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The Chemogenetic Application and Biotherapeutic Potential of Designer Receptors Exclusively Activated by Designer Drugs for Parkinson's Disease

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Abstract

Designer Receptors Exclusively Activated by Designer Drugs, or DREADDs, is a chemogenetic technique which, in recent years, has risen to prominence as a way to activate or deactivate targeted brain areas. This has granted scientists access to new tools in exploring treatment options for several neurodegenerative conditions, including Parkinson's Disease. This review investigates how Parkinson's research has been shaped by DREADD-based techniques, and provides summaries of results found by this method. This includes links between Parkinson's and orexin neurons, α-Synuclein, along with deep brain stimulation and biotherapeutics. The goal of this review is to describe a chemogenetic technique which is vital to several recent studies, discuss its possible limitations, and provide a picture of the path and applicability of the technique moving forward.

Introduction

Chemogenetics is a scientific process where macromolecules, engineered by scientists, precisely target cellular pathways in specific cell populations. Chemogenetics originates from a 1998 study in which researchers created G-protein coupled receptors (GPCRs) that respond only to Receptors Activated Solely by a Synthetic Ligand, or RASSLs (Tocris Bioscience 2022). In that case, the RASSL was based on the human κ opioid receptor (Coward et al. 1998).

Chemogenetic techniques are usually applied within neurons, turning them on or off like a switch via the use of a ligand. *In vivo* expression of a chemogenetic receptor is possible using an Adenovirus encoding the designer receptor, which is then injected into the target brain region. Following this, cell-specific genetic promoters control the cell type that the receptor is expressed in. The technique allows scientists to investigate specific cellular pathways, and collect data on how treatments or disease may affect these pathways and their surroundings. Chemogenetics differs from the similarly named optogenetics, with the latter activating or inhibiting neuronal activity via implanted fiber optics instead of small molecules.

Designer Receptors Exclusively Activated by Designer Drugs, or DREADDs, is one such chemogenetic technique that has been among the most commonly used approaches for transient activation or inactivation of targeted brain areas. In effect, DREADDs use receptor proteins which have been synthetically derived from mutagenesis of endogenous G protein coupled receptor DNA to create synthetic receptors, which are expressed in neuronal membranes but lack a natural ligand to activate them (Smith et. al. 2016). Yet these receptors are sensitive to certain exogenous ligands, like clozapine-n-oxide (CNO). CNO excites neurons that express the chemogenetic receptor protein hMD3q and inhibits the chemogenetic receptor protein hM4Di.

Clozapine is an anti-psychosis medication that can induce sedation in subjects, so studies have stressed the importance of using low doses of CNO in chemogenetic experiments in research animals. Historically, clozapine has been viewed as a treatment option for schizophrenia and other psychiatric disorders, despite the aforementioned risk of sedation due to positive feedback from patients. Thus, clozapine has been confined for use in settings such as institutions where patient responses can be closely monitored.

Clozapine activates hM3Dq and hM4Di DREADDs instead of CNO (Peever 2017). hM3Dq and hM4Di are variants of the human M3 and M4 muscarinic receptors, respectively (English 2022). Studies have suggested the designer receptor hM4D can be used in both *in vitro* and *in vivo* for neuronal silencing via Gq. *In vivo* application includes using GI DREADD activation to inhibit spinal nerve transection in rodents, reducing symptoms such as neuroinflammation, synaptic function, and neuropathic pain (Yi et al. 2021).

One such study, and also one of the earliest established for the deployment of a DREADDs technique, involved muscarinic receptors in yeast to create a family of GPCRs that were activated only by the inert compound CNO (Armbruster et al. 2007). Muscarinic receptors are involved with the transduction of cholinergic signals in the central nervous system (CNS) and other parasympathetic end organs, such as the bronchioles and intestinal tract. Cholinergic signals activate cerebral cortex activation during awake states (Luchicchi et al. 2014). The function of GPCRs was assessed by modifying receptors to preferentially bind synthetic over endogenous substrate-ligand: CNO rather than the native ligand acetylcholine (ACh). Previously, studies were limited to loss of function phenotypes, and a drug's non selectiveness led to difficulty with the interpretation of pharmacological studies. The researchers laid the groundwork for DREADDs by demonstrating a GPCR family can be activated by an inert ligand

over ACh and suggesting the designer receptor hM4D can be silenced by the application of CNO (Armbruster et al. 2007).

