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Roles for Regulator of G protein Signaling (RGS) Proteins in Synaptic Signaling and Plasticity

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Running Title: RGS Proteins in Synaptic Signaling and Plasticity

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Abbreviations:

RGS, Regulator of G protein signaling; GPCR, G protein coupled receptor; GAP, GTPase activating protein; CNS, central nervous system; LTP, long-term potentiation; LTD, long-term depression; GIRK channel, G protein-coupled inwardly rectifying potassium channel; Ca_V channel, voltage-gated calcium channel; MECS, maximum electroconvulsive shock; HFS, high frequency stimulation; VTA, ventral tegmental area; PPF, paired pulse facilitation; KO, knockout; PTX, pertussis toxin; fEPSP, field excitatory postsynaptic potential; GHB, gamma-hydroxybutyrate; MSN, medium spiny neuron; eCB, endocannabinoid; D2DR, D2 dopamine receptor; PD, Parkinson's disease; α2AR, alpha 2 adrenergic receptor; $GABA_BR$, $GABA_B$ receptor; PNC, parvocellular neuroendocrine cell; PVN, paraventricular nucleus; HPA axis, hypothalamic-pituitary-adrenal axis; R7H, R7 homology; GGL, G protein gamma subunit-like; Gβ5, G protein β5; Gαt, transducing; PAG, periaqueductal grey; MOR, μ-opioid receptor; GPR, G protein regulatory; Ca^{2+}/CaM, calcium-activated calmodulin; PSD, postsynaptic density; CGN, cerebellar granule neuron; PTSD, post-traumatic stress disorder.

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ABSTRACT

The ***r***egulator of ***G*** protein ***s***ignaling (RGS) family of proteins serves critical roles in G protein coupled receptor (GPCR) and heterotrimeric G protein signal transduction. RGS proteins are best understood as negative regulators of GPCR/G protein signaling. They achieve this by acting as GTPase activating proteins (GAPs) for Gα subunits and accelerating the turnoff of G protein signaling. Many RGS proteins also bind additional signaling partners that either regulate their functions or enable them to regulate other important signaling events. At neuronal synapses, GPCRs, G proteins, and RGS proteins work in coordination to regulate key aspects of neurotransmitter release, synaptic transmission, and synaptic plasticity that are necessary for CNS physiology and behavior. Accumulating evidence has revealed key roles for specific RGS proteins in multiple signaling pathways at neuronal synapses, regulating both pre- and postsynaptic signaling events and synaptic plasticity. Here, we review and highlight the current knowledge of specific RGS proteins (RGS2, RGS4, RGS7, RGS9-2, RGS14) that have been clearly demonstrated to serve critical roles in modulating synaptic signaling and plasticity throughout the brain, and consider their potential as future therapeutic targets.

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INTRODUCTION

G protein coupled receptors (GPCRs) are necessary for functional neurotransmission throughout the central nervous system (CNS), controlling neurophysiological processes ranging from movement to mood (Betke et al., 2012; Lagerstrom and Schioth, 2008; Rojas and Dingledine, 2013). Receptor activation of heterotrimeric G proteins (Gαβγ) results in release of Gα-GTP and Gβγ that stimulate downstream effectors and second messenger pathways to mediate intracellular physiology (Bourne et al., 1990; Hamm, 1998; Hepler and Gilman, 1992; Simon et al., 1991). GPCR and linked G protein signaling is tightly controlled by the family of *r*egulator of *G* protein *s*ignaling (RGS) proteins. RGS proteins act as GTPase activating proteins (GAPs) on the alpha subunits of the Gαi and Gαq subfamilies of heterotrimeric G proteins, greatly enhancing the intrinsic GTPase activity of the Gα subunit to facilitate the termination of downstream signaling by both the Gα and Gβγ subunits (De Vries et al., 2000; Hollinger and Hepler, 2002; Ross and Wilkie, 2000; Willars, 2006). RGS proteins are a structurally diverse family of signaling proteins with many identified signaling partners distinct from Gα and GPCRs. In this regard, considerable evidence shows that many RGS proteins have cell signaling roles in addition to their shared established roles as GAPs for G protein alpha subunits (Gα) (Abramow-Newerly et al., 2006; Burchett, 2000; Sethakorn et al., 2010).

GPCR signaling regulates key aspects of both pre- and postsynaptic neurotransmission, leading to changes in synaptic plasticity, including long-term potentiation (LTP), long-term depression (LTD), reversal of LTP (depotentiation), and presynaptic vesicle release potential. Various metabotropic GPCRs either positively or negatively regulate presynaptic neurotransmitter release (Betke et al., 2012; Tedford and Zamponi, 2006). On postsynaptic membranes, GPCRs and G protein signaling pathways regulate neuronal excitability, modulating fast acting neurotransmission mediated by ligand-gated ion channels including glutamate (Chalifoux and Carter, 2010; Liu et al., 2006; Rojas and Dingledine, 2013) and gamma-aminobutyric acid (GABA) receptors (Bormann, 1988). Following GPCR activation of

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Gα, released Gβγ directly binds to and activates G protein-coupled inwardly rectifying potassium (GIRK) channels. GIRK channels hyperpolarize the neuron and dampen the overall capacity of the postsynaptic signaling to potentiate (Dascal, 1997), a process known as depotentiation, or the reversal of LTP. As such, GIRK channels are required for depotentiation and many RGS proteins regulate the rate at which GPCR-coupled GIRK channels close following agonist removal (Doupnik et al., 1997; Saitoh et al., 1997; Saitoh et al., 2001; Ulens et al., 2000). Presynaptically, active Gβγ subunits can inhibit voltage-gated calcium (Ca_V) channels necessary for calcium-dependent neurotransmitter release following an action potential (Bormann, 1988; Zamponi and Currie, 2013). In this case, RGS proteins can antagonize the effects of Gβγ on N- and P/Q-type Ca_V channels ($Ca_V2.2$ and $Ca_V2.1$), facilitating neurotransmitter release (Jeong and Ikeda, 2000; Kammermeier and Ikeda, 1999; Mark et al., 2000). Additionally, canonical heterotrimeric G protein signaling through Gα subunits has been shown to affect plasticity via modulation of postsynaptic glutamate receptors (Chalifoux and Carter, 2010; Liu et al., 2006) and multiple other signaling pathways necessary for synaptic plasticity.

