Insight into the roles of CCR5 in learning and memory in normal and disordered states

Deanna Necula[a,1], Cecile Riviere-Cazaux[b,1], Yang Shen[c], Miou Zhou[b,*]

[a] Department of Neuroscience, UCSF, San Francisco, CA.
[b] Graduate College of Biomedical Sciences, Western University of Health Sciences, Pomona, CA.
[c] Neurobiology, Psychiatry and Psychology Departments & Integrative Center for Learning and Memory, UCLA, Los Angeles, CA.
[1] These authors contributed equally.
*Correspondence: mzhou@westernu.edu.

ABSTRACT

As cognitive impairments continue to rise in prevalence, there is an urgent need to understand the mechanisms of learning and memory in normal and disordered states. C-C chemokine receptor 5 (CCR5) has been implicated in the regulation of multiple forms of learning and memory via its regulation on learning-related cell signaling and neuronal plasticity. As a chemokine receptor and a co-receptor for HIV, CCR5's role in immune response and HIV-associated neurocognitive disorder (HAND) has been widely studied. In contrast, CCR5 is less understood in cognitive deficits associated with other disorders, including Alzheimer's disease (AD), stroke and certain psychiatric disorders. A broad overview of the present literature shows that CCR5 acts as a potent suppressor of synaptic plasticity and learning and memory, although a few studies have reported the opposite effect of CCR5 in stroke or AD animal models. By summarizing the current literature of CCR5 in animal and human studies of cognition, this review aims to provide a comprehensive overview of the role of CCR5 in learning and memory in both normal and disordered states and to discuss the possibility of CCR5 suppression as an effective therapeutic to alleviate cognitive deficits in HAND, AD, and stroke.

1. Introduction

More than 16 million Americans suffer from cognitive impairments, and that number

continues to rise as mean life expectancy increases. Cognitive impairments range from mild to severe and can be the result of natural aging or different psychiatric or neurological disorders. There are few targeted therapies available for these cognitive impairments, partly because cognitive decline can rarely be attributed to a single physiological source; instead, it generally arises from the combinatorial effect of multiple neural dysfunctions. Hence, there is a wide therapeutic gap and a significant need for more exploration into the molecular, cellular and systemic mechanisms underlying cognitive deficits (Bredesen, 2014).

One promising avenue of research is in understanding the relationship between neuroinflammation and cognition. Pro-inflammatory biomarkers have been shown to correlate with the severity of cognitive decline both in the context of age-related impairments and in diseases like Alzheimer's (Heyser et al., 1997; Lim et al., 2013). Notably, the hippocampus, a brain region critical for learning and memory, has been shown to be particularly susceptible to neural deficits caused by inflammation because of its high expression of inflammatory markers (Lim et al., 2013). These inflammatory markers include IL-1α, IL-1β, IL-6, TNFα, NFκB and chemokine CCL2 (MCP-1) which are involved in microglia activation, synaptic dysfunction, cognitive impairment, AIDS, epilepsy, and impaired adult neurogenesis (da Cunha et al., 2012; Lin et al., 2020; Valcarcel-Ares et al., 2019; Vallières et al., 2002; Vitkovic et al., 2000).

Accumulating evidence indicates that chemokine receptors play an important role in cognition (Stuart and Baune, 2014). One widely studied chemokine receptor is CCR5 (C-C-chemokine receptor 5). CCR5 is a seven-membrane, G protein-coupled receptor (GPCR) highly enriched in the hippocampal CA1 (Torres-Munoz et al., 2004). CCR5 is expressed on a variety of immune cells, including T lymphocytes, macrophages, and dendritic cells. In the brain, it is highly expressed in microglia and to a lesser extent in astrocytes and neurons (Cartier et al., 2005;

Fantuzzi et al., 2019; Tran et al., 2007; Westmoreland et al., 2002).

CCR5 has multiple ligands, including CCL3 (MIP-1α), CCL4 (MIP-1β) and CCL5 (RANTES). Additional inflammatory chemokines such as CCL8 (MCP-2), CCL3L1 (LD78) and CCL11 (eotaxin) were also found to act as agonists for CCR5 (Bachelerie et al., 2014; Brelot and Chakrabarti, 2018). Through the Gi or Gq subunit of G protein, the binding of chemokine ligands to CCR5 can activate multiple signaling cascades, including phosphoinositide-3 kinase (PI3K), mitogen-activated protein kinases (MAPK) and protein kinase C (PKC) (Brelot and Chakrabarti, 2018; Lorenzen et al., 2018). CCR5 activation also leads to phosphorylation of Janus kinase 2 (JAK2) and the activation of JAK/STAT pathway, which is independent of Gi or Gq activation (Mueller and Strange, 2004). When different chemokines bind to CCR5, the resultant signaling cascades after CCR5 activation lead to calcium flux and chemotaxis of leukocytes, or promote cell survival and cell proliferation. Genetic or pharmacological CCR5 blockade regulates cytokine expression and secretion, including decreased expression of proinflammatory mediators such as interleukin (IL)-1β, tumor necrosis factor (TNF)-α, IL-6 and IL-17A, and increased expression of the anti-inflammatory cytokine IL-10, suggesting that CCR5 could serve as an effective therapeutic target for reducing inflammation (Ahmad et al., 2019; Gu et al., 2016; Tang et al., 2015).

Inflammation has been associated with cognitive decline in various neurological or neurodegenerative disorders, including Alzheimer's disease and HIV-associated cognitive decline (Guha et al., 2019; Paouri and Georgopoulos, 2019; Song et al., 2015). While the studies above have shown a relationship between CCR5 inhibition and decreased inflammation, it must be noted that reduced inflammation is not the only outcome of CCR5 inhibition and therefore not the sole mechanism underlying the effect of CCR5 inhibition on learning and memory and cognitive

deficits.

In addition to its role in immune response, CCR5 is predominantly known as a co-receptor for HIV in the central nervous system, and is widely studied in the context of both the viral infection and its related cognitive disorder (Deng et al., 1996; Ellis et al., 2007). In contrast, the role of CCR5 in normal learning and memory, and in memory deficits in mental or neurological disorders, is not well understood. This review will introduce and discuss the current evidence implicating CCR5 in both normal and disordered learning and memory states to pave the path for future research.

