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Molecular Tools to Study and Control Dopaminergic Neurotransmission With Light

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ABSTRACT

Dopaminergic neurotransmission is involved in several important brain functions, such as motor control, learning, reward-motivated behavior, and emotions. Dysfunctions of dopaminergic system may lead to the development of various neurological and psychiatric disorders, like Parkinson's disease, schizophrenia, depression, and addictions. Despite years of sustained research, it is not fully established how dopaminergic neurotransmission governs these important functions through a relatively small number of neurons that release dopamine. Light-driven neurotechnologies, based on the use of small light-regulated molecules or overexpression of light-regulated proteins in neurons, have greatly contributed to the advancement of our understanding of dopaminergic circuits and our ability to control them selectively. Here, we overview the current state-of-the-art of light-driven control of dopaminergic neurotransmission. While we provide a concise guideline for the readers interested in pharmacological, pharmacogenetic, and optogenetic approaches to modulate dopaminergic neurotransmission, our primary focus is on the usage of photocaged and photoswitchable small dopaminergic molecules. We argue that photopharmacology, photoswitchable molecules of varied modalities, can be employed in a wide range of experimental paradigms, providing unprecedent insights into the principles of dopaminergic control, and represent the most promising light-based therapeutic approach for spatio-temporally precise correction of dopamine-related neural functions and pathologies.

Galyna Maleeva, Carlo Matera, and Pau Gorostiza equally contributed to this study.

1 | Introduction

Class A G protein-coupled receptors (GPCRs) represent the majority of the GPCR superfamily (~85%), and among these, dopamine receptors (DARs) are of particular importance [1]. DARs are widespread in the vertebrate central nervous system (CNS) and are essential to regulate various vital functions including voluntary movement, feeding, reward, motivation, sleep, attention, memory, and cognition. They recognize as primary endogenous ligand the catecholaminergic neurotransmitter dopamine (DA), one of the most behaviorally powerful neuromodulators [2, 3]. DA is the predominant catecholaminergic neurotransmitter both in the CNS and in the periphery. In the former, it is involved in the control of numerous activities (e.g., locomotion, cognition, movements, positive reinforcement, food intake, and endocrine regulation) while in the latter, DA acts as a modulator of cardiovascular functions, catecholamine release, hormone secretion, renal function and gastrointestinal motility [4, 5].

A classical pathway of DA synthesis in the CNS was described by Blaschko in 1939, while recently it was demonstrated that substantial part of DA is biosynthesized outside the brain by mesenteric organs [6, 7]. In the CNS, synthesis of DA starts in the cytosol of catecholaminergic neurons (CAergic neurons) with the hydroxylation of L-tyrosine (which is transported across the blood brain barrier) by tyrosine hydroxylase (TH) to obtain L-DOPA. It is then decarboxylated to DA by the enzyme aromatic amino acid decarboxylase (AADC) also known as DOPA decarboxylase. DA can be further involved in the synthetic pathway of norepinephrine and epinephrine. After completing the synthesis in CAergic neurons, DA is quickly stored into synaptic vesicles by secondary active transport via the vesicular monoamine transporter 2 (VMAT2). This allows to preserve DA from oxidation, as the slightly acidic intravesicular pH allows to stabilize the neurotransmitter. Upon excitation of DAergic neurons, DA is released to the synaptic cleft to activate postsynaptic DARs or regulatory presynaptic DA autoreceptors [6]. To terminate the signaling, extracellular DA is cleared from the synaptic cleft by the DA active transporter (DAT): DA reuptake can lead to either its recycling into synaptic storage vesicles in DAergic neurons or its degradation by glial cells. In the former case, DA accumulated in the cytosol, as a consequence of leakage from synaptic vesicles, is degraded by monoamine oxidase (MAO); in the latter, DA is catabolized by MAO and catechol-O-methyltransferase (COMT) [6, 8]. COMT is predominantly expressed by glial cells, whereas in CAergic neurons it is either missing or found at very low levels [9]. Understanding the diverse components of DA receptor (DAR) signaling and DAergic transmission is crucial both in research and medicine, as dysfunction in these pathways contributes to complex neurological and psychiatric disorders, including Parkinson's and Huntington's diseases, schizophrenia, attention deficit hyperactivity disorder, Tourette's syndrome, substance abuse, and addiction [3].

The architecture and atomic structure of DARs has been determined in extensive biochemical and crystallographic or cryo-electron microscopy studies. Cryo-electron microscopy was used to elucidate the structure of an agonist-bound activated D_2R–G_i complex [10], while X-ray crystallography was used to resolve the crystal structure of D_1R [11], D_2R [12, 13], D_3R [14] and D_4Rs [15]. DARs can be homo- or heterodimers, formed by monomers belonging to the five receptor subtypes that are part of the GPCR family and that share most of their structural characteristics. These receptors have seven transmembrane domains connected by a total of six loops, both intracellular and extracellular, and are characterized by the presence of a disulfide bridge between two cysteine residues in the extracellular loops 2 and 3 to stabilize the structure. Different DARs have variable number of N-glycosylation sites on their NH_2 terminals. DARs are divided in two groups based on the following structural and biochemical features: (1) the C-terminal tail size, which is 7 times longer in D_1-like receptors, than in D_2-like receptors; (2) the length of the third cytoplasmatic loop (link between transmembrane domains 5 and 6), which is responsible for the G protein coupling and thus transmission of the intracellular signaling [16]; and (3) their coupling to either $G_{\alpha s,olf}$ proteins or $G_{\alpha i/o}$ proteins, which respectively stimulate or inhibit the production of the second messenger cyclic adenosine monophosphate (cAMP). D_1-like receptors (D_1, D_5) stimulate cAMP synthesis, while D_2-like receptors (D_2, D_3, D_4) inhibit its production. The binding site of DA and other agonists is predicted to be between transmembrane helices 3, 4, 5 and 6, with tight binding between helices 3 and 5, whereas the binding of the antagonists involves helices 2, 3, 4, 6, 7, with tight binding between helices 3, 6 and minimal contact with transmembrane helix 5 [4].

New insight in the signal transduction mechanism has revealed that DARs can act also through other signaling mechanisms that involve alternative G protein coupling or G protein-independent mechanisms via interactions with ion channels or proteins that are implicated in receptor desensitization, such as G protein-coupled kinases (GRKs) and β-arrestins [17]. Looking deeply into the signal transduction, we can see that the modulation of cAMP, and hence of the protein kinase A (PKA), results in changes in the phosphorylation of a wide range of cytoplasmic, nuclear and membrane proteins affecting significantly the functioning of the cell. DARs can modulate other transduction systems, such as those mediated by phospholipases, arachidonic acid, and MAP kinases, and they can control different channels and transporters including K^+ and Ca^{2+} ion channels, Na^+/H^+ exchange and Na^+/K^+ ATPase. For example, by controlling K^+ and Ca^{2+} channels, DARs affect cell excitability: D_1 receptors enhance neuronal excitability by reducing K^+ conductance and increasing Ca^{2+} currents; instead D_2 receptor activation has an opposite effect, leading to membrane hyperpolarization and reduction of the firing rate [16].

In the CNS, DARs are predominantly expressed in striatum, infundibulum, limbic system and cerebral cortex, hence creating the targets of the four main DAergic pathways: the nigrostriatal, the tuberoinfundibular, the mesolimbic, and the mesocortical (Figure 1A). It was demonstrated that the effects of DAergic signaling in the circuits can shift depending on the extracellular DA concentrations, as D_2-like receptors show a higher affinity for DA than D_1-like receptor. Because of this, DAergic signaling is deeply shaped by the intensity of the activity of DAergic neurons. Phasic release (resulting from burst-firing of DAergic neurons) leads to activation of D_1-like receptors, while tonic release (resulting from population-firing) due to the low concentration of DA liberated can activate only