Our current understanding of roles for RGS proteins in physiology and behavior has been greatly aided by the development and use of RGS-insensitive Gα subunits (DiBello et al., 1998; Fu et al., 2004; Kaur et al., 2011), allowing examination of neurophysiology under conditions that mimic functional uncoupling of Gα-RGS. Studies with these mutants have revealed key roles for RGS proteins in multiple signaling pathways in neurons, as well as pre- and postsynaptic signaling and plasticity specifically (Chen and Lambert, 2000; Goldenstein et al., 2009; Talbot et al., 2010). By examining the role of RGS proteins in synaptic signaling, we can better understand the function of GPCR and G protein signaling in synaptic plasticity as well as diseases associated with RGS protein dysfunction. Here, we highlight and review our current knowledge of the function of specific RGS proteins demonstrated to have a clear role in modulating synaptic signaling and plasticity throughout the brain.

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RGS2

RGS2 is a ~24 kDa protein consisting of a single RGS domain with minimal flanking amino and carboxy terminal regions. RGS2 was first discovered and characterized as a member of the R4 family of RGS proteins (Siderovski et al., 1996), exhibiting selective GAP activity toward Gαq subunits (Heximer et al., 1997), though further studies have reported situational and receptor-dependent modulation of Gαi/o signaling as well (Han et al., 2006; Herlitze et al., 1999; Heximer et al., 1999; Ingi et al., 1998) (Table 1). While many RGS proteins can act as a GAP on Gαq and/or Gαi/o, a key feature of RGS2 is the induction of its expression in response to stimuli capable of evoking plasticity in multiple brain regions, leading to the characterization of RGS2 as an immediate early gene. This phenomenon was first seen when RGS2 mRNA expression was induced in the cortex, striatum, and hippocampus following maximum electroconvulsive shock (MECS), a reliable means with which to induce immediate early gene expression throughout the brain. More targeted induction of expression has been shown to occur in the striatum (caudate putamen and nucleus accumbens) of rats following amphetamine administration (Taymans et al., 2002). In a model more closely related to synaptic plasticity, high frequency stimulation (HFS), which is commonly used to induce hippocampal LTP, has been shown to strongly induce the expression of RGS2 mRNA within the dentate gyrus of the hippocampus (Ingi et al., 1998). Furthermore, stable expression of RGS2 with no induction protocol has been found throughout the brain in the same regions in which its expression is induced: the hippocampus, cortex, striatum, ventral tegmental area (VTA), and amygdala (Grafstein-Dunn et al., 2001; Ingi and Aoki, 2002; Taymans et al., 2002).

Due to its high expression throughout the brain and its unique role as an immediate early gene, functions for RGS2 in neurological diseases and disorders have been extensively studied. Multiple reports have shown a role for this RGS protein in modulating anxiety, with polymorphisms in RGS2 associated with generalized anxiety disorder (Hohoff et al., 2015;

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Koenen et al., 2009; Smoller et al., 2008), panic disorder (Hohoff et al., 2015; Koenen et al., 2009; Otowa et al., 2011), post-traumatic stress disorder (Amstadter et al., 2009), as well as suicide (Cui et al., 2008) in humans. Studies in mice have also shown an association between RGS2 and anxiety (Lifschytz et al., 2012; Okimoto et al., 2012; Oliveira-Dos-Santos et al., 2000; Yalcin et al., 2004) with decreased RGS2 expression causing anxiety (Lifschytz et al., 2012; Oliveira-Dos-Santos et al., 2000) and depression-like (Lifschytz et al., 2012) phenotypes. In order to better treat these diseases associated with RGS2, it is necessary to understand how RGS2 modulates synaptic plasticity and signaling.

Functions for RGS2 in synaptic signaling and plasticity have been examined largely within the hippocampus and VTA. Within the hippocampus, RGS2 regulates short-term synaptic plasticity. High concentrations of RGS2 within the neuron appear to facilitate paired pulse depression, while low expression of RGS2 leads to paired pulse facilitation (PPF). In other words, probability of neurotransmitter release is high in the presence of RGS2 and low in its absence. Notably, pertussis toxin (PTX) blocks the PPF in RGS2-knockout (KO) mice, indicating that RGS2's effects at the presynaptic terminal in this case are due to its modulation of Gαi/o-coupled GPCR signaling as opposed to Gαq (Han et al., 2006). Activation of Gαi/o leads to the dissociation of Gβγ subunits which can inhibit presynaptic voltage-gated $Ca_V2.2$ channels, preventing calcium influx necessary for neurotransmitter release (Ikeda, 1996; Kajikawa et al., 2001) (Figure 1A). Decreased expression of RGS2 leads to increased Gβγ-mediated inhibition of calcium influx and decreased probability of neurotransmitter release from the synapse, hence the observed PPF (Han et al., 2006). This interpretation is strengthened by *in vitro* evidence showing RGS2-mediated facilitation of $Ca_V2.1$ calcium channels, which are also inhibited by Gβγ subunits (Mark et al., 2000).

