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REVIEW ARTICLE

Chemogenetic modulation of astrocytes and microglia: State-of-the-art and implications in neuroscience

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Abstract

Insights into the role astrocytes and microglia play in normal and diseased brain functioning has expanded drastically over the last decade. Recently, chemogenetic tools have emerged as cutting-edge techniques, allowing targeted and spatiotemporal precise manipulation of a specific glial cell type. As a result, significant advances in astrocyte and microglial cell function have been made, showing how glial cells can intervene in central nervous system (CNS) functions such as cognition, reward and feeding behavior in addition to their established contribution in brain diseases, pain, and CNS inflammation. Here, we discuss the latest insights in glial functions in health and disease that have been made through the application of chemogenetics. We will focus on the manipulation of intracellular signaling pathways induced by activation of the designer receptors exclusively activated by designer drugs (DREADDs) in astrocytes and microglia. We will also elaborate on some of the potential pitfalls and the translational potential of the DREADD technology.

KEYWORDS

astrocytes, chemogenetics, CNS disorders, designer receptors exclusively activated by designer drugs, DREADDs, gene therapy, microglia

1 | INTRODUCTION

Glial cells were first described around the mid-1800s by a group of scientists led by Rudolf Virchow (reviewed by Kettenmann & Verkhratsky, 2008) and were long considered as cells of the central nervous system (CNS) with mere supportive and nutritional roles (Allen & Barres, 2009). Astrocytes, microglia, oligodendrocytes, and ependymal cells are the major glial cell types, and a growing body of evidence indicates their importance as active regulators of key

functions in development, metabolism and physiology (reviewed by Herculano-Houzel, 2014; Santello et al., 2012; Vainchtein & Molofsky, 2020). Indeed, astrocytes and microglia are now well-established mediators in synaptic transmission, forming the “quad-partite synapse” (Schafer et al., 2013). Moreover, astrocytes and microglia have been implicated in several CNS diseases (Li & Barres, 2018; Phatnani & Maniatis, 2015; Wolf et al., 2017; Zhang et al., 2021).

Most CNS disorders go accompanied with inflammation and mild to extensive astro and microgliosis. Gliosis refers to the process of molecular, morphological, and functional alterations of glial cells in response to peripheral inflammation, brain or CNS pathologies such as

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trauma, stroke, epilepsy, or neurodegeneration (Elsayed & Magistretti, 2015; Pekny & Pekna, 2016). While microglia respond rapidly within a few minutes after injury via the release of pro-inflammatory cytokines and phagocytosis of debris, astrocytes are generally considered to be activated within days (Gao et al., 2013). Reactive gliosis is a compensatory mechanism aimed to re-establish brain homeostasis and to limit tissue damage after CNS insults (Pekny & Pekna, 2016). However, persistent astro- and microgliosis can result in maladaptive changes, leading to detrimental consequences and contributing to CNS disease mechanisms (Cartier et al., 2014; Sofroniew & Vinters, 2010). Some of the major changes in reactive astrocytes and microglia are altered expression and/or function of receptors, enzymes, and/or transporters (Burda & Sofroniew, 2014). Specifically, several G-protein coupled receptors (GPCRs) expressed on astrocytic and microglial cell surfaces are found to be dysregulated and to contribute to disease progression in various rodent models for CNS disorders. For astrocytes, this includes models for epilepsy (Alvarez-Ferradas et al., 2015; Aronica et al., 2000; Ulas et al., 2000; Umpierre et al., 2019), traumatic brain injury (TBI; Shinozaki et al., 2017), Huntington's disease (HD; Yu et al., 2020), Alzheimer's disease (AD; Delekate et al., 2014; Pannell et al., 2016; Shrivastava et al., 2013), multiple sclerosis (MS; Fulmer et al., 2014), chronic pain (Kim et al., 2016), Parkinson's disease (PD; Morelli et al., 2009), and amyotrophic lateral sclerosis (ALS; Martorana et al., 2012; Vermeiren et al., 2006). For microglia, this includes models for epilepsy (Alves et al., 2019; Avignone et al., 2008), TBI (Shinozaki et al., 2017), AD (Haque et al., 2018; Pannell et al., 2016), PD (Yang et al., 2017), stroke (Costa et al., 2021; Li et al., 2020; Pannell et al., 2016; Webster et al., 2013; Wen et al., 2020; Yang et al., 2013), and neuropathic pain (Kobayashi et al., 2008, 2012). Astrocytic and microglial GPCRs serve as integrators of extracellular signals by regulating intracellular Ca^{2+} signaling, attenuating cyclic adenosine monophosphate (cAMP) levels and/or the release of chemoactive substances (i.e., neurotrophic factors, cytokines, chemokines, and gliotransmitters; Hamby et al., 2012; Haque et al., 2018; Kofuji & Araque, 2021; Zhang et al., 2017). Therefore, it is not surprising that aberrant glial Ca^{2+} signaling (Bosson et al., 2015; Fellin et al., 2006; Jiang et al., 2016; Kim et al., 2016; Kuchibhotla et al., 2009; Liu et al., 2021; Martorana et al., 2012; McLarnon, 2020; Plata et al., 2018; Tian et al., 2005; Yu et al., 2018) as well as altered glial release of chemoactive substances (Gao et al., 2013; Pöyhönen et al., 2019; Zhang et al., 2017) are commonly observed in CNS disorders. This indicates that specific targeting of microglial and astrocytic GPCRs signaling could unravel their role in various pathophysiological conditions and may even be a viable strategy for developing novel disease modifying therapies.

Despite the recent booming interest in astrocytes and microglia, knowledge of neuron–glia communication is limited due to the lack of selective tools that enable direct manipulation of these cells *in vivo* (Eme-Scolan & Dando, 2020a; Yu, Nagai, & Khakh, 2020). However, in the past decade, this issue has been tackled by innovative techniques such as viral gene delivery, *in vivo* live cell imaging, optogenetics, and chemogenetics (reviewed by Hirbec et al., 2020). Both optogenetic

and chemogenetic tools have emerged as cutting-edge technologies that allow selective and spatiotemporal precise modulation of glial cells (Hirbec et al., 2020). Particularly, designer receptors exclusively activated by designer drugs (DREADDs) are an excellent tool to address GPCR-mediated signaling in astrocytes and microglia, as they employ the endogenous GPCR signaling cascades of the targeted cell. Due to the very limited number of studies using DREADDs in other glial cells types, as oligodendrocytes and ependymal cells, we focused on astrocytes and microglia specifically. This review provides a snapshot of the latest discoveries elucidated by DREADD-induced modulation of astrocytes and microglia on blood–brain barrier (BBB) and cerebral blood flow (CBF), metabolism and the release of neuroactive substances in health and several diseases. In addition, we will discuss in detail the intracellular signaling pathways triggered by the most common DREADDs (hM3Dq, hM4Di, and rM3Ds) in both glial cell types. Finally, we will also elaborate on some of the potential pitfalls and the translational potential of the DREADD technology.

2 | PHARMACOGENETICS: THE ERA OF THE DREADDS

Cell-type-specific approaches such as chemogenetics can provide a better understanding of the bidirectional communication between neurons and glia. Since their first development in the early 1990s (Strader et al., 1991), several chemogenetic tools have been engineered that are based on other GPCRs such as the κ -opioid receptor (KOR; Coward et al., 1998), adenosine receptors (Gao et al., 2006; Jacobson et al., 2001), 5-HT_{2A} serotonin receptors (Westkaemper & Glennon, 2002), and Mas-related gene A1 (MrgA1) receptor (Agulhon et al., 2010; Cao et al., 2013; Fiacco et al., 2007; Forsberg et al., 2017; Wang et al., 2013; Xie et al., 2015). However, as with all experimental tools, a few problems remained unsolved. First, some of these receptors showed high constitutive activity. For example, transgenic mice that expressed the Gi-coupled Ro1 receptor in astrocytes, developed hydrocephalus in the absence of a ligand (Sweger et al., 2007). Second, some of the synthetic receptor ligands did not cross the BBB, limiting their use *in vivo*. This was the case for the transgenic mouse model expressing the MrgA1 receptor in astrocytes. However, this does not exclude that very interesting information was reported in a series of high profile papers performed in brain slices with this astrocytic Gq-linked MrgA1 receptor, (Agulhon et al., 2010; Cao et al., 2013; Fiacco et al., 2007; Forsberg et al., 2017; Wang et al., 2013; Xie et al., 2015), which will be discussed further. Finally, some of the synthetic ligands were not well suited for *in vivo* studies because they showed off-target effects (Nichols & Roth, 2009; Sternson & Roth, 2014).

Armbruster et al. (2007) designed a human Gαq-coupled M3 muscarinic receptor with two mutations (Y149C and A239G), termed hM3Dq, that is exclusively activated by a designer drug such as clozapine N-oxide (CNO), is insensitive to the native ligand acetylcholine and has no detectable constitutive activity (Armbruster et al., 2007; Sternson & Roth, 2014). This system was named DREADD (Armbruster et al., 2007). These mutations were used at homologous residues of the

human $G_{\alpha i}$ -coupled M4 muscarinic receptor to form the hM4Di. To create the G_s -coupled hM3Ds, the same mutations as for hM3Dq were applied, but the intracellular loop sequences from the hM3Dq was replaced with that of the G_s -coupled β -adrenergic receptor (Atasoy & Sternson, 2018). However, the most widely used G_s -coupled DREADD is based on the rat M3 muscarinic receptor, rM3Ds (Guettier et al., 2009). The invention of DREADDs was expected to improve the cell-type-specific study of brain function (Jiang et al., 2017). However, while the use of DREADDs has revealed many functions of neurons (Burnett & Krashes, 2016; Sternson & Roth, 2014), it lasted nearly a decade before DREADDs were applied in astrocytes (Aguilhon et al., 2013), and even longer in microglia (Grace et al., 2016).

Until now, DREADDs have already provided substantial progress in the field of glial cell physiology and their role in pathological conditions. However, the signaling pathways downstream of the DREADD-coupled G-proteins remain to be fully elucidated.

