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REGULATION OF MONOAMINERGIC FUNCTIONS BY GPCRS WITH A SPECIAL EMPHASIS ON MENTAL AND MOVEMENT DISORDERS

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Cover illustration: *"Bowl of Neuropharmacology"*: The cover artwork depicts the bowl of Greek Godess of health, Hygeia. This bowl is one of the symbols of pharmacology. The brain that is illustrated into Hygeia's bowl, symbolizes neuropharmacology. G-protein coupled receptors are also known as serpentine receptors, meaning snake-like receptors. Thus, the illustrated snake, which is entwined seven times around Hygeia's arm (cell membrane), symbolizes G-protein coupled receptors. The artwork was inspired from Gustav Klimt's, *Hygeia.* The art model of the illustration is Maria Kalomoiri.

REGULATION OF MONOAMINERGIC FUNCTIONS BY GPCRS WITH A SPECIAL EMPHASIS ON MENTAL AND MOVEMENT DISORDERS.

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Ioannis Mantas M.D.

The thesis will be defended in public at Germinal Center lecture Hall, L800 024, CMM, Karolinska University Hospital, Solna, Wednesday 11th of May at 14:00 p.m.

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POPULAR SCIENCE SUMMARY OF THE THESIS

Monoamines are chemical substances in the brain that are secreted from brain cells and help them to communicate with each other. This group of chemical substances includes dopamine, norepinephrine, histamine and serotonin. This type of communication is accomplished with molecules located in neurons that bind these substances. These molecules are called monoamine receptors and belong to a certain receptor group called G-protein coupled receptors (GPCRs). When monoamines bind to their receptors, they stimulate a cascade of signaling that regulates the activity of each cell. The monoaminergic system is usually affected in neurological and psychiatric diseases such as Parkinson's disease, major depressive disorder and schizophrenia. Interestingly, the drugs that we are using to tackle these diseases are directly or indirectly targeting monoamine receptors. In the current thesis, we used animal models of the aforementioned diseases to study four certain molecules that are associated with GPCRs.

In PAPER I, we examined the role of a molecule called GPR88 in animal models of Parkinson's disease. Our study showed that future development of drugs that block GPR88 activity, may alleviate parkinsonian symptoms, assist the current therapies without side effects. In PAPER II and III, we studied the function of a molecule called TAAR1 in animal models of major depressive disorder. In these papers, we characterize the contribution of TAAR1 in the effects of clinically-used antidepressants with emphasis on monoamine oxidase inhibitors. In PAPER IV, we investigated the TAAR1's influence in the therapeutic action of a newly designed antipsychotic called SEP-856. We revealed that SEP-856's effects are partially mediated due to TAAR1 activation. In PAPER V, we investigated the implication of a molecule called p11 in animal models of stress. We designated that p11 plays a crucial role in mediating stress hyperresponsiveness, which is observed in depressed patients. In PAPER VI, we studied the role of a molecule called NURR1 in animal models of Parkinson's disease. We revealed that NURR1 is might strengthen the therapeutic effects of the current antiparkinsonian treatment. In PAPER VII, we explored the function of NURR1 in a brain region which has been proposed to orchestrate hallucinogenic experience. We provided evidence that NURR1 presence is critical for this brain region's identity. However, absence of NURR1 does not interfere with the brain activity that is correlated with hallucinogenic state.

Taken together, the current thesis gives insights to mechanisms of Parkinson's disease, major depressive disorder and schizophrenia. These findings may aid to the future development of pharmaceutical agents for the efficient tackling of these detrimental diseases.

ABSTRACT

Dysfunction of the brain's monoaminergic system has been implicated in many human neurological and psychiatric disorders, such as Parkinson's disease (PD), major depressive disorder (MDD) and schizophrenia. The monoamines that are most dysregulated in these diseases, are dopamine and serotonin. Monoamines act as signalling molecules through their receptors, which belong predominately to the G-protein coupled receptor (GPCR) superfamily[1]. Most of the clinically employed drugs that are used to tackle these diseases, target directly or indirectly the monoaminergic class of GPCRs. This thesis aims to identify the role of four understudied GPCR-signalling related molecules (GPR88, TAAR1, p11 and NURR1) in animal models of PD, MDD and schizophrenia.

The main findings relate to the functions of GPR88, TAAR1, p11 and NURR1 in relationship to PD, MDD and schizophrenia. GPR88 has been suggested as crucial suppressor of striatal medium spiny neuron activity. We showed that loss of GPR88 facilitates L-dihydroxyphenylalanine treatment for PD by aiding its therapeutic efficacy without worsening its side effects. TAAR1 has been described as negative regulator of dopamine neurons firing rate. Herein, we report that TAAR1 deletion enhances the response of non-selective monoamine oxidase inhibitors but no other classes of antidepressants. Furthermore, we provide evidence that the antipsychotic action of the pioneering drug, SEP-856, depends in part on TAAR1 agonism. P11 is a small GPCR-adaptor protein that has been linked to MDD and antidepressant treatment response. In the current thesis, we demonstrate that loss of p11 causes an overt response to stress by triggering the activity of hypothalamic-pituitary-adrenal and sympathetic-adrenomedullary axes. Finally, NURR1 is a GPCR regulated transcription factor, which is linked to PD and schizophrenia as a consequence of its fundamental role in coordinating midbrain dopamine neuron development. In the present work, we describe the role of NURR1 in extra-dopaminergic brain structures such as striatum and claustrum. In detail, we show that induced striatal NURR1 is crucial for locomotor sensitization to L-DOPA. Moreover, we revealed that NURR1 is important factor for claustral neuron transcriptional identity without affecting the occurrence of hallucinogen states' neural correlates.

Overall, we explored new avenues in the fields of neurology and psychiatry related molecular neurobiology. These findings may support future drug discovery research on PD, MDD and schizophrenia though the identification of novel pharmaceutical agents to treat these detrimental disorders. Thus, this body of work contributes to the better understanding of both the pharmacology and pathophysiology of mental and movement disorders.

LIST OF SCIENTIFIC PAPERS

I. **Mantas I**, Yang Y, Mannoury-la-Cour C, Millan MJ, Zhang X, Svenningsson P. Genetic deletion of GPR88 enhances the locomotor response to L-DOPA in experimental parkinsonism while counteracting the induction of dyskinesia. *Neuropharmacology*, **162**, 107829 (2020).

II. **Mantas I**, Vallianatou T, Yang Y, Shariatgorji M, Kalomoiri M, Fridjonsdottir E, Millan MJ, Zhang X, Andrén PE, Svenningsson P. TAAR1-Dependent and -Independent Actions of Tyramine in Interaction With Glutamate Underlie Central Effects of Monoamine Oxidase Inhibition. *Biol Psychiatry*. **90**, 16-27 (2021).

III. **Mantas I**, Millan MJ, Di Cara B, Groenink L, Veiga S, Cistarelli L, Brocco M, Bertrand M, Svenningsson P, Zhang X. Trace Amine-Associated Receptor 1 Contributes to Diverse Functional Actions of O-Phenyl-Iodotyramine in Mice but Not to the Effects of Monoamine-Based Antidepressants. *Int J Mol Sci*. **22**, 8907 (2021).

IV. Saarinen M, **Mantas I**, Flais I, Ågren R, Sahlholm K, Millan MJ, Svenningsson P. TAAR1 dependent and independent actions of the potential antipsychotic and dual TAAR1/$5HT_{1A}$ receptor agonist SEP-383856. *Manuscript*.

V. Sousa VC, **Mantas I**, Stroth N, Hager T, Pereira M, Jiang H, Jabre S, Paslawski W, Stiedl O, Svenningsson P. P11 deficiency increases stress reactivity along with HPA axis and autonomic hyperresponsiveness. *Mol Psychiatry*. **26**, 3253-3265 (2021).

VI. **Mantas I**, Zhang X, Fridjonsdottir E, Saarinen M, Tiklova K, Gillberg L, Li T, Andrén PE, Perlmann T, Svenningsson P. NURR1 regulates the response of striatal D1-neurons to subchronic treatment of L-DOPA. *Manuscript*.

VII. **Mantas I**, Flais I, Saarinen M, Tiklova K, Yaghmaeian Salmani B, Gillberg L, Ågren R, Sahlholm K, Zhang X, Perlmann T, Hengerer B, Svenningsson P. NURR1 is master-regulator of claustrum identity but not of hallucinogenic-like states. *Manuscript*.

CONTENTS

LIST OF ABBREVIATIONS

2-PCCA	(1R, 2R)-2-(pyridin-2-yl)cyclopropanecarboxylic acid ((2S,3S)-2-amino-3-methylpentyl)-(4′-propylbiphenyl-4-yl)amide
5HT	5-hydroxytryptamine/serotonin
$5HT_XR$	5-hydroxytryptamine receptor X
6-OHDA	6-hydroxydopamine
$A_{2A}R$	Adenosine 2A receptor
AADC	Aromatic amino acid decarboxylase
AAV	Adeno-associated virus
Ach	Acetylcholine
ACTH	Adrenocorticotropic hormone
AD	Adrenaline
AIM	Abnormal involuntary movement
AMPAR	Amino-hydroxy-methyl-isoxazolepropionic acid receptor
Amy	Amygdala
AmyN	Amygdalar neuron
AnxA2	Annexin A2
AP	Anterior pituitary
ARC	Activity-regulated cytoskeleton-associated protein
ASIC1	Acid-sensing ion channel 1
AVP	Arginine-vasopressin
BDNF	Brain derived neurotrofic factor
BF	Basal forebrain
BFN	Basal forebrain neuron
CA1	Cornu ammonis 1
Ca^{2+}	Calcium
cAMP	Cyclic adenosine monophosphate
Cav1.2/3	Voltage-gated L-type calcium channel Cav1.2/3
CBF	Cerebral blood flow
CBV	Cerebral blood volume
Cd	Caudate

Cg	Cingulate cortex
CIN	Cholinergic interneuron
CLA	Claustrum
CNS	Central nervous system
cp11-KO	Conditional p11 knock-out
CPu	Caudoputamen
Cre	Cyclization recombinase
CREB	cAMP response element-binding protein
Cre-ERT	Cyclization recombinase-estrogen receptor T
CRH	Corticotropin-releasing hormone
CRH_1R	Corticotropin-releasing hormone 1 receptor
Ctx	Cortex
DA	Dopamine
DAG	Diacylglycerol
DAT	Dopamine transporter
DBS	Deep brain stimulation
DG	Dentate gyrus
dMSN	Direct pathway medium spiny neuron
DOI	Dimethoxy-iodoamphetamine
DR	Dorsal raphé
DSM-5	Diagnostic and statistical manual of mental disorders-5
D_XR	Dopamine receptor DX
EAAT3	Excitatory amino acid transporter 3
EP_2R	Prostaglandin E2 receptor
EPPTB	N-(3-Ethoxy-phenyl)-4-pyrrolidin-1-yl-3-trifluoromethyl-benzamide
fEPSP	Field excitatory postsynaptic potential
Fg	Fluorogold
fMRI	Functional magnetic resonance imaging
FST	Forced swim test
fUS	Functional ultrasound
GABA	Gamma aminobutyric acid

GAD67	Glutamic acid decarboxylase 67
GDP	Guanine diphosphate
GIRK	G-protein-coupled inwardly-rectifying potassium channel
Glu	Glutamate
GP	Globus pallidus
GPCR	G-protein-coupled receptor
GPe	Globus pallidus externa
GPi	Globus pallidus interna
GPR88	G-protein-coupled receptor 88
GTP	Guanosine triphosphate
Hb	Habenular complex
HCN2	Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 2
HF	Hippocampal formation
HPA	Hypothalamic-pituitary-adrenal
HSP90	Heat-shock protein 90
HTR	Head twitch response
Hy	Hypothalamus
HyN	Hypothalamic neuron
IML	Intermediolateral nucleus
iMSN	Indirect pathway medium spiny neuron
IO	Inferior olive
ISH	In situ hybridization
IT	Intratelencephalic neurons
KO	Knock-out
L5A	Cortical layer 5A
LB	Lewy body
LC	Locus coeruleus
LDB	Light dark box
L-DOPA	L-dihydroxyphenylalanine
LHb	Lateral habenula

LID	L-DOPA induced dyskinesia
loxP	Locus of X-over P1
LSD	Lysergic acid diethylamide
MALDI-MSI	Matrix-assisted laser desorption ionization-mass spectrometry imaging
MAO	Monoamine oxidase
MAOI	Monoamine oxidase inhibitor
MC	Mossy cell
MDD	Major depressive disorder
MDMA	Methylenedioxymethamphetamine
MFB	Medial forebrain bundle
$mGluR_2$	Metabotropic glutamate receptor 2
mito	Mitochondria
mPFC	Medial prefrontal cortex
MR	Median raphé
MSN	Medium spiny neuron
M_XR	Muscarinic X receptor
NA	Noradrenaline
NAc	Nucleus accumbens
NAT	Noradrenaline transporter
NBRE	Nerve growth factor-induced clone B response element
NDRI	Noradrenaline–dopamine reuptake inhibitor
NMDAR	N-methyl-D-aspartate receptor
NOR1	Neuron-derived orphan receptor 1
nor-BNI	Nor-binaltorphimine
NR4A	Nuclear receptor 4A
NRI	Noradrenaline reuptake inhibitor
NTS	Nucleus tractus solitarius
NUR77	Nuclear hormone receptor NUR/77
NURR1	Nuclear receptor related 1 protein
OFT	Open field test
o-PIT	O-phenyl-iodotyramine

PANSS	Positive and Negative Symptom Scale
PAT	Passive avoidance test
PCP	Phencyclidine
PD	Parkinson's disease
PDYN	Prodynorphin
PENK	Proenkephalin
PFC	Prefrontal cortex
Phe	Phenylalanine
PKA	Protein kinase A
PKC	Protein kinase C
PPI	Pre-pulse inhibition
Pu	Putamen
PyN	Pyramidal neuron
RGS9	Regulator of G-protein signaling 9
Rho-GEF	Rho guanine nucleotide exchange factor
RMTg	Rostromedial tegmental area
RST	Restraint stress
SAM	Sympathetic-adrenal-medullary
SERT	Serotonin transporter
SMARCA3	SWI/SNF related matrix associated actin dependent regulator of chromatin subfamily a member 3
SNc	Substantia nigra pars compacta
SNr	Substantia nigra pars reticulata
SNRI	Serotonin-noradrenaline reuptake inhibitor
SP	Substance P
SSRI	Selective serotonin reuptake inhibitor
STN	Subthalamic nucleus
T_1AM	3-iodothyronamine
TA	Trace amine
TAAR	Trace amine associated receptor
TAAR1	Trace amine associated receptor 1

TASK1	TWIK-related acid-sensitive potassium channel 1
TCP	Tranylcypromine
Th	Thalamus
TH	Tyrosine hydroxylase
TJM	Tremulous jaw movement
TM3	Transmembrane part 3
TM6	Transmembrane part 6
TrkB	Tropomyosin receptor kinase B
TST	Tail suspension test
Tu	Olfactory tubercle
Tyr	Tyrosine
$V_{1B}R$	Vasopressin 1B receptor
VGLUT1	Vesicular glutamate transporter 1
VGLUT3	Vesicular glutamate transporter 3
VMAT2	Vesicular monoamine transporter 2
VTA	Ventral tegmental area
α_2R	Alpha 2 adrenergic receptor
α-syn	Alpha-synuclein
β-PEA	Beta phenethylamine
κOR	Kappa opioid receptor
μOR	Mu opioid receptor

1 INTRODUCTION

CNS monoaminergic centres are the brain regions that have the strongest links to human neuropsychiatric and neurodegenerative disease[2]. The pathology and dysfunction of monoaminergic systems has been consistently associated with PD, MDD and schizophrenia[2]. These systems are composed of cells that produce and release monoamine neurotransmitters, such as DA, 5HT, NA and histamine[2]. These cells are located in discrete brain regions and send long-range axons towards critical forebrain areas, which regulate mood and locomotor functions[3]. Following their release, monoamines exert their action through receptors that belong to the GPCR superfamily[1]. The monoaminergic family of GPCRs plays a crucial role in neuropsychiatric and neurodegenerative diseases and represents one of the most important pharmaceutical target class[4,5]. Drugs targeting aminergic GPCRs are widely used for treating both neuropsychiatric and neurodegenerative diseases as they modify the action of crucial monoaminergic connections in the brain[4,5].

1.1 GPCR SIGNALLING

GPCRs exert their effects mainly through coupling to G-proteins, which act as molecular switches by converting GTP to GDP[6]. G-proteins work as protein assemblies of three subunits (Gα, Gβ and Gγ), which dissociate when they interact with the GPCRs[6]. Specifically, Gα-protein contains the GTP binding domain and is recruited to the receptor's active conformation[7]. Subsequently, Gα dissociates with the Gβγ complex and exert their diverse actions. The Gα-subunit is the most diverse component of G-protein complex, with 18 members divided into four distinct groups according to the second messenger signal that they mediate[8]. These groups are named $G\alpha_s$, $G\alpha_i$, $G\alpha_{q/11}$ and $G\alpha_{12/13}$[8]. $G\alpha_s$ and $G\alpha_i$ proteins promote and inhibit the production of cAMP respectively[9]. Subsequently, cAMP activates PKA, which has a crucial role in phosphorylating different protein targets. At the same time, PKA phosphorylates cAMP responsive element CREB which is the main effector of transcriptional changes induced by increased cytosolic cAMP[10]. $G\alpha_{q/11}$ group mediates the stimulation of PKC by the simultaneous activation of DAG production and cytoplasmic Ca^{2+} influx from endoplasmic reticulum[11]. Nevertheless, there are several PKC isozymes, which do not obey the classical PKC activation process[11]. For instance, PKCα-γ follow the conventional PKC activation requirements, while PKCδ-θ action needs only the DAG binding[11]. Furthermore, PKCι and PKCζ belong to the atypical PKC group and neither DAG nor Ca^{2+} is needed for their activation[11]. Like PKA, PKC phosphorylates multiple intracellular protein targets and promotes several transcriptional responses. $G\alpha_{12/13}$ is a unique Gα protein group that cannot be targeted by pertussis and cholera toxins, which are well known for their Gα-binding properties[12]. $G\alpha_{12/13}$ group is featured by the

activation of Rho-GEFs and subsequent stimulation of the small GTPase RhoA[12]. This signalling cascade is crucial for the regulation of actin filament dynamics[12]. Most GPCRs signal through a certain Gα type, while others are able to recruit Gα-proteins from multiple subgroups[8]. Apart from the canonical Gα-protein signalling, all GPCRs are also able to signal through β-arrestin[13]. GPCR-β-arrestin signalling is considered as the pathway that mediates GPCR internalization and subsequent desensitization of the receptor[13].