2. CCR5 in normal learning and memory

Several studies have explored the role of CCR5 in normal learning and memory (Table 1), where it has been shown to function as a potent suppressor for hippocampal and cortical neuronal plasticity, and as a consequence, for learning and memory (Zhou et al., 2016) (Figure 1). Recent evidence suggests that CCR5 may impact learning and memory by acting on CREB signaling, a pathway critical for learning and memory (Josselyn et al., 2004; Sano et al., 2014; Zhou et al., 2009). For example, in a tone conditioning paradigm, neurons with higher CREB levels were more excitable and had greater synaptic efficacy after conditioning, and reversible inactivation of CREB-expressing neurons in the lateral amygdala led to memory disruption (Zhou et al., 2009). Similar results of the role of CREB in learning and memory were also found in the insular cortex following a conditioned taste aversion task (Sano et al., 2014). Studies have found that *Ccr5* knockout has no effect on CREB expression and activation in naïve (home cage) mice. Compared to wild-type (WT) home cage mice, there was no change in CREB expression or CREB phosphorylation (pCREB) in *Ccr5* knockout home cages mice. In contrast, compared to WT mice

after learning, there was a significant enhancement in CREB phosphorylation in the dorsal hippocampus in *Ccr5* knockout mice after learning, suggesting that the effect of *Ccr5* knockout on CREB activation is learning-dependent (Zhou et al., 2016). Consistent with its effect on CREB signaling, *Ccr5* knockout resulted in enhanced long-term potentiation (LTP) in hippocampal CA1, and both *Ccr5* knockout and knockdown enhance performance in contextual fear conditioning, Morris water maze, and social recognition paradigms (Frank et al., 2018; Kalkonde et al., 2011; Zhou et al., 2016). Similar to genetic ablation of *Ccr5*, pharmacological inhibition of CCR5 with its antagonist maraviroc before fear conditioning training enhanced contextual memory in rats (Merino et al., 2020). In line with evidence implicating CCR5 in memory suppression, CCR5 overexpression in excitatory neurons led to memory deficits in both fear conditioning and Morris water maze (Zhou et al., 2016).

Similar to the fact that CCR5 overexpression impairs memory, evidence indicates that CCR5 activation via its ligand CCL3 impaired performance during social recognition, passive avoidance, and in the Y-maze (Kalkonde et al., 2011; Marciniak et al., 2015; Zhou et al., 2016). These studies found that CCR5 activation via CCL3 reduces basal synaptic glutamatergic and to a lesser extent GABAergic transmission in the CA1 (Marciniak et al., 2015). The effects were reversed by the application of maraviroc, a CCR5 antagonist. Therefore, although CCL3 is known to activate both CCR1 and CCR5 (Yanaba et al., 2004), the effects of CCL3 can be predominantly attributed to its binding and activation of CCR5 (Marciniak et al., 2015). Furthermore, CCL3-induced LTP impairment was completely prevented by co-injection with maraviroc. Maraviroc also rescued the short-term memory and long-term memory deficits seen upon CCL3 injection in the Y-maze and passive avoidance tasks, respectively. Importantly, maraviroc had no effect on plasticity when CCR5 was not activated by CCL3, suggesting that without ligand activation, tonic

CCR5 activity does not modulate synaptic plasticity (Marciniak et al., 2015).

In addition to acting as a suppressor for hippocampal plasticity, CCR5 also suppresses cortical plasticity; its knockout leads to enhancements in experience-dependent plasticity in the barrel cortex (Zhou et al., 2016). *Ccr5* heterozygous knockout ($Ccr5^{+/-}$) mice exhibit a more rapid (7 days) response to the spared whisker in neurons deprived of stimulation from the preferred whiskers, while their wild-type (WT) littermates took 18 days to exhibit the same plasticity. This result suggests more rapid plasticity in the partial absence of CCR5. Specifically, $Ccr5^{+/-}$ mice showed lower mEPSP amplitude and frequency, and lower probability of neurotransmitter release in the barrel cortex compared to WT mice, which might provide more "headroom" for the enhanced cortical plasticity observed in CCR5-deficient mice (Zhou et al., 2016).

Moreover, CCR5 is involved in the modulation of dendritic spine turnover and clustered spine addition, both of which correlate with learning and memory performance (Frank et al., 2018). Using two-photon microscopy, Frank et al. tracked spine dynamics in the retrosplenial cortex (RSC) of Thy1-YFP mice before, during, and after contextual fear conditioning or the Morris water maze task, both designed to assess episodic memory. In WT mice, dendritic spine turnover before learning predicted task performance (i.e. higher spine turnover predicts better learning) as well as learning and memory-related spine clustering. In the RSC of $Ccr5^{+/-}$ mice, pre-learning dendritic spine turnover was greatly enhanced. As expected, $Ccr5^{+/-}$ mice showed enhanced learning and memory in both the contextual fear conditioning and Morris water maze tasks. These results suggest that CCR5 may regulate contextual and spatial memory by modulating dendritic spine turnover and clustering (Frank et al., 2018).

However, a study published by Kalkonde et al. found that a lack of CCR5 does not impact spatial and aversive memory. Instead, $Ccr5^{-/-}$ mice showed enhanced social recognition compared

to WT mice (Kalkonde et al., 2011). Different mouse lines and learning protocols were used in this study compared to the studies mentioned above, which might lead to the discrepancies observed with regards to the effects of *Ccr5* knockout on contextual and spatial memory.

