Therapeutic Applications and Targets of Chemogenetic Neuromodulation in Parkinson's Disease

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Abstract

In previous studies, chemogenetics has been utilized as a method of investigation into Parkinson's Disease (PD), and a range of possible therapeutic targets for ameliorating PD have been suggested in terms of engineered receptors of specific neuronal subtypes and pathways. Though chemogenetics has been used on occasions as a tool for investigating brain functions in rodent models, and less often tested as a method of modulating GPCRs in order to mitigate neurodegenerative diseases and their symptoms relative to in vivo studies, it is yet to be tested in humans or recognized as a feasible treatment option for PD in the future. For this reason, this review organizes past research on the topics of chemogenetic neuromodulation, sites linked to Parkinson's that have been successfully targeted using chemogenetics, and targets that may be linked to PD that are viable targets to be regulated using chemogenetics in future studies in order to highlight the therapeutic potential of chemogenetics as a clinical intervention. More precisely, these sources point to the usefulness of chemogenetic technologies like DREADDs and PSAMs in targeting sites previously researched in relation to chemogenetics such as zona incerta GABAergic neurons, the autonomous subthalamic nucleus (STN), and orexin (hypocretin) neurons. Additionally, we extend on current research by suggesting the promising findings on possible targets inclusive of Group I mGluRs, striatal Myf5 cells, and P2Y12R inhibition as it relates to PD progression. Altogether, we conclude that the literature indicates that chemogenetics is a relatively young technology within neuroengineering showing a lot of promise for Parkinson's Disease, and more research must be carried out in order for this technology to be established as a trusted therapeutic and for safe and functional targets for treatment, particularly those discussed in this literature, to be crystallized as tenable for the palliation of PD and its symptoms.

Keywords: chemogenetics; DREADDs; GPCRs; neurodegeneration; neuromodulation; neurosurgery; parkinson's disease; therapeutics

1. Introduction

Chemogenetics has emerged as a relevant approach in translational neuroscience research not only applicable to mechanistic investigation, but to potential clinical therapeutics as well. Chemogenetics is generally defined as a chemical-based intervention applied in research in which engineered receptors, predominantly G-protein coupled receptors (GPCRs), are modified to respond exclusively to a specified exogenous or endogenous ligand in order to modulate neuronal cell function. In the past, the majority of studies implicating the use of chemogenetics have focused on the investigation of circuitry and cellular signals driving cognitive or behavioral activities in nonhuman mammalian models. **[1]** On the contrary, as more work has been done within the field of chemogenetics, there has been a rise in recent literature with an interest in chemogenetics as a strategy for precision medicine targeted towards neuropsychiatric disorders. **[2][3]** This manner of utilizing chemogenetics, as we will discuss,

may be a suitable alternative to more invasive therapies for a mechanistically complex neurodegenerative disorder such as Parkinson's Disease (PD).

Parkinson's Disease can be linked to a plethora of specific neuronal dysfunctions within the brain, such as those of dopaminergic neurons, orexin neurons, cholinergic neurons, GABAergic neurons, glutamatergic neurons, and norepinephrine neurons, all of which pertain to some sort of impairment within a targeted anatomical region of the PD-affected brain. A number of studies to be examined in this review have found favorable outcomes upon the application of DREADDs chemogenetics for the manipulation of relevant targets in mammalian model organisms affected with PD. By precisely, non-invasively, and reversibly modulating receptors of selected neurons, clinically significant improvements can be seen for certain symptoms or functions of PD in model organisms with little adverse or unwanted side effects, distinguishing chemogenetics as an attractive novel therapy applicable for both symptomatic alleviation and early stage PD intervention. Despite this progress, chemogenetic neuromodulation has not yet been offered as a treatment option for any neuropsychiatric disorders in a clinical setting. In addition, there exists many potential therapeutic targets for chemogenetics within the context of PD amelioration which are yet to be explored in research studies, and which consist of various sites to be discussed in this paper, including, but not limited to, the Nrf2/HO-1 signaling pathway, GABA neurotransmission through TGF-β/Smad3, and dopamine synaptic activity within the striatum. Momentarily, we will delve into the next steps in the field of chemogenetics as it relates to Parkinson's by proposing an extension of current targets and considering how to approach the future application of PD-targeted neuromodulation in humans. In this discussion, we put an emphasis on the clinical and therapeutic potential of the examined chemogenetic targets.

2. Parkinson's Disease

Parkinson's Disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease and is frequently referred to as being characterized by several main factors: the degeneration dopaminergic neurons of the substantia nigra pars compacta (SNpc) and the aggregation of α-synuclein in Lewy bodies that leads to cellular dysfunction and toxicity, as well as dysfunction of the basal ganglia. **[4][5][6]** The symptoms that occur in PD may be either motor or nonmotor. According to a review article by Jankovic, the four cardinal features that occur in PD can be described with the acronym "TRAP": Tremor at rest, Rigidity, Akinesia (or bradykinesia), and Postural instability. Akinesia is defined as the inability to perform voluntary movements, while bradykinesia mainly refers to slowness of movement. **[7][8]** It is noted by the author that both flexed posture and freezing have also been included as classic features included in parkinsonism. **[9]** On the other hand, nonmotor symptoms including olfactory and other sensory deficits, sleep abnormalities or disorders, gastrointestinal disturbances, anxiety and/or depression, impaired cognition or cognitive or behavioral abnormalities, and autonomic dysfunction may also be present in PD patients. **[6][9]**

2.1. Parkinson's Disease Pathology

The basis of all PD therapies lies in the knowledge of the pathological functions of PD that have been covered in previous literature. Parkinson's has a complex pathology related to

α-synuclein and Lewy body aggregation as well as environmental and genetic factors, which take on pathways such as neural circuitry dysfunction, impaired autophagy, neuroinflammation, and most notably, the deterioration of dopamine neurons. Perhaps the most relevant of these factors when discussing chemogenetic targets is the state of dopaminergic neurons in the brain **[10]**, specifically those located within the substantia nigra pars compacta (SNpc). A journal article from Kamath et al. provides telling insight on a specific DA neuron population subtype that is especially susceptible to PD-related neurodegeneration; the subpopulation SOX6_AGTR1 was evidence to most significantly upregulate TP53 and NR2F2 gene targets, thus amplifying molecular processes associated with degeneration and neuronal cell death. Although this is only one single pathological outcome associated with PD, it pinpoints the role of DA neurons in the occurrence of PD. In addition to this, it is relevant to discuss the role of oxidative stress, which is commonly defined as a phenomenon caused by an imbalance of free radicals, particularly reactive oxygen species (ROS), and the capacity of a cellular system to detoxify these reactive products, **[11][12]** and its role in the pathogenesis of PD, particularly in relation to dopaminergic neurotoxicity. **[13]** Mitochondrial dysregulation, such as mitochondrial complex I deficiencies of the respiratory chain, perturbation of mitochondrial dynamics and mitophagy such as PINK1–Parkin-dependent mitophagy, **[14]** a mitochondrial dysfunction common in early-onset familial Parkinson's disease. **[15][16]** Moreover, environmental factors such as neurotoxins, pesticides, insecticides and PD-associated genetically-mutated and misfolded proteins bring about mitochondrial dysfunction which antecedes the formation of reactive oxygen species (ROS.) Because PD symptoms can take effect in many different specific brain regions, it is challenging to reduce this condition to a singular factor. Nevertheless, this review touches on a range of brain regions in relation to PD pathology, including the substantia nigra (SN) the basal ganglia pathway, and less commonly targeted areas such as the

lateral hypothalamic area, the hippocampus, and the zona incerta.

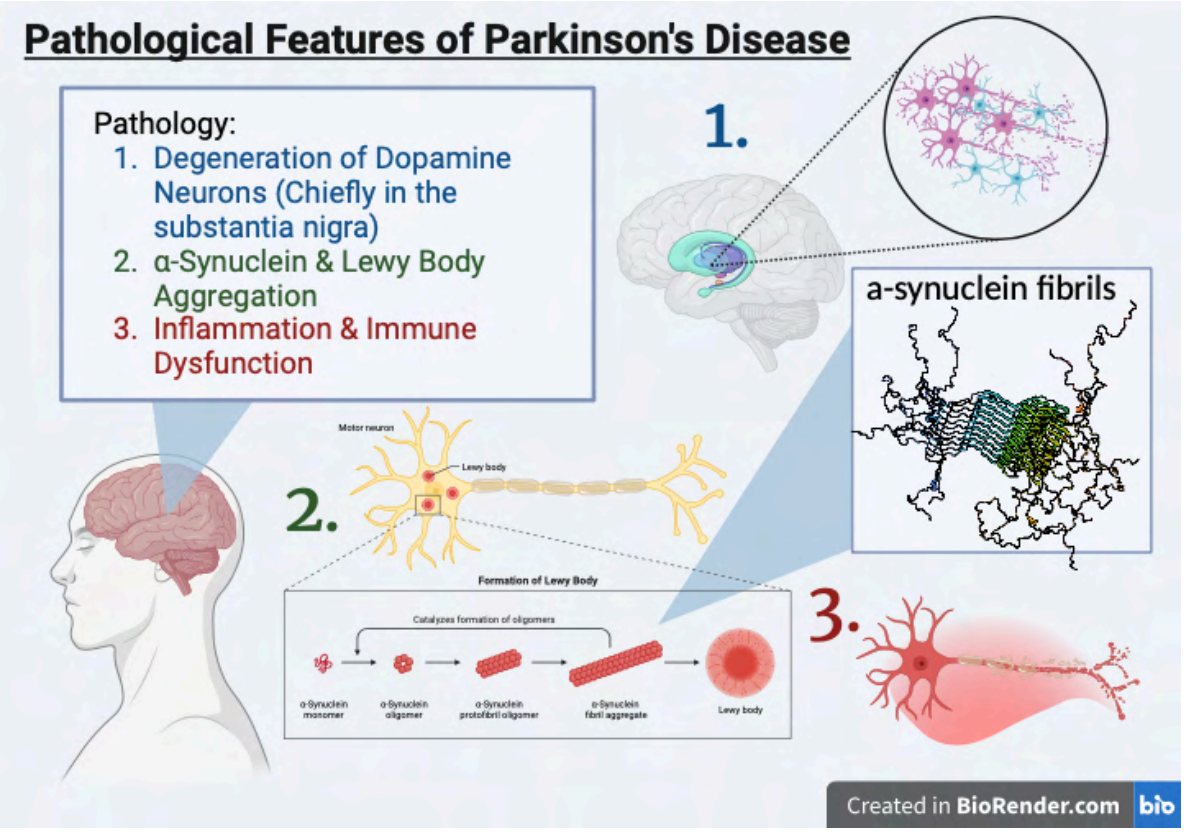

Figure 1. A visual showcasing the pathological features that can be found in Parkinson's Disease including: 1. degeneration of dopamine/dopaminergic neurons, which occurs chiefly in the substantia nigra, 2. aggregation of alpha-synuclein fibrils and lewy bodies in the brain, 3. Central, peripheral, and neuronal inflammation as well as dysfunction of the immune system.

2.2. Current Parkinson's Disease Treatments

Currently, the treatment or relief of PD and its symptoms is primarily addressed by employing a method such as dopamine replacement therapy (DRT) using levodopa, **[5]** physiotherapy, occupational therapy, or speech therapy. Other approaches include the use of medication such as dopamine agonists, monoamine oxidase-B inhibitors, and catechol-O-methyltransferase inhibitors, and in severe cases, surgical procedures such as deep brain stimulation (DBS) implants. **[17]** It is crucial to note, however, that there is no direct cure for PD at present. **[18]** A key method that has become commonplace is the aforementioned symptomatic therapeutic strategy of dopamine replacement, which despite being hailed as a great mechanism for the improvement of core motor symptoms, has a number of limitations that prevent it from being an ideal treatment for many patients. Generally levodopa has proven to be an exceedingly efficacious manner of ameliorating Parkinsonian symptoms when weighed against other drugs, although like any medication, its drawbacks include a number of side effects, which in this case are primarily motor complications taking the forms of motor fluctuations and levo-dopa induced dyskinesia.

Distinctively, a non-pharmacological alternative to consider is the neurosurgical procedure of deep brain stimulation (DBS), which commonly includes the chronic stimulation of the subthalamic nucleus using an implanted device comparable to a cardiac pacemaker that sends and regulates electrical signals through wire electrodes with the goal of lessening motor symptoms including slowness of movement and tremor. In spite of being rather more invasive and costly, this method is seen as a vital breakthrough within the field of PD research that has become the leading surgical approach to the treatment of PD. **[19]** As can be shown by this evidence, there is yet to be a completely ideal solution for PD therapy, though through the analysis of chemogenetic neuromodulation, we can see why this strategy may trump current treatments in certain areas. For example, chemogenetics is regarded as being less invasive than DBS for the manipulation of electrical signals, and is considered to produce less adverse side effects when contrasted with levodopa (DRT).