1.2 MONOAMINERGIC SYSTEMS

1.2.1 Dopaminergic system

The most well studied monoaminergic pathway, which is strongly implicated in many neuropsychiatric, and neurodegenerative disorders is the mesostriatal pathway[14]. The two neuronal hubs that compose this pathway are the ventral midbrain DA neuron group and the striatum[15] (Figure 1). Midbrain DA neurons reside within two major subnuclei, which are called SNc and VTA. These two subnuclei differ primarily in the distribution of their axonal arborizations. Both SNc and VTA send long range projections that pass through diencephalon by forming a thick axonal bundle called the MFB[16]. When SNc and VTA axons reach the ventral telencephalic structures, they arborize densely within the dorsal and ventral striatal nuclei respectively[17]. The striatum is a relatively large brain structure where the predominant cell types utilize GABA as a neurotransmitter[18]. With respect to function, it has been proposed that the striatum is associated with decision making, emotional encoding and habit formation[19]. All these processes are accomplished through a positive and negative feedforward loop between the cerebral cortex and striatum[18]. Specifically, cortical functional units send compact projections to striatum, which in turn will either suppress or reinforce this signal through indirect inhibition or activation of the thalamocortical cells[18]. The fate of the signal (suppress/reinforce) is ruled by two distinct neuronal populations in striatum which are involved in different pathways[18]. Together these populations compose the group of MSNs as they share similar morphological characteristics[18]. However, the functional subdivision of MSNs is based on their gene expression profile and their afferent fibre targets[18]. One of the major separating feature of these cell groups is their connectivity with the GPi and SNr, which constitute the main basal ganglia output structures[18]. The dMSNs reinforce thalamocortical activity by directly inhibiting GPi/SNr[18]. The 2nd MSN population is called iMSN and suppresses thalamocortical input by indirectly activating GPi/SNr through GPe inhibition[18]. Another feature that characterizes these two MSN populations is the type of the DA receptor that they express[18]. Specifically, dMSNs express D_1R whereas the iMSNs express D_2R[18]. In addition with DA receptors, dMSN express PDYN and SP, while iMSNs produce PENK[18]. As mentioned earlier, midbrain DA neurons strongly innervate the two

MSN cell groups. However, because the MSNs express different DA receptor subtypes, striatal DA release has different effects on the activity of each MSN type[18]. It is well known that D_1R displays excitatory actions, while D_2R exerts an inhibitory effect[18]. These differential properties of the two receptors stem from their ability to couple selectively to G-proteins that either stimulate or suppress the cAMP production[18]. Cytosolic accumulation of cAMP in MSNs leads to PKA-dependent phosphorylation of AMPARs and their subsequent translocation to the plasma membrane[20]. Therefore, DA stimulates dMSNs whereas it inhibits the activity of iMSNs[21]. The net effect of striatal DA release results in favouring the action of MSNs that enhance thalamocortical stimulation and subsequently boosting the corticostriatal feedforward connection[18]. Within the group of dMSNs, there is an enigmatic subtype that is distinguished by the expression of μOR and within striatal anatomical structures, referred as striosomes[22]. This dMSN cluster is further characterized by the direct inhibitory projections to midbrain DAergic neurons, thus suppressing striatal DA release[23,24]. At the same time, striatal DA levels are regulated by presynaptic D_2R receptors, which exert a negative feedback upon DA release from SNc and VTA[25]. Apart from D_1R and D_2R receptors, there are three more less abundant types of DA receptors, which are characterized as D_1R-like and D_2R -like according to their Gα coupling properties[20]. D_3R and D_4R belong to D_2R -like subfamily but they do not show similar expression pattern to D_2R receptor[20]. D_3R is enriched in ventral striatum, islands of Calleja and mammillary region[26,27], while D_4R receptor is predominantly expressed in cerebral cortex and HF[28]. D_5R belongs to D_1R-like subfamily and is selectively expressed in hippocampal CA1, parafascicular nucleus of thalamus and striatal CINs[29,30]. Overall, striatal DA release has been linked to positive reinforcement and locomotor stimulation. Consequently, drugs that enhance dopaminergic signalling are associated with positive emotion and locomotor induction while low concentrations of the neurotransmitter are correlated with depressive mood and slowness of movement[31–35]. Even though striatal DA release is reinforcing, aberrant DAergic tone may lead to detrimental effects such as psychosis and addiction[35,36]. In conclusion, mesostriatal pathway is characterized by distinct neuronal subtypes, which directly regulate critical emotional and motor functions.

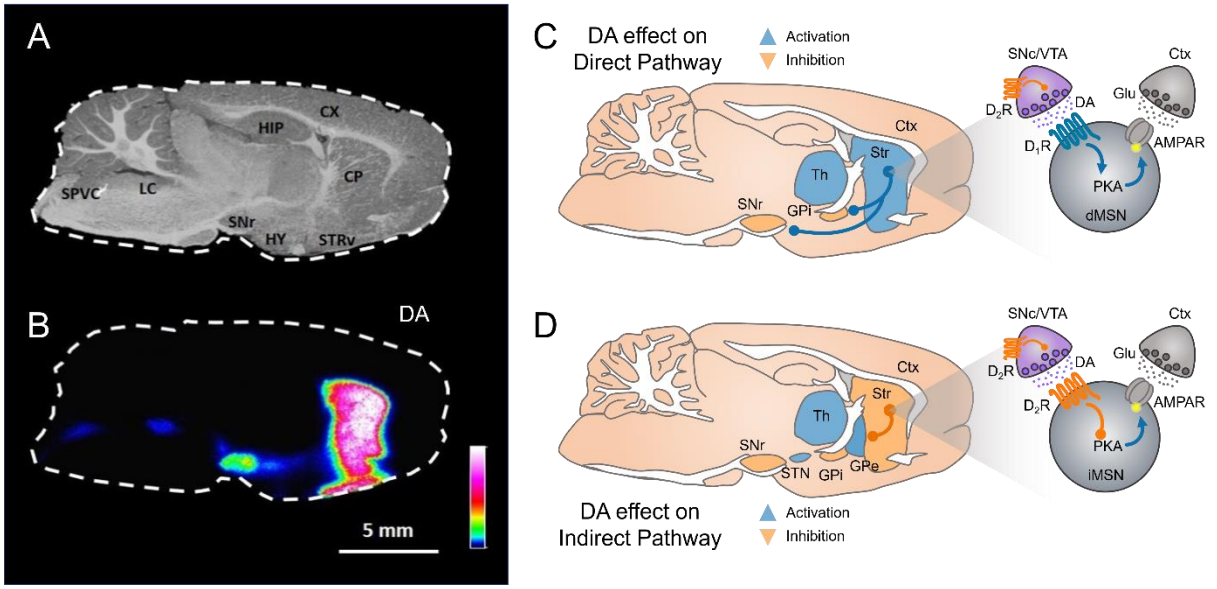

Figure 1. DAergic system and basal ganglia pathways. (A, B) Sagittal slice of mouse brain **(A)** and MALDI-MSI image of DA **(B)** (Adapted from Shariatgorji et al.[37]). **(C)** Illustration depicting the effects of DA on the direct pathway dMSNs. **(D)** Illustration depicting the effects of DA on the indirect pathway iMSNs. Lines with arrowheads depict excitation while lines with circular heads depict inhibition. The yellow circle denotes phosphorylation.

1.2.2 Serotoninergic system

Another crucial monoamine in the brain with great impacts in behaviour is 5HT. A midline nuclear group with a broad rostrocaudal extension in the brainstem, which is called raphé, produces this neurotrasmitter[38]. The different parts of the raphe nuclei display different functions according to their position in the rostrocaudal axis[39]. The caudal parts send descending projections to other brainstem or spinal cord centers while the axon terminals of the rostral parts arborize different regions of the forebrain[40–42]. The major forebrain projecting rostral 5HT groups are the DR and MR[43]. The caudal and rostral portions of DR are co-called B6 and B7, respectively, while MR constitutes the B8 and B5[43]. DR, as a whole, is the largest 5HT nucleus in the brain its function has been strongly connected with neuropsychiatric disorders[40,44]. The rest of 5HT nuclear groups are project mainly to brainstem or spinal cord structures[43]. Unlikely, with the DAergic system, the 5HT system is more complex as it targets many different brain regions and numerous classes of 5HT receptors[45] (Figure 2). DR neurons innervate mainly cortical areas, hippocampus, amygdala, septal nuclei, hypothalamus and basal ganglia[46]. Interestingly, the highest density of 5HT fibers is observed in basal ganglia output structures such as GP and SNr[37,46]. 5HT terminals regulate the function of these brain regions through multiple postsynaptic 5HT receptors which are divided in 7 different classes and each class is further subdivided in subclasses using alphabetical order[45]. The receptor classes are mainly distinguished by their amino-acid sequence and Gα-protein coupling properties[45]. The class that does not contain receptors that do not belong to GPCR family is the 3rd which includes the ionotropic $5HT_3R$ receptor[47]. Class 1 of 5HT receptors are coupling mainly to $G\alpha_i$ and their major representatives

are $5HT_{1A}R$ and $5HT_{1B}R$[48]. Due to their inhibitory nature, $5HT_{1A}R$ and $5HT_{1B}R$ receptors are enriched in 5HT neurons and act as auto-receptors by decreasing presynaptic 5HT release[49]. Interestingly, $5HT_{1A}R$ displays mainly somatodendritic distribution while $5HT_{1B}R$ is positioned in the axonal branches, implying the differential function of the two receptors to regulate 5HT release[50]. Postsynaptically, $5HT_{1A}R$ is densely expressed in the cortex, hippocampal formation and medial septum, while $5HT_{1B}R$ transcripts are concentrated in MSNs and CA1 region of hippocampus[49]. The second class of 5HT receptors recruit primarily $G\alpha_{q/11}$ and includes $5HT_{2A}R$, $5HT_{2B}R$ and $5HT_{2C}R$[48]. However, $5HT_{2A}R$ and $5HT_{2C}R$ are densely expressed in CNS while $5HT_{2B}R$ is located mostly in the periphery[49]. $5HT_{2A}R$ is mostly positioned in the forebrain with predominant expression in cortical layer 5A and striosomes[49,51]. The weak $5HT_{2B}R$ expression in brain is restricted to Hb and HF[49]. $5HT_{2C}R$ displays broad expression in the brain with enrichment in striatal, amygdala, Hb and hypothalamic regions[49]. Intriguingly, the highest $5HT_{2C}R$ levels are found in choroid plexus, indicating a potential role of 5HT system on regulating cerebrospinal fluid production[49]. The 5HT receptor class 4 recruits $G\alpha_s$-protein and exhibits profound expression in HF, striatum and Hb[48]. Similar to class 1, class 5 of 5HT receptors is coupled to $G\alpha_i$ and inhibits neuronal activity[48]. $5HT_{5B}R$ exhibits high expression levels in hippocampal CA1 and Hb[49]. Class 6 of 5HT receptors shares both the coupling properties and striatal distribution with $5HT_4R$[48]. The seventh 5HT receptor class is constituted by $5HT_7R$ which is $G\alpha_s$-coupled receptor and is enriched in hippocampus, thalamus and a distinct subtype of striatal iMSNs[48,52]. Malfunctioning of the 5HT system is largely related to anxiety and depressive mood while drugs that strengthen 5HTergic neurotransmission are alleviating these states[53]. However, similar with DA, excess of 5HT signalling can cause hallucinations or delusions and many potent 5HT agonists are considered as psychotropic agents[54]. In conclusion, the forebrain projecting 5HTergic system of the brain includes a complex receptor landscape, which is crucial for controlling mood changes.

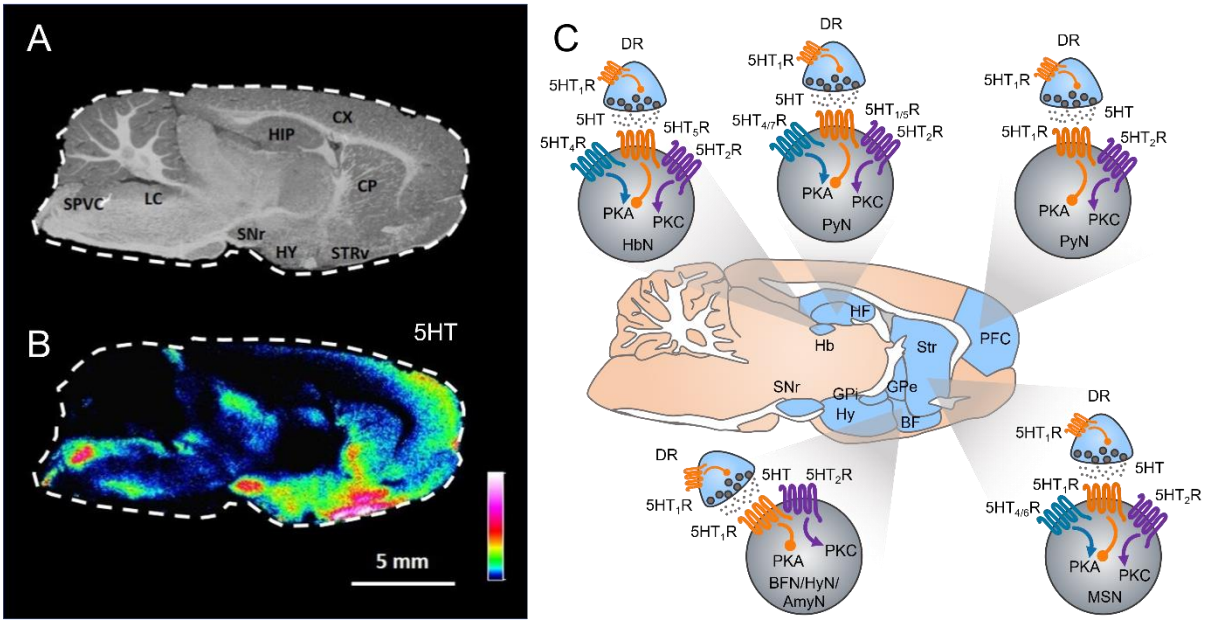

Figure 2. 5HTergic system and 5HTRs in the brain. (A, B) Sagittal slice of mouse brain **(A)** and MALDI-MSI image of 5HT **(B)** (Adapted from Shariatgorji et al.[37]). **(C)** Illustration depicting the effects of 5HT on Hb, HF, PFC, striatum, BF, Hy and Amy. Lines with arrowheads depict excitation while lines with circular heads depict inhibition.

1.3 MONOAMINE RELATED DISEASES

1.3.1 Parkinson's disease

PD is the second most prevalent neurodegenerative disease after Alzheimer's disease worldwide and its diagnosis is based on the incidence of bradykinesia, rigidity and tremor[55]. However, PD symptoms and signs are not restricted to the motor impairment since psychiatric manifestations and vegetative disturbances are prominent features of the disease[55]. Bradykinesia and rigidity is primarily caused by the progressive degeneration of the SNc DA neurons of ventral midbrain[55] (Figure 3). A particular neuropathological feature that characterize PD is the widespread intracellular accumulation of alpha-synuclein[55]. Lewy bodies are the oldest described intracytoplasmic inclusions found in PD in brains and contain large amounts of aggregated alpha-synuclein[55]. Mutations described in familial cases of PD imply that the pathophysiology of SNc neuronal death is largely attributed to alpha-synuclein proteostasis and mitochondrial dysfunction[55]. Nevertheless, it remains unclear whether mitochondrial mutations comprise the same or a distinct disease entity with alpha-synucleinopathy-related PD[56]. In any case, treatments for the motor symptoms in PD have focused on replenishing striatal DA by administration of the DA precursor L-DOPA[55]. The increased concentration of DA in the dorsal striatum following L-DOPA administration alleviates motor symptoms via the simultaneous activation and inhibition of dMSNs and iMSNs, respectively[19]. In patients that do not respond to drug treatment, indirect pathway deactivation through DBS of STN is a highly effective alternative[57]. In the early stages of the disease, L-DOPA treatment shows strong efficacy, but as neurodegeneration progresses

motor complications become gradually evident[58]. Unfortunately, repeated administration L-DOPA often leads to involuntary movements referred as LIDs[59]. The pathophysiology of LIDs is based upon the similarities of the biosynthetic pathway between DA and 5HT[60]. Specifically, the decarboxylation step for the formation of both neurotransmitter is catalysed by the same enzyme which is called AADC[60]. In late PD states, which are characterized by severe DA depletion, L-DOPA is predominately taken up by striatal 5HT terminals and subsequently broken down to DA[60]. This leads to a false striatal DA production, which is released in an uncontrollable manner[60]. The reason for this phenomenon rises from the fact that 5HT boutons lack anticipatory mechanisms for hampering DA release[60]. As a consequence there is a tall and narrow DA wave occurring in the striata which leads to the aberrant activation of D_1R and D_2R[61]. This DAergic rush is followed by a rapid DA depletion, which is usually the cause of higher rate of akinetic states and subsequent decrease in L-DOPA therapeutic efficacy[58]. This excessive DA receptor stimulation produces an excessive locomotor induction which is translated to involuntary dyskinetic movements[61]. Even though, DA released from 5HT terminals stimulates both DA receptors, D_1R stimulation in dMSNs is considered the principal mediator for the occurrence of LID[62]. Overall, PD is treated with DA replacement therapy, which gradually leads to detrimental side effects.