RGS2 also has reported roles in postsynaptic spines within the hippocampus in the context of long-term synaptic plasticity (Figure 1C). However, studies with RGS2-KO mice examining this topic are conflicting. There is no change in canonical hippocampal LTP as

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compared to RGS2 heterozygous mice (Oliveira-Dos-Santos et al., 2000), but increased LTP as compared to wild type (Hutchison et al., 2009). However hippocampal-dependent learning and memory does not appear to be affected in RGS2-KO mice (Oliveira-Dos-Santos et al., 2000), leaving RGS2's role in modulating canonical hippocampal LTP unclear. RGS2 has been shown to modulate signaling through mGluR1a, with increases in RGS2 expression blocking Gαq mediated signaling while leaving Gαi-mediated signaling unaffected (Kammermeier and Ikeda, 1999). Furthermore, mGluR1a has been shown to be responsible for a unique form of postsynaptic NMDA receptor-independent LTP within hippocampal interneurons, which possibly could be regulated by changes in expression of RGS2 (Perez et al., 2001). The numbers of apical and basilar spines of dendrites in CA1 hippocampal neurons are also significantly decreased in RGS2-KO mice (Oliveira-Dos-Santos et al., 2000), with the amount of spines being indicative of overall synapse numbers and synaptic plasticity (Moser, 1999). These changes in spine number could be explained by the discovery that RGS2 binds tubulin directly, stimulating microtubule polymerization (Heo et al., 2006) and potentially aiding in the development of dendritic spines (Gu et al., 2008). Additionally, CA1 hippocampal neurons of RGS2-KO mice show decreased overall basal electrical activity as measured by decreased field excitatory postsynaptic potential (fEPSP) amplitude following stimulation via Schaeffer collaterals (Oliveira-Dos-Santos et al., 2000).

Roles for RGS2 also have been examined in the VTA (Labouebe et al., 2007). RGS2 is selectively expressed postsynaptically in tyrosine hydroxylase-positive dopamine neurons within the VTA. Here, RGS2 associates specifically with GIRK3, one of four GIRK channel subunits, to decrease the coupling efficiency between the $GABA_B$ receptor and GIRK channels (Labouebe et al., 2007) (Figure 1C). These GIRK channels mediate the inhibitory postsynaptic effects of Gαi/o-coupled receptors, including the $GABA_B$ receptor. Because RGS2 is not highly expressed in GABA neurons, $GABA_B$ receptor-GIRK channel coupling efficiency is much higher in GABA neurons of the VTA than those that release dopamine. This allows application of γ-

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hydroxybutyrate (GHB), a $GABA_B$ receptor agonist, to cause disinhibition of dopamine neurons in the VTA, which are typically inhibited by GABA neurons, leading to induction of addictive behavior. However, chronic exposure of mice to GHB reduces the mRNA expression of RGS2 in dopamine neurons, increasing $GABA_B$–GIRK channel coupling and providing a possible mechanism through which tolerance to GHB occurs and demonstrating a novel mechanism through which changes in RGS2 expression mediate signaling at the synapses of dopamine neurons in the VTA (Labouebe et al., 2007).

Roles for RGS2 in the brain also have been examined outside the hippocampus and VTA, but are less well defined. In the amygdala, RGS2 expression is induced upon administration of oxytocin (Okimoto et al., 2012), potentially mediating the anxiolytic effects of the neuropeptide. This may explain the relationship between anxiety and RGS2 although additional studies where RGS2 expression cannot be induced by oxytocin (RGS2-KO mice) must be performed to ensure that RGS2 is necessary to mediate oxytocin's anxiolytic effect. RGS2 also is expressed in olfactory neurons where it inhibits the activity of adenylyl cyclase III downstream of active olfactory receptors, regulating signal transduction and possibly contributing to long-term adaptation to odorants (Sinnarajah et al., 2001). Overall, the status of RGS2 as an immediate early gene highly expressed in multiple types of neurons throughout the brain allows it to play a unique role in modulating G protein signaling at the synapse.

RGS4

Another member of the R4 family of RGS proteins, RGS4, also acts as a GAP on both Gαi/o (Berman et al., 1996; Huang et al., 1997) and Gαq subunits (Hepler et al., 1997; Huang et al., 1997) (Table 1). Similar to RGS2, RGS4 is a ~24 kDa protein consisting of a single RGS domain with modest flanking amino and carboxy terminal regions. Like RGS2, RGS4 is expressed throughout the brain (Gold et al., 1997; Ingi and Aoki, 2002), with expression reported in the prefrontal cortex (Ding and Hegde, 2009; Mirnics et al., 2001; Paspalas et al.,

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2009), hippocampus (Gold et al., 1997; Heraud-Farlow et al., 2013; Saugstad et al., 1998), thalamus (Gold et al., 1997; Ingi and Aoki, 2002; Kim et al., 2014; Ni et al., 1999), and striatum (Larminie et al., 2004). Furthermore, in studies comparing abundance of mRNA coding for RGS proteins, RGS4 has the highest measured levels within the brain (Larminie et al., 2004), though a peculiar property of RGS4 is that its basal protein levels are typically low due to a robustly regulated degradation of the protein (Bodenstein et al., 2007; Davydov and Varshavsky, 2000; Lee et al., 2005). With such a broad expression pattern across brain regions, RGS4 has been widely studied for its role in physiology relating to neuronal signaling and plasticity, as well as in neurological diseases. Here, we will examine the function of RGS4 in the brain regions where its effect on synaptic plasticity and signaling is best characterized: the hippocampus, striatum, hypothalamus, and prefrontal cortex.