Table 1. Treatment approaches for nervous system disorders. Sternson, S. M., & Bleakman, D. (2020). Chemogenetics: Drug-controlled gene therapies for neural circuit disorders. Cell & Gene Therapy Insights, 6(7), 1079–1094. https://doi.org/10.18609/cgti.2020.112 [20]

Pharmacotherapy	► Molecular targeting ► Dose-dependent dynamic range ► Reversible	► Systemic treatment of focal disorders ► CNS access of drugs ► Typically, indirect modulation of neuron electrical activity ► May only work in a specific patient population
Surgical resection	► Eliminate disease tissue	► Permanent tissue loss ► Post-operative side effects ► Patient and physician stigma ► Need for repeated surgeries
DBS	► Local targeting ► Scalable ► Reversible ► Real-time control	► Surgery with permanent implant ► Local targeting reduced by activation axons-of-passage ► gof or lof mechanism of neuromodulation is unclear ► Hardware-related complications
Gene therapy (traditional)	► Replacement of missing or dysfunctional gene product ► Can by locally or broadly targeted	► Static effect, no additional control over neuromodulation ► Irreversible ► Usually cannot non-invasively assess localization and expression
Chemogenetic gene therapy	► Cell-type-specific targeting ► Dose-dependent dynamic range ► Pharmacologically reversible ► Local targeting ► Mechanistically straightforward ► PET to non-invasively assess localization and expression	► Best suited for local brain disorders ► Non-natural elements in engineered proteins ► Three components (small molecule, receptor, AAV)

3. Chemogenetics

Table 1 Overview of chemogenetic systems

Type	Receptor	Genesis	Signaling mechanism	Agonist
Metabotropic	hM3D	directed evolution of hM3 for affinity for CNO/CZP	G_q cascade	DREADD activators
	hM4D	directed evolution of hM4 for affinity for CNO/CZP	$G_{i/o}$ cascade	DREADD activators
	rM3D	chimeric product of rM3Dq and β1-adrenergic receptor	G_s cascade	DREADD activators
	KORD	modified hKOR (D138N) for increased affinity for SalB	G_i cascade	SalB
	Beta arrrestin DREADD	modified hM3Dq that switches Gq with β-arrestin	β-arrestin cascade	DREADD activators
Ionotropic	PSAM-A-5HT	chimeric product of a modified α7 LBD (L141F,Y115F) and the 5-HT3 IPD	increased membrane cation conductance	PSEM89S, PSEM308
	PSAM-A-GlyR	chimeric product of a modified α7 LBD (L141F,Y115F) and the GlyR chloride-selective IPD	increased membrane chloride conductance	PSEM89S, PSEM308
	PSAM-B-5HT	chimeric product of a modified α7 LBD (Q79G,Q139G) and the 5-HT3 cation-selective IPD	increased membrane cation conductance	PSEM22S
	PSAM-C-α7	chimeric product of a modified α7 LBD (Q79G,L141S) and the α7 calcium-selective IPD	increased membrane calcium conductance	PSEM9S
	PSAM4-GlyR	chimeric product of a modified α7 LBD (L131G,Q139L,Y217F) and the GlyR chloride-selective IPD	increased membrane chloride conductance	varenicline, uPSEMs
	GluCl	chloride-specific ion channel sourced from *C. elegans*	increased membrane chloride conductance	ivermectin, glutamate
	eGluCl	modified eGluCl (L9'F) for increased glutamate sensitivity	increased membrane chloride conductance	glutamate

Table 2. "Overview of chemogenetic systems. Descriptions of currently available chemogenetic systems, which include any engineered receptor-ligand system that modulates cell function via altered ligand specificity and affinity. DREADD, designer receptors exclusively activated by designer drugs; hM3D, human muscarinic 3 DREADD; hM4D, human muscarinic 4 DREADD; rM3D, rat muscarinic 3 DREADD; KORD, k-opioid receptor DREADD; hKOR, human k-opioid receptor; PSAM, pharmacologically selective activator modules; LBD, ligand-binding domain; IPD, ion pore domain; PSEM, pharmacologically selective effector modules; GlyR, glycine receptor; uPSEMs, ultrapotent PSEMs; GluCl, glutamate-gated chloride channels; eGluCl, enhanced GluCl." From: Song, J., Patel, R. V., Sharif, M., Ashokan, A., & Michaelides, M. (2022). Chemogenetics as a neuromodulatory approach to treating neuropsychiatric diseases and disorders. Molecular Therapy, 30(3), 990–1005. https://doi.org/10.1016/j.ymthe.2021.11.019 [3]

3.1. DREADDS/Metabotropic Systems

DREADDS, or Designer Receptors Exclusively Activated by Designer Drugs, are a specific type of chemogenetic toolkit that is used to manipulate neuronal activity through engineered G protein-coupled receptors (GPCRs), **[21]** and has emerged as the preeminent platform for research in chemogenetics. In general however, chemogenetics is intended to target metabotropic and ionotropic receptors in order to modulate cellular activity or signaling. **[3]** It is paramount to mention, though, that not all DREADDs are created equal, as will now be reviewed. There are numerous types of DREADDs, each of which falls into three broad categories based on the signaling protein that the receptors couple to; Gq, Gi, and Gs-coupled DREADDs are the classes to which each receptor-ligand complex can be attributed. **[1]** These signaling pathways are known as muscarinic DREADDs, and are systems derived from human

muscarinic receptors. **[22][3]** DREADDs, which reversibly modulate GPCRs, are particularly versatile since GPCRs constitute the largest and most robust family of membranous signaling molecules. It was found by a pair of studies from Allen and Roth and Metzler and Roth that they are characterized structurally by a highly conserved seven-transmembrane domain motif. **[21][23][24]** Moreover, it was shown by these trials that GPCRs are able to recognize an astounding variety of ligands, which includes odorants, photons, neurotransmitters, lipids, hormones, peptides, among other small molecules and also regulate intracellular response in order to adapt to the environment. Articles from Zu and Roth and Allen and Roth point to the astonishing fact that GPCR signaling modulates virtually every known physiological response in the human body. **[21][23][24]** For all of these reasons, modulating GPCRs with DREADDs arises as a promising and incredibly versatile class of chemogenetics for therapeutic purposes.

3.1.1. Gq-DREADDs

Gq-DREADDs make up the first overarching class of DREADDs. The mechanism of action of Gq-DREADDs is through the Gαq/11 G-protein, and this class of DREADDs activates neurons through the stimulation of phospholipase C, as a result releasing intracellular calcium stores. **[2][22][25]** The three original Gq-DREADDs, each of which was based upon its own human muscarinic receptor, hM1Dq, hM3Dq, and hM5Dq, are all activated by low nM concentration of clozapine-N-oxide (CNO), which is pharmacologically inert, mobilizing intracellular calcium. All of these DREADDs are able to be utilized in an excitatory manner, though hM3Dq, a mutant human M3 acetylcholine receptor associated with a Gq-coupled receptor, DREADD is the one most commonly used for activation and enhancing Gq signaling of the three. **[1][20]**

Clozapine-N-oxide (CNO) is the most commonly employed exogenous ligand administered by scientists to modify cellular activity as part of the chemogenetic toolkit in Gq-DREADDs since it is thought to be relatively pharmacologically and behaviorally inert when administered systemically in standard recommended doses, which are generally around 0.1-3 mg/kg. It is possible for CNO to be metabolized via back-transformation into clozapine, particularly in guinea pigs, humans, and nonhuman primates, although the amount back-transformed in humans is relatively low– about 10% or less by mass. **[1]** However, it is nonetheless vital to ensure that clozapine-like side effects, such as hypotension, sedation, and anticholinergic syndrome, do not occur by both keeping the dosage as minimal as possible and by having appropriate controls in place, like for instance, administering CNO to animals expressing irrelevant proteins such as green fluorescent protein (GFP). It is notable, however, that CNO has a host of excellent drug-like properties that make it an ideal chemical actuator. Firstly, CNO exhibits rapid CNS distribution and penetration in mice. Beyond this, CNO seems to have a minimum residence of 60 minutes in vivo in mice when administered intraperitoneally, (within the peritoneal cavity, or the area that contains the abdominal organs,) **[26]** therefore the effects of CNO-mediated activation of hM3Dq result to be both robust and prolonged in nature. For this reason, as CNO is highly potent, it is recommended that the lowest effective dose be

administered unless long-term Gq activation is necessary so that only peak CNO concentrations may activate the desired DREADD.

3.1.2. Gi-DREADDs

Currently, there are three Gi-DREADDs, two of which are based on the human muscarinic receptor, and one of which is based on the human kappa-opioid receptor (KOR). Gi protein activation through DREADDs inhibits neuronal activity by reducing intracellular levels of cyclic adenosine monophosphate (cAMP), which is utilized in a wide range of cellular processes, including metabolism, gene regulation, neurotransmitter synthesis regulation, growth factors, and immune function. The first two, hM2Di and hM4Di, are inhibitory DREADDs that function through the hyperpolarization of cells and that can be activated by CNO, compound 21, and perlapine, though hM4Di is quite more common than other inhibitory DREADDs. **[1][2][3][20][22]** The final type of target within the Gi-DREADDs classification are k-opioid receptors, or KORs, which function by binding salvinorin A (SalvA), a potent psychotropic dissociative hallucinogen KOR agonist. **[27]** Vardy et al. developed an engineered inhibitory DREADD analog of KOR known as KORD, which was activated by salvinorin B (SalB), a metabolite of salvinorin A that has been found to be inert in clinical doses. **[1][2][22][28]** Although SalB has limited solubility and dissolves in 100% dimethyl sulphoxide (DMSO), the use of the SalB/KORD DREADD has various advantages. SalB as a ligand for DREADDs is much faster acting than CNO, having an effect on neural activity within a few minutes following administration and lasting about an hour in vivo. Additionally, KORD can be used as an inhibitory DREADD simultaneously with the use of excitatory DREADDs in the same cell population to provide bidirectional neuronal control. **[2][22]** Moreover, it was shown by Marchant and colleagues that through the systemic injection of SalB in rats with ventral tegmental area expression of KORD, a reduction in locomotor behavior is displayed, thus demonstrating the efficacy of KORD *in vivo* in rodent models. **[22][29]**

3.1.3. Gs-DREADDs & β-Arrestin-DREADDs

The final major class of DREADDs encompasses Gs-coupled DREADDs as well as β-Arrestin-DREADDs, though there only exists one type of Gs-DREADD at present. This Gs-DREADD was synthesized by swapping the intracellular regions of a turkey erythrocyte β adrenergic receptor for equivalent regions of a rat M3 DREADD, creating a rodent DREADD that stimulates neurons through the activation of adenylyl cyclase known as rM3Ds. **[1][2][3][21]**

3.1.4. Alternate chemical actuators for DREADDs

Earlier, CNO was discussed as an ideal chemical actuator, particularly for Gq-DREADDs, and its back-metabolism potential was briefly mentioned. Due to the possibility of back-metabolism of CNO to clozapine as well as other clozapine metabolites in non-rodent species, such as the compound N-desmethyl-clozapine (NDMC) occurring, the discovery of alternate non-CNO chemical actuators to be utilized in lieu of CNO has been necessitated. Clozapine metabolites, however, remain among the most common chemical actuators for DREADDs. Shortly, a plethora of upcoming chemical actuators apart from the aforementioned

CNO will be outlined, including descholoroclozapine (DCZ), a metabolite of clozapine, as well as the non-CNO actuators compound 21 and perlapine.

Deschloroclozapine (DCZ or 11-(4-methyl-1-piperazinyl)-5H-dibenzo(b,e)(1,4)diazepine) is a muscarinic-based DREADD agonist that exhibits high target affinity and selectivity. DCZ is suggested for use as an alternative to CNO due to its off-target effects and sluggish kinetics. According to an investigation from Nagai, et al., it has been found using positron emission tomography that DCZ is able to effectively and selectively bind to DREADDs in both mice and monkey mammalian models, activating neuronal activity by way of hM3Dq within minutes following injection. Nagai and colleagues assert that DCZ, an analog of clozapine, is, based on findings surrounding mice and non-human primates, the most potent, selective, metabolically stable and fast-acting DREADD agonist. **[30]** Nonetheless, DCZ is a relatively new type of DREADD and is much less commonly utilized in comparison to CNO, which was an original DREADD agonist. **[1]**

Compound 21 has been developed as a comparable alternative to CNO without the risk of back-metabolism to clozapine, and is briefly discussed by Roth in the Cell Review titled, “DREADDs for Neuroscientists.” Through a study from Chen, et al., it was found that compound 21 has minimal off-target activity and exceptional selectivity in the activation of hMD3q as opposed to muscarinic receptors and other GPCRs. Not only this, but preliminary investigations point to the promising fact that compound 21 has equivalent potency in *in vivo* studies as does CNO, though these results are based on unpublished data. **[31]** Roth also surmises in this review that compound 21 likely is unable to be back-metabolized to clozapine or any other related compound, and for this reason, this compound appears to be an advantageous actuator to be used as an alternative to CNO.