Parkinson's Disease Overview

Parkinson's disease (PD) is a progressive neurodegenerative disorder which involves the neurotransmitter dopamine in the brain, and leads to both motor and nonmotor symptoms. Examples of the former are things such as resting tremor, bradykinesia, postural instability, and rigidity (Hayes 2019). Nonmotor symptoms include cognitive decline, depression, anxiety, and sleep disturbances (Hayes). PD is the second most common neurodegenerative disease after Alzheimer's, with a low incidence in individuals under 50, but the rate increases significantly with age. The disease affects about 50% more men than women, and has a complex etiology when it comes to determining who is at risk and manifestation of symptoms (Moisan et al. 2016).

PD has a long prodromal phase with several aspects or conditions of a patient's lifestyle relating to the onset of the disease, potentially even 20 years in advance. Recall bias is known to affect studies of this long prodromal period, as patients struggle to recall details such as sleep activity, diet, hyposmia, and physical activity (Ascherio and Schwarzschild 2016). Environmental factors play an important role, as 95% of cases are found to not be due to genetic factors (Dauer and Przedborski 2003).

Pathologically, the depigmentation of the substantia nigra and neuronal loss in the substantia nigra pars compacta and locus coeruleus are defining neurological features of PD (Hayes 2019). PD results primarily from the death of dopaminergic neurons in the substantia nigra, and science currently does not have a definitive solution which explains or halts the death of these neurons (Dauer and Przedborski 2003). Dopaminergic neurons have an important role in

specific brain functions like voluntary movement, and different behavioral functions as well such as mood, stress, addiction, and reward (Chinta and Anderson 2005). Additionally, in PD there is the presence of intraneuronal proteinaceous cytoplasmic inclusions, or “Lewy Bodies” (LB), in the substantia nigra pars compacta (SNpc) (Dauer and Przedborski 2003). The role of LB in neuronal cell death is controversial, but LB has also been observed in Alzheimer's disease.

An important component of LB is the α-Synuclein (α-SYN) protein. It is the product of the first gene identified as associated with PD, *SNCA*. Currently, the normal physiological structure and function of α-SYN is not certain (Xu and Pu 2016). It has roles in storage, and physiological regulation of certain enzymes that could increase the amount of dopamine transporter molecules. There are two hypotheses about the native state of α-SYN. One is that it is a monomeric conformation, and the other is the alpha-helically folded tetramer (Xu and Pu 2016). Furthermore, Braak’s hypothesis is used to describe sporadic PD being caused by a pathogen which enters the body from the nasal cavity and induces LB pathology in the nose and eventually the digestive tract. Clinical and experimental evidence back up Braak’s hypothesis. Gastrointestinal problems such as dysphagia and constipation, and loss of smell have been associated with PD (Rietdijk et al. 2017).

All known mutations associated with familial PD are found in the N-terminal domain of α-SYN, with mutations Glu46Lys, His50Gln, and Ala53Glu being able to promote α-SYN to form insoluble aggregates and create oligomers (Xu and Pu 2016). Experiments with PD are limited by how rodents such as mice and rats do not get the disease. Instead, researchers utilize specially bred mice with mutations to their genetic code in order to induce symptoms of movement disorders after a given amount of time. α-SYN A53T is a prominent example of a transgenic mouse. Mice overexpress mutant α-SYN in the brain at levels which are six times as

high as endogenous mouse α-SYN (Lee et al. 2002). Severe progressive motor impairments are observed about ten months to a year after birth in such mice (Lee et al. 2002). Wobbling and posturing are some of the earliest symptoms, followed by the slowing of spontaneous movement, and end-stage disease is reached about 2 weeks after initial onset of symptoms (Lee et al. 2002).

Parkinson's Disease Treatments

Dopamine replacement therapy (DRT) has been the primary method to alleviate symptoms, though in recent years there has been a growing push to utilize physical rehabilitation treatments in treating PD. DRT can be in the form of levodopa, the precursor to dopamine, dopamine agonists, monoamine oxidase B inhibitors or catechol-O-methyltransferase inhibitors (Ferrazzoli et al. 2016). DRT can restore physiological plasticity of synapses in the striatum but can also cause side effects, which will be investigated later.