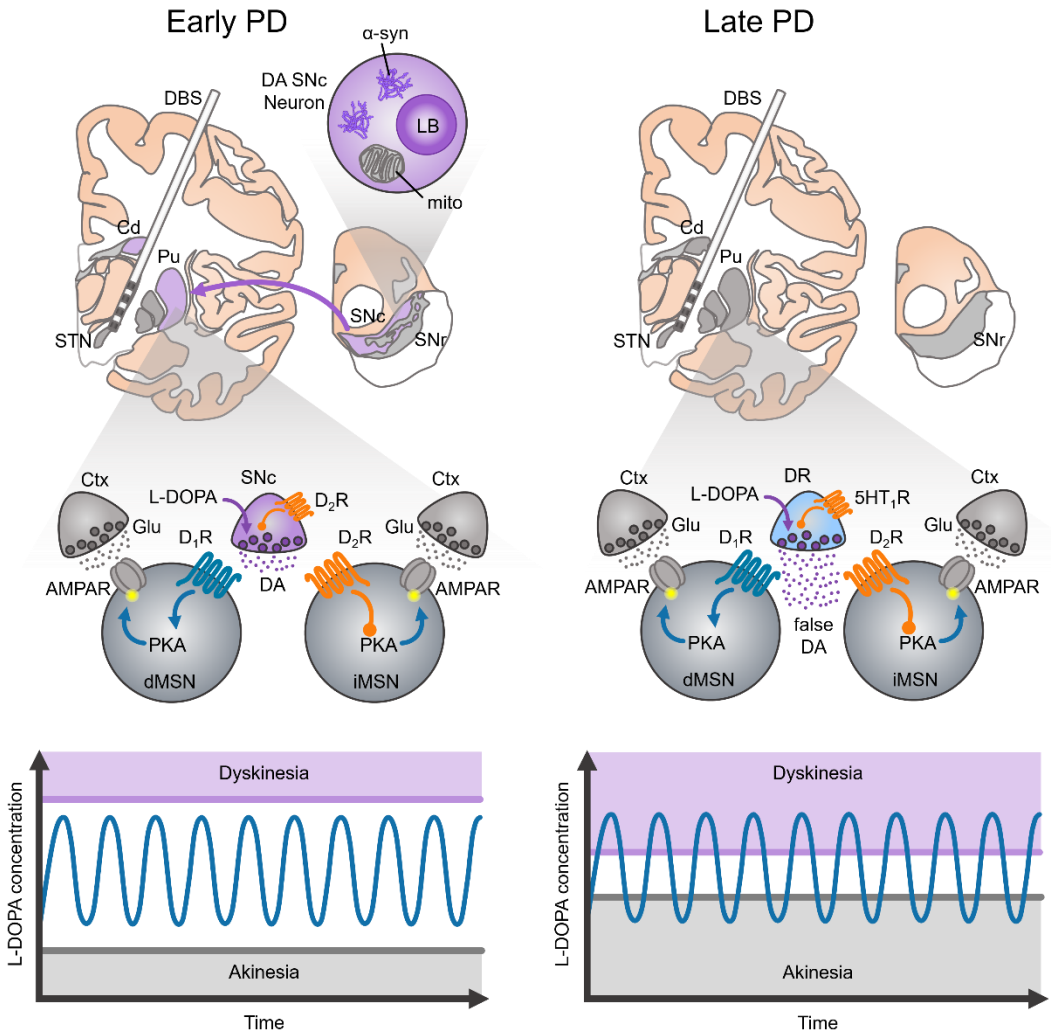

Figure 3. Schematic overview of PD. The illustration shows the pathophysiology of PD motor symptoms in early stage (left panel) and late stage (right panel). Lines with arrowheads depict excitation while lines with circular heads depict inhibition. The yellow circle denotes phosphorylation.

1.3.2 Major depressive disorder

MDD is a serious mental disorder which affects 3% of the global population[63]. The working model for the pathophysiological mechanism of the disease includes alterations in neuronal circuits after stressful stimuli in genetically susceptible individuals[63]. MDD diagnosis is based on DSM-5 criteria which are including dysphoric and anhedonic mood states combined with cognitive and autonomic disturbances[64] (Figure 4). MDD is associated with numerous changes which are the end-product of prolonged stimulation of HPA stress axis[65]. This sustained HPA excitation is believed that is causing the MDD neuropathological changes seen in depressed patients such hippocampal and prefrontal cortical atrophy[63]. Since HPA activity is sensitive to numerous aspects, it is quite puzzling to pinpoint a single factor that may cause aberrant stress response. However, it is believed that the interaction of certain genetic vulnerabilities with specific environmental factors may be the source of HPA-hyperactivity and subsequent development of MDD[63]. HPA axis is an interconnected system with feedback interaction, which

is constituted by three components: hypothalamus, pituitary and adrenal gland[66,67]. The hypothalamic cells that participate in HPA system are located in a relatively small nuclear group that outlines the dorsal ridge of the 3rd ventricle, which is called PVN[68]. This nucleus is divided from to a lateral parvocellular part and a medial magnocellular part. The parvocellular and magnocellular subnuclei contain cells that express CRH and AVP respectively[69]. These neuropeptides are released into the capillary network of median eminence and are transferred to the anterior pituitary through hypophyseal portal system[69]. Subsequently, they stimulate pituitary corticotrophin cells through the $G\alpha_s$-coupled CRH_1R and $V_{1B}R$[69]. Therefore, corticotrophin cells release ACTH in the bloodstream, which stimulate the cells from the fasciculate zone of adrenal cortex to release cortisol[69]. Another parallel system that orchestrates the acute stress response is the SAM axis[70]. This system is mainly composed by NA/AD producing cells in the adrenal medulla and the cholinergic cells located in IML of the spinal cord[70]. This nucleus is occupies the lateral horn grey matter of the thoracic and upper lumbar segments of the spinal cord[70]. These cells project to the adrenal medulla and stimulate the release of NA and AD in the bloodstream[70]. The subsequent plasma elevation of NA and AD activates several adrenergic receptors, which are responsible to elevate the heart rate and blood pressure[71]. Conclusively, HPA and SAM axes are the chief components that orchestrate the stress response, which is dysregulated in MDD.

MDD is characterized by alterations that are taking place in brain areas, which encode the positive or negative emotional value of certain stimuli[72]. Recent advances in functional neuroanatomy research have brought light to the brain areas involved in regulating mood[72]. It has been observed that the rewarding stimuli are encoded by the feedforward interconnectivity between the PFC and NAc[72]. Since NAc is the ventral part of striatum, it shares similar cellular architecture and composition with rest of the nucleus[73]. This implies that NAc harbours both dMSNs and iMSNs which have differential impact upon emotion[74]. It is considered that dMSNs encode is positive reinforcement, while iMSNs are opposing this action[74]. However, recent evidence has shown that the dMSN-iMSN interplay in controlling mood is far more complicated and cannot be explained by the previous simplistic model. Despite that, it is repeatedly shown that rewarding stimuli drive an intra-NAc DA surge, produced by VTA axon terminals[72]. At the same time, interruption of VTA-NAc DA pathway has been shown to create depressive-like phenotype in animal models[75]. Interestingly, both hippocampus and PFC, which are the main areas where MDD related neuropathology is observed, are directly connected with NAc[76,77]. A newly MDD associated area that is highly implicated with the VTA-NAc rewarding system is LHb[78,79]. This glutamatergic nucleus lies in the epithalamic region of diencephalon and sends its axons with a ventrocaudal direction targeting RMTg[79]. This area serves strong GABAergic input to VTA and suppresses DA release in NAc. The LHb-RMTg pathway is considered the aversion

encoding center of the brain as it is strongly opposing the functions of the VTA-NAc reward system[72,79]. Supporting this fact, LHb bursting firing has been correlated to depressive-like behavioral traits in animals. Moreover, the fast-acting antidepressant, ketamine, has been shown to reduce LHb bursts by antagonising NMDAR[80]. Conclusively, MDD is characterized by disturbances in neural circuits that control the reward and punishment perception.

Even though DA shows a direct critical role in alleviating depressive mood, it is evident that the rest of the monoaminergic system has a strong contribution to this process. The link between monoamines and depression was discovered more than half century ago when drugs that interfere with the monoamine breakdown process by inhibiting MAO, displayed antidepressant effects[63,81]. Most of the drugs that alleviate depressive mood, reduce the activity of presynaptic monoamine reuptake transporters (NDRIs, SNRIs, NRIs), monoaminergic autoreceptors (α_2R, $5HT_1$R antagonists) and MAO[63,82] (Figure 4). Even now, the first line antidepressant treatments enhance monoaminergic neurotransmission with emphasis in 5HT (SSRIs)[63]. Although, the exact 5HT mechanism of antidepressant action is not fully unravelled, it is tightly connected with the improvement of depressive mood[63]. Together with the alleviation of depressive-like behaviour, SSRIs restore deficits in hippocampal neurogenesis and synaptic plasticity by increasing BDNF production[83,84]. DR 5HT neurons show similar firing pattern with VTA DA cells in response to salient rewarding stimuli, while they display activity differences in reward prediction tasks[85]. This phenomenon may designate that both cell types are important in reward encoding and affect positive emotional states. It has been described that VGLUT3+/SERT+ DR cells provide an excitatory input to VTA mesoaccumbal neurons, indicating that DR neurons may directly stimulate NAc DA release[86]. At the same time, LHb express multiple 5HT receptors such as $5HT_{2C}$R, $5HT_4$R and $5HT_5$R, which may contribute to VTA-NAc pathway stimulation[87–89]. Nevertheless, it is quite puzzling to pinpoint one brain structure responsible for SSRIs' effects due to 5HTergic system's great receptor variety and broad axonal fields in PFC, hippocampus, amygdala and striatal output structures[46,90]. It is well known that most of the SSRIs require a notable amount of time to exert their antidepressant effects. Nowadays, the research field of fast-acting antidepressants is expanding rapidly. It has been reported that the 5HTergic psychedelic drugs display strong and rapid antidepressant action[91,92]. Most of psychedelics, such as LSD and psilocin share their ability to stimulate $5HT_{2A}$R, which is considered the source of hallucinations[92]. In particular, the "magic mushroom" (e.g. *Psilocybe cubensis*) derived psilocin, which is the dephosphorylated end-product of psilocybin, has been described to exert antidepressant effects in patients[93]. It is believed that antidepressant and hallucinogenic properties of psychedelics derive from biased $5HT_{2A}$R signalling with $G\alpha_q$ and β-arrestin respectively[94]. Nevertheless, apart from $5HT_{2A}$R, psilocin acts as a high affinity agonist for most of the 5HT

receptors such as $5HT_{1A}R$, $5HT_{1B}R$, $5HT_{1D}R$, $5HT_{2B}R$, $5HT_{2C}R$, $5HT_5R$, $5HT_6R$ and $5HT_7R$[95]. Consequently, there is possibility that other 5HT receptors may participate in psilocin's antidepressant effects. Interestingly, both psychedelics and ketamine increase BDNF and promote synapse formation[96]. Similar to SSRI's, further studies are needed to clarify which 5HT receptors are crucial for alleviating depressive symptoms. In brief, monoaminergic system has a pivotal role in emotional states and is the most commonly used drug target to battle MDD.

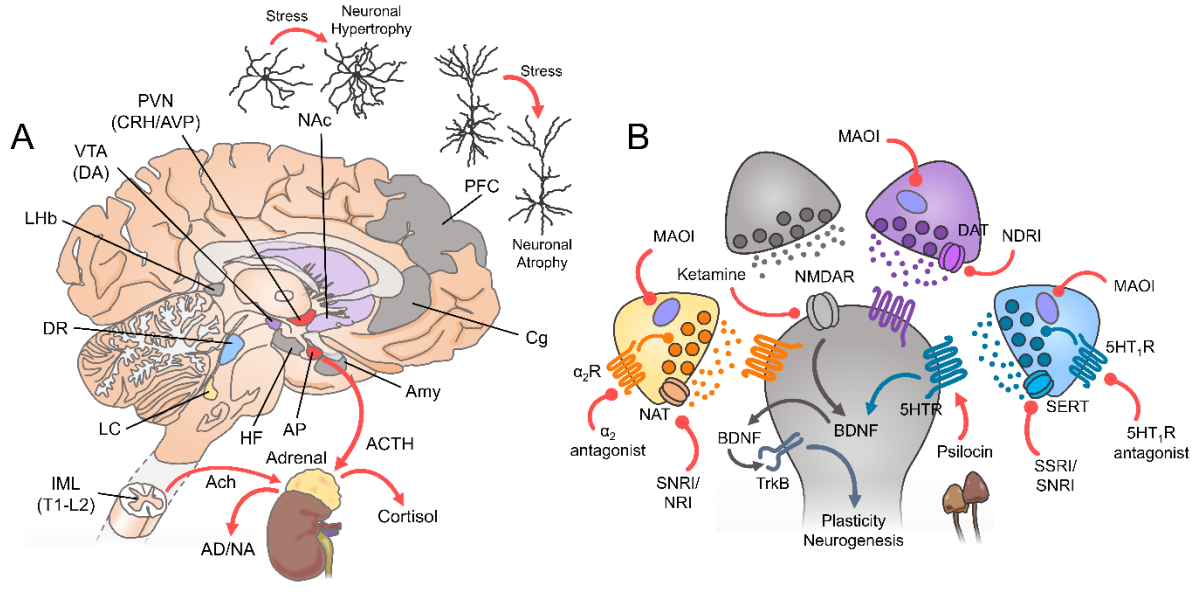

Figure 4. Schematic overview of MDD and antidepressant treatments. (A) The illustration shows the crucial brain regions that affect stress response and are implicated in MDD pathophysiology. **(B)** The illustration shows the antidepressants' mechanism of action in synaptic level. Lines with arrowheads depict excitation while lines with circular heads depict inhibition.

1.3.3 Schizophrenia

Schizophrenia is a debilitating neuropsychiatric disorder that affects approximately the 1% of the world population and is characterized by the incidence of recurrent psychotic episodes[97]. The episodes are described mainly by the occurrence of hallucinations (auditory or visual) and delusional thoughts[97] (Figure 5). Even though, psychosis is the main feature of the disease schizophrenic patients exhibit anhedonia, lack of motivation and cognitive deficits[97]. Hence, the symptoms of the disease are categorized in positive (hallucinations, delusions), negative (anhedonia, apathy) and cognitive (attention, memory deficits)[97]. The disease shows a complex and still obscure pathophysiology which includes neurodevelopmental defects accompanied by with increased and decreased volume in striatum and cortex respectively[97]. The emerging picture of schizophrenia genetics reveals genes associated with glutamatergic synapse but also genes involved in monoaminergic system. The first link of monoamines with schizophrenia was early on when DA was firstly described as a neurotransmitter in CNS[98]. Intriguingly, the observation that connected DA with the disease was the antipsychotics' ability to bind D_2Rs and antagonize

its signalling[99]. The DA hypothesis for positive symptoms of schizophrenia is further supported by the fact that DA-releasing agents such as amphetamine, methamphetamine and phenethylamine can mediate drug-induced psychosis[98]. In detail, it is believed that phasic DA release from VTA neurons activate D_2Rs located in NAc, which, subsequently, mediate psychotic episodes of schizophrenia[100]. Whereas D_2R hyperactivity is attributed to psychotic episodes[101], it is believed that there is D_1R hypofunction in PFC the negative and cognitive symptomatology[102,103]. The D_2R antagonism by typical antipsychotic regimes is sufficient to reduce positive symptoms[104]. Nevertheless, high striatal D_2R occupancy can cause detrimental side effects such as drug induced parkinsonism[105]. The direct link of DA to psychosis is under discrepancy since there are several schizophrenia-related genetic factors that do not involve the DAergic system[97]. In line with the schizophrenia risk genes, drugs that interfere with glutamatergic neurotransmission display psychotomimetic properties[106]. In detail, NMDAR antagonists such as ketamine, PCP and dizocilpine (MK-801), are considered as dissociative hallucinogens and they are commonly used to mimic psychotic states[107]. Concisely, schizophrenia is a mental illness portrayed by the occurrence of psychotic events which are ascribed to disturbances in DAergic and glutamatergic receptor system.

Several naturally occurring psychotropic compounds recruit other GPCRs than D_2R receptors to mediate psychosis. There is evidence that 5HTergic system contributes to the pathophysiology of the schizophrenia. This is supported by the fact that psychotogenic and antipsychotic properties of 5HT receptor agonists and antagonists respectively. Psychedelics, such as DOI, LSD and psilocin, are psychotomimetic drugs that behave as high affinity $5HT_{2A}$R agonists[108–110]. Accordingly, the so-called atypical antipsychotics are evenly capable to inhibit hallucinations and delusions with typical selective D_2R antagonists but exhibit higher affinity for $5HT_{2A}$R[111]. Another 5HT receptor, which is interestingly linked to atypical antipsychotic agents, is $5HT_{1A}$R[112]. Aripiprazole, which shows partial agonist activity on $5HT_{1A}$R, display efficient antipsychotic action[112]. Interestingly, atypical antipsychotic medications are preferred over typical antipsychotics due to their inability to induce drug induced parkinsonism[105]. However, almost all efficacious atypical antipsychotics that act on $5HT_{2A}$R and $5HT_{1A}$R, display some affinity to D_2R receptor, indicating that D_2R blockade might be an essential drug trait for treating schizophrenia[113,114]. Another group of naturally occurring agents that are renowned as atypical hallucinogens are the κOR agonists[115]. The plant-derived (*Salvia divinorum*) Salvinorin A induces strong dissociative hallucinations in humans[116]. Even though it is well established that $5HT_{2A}$R and κOR agonism shows psychotogenic actions, the exact neural substrate that mediates the hallucinogenic state is obscure. In contrast to the clear connection between psychosis and striatal D_2R, it is unclear which $5HT_{2A}$R or κOR expressing region is associated with altered states

of consciousness. $5HT_{2A}R$ is enriched in the cortical L5A[108], while κOR is highy expressed in striatal MSNs and midbrain DA neurons[117]. Another structure in the brain that displays relatively strong expression of both $5HT_{2A}R$ and κOR is the claustrum[118,119]. The evolving neuronal tracing techniques have revealed that the claustral neurons send ipsilateral broad axonal arborizations that entangle almost the entire cortical mass[120]. This phenomenon in combination with the high density of $5HT_{2A}R$ and κOR, led several scientists to hypothesize that the claustrum might be the neuronal substrate of hallucinogens[121–123]. Conclusively, psychotic state does not necessarily involve DAergic or glutamatergic systems but extends to 5HTergic and opioidergic neurotransmission.