An alternative compound that can be used to activate hMD3q is perlapine, which has traditionally been used in Japan as a drug intended for the alleviation of insomnia. **[1]** This compound was identified as a potential DREADD actuator in a broad library screen of existing compounds where it was found to exhibit both binding and Ca2+ mobilization at the hM3Dq receptor. **[32]** Unfortunately, however, perlapine has been found to have off-target effects, thus making it less ideal as an alternative to CNO, especially when compared to a more selective compound like compound 21. **[25]**

This is by no means an exhaustive list of alternative DREADDs actuators, since others such as olanzapine, JHU37152, and JHU37160 have been studied, and it is highly likely that many more DREADDs-compatible compounds will be uncovered in future studies. **[33][34]**

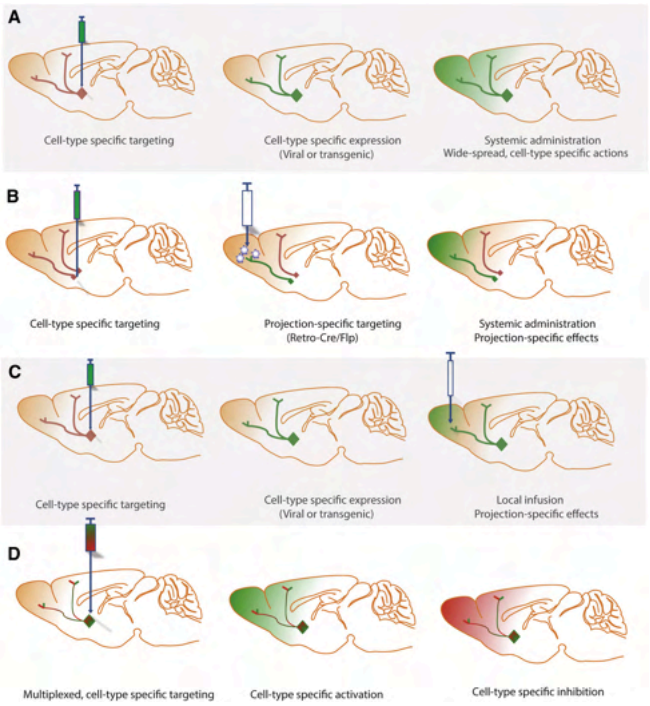

Figure 2. Roth (2016): Potential Approaches for Cell- and Projection-Specific Modulation of Neuronal Activity Using DREADDs

3.2. PSAMs/Ionotropic Systems

Alternatively to GPCR-based tools like DREADDs, a distinct mechanism of action that can be utilized for chemogenetic processes is an ion channel-based system called Pharmacologically Selective Actuator Module/Pharmacologically Selective Effector Molecule (PSAM/PSEM). This technique, developed by the Sternson laboratory, was created to engineer the ligand-binding domain of the alpha 7 nicotinic acetylcholine receptor (nAChR) to bind to pharmacologically selective effector molecules (PSEMs) in lieu of endogenous acetylcholine. **[2][35][36]** Ligand gated ion channels (LGICs) are the mechanism through which this class of system operates, allowing the control of ion conduction to lead to either neuronal activation or inhibition. **[22]** The engineered sites that the PSEMs bind to are known as pharmacologically selective actuator modules, or PSAMs, which are able to modulate both cell activation and inhibition depending on the specific ion pore utilized. For instance, a pair of particularly notable PSAMs combinations in existence thus far includes PSAMs spliced with the serotonin receptor 3a and that spliced with the GlyR. PSAM-5HT3 activation leads to cation (positively charged) influx which in turn induces neuronal depolarization On the other hand, PSAM-GlyR activation allows anion (negatively charged) influx, leading to hyperpolarization and in turn neuronal inhibition.

As for the ligands, PSEMs, that PSAM ligand binding domains (LBDs) have been produced for, various drugs have been utilized for this specified purpose, including ivermectin, a drug for intestinal strongyloidiasis and onchocerciasis, tropisetron, an anti-nausea and vomiting

drug for use during chemotherapy, and varenicline, a smoking cessation aid. **[20][22][37][38][39]** A host of ultrapotent PSEMs have been engineered from agonists of the drug varenicline, and they have proven to be very effective as neuronal modulating ligands in animals. Varenicline is accepted to be well tolerated in low doses and has outstanding central nervous system (CNS) penetrance. For this reason, this drug is an attractive and viable candidate for clinical translation. Campbell and Marchant note that ivermectin, in comparison to muscarinic receptor DREADDs, may be better suited for translation in clinical trials due to its FDA approval as an anti-parasitic drug, however, it is also mentioned that neuronal silencing and reversal succeeding its administration is somewhat delayed compared to other agonists, with its onset occurring within hours rather than minutes and its effects lasting for up to several days. **[22]** According to a 1988 study by Edwards, et al., the $t_{1/2}$ (half-life) of ivermectin in humans is about 24 hours. **[22][40]**

Overall, though PSAM/PSEM complexes are much less commonly utilized than GPCR-based DREADDs, this system still offers many advantages such as the use of FDA approved drugs as activating ligands. Yet another therapeutic benefit of PSAMs/PSEMs is that multiple complexes can be applied concurrently in order to provide bidirectional modulation. Nevertheless, not unlike most therapeutic solutions, PSAM-based systems have their downsides as well, including limited ligand bioavailability and the possibility of functional changes to axonal circuits. **[2]** As a result, further investigation on PSAM/PSEM system possibilities must be carried out before their debut as a clinical therapeutic form of chemogenetics.

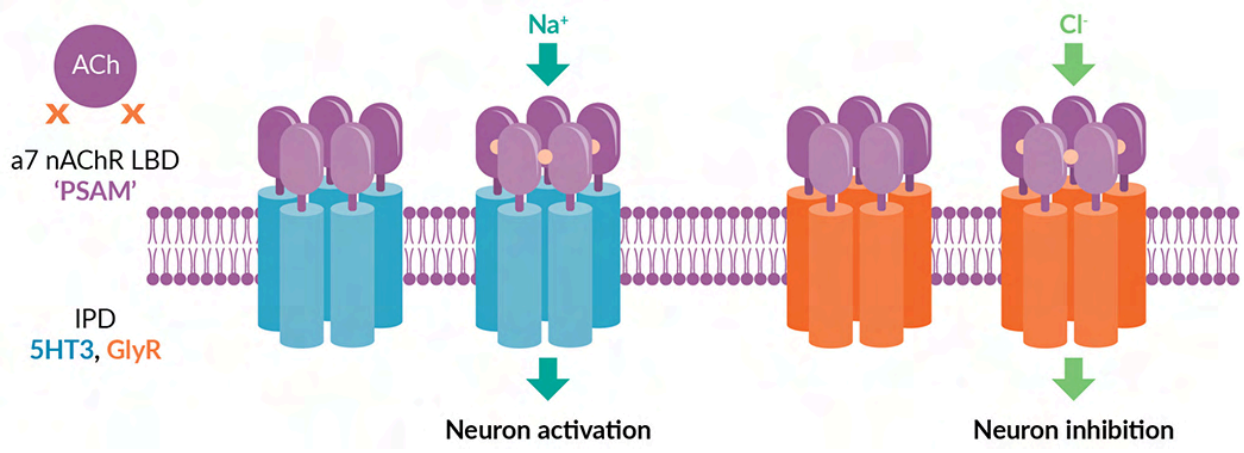

Figure 3. Sternson & Bleakman, 2021: PSAM chimeric ion channels.: PSAMs developed from the ligand binding domain (LBD) of the α7 nAChR are spliced to either the IPD of 5HT3 or GlyR to produce chimeric channels for neuron activation or inhibition, respectively. The same PSAM and its cognate agonist (yellow circle) are used for both types of channel. Mutations in the LBD increase drug-potency and reduce ACh sensitivity. PSAM chimeric channels are homomeric pentamers. **[20]**

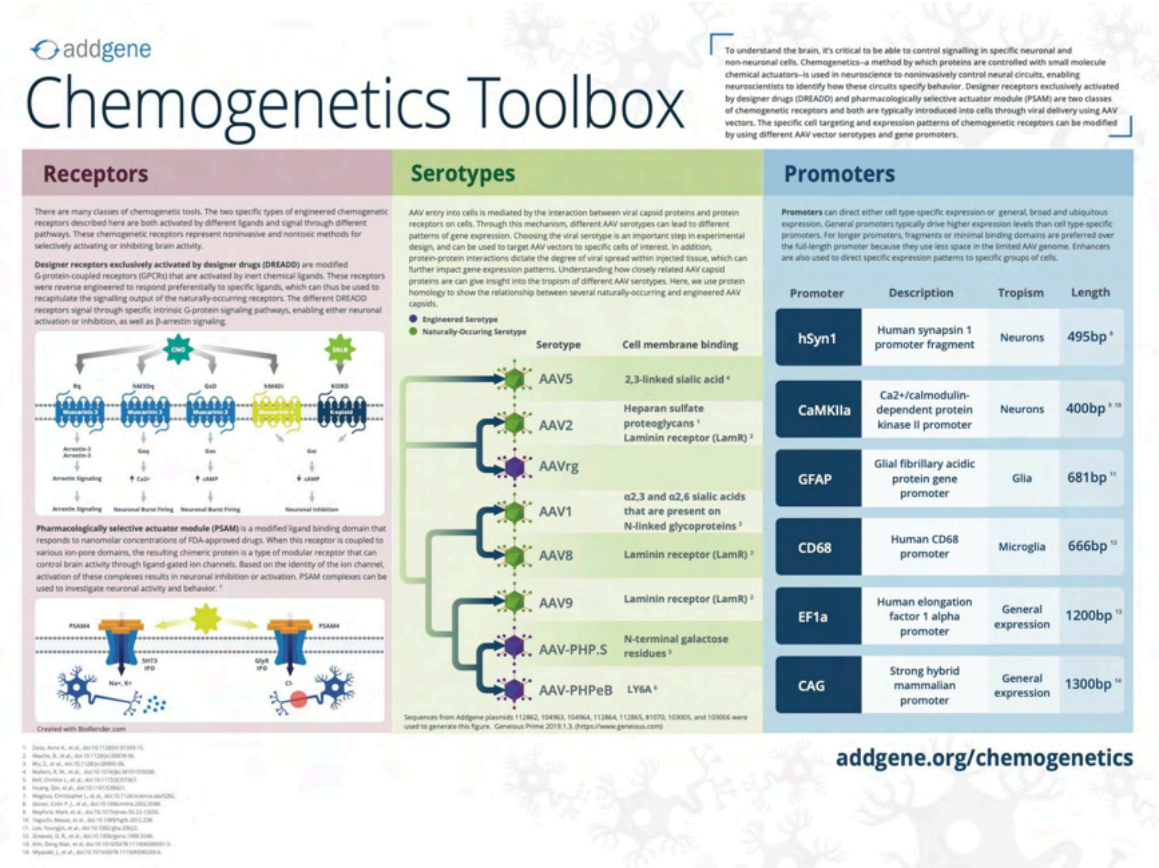

Figure 4. Addgene: Chemogenetics Guide. (n.d.). Retrieved October 13, 2023, from https://www.addgene.org/guides/chemogenetics/#

4. Existing Targets

4.1. Zona incerta GABAergic Neurons

A study carried out by Chen et al. using a 6-OHDA-lesioned PD model mice yielded the findings that motor impairment is significantly improved by both chemogenetic and optogenetic activation of GABAergic neurons in the zona incerta (ZI) and that repeated chemogenetic activation of ZI GABAergic neurons caused an increase in dopamine content in the striatum. **[41]** The chemogenetic targets and tools utilized in this experiment were genetically modified muscarinic receptors (DREADDs) with CNO (clozapine-N-oxide) functioning as a ligand. This study provides information relevant to the exploration of various targets for PD therapy by identifying the role of ZI GABAergic neurons in regulating motor behaviors in 6-OHDA-lesioned PD model mice. This raises the question for future investigations on the role ZI GABAergic neurons play in human PD pathology and whether this class of neurons is a suitable target for possible therapeutic solutions in the future. The evidence presented by this study establishes the potential of GABAergic neurons within the ZI as a viable target for PD therapeutics outside of the SNpc and basal ganglia network. **[42]** Although SN dopaminergic neurons and their function as part of the basal ganglia circuitry have historically been seen as the primary PD culprit, studies such as this one exhibit the many possible pathways and locations in which the

presence of PD manifests. The ZI acts as a diverse processing center in the subthalamic region of the brain, carrying out global behavioral modulation tasks among a range of other processes such as processing sensory information and participating in neural plasticity related to fear learning and extinction. **[43]** The significance of this work is the basis it provides regarding the role of the ZI in PD motor symptoms and how this information may support progress in terms of the use chemogenetics and optogenetics with a focus on PD.

4.2. Autonomous Subthalamic Nucleus Restoration

In a 2018 preprint authored by McIver et al., chemogenetic restoration of autonomous subthalamic nucleus (STN) firing was shown to reduce synaptically-driven synchronization of STN neuronal activity and effectively alleviate Parkinsonian motor dysfunction. **[44]** This finding points to the possibility of the chemogenetic-induced increase of intrinsic STN activity as a therapeutic pathway for symptom-based PD treatment. This study focuses on symptoms appearing in PD with a pathophysiological connection to irregular, synchronized neuronal spiking in the STN, such as akinesia, bradykinesia, and rigidity. A focal point described in this preprint from McIver and colleagues was that autonomous activity of the STN neurons, which aid to prevent synaptic synchronization, was downregulated in both toxin and genetic PD models. This abnormality was found to be due to increased transmission of D2-striatal projection neurons, which led to a cascade of changes and as a result promoted KATP channel opening. The STN region, which is emphasized for its involvement in action control, as well as motor and cognitive functions, **[45]** is a common target for PD-targeted deep brain stimulation (DBS) therapeutics aiming to improve PD symptoms like those aforestated. Chemogenetic modulation of the STN may provide us with a less invasive route not requiring a chronic intracranial implant procedure when contrasted with DBS.

4.3. Activation and Inhibition of Orexin Neurons

Orexin (also called hypocretin) neurons present a fascinating avenue for disparate neuromodulatory actions, as has been found by numberable publications from Stanojlovic et al. **[46][47][48][49]** Firstly, it is imperative to take into consideration what is generally considered the primary role of orexin neurons, which are a class of neurons localized within the lateral hypothalamus. These neurons are known for being heavily implicated in sleep/wakefulness cycle regulation, though they also contribute to neuroendocrine cell function and effect systems that regulate emotion, reward system, arousal, and energy homeostasis **[50]**. A review article published by Liu, et al. defines the neuropeptide orexin as being involved in the regulation of motor control, the sleep/wake cycle, learning and memory, gastric motility and respiratory function, as well as being instrumental in the non-motor manifestations of PD **[51]**. Liu and colleagues detail the connections between low levels of CSF orexin and sleep disorders such as EDS (Excessive daytime sleepiness) and RBD (Rapid eye movement behavior disorder), in PD, as well as how damage of orexinergic neurons in the hypothalamus is associated with PD, and how orexins have been found to have therapeutic and protective effects on PD, on animal and cell models, respectively. This evidence, in conjunction with the mentioned work from Stanojlovic et al., establishes orexin neurons and the orexinergic system as a significant focal

point for chemogenetic PD therapy. Stanojlovic and colleagues explore the link orexin neurons have been found to have with PD by using DREADDs to implement the modification of Parkinsonian orexinergic neuronal signaling in various ways, and observe a range of orexinergic-related PD pathological and symptomological functions, such as Hipp-dependent memory impairment, social cognition impairment, locomotor regulation, anxiety-like behaviors, spontaneous physical activity, and energy expenditure. As will be discussed momentarily, orexin neurons take part in a multitude of processes affected by Parkinson's, and appear to be a strikingly less orthodox, yet promisingly viable alternative target set in contrast to their dopaminergic counterparts.