Potential roles for RGS4 in the modulation of GPCR signaling in brain were first described in the hippocampus. In the CA1 region, the group I metabotropic glutamate receptor, mGluR5, is localized perisynaptically in dendrites (Ottersen and Landsend, 1997). Here, activation of mGluR5 mediates suppression of the afterhyperpolarization current that follows action potential firing as well as potentiation of NMDA receptor currents (Mannaioni et al., 2001), thereby increasing neuronal excitability by intensifying both firing and depolarization, respectively. RGS4 has been shown to inhibit signaling through group I mGluRs (mGluR1 and 5), blocking mGluR5-mediated inhibition of the afterhyperpolarization current in CA1 neurons (Saugstad et al., 1998) (Figure 1C). However, the effect of RGS4 on mGluR5's potentiation of NMDA receptor currents as well as its effect on signaling through mGluR1 within the hippocampus have not been examined. While the role of endogenous RGS4 within the hippocampus is not fully understood, changes in RGS4 expression potentially could regulate group I mGluR-mediated changes in neuronal excitability within the CA1 region as well as other regions of the hippocampus.

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While the precise role of RGS4 in modulating synaptic signaling and plasticity in the hippocampus remains poorly defined, roles for RGS4 in the striatum are better understood. In Parkinson's disease (PD), decreased striatal dopamine leads to increased striatal acetylcholine release, which exacerbates the motor symptoms of the disease. Notably, dopamine depletion in the striatum also upregulates RGS4 expression specifically in cholinergic interneurons (Ding et al., 2006). Here, RGS4 diminishes signaling through presynaptic M4 muscarinic acetylcholine autoreceptors (Figure 1B). Activation of Gαo-coupled M4 receptors in striatal cholinergic interneurons causes Gβγ-mediated inhibition of voltage-gated $Ca_V2.2$ (N-type) channels, leading to decreased acetylcholine release into the synapse. Increased RGS4 expression blocks this inhibition and allows more acetylcholine release, exacerbating Parkinsonian motor symptoms (Ding et al., 2006).

Within the striatum, RGS4 also regulates dopaminergic control of striatal LTD. Here, RGS4 modulates G protein signaling postsynaptically in indirect pathway medium spiny neurons (MSNs), the primary projection neurons of the striatum. Striatal endocannabinoid-dependent LTD (eCB-LTD) is induced by postsynaptic production of endocannabinoids (eCBs), which act on presynaptic CB1 receptors to lower the probability of neurotransmitter release (Figure 1D). Activation of group I mGluRs (Gq-coupled) is necessary for eCB-LTD, while activation of Gαi-coupled D2 dopamine receptors (D2DRs) positively modulates this form of synaptic plasticity (Kreitzer and Malenka, 2005). Activation of Gαs-coupled adenosine A2A receptors antagonizes LTD induction (Lerner et al., 2010). Interestingly, in RGS4-KO mice, eCB-LTD can be induced even in the presence of D2DR antagonist and adenosine A2A receptor agonist (Lerner and Kreitzer, 2012). Activation of the A2A receptor increases PKA activity, which has been shown to induce RGS4 activity via phosphorylation (Huang et al., 2007). This allows RGS4 to inhibit mGluR1/5 and D2DR-mediated release of eCBs, blocking LTD. Furthermore, when RGS4's block on eCB-LTD is removed, mice in a model of Parkinson's exhibit fewer behavioral deficits (Lerner and Kreitzer, 2012). In this case as well as in the case of M4 receptor signaling in

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striatal cholinergic interneurons, inhibition of RGS4 could be a valuable non-dopaminergic therapeutic option that targets multiple signaling pathways within the striatum in the treatment of PD.

Outside of its links to PD in striatum, RGS4 has been studied most extensively in the context of neurological disease in the prefrontal cortex. Polymorphisms in RGS4 and decreased protein expression in the dorsolateral prefrontal cortex are strongly implicated in schizophrenia (Ding and Hegde, 2009; Gu et al., 2007; Mirnics et al., 2001; Paspalas et al., 2009; Prasad et al., 2010; Prasad et al., 2005; Vrajova et al., 2011). One approach to understanding RGS4 roles in signaling in the prefrontal cortex has been to examine its subcellular localization in pyramidal neurons there. In macaques, postsynaptic RGS4 immunoreactivity appears high in extrasynaptic and perisynaptic regions of asymmetric synapses, which are typically excitatory. Furthermore, at inhibitory symmetric synapses, RGS4 expression is high within presynaptic regions of axons (Paspalas et al., 2009), indicating a role for RGS4 modulation of G protein signaling both pre- and postsynaptically in the prefrontal cortex. In the context of signaling at the synapse, RGS4 has been shown to specifically modulate 5-HT_{1A} serotonin receptor signaling, blocking postsynaptic serotonin-mediated inhibition of NMDA receptor current (Figure 1D), thereby providing a possible functional role for RGS4 in the pathogenesis of schizophrenia (Gu et al., 2007). Activation of other GPCRs in the prefrontal cortex can also modulate postsynaptic glutamate receptor currents. Activation of α2 adrenergic receptors (α2ARs) reduces AMPA receptor currents while activation of $GABA_B$ receptors ($GABA_B$Rs) reduces NMDA receptor calcium influx. Both α2ARs and $GABA_B$Rs are Gαi-coupled, decreasing PKA activity and modulating glutamate receptor activity. Even when all of these receptors are expressed in the same spine, RGS4 appears capable of limiting crosstalk between the two Gαi-coupled receptors, aiding the inactivation of the G proteins and preventing interference between the two receptors' neuromodulatory functions despite their close proximity (Lur and Higley, 2015).

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Decreases in RGS4 expression in schizophrenia could lead to increased crosstalk between different signaling pathways, leading to aberrant function.