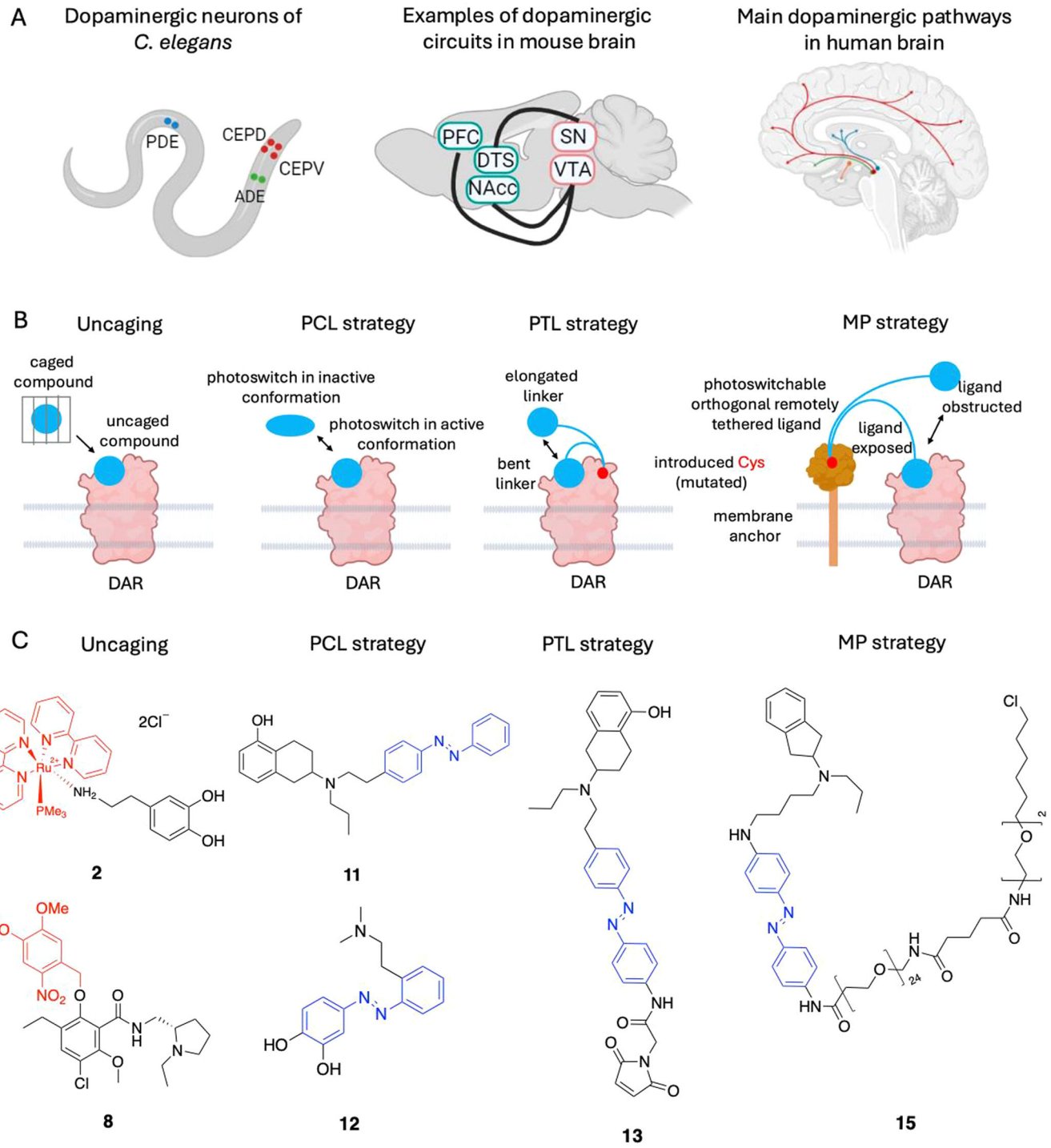

FIGURE 1 | Legend on next page.

D_2-like receptors [18]. This property of DAergic signaling is considered to be the key for the complex patterns of the activity generated within DAergic circuits.

The role of DARs in different neuronal populations in the striatum exemplifies the complexity of DAergic signaling: studies show that D_1 and D_2 receptors are present in striatal GABAergic medium spiny neurons (MSNs) and respond to a different range of stimuli, as D_1Rs react mostly to DA burst signals (neurotransmitter phasic releases), whereas D_2Rs effects are influenced by both phasic and tonic release of DA, and even these responses are conditioned by the environment created by other neurotransmitters [19]. A similar complexity can be observed in cholinergic interneurons in the striatum: DA phasic release can control these cholinergic neurons as they express D_5 (D_1-like) and D_2-like receptors. In particular, cholinergic interneurons firing is controlled by both DA and glutamate co-release, creating a specific pattern of activity in these interneurons [20]. All these evidence show that, while DAergic neurons are limited in numbers and confined in specific and small areas, they can project in many other brain areas exerting powerful effects on their targets. As stated before, DAergic signaling is extremely

complex and its alteration can lead to the development of a wide range of neurodegenerative and psychiatric states such as Parkinson's disease, attention deficit and hyperactivity disorder, bipolar disorder, Huntington's and Tourette's syndrome, among others. Moreover, alterations in DA levels have been reported also in age-related decline symptoms, multiple sclerosis, and even in pain processing [3, 21].

To date, hundreds of compounds have been developed as DAR agonists (including full and partial agonists) and antagonists (neutral antagonists or inverse agonists) to modulate DAergic transmission. They represent important pharmacological tools to study DAergic system and the mechanisms underlying the DA-dependent disorders. Many of them are also used clinically to treat neurological and psychiatric disorders [22]. For instance, it was discovered that the blockade of D_1Rs in monkey lateral prefrontal cortex (PFC) impairs associative learning, making more difficult to learn novel associations: low stimulation and/or blockade of D_1Rs contribute to cognitive deficits in aging and in neurological and psychiatric disorders [23]. Furthermore, the blockade of D_2R, usually associated with antipsychotic treatments, are congruent with the poor capacity of antipsychotic treatments to improve the cognitive and negative symptoms in schizophrenia. In fact, they may further deteriorate PFC-dependent associative learning, cognitive flexibility, and motivation [24].

1.1 | Pharmacological Approaches

Over the last century, DAergic agonists and antagonists that display a high degree of D_1 like or D_2-like selectivity have been developed. Despite sharing DA as endogenous ligand, the subtype differences between orthosteric binding sites (OBSs) enable ligand selectivity. However, seeking selectivity within the same receptor subgroup is more challenging due to the extremely high similarity in their OBSs in a way that these are completely conserved between two or more subtypes. New insights in structural studies led to the development of allosteric ligands as compounds able to recognize secondary binding pockets (SBPs), allowing to obtain an improved selectivity within receptors subgroups. SBPs are less conserved, hence they allow to obtain higher selectivity for different DARs [25]. Another way to take advantage of SBPs is to synthesize a "bitopic" ligand by linking together orthosteric and allosteric pharmacophores or extending the orthosteric moiety to improve subtype selectivity. Both strategies have a great potential to provide new opportunities to target pathological processes with minimal propensity for developing side effects [22]. However, they present certain difficulties: for example, subtype-selective bitopic ligands were successfully created for muscarinic acetylcholine receptors (another class A GPCR), but not for D_2-like receptors due to their high homology, in particular between D_2Rs and D_3Rs [25].

With few exceptions, most conventional compounds lack the ability to recognize and differentiate among various subtypes of DARs and specific neuronal subpopulations. This is the most likely cause of the poor safety and efficacy of many DAergic drugs, which simultaneously activate different DARs that mediate distinct or even opposing physiological functions. Example adverse effects of approved DAergic drugs include hallucinations, delusions, confusion, depression, nausea, vomiting, tachycardia, hypotension, hyperprolactinemia, excessive gambling or shopping [26]. To overcome the side effects derived from poor selectivity, DAergic drugs are often administered at a suboptimal dosage, which leads to a narrow therapeutic window [27]. Thus, despite their clinical usefulness, DAergic drugs display multiple side effects produced by systemic administration, and the localized and on-demand control of DAergic activity remains an unmet need.

1.2 | Pharmacogenetic Approaches

Genetic approaches (overexpression, knockdown, or knockout of individual proteins) can be used to gain a better temporal control

FIGURE 1 | Endogenous DAergic circuits and strategies to control them with light. (A). Examples of DAergic pathways from the nervous systems of *C. elegans*, mice, and humans. Left panel. *C. elegans* has 4 pairs of DAergic neurons – CEPV, CEPD, ADE, and PDE – that release DA synaptically and extrasynaptically to mediate different behaviors of the nematode, such as memory, locomotion, and learning. Middle panel. Examples of DAergic pathways in the nervous system of mice: (i) projections from substantia nigra (SN) to dorsal striatum (DS); (ii) projections from ventral tegmental area (VTA) to nucleus accumbens (NAcc); (iii) projections from VTA to prefrontal cortex (PFC). Right panel. Main DAergic circuits of human brain: nigrostriatal pathway – from substantia nigra pars compacta to dorsal striatum; mesocortical pathway – from ventral tegmental area to prefrontal cortex; mesolimbic pathway – from ventral tegmental area to limbic system (amygdala, nucleus accumbens, hippocampus); tuberoinfundibular pathway – from hypothalamus to pituitary gland. (B). An overview of various strategies of light-dependent activation of DARs. Left panel: Caged DAergic compounds are freely diffusible; the cage undergoes photolysis, and the caged molecule can interact with DAR and activate or inhibit it. Left middle panel: PCL (photochromic ligand) strategy is based on freely diffusible photoswitchable molecules that can be reversibly toggled between two conformations (e.g., based on *cis*- and *trans*-azobenzene, one of them being more active than the other) by illuminating with light of two specific wavelengths. Right middle panel: PTL (photochromic tethered ligand) strategy is based on the ability of photoswitchable molecules carrying specific linker to tether to endogenous or introduced cysteine residues (like in the case of DARs). When illuminated with a specific wavelength, the photoswitchable linker changes its conformation and enables the interaction between the pharmacologically active moiety with the receptor binding site. Right panel: Another approach combines (1) a genetically overexpressed membrane anchor protein and (2) the application of a photoswitchable orthogonal remotely tethered ligand compound (MP strategy). This compound first conjugates to the cysteine introduced in the membrane anchor and then illumination is used to change the compound conformation between ligand-obstructed to ligand-exposed to allow the interaction with the receptor. (C). Examples of DAergic light-dependent modulators reported in literature. Uncaging strategy: 2, RuBi-Dopa, DA caged in [Ru(bpy)2(PMe3) (Dopa)] (PF6)2 (bpy = 2,2′ bipyridine, PMe3 = trimethylphosphine). 8, Caged inverse agonist of D_2R/D_3R (dechloroeticlopride-based). PCL strategy: 11, PCL *cis*-on DAergic agonist. 12, Azodopa, PCL *trans*-on DAergic agonist. PTL strategy: 13, MAP, PTL *trans*-on inverse DAergic agonist or neutral antagonist. MP strategy: 15, MP-$D2_{ago}$, PTL membrane anchored D_2R *cis*-on agonist. [Color figure can be viewed at wileyonlinelibrary.com]

over DAergic neurotransmission than with conventional pharmacology. These modifications allow a better understanding of the DARs' activation and functions in specific cell types. Examples of these useful pharmacogenetic tools are receptors activated solely by synthetic ligands (RASSLs) and designer receptors exclusively activated by designer drugs (DREADDs). RASSLs are engineered GPCRs obtained by directed mutagenesis or directed molecular evolution and have proven useful to study GPCR signaling, for example, to control growth and ensure appropriate control of function for experimental or therapeutic tissue engineering when expressed at different levels of constitutive activity. Unfortunately, RASSLs are far from being optimized, however they show a potential to be used as therapeutic approach in many neurological diseases, such as Parkinson's disease, by correcting the imbalance of neural pathways, in a manner that could complement the surgical/electrical approaches in current clinical practice [28]. DREADDs were directly developed from RASSLs as these showed inherent problematics such as low affinity for chemical compounds, low constitutive activity, and eventually they still responded to endogenous ligands [28]. As a matter of fact, initially RASSLs and DREADDs were distinguished by the approach used to engineer them, but nowadays the two terms can be used interchangeably as they both represent engineered receptor-ligand systems. Recent studies have applied the DREADD technology to identify GPCR signaling processes that control pathways relevant to the treatment of eating disorders, obesity, and metabolic abnormalities [29]. All in all, although such tools offer powerful means to engage GPCR-dependent processes, they require overexpression of nonnative proteins that lack critical aspects of DARs, including their endogenous ligand binding site for DA, and/or receptor elements that control signaling, localization, and trafficking. Thus, current pharmacogenetic tools may provide an incomplete, or even inaccurate, view of DAR function In Vivo [30]. In addition, genetic modifications affect receptor function over long time scales, which limits our understanding of the temporal aspects of DAR activation and can result in confounding compensatory effects on neuronal physiology. Moreover, genetic modification of an organism for overexpression of the proteins is a highly invasive procedure that hampers development of DREADDs and RASSLs into viable therapeutic applications. Hence, methods to selectively and noninvasively activate DARs with high spatiotemporal resolution are required both for research and therapeutic purposes.