3 | DREADD-INDUCED SECOND MESSENGER ALTERATIONS IN ASTROCYTES AND MICROGLIA

Glia express receptors for various neurotransmitters, many of which are GPCRs (Farhy-Tselnick & Allen, 2018). It is assumed that the GPCR signaling pathways described for neurons largely overlap with those in astrocytes and microglia. In neurons, and many other cell types, Gq signaling leads to the activation of phospholipase C (PLC), which cleaves phosphatidylinositol 4,5-bisphosphate (PIP₂) into inositol 1,4,5-trisphosphate (IP₃) and diacylglycerol (DAG). IP₃ binds IP₃ receptors on the endoplasmic reticulum, leading to the release of Ca²⁺ from internal storage. Moreover, DAG and Ca²⁺ activate protein kinase C (PKC), which further engages multiple additional intracellular signaling processes (Atasoy & Sternson, 2018), resulting in, among others, neurotransmitter release from neuronal cells (Huang, 1989). Gi signaling results in decreased activity of adenylyl cyclase (AC; Simonds, 1999), lowering cAMP levels, which in neuronal cells leads to decreased neurotransmitter release. Moreover, due to the presence of $G_{\alpha i}$ /o protein levels in the cell, activation of this G protein is considered to be key in inducing $\beta\gamma$ -mediated signaling processes. In this case, the $\beta\gamma$ subunit-complex activates G protein-regulated inward rectifier K⁺ channel (GIRKs) and/or inhibits Ca²⁺ channels (Atasoy & Sternson, 2018). G_s signaling leads to the activation of AC and increased intracellular cAMP levels (Atasoy & Sternson, 2018). The latter increases protein kinase A (PKA) activity, which, on its turn, induces neurotransmitter release (Leenders & Sheng, 2005). The pathways of the hM3Dq, rM3Ds, and hM4Di DREADDs (from here on referred to as Gq-, G_s -, and Gi-DREADD respectively, specifically G_s -DREADD nomenclature in this review refers to rM3Ds and not the M3Ds, as is the case in most papers; Chai et al., 2017; Oe et al., 2020) have been well documented for neurons. However, modulation of astrocytes and microglia using exogenous GPCRs has shed light on some striking differences in downstream signaling upon G-protein activation (Durkee et al., 2019; Schulz et al., 2022;

Vaidyanathan et al., 2021), emphasizing the need to clarify G protein-associated downstream effects in each specific cell type (Figure 1).

3.1 | Astrocytes

Astrocytes express a large amount of GPCRs that can be coupled to the Gq protein, such as the serotonergic 5-HT_{2A}, 2B receptors (Verkhatsky & Nedergaard, 2018; Xu & Pandey, 2000; Zhang et al., 2015); to the Gi protein, for example GABA_B receptors (Nagai, Rajbhandari, et al., 2019), adrenergic α_2 -AR (Hertz et al., 2010; Verkhatsky & Nedergaard, 2018), adenosine receptors A₁ and A₃ (Horvat & Vardjan, 2019; Verkhatsky & Nedergaard, 2018), dopamine D_{2/4} receptors (Miyazaki et al., 2004; Qiu et al., 2016; Verkhatsky & Nedergaard, 2018); or to the G_s protein as A_{2A} and A_{2B} receptors (Horvat & Vardjan, 2019; Verkhatsky & Nedergaard, 2018; for a detailed review on the different GPCRs expressed in astrocytes see Verkhatsky & Nedergaard, 2018).

Gq-GPCR activation in astrocytes can induce Ca²⁺ increases in various brain regions (Ding et al., 2013; Duffy & MacVicar, 1995; Shao & McCarthy, 1995). As expected, activation of astrocytic Gq-DREADDs, increased Ca²⁺ levels in astrocytes in the hippocampus (Adamsky et al., 2018; Aguilhon et al., 2013; Durkee et al., 2019; Van Den Herrewegen et al., 2021), but also in other brain regions, such as the striatum (Chai et al., 2017), hypothalamus (Chen et al., 2016), and amygdala (Martin-Fernandez et al., 2017). With the transgenic mouse model expressing the Gq-GPCR MrgA1 receptor in astrocytes, similar results were obtained. Ca²⁺ levels were increased after application of the MrgA1 receptor agonist Phe-Leu-Arg-Phe amide in hippocampal slices (Aguilhon et al., 2010; Fiacco et al., 2007; Wang et al., 2013), brain stem slices (Forsberg et al., 2017), or local administration via a cannula (Cao et al., 2013). Specifically, Gq-DREADD activation was shown to induce Ca²⁺ increases through PLC activation and subsequent IP₃-mediated Ca²⁺ release, that is, the canonical signaling of Gq-GPCRs (Durkee et al., 2019; see Table 1). Recently, the effect of Gq-DREADD activation on astrocytic Ca²⁺ signaling was assessed for the first time in awake mice (Vaidyanathan et al., 2021). In this study, a long-lasting increase in intracellular Ca²⁺, but disrupted Ca²⁺ dynamics, in cortical astrocytes were observed upon CNO administration (Vaidyanathan et al., 2021; see Table 1).

Gi-DREADD activation in astrocytes was found to inhibit cAMP signaling (Jones et al., 2018; Oe et al., 2020). This is in agreement with what has been observed for multiple endogenous Gi-GPCRs (Eriksson et al., 1991; Lauritzen et al., 2014; Peakman & Hill, 1996; Woods et al., 1989). Nevertheless, there are some inconsistencies reported on the effects of Gi-GPCR signaling in astrocytes on intracellular Ca²⁺ (Gould et al., 2014; Meier et al., 2008). Some observed a reduction in intracellular Ca²⁺ (Gould et al., 2014), which is in line with the canonical Gi-GPCR pathway. However, others found no apparent effect on Ca²⁺ levels (Nam et al., 2019), or even more strikingly, increases in Ca²⁺ levels (Andersson et al., 2007; Gould et al., 2014; Mariotti et al., 2016; Perea et al., 2016; Serrano et al., 2006), suggesting non-canonical Gi-GPCR signaling in astrocytes. These different effects on

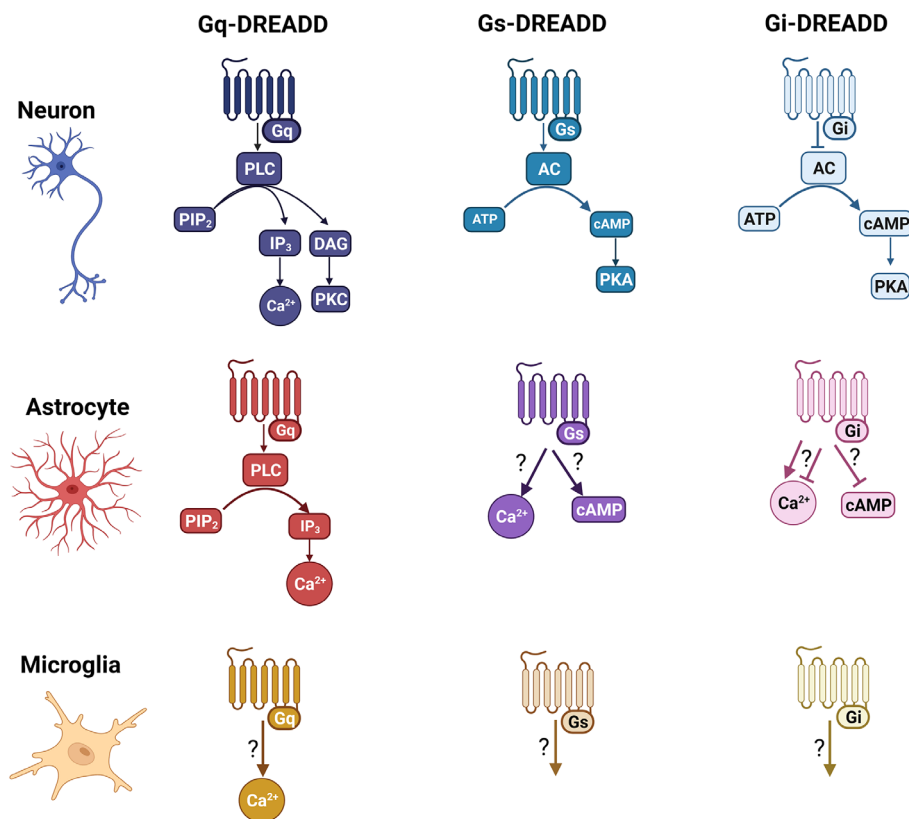


FIGURE 1 Schematic overview of the downstream signaling of the Gq, Gs, and Gi-DREADD signaling pathways in neurons, astrocytes, and microglia. In neurons, the Gq-DREADD activates phospholipase C (PLC) which cleaves phosphatidylinositol 4,5-bisphosphate (PIP₂) into inositol 1,4,5-trisphosphate (IP₃), which affects intracellular calcium signaling, and diacylglycerol (DAG), leading to protein kinase C (PKC) activation. The Gs-DREADD activates adenylyl cyclase (AC), while the Gi-DREADD inhibits this enzyme. AC converts adenosine triphosphate (ATP) to cAMP, which induces protein kinase A (PKA). In astrocytes, the Gq-DREADD induces PLC, which cleaves PIP₂ to IP₃, which induces increases in intracellular calcium signaling. The Gs-DREADD showed to induce increases in intracellular calcium and in cAMP. However, the pathways are not fully unraveled. The Gi-DREADDs in astrocytes have shown to reduce cAMP levels. Different results were obtained on changes in calcium signaling after Gi-DREADD activation. For microglia, the Gq-DREADD induces increases in intracellular calcium, without knowledge of the inducing pathway. For Gs-DREADD and Gi-DREADDs, the signaling pathways remain unknown. Figure created with [BioRender.com](#).

Ca²⁺ events after Gi-GPCR signaling were also observed in astrocytes of the ventral tegmental area (either increase, decrease or no visible effect), which could be explained by differences in duration of application of the GABA_B agonist, baclofen (Gould et al., 2014). Discrepancies on Ca²⁺ levels have also been observed with Gi-DREADD activation in astrocytes. Likewise, the duration and concentration of the DREADD agonist CNO seemed to differentially affect the Ca²⁺ levels in hippocampal astrocytes either increase (Durkee et al., 2019), decrease (Kol et al., 2020), or no visible effect (Chai et al., 2017; Van Den Herrewegen et al., 2021; see Table 1). Further research is necessary to clarify the fundamental mechanisms of astrocytic Ca²⁺ signaling after Gi-DREADD activation.

Finally, astrocytic Gs-DREADD activation, has been shown to increase cAMP signaling (Oe et al., 2020), in line with the effects of the activation of endogenous Gs-GPCR on astrocytes (Horvat et al., 2016; Kubo et al., 1991; Peakman & Hill, 1994; Woods et al., 1989). However, activation of the Gs-DREADD expressed in astrocytes has also been shown to increase Ca²⁺ levels (Ding et al., 2013). Furthermore, even in case of identical DREADD agonist application conditions (i.e., equal concentration and duration of CNO),

individual astrocytes have been found to respond differently to either Gi or Gs DREADD activation between brain regions (Chai et al., 2017; see Table 1), indicating that astrocyte heterogeneity is likely to play a pivotal role in the diverse actions of Gi and Gs protein-associated signaling. This emphasizes that astrocytic Ca²⁺ signaling is extremely complex and still poorly understood (Guerra-Gomes et al., 2017). In conclusion, these results suggest that each DREADD receptor is coupled to the expected G-protein, but that the $\beta\gamma$ subunits of the Gi and Gs protein exert diverse effects depending on the duration and concentration of DREADD receptor agonist.