Finally, RGS4 also has been shown to play a role in synaptic signaling and plasticity in the hypothalamus. Parvocellular neuroendocrine cells (PNCs) in the paraventricular nucleus (PVN) of the hypothalamus are at the head of the hypothalamic-pituitary-adrenal (HPA) axis, both mediating glucocorticoid release and responding to negative feedback (Wamsteeker and Bains, 2010). Sustained stress unmasks presynaptic LTD_{GABA} in which the probability of GABA release onto these neurons is decreased by retrograde opioid release (Wamsteeker Cusulin et al., 2013). Notably, due to the decreased capacity for chloride extrusion of PNCs during stress, GABA is actually excitatory (Hewitt et al., 2009), meaning that in this case LTD_{GABA} is decreasing excitation and potentially imposing a ceiling on HPA activation during prolonged glucocorticoid release. This LTD_{GABA} requires activation of postsynaptic mGluR5 in the PNC, which mediates the retrograde release of opioids from the somatodendritic compartment. RGS4 typically inhibits mGluR5 signaling here (Wamsteeker Cusulin et al., 2013). However, glucocorticoid receptor activation suppresses RGS4 expression (Ni et al., 1999), which increases mGluR5 signaling, thereby unmasking LTD_{GABA} (Wamsteeker Cusulin et al., 2013) and presenting a role for RGS4 as an inhibitor of synaptic plasticity in this system (Figure 1C). Overall, RGS4's expression in multiple brain regions as well as multiple subcellular compartments provides insight into the many ways in which the duration, location, and intensity of G protein activation can affect synaptic signaling and plasticity throughout the brain.

RGS7 and RGS9-2

Shared Signaling properties: RGS7 and RGS9-2 are closely related proteins within the R7 subfamily of RGS proteins that share structural similarities and binding partners (Table 1). Unlike RGS2 and RGS4, which are small, simple RGS proteins containing a single RGS domain, RGS7 and RGS9 are larger, more complex proteins containing multi-domains that bind

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various common binding partners. Both proteins serve as a GAP for Gαi/o family members with varying degrees of selectivity for the different Gαi and Gαo subunits (Hooks et al., 2003). In addition to the canonical RGS domain, both proteins also contain a DEP (disheveled, Egl-10, pleckstrin) domain, an R7H (R7 homology) domain, and a GGL (G protein gamma subunit-like) domain. The GGL domain shares close homology with G protein gamma subunits and specifically binds G protein β5 (Gβ5) with high affinity (Snow et al., 1999). Therefore, RGS7 and RGS9-2 each exist as obligate heterodimers in complex with Gβ5 (Hollinger and Hepler, 2002; Snow et al., 1999; Witherow et al., 2000). Binding partners and functions for the R7H domain remain elusive. The DEP domain binds R7BP, which can form a reversible complex with either RGS7:Gβ5 or RGS9-2:Gβ5 (Drenan et al., 2005; Grabowska et al., 2008; Martemyanov et al., 2005). R7BP is a regulatory protein that, when palmitoylated, anchors the RGS7:Gβ5 and the RGS9:Gβ5 complexes at the plasma membrane (Drenan et al., 2005; Drenan et al., 2006; Jia et al., 2011), and protects these RGS proteins from degradation. R7BP palmitoylation is regulated by Gαi/o signaling, and R7BP facilitates R7:Gβ5 complex association with GIRK channels, which speeds up deactivation kinetics (Jia et al., 2014). Roles of R7BP in R7 family RGS signaling have been thoroughly reviewed (Jayaraman et al., 2009).

R7BP can serve to regulate the balance between RGS7 and RGS9-2 signaling by preferentially anchoring either protein at the membrane, leaving the other unprotected in the cytosol and subject to degradation. For example, RGS7 and RGS9-2 are both expressed within the same postsynaptic dendritic compartments of striatal neurons. R7BP binding (or lack thereof) dictates RGS protein plasma membrane localization versus degradation, and therefore the cellular ratio of RGS9-2 versus RGS7. Under basal conditions, R7BP preferentially couples to RGS9-2 at the plasma membrane. This system is tightly regulated by increased neuronal activity, increased cellular oxygen and calcium levels, and activation of protein kinase C, all of which can shift the ratio toward degradation of RGS9-2 (Anderson et al., 2009). In this case,

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R7BP uncouples from RGS9-2, is released into the cytosol, and binds RGS7 to recruit it to the membrane, where it is protected from degradation. Of note, recent reports suggest that RGS7 is membrane-recruited and stabilized by the orphan GPCR, GPR158 (Orlandi et al., 2012). Interestingly, GPR158 contains a C terminal region that is homologous with R7BP, which competitively binds the DEP domain of RGS7, and binding of RGS7 to GPR158 potentiates GAP activity of RGS7 (Orlandi et al., 2012; Orlandi et al., 2015). Though GPR158 and R7BP form a mutually exclusive complex with RGS7 (Orlandi et al., 2012), future studies are needed to establish whether these binding partners are functionally redundant, divergent, or synergistic. In summary, RGS7 and RGS9-2 have very similar signaling properties despite being divergent in their brain expression pattern and regulation.

***RGS7*:** RGS7 is found throughout the brain (Khawaja et al., 1999), with reports indicating dense mRNA expression in the cerebellum, hypothalamus, thalamus (Lopez-Fando et al., 2005), and mRNA and protein expression in hippocampus (Fajardo-Serrano et al., 2013; Shelat et al., 2006) and striatum (Anderson et al., 2009; Larminie et al., 2004) (Table 1). Within the hippocampus, RGS7 is found mostly extrasynaptically in dendrites at asymmetric (primarily excitatory) synapses (Fajardo-Serrano et al., 2013), with Gβ5 regulating the cellular distribution of RGS7 (Rose et al., 2000). RGS7 acts as a GAP for Gαo and Gαi subunits (Lan et al., 1998; Posner et al., 1999; Rose et al., 2000; Shuey et al., 1998) and negatively modulates $GABA_BR$ signaling (Fajardo-Serrano et al., 2013; Ostrovskaya et al., 2014). Of note, RGS7 interacts in a phosphorylation-dependent manner with the regulatory protein 14-3-3, which binds the RGS domain of RGS7 to inhibit its GAP activity (Benzing et al., 2000). TNF-α negatively regulates this interaction (Benzing et al., 2002), and simultaneously prevents proteasomal degradation of RGS7, consequentially leading to stabilization and upregulation of RGS7 protein (Benzing et al., 1999).