1.3 | Optical Methods

Light serves as an exceptional noninvasive tool for manipulating biological systems and offers high spatial and temporal precision. Its advantages include remote, reversible, and rapid action, along with simple modulation of energy both qualitatively and quantitatively [31]. Additionally, light has low or negligible toxicity, depending on the intensity and wavelength. These properties enable precise control and investigation of rapid, microscopic processes at the cellular or subcellular level.

Optical methods include optogenetics, photocaged or photocleavable compounds, and photoswitchable or photoisomerizable compounds. They are reviewed separately in the next sections. Optogenetics has greatly contributed to the establishment of light-mediated methods as tools of choice for precision control of neuronal activity; however, it relies on gene delivery and overexpression of exogenous proteins, which complicates its application to humans. A previous and alternative approach involves using photolabile "caged" compounds, which are delivered in an inactive form and, upon irradiation, release biologically active ligands with spatiotemporal precision. Today, caged ligands are among the most widely used tools in photopharmacology, with ongoing innovations in photocage varieties. Reversible photopharmacology was reported in the late 1960s and has recently gained increasing prominence, enabling the modulation of small molecules' biological activity through light-induced changes in their geometry.

1.4 | Optogenetic Tools

Optogenetic tools have deeply influenced the progress of neuroscience, allowing to study the activation of neurons and circuits by using cell targeted expression of photoreceptors and photosensitive proteins to obtain cell selective activation or inhibition with light. The modern optogenetic toolbox includes fluorescent sensors to visualize signaling events in living cells and optogenetic actuators enabling manipulation of numerous cellular activities [32].

DA controls diverse behaviors, and dysregulation of DAergic system contributes to many disorders. Optogenetics has helped to understand DA functions, overcoming the molecular and anatomic complexity of the signaling network and DAergic projections, as they have various, sometimes overlapping targets. For example, the relationship between DA release and the activation of specific striatal DARs expressed by different cells is not well understood yet: optogenetic stimulation of DAergic cell bodies or their axons can provide a certain level of control over DA release and hence a better chance to unravel the DAergic system [19].

Corkrum et al. found a way to explore in depth the role of astrocytes in the nucleus accumbens (NAc) as active components of DAergic signaling and the brain reward system. They discovered that astrocytes in the NAc respond to synaptic DA In Vivo and mediate the synaptic regulation induced by DA and amphetamine, using DAT-IRES-Cre mice to selectively express into the ventral tegmental area (VTA) ChrimsonR (red shifted channel rhodopsin) in DAergic neurons projecting to the NAc [33]. Previously, Cho et al. demonstrated that the activity of the dorsal raphe nucleus DAergic neurons (DRN^{DA}) correlates with sleep-wake states and can bi-directionally modulate arousal. The relationship between DRN^{DA} activity and wakefulness was tested using a Cre-dependent excitatory opsin to stimulate DRN^{DA} cells [34]. Authors have shown that both endogenous and optogenetically driven DRN^{DA} activity were associated with waking from sleep, and that DA signal strength can predict wake duration. Another example is seen in the work of Yang et al., in which they defined the networks involved in the control of mesolimbic DA neurons. In particular, the authors used channelrhodopsin-2 (ChR2) as an optogenetic tool to determine whether distinct NAc subregions directly target different mesolimbic DA subpopulations [35].

The mesolimbic DA system has a central role in motivated behaviors, reinforcement learning and reward processing with different functional and organizational principles that lead to a

specific control of the DA neurons: the signal complexity is seen in distinct NAc inputs that can promote excitation or inhibition of mesolimbic DA subtypes involving interaction with distinct GABA receptor classes. Salinas-Hernandez et al. studied the involvement of DA neurons in the VTA in extinction of fear responses, as it is a critical aspect in adaptive behavior and its deficit in safety learning represents a trait in anxiety disorder. Using optogenetic approach, authors have found that inhibition of DA neurons firing at the time of the unconditioned stimulus (US) omission is necessary for the fear extinction learning, while enhancing DA neurons firing at the time of the US omission accelerates fear extinction learning [36].

Lohani et al. used optogenetics in Th-Cre rats (expression of Cre recombinase under tyrosine hydroxylase TH promoter) to selectively stimulate VTA DA neurons, while measuring global hemodynamic changes using blood-oxygenation-level–dependent imaging (BOLD) and cerebral blood volume-weighted (CBVw) fMRI. This study suggests a functional interaction between phasic activation of VTA DA neurons and basal ganglia systems, providing a novel view point that may be used for reevaluation of existing theoretical models of striatal-dependent brain functions in healthy and pathological conditions, such as schizophrenia and substance abuse disorder [37]. Furthermore, Saunders et al. demonstrated that brief phasic optogenetic excitation of DA neurons associated with sensory cues, is sufficient to turn those cues into conditioned stimuli that subsequently are able to both evoke DA neuron activity and elicit cue-locked conditioned behavior. Using Th-Cre rats expressing ChR2 in ventral midbrain, the authors were able to target TH + DA neurons with ~97% specificity and to demonstrate highly parcellated functions for DA neuron subpopulations projecting to different regions of striatum [38].

Optogenetics is a useful tool to study and understand the DA function and involvement of DA neurons in movement, behavior, and in numerous diseases. Although optogenetic constructs are expressed at the cellular level, this method can control DA release in a specific manner by illuminating different locations. However, it is difficult to identify the specific behavioral outcomes due to the neuron's localization, distinct signaling properties and downstream targets of DARs. Moreover, it is known that DA signals can operate over different temporal domains, and DA can be co-released with glutamate and/or GABA [39]. Despite all the progress, current optogenetic method cannot clearly define the role of specific receptors, hence the need for new methods that allow the precise control of specific DARs. Furthermore, the achievements of optogenetics rely on the overexpression of exogenous proteins that lack critical aspects of endogenous GPCR signaling, including their native ligand binding sites, downstream molecular interactions, and other elements that affect receptor dynamics. Besides, the application of genetic manipulation techniques to human subjects is still hampered by safety, regulatory, and economic issues. These limitations can be partially overcome by alternative approaches.

1.5 | Photocleavable Tools

Photopharmacology offers powerful methods to manipulate DAergic transmission that can overcome some of the limitations of optogenetics. They are based on synthetic chemical compounds that bind and activate, inhibit, or regulate DARs endogenously expressed in tissue, using illumination (see Table 1). These compounds can be broadly classified in photocleavable or caged derivatives that irreversibly release DAergic ligands, and photoswitchable or photoisomerizable derivatives that reversibly toggle between conformations bearing different DAergic activity (Figure 1B). Caged compounds, also referred to as photo-triggers, are light-regulated probes that encapsulate the ligand (DA or other active small molecules) in an inactive form. They are able to absorb light and undergo an irreversible reaction, usually a photolysis, to release the active compound, enabling its activity: the irradiation liberates the trapped molecule, permitting targeted perturbation of a biological process [40–42]. Light can be directed in a precise manner and modulated in time and amplitude, allowing control of the target of choice (specific synapses or many neurons simultaneously). The uncaging technology is usually paired with fluorescence microscopy or electrophysiological methods as ways to evaluate the effect on the receptor of interest: in particular, photo-manipulation with caged compounds was revealed as an exceptional way to perform kinetic experiments on neurotransmitter release and clearance, mapping the receptors' distribution and establishing their role in neuronal circuits activity [43].

DA signaling in the brain is extremely complex and involves also other neurotransmitters, hence it is fundamental to simulate a presynaptic release with a tight temporal and spatial precision to mimic the natural release of the neurotransmitter. However, how DARs modulate information flow in neuronal circuits, neurons, and synapses is still poorly understood. Photocleavable agonists and antagonists offer a powerful tool for detailed investigation of DARs, their interactions with other GPCRs, and the physiology of DA signaling in the brain [44].

In one of the earliest applications of caged compounds, Lee et al. investigated DA release and clearance in the caudate nucleus and PFC [45]. They explored how DA autoreceptors regulate release and specific transporters facilitate DA uptake, key mechanisms that influence the temporal and spatial dynamics of DA within synapses, although these processes were not fully understood at the time. By synthesizing and testing "caged-DA" (**1**, Figure 2), the authors demonstrated that photoreleased DA inhibited the release of endogenous DA in the caudate nucleus [45].