3.2 | Microglia

Microglia express several GPCRs, which are either Gq-coupled (e.g. purinoceptors P2Y₆ receptors (Calovi et al., 2019; Xu et al., 2016, metabotropic glutamate receptor mGluR5 (Byrnes et al., 2009; Spampinato et al., 2018), and the M3R (Allen et al., 2023; Pannell et al., 2016)), Gi-coupled (as the α_2 -AR (Gyoneva & Traynelis, 2013), P2Y_{12,13} receptors (Calovi et al., 2019; Hammond et al., 2019; Zhang

TABLE 1 Designer receptors exclusively activated by designer drug (DREADD) downstream signaling pathways in astrocytes.

Downstream signaling	Gq-DREADD	Gi-DREADD	Gs-DREADD
Ca ²⁺ signaling			
<i>Acute activation</i> Puff application: 200 ms – 5 s; 1 mM CNO (Durkee et al., 2019; Martin-Fernandez et al., 2017), 10 mM CNO (Adamsky et al., 2018; Chen et al., 2016) < 3 min bath application 1 μM CNO (Nagai, Rajbhandari, et al., 2019), 10 μM CNO (Agulhon et al., 2013)	Hippocampus: ↑ Ca ²⁺ events (Adamsky et al., 2018; Agulhon et al., 2013; Durkee et al., 2019) Arcuate nucleus: ↑ Ca ²⁺ events (Chen et al., 2016) Central amygdala: ↑ Ca ²⁺ events (Martin-Fernandez et al., 2017)	Hippocampus: ↑ Ca ²⁺ events (Durkee et al., 2019) Striatum: ↑ Ca ²⁺ events (Nagai, Rajbhandari, et al., 2019)	?
<i>Moderate activation</i> > 3 min and < 10 min bath application; 1 μM CNO (Chai et al., 2017)	Hippocampus: ↑ Ca ²⁺ events (Chai et al., 2017)	Hippocampus: = Ca ²⁺ events (Chai et al., 2017) Striatum: ↑ Ca ²⁺ events (Chai et al., 2017)	Hippocampus: = Ca ²⁺ events (Chai et al., 2017) Striatum: ↑ Ca ²⁺ events (Chai et al., 2017)
<i>Long-term activation</i> 10 min (Kol et al., 2020), 35 min (Van Den Herrewegen et al., 2021) or 40 min (Adamsky et al., 2018) bath application of 10 μM CNO	Hippocampus: ↑ Ca ²⁺ events (Adamsky et al., 2018; Van Den Herrewegen et al., 2021)	Hippocampus: ↓ baseline Ca ²⁺ (Kol et al., 2020), or = Ca ²⁺ events (Van Den Herrewegen et al., 2021)	?
<i>In vivo activation</i> i.p. injection 1 mg/kg CNO (Nagai, Rajbhandari, et al., 2019; Vaidyanathan et al., 2021)	Layer 2/3 V1 Cortex: Initial ↑ Ca ²⁺ (10 min) followed by a long-lasting (2–3 h) ↓ Ca ²⁺ dynamics, but consistent ↑ in baseline Ca ²⁺ (Vaidyanathan et al., 2021)	Layer 2/3 V1 Cortex: Long-lasting (2–3 h) ↑ in Ca ²⁺ events (Vaidyanathan et al., 2021) Striatum: Long-lasting (2 h) ↑ in Ca ²⁺ events (Nagai, Rajbhandari, et al., 2019)	?
Cascade involved in Ca ²⁺ release	Classic PLC-mediated cleavage of PIP ₂ to IP ₃ , which releases Ca ²⁺ from internal stores (Durkee et al., 2019)	Direct binding of the GPCR's βγ subunit to IP ₃ R ₂ receptor in hippocampal astrocytes (Durkee et al., 2019)	?
cAMP	/	↓ cAMP (Jones et al., 2018; Oe et al., 2020)	↑ cAMP (Oe et al., 2020)

et al., 2014), GABA_B receptors (Kuhn et al., 2004) or Gs-coupled (e.g. A_{2A} receptors (Colella et al., 2018; Orr et al., 2009 and β-ARs Gyoneva & Traynelis, 2013; Tanaka et al., 2002)).

Multiple studies have shown that Gq-GPCR activation increases intracellular Ca²⁺ in cultured human and murine microglial cells. For example, activation of muscarinic (Zhang et al., 1998), Gq-coupled P2Y₁ and P2Y₆ (Langfelder et al., 2015; Orellana et al., 2013) or mGluR5 (Biber et al., 1999) receptors increased intracellular Ca²⁺ in microglia. Indeed, Gq-DREADD resulted in increased intracellular Ca²⁺ levels in microglia (Binning et al., 2020; Császár et al., 2022). However, Császár et al. (2022) found that repeated administration of a DREADD agonist reduced Ca²⁺ responses and impaired microglial responses to ATP (Császár et al., 2022). A possible explanation for the latter phenomenon could be that repeated Gq-DREADD activation in microglia results in depletion of the intracellular Ca²⁺ stores, which results in diminished Ca²⁺ responses and the inability of ATP administration to induce an increase in Ca²⁺ levels.

Gi-protein signaling has also been shown to increase intracellular Ca²⁺ levels in microglia. For example, activation of the Gi-coupled chemokine receptor CCR5, which was a result of a multistep cascade involving Bruton's tyrosine kinase, phosphoinositide 3-kinase (PI3K) and PLC activation, and a plasma membrane Ca²⁺ channel, resulted in elevated intracellular Ca²⁺ concentrations (Shideman et al., 2006). Moreover, Gi-coupled P2Y₁₂ receptor activation induces microglial chemotaxis, exerted via different signaling pathways, such as, among others, the PLC-mediated increase in intracellular Ca²⁺ (Irino et al., 2008; Ohsawa et al., 2007). The knowledge on the Gi-DREADD induced signaling in microglia is very limited. It is known that Gi-DREADD activation in microglia attenuates levels of pro-inflammatory signaling mediators (Ding et al., 2022; Grace et al., 2018; Yi et al., 2020). A suggested underlying mechanism for these findings is microglial Gi signaling inhibition of increasing Ca²⁺ levels, which are necessary for the release of pro-inflammatory mediators (Parusel et al., 2023). Another possible mechanism was suggested

by Yi et al. They found that interleukin 1 beta (IL-1 β) was reduced via the Gi-DREADD-induced downregulation of the transcription factor interferon regulatory factor 8 (IRF8) (Yi et al., 2020).

Gs protein-coupled signaling in microglia is less investigated, but seems to affect neuroinflammation. Activation of the Gs-coupled β_2 -AR or A_{2A} receptor reduce microglial activation (Gyoneva et al., 2014; O'Neill et al., 2020). Moreover, β_2 -AR activation increases cAMP levels, which suppresses microglial proliferation (Fujita et al., 1998). β_2 -AR activation can drive microglia to a more anti-inflammatory phenotype via activation of the classical cAMP/PKA/cAMP-response element binding protein (CREB) as well as the PI3K and p38 mitogen-activated protein kinase (MAPK) signaling pathways (Sharma et al., 2019). However, in spite of the increasing interest, the specific signaling pathways induced by DREADDs, and especially Gi- and Gs-coupled DREADDs, in microglia remain largely undiscovered.

4 | APPLICATION OF DREADDS IN ASTROCYTES

4.1 | Blood-brain barrier and cerebral blood flow

Astrocytes are key players in the maintenance of the BBB or blood-spinal cord-brain barrier (BSCB), which is known to be compromised in various CNS disorders (Sweeney et al., 2018). Recently, astrocytic Gq- and Gi-DREADD, but not Gs-DREADD, activation restored BSCB in a presymptomatic stage of the SOD1^{G93A} disease model for ALS (Ouali Alami et al., 2020), a progressive disease affecting motoneurons originating in the CNS and spinal cord (Valori et al., 2014). After a 7-day CNO treatment, activation of either Gq- or Gi-DREADDs increased vascular end-feet coverage in this model. Moreover, Gq-DREADD activation directly reduced burden of disease markers in surrounding motoneurons, while Gi-DREADD did not (Ouali Alami et al., 2020). Prolonged activation of Gi-DREADDed astrocytes resulted however in decreased disease markers in motoneurons at a later stage of the ALS model (2020). As BBB dysfunction is also considered to contribute to disease progression in epilepsy (Bar-Klein et al., 2014; Loscher et al., 2020), AD, PD, and HD (Sweeney et al., 2018), DREADD-based modulation aimed at re-establishing BBB properties might have disease modifying effects in models for these illnesses as well and awaits to be investigated. Furthermore, astrocytes also regulate CBF (Attwell et al., 2010). Activation of Gi-DREADDs on astrocytes diminished cocaine-induced decrease in total hemoglobin concentration in the brain, a measure for CBF, oxygenated hemoglobin concentration and vessel diameter (Liu et al., 2022). Reduced CBF is also found in several diseases, as epilepsy (Joo et al., 2008), AD (Korte et al., 2020), PD (Borghammer et al., 2010), MS (D'haeseleer et al., 2015), major depressive disorder (MDD; Duschek et al., 2021), and it was found that increased CBF improves cognition in AD mice (Bracko et al., 2020). Therefore, further research is necessary to determine if astrocytic DREADD activation could also modulates the CBF reductions and thereby also modulate cognitive impairments in these diseases.

4.2 | Metabolism

Like neurons, astrocytes generate most of their required energy via oxidative metabolism of glucose in their mitochondria containing parts, that is, soma and large processes. Nevertheless, glycolysis and glycogenolysis constitute an important part of the astrocytes' energy metabolism. Specifically, during prompt increases in energy demand, glycolysis, and glycogenolysis provide most of the required energy in peripheral astrocytic processes, which are too narrow to accommodate mitochondria. Several astrocytic GPCRs have been shown to affect glycogenolysis (Hertz et al., 2015), such as 5-HT_{2B} receptor (Kong et al., 2002), β_1 and β_2 -AR (Xu et al., 2014), α_2 -AR (Subbarao & Hertz, 1990), P2Y receptor (Sorg et al., 1995), and A_{2B} receptor (Allaman et al., 2003). Glycogen can be converted to L-lactate, which in turn may be released via monocarboxylate transporters or gap junctions (Hertz et al., 2007). Transfer of L-lactate from astrocytes to neurons appears crucial for memory consolidation (Suzuki et al., 2011). In particular, activation of astrocytic Gs-coupled β_2 -AR has been linked to glycogenolysis, promoting lactate transfer to neurons, and stimulating learning and memory consolidation (Gao et al., 2016; Suzuki et al., 2011). However, while short-term activation of β_2 -AR improves learning and memory consolidation, long-term activation has a deleterious effect, supposedly by depletion of intracellular glycogen stores and, thus reducing glycogenolysis and astrocytic L-lactate supply to neurons (Dong et al., 2017). Interestingly, activation of Gs-DREADDs in astrocytes was recently shown to decrease glycogen levels (Oe et al., 2020), further showing a link between astrocytic Gs-GPCR signaling, glycogenolysis, L-lactate supply to neurons and the subsequent effect on learning and memory consolidation.