Within postsynaptic membranes of neurons, the RGS7:Gβ5 complex regulates GIRK channels (Fajardo-Serrano et al., 2013; Kovoor et al., 2000; Ostrovskaya et al., 2014; Saitoh et

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al., 1999; Xie et al., 2010), which serve important roles in mediating hyperpolarization of the neuron (Figure 1C). This process can be regulated by 14-3-3. RGS7 greatly accelerates the deactivation GIRK in oocytes, and the introduction of 14-3-3 reduces the RGS7-mediated deactivation of GIRK currents, consistent with its negative regulatory effects on RGS7 GAP activity (Benzing et al., 2002). Co-expression with R7BP enhances the capacity of RGS7 to regulate GIRK channel activity, presumably by stabilizing RGS7:Gβ5 at the plasma membrane (Drenan et al., 2005). Compelling evidence suggests that RGS7's effect on GIRK channels is due to its action at the $GABA_BR$. RGS7, Gβ5, $GABA_BR$ and GIRK all coexist in a macromolecular complex (Fajardo-Serrano et al., 2013), and genetic knockout of either RGS7 (Ostrovskaya et al., 2014), Gβ5 (Xie et al., 2010), or R7BP (Ostrovskaya et al., 2014) delays deactivation of $GABA_BR$-coupled GIRK currents. Furthermore, ablation of RGS7 or R7BP increases coupling efficiency between $GABA_BR$ and GIRK (Ostrovskaya et al., 2014), in that lower doses of the $GABA_BR$ agonist baclofen produce a stronger GIRK current. Additionally, ablation of RGS7 decreases the intrinsic excitability of hippocampal pyramidal neurons, and impairs LTD and depotentiation (which is reliant on GIRK channels). This was shown to be a postsynaptic mechanism, as paired pulse facilitation was unaltered in the RGS7 knockout mice, which is consistent with RGS7's subcellular expression pattern. In summary, RGS7 is tightly coupled with $GABA_BR$-GIRK signaling on postsynaptic dendrites and spines, which serves as a major player in the modulation of depotentiation of LTP.

Though defined roles for RGS7 in human diseases are unclear, mouse models have begun to elucidate roles for RGS7 in plasticity and behavioral output. Consistent with RGS7 regulation of hippocampal excitability, RGS7 knockout mice show deficits in several tasks of learning and memory, including contextual fear learning, and spatial/contextual learning and memory (Morris water maze, and novel object recognition) (Ostrovskaya et al., 2014). Evidence for RGS7 mediating the effects of the $GABA_BR$ on neuronal excitability extends to behavior. As discussed, Gβ5 is an obligate dimer with RGS7, and loss of Gβ5 results in loss of R7 family of

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RGS proteins, including RGS7 (Chen et al., 2003). Gβ5 knockout mice show a dose-dependent decrease in locomotion in response to the $GABA_B$ agonist baclofen (Xie et al., 2010). Within the striatum, RGS7 knockdown enhances locomotor sensitization to cocaine (Anderson et al., 2010), but not in R7BP knockout mice, suggesting a unique role, separate from RGS9-2, in psychostimulant-induced behavior. RGS7 also has been associated with panic disorder (Hohoff et al., 2009) as well as ischemia (Shelat et al., 2006), though RGS7's roles in these neurological disorders, if any, need further elucidation.

***RGS9-2*:** RGS9 was originally cloned from retina (now identified as RGS9-1), and characterized as a retina specific GAP for the resident G protein, transducin (Gαt) (Cowan et al., 1998; He et al., 1998). However, this sequence was recognized as a shorter splice variant of a longer, striatum-specific isoform of RGS9, which was named RGS9-2 (Rahman et al., 1999). Subsequent work demonstrated that RGS9-2 also is found in other brain regions, specifically periaqueductal grey (PAG) (Zachariou et al., 2003) and thalamus (Lopez-Fando et al., 2005) (Table 1). RGS9-2 was initially characterized as a GAP for Gαi/o coupled to the μ-opioid receptor (MOR) (Rahman et al., 1999), and was later found to modulate D2 dopamine receptor (D2DR) signaling and trafficking. Consistent with these findings, RGS9-2 colocalizes with D2DR and enkephalin in medium spiny neurons (Kovoor et al., 2005; Rahman et al., 2003). The DEP binding protein, R7BP, which protects RGS9-2 from proteasomal degradation (Anderson et al., 2007), is reported to mediate RGS9-2 interaction with the D2DR at the plasma membrane. In one study, an expressed DEP motif alone was recruited to the membrane by D2DR, whereas DEP-less RGS9-2 remained in the cytosol, indicating that the DEP domain is both necessary and sufficient for targeting RGS9-2 to the D2DR and the plasma membrane (Kovoor et al., 2005).