Some studies have postulated a role for glial-expressed CCR5 in mediating changes in synaptic plasticity. In the central nervous system, CCR5 is mainly expressed on the surface of microglia, and to a lesser extent, on astrocytes, oligodendrocytes and neurons (Klein et al., 1999; Vallat et al., 1998; Xia et al., 1998). Microglial CCR5 has been widely implicated in the pathogenesis and progression of various neurological disorders, but evidence also points to a role for microglial CCR5 in non-pathological cognition. For example, CCR5 is known to facilitate the migration of microglia across brain microvasculature towards a chemotactic gradient of the ligand CCL5 (also known as RANTES), after which they may engage in synaptic remodeling, modulation, and pruning (Ekdahl, 2012; Hong et al., 2016; Louboutin and Strayer, 2013; Sisková et al., 2009; Ubogu et al., 2006). Additionally, astrocytes are known to secrete chemokines like CCL5 and CCL3 that may bind CCR5 on neurons and mediate synaptic transmission and plasticity through mechanisms ranging from the prevention of extrasynaptic neurotransmitter diffusion and neurotransmitter clearance to the modulation of synaptic release probability (Ambrosini et al., 2005; De Pittà et al., 2016; Kim et al., 2004). Astrocytes may also indirectly participate in cognition by releasing CCR5 ligands that act on and recruit CCR5-expressing microglia to neighboring neurons, after which the microglia engage in functions related to synaptic remodeling and modulation. Although the exact mechanism of CCR5-regulated microglial pruning remains unknown, microglial CCR5 is well-positioned to contribute to synaptic plasticity and resultant regulation of cognitive function.

3. CCR5 and its implication in various disorders

3.1. CCR5 and HIV-associated neurocognitive disorder (HAND)

The relationship between CCR5 and HIV is well-studied. CCR5 is the coreceptor used by HIV for entry into the host cell following binding of the viral envelope glycoprotein gp120 to the CD4 receptor. However, less understood is how CCR5 contributes to HIV-associated cognitive deficits, which affect about 50 percent of all HIV-infected individuals, even those undergoing antiretroviral therapy (Clifford and Ances, 2013; Deng et al., 1996; Ru and Tang, 2017). Despite these knowledge gaps, there is some evidence that CCR5 contributes to and aggravates the cognitive dysfunctions comorbid with HIV infection (Table 2). For example, *Ccr5* knockout reversed impaired performance of HIV gp120 transgenic mice in the Barnes maze task, which assesses spatial learning and memory (Barnes, 1979; Maung et al., 2014). *Ccr5* knockout and knockdown in mice were shown to protect against deficits in contextual memory acquisition caused by hippocampal gp120 V3 peptide infusion (Zhou et al., 2016). When the endogenous CCR5 ligands CCL3, CCL4 and CCL5 were measured in cerebral spinal fluid (CSF) samples from HIV-infected patients, the three chemokine concentrations not only correlated with each other, but were also higher in demented patients (Letendre et al., 1999). Additional studies have found that in virally-suppressed HIV-infected patients, supplementation with maraviroc or cenciviroc (a dual CCR3/CCR5 antagonist) improved neurocognitive performance (Barber et al., 2018; D'Antoni et al., 2018; Gates et al., 2016; Ndhlovu et al., 2015; Ndhlovu et al., 2014). Lastly, individuals with a natural 32 base pair loss-of-function deletion in CCR5 experienced a milder HIV progression and had reduced HIV-associated cognitive deficits (Kaul et al., 2007).

The exact mechanisms by which CCR5 exacerbates or contributes to these observed HIV-associated cognitive deficits are not clear. One mechanism by which CCR5 may contribute to HIV-

associated cognitive impairments is by causing neuronal death and damage (Saylor et al., 2016), due in part to neuronal apoptosis resulting from the interaction of gp120 and CCR5 (Mocchetti et al., 2013). However, neuronal apoptosis does not necessarily correlate with the severity of cognitive deficits, as the degree of neuron death might not be sufficient to manifest in noticeable impairments and compensatory mechanisms might exist to mitigate the cognitive impact of neuron loss. For example, post-mortem brain sample analysis of AIDS patients showed that the severity of cerebral cortex neuronal apoptosis correlated with the presence of cerebral atrophy, but not with a history of HIV dementia. Rather, cognitive deficits of HIV-infected patients correlated with microglia activation and axonal damage (Adle-Biassette et al., 1999). Therefore, it should be noted that while in early HIV studies viral protein overexpression induced neuronal loss may contribute to cognitive deficits, HIV patients on combination antiretroviral therapy (cART) have minimal neurodegeneration and neuronal loss may no longer be the main contributor to HAND (Kelschenbach et al., 2019). Rather, neuronal dysfunction and synaptodendritic injury and atrophy could be a more plausible source of cognitive impairment, and they correlate better with cognitive deficits (Ellis et al., 2007; Kelschenbach et al., 2019).

Because CCR5 functions as a memory suppressor in normal cognition (Zhou et al., 2016), it is reasonable to suspect that activation of CCR5 might impair synaptic plasticity and learning and memory in the context of HIV infection (Figure 1). Similar to CCR5's endogenous ligands CCL5 and CCL3, the HIV gp120 V3 peptide can bind and activate CCR5 (Shen et al., 2000). Gp120 V3 peptide treatment led to severe long-term potentiation (LTP) impairments in both the dorsal hippocampal CA1 and barrel cortex. Furthermore, whereas control mice exhibited LTP in the barrel cortex in response to a spike-timing dependent protocol, that same protocol generated LTD instead in mice treated with the V3 peptide. This effect was reversed by *Ccr5* KO (Zhou et

al., 2016). Genetic ablation of *Ccr5* also rescued the deficits caused by the V3 peptide in both CA1 LTP and hippocampus-dependent memory, indicating that CCR5 activation by HIV gp120 may lead to cognitive deficits by compromising synaptic plasticity. Similar to *Ccr5* knockout, neuronal *Ccr5* knockdown in the hippocampus was protective against V3-induced MAPK signaling and memory deficits, suggesting a critical role for neuronal CCR5 activation in HIV-associated memory deficits. Nevertheless, while the inhibition of CCR5 function either by genetic ablation or pharmacological inhibition reduces inflammation (Ahmad et al., 2019; Gu et al., 2016; Tang et al., 2015), these results above do not reveal whether cognitive deficits result from the neuroinflammation caused by V3-induced CCR5 activation, or from a direct interaction between the V3 peptide and CCR5 that compromises synaptic plasticity and suppresses memory. Further study is required to determine whether CCR5 is directly responsible for producing some of the cognitive deficits that arise from HIV infection.