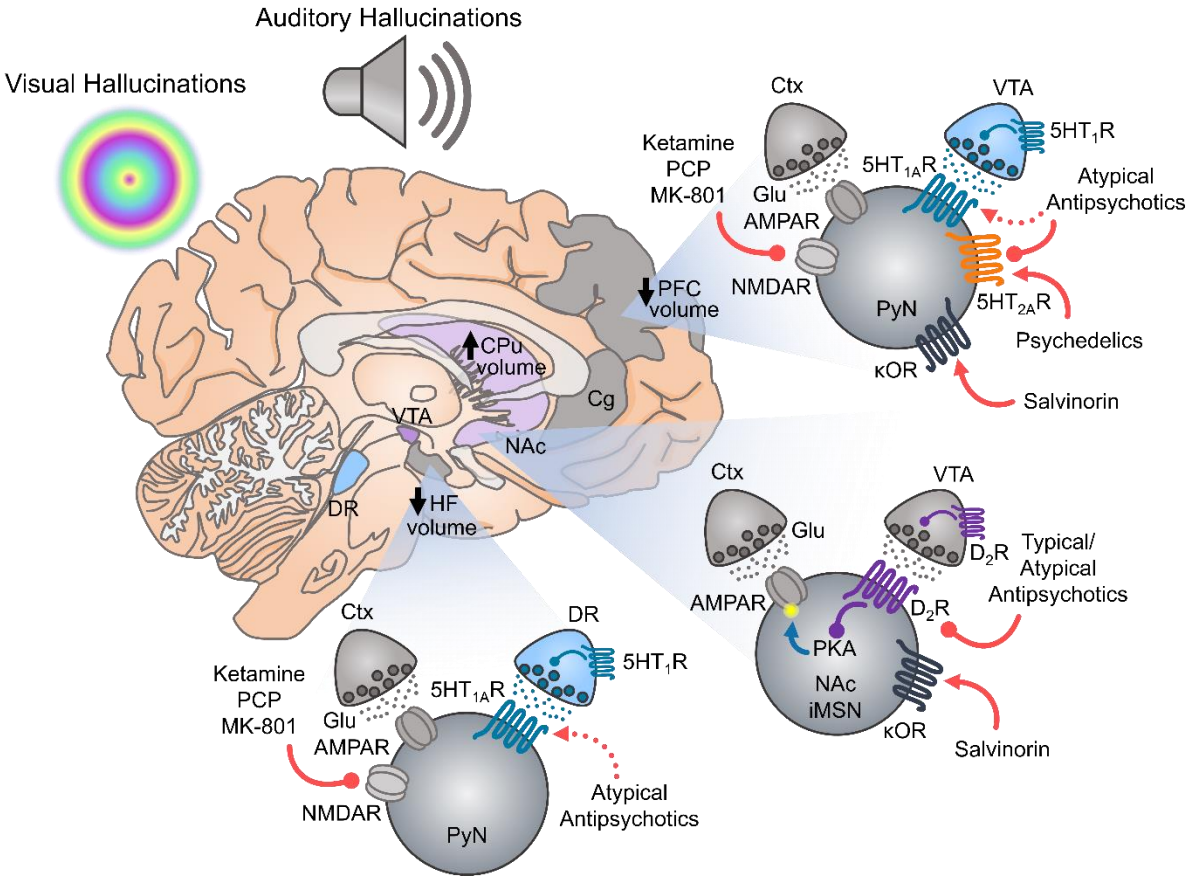

Figure 5. Schematic overview of schizophrenia, psychotropics and antipsychotics. The illustration depicts the key brain regions, cells and receptors that are implicated in schizophrenia. Additionally, the illustration depicts the proposed action mechanism of psychotropic and antipsychotic medications. Solid lines with arrowheads depict excitation, dashed lines with arrwoheads depict partial agonism and lines with circular heads depict inhibition. The yellow circle denotes phosphorylation.

1.4 GPCR PATHWAY RELATED MOLECULES

As we pointed out previously, PD, MDD and schizophrenia are robustly connected with the monoaminergic neurotransmitters of the brain and particularly with the GPCRs that are associated to them. However, recent advances in GPCR research have revealed novel members of the GPCR related signalling with largely unexplored mechanism of action. Data from transgenic animals have revealed that several of these recently discovered molecules are highly implicated to PD,

MDD and schizophrenia. Four representatives of this group of proteins are GPR88, TAAR1, p11 and NURR1.

1.4.1 GPR88

GPR88, is an orphan GPCR that attracted the attention of neuroscientific community is the almost exclusively localization of the receptor in the brain and particularly in striatum[124] (Figure 6). Broadly, GPR88 mRNA shows an extraordinary preference for the striatal mass whereas modest transcript levels are observed in the olfactory tubercle, thalamus, inferior olivary nucleus, amygdala and cerebral cortex[125,126]. GPR88 transcripts are by far the most abundant GPCR mRNA molecules in striatum, surpassing even the well-known D_1R and D_2R[127]. In striatum, the receptor is solely expressed in MSNs with equal distribution in both dMSNs and iMSNs[128]. Furthermore, GPR88 is strategically located proximal to postsynaptic densities of VGLUT1 positive synapses, in order to control the corticostriatal afferent input[128]. MSNs lacking GPR88 exhibit robust augmentation of firing rate and surface expression of AMPA receptors[125]. Considering that GPR88 is $G\alpha_i$ coupled, genetic deletion of the receptor might lead to a rise in cAMP concentration and a subsequent activation of PKA which orchestrates the AMPAR membrane docking through phosphorylation (Figure 1)[125,129]. Even though, there is no known endogenous ligand for GPR88, the receptor shows high amino-acid sequence similarity with monoaminergic receptors and particularly $5HT_{1D}R$[124]. It is proposed that the receptor may be constitutive active due to the fact that lacks the TM3 Aspartate-Arginine-Tyrosine (DRY) motif, which forms salt bridges with TM6 and maintain the inactive conformation of the receptor[124]. Additionally, GPR88 was recently found to interfere with the signalling of multiple striatal receptors[130]. Broadly, GPR88 hinders G-protein signalling of μOR, D_2R, M_1R and M_4R[130]. Moreover, GPR88 diminishes β-arrestin recruitment of all striatal enriched receptors such as μOR, $A_{2A}R$, D_1R, D_2R, M_1R and M_4R[130]. These findings may imply GPR88-mediated actions that extend beyond the $G\alpha_i$-properties of the receptor. Interestingly, it is reported that GPR88-KO mice display reduced levels of RGS9, which is considered an MSN-specific marker[125,131]. RGS9 is described to regulate μOR and D_2R signalling[132]. Consequently, GPR88 is an orphan GPCR with great impact in MSN's electrophysiology and signalling.

GPR88's abundancy and importance in MSNs' function sets the receptor as a candidate novel pharmaceutical target for PD, MDD and schizophrenia[133]. Striatal GPR88 mRNA expression is shown to be regulated by DA given that MFB 6-OHDA lesions significantly reduce the receptor's transcript density[128]. This phenomenon is further supported by the fact that repeated L-DOPA administration rescued the effect of 6-OHDA[128]. These results indicate that GPR88 might be implicated in the locomotor deficits observed in PD. GPR88-KO mice show robust hyperactivity

in OFT, higher sensitivity to psychostimulants but also learning deficits. Interestingly, conditional GPR88-KO in the two populations of MSNs, showed that the increased locomotor activity is attributed to the receptor loss in iMSNs and not dMSNs[134,135]. This phenotypic profile makes GPR88-KO mouse line as a good candidate transgenic model for schizophrenia. Even though, it is known that GPR88-KO animals perform poorly in anxiety assessment tasks, the receptor's role in MDD remains largely unexplored[135]. However, there is evidence that GPR88 mRNA levels are regulated by antidepressants[136]. In summary, GPR88 related research in animals have revealed the fundamental role of the receptor in controlling PD, schizophrenia and MDD animal models.

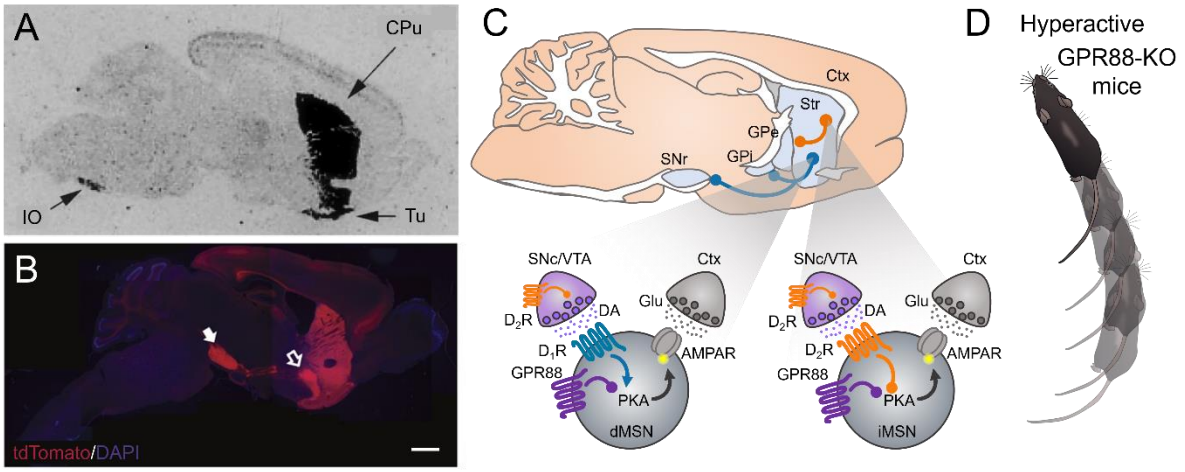

Figure 6. Schematic overview of GPR88 actions. (A, B) Radioactive ISH targeting GPR88 mRNA **(A)** and fluorescent picture depicting tdTomato expression driven by GPR88 promoter **(B)** (Adapted from Mizushima et al.[124] and Quintana et al.[125]). **(C)** Schematic depiction of proposed GPR88 effects in MSNs. **(D)** Illustration showing the hyperactive phenotype of GPR88-KO mice. Lines with arrowheads depict excitation while lines with circular heads depict inhibition. The yellow circle denotes phosphorylation.

1.4.2 TAAR1

Except for the main members of monoaminergic system, the brain hosts other monoaminergic compounds which are found tremendously low concentrations compared to their abundant counterparts such as DA, 5HT and NA[137]. These molecules are known as TAs as they are detected in trace quantities in the brain and the rest of the body[137]. TAs are occurring naturally through the action of aromatic amino-acid decarboxylase as side products of the DA and 5HT synthesis pathway[138]. Until the beginning of 21st century, TAs' function was majorly investigated in flies and worms since there are several invertebrate GPCRs which are known to bind TAs[137]. Regarding the mammalian CNS, TAs were renowned to display amphetamine-like properties and deplete intracellular DA stores, without acting to any specific type of receptor[137]. On the other hand, due to the discovery of the mammalian TAARs, the scientific community has shifted the attention towards the function of TAs in mammalian brain[137]. Most of the TAARs are olfactory receptors, whereas the only non-olfactory member which is predominately expressed in the brain is TAAR1[139]. The main chemicals that act as endogenous TAAR1-ligands, are tyramine, β-PEA,

3-MT and tryptamine[139]. Nonetheless, there is another endogenous TAAR1 acting compound, called T_1AM, which does not occur from classical monoamine metabolism[139]. Interestingly, this compound belongs to a special group of TAs, called thyronamines, which are naturally occurring from the decarboxylation of thyroid hormones[139]. In addition to TAs, TAAR1 is activated by most of the aminergic psychostimulants, such as amphetamine, methamphetamine and MDMA[140]. TAAR1 displays very low mRNA levels in the brain and for that reason knock-in studies have been taken place to describe its localization in different brain regions[141,142]. The receptor can be found in principal monoaminergic brain areas such as VTA, DR and NTS, but also in PFC, entorhinal cortex, hypothalamus and amygdala[141]. Despite its low levels, TAAR1 activation or blockade produces dramatic changes in VTA DA neuron firing rate[142,143] (Figure 7). For instance the selective TAAR1 agonist RO516607 robustly suppressed DA neuron action potential frequency[142,144]. Simultaneously, application of TAAR1 antagonist, EPPTB or genetic deletion of the receptor creates an extremely dense spike train resembling that one of the fast-spiking GABAergic interneurons[142,143]. Even though, TAAR1 is described mediate some of its effects through $G\alpha_{q/11}$ and $G\alpha_{12/13}$, it has been repeatedly shown that the receptor displays strong $G\alpha_s$-coupling properties (Figure 1)[145–147]. Another interesting aspect about the receptor is that it mainly resides inside the cell and its localization to different intracellular compartments may determine the G-protein coupling properties[145,148]. However, there are reports that localize minor TAAR1 levels on the plasma membrane, implying a complex regulatory mechanism behind the receptor's trafficking. Moreover, it has been reported that it interacts with DAT, D_2R and glutamate transporter[145,149]. Concisely, TAAR1 binds to the trace amines produced in the brain and suppresses DAergic neuron activity.

Owing to the fact that TAAR1 affects intensively DA cell electrophysiological properties, it is possible that the receptor is able to modify the course of PD, MDD and schizophrenia. TAAR1 is mostly associated with schizophrenia, since TAAR1-deficient animals show a behavioural phenotype that fits to the animal equivalent of psychosis[141]. TAAR1-KO animals show enhanced susceptibility to both DAergic and glutamatergic psychostimulants[141,144]. In line with this phenomenon, TAAR1 agonists produce efficient suppression of locomotor induction after cocaine and the NMDAR-antagonist, L-687,414 administration[144,150]. The ability of TAAR1 to rescue schizophrenia-like behaviours in animals, was exploited by clinical studies where TAAR1 agonists were tested for their antipsychotic properties[150,151]. Notably, the dual $5HT_{1A}R$ partial agonist/TAAR1 agonist, SEP-856, displayed significant decrease in PANSS score in patients that received the drug, compared with the placebo[152]. Additionally, there is evidence that TAAR1 is involved in PD since TAAR1 KO mice display higher rotational and dyskinetic response to L-DOPA after 6-OHDA lesions in MFB[153]. Even though, TAAR1 implications to MDD are still

obscure there is evidence that TAAR1-agonist improve depressive-like states in animal models. To sum up, TAAR1 is tightly connected to schizophrenia, even though there are indications for its association to PD and MDD.

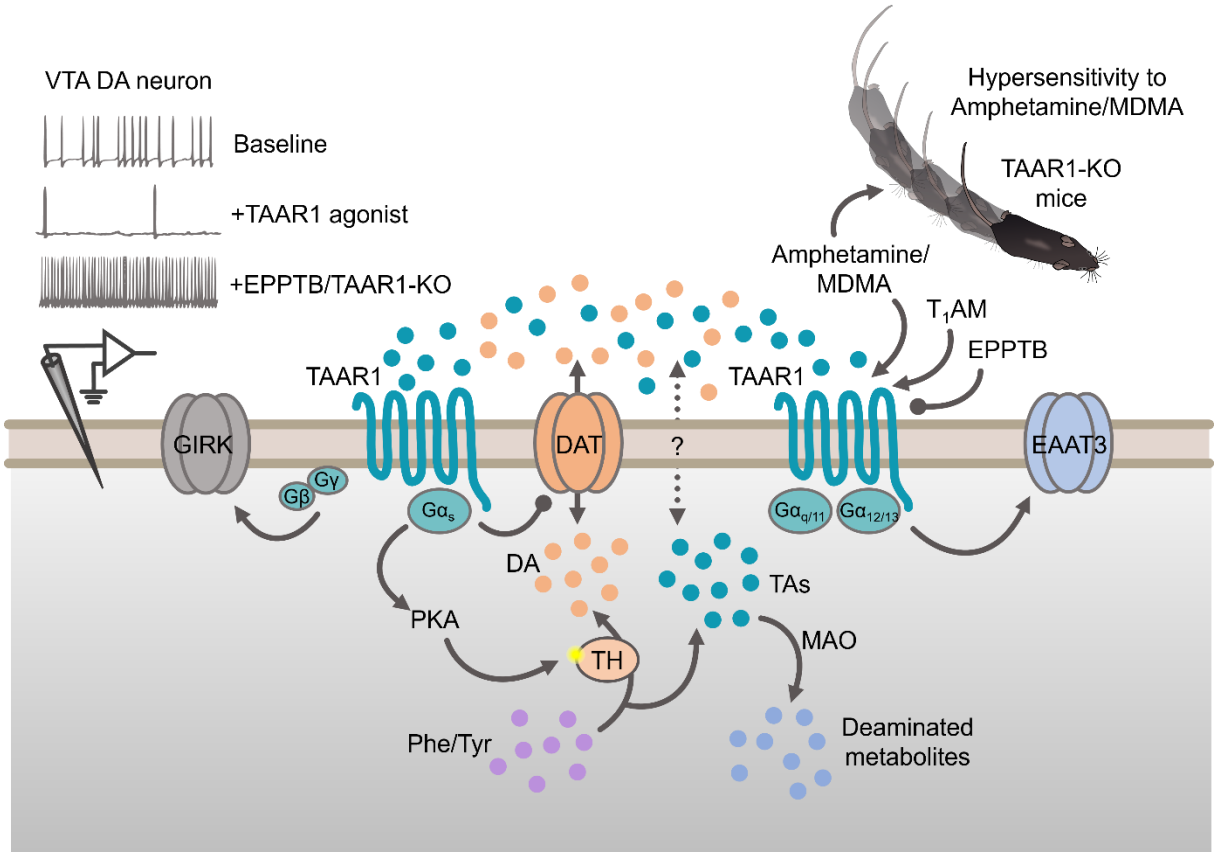

Figure 7. Schematic overview of TAAR1 actions. The illustration depicts the proposed TAAR1 actions in DA neurons. Lines with arrowheads depict excitation while lines with circular heads depict inhibition. The yellow circle denotes phosphorylation.

1.4.3 P11

P11 (also known as S100A10, annexin II light chain and calpactin I light chain) is an adaptor protein that belongs to S100 protein family and is implicated in 5HT and glutamate signalling[154,155]. Even though S100 proteins are well-known for their Ca^{2+} binding properties, p11's EF-hand motifs are not capable of binding Ca^{2+}[156]. P11 was firstly described to form an heterotetramer with the Ca^{2+}/lipid binding protein AnxA2[156]. Later, p11 was found to downregulated in the cingulate cortex from MDD patients, suicide victims and learned helplessness mice[157] (Figure 8). Moreover, rodent cortical p11 mRNA is upregulated with antidepressant treatment and electroconvulsive therapy[157]. Global p11-KO mice exhibit a depression-like behavioral phenotype in several well-established models that measure behavioral despair (e.g., FST and TST) or anhedonia (e.g., SPT)[155]. In addition, p11 KO mice show diminished responses to antidepressants in various behavioural paradigms that measure antidepressant efficacy[155]. The dynamic p11's transcriptional response to antidepressant and chronic stressors is claimed to be partially dependent upon 5HT-BDNF signalling[155]. P11 is a

multifunctional protein that is mainly involved in chromatin remodelling and transmembrane protein docking[154,155]. Regarding chromatin remodelling, p11-AnxA2 complex has been described to strengthen SMARCA3's nuclear matrix anchoring and DNA binding affinity[158]. As for transmembrane protein docking, p11 has been found to increase surface expression of multiple ion channels, such as ASIC1, TASK1 and Cav1.2/3, but also GPCRs[154]. Concerning the latter, p11's interaction with third intracellular loop of serotonin receptors $5HT_{1B}R$, $5HT_{1D}R$ and $5HT_4R$, supports its relationship with MDD[154,155]. Broadly, p11 increases the membrane localization and signalling of $5HT_{1B}R$ and $5HT_4R$[154,155]. In accordance, it is described that the antidepressant actions of $5HT_4R$ agonists, are partially dependent on p11[159].