Similar to RGS7, RGS9-2 accelerates the off-kinetics for D2-coupled GIRK channels when co-expressed in oocytes (Rahman et al., 2003) (Figure 1D), an effect that is dependent on the DEP domain (Kovoor et al., 2005), and a functional RGS domain (Celver et al., 2010). In

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medium spiny neuron (MSN) slice preparations of RGS9-2 knockout (RGS9-2-KO) mice, glutamate-evoked inward currents are inhibited by a D2DR agonist, suggesting a functional role of RGS9-2 in postsynaptic excitability. Indeed, RGS9-2-KO mice show severe abnormal movements following administration of a D2DR agonist (Kovoor et al., 2005). RGS9-2 also regulates receptor internalization, a method of modulating neuronal excitability. Overexpression of the RGS9-2:Gβ5 complex (but not full length RGS9-2 alone or DEP-less RGS9-2) inhibits agonist-dependent D2DR internalization (Celver et al., 2010). MOR signaling and sensitization is also heavily dependent on RGS9-2 activity (Figure 1D). RGS9-2 depletion (and Gβ5 depletion, consistent with the requisite dimer) enhances the analgesic potency and duration of morphine (Sanchez-Blazquez et al., 2003) as well as MOR endocytosis (Psifogeorgou et al., 2007), likely due to unabated stimulation of MOR-activated signals. Further supporting evidence for RGS9-2 functional interactions with MOR signaling includes the finding that RGS9-2 inhibits morphine-induced ERK activation (while a DEP-less RSG9-2 enhances pERK), along with the observation that RGS9-2 translocates to the plasma membrane following MOR activation (Psifogeorgou et al., 2007). In summary, RGS9-2 modulates receptor-membrane localization as well as postsynaptic neuronal excitability by regulating Gαi/o-linked GPCRs coupled to GIRK channels.

Beyond the synapse, RGS9-2 protein expression is both influenced by (Burchett et al., 1998; Rahman et al., 2003), and regulates (Rahman et al., 2003) psychostimulant-induced behavior. These observations are not unexpected given the striatal expression of RGS9-2. In these studies, RGS9-2 overexpression inhibits cocaine-induced hyperactivity, whereas RGS9-2 knockout mice show enhanced sensitivity to cocaine-induced hyperactivity and place preference (Rahman et al., 2003). Perhaps due to its role in dopaminergic signaling in the striatum, RGS9-2 also has been implicated in both schizophrenia (Seeman et al., 2007) and dyskinesias (Kovoor et al., 2005). Activation of opiate receptors alters and is affected by RGS9-2 expression. For example, acute morphine treatment increases RGS9-2 protein expression, whereas chronic

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morphine treatment decreases RGS9-2 in the nucleus accumbens (Psifogeorgou et al., 2007; Zachariou et al., 2003). This finding correlates with behavioral output driven by opiates. RGS9-2 knockout mice have a 10-fold greater sensitivity to the rewarding effects of morphine, as measured by place preference (Zachariou et al., 2003), a phenotype that is rescued by local, virally-driven overexpression of RGS9-2. RGS9-2-KO animals also are more sensitive to morphine analgesia (Garzon et al., 2003), and have delayed tolerance to, and enhanced physical dependence on, morphine (Zachariou et al., 2003). In summary, RGS9-2 plays a prominent role in both psychostimulant- and opiate-induced plasticity, likely by regulating slow acting GPCR modulatory signals at synapses.

RGS14

RGS14, a member of the R12 subfamily of RGS proteins, is a selective GAP for Gαi/o (Cho et al., 2000; Hollinger et al., 2001; Traver et al., 2000), having no effect on the GTPase activities of other Gα. Like RGS7 and RG9-2, RGS14 has a complex domain structure (Snow et al., 1997) that contains additional domains/motifs that interact with both heterotrimeric and monomeric G proteins, modulating their function. The GPR motif (also known as a GoLoco motif) of RGS14 specifically binds inactive Gαi1-GDP and Gαi3-GDP subunits (Hollinger et al., 2001; Kimple et al., 2001; Mittal and Linder, 2004), thereby serving to recruit cytosolic RGS14 to the plasma membrane and anchoring it there (Shu et al., 2007). In so doing, the GPR motif promotes formation of a RGS14:Gαi complex that is capable of interacting with GPCRs in the absence of Gβγ (Vellano et al., 2011b). Furthermore, RGS14 interacts with the monomeric G proteins Rap1 (Traver et al., 2000), Rap2 (Traver et al., 2000), and H-Ras (Shu et al., 2010; Vellano et al., 2013; Willard et al., 2009) at its tandem Rap/Ras binding domains, although H-Ras is likely the functional binding partner in cells (Vellano et al., 2013; Willard et al., 2009).

The first hints of RGS14 function in neuronal signaling came from studies of its protein expression patterns in brain (Table 1), with protein and mRNA expression in adult rodents limited largely to the hippocampus and olfactory cortex (Grafstein-Dunn et al., 2001; Traver et

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al., 2000) (http://mouse.brain-map.org/). Within the hippocampus, RGS14 expression is limited to area CA2, specifically within postsynaptic dendrites and spines of pyramidal neurons (Evans et al., 2014; Lee et al., 2010; Traver et al., 2000). Notably, proximal dendrites of CA2 neurons that receive Schaffer collateral projections from area CA3 are incapable of eliciting LTP under the same conditions that reliably provoke LTP in hippocampal CA1 neurons (Zhao et al., 2007). However, mice lacking RGS14 (RGS14-KO) exhibit robust LTP in CA2 neurons, demonstrating that RGS14 is a natural suppressor of synaptic plasticity within these neurons (Lee et al., 2010). Consistent with this idea, RGS14-KO mice perform markedly better than wild type mice in hippocampal-dependent tasks of spatial/contextual learning (Morris water maze) and memory (novel object recognition) that are associated with LTP (Lee et al., 2010).