Microglial CCR5 is also thought to contribute to HIV-associated cognitive deficits. Microglial activation, which can be triggered by gp120 binding and CCR5 activation, has been shown to be highly correlated with HIV-associated cognitive deficits (Adle-Biassette et al., 1999). For example, microglial activation triggered by gp120 binding to CCR5 can cause the release of reactive oxygen and nitrosative species, viral proteins, proinflammatory chemokines, and cytokines that contribute to neuronal apoptosis (Acquas et al., 2004; Kaul et al., 2007; Kim et al., 2018; Saylor et al., 2016). While neuronal apoptosis alone may not correlate with cognitive impairment, microglial activation and accumulation, which can be enhanced by the presence of apoptotic cells, is thought to contribute to HAND by the microglial release of damaging cytokines and chemokines and excessive synapto-dendritic pruning (Saylor et al., 2016). Altogether, these microglial CCR5-related structural and physiological changes may underlie HIV-associated

cognitive impairments. In HIV mouse models, *Ccr5* knockout or CCR5 antagonists have been shown to reduce the activation and accumulation of microglia and block HIV pathogenesis, preventing the spatial memory deficits caused by gp120 overexpression (Maung et al., 2014). Notably, besides microglial CCR5, astrocyte CCR5 activation can also cause neuronal death in HIV (Kaul et al., 2007). HIV-infected microglia can release neurotoxic proteins that impair normal astrocyte function, leading to glutamate imbalances that can in turn exacerbate or cause neuronal injury, and astrocytes are known to become hyperactivated in the context of HIV (Saylor et al., 2016). Deletion of *Ccr5* from glia rather than neurons in mixed neuron/glia (approximately 90% astrocytes, 8% microglia, and 2% other glial cells) co-cultures significantly reduced neuronal death when cells were treated with the HIV Tat protein plus morphine. This protective effect of *Ccr5* deletion was recapitulated by pre-treatment with CCR5 antagonist maraviroc (Kim et al., 2018). Expression of HIV-Tat protein has also been reported to cause spatial memory deficits in the Barnes Maze task (Carey et al., 2012) and maraviroc ameliorated Tat-mediated spatial memory deficits (Kim, 2019). With the same inducible HIV-1 Tat mice, CCR5 was also found to be involved in morphine tolerance, dependence, and reward behavior after HIV-1 Tat expression. CCR5 antagonist maraviroc not only attenuated Tat and morphine-induced CCL2, CCL3 and CCL4 expression, but also further potentiated morphine-conditioned place preference after Tat expression (Gonek et al., 2018). Because these studies were done with HIV-1 Tat transgenic mice, they suggest that CCR5 can regulate HIV-mediated cognitive deficits without directly interacting with gp120.

3.2. CCR5 and Alzheimer's disease (AD)

In contrast to HIV, in which the role of CCR5 is relatively well-known, CCR5 is poorly

understood in the context of AD. Many studies have cataloged an increase in CCR5 expression in both AD patients and AD rodent models, suggesting a correlation between AD and CCR5 expression (Giri et al., 2004; Goldeck et al., 2013; Li et al., 2009; Reale et al., 2008; Walker et al., 2001) (Table 3). An in vitro study in human peripheral blood mononuclear cells found that Aβ may enhance CCR5 expression in AD patients through cellular signaling pathways involving c-Raf, ERK-1/ERK-2, and c-Jun NH-2 terminal kinase (Giri et al., 2005). Furthermore, CCR5 ligands CCL3 and CCL4 were both found to be upregulated in Aβ-stimulated microglia isolated from the post-mortem brains of AD patients and in APP/PS1 mice, suggesting that besides increased CCR5 expression, increased expression of CCR5 ligands may also enhance the activity of existing CCR5 (Jorda et al., 2019; Walker et al., 2001).

The majority of studies probing the relationship between CCR5 and AD are in vitro cellular studies, which are notably less informative than in vivo work and do not offer the potential to assess memory deficits in AD. More *in vivo* research needs to be done in order to better elucidate the role of CCR5 in AD and to explore possible therapeutic routes through the modulation of this chemokine receptor. One of the few in vivo studies to date to examine the role of CCR5 in AD-associated cognitive deficits reported that CCR5 expression may mediate cognitive impairments in AD. By deleting *Ccr5* in Aβ-injected mice, Passos et al. were able to reduce spatial memory deficits associated with β-amyloid administration and accumulation. Specifically, *Ccr5* deficient mice took significantly less time to find the platform during the training phase of the Morris water maze, and they also spent a greater amount of time in the correct quadrant during the testing phase (Passos et al., 2009). Because genetic deletion of *Ccr5* reduced the number of activated astrocytes and microglia both on a short (e.g. 6 hours) and long time-scale (e.g. 8 days) after treatment with Aβ1-40, the authors attributed the positive cognitive effect of *Ccr5* KO to reduced accumulation

and activation of microglia and astrocytes in response to β-amyloid infusion.

Moreover, while iNOS and COX-2 were found to be upregulated in the inflammatory response that can accelerate neurodegeneration in AD, expression of both enzymes was reduced in the hippocampus of CCR5-deficient mice, suggesting that CCR5 deletion may impair AD pathogenesis by diminishing the production of neuroinflammatory proteins. Furthermore, the mice lacking *Ccr5* had reduced CREB, p65 NF-kB, and c-Jun/AP-1 activation. The inflammatory suppression caused by CCR5 deletion may be a product of CCR5-mediated reduction in glial activation. With fewer active glia present, synapses that would otherwise be prone to degeneration or pruning may be preserved, maintaining cognitive function (Passos et al., 2009). Consistent with the anti-inflammatory protective effects of *Ccr5* knockout, CCR5 antagonist d-Ala-peptide T-amide (DAPTA) dramatically reduced the number of activated microglia and astrocytes as well as the number of immunoreactive cells expressing nuclear factor kappa B (NF-κB) protein, a prominent component of the proinflammatory cytokine signaling pathway, suggesting that CCR5 antagonists may help to attenuate the neuroinflammation associated with AD (Rosi et al., 2005).