Due to p11's strong expression in several forebrain and brainstem regions, it is puzzling to pinpoint a certain brain structure that is responsible for global p11-KO mice' anhedonia and behavioral despair. Nonetheless, several efforts that use conditional p11-KO strategies have been made, which have brought some light to the exact role of p11 in different aspects of MDD animal models. The spontaneous depressive-like behaviour, observed in global p11-KO, has been reproduced by selective deletion of p11 in CINs located in NAc[160]. In detail, it has been shown that p11 stimulates the expression of HCN2 and subsequently, the tonic NAc-CIN firing[161]. Furthermore, $5HT_{1B}R$ is preferentially expressed in the CINs of the ventral versus the dorsal striatum and together with p11 controls acetylcholine efflux[162]. Apart from CINs, p11 transcripts and protein can be found striatal MSNs[163]. Considering the fact that MSNs display one of the highest $5HT_{1B}R$ and $5HT_4R$ transcript density in the brain[164,165], we could hypothesize that part of p11's antidepressant properties arise from striatum. Likewise, p11-KO mice show weaker antidepressant response to $5HT_4R$ agonists[159], which is accompanied by decreased $5HT_4R$ and $5HT_{1B}R$ binding in striatum or striatal projection sites (GPe/SNr)[159,163,166]. It is reported that SSRIs selectively induce p11 expression in cortical L5A IT-PyNs and in hilar MCs located in DG[158,167,168]. Selective deletion of p11 in L5A-IT PyNs and DG MCs blunts antidepressants' effects on mouse performance in paradigms of behavioral despair[167,168]. Especially, conditional p11-KO mice in hilar MCs blunts the antidepressant-induced neurogenesis in DG[158]. Nevertheless, deletion of p11 in these regions does not affect the spontaneous mouse behaviour in the same tests. This phenomenon indicates that cortical and hippocampal p11 does not drive depressive-like phenotype, but it is essential for the antidepressant-induced behavioral improvement.

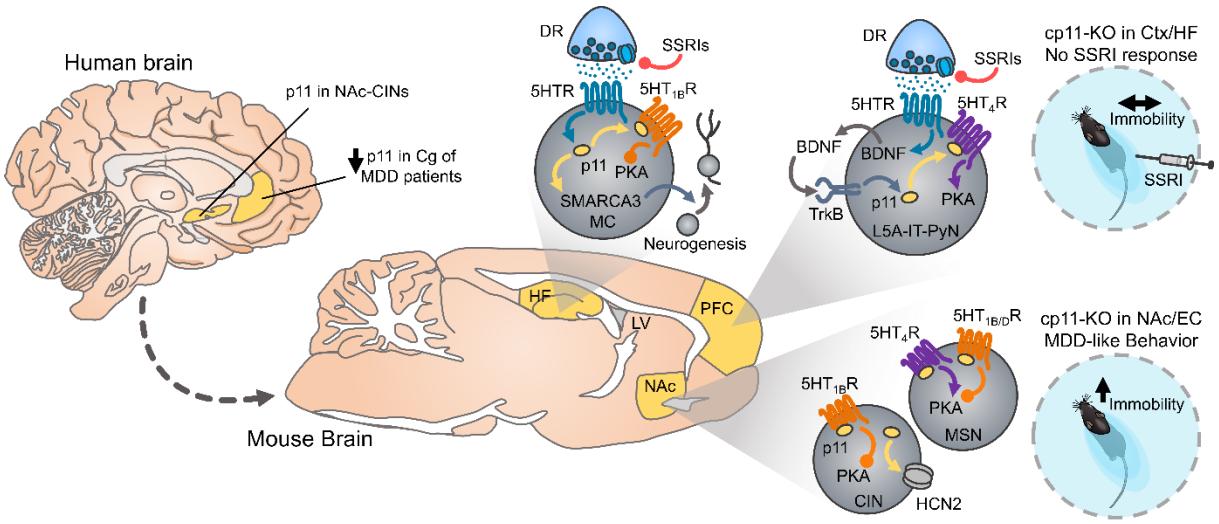

Figure 8. Schematic overview of p11 actions. The illustration depicts the p11 actions in different brain regions and cells. Additionally, the illustration shows the effects on depressive-like behavior after the selective p11 deletion in certain brain areas. Lines with arrowheads depict excitation while lines with circular heads depict inhibition.

1.4.4 NURR1

NURR1 belongs to the NR4A transcription factor family together with NUR77 and NOR-1[169]. All the members of NR4A group display immediate early gene properties, which underlies the inducible nature of their mRNA in certain cell conditions[170]. It is well known that all the genes that belong to the NR4A group are upregulated by CREB and consequently by increased cAMP concentration [171]. Studies performed in CNS have shown that drugs that regulate GPCR signalling affects the mRNA levels of NURR1, NUR77 and NOR1[172,173]. Particularly, it is repeatedly reported that NURR1 gene transcription is induced by the activation of EP_2R[174] (Figure 9). Even though NURR1 is a member of a nuclear receptor family, it does not have any hydrophobic binding pocket[175]. As a result, it is believed that it acts with a ligand independent manner by forming heteromers with other deorphanized nuclear receptors, such as RXR and RAR[176]. NURR1 has gained a lot of attention as a molecule due to its strong connection with the DAergic transcriptional signature[175,177]. The transcription factor plays fundamental role upon ventral midbrain DA neurons development, through induction of several DAergic marker genes such as TH, VMAT2 and AADC[177]. Interestingly, it is reported that there is a NBRE in TH-promoter, which allows the direct binding of NURR1[178]. Notably, NURR1-KO mice are incapable to develop the VTA/SNc complex and die after the first postnatal days[179]. Moreover, selective ablation of NURR1 later in adult stages interferes with DA axon terminal maintenance and integrity[180]. This phenomenon is explained by the downregulation of several mitochondrial genes as a consequence of NURR1 deletion[180]. Apart from its role as a chief factor for DA neuron development, NURR1 has been described to play crucial role in Hb neuron development[181]. Though, it has been reported that NURR1 is not only a transcription factor that controls neuronal

transcriptional identity. High frequency stimultion of the perforant pathway in HF produces an overt NURR1 mRNA upregulation in DG[182]. In accordance, HF NURR1 deletion is associated with learning deficits[183]. Moreover, NURR1 has been detected to postsynaptic sites indicating a possible non-transcriptional role of the protein[184]. Hence, NURR1 is an inducible factor in response to heightened neuronal activity and is crucial for synaptic potentiation.

Due to the fact that NURR1 is an important regulator of DA neuron related genes, it has been linked to several diseases that affect the DAergic system function such as PD and schizophrenia[185]. Additionally, human NURR1 polymorphisms have been associated to both schizophrenia and PD[186,187]. As it is mentioned earlier, NURR1-KO mice are not viable and consequently their behavioural phenotype cannot be studied[179]. However, there are several studies that have been performed upon NURR1 heterozygous mice since they are capable to survive[185,188]. Given that NURR1 demonstrate an important part of DA neuron development, reduced amount of the protein might affect the DAergic cell viability over time. For this reason, the same mouse strain has been used as a progressive animal model of PD[188]. Even though heterozygous NURR1-KO mice do not lose the ability to produce DA neurons, they exhibit an age-dependent DAergic cell degeneration and gradual decline of striatal DA content[188]. The neuroprotective role of NURR1 has been further studied through elegant genetic methods by selective deletion of the gene in DA neurons in postnatal stage[180]. Disruption of NURR1 in DAergic neurons at the 1st month of age leads to a slow and steady axonal and dendritic pathology which is accompanied by a worsened motor phenotype[180]. Moreover, heterozygous NURR1-KO mice show a pronounced schizophrenia-like phenotype as they display hyperactivity and strengthened response to amphetamine and PCP[185]. Nevertheless, it is not well established which brain structure is responsible for the schizophrenic-like behavior of heterozygous NURR1-KO mice. Interestingly, another brain structure that displays extremely high levels of NURR1 expression, is the CLA[189]. This elusive sheet-like brain region is enclosed ("claustrum" means "enclosure", in Latin) by insula and putamen, embedded within the white matter formed by extreme and external capsule[190]. As a brain structure has attracted the attention of the neuroscientific community CLA's peculiar connectivity with the cortex together caught the attention of Francis Crick who devoted his last published paper on the neuroanatomical theories of conciuoussness[121]. Future papers have proposed that CLA might be associated with hallucinations and delusions observed in schizophrenic patients[191,192]. NURR1 is one of the earliest markers that define the claustrum throughout brain development[193]. However, there is no study investigating the role of NURR1 for the claustral function. Overall, decreased NURR1 levels are associated with both schizophrenic and PD-like phenotype.

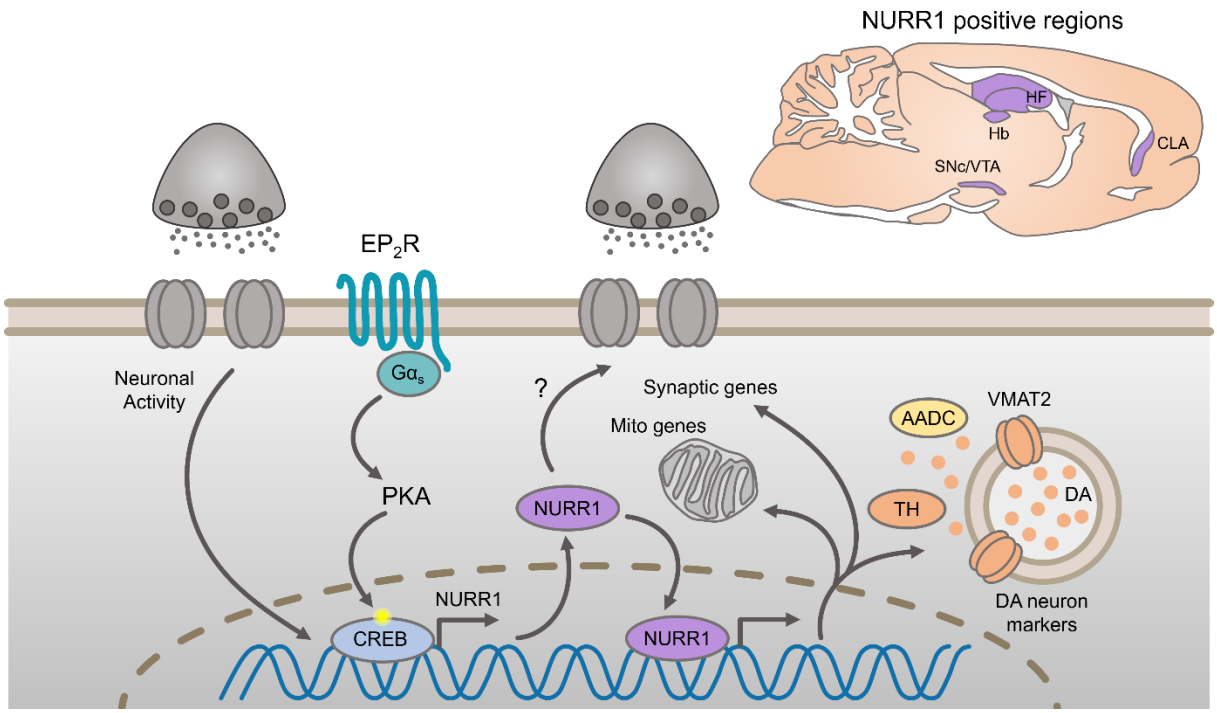

Figure 9. Schematic overview of NURR1 actions. The illustration depicts the factors that control NURR1 gene induction and NURR1's proposed actions. Moreover, the scheme shows that brain regions that the NURR1 is enriched. Lines with arrowheads depict excitation. The yellow circle denotes phosphorylation.

RESEARCH AIMS

The overall aim of the thesis is to provide insights in the action mechanism of several GPCR-related molecules in association with Parkinson's disease, major depressive disorder and schizophrenia. To address these aims we employed transgenic disease animal models and used different methodologies including behavioral studies in combination with stereotactic brain injections and pharmacological treatments, but also histology techniques such as in situ hybridization and immunofluorescence. The specific aims of our studies were to:

I. Elucidate the function of GPR88 in parkinsonian symptoms, L-DOPA therapeutic response and side effects.

II. Unravel the role of TAAR1 in response to monoamine oxidase inhibitors, common antidepressants and the antipsychotic action of SEP-856.

III. Determine the role of p11 in the autonomic response to stress.

IV. Describe the action of NURR1 in D_1R expressing neurons after DA depletion and subsequent subchronic L-DOPA administration.

V. Investigate the impact of claustral NURR1 deletion in the nucleus' cell identity and hallucinogen-induced brain state.

2 MATERIALS AND METHODS

2.1 ANIMAL EXPERIMENTS

2.1.1 Hemiparkinsonian animal model

The most widely used animal model, which simulates the striatal DA depletion of PD patients, is the 6-OHDA-induced Parkinsonism[194,195]. This model requires the unilateral injection of 6-OHDA to brain areas that display densely packed DA fibers[196]. The selectivity of 6-OHDA for catecholaminergic neurons derives from its ability to enter the cell through DAT and NAT[197]. The site of the injection will determine the magnitude of DA neuron loss attributable to the volume differences in axon distribution[198]. Notably, intrastriatal injections produce partial DAergic denervation since the axon terminals occupy relatively large surface area in order to cover the whole striatal mass[198]. At the same time, injections within the nerve tract, which conveys the DAergic fibers to the striatum, create total DA neuronal loss[199]. In particular, this fiber tract is MFB, and injections in that region are often used to mimic the late PD stages[200]. In the current thesis, both MFB and striatal 6-OHDA were performed by using stereotactic frame. Hence, for MFB targeted 6-OHDA injections, we used the coordinates: -1.1 mm anteroposterior and -1.1 mm mediolateral from the bregma and -4.8 mm dorsoventral from the surface of the brain. For intrastriatal 6-OHDA injections, we used the coordinates: +0.6 mm anteroposterior and -2 mm mediolateral from the bregma and -3.2 mm dorsoventral from the surface of the brain. Due to its tendency to oxidize rapidly, 6-OHDA was dissolved in a saline based solution containing 2% ascorbic acid[197]. In order to maximize 6-OHDA uptake by DA producing terminals, the animals were treated 30 minutes before the surgery with a combination of the NAT inhibitor, desipramine and the MAOB inhibitor, pargyline.

6-OHDA hemiparkinsonian animal model is commonly used to assess both anti-parkinsonian and dyskinetic actions of L-DOPA and other DA mimetic compounds[201]. Due to the unilateral 6-OHDA induced degeneration, administration of DA releasing agents such as amphetamine produce an asymmetric locomotor induction which causes the animals to rotate[202]. As a result, the experimental subjects tend to turn towards the side of the injection as each striatum controls the contralateral side of the body[203]. Even though, drugs that enhance DAergic release produce the rotation, L-DOPA and DA agonists generate an opposite rotational bias[203,204]. This phenomenon is credited to the higher sensitivity to DA of the dennervated striatum, which leads the animals to circle towards the contralateral direction of the lesion[203,204]. The therapeutic effect of L-DOPA and DA mimetic compounds is evaluated by measuring animal's rotational rate and distance travelled in an OFT[205,206]. Prolonged exposure of the animals to either L-DOPA or DA agonists creates an enhanced locomotor response to the drugs, which is accompanied by dyskinetic-like movements[207]. These behavioural changes are accompanied by massive transcriptional changes

within lesioned striata, which are mostly related with genes that are connected to heightened neuronal activity[208]. Most of the transcriptional changes have been described to take place within D_1R MSNs[208]. These movements are given the term AIMs and are very well characterized in rodents[206,207]. In our studies, four weeks after 6-OHDA surgery, mice were treated with saline or a combination of LDOPA (10 mg/kg) and benserazide (7.5 mg/kg) once daily for 21 days. The number of ipsilateral and contralateral rotations were video tracked for 30 minutes on days 1, 7, 14 and 21, with a video camera mounted in the ceiling. Immediately after each rotation recording, mice were video tracked using a side camera for 5 minutes. During these 5 minutes individual AIM subtypes (forelimb, orofacial, axial and locomotive) were scored according to validated rodent AIM scales[206,207].

2.1.2 Tests measuring depression-like and anxiety-like behavior

Even though, the clinical MDD features, such as sadness and suicidal thoughts are considered unique for human species, there are some characteristics of the disease that have been successfully modelled in animals[209]. For instance, there are tests which measure helplessness and anhedonia in rodents that are alleviated with antidepressant treatment[209]. MDD animal model research includes studies to investigate the response in acute or chronic stressors, genetic MDD factors, and the neurophysiological mechanisms that govern the action of antidepressant drugs[209]. Rodent behavioural despair, due to a stressful unescapable event, has been an extremely useful tool to evaluate depression in animals[209]. Two behavioural tests that are used to measure despair are the FST and the TST[209]. Both tests set the rodents in an unescapable unpleasant environment such as water (FST) and tail hanging (TST) and measure the willingness of the animal to escape[209]. The rodents that spend less time in making escaping attempts and float motionless are considered to exhibit depressive like behaviour[209]. Immobile behaviour in these tests is attributed to the rodent hopelessness emotional state which drives them to surrender from evading[209]. Notably, administration of antidepressant drugs prior testing increases the number of fleeing efforts and thus decrease the time that the animals stay still[209]. Apart from behavioural measurements, these tests can be used to evaluate the hormonal response to such an acute stressful stimulus. It has been repeatedly shown that mice, which are acutely exposed to FST, exhibit higher levels of plasma ACTH. To evaluate depressive-like behaviour in mice we used the FST. Briefly, the mice were placed into 50 cm tall transparent Plexiglas cylinders filled with water (temperature 24±1°C) to 20 cm depth for a 7 minutes period. Anhedonia is another behavioral trait that can be measured in animals. For measuring anhedonia, researchers perform the SPT, which measures the innate tendency for rodents to prefer sweet solutions over water[209]. Animals that display low preference for sucrose containing solution are considered as anhedonic[209]. In our studies, the mice were single

caged with two bottles containing either water or a 1% sucrose solution for 48 hours. The position of the two bottles was shifted after 24 hours. Sucrose preference was calculated as the percentage of sucrose solution consumption (g) over the total volume consumed (water (g) + sucrose (g)). To sum up, MDD animal models exploit depression-like behavioural traits observed in animals while are used to validate new MDD treatments and genetic factors.