The use of chemogenetics in astrocytes also uncovered a link between lactate metabolism and chronic pain (Miyamoto et al., 2019). Gq-DREADD activation in spinal dorsal horn astrocytes was shown to stimulate L-lactate release in the partial sciatic nerve ligation mouse model for neuropathic pain (Miyamoto et al., 2019). Furthermore, activation of Gq-DREADDs in spinal dorsal horn astrocytes is sufficient to induce mechanical allodynia in naïve rats, which was accompanied by increased L-lactate levels (Huang et al., 2022). The excess of L-lactate may induce continuous synaptic transmission in surrounding neurons (Jourdain et al., 2018; Yang et al., 2014), contributing to hyperalgesia in neuropathic pain (Sandkuhler & Gruber-Schoffnegger, 2012). Since Gi-DREADD activation in astrocytes can decrease intracellular cAMP (Oe et al., 2020), a regulator of glycogenolysis (Zhou et al., 2019), this could also alleviate mechanical hypersensitivity by reducing L-lactate levels after high-frequency stimulation of the sciatic nerve (Huang et al., 2022). Another study found similar results on DREADD modulation in astrocytes and neuropathic pain. Gq-DREADD activation of astrocytes in the ventrolateral periaqueductal gray was sufficient to induce mechanical allodynia and pain-related aversion in naïve rats, whereas Gi-DREADD activation alleviated these effects in a streptozotocin-induced type 1 diabetes rat model for diabetic neuropathic pain (Yang et al., 2022). The underlying mechanism was not investigated, but this might also be explained by changes in L-lactate release by astrocytes. Moreover,

these data suggests that DREADDs might be an interesting strategy to explore the role of spinal cord reactive astrocytes in chronic pain, but also in multiple other CNS disorders in which aberrant lactate metabolism has been described, such as AD (Zhang et al., 2018), epilepsy (Liu et al., 2014), MS (Zeis et al., 2015), and depression (Ernst et al., 2017).

4.3 | Release of neuroactive substances

Besides the release of metabolic substrates, astrocytes are known to release various other substances, such as gliotransmitters (e.g., glutamate, ATP, D-serine, GABA), neurotrophic factors (e.g., brain-derived neurotrophic factor [BDNF], glial-derived neurotrophic factor [GDNF]), peptides (e.g., thrombospondin-1 [TSP-1], neuropeptide Y), inflammatory factors (e.g., IL-1, tumor necrosis factor- α [TNF- α]) and prostaglandins (e.g., prostaglandin E2 [PGE2]; for review see Verkhratsky et al., 2016). The DREADD technology can be used to mimic the endogenous GPCR-mediated signaling conditions specifically in astrocytes (Durkee et al., 2019), making it an interesting tool to help further characterize how signaling pathways in astrocytes modulate the release of bioactive soluble substances. This paragraph will focus on the possible bioactive substances released upon DREADD-based astrocyte modulation.

It remains the subject of debate whether or not gliotransmitter release occurs in physiological conditions (Fiacco & McCarthy, 2018; Savtchouk & Volterra, 2018) but it is widely accepted that astrocytes are able to release gliotransmitters through Ca^{2+} -dependent and Ca^{2+} -independent mechanisms (for review see Bazargani & Attwell, 2016). Research showed that *in vivo* activation of Gq-DREADDs, expressed in astrocytes of the nucleus accumbens core, modulated activity of the surrounding neuropil via release of glutamate (Schofield et al., 2015). Furthermore, glutamate concentrations were elevated in the amygdala after activation of Gq-DREADDs expressed on astrocytes, which in turn reversed the reduced glutamate levels after ethanol consumption in mice (Nwachukwu et al., 2021). Likewise, activation of Gq-DREADDs expressed in astrocytes of hippocampal and striatal brain slices was proposed to elicit glutamate release (Chai et al., 2017; Durkee et al., 2019). More specifically, Gq-DREADD activation in astrocytes induced slow inward currents (SICs) in surrounding neurons (Durkee et al., 2019), which is typically attributed to the activation of extrasynaptic neuronal N-methyl-D-aspartate (NMDA) receptors via astrocytic released glutamate (Shigetomi et al., 2008). Surprisingly, activation of Gi-DREADDs expressed in hippocampal astrocytes also increased SIC frequency in surrounding neurons indicating extrasynaptic glutamate release (Durkee et al., 2019). In contrast to these findings, Chai et al. (2017) did not observe changes in SIC amplitude or frequency upon Gq-DREADD activation in hippocampal astrocytes (Chai et al., 2017). Further characterization is thus necessary to establish under which conditions precisely Gq-DREADD activation in hippocampal astrocytes is capable of inducing SICs in brain slices and/or glutamate release *in vivo*.

Interestingly, Gq-DREADD activation in striatal astrocytes has been described to induce co-release of glutamate and ATP/adenosine (Cavaccini et al., 2020). This co-release can have opposing effects on neuronal glutamate release, as glutamate-induced activation of Group I mGluRs increased neuronal glutamate release, whereas ATP-induced stimulation of A1 receptors mediated a decrease in glutamate release (Cavaccini et al., 2020). Interestingly, Gq-DREADD activation of striatal astrocytes resulted in a predominance of adenosine-mediated inhibition over glutamate-mediated potentiation, inducing A1 receptor-mediated long-term depression (LTD) at corticostriatal synapses (Cavaccini et al., 2020). In addition, the release of ATP/adenosine alone has been demonstrated in multiple brain regions following activation of Gq-DREADDs on astrocytes, including the striatum (Cavaccini et al., 2020; Kang et al., 2020), hypothalamus (Yang et al., 2015), prefrontal cortex (Erickson et al., 2020), suprachiasmatic nucleus (Hablitz et al., 2020), and amygdala (Martin-Fernandez et al., 2017). Gq-DREADD-induced ATP/adenosine release from astrocytes has been implicated in goal-directed reward-seeking behavior, food intake, substance abuse, timing of circadian clock, and fear memory retrieval, respectively.

The concept of D-serine as gliotransmitter is still controversial (see Wolosker & Balu, 2020), but Gq-DREADD activation was shown to trigger D-serine release from hippocampal astrocytes (Adamsky et al., 2018). Indeed, activation of Gq-DREADDs in hippocampal CA1 astrocytes was sufficient to induce *de novo* long-term potentiation (LTP), a crucial mechanism underlying learning and memory. The effect on LTP was dependent on NMDA-receptors and the co-agonist D-serine (Adamsky et al., 2018). In addition, activation of Gi-DREADDs in CA1 hippocampal astrocytes reduced the threshold of LTP induction (Nam et al., 2019). The authors suggest that this reduction of LTP threshold occurs through astrocytic release of glutamate, which in turn activated presynaptic mGluR1 (Nam et al., 2019). Yet, the activation of the MrgA1 receptor on astrocytes, which is also Gq-coupled, did not alter excitatory synaptic transmission and short- or long-term excitatory synaptic plasticity, such as LTP, in CA3-CA1 synapses (Agulhon et al., 2010; Fiacco et al., 2007). Future side-by-side comparisons would be interesting to understand whether the differences are due, for instance, to non-canonical signaling, different cellular localization of the receptors, different tissue penetration of the ligands or other experimental set-up aspects. Notably, Gq-DREADD activation in CA1 dorsal hippocampal astrocytes improved recent contextual memory retrieval (Adamsky et al., 2018), while Gi-DREADD activation in CA1 dorsal hippocampal astrocytes decreased remote memory recall (Kol et al., 2020). To the best of our knowledge, information on the effects of Gs-DREADD activation on gliotransmitter release is still lacking.

Astrocytes do not only interact with their environment by the release of gliotransmitters but are also known to boost plasticity and impart in trophic support by secretion of several trophic factors such as BDNF and GDNF (Marathe et al., 2018). In addition to neurotrophic factors, astrocytes can release multiple neuroinflammatory molecules, such as IL-1 β and TNF- α (Pearson-Leary et al., 2015). Recently, it was shown that Gi-DREADD activation in hippocampal

astrocytes attenuated lipopolysaccharide (LPS)-induced upregulated levels of *Lipocalin-2* (*Lcn2*), *Il-1 β* , *Tnf- α* , and *nitric oxide synthase 2* (*Nos2*) mRNA and alleviated the cognitive impairment in mice (Kim et al., 2021). Moreover, CNO administration to cultured astrocytes with either Gi-DREADD or Gq-DREADD expression resulted in either a decrease or an increase of nitric oxide (NO) release, respectively (Kim et al., 2021). Additionally, Gi-DREADD activation in medial basal hypothalamus astrocytes resulted in reduced IL-1 β , TNF- α , chemokine (CC motif) ligand-2 (CCL2), and CCL5 (Cansell et al., 2021). Further research on the modulation of the release of these pro-inflammatory mediators by DREADDs could be interesting for many CNS diseases. For instance, elevated levels of pro-inflammatory cytokines is often found in patients with AD, PD (Alam et al., 2016), MDD (Khairova et al., 2009), epilepsy (Kamali et al., 2021), MS (Nasi et al., 2020), and ALS (Thonhoff et al., 2018). Reducing the levels of pro-inflammatory cytokines via Gi-DREADD activation in astrocytes could be a new therapeutic strategy for these diseases.

Finally, one secreted protein, other than the above-discussed ones, is also released upon DREADD activation in striatal astrocytes (Nagai, Rajbhandari, et al., 2019), is the synaptogenic cue and matrix glycoprotein TSP-1. Gi-DREADD activation in striatal astrocytes resulted in upregulation of TSP-1, which in turn increased corticostriatal synaptic formation, increased striatal medial spiny neuron firing and led to a hyperactive behavioral phenotype (Nagai, Rajbhandari, et al., 2019). These findings suggest that Gi-DREADD activation in striatal astrocytes can aid in reducing maladaptive actions as seen in attention deficit hyperactive disorder. Moreover, as mechanical allodynia in a rodent model of peripheral nerve injury was previously found to be linked to primary somatosensory (S1) cortical astrocytic Ca²⁺-dependent TSP-1 release (Kim et al., 2016), it would be interesting to investigate the effects of DREADD activation in S1 cortical astrocytes on mechanical allodynia.

5 | APPLICATION OF DREADDS IN MICROGLIA

5.1 | Blood-brain barrier and cerebral blood flow

Microglia can play a detrimental role in the regulation of BBB permeability, as microglial release of proinflammatory cytokines, resulting in increased inflammation and oxidative stress, causes BBB dysfunction, whereas microglial release of anti-inflammatory mediators entail in BBB protection (Ronaldson & Davis, 2020). The Gi-coupled P2Y₁₂ receptor on microglia plays a crucial role in BBB integrity improvement, and was shown to have neuroprotective effects after ischemic stroke (Li et al., 2020; Webster et al., 2013) but also in neurovascular coupling (Császár et al., 2022). Therefore, a Gi-DREADD based approach in microglia could be interesting to further investigate in diseases where BBB dysfunction occurs, such as epilepsy (Bar-Klein et al., 2014; Loscher et al., 2020), AD, PD, ALS, and HD (Sweeney et al., 2018). More recently, it was also found that this P2Y₁₂ receptor plays a crucial role in the modulation of neurovascular structure and

function by microglia. Both after microglial depletion and in P2Y₁₂-KO mice, capillary dilation, increased CBF, and impaired vasodilation were observed (Bisht et al., 2021). On the other hand, it was found that microglial Gq-DREADD activation resulted in diminished CBF and changes in microglial process dynamics (Császár et al., 2022). These results indicate that both Gq- and Gi-GPCR signaling in microglia are important for the modulation of the CBF.