Etchenique, Yuste and collaborators developed a caged DA compound based on ruthenium photochemistry, showing enhanced photochemical characteristics and allowing a high uncaging efficiency with visible light (blue-green) and with infrared light using two-photon excitation (2PE) [46]. Using RuBi-Dopa (**2**, Figure 1C) 2PE uncaging, the presence of functional D_1 receptors in mouse prefrontal cortex (PFC) spines was demonstrated. This study served as a proof of concept for the possibility of reliable manipulation of DAergic receptors on dendritic spines with high spatial resolution ($1\ \mu m^2$) using two-photon excitation. RuBi-Dopa was later used to better investigate the physiological mechanisms underlying DA action in the PFC since its effect on different cell types can be different and even opposite. It was shown that DA release in the medial PFC shifts phase-amplitude co-modulation from theta-gamma to

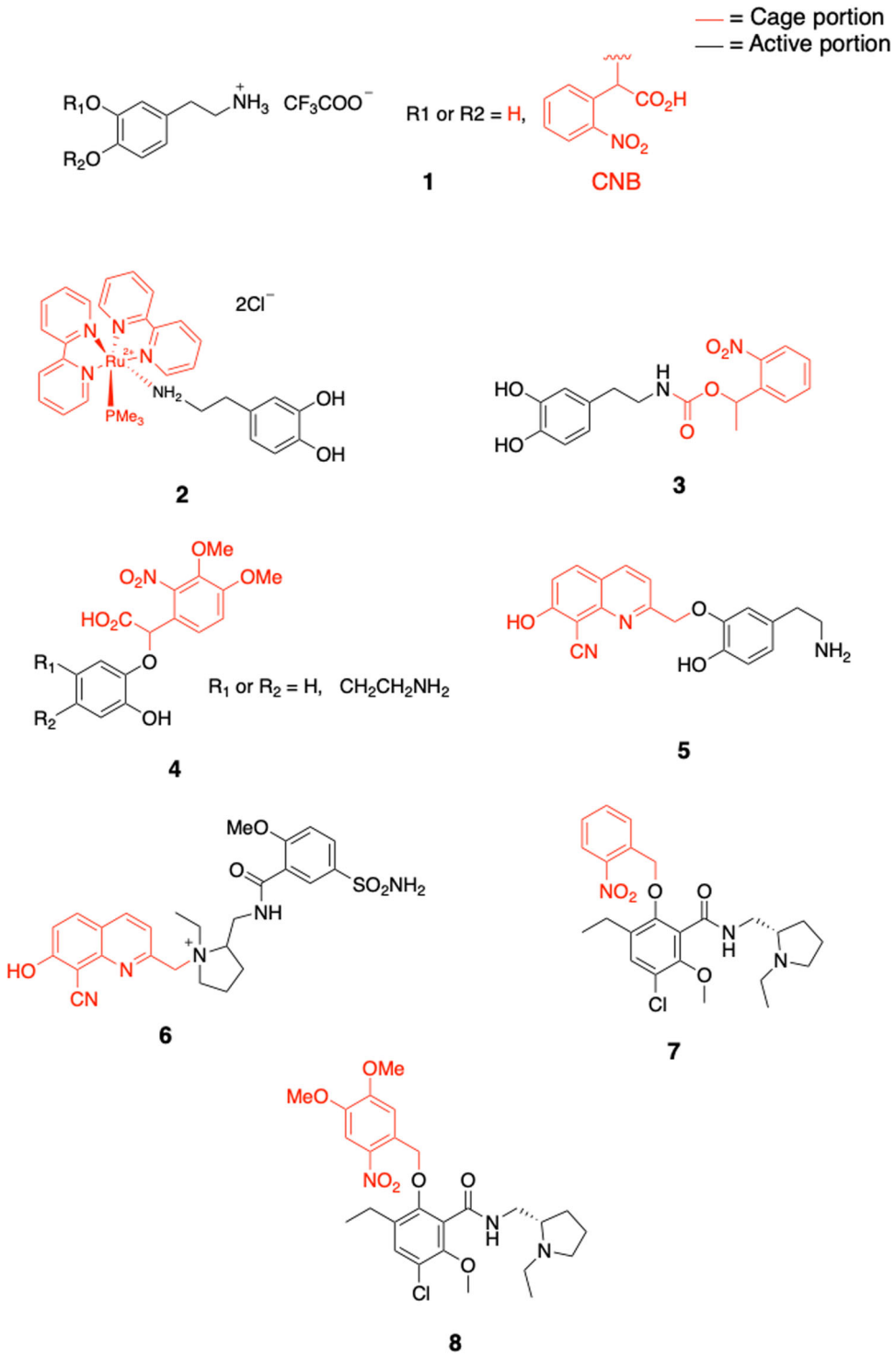

FIGURE 2 | Literature reported caged-DAergic modulators. [Color figure can be viewed at wileyonlinelibrary.com]

delta-gamma and can potentially be a key mechanism through which DA influence the functioning of mPFC [47].

Castro et al. used commercial NPEC-caged DA (**3**, Figure 2) and genetically encoded sensors of cAMP and PKA activity to measure the differences in intracellular molecular dynamics triggered by activation of D_1 receptors between cortical and striatal neurons [48]. They demonstrated that MSNs in the striatum are more sensitive to short phasic exposures to DA than cortical neurons. This property of striatal neurons might be tightly connected to the specific role that striatum plays in DAergic signaling. Later NPEC-caged DA was used to study the dynamics of the DAergic responses in D_1 and D_2 expressing MSNs [49]. The authors paired biosensor imaging (genetically encoded FRET biosensors in striatal brain slices) with photo-release of caged-DA to investigate the effect of transient DA on cAMP or PKA-dependent phosphorylation levels in D_1R and D_2R neurons. They demonstrated that while phasic DA strongly

affects cAMP levels in D_1R and D_2R expressing neurons, the PKA-dependent phosphorylation level remains unchanged in D_2R neurons.

Robinson et al. have examined the signaling kinetics and mechanisms underlying adaptive processes such as desensitization-induced internalization of D_2Rs using carboxynitroveratryl-DA (CNV-DA, **4**, Figure 2), replacing the previous carboxynitrobenzyl (CNB) photolabile group from other caged compounds to improve photostability and shifting the wavelength of absorption to 365 nm [50].

Asad et al. synthesized two caged DA compounds in which the 8-cyano-7-hydroxyquinolinyl (CyHQ) photoremovable protecting group was conjugated to DA and the DAR antagonist sulpiride (CyHQ-O-DA **5** and CyHQsulpiride **6**, Figure 2), that could be released with 365 or 405 nm light or through 2PE at 740 nm. The CyHQ caging group has improved physical and photo-chemical properties for In Vivo use. CyHQ-O-DA and CyHQ-sulpiride have fast release kinetics, favourable 2PE cross section and high stability in aqueous buffers. However, the efficiency of these molecules has been demonstrated only in brain slices [44].

The same year, Gienger et al. described two caged DAR antagonists, composed of dechloreticlopride as the active moiety and 2-nitrobenzyl and dimethoxynitrobenzyl groups as cages (**7** and **8**, Figure 1C; Figure 2), that can serve as valuable tools for light-controlled blocking of D_2/D_3 receptors [51]. In Vitro testing on heterologously expressed receptors demonstrated that photolyzing both compounds efficiently inhibited intracellular DAergic cascades.

Caged compounds with photocleavable groups constitute molecular tools to investigate a biological target with high spatiotemporal resolution, potentially enabling new therapeutic strategies. However, relatively few caged bioactive molecules have the potential to be directly applied to living animals due to several properties: (i) short absorption wavelength (biological tissues are generally highly scattering to light, especially at short wavelengths); (ii) these probes are activated unidirectionally: even with the great spatial and temporal control for its activation, after irradiation the active ligand diffuses from the uncaging sites and follows the usual pharmacodynamic steps; (iii) photouncaging leads to release of byproducts with strong biological activities in other neuronal receptors (caged glutamate displays $GABA_AR$ activity regardless of the type of cage, and has limited applicability In Vitro and In Vivo for that reason); (iv) the low efficacy of photorelease requires very high working concentration which is expensive, aggravates toxicity issues, and limits bioavailability to apply them systemically. For these reasons caged molecules are rarely used In Vivo [52] and have not yet been tested clinically [53]. To the best of our knowledge none of the caged DAs have been used In Vivo so far.

1.6 | Photoswitchable Tools

Notwithstanding its virtues, uncaging is an irreversible chemical process that leaves byproducts of the photolysis in the medium, whereas photopharmacological modulation based on reversible molecular photoswitches has important advantages In Vivo. Reversible photoswitchable compounds were developed more than 50 years ago [54] and have recently obtained significant attention at biological and pharmacological levels, due to their high therapeutic potential, which led to the first clinical trial in 2022 that is currently in phase 2 [55, 56]. Photopharmacology uses light-regulated small molecules to control the response of endogenously expressed biological targets with light. Photoswitches are chemical entities composed of biologically active molecule and a photochromic group that can toggle between two or more stable states upon illumination with light of a specific wavelength in a reversible manner. The photochromic groups are various, but they need to display certain characteristics to be applicable to biological systems: the molecular switch must have favourable pharmacokinetics and metabolic stability, it must be nontoxic (to avoid cellular damage), and it must allow fast photoconversion and a high quantum yield at biologically compatible wavelengths of light. Azobenzenes exhibit many of these properties, making them the most extensively used photochromic molecules, alongside dithienylethenes and spiropyrans. Other structures, such as stilbenes, fulgides, hemithioindigos, chromenes, and donor-acceptor Stenhouse adducts have also been explored as photo-reversible switches; however, certain key chemical and physical characteristics require further development. Each photochromic group differs in the properties that change in response to light, including molecular shape, dipole moment, thermal stability, fluorescence intensity, redox potential, pH sensitivity, and others.

Molecular photoswitches can be classified depending on the mode of interaction with their target (Figure 1B) [31]. Photochromic ligands (PCLs) are freely diffusible molecules that can adopt two different isomeric forms upon irradiation: in this case switching can affect pharmacodynamics (binding affinity, efficacy, and potency) as well as pharmacokinetics of the compound. Covalent ligands anchored to the target protein represent another big class of photoswitches. They can be further divided into three subcategories: photoswitchable tethered ligands (PTLs), photoswitchable orthogonal remotely tethered ligands (PORTLs), and membrane-anchored photoswitchable remotely tethered ligands (MPs) [57], depending on the position of the covalent attachment site in relation to the ligand binding site. In PTLs the switching primarily affects the local effective concentration of the pharmacophore [58], while in PORTLs it affects mostly the efficacy of the tethered ligand [59]. Often, tethered ligands require genetic modifications of the target proteins (e.g., introducing cysteine residues for PRL conjugation) and their overexpression, making this technique invasive and decreasing its therapeutic potential. However, direct conjugation to endogenous receptors has also been reported, as in targeted covalent drugs [60]. In all cases, PTLs generate a fast response due to their high local concentration and the inability to diffuse away from the binding site. MPs combine a photoswitchable ligand (P) with a membrane-bound genetically overexpressed protein tag (M), allowing the ligand to tether near specific receptors and activate or inhibit them upon illumination.