Since chronic stress and anxiety are important risk factors for the development of MDD in patients, it is essential for the establishment of animal tests that mimic anxious behaviour. Classical rodent models of anxiety-like phenotype are focusing on tests that evaluate approach-avoidant behaviour[210]. These tests are imitating environmental threatening situations in which the rodents are naturally avoid[211]. For example, rodents tend to avoid open luminous spaces while they prefer dark wall-enclosed places[211]. This is an innate rodent behaviour that decreases the exposure time to potential predators[211]. A simple assay that simulates that condition is the light-dark box test, which is consisted by a bright and a dark black compartment interconnected with an opening[210]. The mice that spend more time in the minimally lit compartment compared to the control counterparts are considered to display anxiety-like behaviour[210]. Likewise, commonly used anxiolytic drugs, such as benzodiazepines, cause the mice to attempt more entries to the brightly illuminated part of the box[212,213]. In our experiments, the mice were initially placed to the dark part and were freely to explore the two compartments for 15 minutes. The total time spent by mice in the bright vs. dark compartment over the session was recorded and analyzed using an automated video tracking system (Noldus EthoVision XT 8). Other typical assays that evaluate rodent anxiety-like phenotype, measure the ability of certain cues to produce defensive behaviours[214]. In these behaviours are included the freezing, fleeing or aggressiveness and are usually observed as a reflection to the presence of predator[214]. Another similar behaviour is the defensive burying, which refers to the displacement of bedding material by using the snout and the forepaws[215]. This behaviour is often observed in response to aversive stimuli such as electric shocks and air puffs[215]. The same type of behaviour is employed when rodents are exposed to non-aversive stimuli such as glass marbles[213]. However, it is argued if this type of digging is equivalent to defensive burying[215]. Nevertheless, marble-burying assay it is used to measure anxiety-like behaviour by counting the number of glass marbles a rodent would bury beneath the bedding material[213]. Mice that display anxiety-like phenotype tend to hide more marbles than the control counterparts, while anxiolytics cause a decrease in the number of buried marbles[213]. In our studies, mice were placed in transparent cages containing a 5 cm layer of bedding material together with 24 evenly spaced glass marbles (1.5 cm in diameter) and recorded for 30 minutes. A hidden marble was considered when at it was dipped at least two-thirds in the bedding material.

2.1.3 Psychosis and cognition related animal behavioral tests

Modelling psychosis in animals is a quite puzzling and is largely based on the animal response to psychotomimetic drugs[216]. For instance, it is well established that amphetamine, PCP, ketamine and MK-801 produce robust locomotor stimulation in a square field[216]. Thus, the distance travelled by the animals in an OFT after the administration of the aforementioned drugs is simulating the positive symptoms of the disease[216]. Supporting the validity of this test, both typical and atypical antipsychotics are quite efficient at blocking this drug-induced hyperactivity[216,217]. In our studies, the mice were allowed to explore for 60 minutes in a square field arena (46 × 46 cm^2) in separate sessions. The first control session the mice were treated with vehicle. On the second session the mice were treated with MK-801 (0.4 mg/kg.), d-amphetamine (5 mg/kg.) or PCP (8 mg/kg.) immediately before the test. In case of testing antipsychotic drug efficacy, the mice were subjected to a third session where they were treated with the antipsychotic (30 minutes before testing) in combination with the psychostimulant (immediately before testing) drug. In PAPER II, we used the same square arena to assess the psychostimulant effects of MAO inhibitors. In this case, different groups of mice were treated with saline, rasagiline (3 mg/kg), clorgiline (10 mg/kg), tranylcypromine (10 mg/kg) or a combination of rasagiline (3 mg/kg) and clorgyline (10 mg/kg) and recorded for 210 minutes in the squared arena. Video tracking was performed with an overhead video camera and analyzed by EthoVision XT11.5 (Noldus) software. Another test, which is believed to model hallucinations in mice and exploits motor behavioral aspects, is the HTR[218]. This test involves the quantification of a rhythmic sudden rotational head movement, which is elicited after the administration of psychedelic drugs[218]. Nevertheless, it is debatable if this test can reflect the hallucinogenic experience in mice, since HTR is almost entirely depending on $5HT_{2A}R$ agonism[108]. Though, there is an interesting association between HTR and glutamatergic drugs such as $mGluR_2$ agonists, which have been found to block the $5HT_{2A}R$ agonist induced HTR[219]. In our experiments, mice were administered saline or DOI (8 mg/kg) and immediately placed in an empty cage. Mouse behaviour was recorded for 10 minutes with an overhead camera. HTR count was quantified manually by counting the clear, rapid, oscillatory movements of the mouse's head.

As mentioned earlier, schizophrenia symptomatology is not restricted to psychosis, thus there are animal models approaching both negative and cognitive symptoms. Classical behavioural task to assess cognitive deficits in animals are the Y-maze, the PAT and the PPI. These tasks require intact the neuronal hubs of working memory such as PFC and hippocampus which are strongly affected in schizophrenic patients[97,220]. NMDAR antagonists affect negatively the performance in these tests and simulate the cognitive deficits observed in schizophrenic patients[217]. Y-maze spontaneous alternation test measures the novelty-induced exploration ability of a mouse.

Normally, when the rodents are placed to a Y-shaped maze with equal sized arms and 120° angle, choose to investigate a new arm than returning to the already visited one. NMDAR antagonists disrupt the normal innate alternation behavior of a mouse between the three arms[221]. In our experimental setup, mice were placed at the center of a 40 cm long, 8 cm wide and 20 cm high Y-maze and recorded with ceiling-mounted camera for 10 minutes. A correct alteration is measured when the mouse entered the 3 different arms over a triad on overlapping triplet sets (e.g. in the sequence ACBCBACBAA, four alternations were counted). Spontaneous alternation was quantified by the number alternations as percentage of the total arm entries minus 2. PAT is evaluating rodent fear conditioning by measuring the latency of the animal to enter a compartment, which is conditioned with an electric shock. Usually, the non-shock compartment is brightly lit compared to shock-conditioned part, so as to produce a reward-conflict situation for the mouse. Similar to Y-maze, PAT performance is highly is dependent on NMDARs, since dugs such as MK-801 diminish retention latency in the test[222]. Herein, we placed mice in the luminous compartment of the PAT apparatus (Ugo Basile) for 60 s. Immediately afterwards, a sliding door opened, and the mouse was allowed to enter the dark part (training session). When all the four paws of the animal stepped into the dark compartment, the sliding door automatically closed and a weak electrical shock (0.3 mA, 2 s duration, scrambled current) was given through the grid floor. After a 24-h delay, the mouse was again placed in the light compartment and the step-through latency to enter the dark part was counted automatically (test session). Finally, PPI is assessing the ability of the animal to suppress its response to a potent startle-producing stimulus, followed by a weaker prestimulus[217]. The amplitude reduction of the startle response depicts the ability of the animal to predict the warning pre-pulse and adapt its response to the succeeding powerful stimulus[217]. Similar to the aforementioned tests, PPI is significantly impaired after the administration of NMDAR antagonists[217,221]. This impairment is restored if NMDAR antagonists are combined with antipsychotic medications[217]. Herein, we used two startle chambers (San Diego Instruments) that contained a Plexiglas cylinder attached on a piezoelectric transducer and a loudspeaker that produced both a continuous background noise of 65 dB and the different acoustic stimuli. The test session included four blocks of trials, with an average interval of 12 seconds. The first and the last block consisted of five Startle (a 40-ms 120 dB burst) trials. Four trial types were introduced during the second and the third block in a pseudo-randomized sequence. The trial types included a Startle and three separate pre-pulses with intensities 68, 71 and 77 dB that preceded the startle stimulus by a 100 ms interval. The overall test session duration was 23 min and included 60 trials. Finally, schizophrenia animal modelling involves psychostimulant induced hyperactivity and working memory deficits as an attempt to approach the schizophrenic behavioural aspects.

2.1.4 Conditional knock-out strategies

Advances in the field of genetical mouse model engineering have provided new tools for the comprehensive investigation of molecules' function in certain brain regions. Conditional KO technologies provide elegant strategies for the study of brain's molecular biology in neuroanatomical detail. Classical conditional KO methods exploit a DNA recombination system, which requires the presence of Cre recombinase together with two 34 base pair sites, called loxP sites[223]. Each Cre recombinase recognizes two repeated loxP sites and excises the DNA located within the two sites (flanked-DNA, floxed) by forming a circular inactivated DNA molecule[223]. This system of recombinase is an extremely powerful tool for gene inactivation[223]. For the conditional gene deletion in certain neuron clusters in mice, requires a mouse strain with a cell-type specific promoter driven Cre and a mouse line that contains a floxed gene[223]. Breeding of those two strains will lead to the generation of conditional mutant mice that lack the gene of interest in Cre expressing brain cells. Inducible Cre-loxP system provides precise temporal control of gene deletion[223]. This inducible system requires a Cre protein fused with a mutated form of estrogen receptor ($CreER^T$)[223]. This fused protein is present in the cytosol where it is bound to the HSP90[223]. Tamoxifen administration dissociates HSP90 from $CreER^T$, which subsequently translocates to the nucleus where it interacts with the loxP DNA sites[223]. In our studies (PAPER VI) we used a newer version of $CreER^T$ ($CreER^{T2}$), which is 10-times more sensitive to tamoxifen in vivo[223]. Another method that allows temporal and cell-type specific control of gene deletion, is the AAV mediated Cre expression in mice containing a floxed gene[224]. Additionally, AAV vectors containing Cre allow the spatial control of gene deletion, which is not provided by the aforementioned methods[224]. In our studies we used generated a conditional p11 KO mouse in 5HT producing neurons by crossing a floxed p11 mouse line with a SERT-Cre mouse strain. For conditional NURR1 deletion in D_1R expressing cells we crossed a floxed NURR1 mice with mice expressing Cre under D_1R promoter. Moreover, we generated inducible conditional NURR1-KO in DA neurons by crossing a floxed NURR1 mouse line with a DAT- $CreER^{T2}$ strain. Finally, for selective deletion of NURR1 in CLA, we stereotactically injected an AAV that contained a synapsin-1 driven Cre in bilateral CLAs (coordinates: anteroposterior: +1 mm, mediolateral: ±2.8 mm, dorsoventral: −2.4 mm and anteroposterior: 0 mm, mediolateral: ±3.5 mm, dorsoventral: −2.6 mm).

2.2 HISTOLOGY TECHNIQUES

2.2.1 Radiolabelled ligand autoradiography

Radiolabelled ligand autoradiography is a simple method to determine the tissue distribution of the ligand binding after the direct application of the ligand on tissue sections[225]. The tissue

sections used for this method were fresh frozen thaw-mounted brain sections (12 μm thick). Briefly, the procedure is initiated with a preincubation step by incubating the sections to the solvent of the radiolabelled ligand. Afterwards, the radiolabelled ligand solution is applied. At the same time, some sections are incubated with a combination of the radiolabelled ligand together with a high concentration of another non-labelled ligand so as to determine the non-specific binding. When the procedure is completed, the slides are exposed by using autoradiographic films for several days depending on the isotope.

2.2.2 Immunofluorescence

Immunofluorescence aims the visualization of a protein target on tissue by using a set of specific antibodies. The tissue sections used for this method were either fresh frozen thaw-mounted (12 μm thick) or perfusion-fixed frozen free-floating (30-40 μm thick) brain sections. This assay is divided in three major steps: blocking step, primary antibody step and secondary antibody step. The blocking step requires a solution containing serum proteins and it is crucial for eliminating any non-specific binding of the antibodies to the tissue. The primary antibody step includes the incubation of the brain sections with antibodies that are specific against the protein of interest. The secondary antibody step includes the incubation of the brain sections with fluorescently tagged antibodies that are specific against the Fc fragment of the primary antibodies. This last step allows the direct visualization of the protein target by using fluorescent or confocal microscope. When the procedure is completed, the slides were imaged on a Carl Zeiss LSM 880 confocal microscope by using a 20 × 0.8 numerical aperture objective.

2.2.3 Radioactive and Fluorescent (RNAscope) in situ hybridization

ISH is a molecular technique that uses a radio-labelled complementary RNA riboprobe to detect a specific mRNA sequence in a tissue section (in situ)[226]. The tissue sections used for this method were fresh frozen thaw-mounted brain sections (12 μm thick). The riboprobes (250-1000 bases) are labelled with radioactivity during an in vitro transcription from cDNA clones that correspond to the complementary fragments of the desired mRNA. The in vitro transcription is performed with the presence of RNA polymerase (SP6, T3 or T7), ATP, GTP, CTP and radiolabelled UTP-S^{35}, which subsequently radiolabels the entire riboprobe. The procedure is divided in three major steps: the pre-hybridization, hybridization and post-hybridization. The importance of these divisions is majorly arising from the usage of RNAse. It is crucial to avoid contamination with RNAse in the pre-hybridization because there is a risk of riboprobe digestion. As a result, pre-hybridization procedure requires washes with concentrated solution of sodium citrate, which is chelating magnesium ions that are crucial for RNAse function. Moreover, all the

pre-hybridization solutions were prepared by using water containing diethylpyrocarbonate, which inactivates RNAse. Hybridization is the step where the riboprobe hybridizes with the targeted mRNA on the tissue. The post-hybridization step requires both RNAse and high temperature washes in order to eliminate any non-specifically hybridized riboprobe. For this reason, the washing solutions contain graded concentrations of sodium citrate. When the procedure complete, the slides are exposed by using autoradiographic films for 4 to 28 days.

Classical fluorescent ISH differs to radioactive ISH on the probe labelling process. In vitro transcription step for fluorescent probes requires the presence of fluorescently labelled nucleotides[226]. An alternative could be digoxigenin tagged nucleotides which can be subsequently targeted with fluorescently labelled anti-DIG antibodies. Nevertheless, in the present studies, we used a commercially available novel mRNA FISH technology, called RNAscope[227]. This assay consists of target oligonucleotide "Z"-shaped probes and a signal amplification system with preamplifier, amplifier and a fluorescently labelled probe[227]. The "Z"-shaped probes contain 18-25 bases of that are complementary to the mRNA target, a spacer sequence and a 14 bases tail sequence[227]. The unique feature of the technology is that oligonucleotide probes are hybridized in pairs ("ZZ", ~50 bases) to multiple complementary targets of the mRNA molecule[227]. Since each commercially available probe mix usually contains 20 "ZZ" hybridizing pairs, RNAscope technology allows high yield labelling of the entire mRNA molecule[227]. Moreover, due to the fact that pair hybridization is highly unlikely to happen in non-specific regions, the technology offers greater specificity and signal to noise ratio compared to classical FISH[227]. The paired tail-sequence provide a 28-base template for the preamplifier to bind and orchestrate the amplification process[227]. The tissue sections used for this method were fresh frozen thaw-mounted brain sections (12 μm thick). When the procedure is completed, the slides were imaged on a Carl Zeiss LSM 880 confocal microscope by using a 63 × 1.4 numerical aperture oil immersion objective.

2.3 STATISTICAL ANALYSIS

Statistical analysis was carried out by using GraphPad 7, GraphPad 8 or Statistica program version 13.4.0.14. Statistical significance ($p < 0.05$) of the results was assessed by using Student's t-test or Mann-Whitney U test. For comparing the means of multiple groups, we used one-way, two-way or three-way ANOVA depending on the number of independent factors.

3 RESULTS AND DISCUSSION

3.1 PAPER I

As mentioned earlier in the thesis, GPR88 is an orphan Gαi-coupled receptor, which displays predominant expression in striatum.[125] Since, striatal D_1R and D_2R signalling is the main source of parkinsonian symptoms, we desired to investigate the role of GPR88 in PD related states. In detail, we aimed to determine GPR88's impact on L-DOPA's therapeutic efficiency and side effects, but also on PD-like tremor. To address these questions, we employed mice that lack GPR88 (GPR88-KO mice). Chronic L-DOPA administration to GPR88-KO mice, subjected to unilateral 6-OHDA lesions of the MFB, resulted in more rotations than in their WT counterparts (Figure 10). Interestingly, GPR88-KO mice had a significant lower dyskinesia severity score in axial, limb and orofacial AIMs. These behavioral responses were accompanied by changes in L-DOPA-induced transcriptional response. In detail, we observed that the lesioned striata of GPR88-KO mice display lower PDYN and GAD67 mRNA upregulation, but higher induction of ARC transcripts, when compared to WT counterparts (Figure 10). In accordance with a role for 5HT terminals in LID development, WT but not GPR88-KO striata exhibited 5-hydroxytryptamine displacement upon subchronic L-DOPA administration. Furthermore, we evaluated the GPR88's implication in drug induced PD-like tremor. Unlesioned GPR88-KO mice showed reduced tacrine-induced TJMs. Due to the fact that GPR88 is heavily involved in AMPAR membrane availability and MSN excitability[125], we hypothesized that our observations in GPR88-KO mice, could be explained an altered striatal glutamate content. As a result, we performed biosensor recordings, which revealed that GPR88-KO mice display a significantly higher spontaneous and evoked striatal glutamate release compared to WT mice (Figure 10). Furthermore, local application of glutamate in WT and GPR88-KO mice, showed that the observed glutamate release changes do not stem from deficits in glutamate turnover. Conclusively, genetic deletion of GPR88 enhances L-DOPA-induced locomotor induction but diminishes LIDs and PD-like tremor. These data provide evidence that future development of GPR88 antagonists may assist L-DOPA therapeutic effects on PD motor symptoms without worsening the induction of motor side effects