5.2 | Metabolism

Microglia have a high energy demand for maintaining their surveillance function. To meet this demand, their energy metabolism uses either glycolysis or oxidative phosphorylation. Quiescent microglia mainly rely on oxidative phosphorylation and fatty acid oxidation (Yang et al., 2021; Zhao & Xu, 2022). Activated microglia display a metabolic switch to glycolysis (Zhao & Xu, 2022), as glycolysis provides more rapid ATP production, which enables cell growth and cytokine production (Lauro & Limatola, 2020). This metabolic switch has been observed in several neurodegenerative diseases, including PD and AD (Zhao & Xu, 2022). Several GPCRs have been described to affect this metabolic switch of microglia. For example, activation of the Gi-coupled CX3C motif chemokine receptor 1 (CX3CR1), by its ligand CX3CL1, resulted in an increased expression of genes involved in oxidative phosphorylation and decreased the expression of those related to glycolysis, indicating that CX3CL1 switched the metabolic state of microglia from glycolysis to the oxidative pathway (Lauro et al., 2019). Similar results were obtained by activation of the melatonin receptor 1 (MT1), which is highly expressed on microglia in vitro (Olivier et al., 2009). MT1 activation on the murine BV-2 microglial cell line induced a preference to oxidative phosphorylation over aerobic glycolysis (Gu et al., 2021). This indicates that multiple Gi-GPCRs can influence the metabolic state of microglia. Therefore, Gi-DREADD activation in microglia might be an interesting tool to flip the metabolic switch from glycolysis, observed in PD and AD, back to a more oxidative pathway.

Microglia have been associated with obesity pathogenesis, in which glucose intolerance is a typical hallmark (Mendes et al., 2018). A recent study (preprint) has unexpectedly shown that microglial inflammation, induced by Gq-DREADD activation, improved glucose tolerance, both in lean and obese mice. This effect depended on TNF- α signaling in microglia, which increased the activity of hypothalamic glucoreponsive neurons (Douglass et al., 2022). Glucose intolerance is also observed in other diseases as diabetes mellitus (Malone & Hansen, 2019), chronic kidney disease (Spoto et al., 2016), and cystic fibrosis (Kasim et al., 2021). It would be interesting to investigate whether Gq-DREADD activation of microglia could also improve glucose intolerance in these diseases.

5.3 | Release of neuroactive substances

Activated microglia are involved in regulating brain development by the release of gliotransmitters (e.g., glutamate (Barger et al., 2007)

and ATP (Liu et al., 2006)), several trophic factors (as BDNF, insulin-like growth factor I [IGF-1]; (Araki et al., 2021)), basic fibroblast growth factor (bFGF; (Subramanyam et al., 2019)), GDNF and nerve growth factor (NGF; Spielman et al., 2017), but also prostaglandins (as PGE2 (Zhang et al., 2009)) and anti-inflammatory factors (e.g., IL-4, IL-13, and IL-10 interleukins (Orihuela et al., 2016; Pozzo et al., 2019)), and transforming growth factor- β (TGF- β ; Orihuela et al., 2016). However, when microglia become overactivated, they produce of a large array of cytotoxic factors such as several reactive oxygen species (ROS) (as superoxide and NO (Colton & Gilbert, 1987; Orihuela et al., 2016)) and pro-inflammatory factors as, IL-1, IL-6, IL-18, TNF- α , and C-C chemokines CCL2 (Araki et al., 2021; Orihuela et al., 2016).

Microglial activation plays a crucial role in chronic pain promotion, in which the release of several substances, as IL-1 β , IL-6, TNF- α , PGE2, BDNF, and ROS, are key mediators (Zhuo et al., 2011). Grace et al. were the first to chemogenetically modulate microglia to scrutinize their role in nociceptive sensitization and neuropathic pain (Grace et al., 2016). *In vivo* activation of Gi-DREADDs, expressed in the microglia of the lumbar dorsal spinal cord, reversed morphine-induced persistent sensitization (Grace et al., 2016) and reduced chronic constriction injury-induced allodynia (Grace et al., 2018). They confirmed in Gi-DREADD-expressing BV-2 cells that CNO administration attenuated the high mobility group box-1 (HMGB1)-induced increased expression of IkB α , NLRP3, IL-1 β , TNF, and IL-6 (Grace et al., 2016). HMGB1 is a danger-associated molecular pattern (DAMP) also released spinally in chronic pain models (Agalave et al., 2014). Moreover, the expression of *Nos2* mRNA, NO, *Il1 β* mRNA, and IL-1 β were also reduced after both LPS and CCL2 exposure in CNO-treated Gi-DREADD-expressing BV-2 cells (Grace et al., 2018). Similarly, Yi et al. (2020) found reduced mechanical hypersensitivity upon CNO administration in CX3CR1^{CreER(+/-)}-Gi-DREADD transgenic mice. They attributed these effects to reduced expression of IRF8 and IL-1 β , and to weakened C-fiber-evoked field potentials, indicating that suppressing microglial activation reduces nociceptive transmission after spinal nerve transection (Yi et al., 2020). Microglial Gi-DREADD activation also alleviated the pain hypersensitivity in rats with neonatal incision-induced exaggeration of pain, which was accompanied by reduced pro-inflammatory cytokine levels, as IL-1 β , IL-6, TNF- α , and CCL2 (Ding et al., 2022).

Activation of CD68-targeted Gq-DREADDs expressed in spinal microglia induced hind paw allodynia, an effect that was dependent on IL-1 (Grace et al., 2018). This mechanism-of-action was confirmed by *in vitro* experiments where Gq-DREADD activation in BV-2 cells increased levels of proinflammatory cytokines NO, TNF, IL-1 β , and IL-6 release (Grace et al., 2018). Additionally, Saika et al. also demonstrated increased levels of IL-1 β and TNF- α , but also CCL3 and CCL4 after CNO administration in male, but not female, CX3CR1^{Cre}(+/-)-Gq-DREADD mice, which also resulted in mechanical allodynia (Saika et al., 2021). This effect was reversed by administration of the PLX3397, a CSF-1R inhibitor which causes microglial depletion (Elmore et al., 2014), indicating pivotal role of Gq-signaling in microglia themselves (Saika et al., 2021).

Binning et al. further investigated the effects of Gq-DREADD activation in microglia *in vitro* and *in vivo* (Binning et al., 2020). Microglia, harvested from transgenic mice, in which Gq-DREADDs were conditionally expressed in CX3CR1⁺ cells, showed increased phagocytosis and increased levels of pro-inflammatory cytokines TNF- α , IL-1 β , and IL-6 after CNO application. These results were replicated after CNO administration in naïve mice or mice treated with LPS to resemble (neuro)inflammatory conditions. In contrast to these findings, chronic activation of Gq-DREADDs in microglia significantly decreased the LPS-induced increase in TNF- α , IL-1 β , and IL-6 production (Binning et al., 2020). This suggests that repeated stimulation of Gq-DREADDs in microglia leads to immunological tolerance development and even an immunological memory that may ameliorate inflammatory responses in the brain (Binning et al., 2020).

Microglial Gi-DREADD activation also abolished the ethanol-induced increased expression of pro-inflammatory cytokines, which suggested that Gi-DREADD-based microglial modulation could alleviate the primed state of microglia (Coleman et al., 2020). Microglial priming, an increased sensitivity to pro-inflammatory insults leading to an exaggerated inflammatory response, is observed in alcoholism (Crews et al., 2017), depression (Zhang et al., 2020), PTSD (Enomoto & Kato, 2021; Pohl et al., 2021), stress (Niraula et al., 2017), several neurodegenerative diseases (Perry & Holmes, 2014), CNS injury, and aging (Norden et al., 2015). Moreover, microglial priming is associated with prolonged neuroinflammation resulting in cognitive and behavioral deficits (prolonged sickness, depressive-like behavior) and disease progression (Norden et al., 2015). Therefore, it would be interesting to further investigate the effects of microglial Gi-DREADD modulation on neuroinflammatory levels and whether it would also affect cognitive and behavioral complications and pathological developments.

More recently, the role of microglial signaling in mood regulation was investigated. Gq-DREADD activation in striatal microglia, but not in microglia of the barrel cortex, was sufficient to induce an inflammatory response, which in turn caused behavioral changes, as conditioned place aversion and anhedonic-like behavior. More specifically, microglial Gq-DREADD activation triggered an IL-6-mediated autocrine loop and increased production of PGE2, reducing the excitability of the striatal medium spiny neurons (Klawonn et al., 2021). Moreover, Klawonn et al. (2021) found that Gi-DREADD activation in microglia, of CX3CR1^{CreER(+/-)}-Gi-DREADD mice, could counteract LPS-induced negative affective state. Based on these data, it could be interesting to investigate whether microglial Gi-DREADD modulation could influence affective behaviors in various mouse models for mental disorders (Klawonn et al., 2021). The clarification for the inflammatory component in this affective disorder may indicate that microglia are possible druggable targets. It also found that microglial activation decreased the firing of striatal medium spiny neurons (Klawonn et al., 2021). As mentioned before, neurodegenerative diseases characterized by severe motor dysfunctions, such as HD, PD, and ALS, are often associated with elevated activity of medium spiny neurons (Fogarty et al., 2017; Nagai, Rajbhandari, et al., 2019; Ruiz-Calvo et al., 2018). As activation of Gq-DREADDs in microglia could reduce

the excitability of medium spiny neurons, it could possibly also be a potential new therapeutic strategy for these diseases.

Overall, the results from this handful of studies demonstrated anti-inflammatory and anti-nociceptive consequences following acute activation of Gi-signaling in microglia, and pro-inflammatory and pro-nociceptive effects associated with activation of microglial Gq-signaling. However, the recent study by Binning and colleagues highlighted that chronic activation of Gq-DREADD in microglia resulted in decreased pro-inflammatory cytokine expression (Binning et al., 2020). These anti-inflammatory properties of microglial DREADD modulation could become an interesting method to intervene with the neuroinflammation component of many CNS diseases.

