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New tricks for old dogmas: Optogenetic and designer receptor insights for Parkinson's disease

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Abstract

Optogenetics and novel designer receptors have revolutionized the way neuroscientists can interrogate neural circuits. These new tools are being rapidly applied to many facets of neuroscience including the study of Parkinson's disease circuitry and therapies. This review highlights how optogenetics and designer receptors can be applied in the study of Parkinsonian dysfunction to understand the mechanisms behind motor and non-motor symptoms. We discuss how these tools have recently advanced our understanding of basal ganglia function and outline how they can be applied in future to refine existing treatments and generate novel therapeutic strategies for Parkinson's disease.

Keywords

Optogenetic; DREADD; Parkinson's disease; Basal ganglia; Non-motor; Norepinephrine; Dopamine; Striatum

1. Introduction

Parkinson's disease (PD) has been described for nearly two centuries (Parkinson, 1817). The overt neuropathological lesions of nigral dopamine (DA) system associated with this disorder have been known for close to six decades (Carlsson et al., 1958; Ehringer and Hornykiewicz, 1960; Greenfield and Bosanquet, 1953) and become dogmatic as the quintessential, brain dysfunction that causes this disorder. Since this discovery, many dopaminergic treatments for PD have been developed which have permitted management of cardinal motor symptoms (Birkmayer and Hornykiewicz, 1961). Despite an impressive body of research and development, our understanding of the mechanisms that cause PD, and ability to manage the full constellation of motor or non-motor symptoms of this disorder, are quite limited. Thus, there remain many challenges for researchers and unmet needs for patients. Recently, the development of genetically encoded tools for neural control has transformed the way researchers study and control neural circuits in experimental models of disease, including PD. This special issue highlights many advances and developments in optogenetics and designer receptors. This brief review highlights how such tools have

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already extended our understanding of PD, clarifying and confirming principles as well as providing novel insights that may change the dogma of PD as we know it.

PD is highly prevalent: It is the second most common neurodegenerative disorder, currently affecting ~1% of adults over 60 (Lees et al., 2009), and was recently listed by the CDC as the 14th leading cause of death in America (Murphy et al., 2012). Canonically, PD is a motor system disorder caused by the progressive degeneration of substantia nigra compacta (SNc) dopaminergic neurons that innervate the dorsal striatum and regulate basal ganglia function. The loss of these neurons leads to profound motor symptoms including resting tremors, rigidity and bradykinesia (Dickson et al., 2009a). These essential symptoms are the foundation of clinical PD diagnosis, and during early disease stages they can be relatively well managed through DA replacement therapies. As the disease progresses, DA replacement elicits more side effects and becomes less effective as seen by emergence of dyskinesias and on-off effects. As discussed in Sections 3 and 4, recent research with optogenetics and designer receptors can help us to better understand how these therapies interact within the basal ganglia, and assist in identifying ways to better treat motor dysfunction and on-off effects of DA replacement therapy.

PD is also associated with many symptoms that are not benefitted by DA targeted therapeutics including gastrointestinal disturbances, olfactory dysfunction, sleep disorders, depression, freezing of gait, bradyphrenia, executive dysfunction, and dementia. Although outside the traditional dogma of PD, non-motor symptoms begin early in PD and are particularly detrimental to patients' quality of life, life expectancy as well as being major contributors towards caregiver stress (Diaz and Waters, 2009). Non-motor disabilities in PD patients are met by very few therapeutic strategies because the pathology contributing to different non-motor aspects in PD is poorly understood. There is extensive non-DA pathology in PD with strikingly widespread deposition of characteristic lewy body inclusions throughout the nervous system. In particular, it is important to note the pronounced degeneration in locus coeruleus norepinephrine (LC-NE), serotonergic raphe and basal forebrain cholinergic neurons. These systems have been linked with a variety of non-motor functions lost or compromised in PD (Hawkes et al., 2010). Histopathological analyses support a caudo-rostral progression of PD pathology within the CNS, with degeneration of some of these regions, particularly LC-NE and raphe, occurring prior to that of SNc-DA neurons and perhaps even contributing to DA pathology (Del Tredici et al., 2002). We propose that optogenetics and designer receptors afford new approaches to elucidate pathological brain networks that contribute to non-motor as well as motor symptoms of PD, and to target non-DA populations in particular to provide therapeutic benefit.

In this review we summarize how these tools have already been used to great advantage in PD research, and how they can continue to unravel mysteries, redefine the classic dogma related to PD, and aid development of new therapeutic strategies.