4.3.1. Activation/Stimulation of Orexin Neurons in A53T Mice for the Amelioration of Hipp-dependent Early Memory

In a research publication carried out by Stanojlovic et al., orexin (hypocretin) neurons were targeted using DREADDs technology with the intent of activating these cells, and this study was carried out utilizing the A53T (induced) mice model of PD **[48]**. As stated by the author of this publication, the hippocampus (Hipp) is a brain region that is commonly known for its role in cognition and memory. Correspondingly, the neuropeptide orexin has been found to enhance learning and memory. In the primary procedure of this study, mice of 3, 5, and 7 months of age were subjected to a Barnes maze and contextual object recognition test in order to determine Hipp-dependent memory. Subsequently, inflammation and astrogliosis markers in the Hipp were assayed using immuno-fluorescence densitometry. The results of this initial study revealed that cognitive impairment, marked by learning and recognition and abilities significantly incommensurate with those of a healthy mouse, appeared to coincide with an increase in expression of inflammatory and astrogliosis markers. **[48]**

A separate pair of further experiments conducted by the scientists, Hipp-dependent early memory impairment was ameliorated in two separate manners, the first in which DREADDs specific to orexin neurons were given via chemogenetic viral injections, whereas in the second, the mice were given intra-hippocampal injections of exogenous orexin A. In the chemogenetic trial, CNO was used as the actuator ligand to bind to orexin neurons. The results showed that both orexin A intervention and the chemogenetic activation of orexin neurons served to mitigate Hipp-dependent early memory impairment in A53T mice models. **[48]** From these results, it is logical to conclude that in terms of the alleviation of this class of memory impairment in PD, it may be advisable to look into not only exogenous pharmacological orexin treatment in a clinical setting, but to further investigate the chemogenetic activation of orexin neurons as a therapy intended for patients of PD with this impairment.

4.3.2. Inhibition and Activation of Orexin Neurons in A53T Mice for Affected Locomotor Activity and Anxiety-like Behaviors

Likewise, the authors also conducted an investigation into the effects of chemogenetically modulating orexin neurons using DREADDs in order to possibly lessen the presence of unwanted spontaneous locomotor activity and anxious behaviors. The purpose of this study was to examine whether behavioral changes in A53T mice were coupled with increased

inflammation and astrogliosis in the hippocampus and motor cortex, the former which is involved in the regulation of anxiety and the latter which is involved in locomotor regulation. A53T mice show an early reduction in anxiety-like behavior coupled with an increase in locomotor activity, symptoms that occur alongside the aggravation of inflammation and astrogliosis in the hippocampus and motor cortex. Stanojlovic, Pallais, and Kotz hypothesized that through chemogenetic modulation of orexin neurons, it would be possible to reverse the changes of lessened anxiety-like behavior and augmentation of locomotor activity. It was found that this hypothesis was accurate to the results, with the chemogenetic activation of said neuron population leading to the restoration of control levels of anxiety-like behavior from the decreased levels without affecting locomotor activity, and the inhibition of orexin neurons leading to a reverse in the increased locomotor activity without affecting anxiety-like behavior. **[46]**

In all, the key points suggested by this publication are that orexin neurons play a complex role in PD, shifts in locomotor activity and anxiety-like behavior are coupled with inflammation and astrogliosis, and that, perhaps most centrally, that the data points to the orexin system as one that has a significant role within PD in both its early and late stages. **[46]** Nonetheless, it is not yet completely determined whether orexinergic neurons would be an ideal selection for chemogenetic neuromodulation for therapeutic purposes aimed at upregulating anxiety-like behavior back to control levels and downregulating locomotor behavior to its control levels in humans. Further experimentation must be carried out in future studies to ascertain this target's potential for chemogenetic treatment in a clinical setting.

4.3.3. Stimulation and Inhibition of Orexin Neuronal Activity for the Modulation of Sociability and Social Memory in A53T Mice

In addition, another subject of investigation that was targeted by DREADDs in this series of experimental trials looking at orexinergic neuronal modulation in A53T induced-PD mice was how both the stimulation and inhibition of orexin neuronal activity allows for the modulation of sociability and social memory that may be impaired in these PD model mice. **[49]** Though this particular research study from Stanojlovic and colleagues comprised of many conclusions, the most key of these which is relevant to this discussion is that through the use of DREADDs for both the stimulation and inhibition of orexin neurons in A53T PD model mice to modulate sociability and social memory, it was found that the activation of orexinergic neurons via DREADDs is able to restore the original social cognition in this rodent model of PD. **[49]**

Similarly to the above experiment on locomotor activity and anxiety-like behaviors, it was discovered that this social cognition impairment in A53T mice is correlated with an increase in astrogliosis and inflammation markers, though in this particular study this impairment was also linked to a loss of parvalbumin GABAergic neurons and inhibitory presynaptic terminals within the medial prefrontal cortex, a region involved in the regulation of emotion, motivation, and sociability. **[49][52]**

Although the central conclusions of this investigation were not focused primarily on the chemogenetic modulation of orexin neuronal activity to affect sociability and social memory in a

therapeutic or clinical manner, it is certainly possible for this topic to be relevant for future translational studies looking to reverse PD-related social impairments through neuromodulation.

4.3.4. Inhibition of Orexin Neurons for the Amelioration of Elevated Physical Activity and Energy Expenditure A53T Mice

Contrariwise to the earlier studies discussed, the aforementioned researchers, Stanojlovic et al., conducted a similar investigation into orexin neurons of A53T mice using DREADDs, but on this occasion inhibiting the targeted neurons for distinct purposes. In this case, the aim of this experiment was to ameliorate PD-associated elevated physical activity and energy expenditure in the A53T mouse, given that orexin neurons, which are implicated in exploratory locomotion, spontaneous physical activity, and energy expenditure, are impaired in Parkinson's. Analogously to the above study surrounding sociability and social memory, though chemogenetic modulation via DREADDs was not the only key finding observed in the research, it is one of the most relevant findings to this review. The experimental results indicated that the inhibition of orexin neurons via CNO with DREADDs in orx-Cre/A53T mice was able to reduce both locomotion and spontaneous physical activity, though neither of these functions were lowered to levels typical in orx-Cre (non-PD model) mice. Moreover, chemogenetic inhibition of orexin neurons did not appear to have any effect on energy expenditure in the light phase of the experiment, though in both the dark phase and in total, reduced energy expenditure was observed. **[47]**

In general, it appears that to alleviate exacerbated levels of physical activity and energy expenditure in these PD model mice, the chemogenetic inhibition of orexin neurons might not be the most effective procedure. For future studies with the goal of ameliorating elevated physical activity and energy expenditure, it would be advisable to modify a variable, such as the ligand, target receptor type, neuronal cell type, etcetera in order to optimize outcomes of potential preclinical trials.

4.4. The Pedunculopontine Nucleus and Cholinergic Neuron Receptors

There are four areas within in the mammalian brain which are responsible for the production of the majority of cholinergic projections, namely in the pedunculopontine nucleus (PPN), the laterodorsal tegmental nuclei, thalamic nuclei, and the striatum. **[53]** Other regions that comprise the brain's cholinergic projection system are the basal forebrain cholinergic nuclei (BFCN), which send cholinergic inputs to the prefrontal cortices, the hippocampi, and the amygdala, helping in the regulation of functions such as attention and memory. **[54]** The cholinergic system, which modulates the striatal cells that regulate cognitive and motor functions, contains two chief classes of receptors expressed in the striatum that relate to these functions which are impaired in PD. These receptors are muscarinic acetylcholine receptors (mAChR) and nicotinic acetylcholine receptors (nAChRs).

A review article from Iarkov, et al. details cholinergic receptor modulation, or the cholinergic system, as a target to ameliorate cognitive symptoms and dementia in PD. This article focuses on many relevant components of the cholinergic system, including mAChrs,

nAChRs, and the pedunculopontine nucleus, each of which will be discussed in detail within the following subsections.

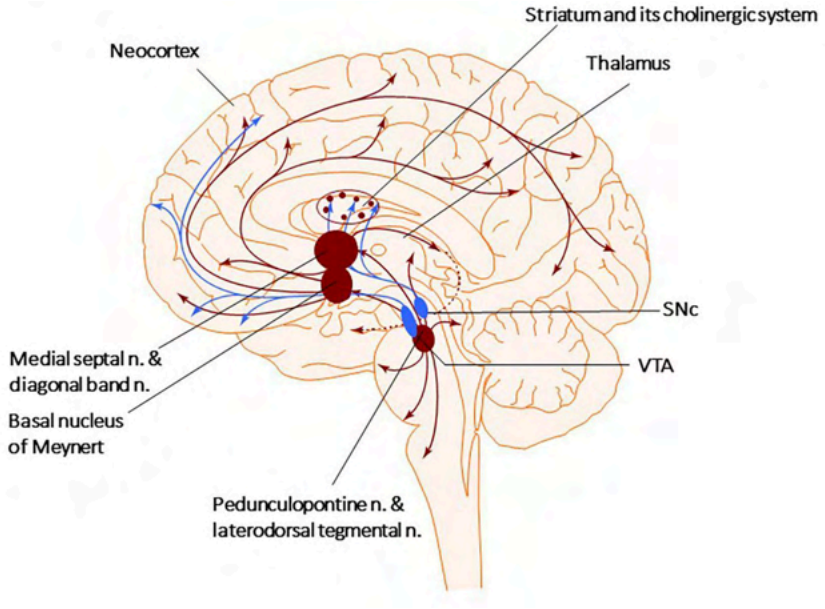

Figure 5. Iarkov et al.: Cholinergic and Dopaminergic systems. The diagram describes the Cholinergic (brown) and Dopaminergic (blue) systems. There are four primary sources of cholinergic projections in the mammalian brain. These include pedunculopontine nucleus and laterodorsal tegmental nuclei; a set of thalamic nuclei; striatum, where few cholinergic neurons are local interneurons; and the basal forebrain nuclei, which collectively serve as the primary sources of cholinergic projection neurons in the neocortex, hippocampus, and amygdala. SNc-Substantia nigra pars compacta, VTA, ventral tegmental area. (Iarkov, A., Mendoza, C., & Echeverria, V. (2021). Cholinergic receptor modulation as a target for preventing dementia in Parkinson's disease. Frontiers in Neuroscience, 15. https://doi.org/10.3389/fnins.2021.665820

4.4.1. Nicotinic Acetylcholine Receptors

Nicotinic acetylcholine receptors (nAChRs) are receptors whose signaling has a variety of functions, including reducing neuroinflammation, facilitating neuronal survival, releasing neurotransmitters, and aiding in synaptic plasticity. **[53]** Beyond this, these receptors have the ability to stimulate other brain cells that support cognitive and motor functions, both of which are regularly impaired in PD. In Parkinson's, there is a notable deficit of these nAChRs, and it it hypothesized that somehow hindering the loss of these receptors may help avert the depletion of dopaminergic neurons in the striatum, and therefore the pathological consequences that come with this loss. It has been suggested that drugs that act on these receptors, such as nicotine, may be used to ameliorate PD symptoms and diminish levodopa-induced dyskinesias that often result from treatments like dopamine replacement therapy (DRT). **[55]** In addition, it has been found that nAChR-affecting drugs might in fact retard the process of neurodegeneration itself. Intriguingly, a plethora of epidemiological studies have presented the data stating that there exists a ~50% reduced incidence of Parkinson's disease in smokers.

Though smoking tobacco presents a host of undesirable side effects, there are other ways we can assert influence on nAChRs, such as chemogenetic modulation.

A specified nAChR that has been studied in depth as a therapeutic target for PD is the alpha7 nicotinic receptor type. A study by Quik and colleagues highlights the utility of targeting CNS α7 nicotinic acetylcholine receptors (nAChRs) in order to garner both neuroprotective and antidyskinetic effects. **[56]** Alpha7 nAChRs in particular are fascinating firstly, because they are structurally, phylogenetically, and functionally distinct from all other nAChR subtypes, and secondly, the possess both the lowest nicotine sensitivity and fastest desensitization kinetics of al nAChR subtypes. Along with this, they also have an exceedingly high calcium permeability, thus allowing them to regulate multitudinous calcium-dependent cellular mechanisms vital to ideal CNS functioning. A7 nAChRs are located in diverse regions of the brain, but are densely located in brain regions including the hypothalamus, geniculate nuclei, colliculi, hippocampus, medial habenula, thalamus, cortex, amygdala, forebrain, and medulla as well various brain nuclei and existing in sparse expression in the striatum. **[56]** Due to their widespread presence in numerous locations within the brain, they are consequently implicated in a diverse myriad of cellular functions such as development, maintenance, survival, synaptic plasticity, neurotransmitter release and immune responsiveness, functions which eventually end up playing a role in behaviors like anxiety, attention, learning, memory, and movement and sensory gating. These behaviors relate these receptors to a range of neurodegenerative, neurodevelopmental, and neuropsychiatric disorders, including PD, Alzheimer's disease, autism, schizophrenia, addiction, and more. As summarized in this paper, A7 nAChRs have not only been found to have a protective, trophic role against varying toxic insults, but both α7 and β2* nAChRs have been found to assert protection against neurodegenerative nigrostriatal damage in PD-afflicted animal models.