Current cell therapy for PD consists of trying to replace the degenerated dopamine neurons and restore their transmission to denervated targets in the forebrain (Bjorklund and Parmar 2021). A limitation of this grafting approach is how the transplanted neurons are placed in the putamen, not the ventral midbrain where they are typically located. Homotopic transplantation into the substantia nigra is now being explored, where grafts of midbrain dopamine neurons (mDA) derived from human embryonic stem cells can re-establish nigrostriatal and mesolimbic pathways in striatal, limbic, and cortical areas. Transplantation has shown promise in rodent models. However, to work in the human brain, the technique must take into account the larger size of the human brain, where the distance between the substantia nigra and forebrain targets are about 10 times the distance in rodent brains (Bjorklund and Parmar 2021).

Newly established connections are found to be functional and restored over a period of 3 to 4 months, with DREADDs also being useful to apply to intrastriatal grafts. DREADD-induced inhibition of grafted dopaminergic neurons was, in rodent models, shown to reverse the graft induced motor recovery, and DREADD-induced activation of dopaminergic neurons causes an opposite effect (Bjorklund and Parmar, 2021).

Patients who have undergone surgery to transplant embryonic DA neurons can develop a condition, known as graft-induced dyskinesia (GID). The condition has been examined by researchers, but there is still a lack of understanding when it comes to its onset. GID has been suggested to be a distinct neurological entity from the more common drug induced levodopa-induced dyskinesia (Steece-Collier et al. 2012). Levodopa is an amino acid which is a precursor to dopamine, and is used to increase dopamine concentrations as treatment for PD. This is because Levodopa can cross the blood-brain barrier, and dopamine itself cannot. One piece of evidence is that GID often presents as stereotypy and hyperkinesia in the extremities, and lowering of the levodopa dose does not alleviate the dyskinesia (Steece-Collier et al. 2012). Stereotypy is the persistent repetition of movement for no reason, aligning with the definition of dyskinesia as the impairment of voluntary movement.

DREADD-Mediated Activation of Orexin Neurons in A53T Mice

Cognitive impairment has been recognized, in recent years, to be several times more likely to occur in PD patients than in control groups. Researchers have experimented with orexin intervention to ease hippocampus-dependent memory impairment resulting from Parkinson’s. Orexin, also known as hypocretin, is produced by orexin neurons mainly located within the lateral hypothalamus (Berthoud and Münzberg 2011). Orexin is involved with several

physiological functions such as behavior, sleep, and physical activity. Orexin deficiency results in learning and memory deficits and the presence of orexin improves memory and spatial learning (Chieffi et al 2017). Drugs used to treat PD also cause selective loss of orexin neurons, postulated to be from receptor-mediated presynaptic silencing of glutamatergic excitatory inputs to orexin neurons (Stanojlovic).

Chemogenetics have been applied in order to better characterize the relationship between PD-related neurodegeneration and hippocampus-dependent memory. Researchers used intracranial orexin delivery to the CA1 region of the hippocampus, then used a chemogenetic approach to activate orexin neurons in the A53T rodent model of PD. Expression of genetically modified DREADDs was targeted to orexin neurons by using viral DNA containing a DREADD inverted open reading frame which was injected into the lateral hypothalamus (Stanojlovic et al. 2019).

Mice were subjected to a Barnes maze and contextual object test to determine hippocampus-dependent memory. Immuno-fluorescence densitometry was used to assess inflammation and astrogliosis markers in the hippocampus (Stanojlovic et al. 2019). Densitometry is the qualitative measurement of optical density in materials which are light sensitive. Cognitive impairment was suggested by behavioral and histological analyses, especially in the hippocampus. Within the A53T mice, non motor impairments were also observed prior to disease onset, in the form of memory loss. Orexin neuronal loss in A53T mice was not observed while the subjects were younger, suggesting more studies need to be conducted before fully defining the relationship between impaired orexin circuitry and function in PD (Stanojlovic et al. 2019).

Researchers also looked at restoring the impairment of orexin system function to ease cognitive impairment within A53T mice (Stanojlovic et al. 2016). DREADD-mediated activation of orexin neurons has been used to improve the performance of A53T mice in a contextual object recognition test, a behavioral assay which measures spatial memory and contextual dependent memory (Stanojlovic et al. 2016). Overall, it is possible that orexin neurodegeneration could emerge in A53T mouse models at later times corresponding with various environmental triggers.

