization) [y]	 71 (IP-acc.) [n]	
Norfenfluramine		60 (PI) [e] 1.4 (ERK2) [11]	96 (PI) [e] 75 (ERK2) [11]	77 nM/L (unbound, 30 mg/d) [s]

5-HT2$_B$: Affinity	
Compound	**Affinity (Ki nM/L)**
Methylergonovine	0.57
LSD	0.57-3.72
25E-NBOMe	1.11
Cabergoline	1.17
25I-NBOMe	1.4
5-HT	1.54-8.71
25D-NBOMe	2.05
Ergotamine	2.61
Psilocin	4.6
Pergolide	7.08-14
25N-NBOMe	8.7
(+)-norfenfluramine	11.2-27
Dihydroergotamine	24.2
25CN-NBOMe	47
(-)-norfenfluramine	47.8
Bromocriptine	56.2
25H-NBOMe	62.9
(-)-fenfluramine	680
Mescaline	795
(+)-fenfluramine	3920

5-HT2$_B$: ERK2 Potency	
Compound	**Potency (EC_{50} nM/L)**
5-HT	0.4 – 6.2
Pergolide	1
Norfenfluramine	1.4
Methylergonovine	2
Ergonovine	3
Cabergoline	4
Dihydroergotamine	5
Ergotamine	20
Guanfacine	91
LSD	110
5-HT2$_B$: ERK2 Efficacy	
Compound	**Efficacy (%)**
5-HT	100
Pergolide	79
Norfenfluramine	75
Ergotamine	74
Dihydroergotamine	71
Cabergoline	60
Guanfacine	59
Ergonovine	57
Methylergonovine	53
LSD	39

5-HT2$_B$: PI Hydrolysis Potency	
Compound	**Potency (EC_{50} nM/L)**
Methylergonovine	0.8
5-HT	1
Cabergoline	2.57
Pergolide	6.03-53
Dihydroergotamine	30
Psilocin	58
Norfenfluramine	60
5-HT2$_B$: PI Hydrolysis Efficacy	
Compound	**Efficacy (%)**
Cabergoline	123
Pergolide	112
Psilocin	45
Methylergonovine	40
Dihydroergotamine	30
(-)-norfenfluramine	24

5-HT2$_B$: IP accumulation Potency	
Compound	**Potency (EC_{50} nM/L)**
Methylergonovine	0.063
Cabergoline	0.76
Pergolide	6.3

Dihydroergotamine	7.6
5-HT	9.33
(+)-norfenfluramine	25.9
Ergotamine	43.7
5-HT2$_B$: IP accumulation Efficacy	
Compound	**Efficacy (%)**
Pergolide	94 (95 [a])
Cabergoline	89 (77 [a])
(+)-norfenfluramine	88
Norfenfluramine	84 [a]
(-)-norfenfluramine	71
Dihydroergotamine	64 (77 [a])
Ergotamine	45
Methylergonovine	28 (19 [a])

[a] Cavero and Guillon (2014)

5-HT2$_B$: Calcium flux	
Compound	**Potency (EC_{50} nM/L)**
5-HT	0.29-2.09
Psilocin	2.39
25I-NBOMe	7.3
LSD	8.91-39
25CN-NBOMe	12
Methylergonovine	21.4
(+)-Norfenfluramine	23
Pergolide	66-88.5
Cabergoline	398
Lisuride	537
Bromocriptine	NA
5-HT2$_B$: Calcium flux	
Compound	**Efficacy (%)**
5-HT	100
Cabergoline	98.8
Pergolide	74.1 (-94.2)
25CN-NBOMe	79
25I-NBOMe	65-79
LSD	51-86
Methylergonovine	49.5
Psilocin	39.2
Lisuride	27.9
Bromocriptine	NA