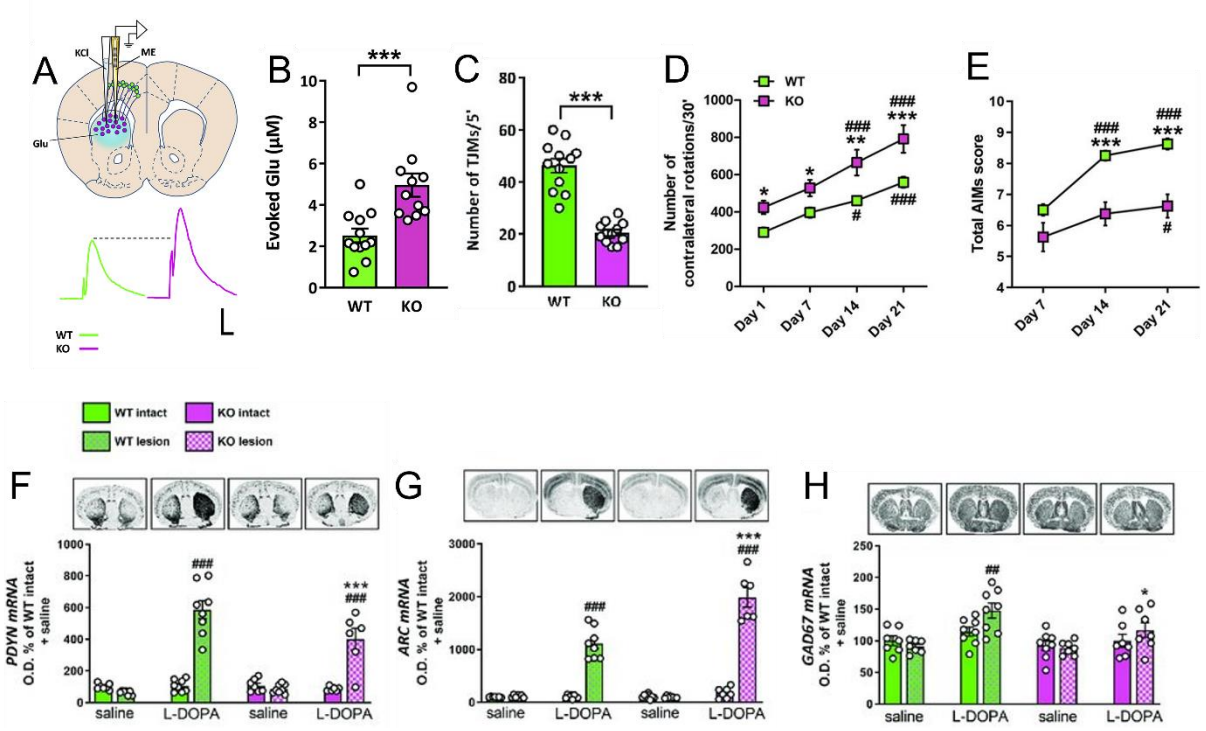

Figure 10. GPR88 deletion effects in PD animal models. (A) Schematic depiction of striatal glutamate recordings and evoked glutamate release traces (vertical scale bar: 1 μM, horizontal scale bar: 2.5 s). **(B)** Bar graph showing the striatal evoked glutamate release concentration in WT and GPR88-KO mice (***p < 0.001, unpaired t-test). **(C)** Bar graph showing the TJM count after the administration of tacrine in WT and GPR88-KO mice (***p < 0.001, unpaired t-test). **(D, E)** Line graphs showing the contralateral rotation count **(D)** and total AIM score **(E)** in hemiparkinsonian WT and GPR88-KO mice over the course of L-DOPA treatment (#p < 0.05, ###p < 0.001, Day 1 vs Day 21; *p < 0.05, **p < 0.01, ***p < 0.001, WT vs GPR88-KO; Fischer's LSD post-hoc test). **(F-H)** Radioactive ISH pictures and bar graphs showing the L-DOPA-induced upregulation of PDYN **(F)**, ARC **(G)** and GAD67 **(H)** in hemiparkinsonian WT and GPR88-KO mice (##p < 0.01, ###p < 0.001, saline vs L-DOPA; *p < 0.05, ***p < 0.001, WT vs GPR88-KO; Fischer's LSD post-test). Data are expressed as mean±SEM (Adapted from Mantas et al.[228]).

3.2 PAPER II

Endogenous TAs, such as tyramine and phenethylamine, display DA releasing properties while are degraded by MAO enzymes[138]. At the same time, tyramine decrease DA neuron firing rate by activating TAAR1[141]. These facts led us to the hypothesis that TAAR1 may act as TA sensor and mediates a negative feedback loop that limits TAs' amphetamine-like effects in MAO inhibition states. Accordingly, we questioned if TAAR1 is able to hinder the effects of MAOIs. For this reason, we examined the behavioral and histological response to the non-selective irreversible MAOA/BI, TCP in mice that lack the expression of TAAR1. The TAAR1-KO mouse line that we employed is generated by replacing of TAAR1 genes's single exon (exon 1) with a β-galactosidase gene. In line with our hypothesis, TAAR1-KO show decreased immobility in FST and increased locomotion in response to TCP compared to their WT litermates. These results indicate that TAAR1 may impede MAOIs' actions, which were enhanced in TAAR1-KO mice. To examine if TAAR1 has a direct impact on monoamines' concentration after MAO inhibition, we used a recently developed fluoromethylpyridinium-based MALDI-MSI method. We used WT

and TAAR1-KO mice subchronically treated with TCP and unilateral partial striatal 6-OHDA lesion (Figure 11). The purpose of the 6-OHDA lesions was to identify possible MAO inhibition related alterations that stem from DAergic fibers. This experiment revealed a robust accumulation of monoamines, in TCP treated mice (Figure 11). However, tyramine's accumulation was significantly higher in TCP treated TAAR1-KO versus WT counterparts, suggesting a negative feedback mechanism for TAAR1 in sensing TAs' levels (Figure 11). The accumulation of tyramine was affeced by 6-OHDA lesion, implying that striatal tyramine is derived by DA producing terminals. Accordingly, we showed that TAAR1-KO mice displayed a significantly heigher ARC and PDYN mRNA response to TCP compared to the WT mice. TAAR1's direct effect on tyramine concentration might be explained by the receptor's strong impact on DA neuron firing rate. Though, TAAR1 expression is not restricted to VTA/SNc boundaries and extends to several brain regions that may affect DA neuron excitability. Since the employed TAAR1-KO mouse line is expressing β-galactosidase under TAAR1 promoter, we proceeded to histoensymological detection of the enzyme. Therefore, we unveiled some previously unknown TAAR1-expressing brain sites (medial bed nucleus of stria terminalis, lateral hypothalamic area, zona incerta and dorsolateral parabrachial nucleus) which are known to affect DA neuron activity. Similar to PAPER I, we used an enzyme-based biosensor technology and we showed that both TCP and tyramine reduced glutamate release in the substantia nigra in WT but not in TAAR1-KO mice. We further expanded these studies in freely moving animals treated with TCP, where we demonstrated that TAAR1 prevents glutamate accumulation in the substantia nigra during hyperlocomotive states. Conclusively, these observations suggest that TAAR1 may act as a TA sensor, which limits tyramine accumulation and subsequent behavioral and transcriptional effects in MAO inhibition states. These findings provide insights for the elucidation of TAAR1's function in the brain.

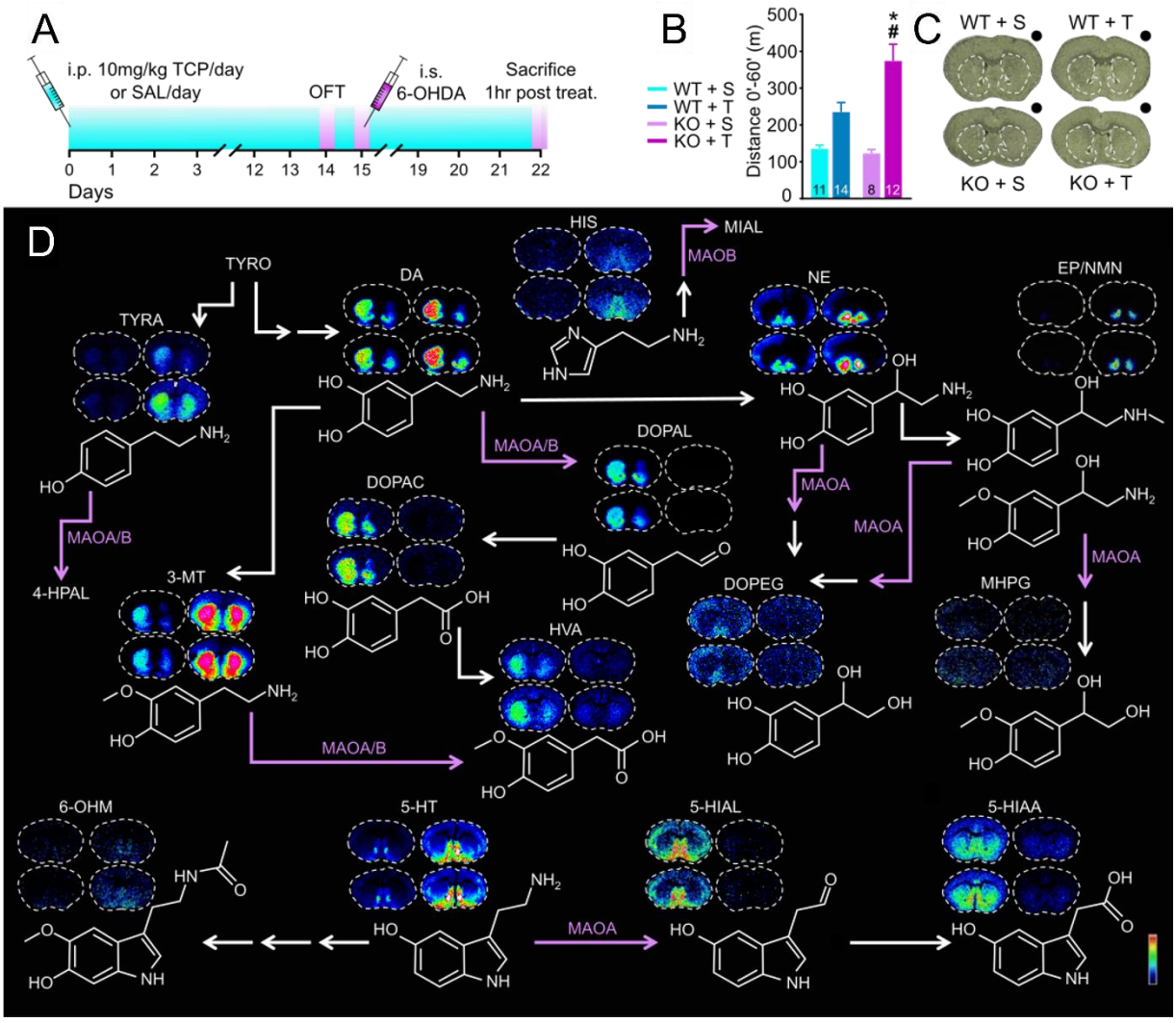

Figure 11. Genetic deletion of TAAR1 enhances the MAOI-induced accumulation of tyramine. (A) Schematic depiction of subchronic TCP administration and intrastriatal 6-OHDA lesions. **(B)** Bar graph showing the locomotor response of WT and TAAR1-KO mice after 14 days of TCP daily treatment (#p < 0.05, S vs T; *p < 0.05, WT vs KO; Tukey's HSD post-hoc test). **(C)** Representative pictures showing the striatal area used for quantification. The 6-OHDA lesioned side is located on the right-hand side of each image (black circle). **(D)** Representative images for each metabolite among the four different groups of animals (left upper: WT+S group; right upper: WT+T group; left lower: TAAR1-KO+S; right lower: TAAR1-KO+T). Each metabolite picture is accompanied by its chemical structure. The arrows denote the metabolic pathways, while magenta arrows indicate a MAO-dependent reaction. Data are expressed as mean±SEM (Adapted from Mantas et al.[229]).

3.3 PAPER III

As mentioned earlier, TAAR1 agonists improve the performance in animal behavioral paradigms of depression-like traits[150,230]. Nevertheless, the exact functional roles of TAAR1 to the effects of clinically used antidepressants remains elusive. Therefore, we desired to address these issues by employing the artificial selective TAAR1 agonist, o-PIT, which is structurally inspired by the endogenous iodothyronamines[231]. Together with o-PIT, we employed that TAAR1-KO mouse line that was used in PAPER II. Regardless the genotype, systemic i.p. administration of o-PIT raised both plasma and brain levels of the compound. Furthermore, we carried out a detailed characterization of brain regions that β-galactosidase in TAAR1-KO mice. Since we observed a

high density of X-gal deposits in the thermoregulatory region, MPOA, we hypothesized that TAAR1 agonists might be capable of regulate body temperature. Thus, we tested the ability of o-PIT to alter core temperature in WT and TAAR1-KO mice. Interestingly, o-PIT induced a hypothermic response which was significantly lower in TAAR1-KO mice. This phenomenon indicates that o-PIT exhibits in vivo effects which are partially dependent on TAAR1. In addition, we showed that o-PIT increased the PPI response of WT but not TAAR1-KO mice. We demonstrated that application of o-PIT (10 mM) on striatal slices, induced a significant increase of striatal TH phosphorylation at serine 31 in WT but not in TAAR1-KO mice. Accordingly, we observed a significant rise of extracellular DA in PFC of WT mice that was absent in TAAR1-KO mice. Similar to other TAAR1 agonists, o-PIT showed antidepressant and anxiolytic-like effects in WT mice. In particular, o-PIT significantly reduced immobity time in FST and decreased the number of buried marbles in MBT. Nevertheless, these actions were significantly reduced in TAAR1-KO mice. Even though it was substantially decreased, o-PIT's ability to diminish the number of buried marbles was maintained in TAAR1-KO mice. Similar to core temperature results, this effect might indicate an alternative o-PIT receptor target. Further MBT experiments with the combination of several monoamine receptor antagonists failed to identify another o-PIT receptor apart from TAAR1. In contrast to o-PIT's effects, the impact on behaviour and PFC dialysis of a broad suite of clinically-employed antidepressants were unaffected in TAAR1-KO mice. In PAPER II, we showed that TAAR1-KO are more sensitive to the effects of non-selective MAOIs. Herein, we showed that selective MAOBIs show similar effects in both genotypes, indicating the importance of combined MAOA and MAOB inhibition to achieve stronger antidepressant effects in TAAR1-KO mice. In conclusion, we reported that o-PIT is a useful tool for the in vivo investigation of TAAR1-dependent aspects in the brain. Moreover, we showed that TAAR1 does not contribute to the behavioral effects of commonly used antidepressants.

3.4 PAPER IV

A recently performed randomized, controlled 4-week trial showed that the SEP-856 administration caused a greater reduction from baseline in the PANSS total score than placebo[152]. If the ongoing clincal trials allow SEP-856 be an FDA approved antipsychotic medication, it is going to be an historic moment for the drug discovery field of schizophrenia. SEP-856 importance arises from the fact that does not display any significant activity on D_2R[230]. Instead, cAMP detection assays have shown that SEP-856 acts as a TAAR1 agonist and $5HT_{1A}R$ partial agonist[230]. However, independent confirmation of these findings and detailed characterization of SEP-856 in vitro and by using TAAR1 or $5HT_{1A}R$-KO mice, are absent. Previous efforts that have been made to evaluate TAAR1's pharmacology in vitro, reported the difficulty of expressing the receptor in

model cell systems[232]. Herein, we highlight the generation of a functional TAAR1 signaling assay by using a mammalian codon optimized human TAAR1 construct expressed in Expi293F cells. We showed that SEP-856, similar to β-PEA, stimulates significantly TAAR1-$G\alpha_s$ recruitment but not $G\alpha_q$, $G\alpha_i$ or $G\alpha_{12}$. We detected TAAR1 in the plama membrane by using flow cytometry, underlying the possibility of TAAR1 stimulation on that site. We showed that SEP-856 is a partial agonist on $5HT_{1A}R$ capable of recruiting mainly $G\alpha_i$, $G\alpha_q$, $G\alpha s$ and arrestin-β2. The SEP-856's $5HT_{1A}R$ partial agonist profile was retained at the level of $G\beta\gamma$-induced GIRK activation in oocyte patch clamp electrophysiology experiments. Furthermore, we show that SEP-856 does not display any pharmacologically significant D_2R partial agonist or antagonist properties, regarding $G\alpha_i$ recruitment or $G\beta\gamma$ signaling like other atypical antipsychotics that act as D_2R partial agonists. By using the TAAR1-KO mouse line from PAPER II and III, we showed that SEP-856 (10 mg/kg) caused a significantly greater decrease in core body temperature of WT than TAAR1-KO mice. Furthermore, SEP-856 (10 mg/kg) significantly increased the PPI response (+12 dB from the background noise) of WT but not of TAAR1-KO mice. Conversely, SEP-856 produced a significant suppression in MK-801-induced hyperactivity of both WT and TAAR1-KO mice, indicating a possible involvement of $5HT_{1A}R$. Our findings give important insights on SEP-856's pharmacology, which might be useful for future development of efficient non-D_2R antipsychotics.

3.5 PAPER V

As mentioned earlier in the thesis, MDD's pathophysiology involve the hyperactivity of HPA and SAM axes. At the same time, there is evidence that lower levels of p11 are associated in depression-like behavior[155]. Even though, there are studies investigating the role of p11 in depressive-like behavioral paradigms, there is no research on p11's function in stress hyperresponsiveness. In PAPER V, we desired to address this issue by employing p11-deficient (p11-KO) mice. We characterized the stress response of these mice in both early postnatal and adult life. Our study showed that p11-KO pups produce more maternal separation-induced ultrasonic vocalizations than WT pups, even though their mothers display similar maternal behavior. Accordingly, adult p11-KO mice respond stronger to painful stimuli and exhibit anxiety-like behavior, which is concomitant with stress axis hyperactivity. Thereafter, we desired to characterize the p11's role in the major hubs of HPA and SAM axes. P11 is highly expressed in hypothalamic PVN, with preference to AVP producing cells (Figure 12). P11-KO mice displayed elevated plasma ACTH, which was more pronounced in female mice (Figure 12). The observed changes in ACTH levels were correlated with the ARC mRNA levels in PVN of p11-KO mice (Figure 12). Nevertheless, RNAscope experiments in female p11-KO mice, showed that ARC mRNA was preferentially expressed in AVP negative neurons in the PVN. The high ACTH levels

observed in p11-KO mice were unaffected by CRH_1R antagonist but suppressed after $V_{1B}R$ antagonist, supporting the importance of p11 in AVP expressing cells. Moreover, we showed that the mRNA of p11's binding partner, $5HT_{1B}R$ was present in p11 expressing PVN neurons, denoting a possible suppressing role of the $5HT_{1B}R$-p11 complex on the activity of AVP cells. Despite that, 5HT neurons display one of the highest densities of p11 in the brain, we showed that they are not important for mediating the HPA hyperactivity of p11-KO mice. Going one-step further, we desired to evaluate the p11-KO mice reactivity of SAM. It is known that p11 is highly expressed in cholinergic cell groups in the brain[233]. In accordance, we showed p11 expression in IML cholinergic neurons in spinal cord. Additionally, p11-KO mice display SAM axis hyperactivity, with heightened adrenal NA and AD levels. In contrast to ACTH levels, the high adrenal NA and epinephrine content is not restored after the administration of CRH_1R or $V_{1B}R$ antagonists. This indicates a possible primary role of p11 in IML neurons to mediate the observed heightened SAM response. By using conditional p11-KO mice in 5HT producing neurons, we demonstrated that this SAM hyperactivity is partially affected by the loss of p11 in Raphé nuclei. SAM axis activity is crucial for regulating heart rate in response to stress. Telemetric electrocardiogram measurements revealed that p11-KO mice exhibited both basal heart rate and delayed heart rate recovery during the extinction period of an auditory trace fear conditioning task. Concisely, we provide evidence that loss of p11 leads to HPA and SAM axes hyperresponsiveness, which may assist to understand the pathophysiology mechanism of MDD.