Although the majority of CCR5 and AD studies reported increased expression of CCR5 or its ligands in AD, and suggested that inactivation or deficiency of CCR5 could reduce inflammation and enhance cognitive performance (Table 3), one study has posited that CCR5 deficiency may in fact accelerate Aβ deposition and exacerbate memory dysfunction. In this study, *Ccr5* deletion impaired mouse performance in the passive avoidance task and Morris water maze concurrent with enhanced amyloidogenesis, astrocyte activation, and neuronal apoptosis. The study found that CCR5 deletion increased CCR2 expression, which subsequently activated astrocytes, increased Aβ production and hippocampal cellular apoptosis (Lee et al., 2009). One explanation for the contradictory findings for the role of CCR5 in AD-related cognition may be

that this study utilized middle-aged mice (12-18 months old) instead of young adult mice. The expression of both CCR5 and CCL5 has been reported to increase with age both in mice and humans (Sieber et al., 2011; Yung et al., 2007; Yung and Mo, 2003), suggesting that the effect of CCR5 deficiency on neuronal plasticity and cognitive function might change with age. Furthermore, B6/129P F2 hybrid mice instead of littermates were used as controls for the *Ccr5*$^{-/-}$ mice which were on the B6J background. This might explain the learning deficits observed in the *Ccr5*$^{-/-}$ mice, because mice on the 129 background more readily learn the Morris water maze spatial reference memory task relative to mice on the B6 background (March et al., 2014). Controlling for genetic background is critical for learning and memory behavioral analysis (Graves et al., 2002).

Several epidemiological studies evaluating the gene polymorphisms of CCR5 in patients with AD found no significant differences in the distribution of CCR5 polymorphisms among AD patients and controls (Balistreri et al., 2006; Combarros et al., 2004; Huerta et al., 2004; Wojta et al., 2020). When the association between AD and various genetic factors was analyzed with the interactions of apolipoprotein E (APOE) status, the association with the CCR5Δ32 mutation was found to be significant only among subjects carrying the APOE ε4 allele, suggesting a synergistic effect of APOE and CCR5 mutation on AD (Rezazadeh et al., 2016). In contrast, a recent study reported that the CCR5Δ32 mutation was associated with an early age of onset of various neurodegenerative diseases including AD (Wojta et al., 2020). Notably, the majority of individuals with the CCR5Δ32 mutation were heterozygous in these studies, and few patients carried the homozygous CCR5Δ32 mutations. A single intact CCR5 allele might negate the impact of the heterozygous mutation on AD progression and cognitive performance.

3.3. CCR5 in stroke and psychiatric disorders

Besides HIV and AD, another field in which CCR5 has been implicated is stroke (Table 4). As a form of neural injury, ischemia is generally associated with an increase in CCR5 expression (Joy et al., 2019; Kremlev et al., 2007; Spleiss et al., 1998). This ischemia-related spike in CCR5 expression has mixed implications. One study found that *Ccr5* deletion exacerbated neuronal death and generated a larger infarct core in mice with induced ischemia, although no differences in morphology and abundance of astrocytes and microglia were observed between WT and *Ccr5*-deficient mice (Sorce et al., 2010). In contrast, a more recent study showed that the absence of CCR5 was neuroprotective against stroke-related deficits (Victoria et al., 2017). After transient cerebral ischemia induced by bilateral common carotid artery occlusion, *Ccr5*-deficient mice had fewer neurological deficits and smaller infarct areas compared to WT mice. CCR5 deficiency also resulted in fewer necrotic cavities, fewer ischemic neurons, and higher expression of the trophic factor BDNF (Victoria et al., 2017).

Another recent study reported that both *Ccr5* knockdown and treatment with the CCR5 antagonist maraviroc improved motor and cognitive recovery in a mouse model of stroke (Joy et al., 2019). In this study, CCR5's neurotoxic effect was attributed to its role in clearing away existing synaptic connections and preventing the formation of new ones after stroke. CCR5 loss prevents such post-stroke outcomes through the DLK and CREB pathways, both of which are implicated in dendritic spine formation and axon regeneration after brain ischemic injury. As the result of reduced synaptic damage, early recovery of motor function was observed both after *Ccr5* knockdown and CCR5 inhibition. Importantly, in a large clinical cohort of stroke patients, patients carrying the naturally occurring loss-of-function CCR5-Δ32 mutation were found to exhibit greater recovery of neurological impairments and cognitive function, including faster motor, language, and sensory recovery, and better performance in memory, verbal function, attention, and

total cognitive scores compared to non-carriers. These findings suggest CCR5 is a translational target for neural repair in stroke, and make *CCR5* the first reported gene associated with enhanced recovery in human stroke (Joy et al., 2019).

Lastly, changes in CCR5 expression have also been implicated in certain psychiatric disorders, such as post-traumatic stress disorder (PTSD) and schizophrenia. In 2015, a study found that men and women with PTSD exhibited higher levels of plasma CCR5, in addition to greater levels of its ligand CCL5. Within the PTSD group, women had greater levels of this receptor than men (Ogłodek et al., 2015). These findings suggest that CCR5 may be exploited as a marker for disorders like PTSD. In a CSF study in patients with treatment-resistant depression, CSF levels of chemokines and interleukins were measured to examine the role of the immune system in the antidepressant effects of electroconvulsive therapy (ECT). Greater reduction of symptoms by ECT correlated with less CSF CCL3 and CCL5, indicating that these CCR5 ligands may play a role in depression and its therapeutic response (Mindt et al., 2020). Furthermore, a study of schizophrenia patients found that the CCR5-Δ32 deletion was associated with a later age of admission into a psychiatric hospital department. However, it failed to find an association between the CCR5-Δ32 deletion and schizophrenia susceptibility (Rasmussen et al., 2006). The authors postulated that the CCR5 deletion may protect against more progressed forms of early onset schizophrenia or that it could increase susceptibility to late-onset schizophrenia.