APPENDIX 3

	5-HT2$_A$		
Compound	Affinity (Ki nM/L)	Potency (EC_{50} nM/L)	Efficacy (% of 5-HT)
Serotonin	66 (ketanserin) [m]	0.575 (Ca-mobilisation) [m]	103 (Ca-mobilisation) [m]
		29.5 (Gq/11) [m]	108 (Gq/11) [m]
	16.8 (^{125}I-DOI) [k]	83 (AA-release) r	100 (AA-release) [r]
		120 (IP-accumulation) [r]	100 (IP-accumulation) [r]
		0.26 (Gq-calcium flux) [x]	100 [x]
LSD	0.32 (ketanserin) [m]	33.8 (Ca-mobilisation) [m]	84.6 (Ca-mobilisation) [m]
		1.81 (Gq/11) [m]	71.2 (Gq/11) [m]
	3.5 [r]	20 (AA-release) [r]	56 (AA-release) [r]
		9.8 (IP-accumulation) [r]	22 (IP-accumulation) [r]
	4.0 [d]	261 [d]	28 [d]
	0.091(^{125}I-DOI) [k]	0.706 (IP-1) [k]	64.5 (IP-1) [k]
	0.73 (^{125}I-DOI) [a]		
	3.29 (ketanserin) [a]		
Bromocriptine	107 (ketanserin) [g]	7.07 (PI-depletion) [f]	69 (PI-depletion) [f]
Cabergoline	6.17 (ketanserin) [g]	7.76 (PI-depletion) [f]	94 (PI-depletion) [f]
Ergotamine	0.98 (^{125I}-DOI) [p]	-	-
Dihydroergotamine	38 (ketanserin) [a]	-	-

Lisuride	3.52 (ketanserin) [a]	8.12 (PI-depletion) [f]	52 (PI-depletion [f]
	7.2 [r]	13 (AA-release) [r]	32 (AA-release) [r]
		41 (IP-accumulation) [r]	13 (IP-accumulation) [r]
Methylergonovine	0.35 (^{125}I-DOI) [p]	-	-
Pergolide	8.32 (ketanserin) [g]	1.62 (PI-depletion) [f]	103 (PI-depletion) [f]
Psilocin	49 [d]	721 [d]	16 [d]
		24 (PI) [l]	43 (PI) [l]
		2.40 (Gq-calcium flux) [q]	98.4 (Gq-calcium flux) [q]
	25 (^{125}I-DOI) [r]	86 (AA-release) [r]	42(AA-release) [r]
		2300 (IP-accumulation) [r]	46 (IP-accumulation) [r]
DMT	237 [d]	76 [d]	40 [d]
	131.5 (^{125}I-DOI) [a]		
4-OH-EPT	-	3.15 (Gq-calcium flux) [q]	99.5(Gq-calcium flux) [q]
4-OH-DPT	-	6.28	
		1.64 (Gq-calcium flux) [q]	103 (Gq-calcium flux) [q]
4-OH-DIPT	728 [d]	93 [d]	74 [d]
		6.28 (Gq-calcium flux) [q]	102 (Gq-calcium flux) [q]
4-OH-MET	57 [d]	37 [d]	72 [d]
Mescaline	6300 [d]	10 000 [d]	56 [d]
	551 (^{125}I-DOI) [v]		
25B-NBOMe (Cimbi-36)	0.5 (ketanserin) [x]	0.95- 1.6 (Ca-assay) [h]	88–83 (Ca-assay) [h]
		40 [c]	28 [c]
		2.0 (IP_{1-3} assay) [h]	80 (IP_{1-3} assay) [h]

25C-NBOMe	0.7 [c]	150 [c]	32 [c]
25D-NBOMe	0.22 (^{125}I-DOI [j] [k]	1.53 (IP-1) [k]	95.1 (IP-1) [k]
	1 [c]	90 [c]	27 [c]
25E-NBOMe	0.127 (^{125}I-DOI) [k]	0.50 (IP-1) [k]	87.1 (IP-1) [k]
25H-NBOMe	4.9 (^{125}I-DOI) [k]	40.7 (IP-1) [k]	85.9 (IP-1) [k]
25I-NBOMe	0.6 [c]	240 [c]	27 [c]
	0.16 (^{125}I-DOI) - 0.52 (ketanserin) [j]	0.44 (Ca-assay) h	86 (Ca-assay) [h]
	0.044 (^{125}I-DOI) [i]	0.72 (IP1-3 assay) h	90 (IP1-3 assay) [h]
25I-NBOH	0.169 (^{125}I-DOI) [k]	0.76 (IP-1) [k]	87.5 (IP-1) [k]
25N-NBOMe	0.144 (^{125}I-DOI) [k]	0.51 (IP-1) [k]	87.9 (IP-1) [k]
	0.8 [c]	70 [c]	34 [c]
25P-NBOMe	1.1 [c]	220 [c]	42 [c]
25T2-NBOMe	0.6 [c]	100 [c]	38 [c]
25T4-NBOMe	1.6 [c]	130 [c]	46 [c]
25T7-NBOMe	1.1 [c]	260 [c]	41 [c]
25CN-NBOH	0.81 ([^{3}H]LSD) - 1.7(Cimbi36) [h]	0.41 – 1.2 (Ca-assay) [h]	77–80 (Ca-assay) [h]
		2.1 (IP_{1-3} assay) [h]	86 (IP_{1-3} assay) [h]
25CN-NBOMe	1.8 ([^{3}H]LSD) – 2.1 (Cimbi36) [h]	0.4 – 1.2 (Ca-assay) [h]	81-87 (Ca-assay) [h]
		3.5 (IP_{1-3} assay) [h]	86 (IP_{1-3} assay) [h]
25CN-NBF	31([^{3}H]LSD) - 130 (Cimbi36) [h]	17–32 (Ca-assay) [h]	54-82 (Ca-assay) [h´]
		81 (IP_{1-3} assay) [h]	70 (IP_{1-3} assay) [h]
25CN-NBMD	2.0 ([^{3}H]LSD) - 6.9 (Cimbi36) [h]	5.6–5.8 (Ca-assay) [h]	56-83 (Ca-assay) [h]
		19 (IP_{1-3} assay) [h]	84 (IP_{1-3} assay) [h]
Mescaline-NBOMe	140 [c]	3000 [c]	33 [c]