2. Adding to the modern toolbox for PD research

2.1 Optogenetics

Many articles in this issue discuss optogenetics, the use of light responsive, membrane bound proteins for temporally precise and reversible neural control (Deisseroth, 2011). The most common of such light reactive ion channels (opsins) include channelrhodopsin (ChR2) and its variants as well as ion pumps such as halorhodopsin or archaerhodopsin; other opsin variants including light responsive G protein coupled receptors for targeting intracellular signaling pathways are also available (as reviewed in Bernstein and Boyden, 2011; Yizhar et al., 2011). Remotely regulating neurons with light allows unprecedented millisecond-scale

upregulation (ChR2) or downregulation (halo- and archaerhodopsins) of neural activity to the level of single action potentials. This technology has been applied widely, from *ex vivo* preparations to targeted *in vivo* manipulations of deep brain structures. Importantly, such manipulations include local regulation of terminals of transduced neurons in specific projection targets, due to anterograde trafficking of opsins along axons. In the next sections we will discuss several studies that have applied optogenetic technology to models of PD to both validate and to substantially extend our understanding of PD and PD therapies. This ability to specifically control selected neural circuitry is likely to lead to many more PD insights in the future.

2.2 Designer receptors

Designer G protein-coupled receptors reversibly control gain- or loss- of function by targeting specific intracellular signaling pathways (Conklin et al., 2008). Designer receptors are an alternative strategy to remotely control neural activity that is gaining in popularity (see Farrell and Roth, 2012 in this issue). DREADDs (designer receptors exclusively activated by designer drugs) have been particularly versatile in this realm. Initially developed by Bryan Roth and colleagues, DREADDs have minimal (Gs) or no (Gi, Gq) constitutive activity, and importantly no endogenous compounds at physiological concentrations are capable of activating DREADDs (Armbruster et al., 2007; Guettier et al., 2009). The DREADD binding sites have been specifically designed (by site directed mutagenesis - see Dong et al., 2010) to be exclusively activated with high affinity by the otherwise biologically inert ligand clozapine-N-oxide (CNO). This system is referred to as a biological 'lock-and-key' for regulating cell activity through G protein-coupled intracellular signaling pathways. As reviewed elsewhere in this special issue (see Farrell and Roth, 2012), several other DREADDs targeting additional intracellular signaling mechanisms (e.g. β -arrestin specific DREADD by Nakajima and Wess, 2012) are currently in development. The specific ligand CNO rapidly enters the brain after systemic administration and is also orally bioavailable, making it well suited for repeated non-invasive manipulation of neural circuitry. In neurons, DREADDs are trafficked to dendrites and axon terminals (Alexander et al., 2009), allowing local intraparenchymal injections of CNO to manipulate specific projections. DREADD based strategies are particularly useful for dose titrations in individual patients, and for regulation of widespread/diffuse neural populations that could be difficult to effectively cover with light using optogenetics. Although DREADDs do not provide the temporal resolution of many optogenetic approaches, they are particularly useful for tonic regulation of specific circuits across a range of timescales - minutes, hours or weeks without risk of accessory artifact from mechanical or heat damage. DREADDs have the potential to provide highly selective neuroprotective or therapeutic strategies in PD clinical populations, in addition to numerous uses in circuit and signaling studies in animal models.

2.3 Delivering genetically designed neuroregulatory tools

2.3.1 Viral vectors—The opsin or DREADD proteins discussed here for neural control are each encoded by a single small gene, a great advantage that makes them amenable to the wide range of genetic targeting strategies that has been established over recent decades. Viral vector technologies similar to those developed for gene therapy using adeno associated virus (AAV), herpes simplex virus (HSV), or lentiviral (LV) vectors are familiar to many PD researchers and have been the most popular methods for delivering genetically designed regulatory tools to complex neural circuits (Deisseroth, 2011; Farrell and Roth, 2012). However, given that HSV provides only ectopic and therefore temporary expression of genes, longer lasting AAV or LV vectors may be better suited for future clinical application. Virally delivered tools allow regional targeting and selective control over phenotypically specific populations of neurons or glia based on a variety of small promoter

sequences. Furthermore, the range of small cell-type specific promoters is soon to be rapidly expanded through programs such as the Pleiades project (Portales-Casamar et al., 2010). Viral delivery of opsins and DREADDs also makes them applicable to a wide range of neural structures, species and preparations. The pronounced acceleration of opsin and DREADD tools has been greatly facilitated by the openness of early developers in sharing resources (Farrell and Roth, 2012; Yizhar et al., 2011); this has allowed rapid application of these technologies in many neural cell types and disease models across hundreds if not thousands of laboratories. At present, a number of broad spectrum constructs are available in ready-to-use AAV vectors at modest cost from large institutional core facilities including University of North Carolina (<http://genetherapy.unc.edu/services.htm>) and University of Pennsylvania (<http://www.med.upenn.edu/gtp/vectorcore/Catalogue.shtml>) vector cores.

