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Review

Pharmacogenetic and optical dissection for mechanistic understanding of Parkinson's disease: Potential utilities revealed through behavioural assessment

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ABSTRACT

The technology toolbox by which neural elements can be selectively manipulated in vertebrate and invertebrate brains has expanded greatly in recent years, to now include sophisticated optogenetics and novel designer receptors. Application of such tools allow for ascertaining whether a particular behavioural phenotype associates with interrogation of a specific neural circuit. Optogenetics has already found application in the study of Parkinson's disease (PD) circuitry and therapies, whereas novel designer receptors hold promise for enlightening on current understanding of the mechanisms underlying parkinsonian motor and non-motor symptoms. In particular, this new generation of research tools provide a method by which significant insights can be gained on brain networks implicated in brain diseases such as PD. These tools also promise to assist in the development of novel therapies for targeting degenerated dopaminergic and non-dopaminergic neurons in the diseased basal ganglia system of PD patients, for providing symptomatic relief or even reverse neurodegenerative processes. The present review discusses how such technologies, in conjunction with application of sensitive behavioural assays, continue to significantly advance our knowledge of circuit and signalling properties inherent to PD pathology. The discussion also highlights how such experimental approaches provide additional explorative avenues which may result in dramatically improved therapeutic options for PD patients.

Contents

Abbreviations: ACh, acetylcholine; AAV, adeno-associated virus; AgRP, agouti-related protein; ArchT, archaerhodopsin; BAC, bacterial artificial chromosomes; ChRs, channelrhodopsins; DBS, deep brain stimulation; DREADDs, designer receptors exclusively activated by designer drugs; DA, dopamine; dlVP, dorsolateral subcompartment of the ventral pallidum; eNpHR, enhanced halorhodopsin; EN, entopenduncular nucleus; GPe, external globus pallidus; GPi, globus pallidus internus; GFAP, glial fibrillary acidic protein; GPCRs, G protein coupled receptors; HSV, herpes simplex virus; HFS, high-frequency stimulation; LV, lentiviral; L-dopa, levodopa; LBs, Lewy bodies; LC, locus coeruleus; LFS, low-frequency stimulation; MSN, medium spiny neurons; PD, Parkinson's disease; PPN, pedunculopontine nucleus; REM, rapid eye movement; 6-OHDA, 6-hydroxydopamine; SNpc, substantia nigra pars compacta; SNr, substantia nigra reticulata; STN, subthalamic nucleus; TH, tyrosine hydroxylase.

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1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disease that is clinically characterised by bradykinesia (slowness of movement), extrapyramidal rigidity, postural instability and resting tremor. With the discovery that such symptoms result from the large-scale, progressive degeneration of dopamine (DA)-synthesising neurons that principally reside in the Substantia nigra pars compacta (SNpc), various DA-based pharmacological interventions have been developed. Although permitting for the effective clinical management of the cardinal symptoms of the disease, long-term usage results in the onset of progressively worsening side-effects, including dyskinesias and medication on-off effects. A surgical intervention therapy, deep brain stimulation (DBS), has been developed partly to overcome the limitations inherent to current pharmacotherapeutics (Limousin et al., 1998; Stefani et al., 2007; Ngoga et al., 2014).

A variety of other non-motor symptoms, including cognitive disturbances, hallucinations and rapid eye movement sleep disturbances form additional symptoms of the disease (reviewed by Schapira and Jenner, 2011). Such symptoms often comprise the most frustrating for attending clinicians to treat, as they remain non-responsive to DA-based interventions. This is due to underlying neural substrates rather comprising non-dopaminergic neuronal populations, such as degeneration of the locus coeruleus (LC's) norepinephrine-, raphe nucleus' serotonergic- and the pedunculoponine nucleus' (PPN's) and forebrain's cholinergic neurons (Hirsch et al., 1987; Chan-Palay and Asan, 1989; Politis et al., 2012). In this regard, work suggests that PD-related neuropathology progresses caudal-rostrally, with non-dopaminergic cell death that occurs prior to that of SNpc dopaminergic neurons (Del Tredici et al., 2002).

An additional diagnostic hallmark of both idiopathic and familial PD is the presence of widespread accumulation and aggregation of proteins such as α-synuclein (αSYN) and ubiquitin, which appear within eosinophilic intracytoplasmic neuronal inclusions, termed Lewy bodies (LBs) and Lewy neurites (Kuzuhara et al., 1988; Spillantini et al., 1997). Hypotheses relating to the mechanism of LB formation include a failure of the proteasome (McNaught et al., 2001; Pienaar et al., 2013a) and that αSYN monomers join to generate oligomers (protofibrils) in the neuronal cytoplasm, during a process referred to as fibrillization. Protofibrils then coalesce to form fibrils, which in turn aggregate into LB neuronal inclusions (Conway et al., 1998; Wood et al., 1999). Whereas monomers and oligomers are soluble, fibrils and LBs remain insoluble within the neuronal cytoplasm. In seminal work, the A53T αSYN mutation, responsible for familial PD, was shown to increase the rate of protofibril formation during fibrillization (Conway et al., 2000). However, the precise role of these abnormal aggregates of protein found in neurons of post-mortem brains of parkinsonian patients remains contentious.