(+)-Fenfluramine	2470 (^{125}I-DOI) [y]	>10 000 [n]	NA [n]
(-)-Fenfluramine	1430 (^{125}I-DOI) [y]	5279 (IP-acc.) [n]	
(+)-Norfenfluramine	187 (^{125}I-DOI) [y]	3100 (IP-hydrolysis) [y] 720 (Ca-mobilisation) [y] 630 (IP-acc.) [n]	88 (IP-acc.) [n]
(-)-Norfenfluramine	267 (125I-DOI) [y]	26000 (IP-hydrolysis) [y] 1990 (Ca-mobilisation) [y] 1565 (IP-acc.) [n]	93 (IP-acc.) [n]

5-HT1$_A$ (Ki nM/L)		
Compound	Affinity (Ki)	[35S]GTPS: EC50 (nM) / of maximum (%)
Serotonin	3.32 (8-OH-DPAT) [a] 1.71 (8-OH-DPAT) [k]	2.88 / 100% [k]
LSD	1.1 (4) 3 [d] 2.29 (8-OH-DPAT) [a]	1.31 / 93.2% [k]
Bromocriptine	12.9 (8-OH-DPAT) [g]	57.5 / 72% [f]
Cabergoline	20 (8-OH-DPAT) [g]	372 / 93% [f]

Dihydroergotamine	1.5 (8-OH-DPAT) [a]	-
Ergotamine	0.17 (8-OH-DPAT) [a]	-
Lisuride	0.13 [g] – 0.32 [a] (8-OH-DPAT)	1.26 / 98% [f]
Pergolide	1.9 (8-OH-DPAT) [g]	112 / 63% [f]
Psilocin	123 [d]	-
	49 [a]	
DMT	75 [d]	-
4-OH-DIPT	5700 [d]	-
4-OH-MET	228 [d]	-
Mescaline	4600 [d]	-
25B-NBOMe	3600 [d]	-
25C-NBOMe	5000 [d]	-
25D-NBOMe	7100 [d]	5900 / 55% [k]
	4510 (8-OH-DPAT) [k]	
25E-NBOMe	3500 [d]	13700 / 38% [k]
	1680 (8-OH-DPAT) [k]	
25H-NBOMe	6000 [d]	28400 / 52% [k]
	4520 (8-OH-DPAT) [k]	
25I-NBOH	2220 (8-OH-DPAT) [k]	37000 / 74% [k]
25I-NBOMe	1033 [j]	
	1969 (8-OH-DPAT) [i]	(8-OH-DPAT) [i]
	1800 [d]	
25N-NBOMe	4200 [d]	4800 / 35% [k]
	2260 (8-OH-DPAT) [k]	

25P-NBOMe	1800 [d]	-
25T2-NBOMe	2200 [d]	-
25T4-NBOMe	2500 [d]	-
25T7-NBOMe	1800 [d]	-
Mescaline-NBOMe	21000 [d]	-

a: (Psychoactive drug screening program 2021), b: (Porter et al. 1999), c: (Rickli et al. 2015), d: (Rickli et al. 2016), e: (Setola et al. 2003) f: (Newman-Tancredi et al. 2002), g: (Millan et al. 2002), h: (Jensen et al. 2017), i: (Elliot and Brandt 2014), j: (Nichols et al. 2015), k: (Eshleman et al. 2018, l: (Sard et al. 2005), m: (Cussac et al. 2008), n: (Rothman et al. 2000), o: (Hutcheson et al. 2011), p: (Knight et al. 2004), q: (Klein et al. 2020), r: (Kurrasch-Orrbaugh et al. 2003), s: (Unett et al. 2013), t: (Halberstadt et al. 2011), v: (Monte et al. 1997), x: (Hansen et al. 2014), y: (Fitzgerald et al. 2000), z: (Saper and Silverstein 2006)