The first DAergic reversible photoswitches (PCLs) were reported in 2017 by Lachmann et al. [61] and Donthamsetti et al. [30] almost simultaneously. Lachmann et al. described the

synthesis and characterization of DAR ligands incorporating photochromic switches that enabled reversible control over D_2Rs, implicated in CNS disorders like schizophrenia and Parkinson's disease. Two classes of photochromic scaffolds, dithienylethenes (DTEs) and fulgides, were incorporated into known DA ligands. DTE ligands were further subdivided into cyclopentene-DTEs and diarylmaleimides. Cyclopentene-DTEs showed high photostationary states (PSS) but degraded quickly into an irreversible byproduct, while diarylmaleimides exhibited high fatigue resistance but poor ring closure in polar solvents, attributed to twisted intramolecular charge transfer. Fulgide-based ligands, however, performed well in polar environments and demonstrated stable switching between open and closed isomers with high PSS values. Compounds like DTE *29* (**9**, Figure 3) and fulgide *52* (**10**, Figure 3) displayed significant differences in DAR activity between two different photostates. In IP (inositol phosphate) accumulation assay in HEK293T cells at 1 nM DTE *29*-open showed an 11-fold increase in D_2Rs activation compared to its closed form, while fulgide *52*-closed exhibited a fourfold higher activity than its open form. The efficacy of DTE *29* and fulgide *52* depended on specific functional groups and spacer attachments that allowed selective receptor activation by the open or closed forms, enabling precision control over GPCR-mediated signaling. Despite the remaining challenges in improving DTE stability in polar solvents, this study demonstrated for the first time that integrating photochromic scaffolds into DAR ligands enables reversible control of receptor activity, highlighting potential applications for studying their functionality and targeting related disorders.

Donthamsetti et al. described a PCL agonist of D_1Rs and D_2Rs – AP (**11**, Figure 1C; Figure 3) based on 2-(N-phenethyl-N-propyl) amino-5-hydroxytetralin (PPHT). The activity of this molecule (*cis*-on) was demonstrated in cAMP accumulation and BRET-based β-arrestin recruitment assays [30]. This molecule was used as a basis for the development of MAP (**13**, first DAergic covalent photoswitch, see below), but its properties were not studied in detail.

In 2022, Matera et al. presented a novel DAergic azobenzene-based PCL, named azodopa (**12,** Figure 1C; Figure 3) [62]. Azodopa was designed to activate preferentially D_1-like receptors, as demonstrated with cAMP production and ERK1/2 phosphorylation assays, key indicators of D_1Rs activation, as well as intracellular calcium mobilization, a secondary pathway triggered by these receptors [63]. In Vivo experiments in zebrafish larvae proved that azodopa increases swimming activity in its active *trans*-configuration and that its effect can be transiently reduced or halted by photoisomerization to *cis* under 365 nm light, in agreement with In Vitro results. Additional experiments with blinded zebrafish showed that the effects of azodopa are independent of visual cues, confirming its action on CNS DAergic circuits, and allowed to separate the retinal component of DAergic transmission. Finally, electrophysiological recordings in anesthetized mice showed that azodopa increases cortical activity and oscillatory power in the 1–10 Hz frequency range in a light-dependent way, although the effects varied among individual neurons possibly due to differential DAR expression across neuronal types. Overall, these results highlighted the potential of azodopa as an In Vivo nongenetic tool to study endogenous DA-related pathways and conditions, with applications extending to therapeutic contexts where light-based interventions would be beneficial to selectively control DAergic activity.

The first DAergic covalent photoswitch (PTL) was described in 2017 by Donthamsetti et al. [30] The PTL, named maleimide–azobenzene–PPHT (MAP, **13**, Figure 1C; Figure 3), was designed by combining a maleimide-azobenzene to a potent DA receptor agonist (PPHT) and DAR with cysteine mutations in proximity of the binding site to enable covalent binding. The engineered receptors, termed LiD1R and LiD2R (light-activated D_1Rs and D_2Rs), allowed spatiotemporal photocontrol over signaling pathways. The authors showed that MAP can photomodulate DARs in different ways: in combination with the D_1R mutation I183C in extracellular loop 2, MAP acted as a light-controlled antagonist; similarly, at the D_2R I184C site, MAP displayed inverse agonism in its *trans*-configuration. These findings indicated that the effectiveness and behavior of this covalent photoswitch depend on its tethering location on the receptor, affecting how MAP occupies the DA binding site.

A few years later, Donthamsetti et al. introduced another method for light-activated control of DARs based on membrane-anchored photoswitchable ligands (MPs) [57]. The MP-$D1_{ago}$ compound was designed to attach to a genetically encoded membrane anchor overexpressed by the target cells. This modality allowed for the selective localization of MP-$D1_{ago}$ to the desired cell type and confined its effect to specific DARs. The agonist component of MP-$D1_{ago}$ (**14**, Figure 4) was tethered to the anchor via a photoswitchable azobenzene linker responding to UV and blue light, allowing for precise temporal control of receptor activation (*trans*-active and *cis*-inactive). The authors used MP-$D1_{ago}$ to specifically activate D_1Rs in dorsal striatal medium spiny neurons, which play a critical role in movement initiation, showing that blue light increased locomotion initiation, and that the effect was reversed by UV light. This novel method could be in principle adapted to other GPCRs, expanding its applications in neuroscience research and therapeutic interventions.

The same approach was later used by Hetzler et al. to build a toolkit of photoswitchable ligands targeting D_2-like receptors (D_2R, D_3R, and D_4R) [64]. These researchers designed three types of MP ligands: a full agonist (MP-$D2_{ago}$, **15**, Figure 1C; Figure 4), a partial agonist (MP-$D2_{p.ago}$ **16**, Figure 4), and an antagonist (MP-$D2_{block}$ **17**, Figure 4). The functionality of these photopharmacological tools was demonstrated in HEK293T cells by measuring their effect on D_2R signaling through a co-expressed potassium channel, which acts as a downstream target. MP-$D2_{ago}$ produced near-complete activation of D_2R under blue light, and its effect was reversible in the dark. A branched variant of the ligand, MP-$D2_{ago}$-2X (**18**, Figure 4), showed enhanced sensitivity and faster response due to increased local concentration of the ligand. The authors also screened for the effect of MP-$D2_{ago}$-2X on other receptor subtypes to assess its specificity: MP-$D2_{ago}$-2X resulted to be a strong partial photo-agonist of D_3R, and a weak partial photo-agonist of D_4R; in contrast to its effect on D_2-like receptors, it had no effect on D_1-like receptors. Potential applications of

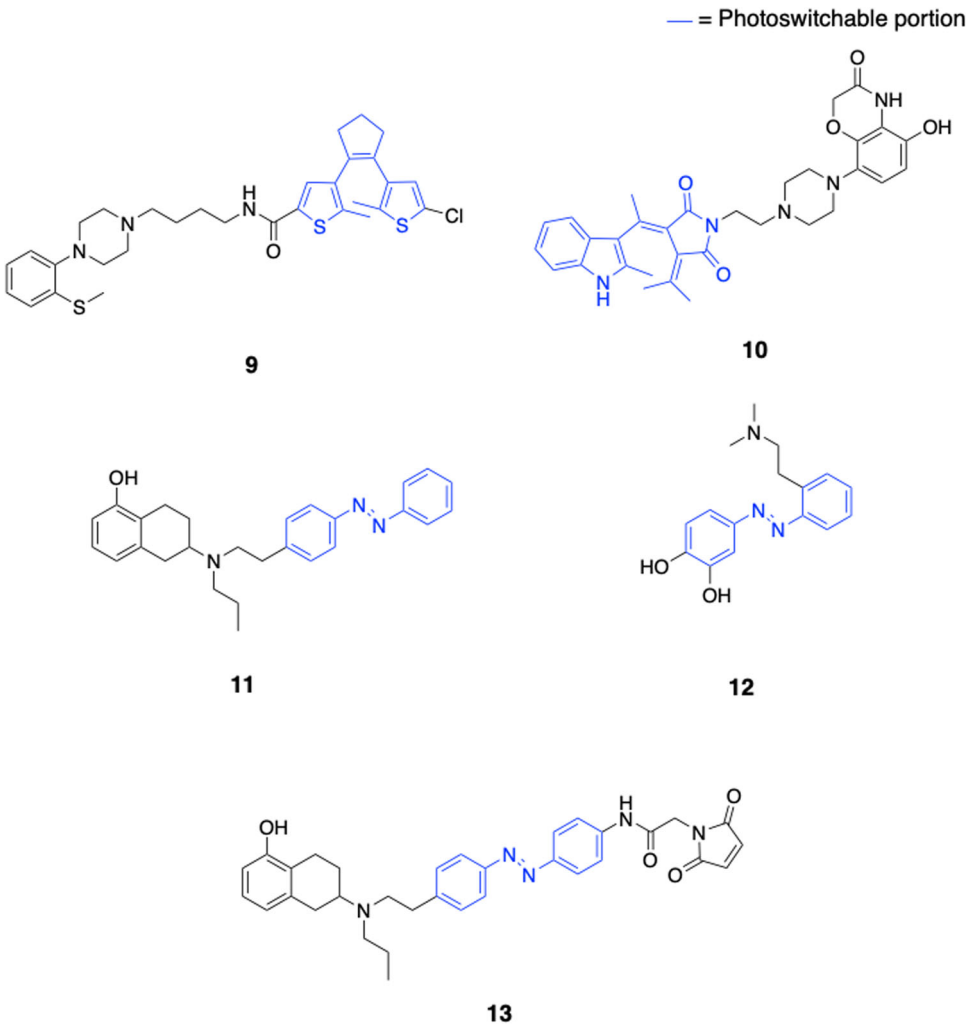

FIGURE 3 | Literature reported PCL and PTL DAergic modulators. [Color figure can be viewed at wileyonlinelibrary.com]

these and other MP tools include the investigation of receptor signaling In Vivo, where membrane anchors could be targeted to specific brain regions or cell types using viral vectors.