2.3.2 Pathway specific targeting—Cell-type specific promoters imbue these regulatory tools with great selectivity, however, many neuronal populations project to multiple target regions. If interested in a specific pathway, both opsins and DREADDs can be used to activate terminals of transduced neurons in a specific target region by local photostimulation (Gradinaru et al., 2009) or CNO microinjection (Mahler et al., 2012; unpublished observations) at axon terminals, as opposed to broadly targeting the source cell bodies that were transduced by vector injection. Terminal stimulation can be effective in altering post-synaptic neural activity and eliciting behavioral effects (Gradinaru et al., 2009; Mahler et al., 2012; Stuber et al., 2010). However use of terminal targeting should be cautiously evaluated for potential unexpected effects due complex negative feedback mechanisms at stimulation sites. With optogenetics, antidromic activation of cell bodies could lead to additional stimulation of alternative afferent paths, potentially confounding experimental interpretation.

Particularly useful in conjunction with systemic activation of DREADDs, a convergent dual virus approach can be used to specifically restrict expression to only a given set of projection neurons. Dual virus approaches can utilize the cre-lox (or other enzyme) recombination strategies with retrograde transport of a vector expressing cre recombinase. To complete pathway specific expression it is necessary to deliver a second complementary cre dependent floxed or flip excision vector containing the opsin or DREADD at the origin of the projection neurons. AAVs expressing the wheat germ agglutinin (WGA) tracer conjugated to cre are available to facilitate pathway specific targeting. Additionally, vectors such as canine adeno virus (CAV) are inherently transported in a retrograde manner (Peltekian et al., 2002), a CAV vector encoding cre facilitates excellent projection-specific expression using a dual vector strategy as demonstrated by Nair et al (2012) in this issue. AAV6 has also been reported to undergo efficient retrograde transport and may be amenable to recombinase delivery for dual vector pathway specific targeting (Salegio et al., 2012).

2.3.3 Transgenics and cre driver lines—Even in smaller animal models, achieving high levels of transgene expression in widespread populations of neurons using viral vectors can be challenging. Also, the number of small cell-type specific promoters is rapidly expanding but limited; for example, currently no small promoter conveys good specificity for DA neurons. These limitations have been overcome by several genetic rodent lines that express opsins or DREADDs, including transgenic, knock-in and intersectional genetic models expressing excitatory or inhibitory opsins or DREADDs in various neuronal subtypes or glia (Farrell and Roth, 2012; Ray et al., 2011; Zhao et al., 2011). Even more versatile is the already extensive range of cre driver mouse lines, and more recently cre driver rat lines (Madisen et al., 2012; Smedley et al., 2011; Weber et al., 2011; Witten et al., 2011). As with the dual virus strategy described above, transgenic cre recombination can be utilized with cre-dependent vectors to control specific expression. Cre driver lines allow very small and strong promoter sequences to be used for the cre-dependent vector containing the protein of

interest. This is a particularly useful strategy for preclinical PD research as both TH-cre rat and mouse lines are available for controlling DA and NE neurons with microinjection of floxed opsin or DREADD vectors into targeted TH cell areas (Lindeberg et al., 2004; Witten et al., 2011). Given the utility of this strategy and the wide base of existing cre driver lines, novel opsin and DREADD constructs are frequently being developed in cre-dependent form with a strong preference to the flip excision inverted open reading frame style construct which is particularly resistant to non-specific leakage (Atasoy et al., 2008).

In the last 15 years several genetic mutations that increase PD susceptibility in humans have been translated to *in vivo* and *in vitro* models (Lee et al., 2012). Opsin and DREADD tools can be used *in vitro* for elucidating activity-dependent effects of PD related genes. Such studies can aid development or validation of future therapeutic strategies involving targeted control of intracellular signaling mechanisms. In *in vivo* studies in PD animal models, the expression of opsins or DREADDs in specific neurons using viral vectors and cell-type specific promoters will allow powerful tests of the necessity or sufficiency of identified brain circuits in the behavioral dysfunctions associated with PD in translationally relevant models.