Altogether, the studies above provide convincing evidence that CCR5 may modulate cognitive function in neurological or psychiatric disorders that exist outside the more widely-explored fields of HIV and AD and lay promising groundwork for CCR5 to be exploited as a therapeutic tool.

4. Conclusion

A growing body of evidence points towards chemokine receptors as playing important roles in mediating various types of cognition including learning and memory. CCR5 has been well-studied in the scope of immune response and HIV infection (Ellis et al., 2007; Zhou and Saksena, 2013). However, its role in the context of normal and disorder-associated cognition, is less understood. The existing literature points to a general role of CCR5 in limiting cognitive capabilities in both normal learning and cognitive dysfunctions associated with HIV, AD, stroke and psychiatric disease. CCR5 activation can directly regulate neuronal function, including CREB and MAPK inactivation, decreased synaptic plasticity, and impaired axonal regrowth after neuronal damage (Joy et al., 2019; Zhou et al., 2016). Microglia are the main cell type expressing CCR5 in the nervous system (Joy et al., 2019), although how CCR5 is involved in neuron-glial interactions during learning is still unclear. While most studies have found that CCR5 negatively regulates cognition, some exceptions can be found in AD and stroke studies, where *Ccr5* knockout in mice impaired memory (Lee et al., 2009; Sorce et al., 2010). While it's unclear why these studies reported contradictory results, the use of different animal backgrounds may partly explain these discrepancies. These conflicting studies raise the possibility that divergent roles of CCR5 in stroke or AD could arise from unexplored functions for CCR5 during development and aging.

Therefore, it is important that future studies examine CCR5 and other chemokine receptors in cognition using conditional or inducible transgene animal models. By assessing the role of CCR5 in various cell types and without any influence on development, one can more precisely understand how CCR5 contributes to learning and memory. Furthermore, few studies to date have sought to understand the role CCR5 plays in the aging brain. Aging remains the primary risk factor for cognitive decline, and it is important to investigate how CCR5 may participate in the process

of age-related cognitive decline, particularly since the expression of CCR5 and its ligands increases with age (Sieber et al., 2011; Yung et al., 2007; Yung and Mo, 2003). CCR5 is also a notable subject of HAND study because of its critical role in HIV infection in the brain. As an HIV coreceptor, it has received a great deal of attention as a candidate for pharmacological intervention, and is the primary target for the FDA-approved antagonist maraviroc. Studies that examine how transient or long-term blockade of the receptor might rescue cognitive deficits have particular translational significance. Unfortunately, relatively few animal studies have used maraviroc to study CCR5 in the context of cognition. More cognitive studies with maraviroc would allow us to better understand how CCR5 inhibition might be applied in clinical treatment. Lastly, while this review was primarily concerned with the role of CCR5 in learning and memory, it is by no means the only chemokine receptor to regulate cognition. CCR5 and other chemokine receptors are known to share chemokine ligands and similar intracellular signaling pathways. Therefore, understanding the role of CCR5 and other chemokine receptors in learning and memory will facilitate the design of effective therapeutics to ameliorate cognitive deficits in HAND, stroke, AD and age-associated cognitive decline.

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Table 1: Role of CCR5 in learning and memory

Title	Author, Date	Model	Main Discovery	CCR5 Role
Hippocampal CCR5/RANTES Elevations in a Rodent Model of Post-Traumatic Stress Disorder: Maraviroc (a CCR5 Antagonist) Increases Corticosterone Levels	Merino et al., 2020	Rat	•Both CCR5 and CCL5 expression increased after fear conditioning •Maraviroc treatment before fear conditioning enhanced contextual memory	Disadvantageous
Hotspots of dendritic spine turnover facilitate clustered spine addition and learning and memory	Frank et al., 2018	Mouse	•*Ccr5* heterozygous (het) knockout (KO) enhanced spine turnover and spine clustering •*Ccr5* het KO enhanced both water maze memory and contextual fear memory	Disadvantageous
CCR5 is a suppressor for cortical plasticity and hippocampal learning and memory	Zhou, M. et al., 2016	Mouse	•*Ccr5* KO increased MAPK/CREB signaling •*Ccr5* KO enhanced LTP and hippocampus-dependent memory •*Ccr5* KO enhanced experience-dependent plasticity in the barrel cortex •Neuronal CCR5 overexpression caused memory deficits	Disadvantageous
The chemokine MIP-1 α/CCL3 impairs mouse hippocampal synaptic transmission, plasticity and memory	Marciniak, E., et al., 2015	Mouse	•CCR5 activation by its ligand CCL3 reduced basal synaptic transmission and LTP in CA1 hippocampal synapses •CCL3 impaired spatial memory which was blocked by maraviroc (CCR5 antagonist)	Disadvantageous
The CC chemokine receptor 5 regulates olfactory and social recognition in mice	Kalkonde et al., 2011	Mouse	•*Ccr5* KO enhanced social recognition • Administration of CCR5 ligand CCL3 impaired social recognition	Disadvantageous