The activity of DARs has also been controlled with light using photoswitchable nanovesicles containing azobenzene-phosphatidylcholine (azo-PC) moiety that encapsulate SKF-81297 D_1-like DAR agonist. Xiong et al. developed "azosomes", a novel class of vesicles for highly photo controllable molecular release. By incorporating azo-PC into liposomes, the system undergoes reversible isomerization from *trans*-azo-PC to *cis*-azo-PC using short light pulse of 365 nm UV light.

Irradiation led to rapid reduction of lipidic bilayer thickness, increased permeability, and fast (less than 3 s) photocontrolled release of DAR agonist. Blue light of 455 nm immediately reversed thickness and cargo discharge of the system, demonstrating good photoswitchable release properties. The authors also demonstrated SKF-azosome efficacy by evaluating the effect of D_1 receptor activation mediated by SKF-81297 release on striatal neurons observing an increase in intracellular Ca^{2+}. Almost 20–30% of the treated neurons population showed higher levels of calcium after a single irradiation cycle. Thus, this new tool for DAergic neurotransmission manipulation offers light-controllable and repeatable release of DAR agonist cargo In Vitro in cultured striatal neurons [65].

Photoswitchable ligands have been successfully used to bi-directionally control activity of DARs in spatially and temporally precise manner In Vitro and In Vivo. They are chemically stable under physiological conditions, easily adaptable to various applications and formulations, and exhibit high selectivity for DARs. Moreover, most of these compounds (except certain tethered variants) do not require genetic modifications. Because they are synthetic compounds, like clinical drugs, and are subject to well defined pharmacokinetics and safety studies for approval, in our opinion photoswitchable ligands hold the greatest potential for In Vivo applications, both in the intricate study of neuronal circuits and in future therapeutic strategies targeting DARs.

Localized noninvasive activation of DARs will provide unprecedented opportunities for personalized treatment of neurological disorders caused by dopaminergic dysfunction. For instance, it was shown that agonists of DARs demonstrate better results in treatment of Parkinson's disease than L-DOPA and with later onset of side effects [66]. However, they still can cause valvulopathy and DA dysregulation syndrome. Using light-switchable agonists will allow to trigger activation of

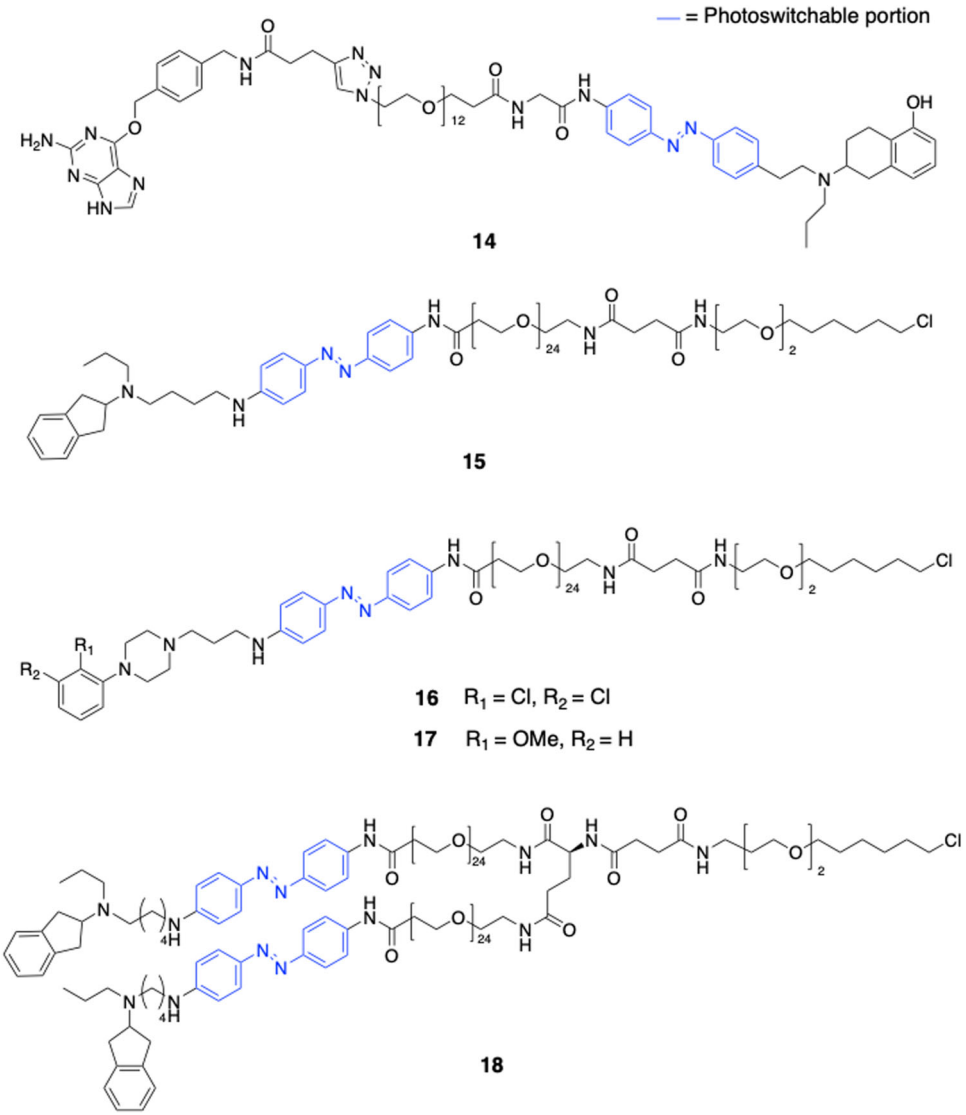

FIGURE 4 | Literature reported PCL and MP DAergic modulators. [Color figure can be viewed at wileyonlinelibrary.com]

DARs only in specific brain regions, or even specific neuronal circuits, without disturbing dopaminergic signalling in the rest of the brain and body. Similar approach will be beneficial for the treatment of other disorders connected to the dopaminergic dysfunction, such as schizophrenia [67], addictions [68], attention-deficit/hyperactivity disorder [69], and narcolepsy [70].

Currently existing photoswitchable DAR ligands have some limitations that should be overcome for their further adaptation to In Vivo and therapeutic usage. First, it is essential to develop novel photoswitches that can be activated by red or infrared light, which offers superior tissue penetration compared to blue or UV light. Second, systematic studies on the pharmacokinetics and blood-brain barrier permeability of photoswitchable compounds should be conducted to identify those with optimal properties and the greatest potential for therapeutic applications. Third, development of subtype-selective compounds, that would discriminate for instance between D_1Rs and D_5Rs, would greatly advance our understanding of how DAR control activity of neuronal circuits and allow to target specific neurological processes that are dysregulated in pathological conditions.

2 | Final Remarks

In the last few years, great strides have been made in the field of optical control of DAergic transmission, bringing to the fore sophisticated techniques for spatiotemporal modulation of DARs with exceptional specificity. The development of photoswitchable ligands has enabled the precise control of DAergic signaling pathways implicated in both physiological and pathological processes. Overcoming limitations of traditional pharmacology and genetic approaches, these light-activated tools allow selective, reversible, and fast modulation of function without the need for permanent genetic manipulations. The capability of controlling DARs on demand at localized regions of the CNS allows a deeper understanding of DAergic signaling and opens the way to minimize the side effects produced by systemic administration of DAergic drugs.

This review underlined several promising applications of DAergic photopharmacology and optogenetics for addressing critical questions in neuropsychiatry and neurodegenerative diseases. Photoswitchable DA agonists and antagonists hold promise for the advancement of research into Parkinson's

TABLE 1 | Photoswitchable (first part of the table) and photocleavable (second part of the table) molecules, that were developed for the control of DARs in chronological order.

Target	Type of (main) activity	Biological effects	Switching wavelengths	Compound name	Reference
D_1R and D_2R	PCL *cis*-on agonist	In Vitro: for D_1R - cAMP accumulation assay was used; for D_2R - assessing agonist-induced conformational changes within the G protein itself and arrestin recruitment assay.	360 nm/460 nm	AP **(11)**	Donthamsetti et al., 2017 [30]
D_2R	PCL open-form agonist (compound *29*) and PCL closed-form agonist (compound *52*)	In Vitro: IP (inositol phosphate)-accumulation, β-arrestin accumulation, competitive binding to the receptor.	For *29* – 259 nm and 489 nm; for *52* – 363 nm and 563 nm.	Ligands *29* (cyclopentene-DTE **9**), and *52* (fulgimide-based **10**).	Lachmann et al., 2017 [61]
D_1R and D_2R	PTL *trans*-on inverse agonist or neutral antagonist	In Vitro: inverse agonist and neutral antagonist effects on the currents mediated by G protein-activated inwardly rectifying K^+ (GIRK) channels co-expressed with D_1Rs or D_2Rs.	360 nm/460 nm	MAP **(13)**	Donthamsetti et al., 2017 [30]
D_1R	PTL membrane anchor tethered *trans*-on agonist	In Vitro: activation of GIRK channels co-expressed with D_1Rs and anchor construct. In Vivo in mutant mice: after the delivery of membrane anchor and P-$D1_{ago}$ to dorsal striatum medial spiny neurons, blue light was producing movement initiation in mice.	370 nm/460 nm	P-$D1_{ago}$ **(14)**	Donthamsetti et al., 2021 [57]
D_1R (D_2R)	PCL *trans*-on agonist	In Vitro: competitive radioligand displacement; activation of $G_{\alpha s}$ and consequent increase of cAMP accumulation, and phosphorylation of ERK1/2; activation of $G_{\alpha q}$ and consequent increase of Ca^{2+} mobilization. In Vivo in wildtype zebrafish: increase of motility. In Vivo in wildtype mice: increase in neuronal activity in anesthetised animals.	365 nm/fast relaxing	Azodopa (12)	Matera et al., 2022 [62]