3. New insights into neural mechanisms of PD from optogenetics

3.1. Confirmation of direct and indirect basal ganglia output pathways and a new potential treatment for PD

Many decades of PD research have been devoted to dopaminergic regulation of striatum with the goal of controlling motor dysfunction in PD. The striatum is a critical site for integrating cortical outputs to regulate motor planning and movement through the basal ganglia. The majority of striatal neurons (>95%) are GABAergic medium spiny projection neurons (MSNs), dorsal MSNs are heavily innervated by nigral DA inputs. Distinct subpopulations of MSNs express D1 vs D2 receptors (among other histological markers), respectively forming the direct and indirect basal ganglia output pathways. Distilled to a very basic overview in relation to motor function, D1 receptor-expressing MSNs are activated by DA to directly inhibit SNr neurons, which facilitates movement; this is denoted as the 'direct' pathway. In finely balanced opposition, D2 receptor-expressing MSN neurons are inhibited by DA and project to the globus pallidus leading to excitation of SNr neurons and inhibition motor output; this circuit forms the 'indirect' pathway (Albin et al., 1989). Anatomical and lesion studies indicate that loss of SNc DA function decreases activity in the direct pathway and disinhibits indirect pathway, creating an imbalance in basal ganglia regulation that produces the bradykinesia or freezing seen in PD (Wichmann et al., 2011). In the anesthetized rat, direct pathway MSNs are inhibited and indirect MSNs activated in response to cortical stimulation after 6-OHDA lesion of SN DA projections, additionally compounding basal ganglia imbalance and motor symptoms of PD (Mallet et al., 2006).

With the advent of optogenetics and advantage of D1 vs D2 receptor cre-driver mice, Kravitz et al. (2010) were able to empirically test the proposed opposing functions of direct and indirect basal ganglia circuits. Bilateral ChR2-mediated activation of a small number of MSNs in dorsomedial striatum targeted to the direct pathway (D1-cre mice) increased fine movements and ambulation time in freely moving animals, whereas bilateral indirect pathway (D2 cre-mice) stimulation decreased movement initiation, and produced bradykinesia and freezing bouts, essentially eliciting a parkinsonian like state. In a classical bilateral striatal 6-OHDA model of PD, optogenetic stimulation of direct pathway MSNs restored locomotor initiation, bradykinesia and freezing to pre-lesion levels. These findings not only causally demonstrated the opposing roles of direct and indirect pathways in basal ganglia motor output, but they also identified a novel mechanism for ameliorating PD motor deficits by specifically stimulating direct pathway activity.

Kravitz et al. (2010) also provided insight to which PD motor symptoms are basal ganglia-mediated as opposed to potentially regulated by other dysfunctions in the PD brain. In contrast to popular dogma, neither direct nor indirect pathway stimulation had an effect on gait. However, it should be noted that the stimulation region was restricted to dorso-medial striatum, which calls into question but does not exclude the possibility of MSN regulation of gait disturbances in PD.

3.2. GABA co-release from nigral DA neurons

Adding further intricacy to striatal DA regulation, a recent publication from Tritsch et al. (2012) used optogenetic stimulation of SNc DA terminals in slice preparations. This uncovered a rapid inhibition of both direct and indirect MSNs elicited by co-release of GABA from DA terminals. The functional implication of GABAergic co-release from SNc DA neurons is not yet understood; however, the absence of such GABAergic fine tuning of MSNs could potentially be involved in the development of dyskinesias seen with classic pharmacological DA replacement strategies for PD.