6 | STRENGTHS AND SHORTCOMINGS OF CHEMOGENETIC APPROACHES IN GLIAL CELLS

DREADDs are a powerful cell-specific tool for modulating glial activity and have several advantages, not in the least of which is their temporal and spatial precision. Table 2 gives an overview of the discussed advantages and limitations of DREADDs. DREADDs are designed to be insensitive to endogenous ligands and only respond to designer drugs that are otherwise presumed to be inert (Roth, 2016). This results in a system where the signaling pathways coupled to the DREADDs can be controlled reversibly in specific cell types (Walker & Kullmann, 2020), unlike other methods such as cell ablation. Most DREADD ligands, such as deschloroclozapine (DCZ; 2020), DREADD agonist 21 (Jendryka et al., 2019) and olanzapine (Loryan et al., 2016), are able to cross the BBB with ease (Chen et al., 2015). For CNO, it has been suggested that it is not CNO itself, but its metabolized form, clozapine, that crosses the BBB (Gomez et al., 2017; Manvich

et al., 2018). Moreover, as DREADDs are GPCRs, they are subjected to the “receptor reserve” phenomenon, meaning that if these receptors are expressed at high levels, only a small fraction of those need to be activated to have a maximal downstream effect (Wacker et al., 2017). This implies that DREADD ligands can potentially be used at very low doses to produce clinically relevant pharmacological effects (Gomez et al., 2017).

It must also be taken into account that DREADDs, like all GPCRs, can possibly undergo desensitization, internalization, and degradation. Initially, it was argued that DREADDs do not undergo significant desensitization (Roth, 2016). However, desensitization of receptors can cause rebound effects (Lerner & Klein, 2019) and some evidence has been gathered suggesting rebound effects for DREADD-based modulation of neurons (Desloovere et al., 2019) and astrocytes (Yang et al., 2015). However, in the latter study, a rather high dose of CNO (5 mg/kg) was used (Yang et al., 2015), which was later discussed to cause substantial non-specific effects (Chen et al., 2016; Gomez et al., 2017). Therefore, it cannot be ruled out that these observed “rebound” effects were in fact CNO-induced side-effects and could be prevented by the use of a lower dose. Generally speaking, the used dose for *in vivo* modulation of DREADDs in glia ranges between 0.5 and 3 mg/kg CNO (Adamsky et al., 2018; Binning et al., 2020; Császár et al., 2022; Grace et al., 2018; Jones et al., 2018; Klawonn et al., 2021; Nam et al., 2019; Xie et al., 2017).

All DREADD receptors are responsive to the same synthetic ligands limiting their use for bidirectional and multiplexed control of a cell-type (Armbruster et al., 2007; Vardy et al., 2015). However, the problem could be addressed by using DREADDs that depend on a different ligand, such as the kappa opioid receptor-based DREADD (KORD). The Gi-coupled KORD silences neuronal firing in the presence of Salvinorin B (SalB), a metabolite of Salvinorin A (Vardy et al., 2015). Behavioral effects induced by SalB appear shortly after

TABLE 2 Overview of the main advantages and limitations of designer receptors exclusively activated by designer drugs (DREADDs).

	Advantages	Limitations
DREADD are GPCRs	<ul style="list-style-type: none"> Receptor reserve phenomenon (Wacker et al., 2017) Several DREADDs (Gq, Gi, Gs) available 	<ul style="list-style-type: none"> Possibly could undergo desensitization (Desloovere et al., 2019; Lerner & Klein, 2019; Roth, 2016; Yang et al., 2015)
Use of designer drugs	<ul style="list-style-type: none"> Only responsive to designer drugs (Roth, 2016) Reversible control possible (Walker & Kullmann, 2020) Negligible constitutive activity (Armbruster et al., 2007; Sternson & Roth, 2014), except for Gs-DREADD (Guettier et al., 2009). 	<ul style="list-style-type: none"> All DREADDs are activated by the same ligands
DREADD expression via transgenic animals	<ul style="list-style-type: none"> No difficulties with obtaining high transduction efficiency of cells No tissue damage or inflammation of stereotaxic injection of viral vectors itself (Stoica et al., 2013) 	<ul style="list-style-type: none"> Cell type specific promotor necessary No spatial control of expression Limited value in relation to clinical translation
DREADD expression via viral vectors	<ul style="list-style-type: none"> Spatial specific expression 	<ul style="list-style-type: none"> High specificity and high transduction efficiency can be challenging Tissue damage and inflammation induced by stereotaxic injection or viral vectors Cell type specific promotor necessary
Others		<ul style="list-style-type: none"> Toxicity of membrane-associated overexpression

injection and last about 1 h (Vardy et al., 2015), whereas DREADD ligands, such as CNO, are known to activate the receptors within 15 min and lasts for several hours with a single drug administration (Vlasov et al., 2018). However, whether or not the same conditions apply in glial cells has yet to be determined, as the KORD system has not yet been employed in astrocytes or microglia. The combination of hM3Dq/CNO and KORD/SaIB could provide more insights into bidirectional chemogenetic control of the same glial cell-type, in which CNO can induce Gq-mediated modulation over a longer timeframe, which can be rapidly attenuated by SaIB-induced Gi-mediated effects. This could be particularly interesting in astrocytes to determine whether Gi-signaling reinforces or suppresses the Gq-mediated effects, as it is not yet clear if Gq- and Gi-DREADD induce opposite actions in the same cell and if this varies across the brain.

Specific targeting of astrocytes or microglia is challenging in general, and thus also for inducing DREADD expression. To this end, genetically engineered animal models have been used (Agulhon et al., 2013; Binning et al., 2020; Porter-Stransky et al., 2019; Saika et al., 2020; Xie et al., 2017, 2020; Yi et al., 2020; see Table 3). The first transgenic model with DREADD-containing glia, was the GFAP-hM3Dq expressing mouse line driving Gq-DREADD expression in their astrocytes (Agulhon et al., 2013). This causes DREADD expression to occur in astrocytes in the entire CNS system, as such these transgenic models are valuable to discover the effect of widely distributed GFAP+ astrocyte populations in a noninvasive manner (Xie et al., 2015). Brain region-selective modulation can be achieved in these models via local infusion of the agonist (Porter-Stransky et al., 2019). However, this nullifies the non-invasiveness of this technique. Importantly, it should be noted that GFAP has been reported to be also expressed by neuronal progenitor cells (Kriegstein & Alvarez-Buylla, 2009) and that neuronal GFAP expression is observed in various brain pathologies (Zwirner et al., 2021), and thus GFAP+ neurons would also express Gq-DREADDs in this GFAP-hM3Dq mouse line. This abolishes the cell specificity of the mouse line toward GFAP+ astrocytes. This is why others have used transgenic mouse lines expressing a DREADD receptor using Cre and flippase-mediated recombination, especially when the recombination is inducible. For microglia, the Cre or CreER genes were expressed under the CX3CR1 promoter to drive DREADD expression (Binning et al., 2020; Bolton et al., 2022; Coffey et al., 2022; Császár et al., 2022; Khan et al., 2023; Saika et al., 2020, 2021; Yi et al., 2020). It has been described that the CX3CR1-Cre mouse line can drive expression of reporters, as YFP, in neurons (Haimon et al., 2018). This is hypothesized to be caused by the fact that neurons express CX3CR1 during development, which may result in gene rearrangements in a considerable fraction of neurons when using CX3CR1-Cre mice, even if CX3CR1 is no longer expressed in adult neurons. However, with the tamoxifen inducible CX3CR1-CreERT2 mice, no expression of the YFP reporter was detected in neurons (Haimon et al., 2018). For astrocytes, GFAP-CreER transgenic mice expressing CreER under the GFAP promoter are available as well, and have been used to drive DREADD expression (2020). However, the use of transgenic mouse models has a high cost and is also time consuming (Maes et al., 2019).

To circumvent the disadvantages associated with transgenic mice, viral vectors are often used to deliver the DREADD constructs (see Table 3). Adeno-associated vectors (AAVs) are the most commonly used viral vector system to deliver the DREADD gene constructs to glial cells but their cargoes are restricted to 4.5 kb, which limits the use of some cell-specific promoters (Hirbec et al., 2020). Therefore, occasionally lentiviral vectors are used that allow larger packaging size (up to 8 kb), for example to transfect BV-2 microglial cells using the CD68 promoter (Grace et al., 2018), but these have the disadvantage of genome integration. The entry of AAVs depends on the interaction of specific surface glycans on the viral particle and receptor/co-receptor(s) on the cell membrane. There are several serotypes, all differing in their interaction with the cell surface. For astrocytes AAV5, AAV8, or AAV9 with the *GfaABC₁D* or *Gfa2* promoter seem to have the highest and most selective transduction rates (Yu, Nagai, & Khakh, 2020). Microglia have been more challenging to transfect. Rosario et al. modified the rAAV6 serotype with three mutations Y731F/Y705F/T492V, which prevented proteasomal degradation and resulted in increased transduction efficiency of microglia (Rosario et al., 2016). In addition, microglia can detect, engulf, and destroy the viral vectors used for their transduction since they are the macrophages of the CNS (Maes et al., 2019). However, several papers have described successful expression of Gi-DREADDs and Gq-DREADDs after transduction with AAVs or lentiviral vectors (Coleman et al., 2020; Ding et al., 2022; Grace et al., 2016, 2018; Zou et al., 2022). Moreover, Klawonn et al. and Khan et al. combined the use of a transgenic mouse expressing CreER in CX3CR1+ microglia in the CNS, with transduction of DREADD encoding viral vectors to obtain specific expression of the DREADDs in striatal (Klawonn et al., 2021) and dorsal raphe microglia (Khan et al., 2023). Yet, viral vectors have drawbacks, if not properly controlled for, which can lead to ambiguous interpretation of the obtained results. First, stereotaxic injection of viral vectors itself can lead to tissue damage and inflammation (Stoica et al., 2013), therefore both astrocyte and microglial reactivity resulting from viral transfection should be assessed and appropriate controls included. Second, even though stereotactic injection of viral vectors permits anatomically restricted expression; the virus can spread into surrounding brain areas to cause potential off-target effects (Jiang et al., 2017). Additionally, viral vector injection often elicits considerable interindividual variation in the amount of transduction efficiency (Atasoy & Sternson, 2018). Therefore, systematic post hoc evaluation of DREADD expression is definitely required.

Another point of attention is the selection of a cell-specific promoter to specifically target astrocytes and microglia. For astrocytes, the GFAP promoter is commonly used to drive DREADD expression in both viral vector approaches as in transgenic mouse models. GFAP is expressed in astrocytes throughout different brain regions and during development (Guttenplan & Liddel, 2019). However, some aspects must be taken into consideration when using the GFAP promoter, for example *Gfap* mRNA and GFAP protein levels can vary with age and between brain regions (Boisvert et al., 2018; Cahoy et al., 2008; Zhang et al., 2019). Moreover, GFAP does not identify all astrocytes throughout the CNS, nor is GFAP expression alone sufficient to identify a cell



TABLE 3 Overview of the specificity and the transduction efficiency of designer receptors exclusively activated by designer drug (DREADD) expression in astrocytes and microglia obtained via either animal models and/or viral vectors.