(Continues)

TABLE 1 | (Continued)

Target	Type of (main) activity	Biological effects	Switching wavelengths	Compound name	Reference
D_2R	PTL membrane anchored D_2R *cis*-on agonist, partial agonist, antagonist	In Vitro: activation or modulation of GIRK channels co-expressed with D_2Rs. Blue light induced agonism, partial agonism and antagonism.	440 nm/fast relaxing	MP-$D2_{ago}$ **(15),** MP-$D2_{p.\ ago}$ **(16)**, MP-$D2_{block}$ **(17)**	Hetzler et al., 2023 [64]
D_1R and D_2R	DA caged by α-carboxy-2-nitrobenzyl (NCB)	Ex Vivo in rat brain slices: the clearance of photo-released DA and its effect on the release of endogenous DA were compared between the caudate nucleus and prefrontal cortex.	300-350 nm	Caged-DA **(1)**	Lee et al., 1996 [45]
DRs located in spines; specific D_1R inhibitor blocked the response	DA caged in [Ru(bpy)2(PMe3) (Dopa)] (PF6)2 (bpy = 2,2′ bipyridine, PMe3 = trimethylphosphine)	Ex Vivo in mice brain slices: two-photon calcium imaging and two-photon induced release of DA to demonstrate that spines can express functional DARs.	Visible blue-green and infrared 820 nm 2PE.	RuBI-Dopa **(2)**	Araya et al., 2013 [46]
D_1R (D_2R)	DA caged by (N)-1-(2-nitrophenyl) ethyl (NPEC)	Ex Vivo mice brain slices: striatal neurons are more sensitive to fast (0,1 s) stimulation with DA, than cortical neurons (Castro et al., 2013). D_1Rs and D_2Rs on striatal neurons have similar sensitivity to DA (Yapo et al., 2017).	360 nm	NPEC-caged-DA **(3)**	Castro et al., 2013 [48]; Yapo et al., 2017 [49]
D_1R and D_2R	DA caged by carboxynitroveratryl	Ex Vivo mice brain slices: activation of D_2Rs in live brain slices does not induce receptors' internalization.	365 nm	CNV-caged DA (**4**)	Robinson et al., 2017 [50]
D_1R and D_2R	DA and DAR antagonist sulpiride caged by 8-cyano-7-hydroxyquinolinyl	In Vitro: activation of D_1Rs on MDA-MB-231 cells. Ex Vivo mice brain slices: activation of D_2Rs on substantia nigra DA neurons manifested by activation of GIRK channels.	365 nm, 405 nm, 2P 740 nm	CyHQ-O-DA (**5**) and CyHQ-sulpiride **(6)**	Asad et al., 2020 [44]
D_2R (D_3R)	Caged inverse agonist of D_2R/D_3R (dechloroeticlopride)	In Vitro: radioligand binding assay; IP accumulation.	365 nm	Compounds 4, 5, 6 **(7-8)**	Gienger et al., 2020 [51]

disease, schizophrenia, and addiction, where DAergic dysfunction is at the core. Together, these tools confer on the researcher an unprecedented opportunity to differentially manipulate DAR subtypes implicated in disease mechanisms, thus facilitating the unraveling of complex issues concerning receptor-specific signaling and neural circuitry activation. However, translation into therapeutic contexts requires overcoming technical challenges to enhance light penetration and response time for successful application in live tissue and deep brain structures. Besides this, more powerful delivery systems are required for the practical use of light-sensitive ligands in clinical research, such as minimally invasive optical systems or even wireless devices. Continued advances in the chemistry of drugs, light, and drug delivery, as well as In Vivo animal models of neurological disorders, will be critical for the fulfillment of the potential of optical tools in furthering our knowledge and control of DAergic signaling. This convergence of multiple disciplines represents an exciting phase of neuromodulation research intended to better define the framework for future work on precision medicine and targeted therapies.

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Data Availability Statement

The authors have nothing to report.

References

1. C. S. Odoemelam, B. Percival, H. Wallis, et al., "G-Protein Coupled Receptors: Structure and Function in Drug Discovery," *RSC Advances* 10 (2020): 36337–36348.

2. D. Yang, Q. Zhou, V. Labroska, et al., "G Protein-Coupled Receptors: Structure-And Function-Based Drug Discovery," *Signal Transduction and Targeted Therapy* 6 (2021): 7.

3. J.-M. Beaulieu and R. R. Gainetdinov, "The Physiology, Signaling, and Pharmacology of Dopamine Receptors," *Pharmacological Reviews* 63 (2011): 182–217.

4. C. Missale, S. R. Nash, S. W. Robinson, M. Jaber, and M. G. Caron, "Dopamine Receptors: From Structure to Function," *Physiological Reviews* 78 (1998): 189–225.

5. M. Jaber, S. W. Robinson, C. Missale, and M. G. Caron, "Dopamine Receptors and Brain Function," *Neuropharmacology* 35 (1996): 1503–1519.

6. J. Meiser, D. Weindl, and K. Hiller, "Complexity of Dopamine Metabolism," *Cell Communication and Signaling* 11 (2013): 34.

7. H. Blaschko, "The Specific Action of L-Dopa Decarboxylase," *Journal of Physiology* 96 (1939): 50–51.

8. J. A. Best, H. F. Nijhout, and M. C. Reed, "Homeostatic Mechanisms in Dopamine Synthesis and Release: A Mathematical Model," *Theoretical Biology and Medical Modelling* 6 (2009): 21.

9. H. Juárez Olguín, D. Calderón Guzmán, E. Hernández García, and G. Barragán Mejía, "The Role of Dopamine and its Dysfunction as a Consequence of Oxidative Stress," *Oxidative Medicine and Cellular Longevity* 2016 (2016): 9730467.

10. J. Yin, K. Y. M. Chen, M. J. Clark, et al., "Structure of a D2 Dopamine Receptor–G-Protein Complex in a Lipid Membrane," *Nature* 584 (2020): 125–129.

11. B. Sun, D. Feng, M. L. H. Chu, et al., "Crystal Structure of Dopamine D1 Receptor in Complex With G Protein and a Non-Catechol Agonist," *Nature Communications* 12 (2021): 3305.

12. Y. Zhuang, P. Xu, C. Mao, et al., "Structural Insights Into the Human D1 and D2 Dopamine Receptor Signaling Complexes," *Cell* 184 (2021): 931–942.e18.

13. L. Fan, L. Tan, Z. Chen, et al., "Haloperidol Bound D2 Dopamine Receptor Structure Inspired the Discovery of Subtype Selective Ligands," *Nature Communications* 11 (2020): 1074.

14. E. Y. T. Chien, W. Liu, Q. Zhao, et al., "Structure of the Human Dopamine D3 Receptor in Complex With a D2/D3 Selective Antagonist," *Science* 330 (2010): 1091–1095.

15. Y. Zhou, C. Cao, L. He, X. Wang, and X. C. Zhang, "Crystal Structure of Dopamine Receptor D4 Bound to the Subtype Selective Ligand, L745870," *Elife* 8 (2019): e48822.

16. M. Habibi Dopamine receptors. (2017).

17. R. R. Gainetdinov, R. T. Premont, L. M. Bohn, R. J. Lefkowitz, and M. G. Caron, "Desensitization of G Protein–Coupled Receptors and Neuronal Functions," *Annual Review of Neuroscience* 27 (2004): 107–144.

18. J. K. Dreyer, K. F. Herrik, R. W. Berg, and J. D. Hounsgaard, "Influence of Phasic and Tonic Dopamine Release on Receptor Activation," *Journal of neuroscience* 30 (2010): 14273–14283.

19. P. F. Marcott, A. A. Mamaligas, and C. P. Ford, "Phasic Dopamine Release Drives Rapid Activation of Striatal D2-Receptors," *Neuron* 84 (2014): 164–176.

20. S. Wieland, D. Du, M. J. Oswald, R. Parlato, G. Köhr, and W. Kelsch, "Phasic Dopaminergic Activity Exerts Fast Control of Cholinergic Interneuron Firing via Sequential Nmda, D2, and D1 Receptor Activation," *Journal of Neuroscience* 34 (2014): 11549–11559.

21. A. Mishra, S. Singh, and S. Shukla, "Physiological and Functional Basis of Dopamine Receptors and Their Role in Neurogenesis: Possible Implication for Parkinson's Disease," *Journal of Experimental Neuroscience* 12 (2018): 1179069518779829.

22. J. M. Beaulieu, S. Espinoza, and R. R. Gainetdinov, "Dopamine Receptors–IUPHAR Review 13," *British Journal of Pharmacology* 172 (2015): 1–23.

23. M. V. Puig and E. K. Miller, "The Role of Prefrontal Dopamine D1 Receptors in the Neural Mechanisms of Associative Learning," *Neuron* 74 (2012): 874–886.

24. M. V. Puig and E. K. Miller, "Neural Substrates of Dopamine D2 Receptor Modulated Executive Functions in the Monkey Prefrontal Cortex," *Cerebral Cortex* 25 (2015): 2980–2987.

25. M. Michino, T. Beuming, P. Donthamsetti, A. H. Newman, J. A. Javitch, and L. Shi, "What Can Crystal Structures of Aminergic Receptors tell us About Designing Subtype-Selective Ligands?," *Pharmacological Reviews* 67 (2015): 198–213.

26. J. Choi and K. A. Horner Dopamine agonists. (2019).

27. W. A. Velema, W. Szymanski, and B. L. Feringa, "Photopharmacology: Beyond Proof of Principle," *Journal of the American Chemical Society* 136 (2014): 2178–2191.

28. D. J. Urban and B. L. Roth, "DREADDs (Designer Receptors Exclusively Activated by Designer Drugs): Chemogenetic Tools With Therapeutic Utility," *Annual Review of Pharmacology and Toxicology* 55 (2015): 399–417.