3.3. A new mechanism for efficacy of deep brain stimulation in subthalamic nucleus

In a seminal functional demonstration of the power of the optogenetic approach, Gradinaru et al (2009) undertook a series of experiments designed to elucidate the mechanism underlying the efficacy of deep brain stimulation (DBS) in the subthalamic nucleus (STN) for treating PD related motor deficits. It had been widely assumed that local DBS inhibited hyperactive STN neurons and that this inhibition produced beneficial effects on motor symptoms in a manner similar to therapeutic subthalamic lesions in PD (Alvarez et al., 2001). However, Gradinaru and colleagues found that specific optogenetic inhibition of STN neurons (using the light activated chloride pump NpHR) produced no effect on PD-related motor deficits in hemiparkinsonian rats (motor activity, rotational behavior or head tilt). They also found that neither inhibition of STN neurons through optogenetically triggered glial derived factors, nor specific optogenetic β or high gamma frequency excitation of STN neurons, was able to ameliorate parkinsonian behaviors. They also employed the transgenic Thy1-ChR2 mouse line that expresses ChR2 primarily in cortical projection neurons and not in STN neurons. Photostimulation of ChR2-containing terminals in STN of hemiparkinsonian Thy1-ChR2 mice at 130Hz prevented pathological burst activity in STN neurons and robustly reversed parkinsonian-like rotational activity and head tilt. By stimulating ChR2 positive cell bodies in layer V of primary motor cortex that project directly to STN, they were able to reproduce the beneficial effects of local high frequency activation of STN afferents, demonstrating that M1 to STN activation is sufficient to attenuate PD-like motor symptoms. These findings identify cortical inputs from M1 to basal ganglia as a novel future therapeutic target for PD. Moreover, this optogenetic approach demonstrated that the prior dogma regarding the mechanism of action of DBS in STN for treating PD motor symptoms is likely incorrect.

The optogenetic studies discussed above have provided new insight into current and future therapeutics for PD. As the precision of optogenetic neural control continues to unravel the complexity of innate DA neuron function, we may find further insights into DA function on basal ganglia circuits leading to novel treatments and additional refinement of existing treatment strategies for canonical motor symptoms in PD.

4. Potential uses of optogenetics and designer receptors for researching non-motor symptoms of PD

Optogenetic and DREADD technologies are in their early stages, particularly with respect to PD research or therapy. Many experiments could and likely will take advantage of the

precision and control afforded by opsin and DREADD regulation of neural circuitry. As yet no published studies have applied these tools to the myriad of non-motor symptoms in PD. Non-motor symptoms are a major cause of disability in PD, are poorly understood, and are one of the most pressing therapeutic challenges for PD researchers and clinicians. Non-motor symptoms in PD can be divided into four primary domains; autonomic dysfunction, disordered sleep, sensory disruptions and neuropsychiatric manifestations (as detailed in Dickson et al., 2009b; Langston, 2006; Wolters, 2009). Determining the underlying substrates of non-motor symptoms is particularly important as symptoms from each domain are present in 'prodromal' or 'premotor' phases of PD (Hawkes et al., 2010); therefore, early intervention to treat these symptoms may hold potential for modifying disease progression. For many non-motor symptoms in PD the pathophysiology is not clear, hampering therapeutic development. In the neuropsychiatric domain, mood and cognitive dysfunctions are present from early in disease progression; furthermore, independent evidence and post-mortem investigations in PD have identified several candidate neural systems that may contribute to these symptoms.

4.1 Elucidating substrates of neuropsychiatric dysfunction in PD

Our group is particularly interested in the role of noradrenergic pathology in cognitive dysfunction in PD (Vazey et al., 2012a; Vazey and Aston-Jones, 2012). Significant pathology and loss of LC-NE neurons occurs in PD (Chan-Palay and Asan, 1989). LC-NE dysfunction occurs early in disease progression (Hawkes et al., 2010), and the known functions of LC-NE correlate well with several early symptoms in PD including sleep disorders (Rothman and Mattson, 2012), depression (Weintraub et al., 2010) and cognitive dysfunction (Vazey and Aston-Jones, 2012).

Cognitive dysfunction in early PD primarily manifests as a dysexecutive syndrome including impairments in planning, working memory, attentional control and cognitive flexibility (Kehagia et al., 2010). These functions are associated with prefrontal cortex, which is not subject to direct PD pathology in early stages. The prefrontal cortex is regulated by several neuromodulatory systems including DA, serotonin, acetylcholine and NE (Aston-Jones and Cohen, 2005; Chudasama and Robbins, 2006; Robbins and Arnsten, 2009). NE, serotonin and cholinergic nuclei that project to prefrontal regions are all subject to PD pathology; however, DA inputs to prefrontal regions arise primarily from medial ventral tegmental regions and remain relatively preserved even in late PD (Dickson et al., 2009a).