Moreover, Quik and coauthors recapitulate a telling data set that shows that α7 nAChR agonists, including nicotine, varenicline, ABT-126, and AQW051 are able to reduce L-dopa-induced dyskinesias (LIDs). More precisely, the general nAChR agonist nicotine exhibited a 60% decrease in LIDs in animal models of parkinsonian monkeys and rodents, while a similar general nAChR agonist, varenicline, mitigated LIDs by about ~50%. Likewise, the authors covered a pair of specified drugs that selectively target α7 nAChRs, ameliorating LIDs while experiencing no change in parkinsonism when the animal models were administered L-dopa, thus suggesting a longer-term protective effectiveness offered by these agonists. The first of these two agonists was he α7 agonist ABT-107 applied to *Saimiri sciureus (common squirrel monkey) and resulted in a* ~60% reduction of LIDs, while the second was the α7 nAChR agonist AQW051 to applied to *Macaca fascicularis (crab-eating macaque)* which similarly lead to a ~60% decrease in LIDs. As can be seen from this data, α7 nAChRs may very well be efficacious targets could we find a way to modulate them using chemogenetics.

In a similar manner, an investigation carried out by Zhao, et al. indicated that the activation of α7-nAChRs asserts a neuroprotective effect on the pathology and aggregation of exogenous alpha-synuclein by precipitating the clearance of αSyn and inhibiting apoptotic cell

death. **[57]** The investigators found these results by utilizing both nicotine and the selective α7-nAChRs agonist PNU-282987. As of now, such results related to the amelioration of αSyn-induced damage targeting nAChRs has not been tested or achieved by way of chemogenetic neuromodulation.

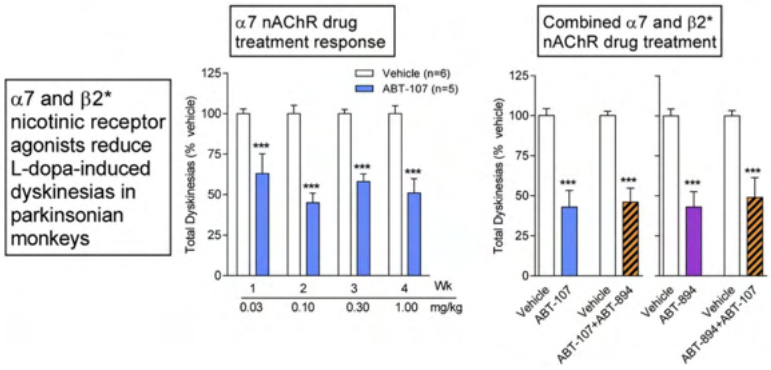

Figure 6. Graphical Abstract: Alpha7 nicotinic receptors as therapeutic targets for Parkinson's disease by authors Maryka Quik, Danhui Zhang, Matthew McGregor, & Tanuja Bordia. (Quik, M., Zhang, D., McGregor, M., & Bordia, T. (2015). Alpha7 nicotinic receptors as therapeutic targets for Parkinson's disease. Biochemical Pharmacology, 97(4), 399–407. https://doi.org/10.1016/j.bcp.2015.06.014)

4.4.2. Muscarinic Acetylcholine Receptors

In terms of muscarinic acetylcholine receptors (mAChRs), it has been determined that subtype specificity is vital in developing a therapeutic **[58]**, and for this reason, chemogenetics are relevant to this issue, since they are known for being specific and causing few adverse side effects. This is due to the fact that non-selective anti-muscarinic acetylcholine receptor therapeutic agents used to lessen tremor often produce a host of adverse effects and are therefore poorly tolerated. In an article by Bickham and colleagues, the use of muscarinic subtype selective antagonists are suggested, as they may ameliorate tremor while producing minimal adverse side effects. Through a series of experimental trials on pre-clinical models, the researchers ascertained that selectively modulating M1, M4, or M5 does not help to reduce tremor in the models used, however, this does point to the conclusion that M2 or M3 receptors of the central or peripheral system, on the other hand, may be the specified receptors that bring about lessened tremor in current non-selective anti-muscarinic therapy, indicating that these receptors may be the ones to target in further trials. On the contrary, a separate report from Brugnoli, et al. reports that via M1 and M4 striatal and nigral receptors, it is possible to mediate L-dopa-induced dyskinesia using endogenous acetylcholine. **[59]** This indicates that despite previously discussed results, it may still be worth investigating the mechanisms of M1 and M4 receptors via chemogenetic methods.

Since these receptors are a robust class of GPCRs that are broadly expressed in both the central and peripheral nervous system and have an effect on tremor, **[60]** a possible next

step would be to attempt to target these receptors using DREADDs technology using a pre-clinical model. Thus far, such an endeavor has not been carried out, but a designer receptor-drug complex may be an efficacious and salubrious way to modulate M2 and M3 while evading harmful side effects.

4.4.3. The Pedunculopontine Nucleus

Yet another cholinergic component to discuss, though not a specific receptor class, is the pedunculopontine nucleus (PPN). The PPN is organized into two subnuclei, each with its own class of neurons and purpose. One of the two is the *cholinergic* pars compacta (PPNc), which is composed of cholinergic neurons and is included in the loop that connects the spinal cord and limbic areas with the basal ganglia and thalamus. On the other hand, the dopaminergic pars dissipatus (PPNd) is implicated in the process of starting and modulating stereotyped movements like gait. **[53]** Interestingly enough, one of the primary motivations for investigating the PPN and its cholinergic neurons in relation to PD was the discovery that in advanced stage PD patients, nearly half of these neurons are gone. **[61]**

One one hand, according to an article by Tubert, et al., despite the fact that the PPN is incontrovertibly implicated in movement control and PD, its specific function in relation to behaviors such as gait, posture, arousal, sleep, and cognition is unclear, and as a result, any efforts to target this region via DBS will likely be in vain due to inconsistent results. However, the authors note that alternative, more targeted treatment methods like chemogenetics and optogenetics could possibly yield significantly more promising results.

On the other hand, a similar report by Pahapill and coauthors suggests that the PPN is a quite promising surgical location, contrary to the findings of Tubert, et al. As per this opposing review, both animal and clinical investigations have proven that surgical interventions aiming to reduce inhibitory basal ganglia output to the thalamus and PPN bring about the amelioration of all major features pertaining to PD. **[62]** Not only this, but it has also been observed that STN lesions as well as chronic electrical stimulation in PD-afflicted primate models and human patients can reduce the entirety of major motor disturbances occurring in PD, such as akinesia, rigidity, and tremor, while similarly, the direct lesions or stimulation to the GPi in both humans and non-human primates has been seen to lessen parkinsonism as well. On the basis of the data provided within the article from Pahapill, et al., electrical stimulation as well as the delivery of neuroactive substances to the PPN facilitate dramatic effects on motor function (or dysfunction) in PD, though the given data does not discuss chemogenetic stimulation. But given this, we are assured about the promising utility of chemogenetics in the PPN due to aforementioned reports chronicling surgical success in alleviating PD-associated motor symptoms.

Based on these disparate findings we can conclude that due to the complexity of the PPN, it has, in the past, been very difficult to target clinically using current methods. However, with this in mind, we now must consider how chemogenetic techniques may be invaluable in the process of discovering more about the PPN and eventually developing a targeted therapeutic

through a system like DREADDs. Where many methods have failed in the PPN, it is hopeful that chemogenetic stimulation may succeed.

4.5. Activation of SNpc Dopaminergic Neurons

In a 2023 study by Seo, et al. published in the International Journal of Molecular Sciences, an investigation examining the manipulation of DOPA decarboxylase (DDC)-positive neuronal activity was carried out. **[63]** To do this, the authors crafted an adeno-associated virus (AAV) vector expressing mCherry under rat DDC promoter (AAV-rDDC-mCherry) established its specificity in the rat substantia nigra pars compacta (SNpc), and thereafter modified said vector to express the Gq-DREADD hM3Dq under the DDC promoter in the SNpc. The functionality of this was then validated electrophysiologically ex vivo, yielding the finding that in the case of A53T-mutated alpha-synuclein overexpression rodent model of PD, chemogenetically activating DDC-positive neurons in the SNpc significantly ameliorated parkinsonian motor symptoms and rescued nigrostriatal tyrosine hydroxylase (TH) loss. This is facilitated by the awakening of dormant DA neurons, which results in the aforementioned recovery of TH expression. **[63]**

Howbeit, there were relevant drawbacks and limitations that prevent chemogenetics from being optimally efficacious in this situation. Although chemogenetic manipulation using hM3Dq and the administration of CNO is very effective short-term, showing immediate activation and polarization as well as efficacy within minutes upon the administration of CNO, at the cessation of CNO administration, it is expected that the affected DA neurons will return to dormancy within a few days, possibly causing PD-associated motor symptoms to recommence. This means that in order for this type of chemogenetic therapy to be effective both short and long-term, it may be necessary to administer CNO to the patient on a regular basis. **[63]**

As can be deduced from the above results, it appears that DDC+ DA neurons in SNpc are a helpful target for chemogenetics, though it is clear that for clinical purposes, the methods tested by the authors must still be refined to reap any long-term benefit.

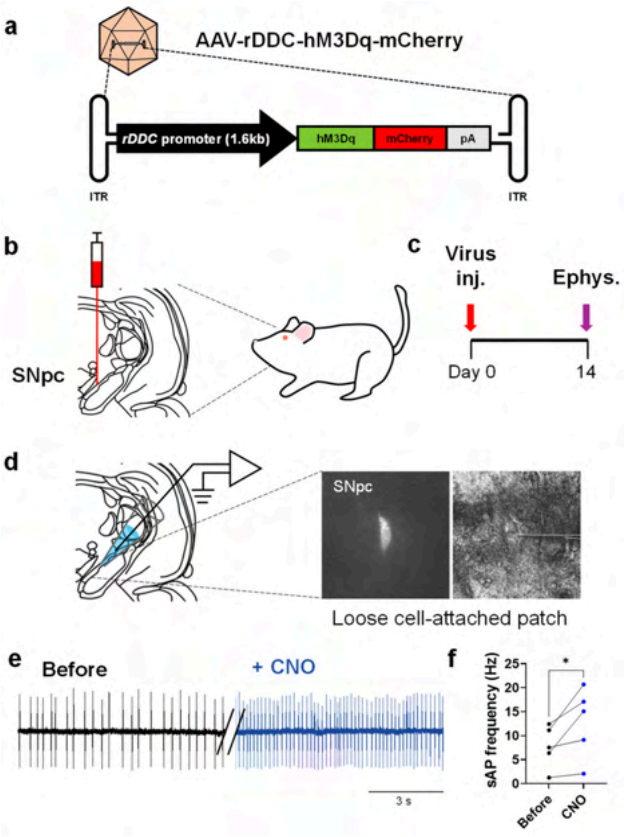

Figure 7. Seo, et al.: Gq-DREADD-mediated chemogenetic activation of DDC+ neurons increases the firing rate in SNpc. (a) The schematic for a viral vector that contains hM3Dq and mCherry; (b) schematic diagram of stereotaxic injection of AAV-rDDC-hM3Dq-mCherry virus; (c) experimental timeline; (d) representative images of the DDC+ neurons with loose cell-attached patch; (e) representative trace of spontaneous action potential firing of DDC+ neuron before and after CNO bath application (5 μM); (f) quantification of firing rate. The data normality was assessed by Shapiro–Wilk normality test. Statistical significance was calculated by two-tailed Student's paired t-tests. The significance levels are represented as asterisks (* $p < 0.05$). (Seo, D., Ju, Y. H., Seo, J., Oh, S., Lee, C. J., Lee, S. E., & Nam, M. (2023). DDC-Promoter-Driven chemogenetic activation of SNPC dopaminergic neurons alleviates parkinsonian motor symptoms. International Journal of Molecular Sciences, 24(3), 2491. https://doi.org/10.3390/ijms24032491)

4.6. Activation of Locus Coeruleus Norepinephrine Neurons

The locus coeruleus norepinephrine system (LC-NE) is one that is involved in regulating a host of behaviors, such as sleep/wake states and arousal, modality-specific sensory processing, attention and memory during cognitive tasks, and stress response. **[64][65][66]** This is because the locus coeruleus (LC), which translates to "blue spot" in Latin, **[67]** though a mere small brainstem nucleus containing less than 50,000 neurons in the human brain **[68] [69]** (the

human brain in total is comprised of about 86 billion neurons and just as many nonneuronal cells **[70]**) and only around 3000 neurons in the rodent brain **[71]**, the LC is the chief synthesizer of the neurotransmitter norepinephrine in a range of anatomically and functionally diverse brain regions. **[64]** Evidence shows that dysregulation of the LC-NE system is implicated in a diverse range of psychiatric pathologies such as depression, anxiety, attention deficit hyperactivity disorder (ADHD), post traumatic stress disorders (PTSD), and most importantly in this context, neurodegenerative diseases, which includes both Parkinson's disease and Alzheimer's disease (AD). **[72][73][74]** Krohn and colleagues point out that not only is the LC a relevant region for healthy aging due to its impact on a handful of crucial cognitive functions connected to the noradrenergic system including verbal intelligence, response inhibition, memory, emotional memory, and attention and processing speed, but the LC is also central to neurodegenerative disease pathology because the dysfunction and generation of the LC-NE system ensues during beginning stages of both PD and AD, and as a matter of fact, it is thought that this dysfunction may contribute to the spread of neurodegenerative pathology. **[75]** A number of investigations demonstrate that LC volume and MRI contrast diminishes considerably in both Alzheimer's and Parkinson's. To add to this, in PD specifically, the LC displays signs of structural degeneration early on in disease onset, an occurrence that might be associated with non-motor psychiatric symptoms such as anxiety, depression, and REM sleep disturbances that precede degeneration of the substantia nigra (SN). **[75]** These facts indicate that the LC could very well be an effective target for the use of therapeutic strategies such as chemogenetics.