As the second most common neurodegenerative disease, affecting approximately 1% of adults aged over 60 years (Lees et al., 2009) and with the ageing population increasing in many societies, a critical need exists for developing novel experimental tools for elucidating to which extent brain networks are altered in Parkinsonism. It is believed that such insights will generate the fundamental insights necessary for designing and developing therapies aimed at preventing or reversing parkinsonian pathology.

2. Neuro-regulatory tools

A revolutionary new set of research techniques, which relies on the use of genetically-encoded tools by which to achieve neural control, has greatly increased opportunities for researchers to study and control neural circuits in validated experimental animal models of PD (Pienaar et al., 2012; Bury and Pienaar, 2013). The overarching term 'optogenetics' refer to a set of techniques which express light-responsive, membrane-bound cation channels, capable of activating neuronal firing in a temporally precise and reversible manner (Deisseroth, 2011). Commonly used light-reactive ion channels (opsins) include channelrhodopsins (ChRs) as well as its variants, capable of upregulating neuronal activity. On the other hand, optical silencers include halorhodopsin (NpHR) that are specific for chloride ions and were first discovered in *Natronomonas pharaonis* (Schobert and Lanyi, 1982), whilst characterisation of the hyperpolarizing proton pump (triggered by yellow-green light, 550 nm) archaerhodopsin (ArchT), with its encoding gene *archaerhodopsin-3* (*Arch3*) that derives from *Halorubrum sodomense* (Chow et al., 2010), followed suit. It was shown that the neuronal pH levels resulting from Arch-mediated proton pumping compared favourably to those triggered either by ChRs or to the spike firing seen *in vivo* (Chow et al., 2010). Moreover, the use of Arch holds advantages over utilisation of NpHR. For instance, continuous illumination instigates the decay of NpHR-mediated hyperpolarization, necessitating the delivery of brief light-pulses instead. In contrast, Arch was shown to resist such inactivation to prolonged illumination (Okazaki et al., 2014). In addition, *in vitro* studies showed that Arch generates a large neural silencing current at a lower light intensity, compared to NpHR (Chow et al., 2010). Han et al. (2011) introduced ArchT, a light-driven proton pump deriving from the Halorubrum strain TP009, which exhibited an action spectrum matching that of Arch, whilst showing an ability to silence mammalian neurons at 3.3× higher light sensitivity compared to Arch (Okazaki et al., 2014). However, the enthusiasm with which ArchT has been met, has been slightly tempered by the suggestion that its use could initiate tissue toxicity, although concrete evidence for this is yet to be given.

Berndt et al. (2014) recently put forward an improved design on existing direct light-triggered inhibition of neuronal activity mediated through inward-pumping Cl^--transporting opsins and outward-pumping proton-transporting opsins. The improved design was intended to overcome several disadvantages associated with ion pumps. These include the inefficiency by which pumps operate in neural systems due to their inherent restriction to only move a single ion per photon, whilst no input resistance decrease is elicited. In addition, since pumps require energy for transporting ions against an electrochemical gradient, there is significant potential for abnormal gradients to form (Mattis et al., 2011). An additional reason for creating an inhibitory channel for use in optogenetics-based experiments is due to pumps not being able to achieve light sensitivity and long-term photocurrent stability (Bamann et al., 2010; Berndt et al., 2014). The authors hypothesised that the cation-selectivity of ChR2 results from negative electrostatic potential surrounding the pore and vestibule and is thus aimed at reversing this polarity and create an inhibitory ChR2 by placing site-direct mutations within the ChR2 chimaera, C1C2 (Kato et al., 2012). Mutations that showed most promise, as assessed by whole-cell patch–clamp recordings for measuring photocurrents, were then combined to finally produce a nine-fold mutant. When expressed in human embryonic kidney cells, functional analyses of the multi-fold mutant revealed that peak and stationary photocurrents remained fast and robust, whilst the spectrum of response to blue light–activation was similar to that of the non-mutated C1C2, emphasising the potential use for this newly engineered opsin in functional optogenetics studies. To meet this demand, the group designed and tested a new class of inhibitory ChR2, by converting their cation-conducting properties into chloride-conducting ones. The authors claimed that this new set of optogenetic proteins enabled more physiological, efficient and sensitive optogenetic inhibition.