Prefrontal reductions in 5-HT and NE, but not in DA, have been demonstrated in PD patients and animal models (Goldstein et al., 2011; Nayyar et al., 2009). Serotonin and NE systems are known to have profound impacts on both cognitive function and mood (Nemeroff, 2002; Robbins and Arnsten, 2009). An intersectional genetic model has been used to express Gi-coupled DREADDs specifically in serotonergic raphe neurons (Ray et al., 2011); this strategy could also be used for investigating the role of serotonin in cognitive dysfunction and depression in PD. Similar intersectional fate mapping applied to the noradrenergic system, as well as TH-cre mouse and rat models (Robertson et al., 2011; Witten et al., 2011), could be used for targeting opsins or DREADDs to noradrenergic neurons to manipulate LC function in models of PD. Such optogenetic manipulations of LC-NE have already been used to manipulate sleep architecture in TH-cre mice (Carter et al., 2010). In our lab we have been using a small synthetic promoter to target opsins and DREADDs to LC-NE neurons to understand their contribution to cognitive dysfunction and as a potential therapeutic avenue in PD (Vazey et al., 2011; Vazey et al., 2012b, unpublished observations). Recent results indicate that photostimulation of LC-NE neurons with optogenetics modulates cognitive flexibility, a prime executive deficit in PD (Cope et al., 2012; Vazey et al., 2011, unpublished observations).

4.1.2 Neuropsychiatric dysfunction in late stage PD—Late stage PD is often dominated by global cognitive decline and dementia in many patients (Kehagia et al., 2010). Dementia is poor prognostic indicator, being a major precipitator of transfer to nursing home care and increased mortality rates (Aarsland et al., 2000; Willis et al., 2012). Dementia in PD is associated with pathology in, and degeneration of, the basal forebrain cholinergic system (Perry et al., 1985). Optogenetic manipulations with Chat-cre mice have been used to elucidate functions of cholinergic striatal interneurons (Witten et al., 2010). Manipulation of basal forebrain cholinergic neurons in Chat-cre mice or rats (Witten et al., 2011) could be used to investigate cholinergic disruptions in models of PD.

In addition to dementia, psychosis is a prevalent neuropsychiatric manifestation in PD which also precipitates nursing home care and poor prognosis (Factor et al., 2003). Psychosis in PD increases with disease duration, particularly after long term dopamine replacement therapy, making it unclear if this facet of late stage PD is drug induced or part of the organic disease process. Given the DA replacement history of most patients, PD-psychosis has been hypothesized to arise from an overdose in mesolimbic dopaminergic systems which are relatively preserved during the degenerative processes in PD (Friedman, 2010). Optogenetic or DREADD stimulation of DA terminals, or postsynaptic D4 receptors in limbic structures could be used to clarify this hypothesis on the generation of psychotic symptoms.

Optogenetic or DREADD investigation of the substrates of psychosis in PD relies on valid preclinical models in which to test candidate perturbations. The perceptual nature of psychotic features of PD, such as visual hallucinations and delusions, are difficult to evaluate in animal models. Sensorimotor gating deficits as demonstrated by impaired prepulse inhibition (PPI) are a translational feature of psychosis that is quantifiable in both patients and rodent models of disease (Geyer and Swerdlow, 2001). Impaired PPI has been correlated with positive symptoms in schizophrenic patients and could be used to evaluate substrates of psychosis in PD models (Braff et al., 1999). Recently, impaired PPI was demonstrated in a preclinical model of PD after bilateral 6-OHDA lesion to the SNc in rats (McFarland et al., 2011).

Delusions and visual hallucinations are particularly common in patients with comorbid REM sleep disorder, depression or anxiety (Friedman, 2010). Interactions of DA replacement with other perturbed systems associated with co-morbid conditions are also an avenue which could be investigated using cell type specific optogenetic or DREADD manipulations in PD models. Candidate interactions include loss of function in LC-NE system mentioned above, which is involved in regulating sensory processing and arousal states. The cholinergic pedunculopontine nucleus, targetable in Chat-cre rodents, degenerates in the course of PD and has been implicated in both REM behavior disorder and PD psychosis (Janzen et al., 2012). Pharmacological studies in PD patients and animal models of PD have identified serotonin, particularly 5-HT_{2A} signaling as a probable mediator of psychotic symptoms in PD (McFarland et al., 2011; Meltzer et al., 2010). The serotonergic system, classically involved in mood regulation and associated with hallucinogens is also tractable with genetic DREADD or opsin manipulations in animal models (Ray et al., 2011).