In terms of applying chemogenetics to the LC-NE system, there have been two principal manners discovered in which chemogenetic activation can be utilized in order to benefit brain function via the LC. The first, though not linked to PD in particular in its relevant literature, investigates how after locus coeruleus activation, the functional connectome reconfigures itself in that brain-wide functional connectivity promptly increases and interestingly, such connectivity changes tend to correlate positively with adrenergic receptor distribution. **[72]** This is telling information given that adrenergic receptors bind selectively to the catecholamines norepinephrine and epinephrine. **[76]** To put this matter more precisely, LC activation drastically shifts the brain's connectivity, exerting a especially significant impact on salience processing and amygdala networks, pointing to increased arousal activity related to the LC-NE system. In the mentioned study from Zerbi, et al., an innovative approach deemed "chemo-connectomics," which integrated activation via chemogenetic technology and resting-state fMRI, was utilized on a mouse model. **[72]** It is quite possible that similar results would be garnered from the application of such a method on humans, though a clinical test or this manner has not yet been looked into. It would be advisable for future research to look into how chemogenetic reconfiguration of the LC could be a viable therapeutic for non-motor PD symptoms.

Apart from reconfiguration, there is another manner in which chemogenetics is able to ameliorate parkinsonian dysfunction by way of the LC. In a research study conducted by Jovanavic, et al., one of the principal objective taken up by the researchers was to evaluate the manner in which central norepinephrine-producing neurons may be involved in Parkinson's by

way of chronic chemogenetic stimulation of catecholaminergic neurons in the LC. **[77]** This study demonstrated firstly, that norepinephrine neurons send intricate axonal projections to dopaminergic neurons in the substantia nigra, one of the primary brain regions implicated in PD, and secondly, that the increased activity of these norepinephrine neurons has actually been found to be a deterrent of dopaminergic neuron depletion in human α-syn A53T missense mutation over-expressing mice. Not only this, Jovanavic and colleagues discovered that this higher activity of norepinephrine neurons prevents motor dysfunction in the mice. Though elevated norepinephrine neuronal activity does not have the ability to ameliorate α-synuclein aggregation and microgliosis in the substantia nigra, the aforementioned results of the experimentation do support the existence of a process in which the elevation of LC norepinephrine neuronal activity protects dopaminergic neurons, thus promoting the survival and preventing the loss of these neurons in the face of synucleinopathy. These findings are promising in the fact that they suggest that the consistent activation of LC norepinephrine-producing neurons by chemogenetics could be tested as a future therapeutic strategy to combat the destruction of dopamine (DA) neurons due to α-synuclein aggregation. Although these results are not conclusive, it is reasonable to conclude that the locus coeruleus is a chemogenetic target that is highly relevant to the alleviation of non-motor symptoms and the protection of DA neurons.

5. An Extension of Current Chemogenetic Targets

5.1. Modulation of Metabotropic Glutamate Receptors

Metabotropic glutamate receptors (mGluRs; members of class C G-protein-coupled receptors), have been found to exhibit irregular patterns of expression in neurodegenerative diseases such as Parkinson's. The issue that arises due to increased mGluR expression in PD is that not only does overexpression of mGluRs cause the poisoning of dopaminergic neurons in the substantia nigra, but the increased glutamate expression can lead to neuronal cell death and degeneration due to an excess concentration of Na+ and Ca2+ molecules. **[78]** Group I mGluRs, specifically mGluR1 and mGluR5, have been connected to PD due to their presence within basal ganglia structures, with an especially high expression in the globus pallidum (GP), substantia nigra pars reticulata (SNr), and striatum. Upregulation of mGluR5 expression has been seen in both human and animal models of PD, and has been shown to have a directly corresponding relationship to α-synuclein (αS) aggregation in the brain. Various research studies, all of which were conducted using animal models, have indicated that pharmacological inhibition by glutamatergic antagonists or negative allosteric modulation of group 1 mGluRs have been shown to protect dopaminergic neurons and lessen dyskinesia. **[79][80][81]** Likewise, these studies determined that targeting mGluR5 enables the mitigation of motor and/or cognitive impairment caused by PD. As can be identified from the information outlined by this 2022 journal publication from Azam et al., **[78]** the antecedently discussed Group I mGluRs provide researchers with valuable targets to be considered for therapeutic interventions using chemogenetics, as the manipulation or inhibition of this class of GPCRs using DREADDs

technology could be an advantageous symptomatic treatment for PD with the ability to generate significant improvement in motor and cognitive impairments.

5.2. Modulation of the Dopamine D1 and D2 Receptors/Activation of D1/D5 (D1-like) Dopamine Receptors

A further receptor subset that is conducive to our discourse surrounding receptors in the striatum are D1/D5 (D1-like) dopamine receptors. The D1 subtype of these receptors are most abundantly expressed within the striatal neurons, **[82]** however D5 receptors are found most plenteously not only in the striatum, but in the substantia nigra-pars compacta, hypothalamus, cerebral cortex, nucleus accumbens and olfactory tubercle as well. **[83]** Dopamine agonists are the existing drugs that bind to dopamine receptors and are used clinically to treat motor symptoms of PD, however, these drugs often cause adverse nonmotor side effects. In a 2023 review article by Isaacson, et al., it is asserted that the activation of D1/D5 (D1-like) receptors may enable the robust activation of motor function while circumventing adverse side effects that are commonplace to dopamine receptor D2/D3 (D2-like) agonism, which is currently the standard for approved dopamine agonist treatments. Levodopa, which has been reviewed in an earlier section, is the most common dopamine agonist in use clinically.

Due to the side effects brought about by current dopamine receptor targeting treatments, it is pressing to introduce a comparable treatment that is able to induce similar ameliorating effects without any adverse effects occurring alongside those benefits. This is exemplified by an excerpt from this article titled, “Dopamine agonists in Parkinson’s disease: Impact of D1-like or D2-like dopamine receptor subtype selectivity and avenues for future treatment,” by Isaacson, et al., where the authors close their discussion with the assertion, *“In conclusion, there remains a major unmet need to identify novel medications that provide robust (levodopa-like) efficacy without increasing risk of dyskinesia and motor fluctuations, and without the neuropsychiatric adverse effects attributable to D2/D3 receptor activation.”* **[82]** Though the authors in this case suggest D1/D5 receptor selective partial dopamine agonists as the clear solution to this research gap, chemogenetics, particularly DREADDs, would be an effective path to providing a solution that is effective and reversible while avoiding negative nonmotor and/or motor side effects that could occur during treatment with levodopa or comparable dopamine agonists. **[82]** However, these particular sites have not yet been targeted in any chemogenetic experiments with the goal of ameliorating PD symptoms or progression. In the future, it may be prudent to determine whether the utilization of chemogenetics is able to reduce adverse effects compared to dopamine agonists and whether D1-like receptors are truly the superior option over D2-like receptors when using DREADDs.

5.3. The Role of Striatal Myf5 Cells

A 2019 thesis from Yeo (UC San Diego) details the instrumental role of striatal myf5 cells in an induced mouse model of PD, and how the insight provided by this model may help provide a basis for the investigation of the striatum and Myf5 cells in Parkinsonian symptoms and pathology in the human brain. **[6]** The mouse model was genetically edited employing the Cre/loxP system to exhibit Myf5-Cre positive, homozygous floxed CDK5.

In this study, the researchers began producing a genetically modified animal model with Parkinsonian symptoms by knocking out a CDK5 gene from the Myf5 cell populations of the organism. It was found that the mutant model exhibited a tremor event at around 18-20 Hz, while its wildtype counterpart displayed a peak at only 10-12 Hz. Likewise, the mutant tremor-displaying animals were found to show a hyperactivity in locomotion, an increase in motor coordination, and a reduction in muscular strength when compared to the wildtype animals.

Following this, the researchers compared the manipulation of striatal Myf5 neurons and that of D1 and D2 MSNs in RGS-Cre animals. First, they utilized chemogenetic DREADD technology in order to activate the striatal Myf5 positive neurons, which effectively imitated a phenotype of resting tremor, one of the most conspicuous symptoms of PD. These results were further supported by the use of two additional methods; the investigators produced in-vitro recordings of DREADD-injected, Myf5-Cre positive organisms as well as made use of the comparable optogenetics method. Furthermore, when looked at in terms of striatal D1 and D2 medium spiny neurons, the researchers found that the mutant animals with CDK5 cKO containing Myf5 cells had a greater quantity of hyperactive D1 MSNs compared to D2 MSNs in a striking ratio of 2:1. These numbers are significant due to the contrasting roles of MSNs based on the type of dopamine receptors they possess. **[84]** MSNs are projection neurons of the striatum that are connected to motor control and both direct pathway MSNs and indirect pathway MSNs are central locations from where abnormal neural signals related to parkinsonism are initiated. **[85][86]** While the D1 MSNs have a projection that targets into the direct pathways that drive action initiation, D2 MSNs project into indirect pathways that drive inhibition. Given that the author found a significantly higher amount of reactivity in the D1 MSNs, it is logical to conclude that the animals may have had an elevated level of motor activity. In accordance with these such findings, it was ascertained that equivalently exciting both D1 and D2 MSNs by way of DREADDs in RGS-Cre animals did not accomplish the generation of Parkinsonian motor dysfunctions.

Therefore, it is apparent that Myf5 neurons and the imbalance between direct and indirect pathways related to MSNs may play a role in the development of motor-related symptoms of Parkinson’s Disease, ergo pointing to Myf5 neurons, and perhaps to D1 and D2 MSNs to a certain extent, as viable targets for future studies related to chemogenetics as a therapeutic for PD.

5.4. Restoration of Subthalamic Nucleus Activity for Akinesia and Dyskinesia Amelioration

A chief contributor to the development of akinesia, dyskinesia, bradykinesia, and rigidity in Parkinson’s disease is the synaptically-driven synchronization of subthalamic nucleus (STN) neuronal activity **[87]**. A variety of experimental techniques, such as electrophysiological, optogenetic, chemogenetic, genetic, 2-photon imaging, and pharmacological methods were employed in order to reveal that the autonomous activity of STN neurons, in opposition to synaptic synchronization of the same neurons, appeared to be downregulated in both

toxin-induced and genetic mouse models of Parkinson's. It was discovered that the the loss of autonomous spiking within the STN in PD is due to the elevated transmission of D2-striatal projection neurons instigating an increase in both the activation of NMDA receptors and the production of reactive oxygen species that upregulate KATP channel opening in the STN. McIver and colleagues concluded their investigation by reporting the results they found when using chemogenetics to restore autonomic activity in STN neurons. It was uncovered that restoring autonomous firing of neurons in the STN lessened synaptic patterning and ameliorated PD-induced motor dysfunction such as akinesia and dyskinesia. As evidenced by these results, chemogenetically stimulating an increase in autonomous neuronal STN activity appears to be an effective method of alleviating motor symptoms of PD that may come in useful as a clinical therapeutic strategy.

On the other hand, the STN is a very commonly targeted area in the presently prevalent PD intervention of deep brain stimulation (DBS.) The STN boasts a myriad of benefits as a symptom-alleviating therapeutic target, however, there is an ongoing debate in the world of DBS about whether the STN or the globus pallidus internus (GPi) region is a more beneficial and practical target area for the application of DBS. In theory, it may be equally, if not more beneficial to focus on the GPi as a target of chemogenetic modulation. Apart from mentions of economic benefits, differences in programming, and side effects, there are a handful of motor differences exhibited between DBS stimulation of the STN versus the GPi. **[88]** Ramirez-Zamora and colleagues point out that while both areas show equal benefit in ameliorating bradykinesia, rigidity, tremor, and general quality of life, only the STN shows a potential mild benefit in nonmotor cognitive domains, while only the GPi displays a potential benefit in gait, a particular potential benefit for *brittle* dyskinesia, and a possible increased benefit for general dyskinesia when compared to the STN. Similarly, Williams et al. also assert that while the STN stimulation is more flexible and economically favorable, GPi stimulation provides more robust dyskinesia suppression **[89]**. Overall, it is evidenced that while DBS of both the GPi and STN have their disparate advantages, they still have their faults, as was mentioned in the earlier section regarding current PD treatments. For this reason, it would be favorable to delve further into how chemogenetics may function as an alternative to DBS for STN and GPi regions.

In this manner, I suggest the comparative investigation of STN and GPi targets using chemogenetic DREADDs technology. Though McIver and others' paper effectively outlines the use of chemogenetics on the STN to alleviate motor symptoms, there lacks yet a comparison of the employment of chemogenetics in a therapeutic manner between the STN and GPi, or conversely, an investigation into the GPi alone as a therapeutic target for chemogenetics in a clinical setting. Despite these shortcomings, the above findings from McIver et al. are incredibly promising and must be carried on into future studies. As of now, chemogenetics are proving to be a much less invasive alternative to DBS in the STN with many less possible negative side effects, so factors such as feasibility, cost, ethics, and applicability in human patients must be looked into to determine its true clinical usability.