DREADD Stimulation of Striatal Projection Neurons

As mentioned previously, levodopa is often used to treat PD, as it can cross the blood-brain barrier and increase dopamine production and storage in the SNpc. Unfortunately, the majority of PD patients who are treated with levodopa often develop involuntary movements in the form of hyperkinesia and dyskinesia. It is estimated that 80% of levodopa patients develop hyperkinesia or dyskinesia within 10 years of starting DRT (Manson et al. 2012).

Levodopa-induced dyskinesia (LID) can be investigated by examining the activity of striatal projection neurons forming the indirect or direct pathway (Alcacer et al. 2017). Indirect striatal projection neurons (iSPNs) are associated with poverty of movement in PD, while direct striatal projection neurons (dSPNs) are associated with the tremors of PD. Transgenic mice expressing Cre recombinase under the control of signaling pathway-specific promoters have been used to chemogenetically control iSPNs and dSPNs *in vivo*. Adeno-associated viral vectors (AAVs) coding for the activating Gq-coupled human M3 muscarinic receptor hM3Dq DREADD were injected into the dorsolateral striatum (Alcacer et al. 2017).

An open field test was used to study the effects of chemogenetic striatal projection neurons (SPN) stimulation on whole body movements. Activation of iSPNs by CNO notably

decreased horizontal and vertical activity. Opposite effects were observed following M3Dq mediated activation of dSPNs. Chemogenetic stimulation of dSPNs caused larger motor changes in Drd1a-Cre (D1-Cre) and Adora2a-Cre (A2a-Cre) mice versus the control animals (Alcacer et al. 2017). This is in contrast to iSPN stimulation, which did not mitigate motor changes produced by the nigrostriatal lesion. Researchers found that dyskinetic behaviors induced by L-DOPA in a rodent model of PD can be modulated by activating indirect or direct pathway SPNs via the use of hM3Dq DREADD (Alcacer et al. 2017). dSPN stimulation from hM3Dq DREADD was found to have a beneficial, therapeutic-like effect and did not cause dyskinesia (Alcacer et al. 2017).

Activating dSPNs and iSPNs oppositely modulates whole body motor activity in mice subjects, and iSPNs heavily modulate LID (Alcacer et al. 2017). iSPN stimulation attenuates all types of L-DOPA induced involuntary abnormal movements. There are implications that treatments adapting both dSPNs and iSPNs will be more effective in the future compared to pathway-specific interventions in treating PD.

Graft-Induced Dyskinesia and the Serotonin 5-HT6 Receptor

GID is dependent on serotonergic neurotransmission and can be suppressed by drugs targeting autoreceptors on serotonin neurons (Politis et al. 2010). The functional link between the serotonin system and dysregulated DA neurotransmission that causes dyskinesia is still difficult to understand, however. This is because attempts to replicate GID in rodent or primate models of PD have all failed so far.

In one such attempt, a rat model was used in which DA neurons derived from fetal midbrain neuroblasts were selectively modulated for graft activity *in vivo* (Alden-Kirk et al.

2016). Grafted DA neurons displayed an increase in cyclic AMP (cAMP) via activation of Gs-coupled DREADDs, done through stereotactic, intraparenchymal injection of a AAV-8 vector (rM3Ds). The increase in cAMP was associated with GIDs in animals without any L-DOPA treatment (Alden-Kirk et al. 2016). Additionally, the serotonin 5-HT6 receptor was identified as an important activator of DA neurons in the graft, and that selective activation of this particular receptor can cause significant GIDs in a phenotype extremely similar to LIDs in animals unexposed to Levodopa (Alden-Kirk et al. 2016).

In order to better understand endogenous expression of 5-HT6 receptors in DA neurons, human fetal ventral mesencephalon was transplanted to the DA-depleted striatum of immunosuppressed rats (Alden-Kirk et al. 2016). DA neurons from the transplant were found to have a strong expression of the 5-HT6 receptor. Tyrosine hydroxylase (TH), was used as a marker for dopamine and epinephrine containing neurons and endocrine cells (Weihe et al. 2006). The researchers analyzed a brain from a patient with a functional graft who had lived for 24 years post-graft, and found 5-HT6 receptors being expressed in TH+ neurons at a much higher rate than inside the host striatum or other transplanted neurons (Alden-Kirk et al. 2016). 5-HT6 activation alone is enough to induce GIDs through a strong DA release in the reinnervated host striatum, which ultimately points to a strong role that this particular serotonin receptor has with GID (Alden-Kirk et al. 2016).