Table 2: Role of CCR5 in HIV-Associated Neurocognitive Disorder

Title	Author, Date	Model	Main Discovery	CCR5 Role
CSF inflammatory markers and neurocognitive function after addition of maraviroc to mono-therapy darunavir/ritonavir in stable HIV patients: the CINAMMON study	Barber, T.J., et al., 2018	Human	•Addition of maraviroc to darunvair/ ritonavir monotherapy in HIV-infected patients led to improvements in executive function	Disadvantageous
Improved cognitive performance and reduced monocyte activation in virally suppressed chronic HIV after dual CCR2 and CCR5 antagonism	D'Antoni, M.L., et al., 2018	Human	•Patients given cenicriviroc (dual CCR2 and CCR5 antagonist) showed improved neuropsychological test performance and lower plasma markers for monocyte immune activation	Disadvantageous
CCR5 mediates HIV-1 Tat-induced neuroinflammation and influences morphine tolerance, dependence, and reward	Gonek, M., et al., 2018	Mouse	•Morphine and Tat increased CCL2, CCL3 and CCL4 expression in dorsal striatum, which was attenuated with maraviroc pre-treatment •HIV-1 Tat significantly potentiated morphine-conditioned place preference and maraviroc further potentiated these effects	Disadvantageous
Maraviroc-intensified combined antiretro-viral therapy improves cognition in virally suppressed HIV-associated neurocognitive disorder	Gates, T.M., et al., 2016	Human	•Improved global neurocognitive performance in patients with maraviroc and cART	Disadvantageous
CCR5 is a suppressor for cortical plasticity and hippocampal learning and memory	Zhou, M., et al., 2016	Mouse	•*Ccr5* KO or KD in hippocampus prevented LTP, signaling and memory deficits induced by HIV V3 peptide •*Ccr5* KO prevented LTP deficits induced by V3 peptide in the barrel cortex	Disadvantageous
Treatment intensification with maraviroc (CCR5 antagonist) leads to declines in CD16-expressing monocytes in cART-suppressed chronic HIV-infected subjects and is associated with improvements in neurocognitive test performance: implications for HIV-associated neurocognitive disease (HAND)	Ndhlovu, L., et al., 2014	Human	•After addition of maraviroc to cART regimen, significant improvement was observed in neuropsychological performance among subjects who had mild to moderate cognitive impairment	Disadvantageous
CCR5 knockout prevents neuronal injury and behavioral impairment induced in a transgenic mouse model by a CXCR4-using HIV-1 glycoprotein 120	Maung, R., et al., 2014	Mouse	•Genetic ablation of *Ccr5* prevented microglial activation and neuronal damage in gp120-transgenic mice •*Ccr5* KO rescued spatial learning and memory in gp120-transgenic mice	Disadvantageous

Table 3: Role of CCR5 in Alzheimer's Disease

Title	Author, Date	Model	Main Discovery	CCR5 Role
Lack of Association Between the CCR5-delta32 Polymorphism and Neurodegenerative Disorders	Wojta, K., et al., 2020	Human	•No significant association was observed between the CCR5Δ32 polymorphism and neurodegenerative diseases (including AD) •An early age of onset among neuro-degenerative disease patients carrying the CCR5Δ32 allele	No association; Disadvantageous for age onset
Changes in Chemokines and Chemokine Receptors Expression in a Mouse Model of Alzheimer's Disease.	Jorda, A., et al., 2019	Mice	•Compared to control mice, CCR5 expression was decreased and both CCL3 and CCL4 were highly expressed in the cortex of APP/PS1 mice	Uncertain
Genetic Factors Affecting Late-Onset Alzheimer's Disease Susceptibility	Rezazadeh, M., et al., 2016	Human	•The association between late-onset Alzheimer's disease and CCR5Δ32 was significant only among subjects carrying the APOE ε4 allele	Association among patients carrying APOE ε4
Role of the macrophage inflammatory protein-1α/CC chemokine receptor 5 signaling pathway in the neuroinflammatory response and cognitive deficits induced by β-amyloid peptide	Passos, G.F., et al., 2009	Mouse	•*Ccr5* KO mice had reduced astrocytosis and microgliosis after Aβ1-40 administration •Cognitive deficits and synaptic dysfunction after $A\beta_{1\text{-}40}$ administration were improved by *Ccr5* KO	Disadvantageous
Amyloid interaction with receptor for advanced glycation end products up-regulates brain endothelial CCR5 expression and promotes T cells crossing the blood-brain barrier	Li, M., et al., 2009	Human	•Intracerebral Aβ interaction with RAGE at BBB up-regulated endothelial CCR5 expression and caused circulating T cell infiltration in the brain in AD	Disadvantageous
CCR5 deficiency induces astrocyte activation, Aβ deposit and impaired memory function	Lee, Y.K., et al., 2009	Mice	•Spatial memory impaired in middle-aged (12-18 months old) *Ccr5* KO mice •Middle-aged *Ccr5* KO mice showed higher CCR2 expression, leading to activation of astrocytes which caused Aβ deposit and impaired memory	Advantageous
The chemokine receptor CCR5-32 gene mutation is not protective against Alzheimer's disease	Combarros, O., et al., 2004	Human	•AD patients and healthy controls showed no differences in frequency of CCR5Δ32 deletion	No association
Immunohistochemical study of the β-chemokine receptors of CCR3 and CCR5 and their ligands in normal and Alzheimer's disease brains.	Xia, M., et al., 1998	Human	•Increased expression of CCR5 on some reactive microglia in AD •$CCR5^{+}$ reactive microglia associated with amyloid deposits	Disadvantageous

Table 4: Role of CCR5 in stroke and psychiatric disorders

Title	Author, Date	Model	Main Discovery	CCR5 Role
CCR5 is a therapeutic target for recovery after stroke and traumatic brain injury	Joy, M.T., et al., 2019	Mouse and human	•*Ccr5* KD showed preservation of dendritic spines, new axonal projections to contralateral premotor cortex, upregulation of CREB and DLK signaling, and faster recovery of motor control •In human stroke cohort, those carrying the CCR5Δ32 mutation had better recovery of neurological impairments in multiple assessments	Disadvantageous (stroke)
Knockdown of C-C Chemokine Receptor 5 (CCR5) is Protective Against Cerebral Ischemia and Reperfusion Injury	Victoria, E., et al., 2017	Mouse	•CCR5-deficient mice had greater improvement in neurological deficits •CCR5 deficiency associated with decreased percentage necrotic cavities areas and ischemic neurons	Disadvantageous (stroke)
Increased brain damage after ischaemic stroke in mice lacking chemokine receptor CCR5	Sorce, S., et al., 2010	Mouse	•Size of infarct, greater neuronal death, and motor deficits after permanent cerebral ischemia increased in CCR5-deficient mice	Advantageous (stroke)
Minocycline modulates chemokine receptors but not interleukin-10 mRNA expression in hypoxic-ischemic neonatal rat brain	Kremlev, S.G., et al., 2007	Rat	•Following hypoxic-ischemic brain injury in the ipsilateral hemisphere, there was a significant increase in CCR5 mRNA levels	Disadvantageous (stroke)
Cloning of rat HIV-1-chemokine coreceptor CKR5 from microglia and upregulation of its mRNA in ischemic and endotoxinemic rat brain	Spleiss, O., et al., 1998	Rat	•*Ccr5* mRNA showed a sustained rise until 96 hours after middle cerebral artery occluded (MCAO) surgery, which may impact on microglial activation	Disadvantageous (stroke)
Cytokine-mediated cellular immune activation in electroconvulsive therapy: A CSF study in patients with treatment-resistant depression	Mindt, S., et al., 2020	Human	•Greater reduction of depressive symptoms by electroconvulsive therapy (ECT) correlated with less CSF CCL3 and CCL5	Disadvantageous (depression)
Serum concentrations of chemokines (CCL5 and CXCL12), chemokine receptors (CCR5 and CXCR4), and IL-6 in patients with posttraumatic stress disorder and avoidant personality disorder	Ogłodek, E., et al., 2015	Human	•Plasma levels of CCR5 and CCL5 were greater in men and women with PTSD; women had higher levels than men	Role unknown - marker for detection (PTSD)
Association between CCR5 32-bp deletion allele and late onset schizophrenia	Rasmussen, H., et al., 2006	Human	•Increased frequency of CCR5Δ32 deletion allele in late-onset schizophrenic group	Possibly disadvantageous (SZ)