5.5. Modulation of Nrf2/HO-1 Signaling Pathway

A 2021 review article from Wang et al. summarizes a series of studies that point to different bioactive compounds as being able to activate the transcription factor nuclear factor erythroid 2-related factor (2Nrf2)/antioxidant response element (ARE), thus ameliorating PD-associated neurotoxin, a phenomenon that has been seen in both animal models and in tissue culture. **[90]** This is due to the common existence of oxidative stress (OS) as a central culprit in the progression of PD through dopaminergic degeneration. Antecedently, we defined OS as being, “a phenomenon caused by an imbalance of free radicals, particularly reactive oxygen species (ROS), and the capacity of a cellular system to detoxify these reactive products.” According to said 2021 review from Wang et al., Nrf2 has been found to exert anti-inflammatory effects as well as modulate both mitochondrial function and biogenesis. The authors point out that the Keap1/Nrf2/ARE pathway is becoming a cogent candidate for a target in relation to therapy for neurodegenerative diseases such as Parkinson’s. Nrf2 in particular is a basic region leucine-zipper (bZIP) transcription factor that carries out tasks such as orchestrating the cytoprotective pathway **[91]**, coordinating the genetic expression of a handful of protective genes containing antioxidant response elements (AREs) within their promoters that serve in restoring homeostasis after countering OS **[92]**, as well as antioxidant and detoxifying enzymes They then elucidate that in Parkinson’s in vivo or in vitro models induced by experimental the neurotoxins 6-OHDA, MPP^+, MPTP, paraquat, and rotenone, the Nrf2/ARE shows significant resistance to PD-associated neurotoxicity, and that the activation of Nrf2 by way of pharmacological compounds was ascertained to exert neuroprotection on affected neurons. Likewise, a deficiency of Nrf2 was evidenced to elicit augmented neurotoxicity to aforesaid neurotoxin. Accordingly, it is suggested by Wang and coauthors that based on a breadth of published literature regarding this topic, it is apparent that the the activation of Nrf2 is able to mitigate the harm in produced by PD-related neurotoxin-induced neurotoxicity when activated either before or coincident with exposure to neurotoxin. Despite the fact evidence is not provided by the authors on whether or not the activation of Nrf2 has any considerable benefit if activated subsequent to the exposure to neurotoxin, Nrf2 is continuing to be studied as a possible treatment for PD and is becoming quite a strong candidate for the task. Though not explicitly implied by the article, it is crucial to add that with the use of *chemogenetics*, it may be possible to noninvasively impart activation upon Nrf2 as a therapeutic for Parkinson’s influenced by the presence of OS.

Though Nrf2 has *not* yet been studied with chemogenetics in regards to the amelioration of PD and its symptoms, based on the findings from the aforementioned studies, the Nrf2/HO-1 signaling pathway may be tenable option for futures studies, of course starting with experiments utilizing either in vitro tests or in vivo animal models. It may be possible to ameliorate the negative impacts of OS in PD through the modulation of Nrf2 and its associated pathways.

From this point, the Heme oxygenase-1 (HO-1), a metabolic antioxidant enzyme regulated by Nrf2 **[93]**, is elaborated on in more detail. Precisely, HO-1 functions by degrading heme, an iron-coordinated tetrapyrrole molecule that is involved in a range of biological processes including respiration, oxygen metabolism, oxygen transfer, photosynthetic electron

transportation, and most importantly in this particular case, oxidative stress responses, **[94]** into the products of carbon monoxide, free iron, and biliverdin. Most crucially, HO-1, which has in fact been observed in elevated concentrations in serum of PD patients, **[95]** plays a key neuroprotective role in allaying OS-dependent damage, thus designating HO-1 as a possible novel therapeutic target for PD. Wang and colleagues point out that the induction of HO-1 has been evidence exert neuroprotective properties against the exposure to a medley of PD-related neurotoxic compounds in both animal models and tissue culture, and that the pharmacological induction of HO-1 has been shown to have therapeutic effects on the aforementioned experimental neurotoxins 6-OHDA, MPP^+, MPTP, paraquat, and rotenone within *in vivo* and *in vitro* models of Parkinson's.

As HO-1 is regulated by Nrf2, it may be useful in future investigations to look into the pathways of Nrf2 and concentrations of HO-1 may me chemogenetically manipulated to exert an ameliorating effect in relation to OS and neurotoxin-related damage in cases of PD. Since oxidative stress and a surplus of ROS, and correspondingly a neuroinflammation and environmental factors, are thought to play a role in both familial and sporadic forms of PD, **[96]** it is crucial to investigate novel solutions that can help alleviate neurotoxicity and OS in the brain. Though we do not yet have knowledge of particular GPCR targets for DREADDs within the Nrf2/HO-1 signaling pathway, the next actionable step would likely be uncovering a particular cellular target affecting Nrf2 and HO-1 with the goal of upregulating antioxidant protection to combat OS and the surplus of ROS. At this point, these targets have not been investigated using chemogenetic investigation methods, nor have they been considered as a therapeutic target for chemogenetic neuromodulation, but with further *in vitro* and *in vivo* investigation, it may be determined whether or not such an approach would be beneficial for clinical settings in the future.

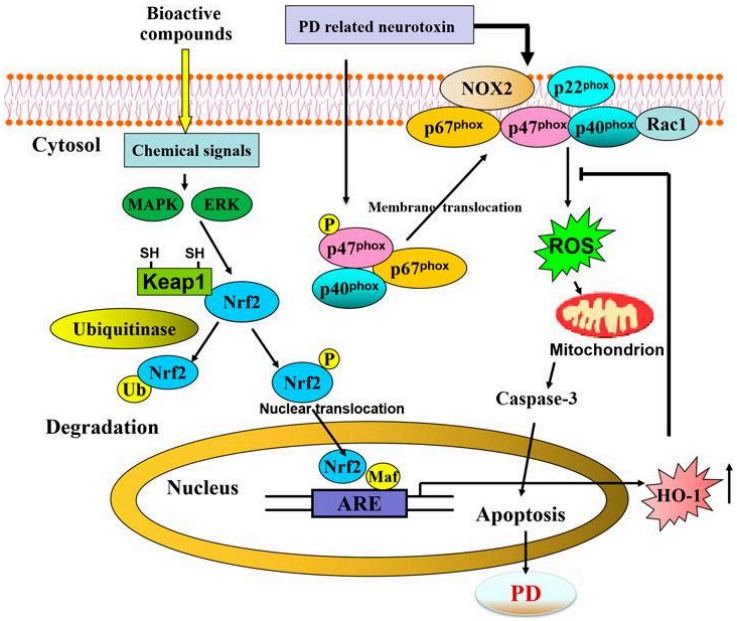

Figure 8. Wang et al.'s schematic representation of bioactive compounds-mediated neuroprotective against PD through activating Nrf2/ARE/HO-1 pathway. DOI: https://doi.org/10.3389/fphar.2021.757161

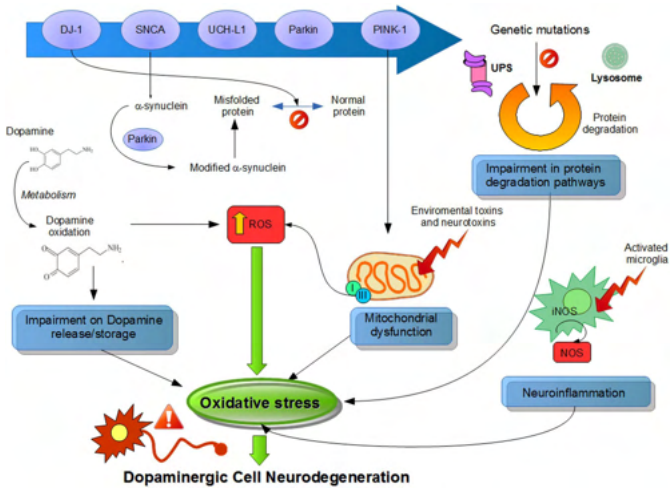

Figure 9. Blesa et al.'s suggested physiological processes related to pathogenesis of Parkinson's disease (PD), in particular how these processes are related to oxidative stress: https://doi.org/10.3389/fnana.2015.00091

5.6. Modulation of GABA neurotransmission in relation to TGF-β/Smad3

Yet another suggested chemogenetic target in parkinsonism is affecting the mechanism of the transforming growth factor β (TGF-β)/Mothers against decapentaplegic homolog 3 (Smad3) intracellular signaling, which has been discovered to modulate γ-Aminobutyric acid

(GABA) signaling in relation to PD. It has been observed that in Parkinson's disease, there is an augmentation of GABA neurotransmission that is believed to actuate bradykinesia and l-DOPA-induced side effects. GABA and GABAergic neurons occur in a plethora of locations within the brain, particularly within the basal ganglia, and as a result are widely implicated in the pathology of PD. **[97]** GABA is highly prevalent in PD-implicated brain nuclei such as the basal ganglia and hippocampus. In fact, the basal ganglia, which includes the striatum, glucose pallidus, subthalamic nucleus, substantia nigra, and midbrain, is composed primarily of GABAergic neurons, which is key given the fact that this collection of subcortical nuclei is altogether involved a range of functions hindered in PD, including voluntary movement and motor control, executive functions and behaviors, cognition, reward and aversion, and mood and emotional regulation. **[97] [98] [99]** TGF-β/Smad3 specifically is also said to be correlated with a handful of pathological features occurring in PD, including dopaminergic degeneration and the the decline of dopaminergic axons and dendrites, as well as alpha-synuclein aggregation.

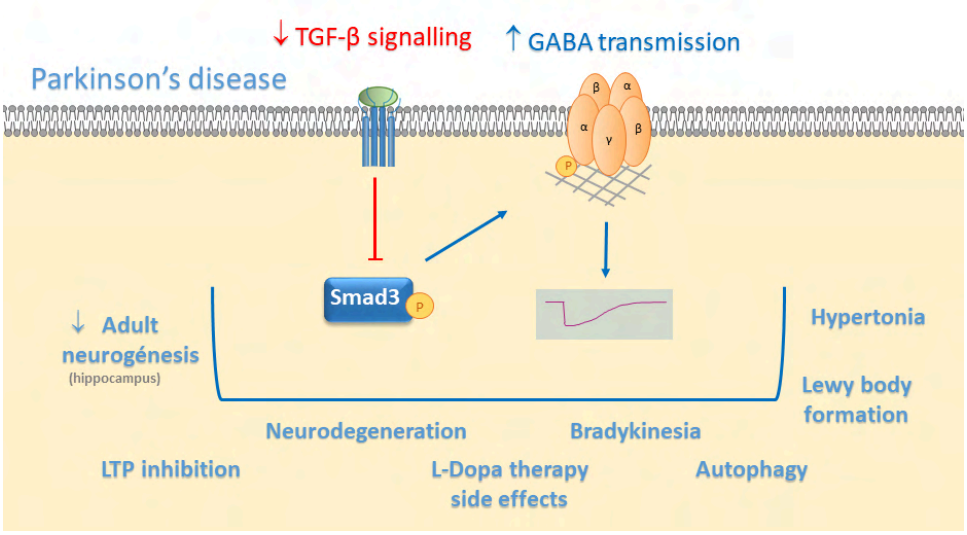

Figure 10. Muñoz, et al.: Graphical Abstract: TGF-β/Smad3 Signalling Modulates GABA Neurotransmission: Implications in Parkinson's Disease: Muñoz, M. D., De La Fuente, N., & Sánchez-Capelo, A. (2020b). TGF-B/SMAD3 signalling modulates GABA Neurotransmission: Implications in Parkinson's Disease. International Journal of Molecular Sciences, 21(2), 590. https://doi.org/10.3390/ijms21020590 [97]

In terms of the modulation of TGF-β/Smad3 signaling and its relation to PD and its symptoms, there are many functions that are thought to contribute to PD-related pathological characteristics. For starters, the extracellular growth factor TGF-β1 is significantly upregulated in both the striatal regions and in the cerebrospinal fluid (CSF) of PD patients, and chronic overexpression of this cytokine may play a part in exacerbating the disease pathology, though deficiencies in TGF-β1 signaling have been proven to have a link to the developments of a number of brain disorders. Similarly, a deficiency of Smad3 in mice models has been shown to

lead to the developments of α-synuclein aggregates as well as dopaminergic and hippocampal dysfunction. Beyond this, various studies have explored other interactions between TGF-β/Smad3 and α-synuclein, demonstrating that α-synuclein oligomers, which are a class of neurotoxic α-synuclein that occurs in PD, induce striatal TGF-β1 secretion with the goal of protecting them from PD-associated neurotoxicity.

From a chemogenetic perspective, it would firstly be necessary to investigate the precise workings of TGF-β/Smad3 signaling in relation to PD, since although an abundance of investigations have definitively linked this target to PD pathological features such as α-synuclein aggregation, the specifications of the pathways and mechanisms used by TGF-β/Smad3 to either exacerbate or alleviate PD pathology is not quite clear. Given that through such investigations the most advantageous manner of chemogenetically affecting TGF-β/Smad3 can be decided, it would thereafter be prudent to bring this area and its role in GABA neurotransmission into the equation of PD-ameliorating chemogenetic therapy possibilities. As can be seen from this information, the promise of TGF-β/Smad3 is rather preliminary, so extensive studies must be conducted for it to be validated as a realistic clinical target.