DREADD Chemogenetics to Study Chronic Neuromodulation in α-SYN Rat Models

As previously discussed, α-SYN deposits, or LB, within neurons represent a distinctive feature of PD. The exact conformation of α-SYN in physiological conditions has been heavily

debated among scientists. Braak's hypothesis states disease progression falls along lines of topographical spreading of LB pathology throughout the brain (Torre-Muruzabal et al. 2019). Yet there have been divisions among scientists on the validity of the hypothesis, as not all patients fit within its claims (Rietdijk et al. 2017).

To study the link between neuronal activity and α-SYN pathology, hM3Dq DREADD receptor was used to activate a Gq-cascade and increase neuronal activity in mutant A53T α-SYN rat models (Torre-Muruzabal et al. 2019). The modified GPCR receptors were activated only when ligand CNO was administered. Rats were treated daily with CNO for three weeks, and all groups had decreased use of the left paw, caused by unilateral overexpression of α-SYN (Torre-Muruzabal et al. 2019).

Changes in behavior or weight were not observed in response to CNO treatment, which suggests behavioral deficits were caused by chronic modulation of neuronal activity in neurons over expressing α-SYN (Torre-Muruzabal et al. 2019). Chronic stimulation of nigral dopaminergic neurons resulted in a decline in motor function, meaning that the data obtained in this particular study could potentially be used to inform research on neuronal stimulation as a therapeutic approach to neurodegenerative diseases.

An important point found in this study was that control animals treated with CNO presented with a smaller striatal lesion after a-SYN overexpression (Torre-Muruzabal et al. 2019). This was found in two other studies too, one unrelated to DREADDs technology, suggesting work could be done to investigate the link between neuroprotection and using CNO or its predecessor Clozapine as a DREADDS activator (Schultz et al. 2018).

DREADD Stimulation Within the Pedunculopontine Nucleus (PPN) and Deep Brain Stimulation (DBS) to Treat Parkinson's

DREADDs can be utilized within PPN cholinergic neurons of a rat model to yield data. This data can then be analyzed via PET brain scans, to study the relationship between CNO-induced motor impairment and PPN-cholinergic induced DA release from nigrostriatal DAergic terminals in the striatum. The PPN is a heterogeneous neuronal structure in the upper pons of the dorsolateral portion in the brainstem (French and Muthusamy 2018). Low frequency deep brain stimulation can target the PPN, leading to improvement of motor impairment.

In one study, PPN cholinergic projection neurons were stimulated by overexpressing an excitatory DREADD, made up of hM3 and a fluorescent marker mCherry (Sharma et al. 2020). The Adeno-associated virus, serotype 2 (AAV2), allowed restricted expression of the DNA encoding hM3Dq in rats' cholinergic neuronal population, and spatial restriction to the PPN.

Researchers found that in both lesions and sham-controlled animals, no hM3Dq-mCherry expression was observed in any of the stained sections. This indicates the use of Cre-dependent viral vectors is an effective strategy for targeting the PPN cholinergic neurons of rats in order to enable cell-type specific manipulations (Sharma et al. 2020).

The researchers found that DREADDs can be used to single out the functional role of cholinergic pathways with a PPN origin, for explaining the clinical benefits seen with PPN-DBS treatment used to relieve Parkinson's-related disability. Via a previously established rat model of PD developed by the same researchers, it was found the reversal of motor deficits is mainly due to PPN cholinergic neurons' innervation of the nigrostriatal pathway (Sharma et al. 2020). It was further discovered that heightened activation in both the striatum and SNpc following PPN cholinergic-specific activation upholds the idea that the PPN-nigrostriatal pathway is a key

player in neural pathways which allow the behavioral recovery for the particular rat model of PD (Sharma et al. 2020).

With therapies moving forward, the researchers suggested the most effective plans would involve targeting both the dopaminergic and ACh modulatory systems. Cell-type specific modulation could be more useful in alleviating axial-related motor dysfunction which is seen in Parkinson's, rather than more conventional DBS which does not have any cell-type specific modulation (Sharma et al. 2020).

Like the study by Torre-Muzurabal et al., the researchers found CNO is not completely inert, and reverse-metabolizes to clozapine, which crosses the blood-brain barrier to induce central nervous system effects (Sharma et al. 2020). The researchers emphasized future studies establishing significant control conditions to show observed effects are not due to the binding of CNO's predecessor, Clozapine.