Figure 1

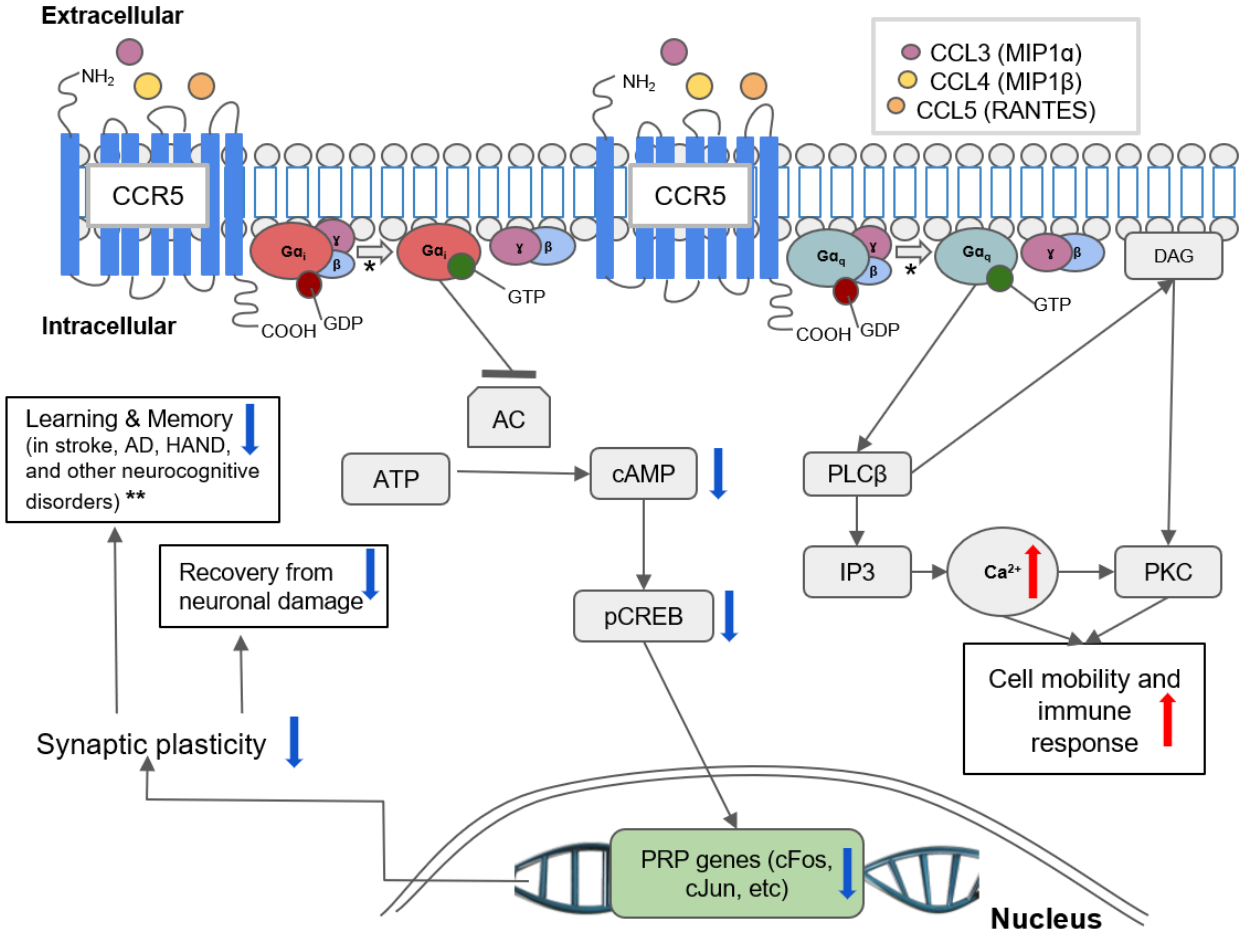

Figure legend

Figure 1. Binding of ligands (CCL3, CCL4, and CCL5) to CCR5 activates two major pathways, one under the control of $G\alpha_i$ and the other under $G\alpha_q$. The $G\alpha_i$ pathway results in decreased cAMP and pCREB levels, which decreases the transcription of plasticity-related protein (PRP) genes, causing a reduction in synaptic plasticity. This subsequently causes reduced learning and memory and worsened recovery from neuronal damage, which are associated with the plasticity and memory deficits seen in stroke, AD, HAND, and other neurocognitive disorders. In the other pathway, activation of $G\alpha_q$ results in activation of PLCβ, splitting into IP3 and DAG. IP3 results in increased calcium levels, activating PKC and also directly resulting in greater cell mobility and immune response via intracellular calcium signaling pathways. * Upon ligand binding; ** From multiple studies, the role of CCR5 is most disadvantageous for L&M.