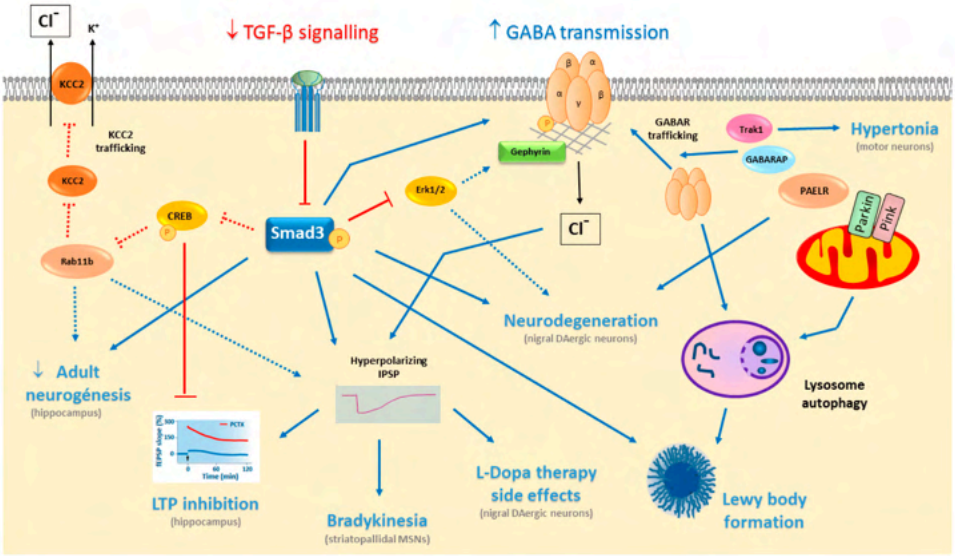

Figure 11. Muñoz, et al.: Illustration of the working model of TGF-β signaling and GABA neurotransmission interaction in the context of PD. Arrows indicate induction and T-bars inhibition. Dotted arrows and dotted T-bars suggest possible interactions, not yet shown experimentally and derived from the bibliographic analysis. Muñoz, M. D., De La Fuente, N., & Sánchez-Capelo, A. (2020b). TGF-B/SMAD3 signalling modulates GABA Neurotransmission: Implications in Parkinson's Disease. International Journal of Molecular Sciences, 21(2), 590. https://doi.org/10.3390/ijms21020590 [97]

5.7. P2Y12R Inhibition to Influence PD Progression

In a 2022 paper authored by Iring, et al., the dualistic role that the purinergic P2Y12-receptor, a member of the G-protein coupled subfamily of nucleotide receptors, plays in

Parkinson's Disease is explored with the use of an in-vivo rodent model. **[100]** Though the P2Y12R ATP receptor has been heretofore recognized for its appearance on the microglia in the CNS and linked to the regulation of microglial activity and responses, it was not yet clear to scientists how its function might be altered in PD. The aim of this research project was therefore to clearly establish the role of P2Y12Rs related to Parkinsonian symptoms, neuroinflammatory changes, and their activation of microglia, ultimately pointing to their potential as a target site for PD-ameliorating therapies. The highlights of the authors' findings surrounding the Gi-coupled P2Y12-receptor's specific roles in relation to PD include, in general, how the inhibition of P2Y12R could be employed as a means of protecting against neurodegenerative cell loss during Parkinsonism and the regulatory role p38 MAPK activity via ROCK1 plays in reducing pro- and anti-inflammatory cytokine production.

A 2014 journal article published in Wiley's *GLIA* from Tatsumi, et al. elaborates on the RhoA/ROCK pathway and its mediation of p38 MAPK activation and morphological changes downstream of P2Y12/13 receptors in spinal microglia, emphasizing how manipulation of the ROCK pathway is a viable candidate for the alleviation of neuropathic pain. **[101]** Though this research study was not explicitly focused on these protein kinases' role in *Parkinson's*, the related report on neuropathic pain amelioration via this pathway provides us with invaluable insight into how the protein kinases ROCK and p38 MAPK as well as the P2Y12R receptor may be involved in the pathology of certain symptoms of PD related to pain and peripheral neuropathy. On a similar note, this evidence also provides us with insight into the role of cytokines within PD and how we may be able to regulate these factors. Because immune system dysfunction has been evidenced to play a role in the perpetuation of neurodegeneration in PD, **[102][103]** it may prove useful to investigate more deeply into the how the levels of cytokines, which are generally known for their role in controlling inflammation and the body's response to cellular damage, can be modulated for the purpose of preventing the progression of neurodegeneration and its symptoms.

On the effects of inhibition of the Gi-coupled P2Y12-receptor on PD, the author concludes, "Blockade of P2Y12R is harmful in the acute phase of MPTP-induced Parkinsonism, presumably due to the impaired phagocytic activity of activated microglia; but prevents MPTP-induced dopaminergic neuron loss and the development of Parkinson's disease at later time points. Furthermore, inhibition of the receptor abrogates disease progression, reduces motor function impairment and mitigates neuronal cell death." To this extent, it is shown that further investigation of the mechanisms and products of P2Y12R signaling in the CNS is pharmacologically relevant and certainly advisable in order to influence, and most ideally significantly impede, disease progression during Parkinson's Disease.

5.8. Regulation of GPR143 in Relation to L-DOPA

The receptor GPR143 (OA1), also known as human G protein-coupled receptor 143, which is the gene product of ocular albinism and is known to be widely expressed in the central and peripheral nervous system, has an ostensible link to l-3,4-Dihydroxyphenylalanine (l-DOPA) as a receptor candidate, as reported by Goshima, et al. in a October 2019 mini review published

in Frontiers in Pharmacology **[104]**. The authors concluded that GPR143 immunoreactivity is colocalized with phosphorylated α-synuclein within Lewy bodies in PD-affected brains, thus suggesting that GPR143 may contribute to the therapeutic effectiveness of l-DOPA in Parkinson's and might be related to pathogenesis of PD. GPR143 is a prevalent receptor in the nervous system that, in general, serves a range of purposes beyond its implications with l-DOPA and PD pathogenesis. This receptor is most widely recognized for its involvement in the encodement of a protein targeted to melanosomes in pigment cells **[105]**, or its expression in retinal pigment epithelial cells **[106]**, however, in this review from Goshima, et al., it is reported that changes in the properties, such as expression and sensitization, of GPR143 may be seen during the progression of PD, and that GPR143 may be involved in the pathogenesis of PD. It was discovered in a disparate, previous research study from 2018 by Goshima, et al., that GPR143 was colocalized with phosphorylated α-synuclein in Lewy bodies in PD-affected brain tissue **[104][107]**, and additionally, it was speculated by the author that the accumulation and localization of GPCRs such as GPR143 could be related to occurrences such as the pathogenesis of PD or the efficacy and/or adverse effects of l-DOPA.

In a similar manner, it was observed by this group of researchers that much evidence has pointed to the fascinating similarities held by Parkin-associated endothelin receptor-like receptor (Pael-R), or GPR37, and the aforestated receptor GPR143. Analogous to GPR143, to which GPR37 shows overlapping expression patterns, GPR37 was found to be localized within Lewy bodies, to exhibit poor trafficking to the plasma membrane, and to possess high basal activities. Not only this, but it was uncovered in a 2017 paper by Leinartaite and Svenningson that that the overexpression of the receptor GPR37 resulted in the death of dopaminergic neurons **[108]**, although a distinct piece of research from 2013 by Meyer, et al. concluded that GPR37 contributes to the signaling of certain neuroprotective factors, thus pointing to the quite intriguing juxtaposing role GPR37 might play in the pathology of PD. **[109]** On the whole however, there is not yet enough information regarding the relationship of this receptor to the mechanisms of PD to designate it as an ideal target for the use of DREADDs in translational clinical medicine. Nonetheless, it would likely be desirable to advance research on this receptor by viewing its activity and properties through the tool of chemogenetics, and later assessing its therapeutic potential as a target.

From the previously discussed evidence, we are able to surmise the possible potential of receptor GPR143 as a chemogenetic target for the amelioration of PD and its symptoms. GPR143 in this sense appears to be a suitable candidate for chemogenetic neuromodulation using the DREADDs toolkit, and its sister receptor GPR37 may also prove to be a worthy target upon further investigation.

On the contrary, a component that points to the dubitability of this argument is the fact that GPR143 is an Orphan G protein-coupled receptor (oGPCR), meaning it falls into the classification of GPCRs that have either endogenous ligands and/or downstream signaling pathways that are, at present, unknown **[110]**. Likewise, in relation to L-DOPA as a neurotransmitter, the specified downstream signaling pathways triggered by L-DOPA in vivo are

currently unknown, therefore leaving our knowledge of GPR143 as a possible PD-associated receptor quite uncertain. **[104][105]** Moreover, this study failed to identify whether GPR143 is truly certain to be involved in the pathogenesis of PD, and whether GPR143 the only functional receptor for L-DOPA, given that the mechanism of L-DOPA as a neurotransmitter is, for the most part, exceedingly murky.

Overall, further investigation of the regulation of GPR143 in relation to the reception of L-DOPA may be a an advisable route for scientists to explore in order to allow for the expansion of chemogenetic techniques in translational and eventually clinical neuroscience, although the first actionable step would presumably be taken by carrying out investigations concerning the mechanism and downstream signaling pathways of GPR143, and how this information may affect our knowledge of the role of this receptor in regards to the Dopamine precursor L-DOPA.

6. **Potential Gaps in Past Research for Future Investigations**

Comprehensively, this review suggests a plethora of research gaps that may be practical starting points for future studies involving chemogenetic neuromodulation to alleviate PD symptoms and progression:

1. The escalation of therapeutically proven or promising receptor targets, such as the autonomous subthalamic nucleus (STN), GABAergic neurons in the zona incerta (ZI), M2 and M3 muscarinic acetylcholine receptors (mAChRs), orexin (or hypocretin) neurons, nicotinic acetylcholine receptors (nAChRs), specifically α7-nAChRs, the pedunculopontine nucleus (PPN), dopamine neurons in the SNpc, and locus coeruleus norepinephrine system (LC-NE) neurons, to clinical trials following further testing.
2. The amelioration of elevated physical activity and energy expenditure in PD-affected mouse models via an alternate strategy for CNO DREADDs to inhibit orexin neurons.
3. The chemogenetic inhibition of Gi-coupled P2Y12-receptor signaling in PD-affected preclinical animal models with the goal of impeding disease progression.
4. The chemogenetic regulation of more uncertain targets including GABA neurotransmission in relation to TGF-β/Smad3, receptor GPR143 (OA1), also known as human G protein-coupled receptor 143, striatal Myf5 cells, and the Nrf2/HO-1 signaling pathway. These targets may require additional experimentation compared to others.
5. Further in the future, once the safest and most effective targets for therapeutic chemogenetics are recognized, clinical trials applying these successful strategies to PD patients.

This list may not be exhaustive, however, the aforementioned targets and research gaps provide an actionable point of reference for what future investigation may be necessary for the eventual utilization of chemogenetics systems as a clinical therapeutic for PD. In a 2021 article from Sternson and Bleakman, the authors outline the 5 criteria that the first therapeutic applications of chemogenetics are likely to meet, and these criteria serve as suitable guidelines to consider in the process of identifying a target to be approved for clinical trials. **[20]** Firstly, it is noted that a localized disease focal point that will provide clinical benefit must be identified.

Within this review, a plethora of particular Parkinson's disease focal points were discussed along with the benefit modulating these regions would provide clinically in terms of symptoms or disease progression. The second criterion mentioned is that the first therapeutic applications may be disorders of either hypo- or hyper-excitability that are also well suited for the mechanism of action of chemogenetic receptors, which is either an engineered GPCR or LGIC paired with an engineered ligand, usually an otherwise inert small molecule drug. The authors specified the third characteristic as being the existence of a significant unmet medical need necessitating the introduction of a novel method of treatment, while the fourth characteristic delineated is that there are existing surgical procedures that are encompassed within the standard care that can be adopted for targeting the affected tissue with adeno-associated virus (AAV), a common viral vector for gene therapy and chemogenetics. The fifth and final necessity in this set is the requirement for tunable control over the activity over the activity in the affected tissue (the chemogenetic target) by adjusting the dosage of the chemogenetic agonist. **[20]** Since chemogenetics is a modality that can be easily manipulated based on clinical needs, it would be ideal for such a type of application. This description from Sternson and Bleakman, I believe, provides adequate and actionable guidelines for the introduction of chemogenetics as a therapeutic tool.

7. Conclusion

It has been over two centuries since the publication of James Parkinson's "An Essay on the Shaking Palsy," and along with it, the discovery of PD. Currently, it is estimated that there are over 10 million people living with PD worldwide, and the cost of treating PD is estimated to be upwards of $14 billion annually in the United States alone. **[6][111][112]** The central hallmarks required for PD diagnosis are its motor symptoms, in particular tremor at rest, rigidity, akinesia or bradykinesia, and postural instability. **[9]** At the moment, the most common and cutting-edge treatments available for PD are dopamine replacement therapy and deep brain stimulation, both of which have a host of their own side effects and risks. For this reason, I suggest chemogenetics such as DREADDs and PSAM/PSEM systems as potential therapeutic methods due to their specificity, reversibility, and lack of off-target effects. As of 2024, chemogenetics has not yet been utilized on humans in a clinical setting to treat, target, or cure any neurodegenerative or psychiatric diseases. On account of this research gap, I have organized past literature on chemogenetic targets into two principal sections within this review. Firstly, we discussed what I have called existing targets, or targets that have been shown experimentally to reap benefit from chemogenetics in PD animal models, though not necessarily intended in a clinical manner. The second major section consisted of an extension of current chemogenetic targets, or targets where experiments were carried out in which chemogenetics was either not a central conclusion of the paper or was not present in said experimentation, meaning that although these targets *may* not have yet been targeted using chemogenetics with the intent of symptomatic or progressive amelioration, they are regions that I deem promising for chemogenetic experimentation due to their roles and properties relative to PD symptoms and progression. Overall, in comparison to current treatments such as deep brain stimulation (DBS)

and dopamine replacement therapy (DRT), both treatments that come with side effects like dyskinesia in DRT and mild gait and/or speech disturbances in DBS, chemogenetics may provide some clinical advantages due to its non invasiveness, reversibility, specificity, and close manipulability. Further preclinical trials must be carried out to determine the viability, risks, and benefits associated with modulating each target.

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