4.2 Non-motor insights from optogenetic and DREADD studies

Although non-DA substrates predominantly underlie many non-motor symptoms in PD, some non-motor symptoms may also arise from the canonical DA loss in basal ganglia circuitry. Basal ganglia function is the substrate for many behaviors beyond motor output. A prominent role of dorsal striatum is facilitation of reinforcement learning and habit formation, more commonly investigated in addiction behavior. The physiological substrates of reinforcement learning also have insights for PD. Among relevant symptomatology, PD patients present with apathy and deficits in motor learning, the etiology of which is likely

related to the loss of appropriate DA regulation in basal ganglia. Two recent studies, one using optogenetic activation, the other DREADD-mediated inhibition of D1 and D2 MSNs, have highlighted the roles of direct and indirect pathway regulation in reinforcement learning and behavioral plasticity (Ferguson et al., 2011; Kravitz et al., 2012). Specifically, disrupting direct pathway transmission in dorsal striatum with DREADDs impaired behavioral sensitization, and optogenetically activating direct D1 MSNs induced persistent reinforcement, leading to the mice self-stimulating for optogenetic direct pathway activation. Given this evidence for mediating reinforcement and behavioral plasticity, reduced DA activation of the direct pathway in PD could contribute to motor learning deficits and apathy. Optogenetically stimulating indirect pathway D2 MSNs led to transient aversive behavior (Kravitz et al., 2012); with the imbalance in indirect activity in PD, this mechanism could further contribute to apathy and negative affect. These studies are also relevant to the subset of patients who develop impulse control disorders with DA replacement therapy. Sensitivity to independent activation of direct pathway by dopamine agonists used to treat motor symptoms likely contributes to development of compulsive behavior through persistent reinforcement of other actions.

5. Optogenetics and DREADDs in current and emerging therapeutic strategies for PD

In addition to insight on the mechanisms of PD pathophysiology, dopamine replacement and DBS therapies, optogenetics and DREADDs could be implemented in the understanding or refinement of many other therapeutic strategies for PD. In this section we describe opportunities for integrating these new tools in several therapeutic avenues.

5.1 Lesions of basal ganglia circuitry

Ablative lesions of basal ganglia components have been used to treat parkinsonism beginning with post-encephalitic patients, even prior to the discovery of dopamine (Meyers, 1940). Although currently out of favor since the advent of DBS, subthalamotomy and pallidotomy are still practiced clinically and provide effective treatment of motor symptoms in PD (Alvarez et al., 2009). The effectiveness of these lesions was the primary driver of the STN inhibition hypothesis surrounding DBS efficacy, until the probable mechanism through afferent stimulation was elucidated with optogenetics by Gradinaru et al. (2009) (discussed above). The use of lesions to treat PD could be refined and superseded with the development of cell type specific inhibition using viral vector-mediated gene therapy with opsins or DREADDs. The advantages of these approaches are dramatically increased selectivity of neuronal manipulations, sparing of fibers of passage, reversibility, and with DREADDs the ability to titrate the dose-effect of therapy to correspond with disease progression or advent of adverse effects. Future therapeutic strategies directly targeting downstream activity in basal ganglia circuitry at a common output point (for example inhibiting SNr with DREADDs or opsins) could also provide refined control over basal ganglia function in PD. In developing countries where cost may prohibit application of DBS or future DREADD or opsin mediated gene therapy, research using optogenetics or DREADDs in this manner can inform us of optimal circuit nodes to target using traditional lesion techniques for improved outcomes and minimized side effects.

5.2 Gene therapy

In an extension of lesion and DBS therapy, virally mediated overexpression of the GABA precursor GAD in STN is currently undergoing clinical trials (Kaplitt et al., 2007). Importantly these trials have demonstrated the safety and tolerability of AAV-mediated gene therapy in PD patients, an critical principle for the advent of any future optogenetic or DREADD mediated therapeutic strategy using viral vectors. By changing the phenotype of

STN neurons to co-release GABA and glutamate in downstream targets, these studies have shown improvements in motor function and brain metabolic function in PD patients after AAV-GAD gene therapy (Kaplitt et al., 2007; LeWitt et al., 2011). It is unclear how this finding fits with the Gradinaru et al. (2009) study that showed inhibition of STN neurons was not effective in improving PD motor symptoms in an animal model (discussed above). However, they may indicate that nuanced DREADD-mediated modulation of STN activity may have benefit for at least some PD patients.