1: (Dolder et al. 2017), 2: (Family et al. 2020), 3: (Henry et al. 2003), 4: (Deleu et al. 2002), 5: (Brown et al. 2017), 6: (Kamińska et al. 2020), 7: (Gee et al. 2016), 8: (Kristofic et al. 2016), 9: (Brabredbergndt and Anderson, 2011), 10: (Knauer et al. 2009), 11: (Cavero and Guillon 2014)

AA = arachidonic acid, IP-1 = inositol monophosphate accumulation, PI = phosphoinositide hydrolysis

5-HT1$_A$: Affinity	
Compound	**Affinity (Ki)**
LSD	1.1
5-HT	1.71-3.32
Psilocin	49-123
25I-NBOMe	1033
25E-NBOMe	1680-3500
25P-NBOMe	1800
25T7-NBOMe	1800

25T2-NBOMe	2200
25N-NBOMe	2260-4200
25T4-NBOMe	2500
25B-NBOMe	3600
25N-NBOMe	4800
25D-NBOMe	4510-7100
25H-NBOMe	4520-6000
Mescaline	4600
Mescaline-NBOMe	21000

5-HT1$_A$: Potency	
Compound	**Potency (EC_{50} nM/L)**
LSD	1.31
5-HT	2.88
25N-NBOMe	4800
25D-NBOMe	5900
25E-NBOMe	13700
25H-NBOMe	4520-6000
5-HT1$_A$: Efficacy	
Compound	**Efficacy (%)**
5-HT	100
LSD	93.2
25I-NBOH	74
25D-NBOMe	55

25H-NBOMe	52
25E-NBOMe	38
25N-NBOMe	35

5-HT2$_A$: Affinites	
Compound	**Ki (nM)**
25I-NBOMe	0.044-0.6
25E-NBOMe	0.127
25N-NBOMe	0.144-0.8
25D-NBOMe	0.22-1
25B-NBOMe	0.5
25T2-NBOMe	0.6
25C-NBOMe	0.7
LSD	0.73-3.29
25P-NBOMe	1.1
25T7-NBOMe	1.1
25T4-NBOMe	1.6
25CN-NBOMe	1.8-2.1
25H-NBOMe	4.9
5-HT	16.8-66
Psilocin	25-49
Mescaline-NBOMe	140

Mescaline	553-6300

5-HT2$_A$: IP-1 Potencies	
Compound	**Ki (nM)**
25E-NBOMe	0.5
25N-NBOMe	0.51
LSD	0.71
25D-NBOMe	1.53
25H-NBOMe	40.7
5-HT2$_A$: IP-1 Efficacies	
Compound	**(%)**
25D-NBOMe	95.1
25N-NBOMe	87.9
25E-NBOMe	87.1
25H-NBOMe	85.9
LSD	64.5

5-HT2$_A$: Rickli et al. (2015, 2016)	
Compound	**Potency (EC_{50} nM/L)**
25B-NBOMe	40
25N-NBOMe	70
25D-NBOMe	90
25T2-NBOMe	100

25T4-NBOMe	130
25C-NBOMe	150
25P-NBOMe	220
25I-NBOMe	240
25T7-NBOMe	260
LSD	261
Psilocin	721
Mescaline-NBOMe	3000
Mescaline	10 000
5-HT2$_A$: Rickli et al. (2015, 2016)	
Compound	**Efficacy (%)**
Mescaline	56
25T4-NBOMe	46
25P-NBOMe	42
25T7-NBOMe	41
25N-NBOMe	34
Mescaline-NBOMe	33
25C-NBOMe	32
25T2-NBOMe	31
LSD	28
25B-NBOMe	28
25D-NBOMe	27
25I-NBOMe	27
Psilocin	16

5-HT2$_A$: Ca-assay of Jensen et al. (2017)	
Compound	**Affinity (Ki)**
25CN-NBOMe	0.41-1.2
25I-NBOMe	0.44
25B-NBOMe	0.95-1.6
5-HT2$_A$: Ca-assay of Jensen et al. (2017)	
Compound	**Efficacy (%)**
25I-NBOMe	86
25B-NBOMe	83-88
25CN-NBOMe	81-87
5-HT2$_A$: Ca-mobilization of Cussac et al. (2008)	
LSD	Potency: 33.8 nM Efficacy: 84.6%