29. B. R. Conklin, E. C. Hsiao, S. Claeysen, et al., "Engineering GPCR Signaling Pathways With RASSLs," *Nature Methods* 5 (2008): 673–678.

30. P. C. Donthamsetti, N. Winter, M. Schönberger, et al., "Optical Control of Dopamine Receptors Using a Photoswitchable Tethered Inverse Agonist," *Journal of the American Chemical Society* 139 (2017): 18522–18535.

31. K. Hüll, J. Morstein, and D. Trauner, "In Vivo Photopharmacology," *Chemical Reviews* 118 (2018): 10710–10747.

32. B. R. Rost, F. Schneider-Warme, D. Schmitz, and P. Hegemann, "Optogenetic Tools for Subcellular Applications in Neuroscience," *Neuron* 96 (2017): 572–603.

33. M. Corkrum, A. Covelo, J. Lines, et al., "Dopamine-Evoked Synaptic Regulation in the Nucleus Accumbens Requires Astrocyte Activity," *Neuron* 105 (2020): 1036–1047.e5.

34. J. R. Cho, J. B. Treweek, J. E. Robinson, et al., "Dorsal raphe Dopamine Neurons Modulate Arousal and Promote Wakefulness by Salient Stimuli," *Neuron* 94 (2017): 1205–1219.e8.

35. H. Yang, J. W. de Jong, Y. Tak, J. Peck, H. S. Bateup, and S. Lammel, "Nucleus Accumbens Subnuclei Regulate Motivated Behavior via Direct Inhibition and Disinhibition of VTA Dopamine Subpopulations," *Neuron* 97 (2018): 434–449.e4.

36. X. I. Salinas-Hernández, P. Vogel, S. Betz, R. Kalisch, T. Sigurdsson, and S. Duvarci, "Dopamine Neurons Drive Fear Extinction Learning by Signaling the Omission of Expected Aversive Outcomes," *eLife* 7 (2018): e38818.

37. S. Lohani, A. J. Poplawsky, S.-G. Kim, and B. Moghaddam, "Unexpected Global Impact of VTA Dopamine Neuron Activation as Measured by Opto-fMRI," *Molecular Psychiatry* 22 (2017): 585–594.

38. B. T. Saunders, J. M. Richard, E. B. Margolis, and P. H. Janak, "Dopamine Neurons Create Pavlovian Conditioned Stimuli With Circuit-Defined Motivational Properties," *Nature Neuroscience* 21 (2018): 1072–1083.

39. C. Liu, P. Goel, and P. S. Kaeser, "Spatial and Temporal Scales of Dopamine Transmission," *Nature Reviews Neuroscience* 22 (2021): 345–358.

40. G. C. R. Ellis-Davies, "Caged Compounds: Photorelease Technology for Control of Cellular Chemistry and Physiology," *Nature Methods* 4 (2007): 619–628.

41. H. A. Lester and J. M. Nerbonne, "Physiological and Pharmacological Manipulations With Light Flashes," *Annual Review of Biophysics and Bioengineering* 11 (1982): 151–175.

42. J. H. Kaplan and A. P. Somlyo, "Flash Photolysis of Caged Compounds: New Tools for Cellular Physiology," *Trends in Neurosciences* 12 (1989): 54–59.

43. J. M. Nerbonne, "Caged Compounds: Tools for Illuminating Neuronal Responses and Connections," *Current Opinion in Neurobiology* 6 (1996): 379–386.

44. N. Asad, D. E. McLain, A. F. Condon, et al., "Photoactivatable Dopamine and Sulpiride to Explore the Function of Dopaminergic Neurons and Circuits," *ACS Chemical Neuroscience* 11 (2020): 939–951.

45. T. Lee, T. H. Lee, K. R. Gee, E. H. Ellinwood, and F. J. Seidler, "Combining Caged-Dopamine Photolysis With Fast-Scan Cyclic Voltammetry to Assess Dopamine Clearance and Release Autoinhibition In Vitro," *Journal of Neuroscience Methods* 67 (1996): 221–231.

46. R. Araya, V. Andino-Pavlovsky, R. Yuste, and R. Etchenique, "Two-Photon Optical Interrogation of Individual Dendritic Spines With Caged Dopamine," *ACS Chemical Neuroscience* 4 (2013): 1163–1167.

47. V. Andino-Pavlovsky, A. C. Souza, R. Scheffer-Teixeira, A. B. L. Tort, R. Etchenique, and S. Ribeiro, "Dopamine Modulates Delta-Gamma Phase-Amplitude Coupling in the Prefrontal Cortex of Behaving Rats," *Frontiers in Neural Circuits* 11 (2017): 29.

48. L. R. V. Castro, M. Brito, E. Guiot, et al., "Striatal Neurones Have a Specific Ability to Respond to Phasic Dopamine Release," *Journal of Physiology* 591 (2013): 3197–3214.

49. C. Yapo, A. G. Nair, L. Clement, L. R. Castro, J. Hellgren Kotaleski, and P. Vincent, "Detection of Phasic Dopamine by D1 and D2 Striatal Medium Spiny Neurons," *Journal of Physiology* 595 (2017): 7451–7475.

50. B. G. Robinson, J. R. Bunzow, J. B. Grimm, et al., "Desensitized D2 Autoreceptors are Resistant to Trafficking," *Scientific Reports* 7 (2017): 4379.

51. M. Gienger, H. Hübner, S. Löber, B. König, and P. Gmeiner, "Structure-Based Development of Caged Dopamine D2/D3 Receptor Antagonists," *Scientific Reports* 10 (2020): 829.

52. J. Noguchi, A. Nagaoka, S. Watanabe, et al., "In Vivo Two-Photon Uncaging of Glutamate Revealing the Structure–Function Relationships of Dendritic Spines in the Neocortex of Adult Mice," *Journal of Physiology* 589 (2011): 2447–2457.

53. B. M. Vickerman, E. M. Zywot, T. K. Tarrant, and D. S. Lawrence, "Taking Phototherapeutics From Concept to Clinical Launch," *Nature Reviews Chemistry* 5 (2021): 816–834.

54. W. J. Deal, B. F. Erlanger, and D. Nachmansohn, "Photoregulation of Biological Activity by Photochromic Reagents, III. Photoregulation of Bioelectricity by Acetylcholine Receptor Inhibitors," *Proceedings of the National Academy of Sciences* 64 (1969): 1230–1234.

55. Fighting Blindness, F., www.fightingblindness.org.

56. Kiora Pharmaceuticals. A Phase II Study of Intravitreal KIO-301 in Patients with Late-stage Retinitis Pigmentosa (ABACUS-2). Preprint at (2024).

57. P. Donthamsetti, N. Winter, A. Hoagland, et al., "Cell Specific Photoswitchable Agonist for Reversible Control of Endogenous Dopamine Receptors," *Nature Communications* 12 (2021): 4775.

58. P. Gorostiza, M. Volgraf, R. Numano, S. Szobota, D. Trauner, and E. Y. Isacoff, "Mechanisms of Photoswitch Conjugation and Light Activation of an Ionotropic Glutamate Receptor," *Proceedings of the National Academy of Sciences* 104 (2007): 10865–10870.

59. A. Acosta-Ruiz, J. Broichhagen, and J. Levitz, "Optical Regulation of Class C GPCRs by Photoswitchable Orthogonal Remotely Tethered Ligands," *G Protein-Coupled Receptor Signaling: Methods and Protocols* 103 (2019): 136.

60. M. Izquierdo-Serra, A. Bautista-Barrufet, A. Trapero, et al., "Optical Control of Endogenous Receptors and Cellular Excitability Using Targeted Covalent Photoswitches," *Nature Communications* 7 (2016): 12221.

61. D. Lachmann, C. Studte, B. Männel, H. Hübner, P. Gmeiner, and B. König, "Photochromic Dopamine Receptor Ligands Based on Dithienylethenes and Fulgides," *Chemistry–A European Journal* 23 (2017): 13423–13434.

62. C. Matera, P. Calvé, V. Casadó-Anguera, et al., "Reversible Photocontrol of Dopaminergic Transmission in Wild-Type Animals," *International Journal of Molecular Sciences* 23 (2022): 10114.

63. J. Jones-Tabah, H. Mohammad, E. G. Paulus, P. B. S. Clarke, and T. E. Hébert, "The Signaling and Pharmacology of the Dopamine D1 Receptor," *Frontiers in Cellular Neuroscience* 15 (2022): 806618.

64. B. E. Hetzler, P. Donthamsetti, Z. Peitsinis, C. Stanley, D. Trauner, and E. Y. Isacoff, "Optical Control of Dopamine D2-like Receptors With

Cell-Specific Fast-Relaxing Photoswitches," *Journal of the American Chemical Society* 145 (2023): 18778–18788.

65. H. Xiong, K. A. Alberto, J. Youn, et al., "Optical Control of Neuronal Activities With Photoswitchable Nanovesicles," *Nano Research* 16 (2023): 1033–1041.

66. S. Hisahara and S. Shimohama, "Dopamine Receptors and Parkinson's Disease," *International Journal of Medicinal Chemistry* 2011 (2011): 403039.

67. P. Li, G. L. Snyder, and K. E. Vanover, "Dopamine Targeting Drugs for the Treatment of Schizophrenia: Past, Present and Future," *Current Topics in Medicinal Chemistry* 16 (2016): 3385–3403.

68. F. A. Moreira and J. W. Dalley, "Dopamine Receptor Partial Agonists and Addiction," *European Journal of Pharmacology* 752 (2015): 112–115.

69. S. V. Faraone, P. Asherson, T. Banaschewski, et al., "Attention-Deficit/Hyperactivity Disorder," *Nature Review Disease Primers* 1 (2015): 15020.

70. C. R. Burgess, G. Tse, L. Gillis, and J. H. Peever, "Dopaminergic Regulation of Sleep and Cataplexy in a Murine Model of Narcolepsy," *Sleep* 33 (2010): 1295–1304.