DREADDs Limitations

Avenues for improvement exist within the field of chemogenetics. Principal concerns lay within utilizing CNO as a main DREADD ligand. Studies have demonstrated that clozapine back-metabolized from CNO may contribute to DREADD activation after peripheral CNO injection (Mahler and Aston-Jones 2018). Effects of clozapine metabolized from CNO have also been found to accumulate over time, making their effects potentially appear long after the initial injection. Undesired effects of back-metabolized clozapine could also depend on behaviors examined and the presence or absence of other pharmacological agents (Mahler and Aston-Jones 2018). There is also the possibility low doses of clozapine could have complex effects from concurrent actions at DREADDs and endogenous receptors which are present in the same

neurons. Readings of clozapine blood and brain levels over an appropriate amount of time should be taken into account in studies with prolonged testing periods.

One solution which has been proposed to get around potential unwanted effects of CNO or clozapine is to use a DREADD agonist other than CNO, with one notable example being the hypnotic compound perlapine. Some qualities of DREADD agonists are that they do not possess active metabolites, but do penetrate the blood brain barrier and selectively activate DREADDs. Perlapine has been used as a sedative antihistamine drug in Japan but is not approved by the FDA in the U.S. (Weston et al. 2019). Some believe that due to perlapine's history of safety within humans, it would be the most likely DREADD ligand for activating CNO-based DREADDs in human models. The question of using CNO in human treatments is in contention for its qualities of back-metabolizing to clozapine, seen in nonhuman trials and the primary studies above (Roth 2016).

Another point of criticism related to DREADD technology is desensitization and subsequent receptor downregulation. Both desensitization and downregulation could be caused by repeated dosing of a DREADD actuator. An actuator is a biological component which produces phenotypic effects from genetic devices within the host cell. GCPRs can be desensitized and then down regulated after agonist-induced activation (Roth 2016). When receptors are desensitized or downregulated, there could be no change in maximum response elicited by the agonist, but there could be a shift in the dose-response curve because of receptor reserve. Receptor reserve is a phenomenon where maximum agonist response can be achieved from less than full occupancy of the receptors of the agonists. When DREADDs are then expressed at high levels relative to native GCPRs from viral or transgenic approaches, the

cellular and behavioral responses will be less sensitive to repeating doses than responses which were observed when expressed at lower levels (Roth 2016).

As briefly mentioned earlier, a major hurdle for the advancement of DREADDs and its use in treating conditions such as PD lies in the use of animal models. Specifically, the application and translational relevance of results from such studies may be limited due to differences in mouse and human genomes, as they are about 15% different. Mouse brains also contain more neurons than glia, and human brains have the opposite ratio (Hendricks 2010). Mice also have an average life span of two years, over forty times less than the average span of a human. This inherently makes it more difficult to utilize mice in studying neurodegenerative diseases linked to aging.

Benefits still exist for using animal models. Chiefly, discoveries within animal models have led to greater understanding of disease mechanisms, also molecular mechanisms of the animal's cells and the chemogenetic technology used (Dawson et al. 2018). While not yet providing much successful transfer of therapies to human models, rodent and non-human primate models have been a great boon for identifying candidates of potential disease-modifying therapies.

Widespread failures in the translation of clinical trials based on therapies which were successful in animal models could be the result of a number of factors. A preclinical intervention focused on preventing the onset of a disease will likely not work in a human study where many of the participants are well within the diseases' progression (Dawson et al. 2018). Other factors include poor study designs, imperfect animal models, optimistic interpretation of results, and clinical trial limitations such as poor biomarkers (Dawson et al. 2018). Using appropriate sample sizes, blinding researchers, proper animal care, and minimizing bias in interpreting results would

all increase the viability of preclinical results and create more hope of finding a consensus within the library of scientific literature dealing with animal studies of neurological disease (Ransohoff 2018).

Conclusion and Future Outlook

DREADDs and its application within treatment plans for neurodegenerative disorders is making good progress. With PD, researchers can look to similar disorders like Alzheimers to compare treatment options and the chemogenetic setup of their laboratory studies. Though there are limitations expressed in the section above, the field has an optimistic future. DREADDs will likely continue to further our understanding of different neural circuits and cell types to establish reliable disease models. CNO or different DREADD activators are a rich field to explore, and as shown, there is still much to be done with minimizing side effects and reducing off-target results. Although clinical trials with DREADDs technology have not quite been achieved, the technique is an undeniably helpful tool in allowing researchers to further this ambition and someday establish a viable, successful human trial.

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