Specificity		Transduction efficiency
Astrocytes		
GFAP-hM3Dq transgenic mouse model (Agulhon et al., 2013)	<ul style="list-style-type: none"> Gq-DREADDs will be expressed in all GFAP+ astrocytes in the whole CNS. Specificity confirmed by immunohistochemistry (Agulhon et al., 2013) GFAP+ neuronal progenitor cells could also express the DREADD. 	Not applicable.
GFAP-Cre induced DREADD expression	<ul style="list-style-type: none"> Either Gq- or Gi- DREADDs will be expressed in all GFAP+ astrocytes in the whole CNS. GFAP+ neuronal progenitor cells could also express the DREADD. 	Not applicable.
AAV	<ul style="list-style-type: none"> DREADD expression will be more spatially defined to the site of injection. Specificity of the DREADD expression will be dependent on the promoter that is used. Specificity confirmed by immunohistochemistry (Durkee et al., 2019; Kol et al., 2020; MacDonald et al., 2020; Nagai, Rajbhandari, et al., 2019; Oe et al., 2020; Scofield et al., 2015), however the percentage of GFAP+ DREADD+ cells is (almost) never communicated. 	<ul style="list-style-type: none"> The transduction efficiency was reported to be between 70% and 90%, however, this is both brain-region dependent and dependent on the AAV subtype that was used (Kol et al., 2020; MacDonald et al., 2020; Scofield et al., 2015).
Microglia		
CX3CR1-Cre induced expression of DREADDs	<ul style="list-style-type: none"> Either Gq- or Gi- DREADDs will be expressed in all CX3CR1+ microglia in the whole CNS. Specificity confirmed by immunohistochemistry (Colton & Gilbert, 1987; Saika et al., 2020; Saika et al., 2021). CX3CR1+ cell of the myeloid cell lineage will also express DREADDs (Saika et al., 2020). CX3CR1-Cre mouse strain also induces expression into neuronal lineage (Haimon et al., 2018). 	Not applicable.
CX3CR1-CreERT induced expression of DREADDs	<ul style="list-style-type: none"> Either Gq- or Gi- DREADDs will be expressed in all CX3CR1+ microglia in the whole CNS. Specificity confirmed by immunohistochemistry (Binning et al., 2020; Császár et al., 2022; Yi et al., 2020) and DREADD-expressing cells were 84% of Iba1+ microglia (Binning et al., 2020) and 95% of P2Y12R+ microglia (Császár et al., 2022). Contribution of CX3CR1+ myeloid cells can be avoided by waiting 1 month between tamoxifen injections and the start of experiments, so myeloid cells turn over (Parkhurst et al., 2013). No neuronal expression was reported with CX3CR1-CreERT2 lineage (Haimon et al., 2018). 	Not applicable.
CX3CR1-CreERT in combination with viral vectors	<ul style="list-style-type: none"> DREADD expression will be more spatially precise at the site of injection. Specificity confirmed by immunohistochemistry (Khan et al., 2023; Klawonn et al., 2021) and DREADD-expressing cells were approximately 72% CX3CR1+ microglia with AAV vector (Khan et al., 2023) and 98% of Iba1+ cells with lentiviral vector (Klawonn et al., 2021) 	<ul style="list-style-type: none"> AAV (AAVDJ-ef1α-DIOhM3Dq-mCherry): transduction efficiency of 52% of Cx3cr1+ cells in the dorsal raphe nucleus (Khan et al., 2023). Lentiviral (Lenti-FLEX-hM3Dq-GFP): transduction efficiency of approximately 28% in Iba1+ cells in striatum (Klawonn et al., 2021).
AAV	<ul style="list-style-type: none"> DREADD expression will be more spatially precise at the site of injection. Specificity of the DREADD expression will be dependent on the promoter that is used. Specificity confirmed by immunohistochemistry, however the specificity is never communicated (Ding et al., 2022; Grace et al., 2016; Grace et al., 2018). 	<ul style="list-style-type: none"> Transduction efficiency is not communicated with the use of pAAV9-CD68-hM3Dq and pAAV9-CD68-hM4Di (Coleman et al., 2020; Ding et al., 2022; Grace et al., 2016; Grace et al., 2018; Zou et al., 2022).

as an astrocyte (Sofroniew & Vinters, 2010), as it is expressed by neuronal progenitor cells (Kriegstein & Alvarez-Buylla, 2009), that give rise to neurons, oligodendrocytes (Casper & McCarthy, 2006), endothelial cells, and vascular smooth muscle cells (Osman et al., 2020). Transcriptome analysis has shown that the astrocyte-specific enzyme aldehyde dehydrogenase 1 family member L1 (*Aldh1l1*) might be a more suitable astrocyte-specific promoter, as it is able to identify a broader range of astrocytes in the brain (Cahoy et al., 2008). However, AAVs using the *Aldh1l1* promoter shows low astrocytic transfection rates and, strikingly, preferentially drives expression in neurons in many brain regions (Koh et al., 2017; Mudannayake et al., 2016). In addition, selecting an appropriate astrocyte marker is ambiguous as they are a heterogeneous group which show broad functional and morphological diversity in the CNS (Zhang et al., 2019). It is argued that rather than searching for a suitable “pan-astrocyte” marker it might be more meaningful to discover markers for specific subsets of astrocytes (Pestana et al., 2020; Zhang & Barres, 2010). For the transduction of microglia, the CD68 promoter can be used (Ding et al., 2022; Grace et al., 2016, 2018). However, CD68 is not cell-specific for microglia, as it is also expressed by macrophages. Moreover, the CD68 expression is often associated with activated or inflammatory phenotypes and elevated antigen presentation (Song et al., 2011) and it is known to have a particular role in phagocytosis (Zotova et al., 2013). So, it is possible that only a subset of microglia express DREADDs when using the CD68 promoter. The use of more generic promoters, such as *Iba1*, may lead to wider expression on microglia, but this promoter is also expressed on macrophages. In pathological conditions, CNS infiltration of macrophages is a frequently observed phenomenon (Stoll & Jander, 1999). Experiments may be designed in such a way to exclude transfection of infiltrating macrophages, for instance DREADD transfection prior to injury, however, this approach does not circumvent microglial proliferation or infiltration of macrophages. Therefore, effects by DREADD-transfected microglia may as such be obscured or minimized because of the contribution of non-transfected macrophages or proliferated microglia in CNS pathologies (Grace et al., 2018). Additionally, Cre or CreER mouse models under the CX3CR1 promoter have been used to drive DREADD to expression in their microglia, however, CX3CR1 is also expressed on myeloid cells. Hence, Binning et al. (2020), Klawonn et al. (2021), and Yi et al. (2020) have found an ingenious way to avoid contribution of myeloid cells. The authors reported 1 month of waiting after tamoxifen treatment of the CreER-DREADD transgenic mice before starting experiments. In doing so, the peripheral myeloid-derived cells could turn over (Binning et al., 2020; Klawonn et al., 2021; Yi et al., 2020) and new-born peripheral myeloid-derived cells would not express the DREADD receptor anymore. Interestingly, recent research has found Transmembrane Protein 119 (TMEM119) to be a specific microglial marker that does not occur in infiltrating peripheral immune cells (Bennett et al., 2016; Eme-Scolan & Dando, 2020b), which makes the TMEM119 promoter interesting to obtain cell-specific expression in microglia.

Furthermore, the membrane-associated character of DREADDs could possibly lead to toxic effects on the target cells. Membrane protein overexpression can lead to toxicity due to the high metabolic

demand or compromised cell function by an overload on the cellular machinery, which can interfere with the assembly, trafficking, and functioning of other proteins important for cell viability (Keifer et al., 2020). To the best of our knowledge, however, there have been no reported cases of toxic effects of DREADDs in glial cells. In neurons, transduction with high titers of AAV vectors for DREADDs resulted in neuronal loss and neuroinflammatory reactions, whereas this was not the case in the mCherry control group, despite an equally high titer (Goossens et al., 2021). Therefore, the possible toxic effects of DREADD expression should be assessed in the future.

Spatiotemporal modulation of glial cells can be achieved by optogenetic approaches. Optogenetic modulation is based on opsins, light-sensitive proteins. These proteins can be ion channels, ion pumps or GPCRs, and intracellular signaling will be initiated by light of a specific wavelength rather than selective ligands (Airan et al., 2009; Ebrahim-Amini et al., 2021; Oceau et al., 2019). Practically, the opsins will be activated by light delivered through an optic fiber implanted at the region of interest. The surgical implantation of the optic fiber in the brain region of interest could be considered as a disadvantage compared to chemogenetics (Geng et al., 2023). This can be especially challenging when the technique has to be combined with another technique requiring implantation in the brain such as a microdialysis probe (Zant et al., 2016). Chemogenetics and optogenetics both rely on transgenic animals or viral vectors for the delivery of the genetically modified proteins to the glia (Forcelli, 2017; Geng et al., 2023). Nevertheless, optogenetics has a superior spatiotemporal resolution compared to chemogenetics as the light is emitted only at the brain location of interest while the designer drug is distributed to all tissues and could be beneficial if modulation of larger areas is desired. Optogenetics has an increased temporal resolution due to the light that can easily be switched on or off and has an immediate effect at the region of interest, while the temporal resolution of chemogenetics is dependent on the pharmacokinetic properties of the chosen designer drug. The latter is only a disadvantage when the temporal resolution is critical for the intended purposes (Forcelli, 2017). Moreover, the designer drugs are not entirely specific for the designer receptor only and thus could have off-target effects (Bærentzen et al., 2019). However, the temporal resolution is not only dependent on the agonist but is also dependent on the interacting target itself. Opsins with varying temporal kinetics have been described (millisecond range; Guru et al., 2015), chemogenetics with GPCRs are described as receptors that signal with intermediate speed (Lohse et al., 2008). On the other hand, the illumination of glial cells itself should not have a considerable effect although it could lead to energy-induced heating of tissue (Bang et al., 2016; Cardozo Pinto & Lammel, 2019). The use of optogenetics in astrocytes and microglia has already been reviewed elsewhere (Bang et al., 2016; Parusel et al., 2023). In the present review, we therefore focused on chemogenetic approaches.

Although DREADD-based astrocyte and microglia modulation still encounters some hurdles, as reviewed here, they really have become an established and state-of-the-art tool for unraveling cell-specific astrocyte physiology or microglial functioning. By implementing the correct control conditions and by taking necessary precautions, as

with each experimental technique, chemogenetic approaches are extremely valuable for elucidating glial cell specific functions in the brain and as future therapeutic strategies to modulate brain diseases. Recent breakthroughs regarding advanced Ca^{2+} imaging (Bindocci et al., 2017) and improved strategies for mRNA sequencing (Boulay et al., 2019; Mazare et al., 2020) combined with chemogenetic technology will help with in-depth characterization of glial signaling and elucidate the underlying mechanism of DREADD-mediated behavioral and functional responses.