5.3 Transplantation therapy

Transplantation strategies, particularly ectopic transplantation of DA neurons into the striatum, has long been pursued as a therapeutic strategy for PD (Freed et al., 2011). Recently, optogenetic control of DA neural transplants, and optogenetic control of striatal neurons surrounding DA neuronal transplants, was used to demonstrate proof-of-concept examination of host-graft synaptic interactions that regulate activity in transplanted neurons (Tonnesen et al., 2011). Optogenetic control of transplanted neurons in animal models may help to understand how ectopic transplants integrate with host circuitry, to identify the importance of developing *de novo* neural circuits in diseased brains through transplantation strategies. This could lead to greater understanding of DA transplantation in PD, separating functional benefits that are facilitated by integration into striatal circuitry and those that result from trophic support provided by the transplant. Additionally, DREADD control over ectopic DA transplants could be used to facilitate controlled DA release optimizing function in these grafts as CNO doses can be titrated to individual levels of DA depletion.

5.3 Intracellular signaling cascades as a therapeutic target

As G protein-coupled receptors themselves, existing DREADDs inherently target secondary messenger signaling cascades (Armbruster et al., 2007), and a number of opsin constructs also target G protein coupled receptors (Yizhar et al., 2011). Intimate control of intracellular signaling pathways through DREADDs or light activated G protein coupled receptors could be a valuable means for investigating how different signaling mechanisms contribute to PD pathogenesis, and indicate potential new therapeutics. For example, β -arrestin signaling in MSNs has been hypothesized to be important in the beneficial effects of L-dopa treatment but distinct from the signaling processes involved in L-dopa induced dyskinesia (Guigoni et al., 2005). Thus, use of a β -arrestin-specific DREADD (Nakajima and Wess, 2012) or opsin that is targeted to striatal MSNs could provide beneficial effects on motor control and potentially avoid dyskinesia development currently associated with long term use of L-dopa.

As optogenetic and DREADD based tools expand, specific manipulations of various intracellular signaling pathways can be elegantly designed on similar principles. Given emerging evidence of mechanisms responsible for cell death in PD (Gupta et al., 2008), we can hypothesize future neuroprotective strategies that target intracellular signaling mechanisms specifically implicated in the pathogenesis of PD. These targeted neuroprotective strategies could dose-dependently interfere with or facilitate intracellular signalling with delivery of exogenous substrates the way DREADD and opsin strategies presently control depolarization and hyperpolarization of neurons.

5.4 Neuroinflammation as a therapeutic target

Mechanisms in astrocytes and microglia are also a potential therapeutic target in PD that could be amenable to modification with new opsin and designer receptor tools. Genetic and toxin models of PD and post mortem human studies have all implicated neuroinflammatory processes in contributing to pathological processes and cell loss in PD (Hirsch et al., 2012). Decreased glial metabolic support with aging and reductions in glial-derived factors likely contribute to oxidative stress in neurons and the pathogenesis PD, for example decreased

levels of the antioxidant glutathione have been identified in PD patients and genetic PD models (Rappold and Tieu, 2010). Although primarily adopted with the physiological properties of neurons in mind, recent studies have used opsins and designer receptors to successfully drive glial functions by driving calcium waves and G protein coupled receptor cascades (Figueiredo et al., 2011; Gourine et al., 2010; Gradinaru et al., 2009; Li et al., 2012; Sweger et al., 2007). The advent of additional precision in the variety of opsins and designer receptors will enable regulation of specific intracellular pathways to control astrocyte and microglial function with targeted expression of these tools. Using these novel tools outside of neurons provides additional opportunities for understanding how neural support networks contribute to cellular dysfunction and loss in PD. Exogenous control over specific glial functions with designer receptors and opsins may also provide neuroprotective strategies by regulating astrocytes and treating neuroinflammation.

5. Conclusions

Optogenetic and designer receptor methodology is in its infancy, but as indicated in this special issue these techniques are already transforming many areas of neuroscience research. For PD, optogenetics and designer receptors offer exciting new abilities to selectively dissect circuits involved in normal motor control as well as dysfunctional circuit elements that give rise to parkinsonian symptomology. This new level of understanding mechanisms that underlie PD behavioral pathology will allow more rational and specific therapeutics to be developed, targeted at the corresponding neural dysfunctions. Moreover, designer receptors delivered by promoter-driven cell type-specific viral vectors may themselves offer a future highly selective and flexible set of therapeutics for treating motor or non-motor deficits of PD. Thus, optogenetic and DREADD tools offer tremendous future opportunities for identifying and treating PD, and further work in this area is certain to bring major gains in PD therapeutics.

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