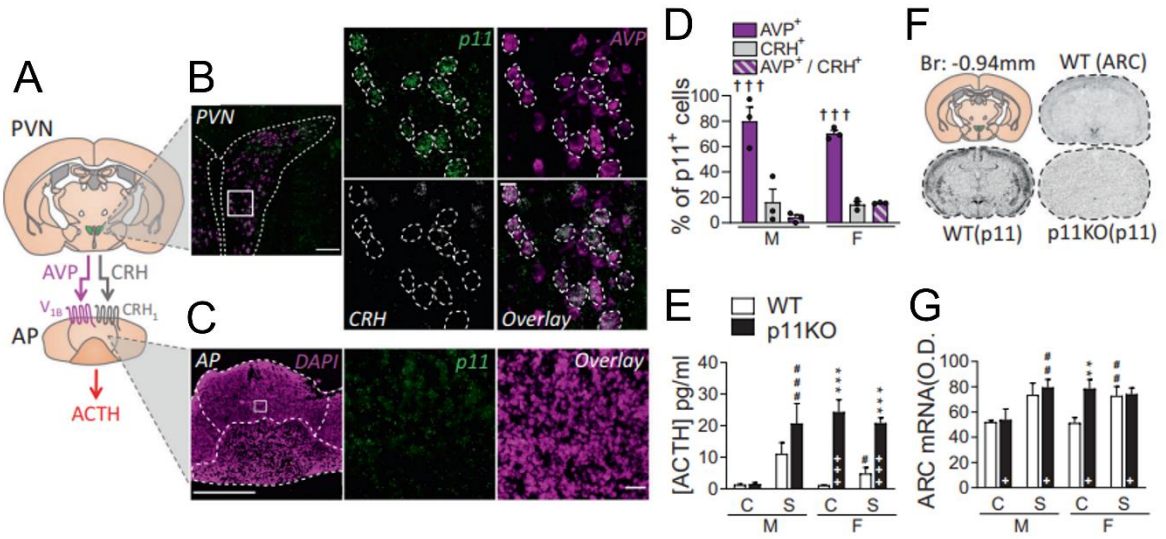

Figure 12. P11 effects in HPA axis. (A) Schematic depiction of hypothalamic pituitary interaction together with CRH and AVP systems. **(B)** Triple RNAscope showing p11, AVP and CRH positive cells in PVN (scale bars: 100 µm on the left, 30 µm on the right). **(C)** RNAscope showing low p11 levels in anterior pituitary gland (scale bars: 500 µm on the left, 30 µm on the right). **(D)** Bar graph showing the percentage overlap of p11+ cells with CRH+ and AVP+ cells (†††$p < 0.001$). **(E)** Bar graph showing the ACTH plasma levels of control and stressed WT and p11-KO mice (#$p < 0.05$, ###$p < 0.001$, C vs S; ***$p < 0.001$, WT vs p11-KO; Fischer's LSD post-test). **(F)** Radioactive ISH showing ARC and p11 mRNA at the level of PVN. **(G)** Bar graph showing the ARC mRNA optic density in control and stressed WT and p11-KO mice (##$p < 0.01$, ###$p < 0.001$, C vs S; **$p < 0.01$, WT vs p11-KO; Fischer's LSD post-hoc test). Data are expressed as mean±SEM (Adapted from Sousa et al.[234])

3.6 PAPER VI

NURR1 is mostly well-known as a transcription factor that is necessary for development of midbrain DA neurons[179]. However, it has been described that NURR1's transcription can be induced by several factors that increase synaptic activity. In PAPER V, we made an effort to describe the role of striatal NURR1 after the subchronic treatment of L-DOPA in hemiparkinsonian animals. Firstly, we showed that the induction of NURR1 after repeated L-DOPA treatment is maintained in both rodents and non-human primates. Moreover, we show that L-DOPA induced NURR1 is not restricted in the DA depleted striatum but extends to cortical layer 6. Both striatal and cortical L-DOPA induced NURR1 mRNA is located mainly in D_1R expressing neurons. Furthermore, we confirmed that D_1R stimulation is crucial for NURR1 upregulation by using HEK-293 overxpressing cell lines. L-DOPA induced NURR1 upregulation was not affected by the deletion of GPR88 but it was completely abolished by blocking false DA release through a $5HT_{1A/B}R$ agonist. Due to this fact, we proceeded to conditional gene-targeted strategies by generating mice with selective deletion of NURR1 in D_1R expressing neurons (cNURR1-D_1RCre). These cNURR1-D_1RCre mice developed significantly less contralateral rotation and locomotor sensitization in response to subchronic L-DOPA. Nevertheless, we did not observe any significant changes in AIMs score in these mice apart from orofacial AIMs score which was significantly higher in cNURR1-D_1RCre mice. The observed behavioural alterations

were accompanied by changes, respectively, in production of specific striatal neuropeptides. In detail, cNURR1-D_1RCre mice showed significantly higher PDYN mRNA levels and lower PENK derived peptide content in the lesioned striata after L-DOPA administration. Moreover, we unveiled that L-DOPA induced NURR1 does not promote striatal expression of TH[178]. Broadly, we showed that both control and cNURR1-D_1RCre display similar extent of striatal TH mRNA upregulation after L-DOPA treatment. Interestingly, L-DOPA increases TH expression in a certain population of dMSNs that does not overlap with the NURR1 expressing PDYN+ cells (Figure 14). Mass spectrometry imaging showed that L-DOPA affects the levels of striatal α-glycerophosphocholine and adenosine diphosphate ribose. Though, these changes were not observed in cNURR1-D_1RCre mice. In conclusion, L-DOPA induced NURR1 plays a crucial role in the control of locomotor sensitization and metabolic response of DAceptive D_1R-expressing neurons.

3.7 PAPER VII

NURR1 has been described as one of the molecular markers that define the CLA as a distinct region in cortical plate[193]. Even though, its pronounced CLA expression has been described in the past[193], NURR1 function in this elusive brain region is still unknown. In PAPER VII, we attempted to investigate the role of NURR1 in CLA and thus, hallucinogenic states. First, we reported that NURR1 is enriched in the CLA of mice, rats, marmosets and humans, denoting the importance of the transcription factor for CLA's identity throughout mammalian species. RNAscope and immunofluorescent studies showed that NURR1 is restricted to CLA projection neurons rather than the GABAergic interneurons. By using heterozygous NURR1-KO mice, we showed that in contrast to VTA/SNc and HF, NURR1 mRNA in CLA is highly depending on NURR1 gene dosage. Homozygous NURR1-KO mice do not survive more than a week after birth and do not allow the investigation of NURR1's role in adult CLA. Considering that D_1R is expressed in the CLA NURR1 cells (~80%) but not in DA neurons, we generated a cNURR1-D_1RCre, which showed diminished expression of NURR1 in CLA. Due to the fact that NURR1 in SNc/VTA neurons is crucial for DA terminal integrity, we questioned if NURR1 in CLA display similar functions. Stereotactic Fg injections in the mPFC of cNURR1 and cNURR1-D_1RCre, showed retrograde labelling in the CLAs regardless the genotype. This indicates that claustrocortical fibers integrity is intact after deletion of NURR1 in CLA. RNAseq data from both insula-striatal region and Fg+ sorted nuclei showed downregulation of several CLA enriched markers in cNURR1-D_1RCre mice. Radioactive ISH experiments revealed that both CLA specific markers and hallucinogen receptors are diminished in cNURR1-D_1RCre mice. Since CLA has been proposed to play a role in hallucinations[191], we examined the performance of cNURR1-D_1RCre mice in

psychosis-related tasks. Nevertheless, cNURR1-D_1RCre mice did not show any impairment in Y-maze and PPI tests. D_1R receptors is expressed quite early on in CLA development, denoting that our observations on cNURR1-D_1RCre may arise from developmental factors. AAV-Cre injections in the CLAs of cNURR1 mice showed diminished NURR1 in CLA, which was accompanied by a concomitant downregulation of both CLA specific markers and hallucinogen receptors. These facts highlight the central role of NURR1 in maintaining the CLA cell transcriptional identity. Furthermore, we examined if the hallucinogen receptor effects are abolished after NURR1 deletion. CNURR1-D1RCre exhibited less C-fos and ARC induction in CLA after acute DOI treatment. Accordingly, CLAs of cNURR1-D_1RCre mice displayed significantly reduced 5HT or U69 induced fEPSP suppression. Nonetheless, do not give information about the hallucinogen-induced states of the brain. FMRI in humans has shown that hallucinogens cause decreased CBF in mPFC[235]. At the same time, there is no test that evaluates both $5HT_{2A}$R and κOR induced hallucinogenic states in mice. Considering these facts, we employed fUS, which is a highly sensitive method in detecting changes in CBF combined with $5HT_{2A}$R and κOR agonists. In WT mice, DOI and U69 induced a decrease in mPFC CBV signal, which was significantly reversed with the pretreatment of ketanserin and nBNI respectively. Even though cNURR1-D_1RCre mice exhibited diminished expression of $5HT_{2A}$R and κOR mRNA in their CLAs, they displayed a similar DOI and U69 induced decrease in mPFC CBV signal. Overall, we show that NURR1 deletion in CLA affects the transcriptional identity of the region but does not interfere with brain's response to $5HT_{2A}$R or κOR agonists.

4 CONCLUSIONS AND PERSPECTIVES

Moving from animal models of CNS diseases to explanations of human brain and behavior is extremely complicated. Below I highlight the main conclusions of our studies and discuss future research avenues that might strengthen the translational value of the molecules presented in the current thesis.

4.1 GPR88 AS A NOVEL TARGET FOR TREATING PD.

In our first study, we unveiled that GPR88 blockade exhibits key role on widening L-DOPA's therapeutic window. This conclusion sheds light on the development novel GPR88 targeting pharmaceutical agents that will assist current PD treatment. Though, it is necessary to consider the limitations of the fact that our studies involved GPR88-deficient mice rather than pharmacological antagonism of the receptor. Since GPR88 is an early marker of developing striatum, it is possible that GPR88 deletion may affect striatal neuron development[128]. Hence, the behavioral and neurochemical alterations that we observed may arise from developmental compensations and not from the ablated GPR88 signaling in adult brain. For this reason, there is a necessity for future studies that would evaluate our observations on GPR88-KO mice by employing inducible GPR88-KO. Interestingly, there is a recent study that confirmed our findings by using AAV-mediated deletion of GPR88 in rats[236], strengthening the non-developmental aspect of our results. For increasing the translational value of our data, it is important for future studies to replicate our data by employing specific GPR88 antagonists. Even though researchers have made efforts to develop selective GPR88 agonists (2-PCCA and RTI-13951-33)[237], there are no synthetic GPR88 antagonists that could be used in such experiments, so far. Another aspect that is necessary to be investigated is the identification of endogenous GPR88 ligands. This information would give the opportunity to indirectly deactivating GPR88 signaling by pharmacological targeting of the compounds that stimulate the receptor. Concisely, it is essential for our results to be reproduced by using pharmaceutical agents that impede GPR88 signaling.

4.2 TAAR1 AND TYRAMINE AS CONTRIBUTING FACTORS TO MAOIS' EFFECTS

In our studies regarding TAAR1, we showed that the receptor sets a threshold to the effects of non-selective MAOIs. It is well-established that the life-threatening side effects of non-selective MAOIs, derive from the peripheral elevation of tyramine[81]. However, there is little research on the effects of MAOI-induced tyramine in the brain. Herein, we showed that tyramine might contribute to the MAOIs' effects on the brain. Moreover, we provide evidence that TAAR1

prevents tyramine accumulation and thus limits MAOIs' effects. Our data imply that prescribing MAOIs' in combination with TAAR1 antagonists, strengthens MAOIs' action in the brain. Nevertheless, like GPR88 study, it is essential that future studies must investigate the developmental aspect of our results with both inducible TAAR1-KO and pharmacological studies. Even though, there have been developed plenty of TAAR1-specific agonists[238], EPPTB is the only synthetic TAAR1-selective antagonist that exists[143]. Nevertheless, EPPTB has questionable BBB permeability as there is no in vivo study confirming its TAAR1 specific properties. Thus, future development of brain penetrant TAAR1 antagonists it is essential for both preclinical and clinical research on the field. Unlike to non-selective MAOIs, we showed that TAAR1 does not contribute to the effects of drugs that target monoamine transporters, underlying the importance of tyramine accumulation. Overall, our results highlight that future development of TAAR1 antagonists would modify the efficacy of currently available pharmaceutical regimes.

4.3 O-PIT AS A NEW TOOL TO STUDY TAAR1 ACTIONS IN VIVO.

In PAPER III, we showed that the synthetic thyronamine derivative, o-PIT is a useful tool to study TAAR1 effects in vivo. Most of TA-derived TAAR1 agonists, such as amphetamine, methamphetamine and MDMA display monoamine-releasing properties[138]. This phenomenon restricts their practicality in studying TAAR1 specific aspects in behavioral tests. Thyronamines do not display amphetamine-like properties and could be used for TAAR1 in vivo research. However, drugs such as T_1AM exhibit low specificity for TAAR1, while the act on several other ion channels and receptors[138]. Hence, o-PIT is a thyronamine synthetic derivative that displays high specificity for TAAR1[231]. By using TAAR1-KO mice we validated that o-PIT is a brain penetrant compound that exhibits TAAR1 specific antipsychotic and antidepressant-like effects. The novel antipsychotic SEP-856, which is gained a lot of attention due to its TAAR1 properties, currently being tested in clinical trials[152,239]. Despite that, SEP-856 is not a solely acting on TAAR1 as it shows $5HT_{1A}R$ partial agonism[230]. Unlike SEP-856, we showed that o-PIT maintains its TAAR1 agonist properties without acting on $5HT_{1A}R$. Hence, future research could focus on developing clinically effective TAAR1 selective agonists, by using o-PIT as a scaffold.

4.4 TAAR1 IS PARTIALLY RESPONSIBLE FOR SEP-856'S ANTIPSYCHOTIC ACTION.

In PAPER IV, we showed that in vivo antipsychotic action of SEP-856 is not fully dependent on TAAR1. SEP-856's FDA approval is going to be a historic moment for psychiatry, as it will be the first antipsychotic medication that does not act on D_2R. Even though it gained a lot of attention about its TAAR1 properties, the original SEP-856 discovery paper does not show if TAAR1 is necessary for the antipsychotic actions of the drug[230]. Our data support that SEP-856

requires both TAAR1 and $5HT_{1A}R$ activity to exert its in vivo antipsychotic actions. Furthermore, we provide a detailed pharmacological description of SEP-856's Gα-protein recruitment properties. The knowledge regarding the in vivo receptor-specific actions and G-protein profile of SEP-856, may shed light for the development of additional non-D_2R antipsychotic with better clinical efficacy.

4.5 P11 AS A RISK FACTOR FOR STRESS HYPER-RESPONSIVENESS

Our study on p11, showed that genetic deletion of this protein can lead to an overall hyperactivity of the stress axes. Interestingly, we showed that this hyperactivity is not driven by loss of p11 in a single brain structure, denoting a direct connection of the molecule with stress reactivity. Considering the fact that p11 is an intracellular adaptor protein, the pharmacological value of our data is relatively low. Even though, there is a synthetic A2tI that blocks p11's interaction with AnxA2, the research field on p11 targeting drugs is still in primitive stages[240]. Additionally, our study provides further evidence that genetic factors can predispose for autonomic hyper-responsiveness to stress. Taking into account that decreased p11 expression is associated with depressive-like behavior[155], our data support the chronic stress hypothesis of MDD. Accordingly, our study suggests that chronic stress and MDD have a common molecular substrate, which could be pharmacologically targeted to tackle both anxiety and depressive mood.

4.6 NURR1 IS CRUCIAL FOR LOCOMOTOR SENSITIZATION TO L-DOPA

In PAPER VI, we showed that NURR1 actions in PD extend beyond DA neuron level to striatum by controlling L-DOPA induced locomotor sensitization. NURR1 has been notoriously difficult to target pharmacologically due to the lack of a canonical ligand-binding domain[241]. Though, numerous researchers have attempted to find a suitable NURR1 agonist for preventing DA neuron degeneration in PD[242]. Nonetheless, there is a lack of evidence that these agents display NURR1-specific effects in vivo. Another pharmacological way of targeting NURR1, is the indirect activation of the molecule through stimulating its heterodimerization with RXR[175]. This alternative approach of targeting NURR1 has been tested in heterozygous NURR1-KO mice, which displayed diminished effects of RXR agonist[243]. Our study implies that drugs that stimulate NURR1 action, facilitate L-DOPA therapeutic effects. Moreover, we show evidence that NURR1 function is not restricted to the regulation of DA-related genes but extends to L-DOPA induced synaptic plasticity. These aspects highlight the overall beneficial effects of future NURR1 stimulating drugs in PD symptom alleviation.

4.7 NURR1 CONTROLS CLA CELL TRANSCRIPTIONAL IDENTITY

In PAPER VII, we described that NURR1 is crucial for the expression of CLA enriched markers including hallucinogen receptors. Even though, NURR1 deletion disrupted the effects of hallucinogen receptors in CLA, we did not observe any alterations regarding the neural correlates of hallucinogenic state. As mentioned earlier, CLA has gained a lot of attention due to its broad cortical connections[121]. Thus, it is proposed that CLA that is the responsible structure for the generation of delusions and hallucinations[191]. Nevertheless, the hallucinogen effects in CLA are largely unexplored. Our findings provide insights in hallucinogen research proposing that CLA is not crucial for the neural correlates of this altered state of concioussness. Moreover, 5HT and opioid based hallucinogens exhibit other beneficial actions. For instance psychedelics show profound fast antidepressant action[244], while κOR agonists display analgesic actions without the potential of addiction[245]. Thus, it is essential to identify the brain structure that mediates the hallucinogenic effects of these drugs, in order to exploit their beneficial actions.

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