7 | TRANSLATIONAL ASPECTS OF THE DREADD-BASED MANIPULATION OF GLIA

DREADDs have an interesting potential for translation as therapies for brain disorders. Indeed, their cell specificity, together with the increasing need for innovative approaches for treating CNS disorders, has captured the attention from the neuroscientific community. Importantly, the recent expansion of gene therapy approaches in the clinic underscores the therapeutic potential of DREADD applications. Gene therapy methods, with local injection of viral vectors can be used as a delivery modality for the DREADDs, allowing cell- and spatial-specificity of the approach. This contributes to an attractive therapeutic profile of the DREADD-mediated strategy. In addition, it is believed that DREADDs can be relatively easily translated to large animals, including humans, especially because they are mutated human GPCRs, and thus would more likely not lead to an immunogenic response (English & Roth, 2015). To date, chemogenetics have not yet been implemented clinically (English & Roth, 2015), but feasibility of this chemogenetic approach has been demonstrated in non-human primates. Nevertheless, these studies are sparse and exclusively focus on modulating neurons to date (Deffains et al., 2021; Galvan et al., 2019; Keifer et al., 2020; Roseboom et al., 2021; Upright & Baxter, 2020).

The ideal treatment for neurological conditions would be spatially precise, noninvasive, not associated with side-effects and providing bidirectional and reversible, on-demand control of specific targeted brain cells (English & Roth, 2015). Although DREADD-mediated modulation of glia still faces stumbling blocks, one major advantage is the reversible nature of DREADD activation in contrast to other gene therapy approaches (Weston et al., 2019). DREADD activation and resulting intracellular signaling is dependent on the administration of the designer drugs and results in only temporary modulation of the cells, as both Gq- and Gi-DREADDs were engineered to have negligible constitutive activity (Armbruster et al., 2007; Sternson & Roth, 2014). However, for the Gs-DREADD, it was shown to have a small degree of constitutive activity in pancreatic β -cells (Guettier et al., 2009). Although constitutive activity of the DREADDs have not yet been reported in astrocytes nor microglia (Oe et al., 2020), basal activity of these receptors has to be thoroughly assessed cell type specifically, before further translation to humans would be possible (Lieb et al., 2019). Nevertheless, DREADD-mediated cell activation is easy to control by the administration of the designer drug, and thus

the agonist can only be administered when therapeutic effects are envisaged, which would limit possible side-effects. Moreover, DREADD ligands have been described to activate their receptors for several hours following a single drug administration (Vlasov et al., 2018). Yet, the most recent designed DREADD agonist was described to be more fast-acting compared others, with the first effect observed 5 min after i.p. injection in mice (Nagai et al., 2020). As is the case for all cell types, the choice of the DREADD agonist has to be carefully evaluated when considering therapeutic use (which has been extensively covered by EbrahimAmini et al., 2021; Geng et al., 2023; Pestana et al., 2020; Zotova et al., 2013).

In addition, more in-depth information needs to be gathered on subcellular localization and density of the DREADDs and the potential interference with the expression and/or function of the endogenous proteins of the targeted cell (Jiang et al., 2017; Keifer et al., 2020). Indeed, only limited information is currently available on the subcellular localization of the DREADD receptors. It was found that in monkey brains and in mouse brains, both Gi-DREADD and Gq-DREADD were localized on the plasma membrane of neurons, but rarely at synapses. However, for Gi-DREADDs, the localization was dependent on the tag, either mCherry or hemagglutinin, which resulted in localization in the intracellular space or the plasma membrane, respectively (Galvan et al., 2019). Moreover, the density of the DREADD receptors on the membrane of glial cells are not yet assessed. It is possible that the density varies across species, across the membrane of microglia or astrocytes and among different subtypes of microglia and astrocytes, as both form heterogeneous groups, potentially affecting the outcome of the desired DREADD modulation. Additionally, as discussed previously, the DREADD-induced downstream signaling pathways are not yet fully elucidated in glial cells and some researchers suggest DREADD-based modulation does not exactly resemble cell location and/or properties of the endogenous GPCR-mediated transients (Savtchouk & Volterra, 2018), potentially inducing unexpected side-effects when translating to the clinic. Therefore, DREADD-associated intracellular signaling pathways needs to be thoroughly investigated in human glial cells and it must be taken into account that activation of DREADDs might differ from native receptors in terms of spatiotemporal patterns (Shchepinova et al., 2020).

As mentioned previously, to fully grasp the therapeutic potential of the DREADD strategy, drawbacks inherent to the DREADD receptor as well as to the DREADD delivery strategy need to be taken into consideration. DREADDs must be delivered to and expressed inside the brain tissue of interest to elicit the desired response. Off-target or weak expression of the DREADD is for obvious reasons undesirable (English & Roth, 2015). The vector of choice in gene therapies currently in the clinic is the adeno-associated viral (AAV) vector, due to its desirable safety profile (Hudry & Vandenberghe, 2019). This has been highlighted by the recent approval of Zolgensma, an AAV9 vector expressing survival motor neuron 1, for the treatment of spinal muscular atrophy (Mendell et al., 2017). Additionally, the route of administration is another critical factor that has to be considered. Direct intraparenchymal rAAV injections result in localized distribution of rAAV and are suited for the treatment of CNS diseases that afflict a

defined region of the brain (Wang et al., 2019), as in mesial temporal lobe epilepsy. As the rest of the brain remains unaffected, this method would minimize the risk of side-effects (Lieb et al., 2019). Other possible routes, as systemic administration are less invasive, but then a BBB-crossing serotype has to be used, such as AAV9, the AAV rhesus isolate (rh.) 8 (Saraiva et al., 2016) or AAV-PHP.B (Rincon et al., 2018). Moreover, AAV9 is known to primarily target astrocytes in both mice and non-human primates, even with the use of constitutive active promoters (Foust et al., 2009; Samaranch et al., 2012). Both intravascular delivery as well as administration into cerebrospinal fluid space (intracerebroventricular, intra-cisterna magna or lumbar intrathecal injections) are expected to result in a widespread CNS distribution and to be more suitable for targeting broad regions or multiple regions of the brain (Saraiva et al., 2016), such as several mental disorders and AD.

Finally, the immunological barriers upon AAV-DREADD delivery need to be discussed. The AAV protein capsid, its DREADD-encoding transgene and the DREADD protein product can all be recognized by the host immune, which can affect the gene delivery and persistent gene expression (Wang et al., 2019). For instance, intra-hippocampal delivery of the AAV2/5 vector, expressing enhanced green fluorescent protein (eGFP) under the GFAP promoter, was shown to induce titer-dependent astrocyte activation in mice, but did not affect microglia activation (Ortinski et al., 2010). Recently, two AAV9 variants were discovered which transduce microglia more effectively and did not induce microglia immune activation after transgene delivery into striatum or midbrain (Lin et al., 2022). Although, intraparenchymal administration is currently the most commonly used administration route for AAV vector delivery into the brain in ongoing clinical trials (Hudry & Vandenberghe, 2019), intraparenchymal transduction of glial cells demands extra caution and further research is necessary to find the right balance between optimal transduction rates and exclusion of possible toxic side-effects. Furthermore, the immune system can produce neutralizing antibodies against the AAV capsid after exposure to the therapeutic AAVs, and some humans even have preexisting antibodies. However, this appears to be less important in determining the efficacy of AAVs to deliver transgenes in the CNS than in other tissues (Hudry & Vandenberghe, 2019). Not only the viral vectors, but also expression of DREADDs themselves can cause immune responses. However, as mentioned before, DREADDs were created by mutating the ligand-binding domain of human muscarinic receptors, an endogenous protein, which expects to reduce their immunogenic properties (English & Roth, 2015; Walker & Kullmann, 2020).

Besides the challenges that still have to be addressed, we strongly believe that patients suffering from CNS diseases could benefit from the DREADD technology in the future. Traditional therapies often have major drawbacks such as the off-target effects of medicines. In contrast, designer drugs are, ideally, only interacting with one receptor type, being the designer receptor and should therefore lack off-target effects. Moreover, it is clear that astrocytes and microglia have crucial functions in the healthy brain. In many neurological and psychiatric brain diseases, however, these glial cells are also closely involved and

reactive gliosis is a typical pathophysiological feature of these diseases. By correcting aberrant glial functions and modulating gliosis, we believe we can define innovative therapeutic strategies with clinical translational potential. DREADD-based modulations of either astrocytes or microglia have proven to result in beneficial effect in preclinical models for several CNS disorders. For example, Gi-DREADD modulation of astrocytes was found to reduce stress enhanced fear learning, a preclinical model to study post-traumatic stress disorder (Jones et al., 2018). The contribution of glial cells in addiction has also been investigated, showing that Gq-DREADD activation in astrocytes crucial for abolishing cue-induced reinstatement of cocaine seeking (Schofield et al., 2015) and of methamphetamine (Siemsen et al., 2019), whereas Gi-DREADD activation of hippocampal astrocytes induced conditioned place preference (Nam et al., 2019). Chemogenetic modulation of microglia has also been studied within the field of MDD (Klawonn et al., 2021) and neurodevelopmental disorders (Bolton et al., 2022). Gi-DREADD activation in astrocytes and in microglia both have been reported to reduce allodynia in several animal models for neuropathic pain (Ding et al., 2022; Grace et al., 2018; Lu et al., 2023; Saika et al., 2020; Yang et al., 2022; Yi et al., 2020). By correcting aberrant glial functions and modulating gliosis, we believe we can define innovative therapeutic strategies with clinical translational potential.

8 | CONCLUSION

In summary, DREADDs are valuable tools for deciphering the specific role of astrocytes or microglia in the CNS. The use of DREADDs has established astrocytes as key players in the modulation of synaptic activity. In microglia, the DREADD technique has been mainly used to modulate pro-inflammatory cytokine levels in the field of neuropathic pain. Yet, several aspects remain to be elucidated before this technology will allow us to further unravel the molecular pathways underlying DREADD-modulated glia-induced behaviors. Additionally, certain features related to glial DREADD-construct delivery, such as promoter specificity and the induction of gliosis after invasive viral delivery, remain hurdles to be dealt with. Still, DREADD-based manipulation of astrocytes and microglia are promising strategies for therapeutic intervention in several brain disorders. In addition, availability of BBB-crossing, market-approved DREADD-agonists, the reversible nature of DREADD activation, together with the increasing clinical use of therapeutic viral vectors, are promising factors for the potential translation to the clinic. These are stirring times for the *glioscientists* among us and tangible applications of glial cell modulation for clinical purposes look promising now more than ever.

AUTHOR CONTRIBUTIONS

Jo Bossuyt, Yana Van Den Herrewegen, Ilse Smolders: Conceptualization. **Jo Bossuyt, Yana Van Den Herrewegen, Liam Nestor:** Writing-original draft preparation. **Jo Bossuyt, Yana Van Den Herrewegen, Liam Nestor, An Buckinx, Dimitri De Bundel, Ilse**

Smolders: Writing-review and editing. **Dimitri De Bundel, Ilse Smolders:** supervision. **Yana Van Den Herrewegen, Dimitri De Bundel, Ilse Smolders:** Funding acquisition. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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