The exact mechanism by which RGS14 suppresses LTP is currently unclear, though RGS14 engages signaling proteins and pathways that are critical for LTP. RGS14 binds active H-Ras to inhibit ERK activation (Shu et al., 2010), which is necessary for AMPA receptor trafficking and LTP in CA1 neurons (Atkins et al., 1998; English and Sweatt, 1997). RGS14 also can bind calcium-activated calmodulin (Ca^{2+}/CaM) (Evans and Hepler, 2012), which is essential for regulating both CaMKII- and ERK-dependent signaling events that underlie induction of LTP. Recent evidence suggests that the RGS domain of RGS14 maintains GAP activity when its GPR motif is bound to inactive Gαi subunits at the plasma membrane (Brown et al., 2015). This supports a model (Brown et al., 2015) in which cytosolic RGS14 could be recruited initially to the postsynaptic density (PSD) through its RGS domain following GPCR and Gαi activation, where its GPR motif captures the resulting inactive Gαi-GDP (Figure 1C). In this way, the newly formed RGS14:Gαi complex is properly placed to GAP other nearby Gαi/o-GTP enabling those resulting Gαi-GDP to recruit and cluster additional RGS14:Gαi complexes to form a signaling node at or near the plasma membrane and possibly the PSD(Brown et al., 2015). In this speculative scenario, RGS14 would be well positioned to intercept local signals necessary for the induction of LTP, such as H-Ras activation of ERK and/or Ca^{2+}/CaM activation of CaMKII.

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Further studies are necessary to confirm this model. In summary, RGS14's modulation of synaptic plasticity in hippocampal area CA2 provides a unique example of RGS protein action at the synapse, with the RGS domain acting in concert with multiple other domains and signaling partners/pathways to tightly modulate downstream synaptic signaling.

Other RGS Proteins

While many RGS proteins are expressed in brain (Gold et al., 1997; Grafstein-Dunn et al., 2001), only those highlighted here have so far been reported to directly affect synaptic plasticity through modulation of G protein signaling at the synapse. The following RGS proteins also have reported roles in modulating synaptic signaling, but further work is necessary to determine their precise function in synaptic plasticity. One such protein is RGS6, another member of the R7 subfamily, which is highly expressed in cerebellar granule neurons (CGNs) (Maity et al., 2012). Similar to RGS7 and RGS9-2, RGS6 forms a stable signaling complex with Gβ5 and is a selective GAP for Gαi/o family members (Hooks et al., 2003; Posner et al., 1999). Studies indicate that RGS6 antagonizes $GABA_BR$-mediated GIRK currents, as shown by ataxia and increased CGN GIRK currents exhibited by RGS6-KO mice that can be rescued by administration of a $GABA_BR$ antagonist (Maity et al., 2012). Additionally, RGS6 is required for adult maintenance of dopaminergic neurons in the ventral substantia nigra (Bifsha et al., 2014) and a recent study has proposed a role for RGS6 as a key mediator of both reward-related behavioral and pathological responses to alcohol (Stewart et al., 2015), though it is unclear whether these effects are due to changes in synaptic signaling or plasticity. Furthermore, RGS6 is expressed in the soma and dendrites of hippocampal and cortical neurons. In both brain regions, RGS6 can promote anxiety and depression-like behavior by inhibiting signaling through the $5\text{-}HT_{1A}R$ (Stewart et al., 2014). These findings would suggest a possible role for RGS6 antagonists not only as novel antidepressants but also as a way to reduce alcohol cravings and

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withdrawal, though such inhibition may be contraindicated due to necessary RGS6 functions in other parts of the brain (Bifsha et al., 2014; Maity et al., 2012).

RGS8 has also been shown to modulate signaling in neurons in the cerebellum. Here, RGS8 is expressed exclusively in the soma and dendrites of Purkinje cells (Itoh et al., 2001; Saitoh et al., 2003; Saitoh and Odagiri, 2003), where it increases both the on and off rate of Gβγ-mediated GIRK channel current following GPCR activation (Saitoh et al., 1997). Interestingly, rapid activation of the GIRK channel appears to be mediated by the N-terminal region of RGS8 as opposed to the RGS domain (Jeong and Ikeda, 2001), demonstrating a function for RGS8 independent of GAP activity.

Little is known about the neuronal function of RGS12, a member of the R12 family of proteins that also contains RGS14 and RGS10. Although RGS12 mRNA is found in the brain (Lopez-Aranda et al., 2006) (http://mouse.brain-map.org/), the protein's known actions are in dorsal root ganglion (DRG) neurons in the spinal cord. In these neurons, RGS12 modulates the presynaptic $GABA_BR$-mediated inhibition of presynaptic $Ca_V2.2$ channels (Schiff et al., 2000) by interacting with the SNARE-binding region of the channel (Richman and Diverse-Pierluissi, 2004; Richman et al., 2005). Further research is needed to determine the function of RGS12 in modulating synaptic signaling and plasticity within the brain, though it is possible that it acts through a similar mechanism as that shown in DRG neurons.

RGS10, the third member of the R12 family, is also expressed highly in the brain. While much of this expression is in microglia, RGS10 immunolabelling is also high within the nuclei of neurons throughout the brain as well as both pre- and postsynaptically (Waugh et al., 2005), indicating a possible role in synaptic plasticity. Another, RGS protein, Axin, is also expressed at the synapse (Chen et al., 2015). Here, Axin, has a clearly defined role in modulating neuronal differentiation and synapse development, acting as a scaffold for GSK-3β and β-catenin to manipulate canonical Wnt signaling at the both pre- and postsynaptic compartments (Chen et al., 2013). Notably, GSK-3β has been shown to play a role in the induction of LTD (Peineau et

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al., 2007), potentially implicating Axin in modulation of synaptic plasticity as well. Additionally, RGS19 (GAIP) has been shown to be expressed in striatal neurons where it may attenuate D2DR downstream signaling, though synaptic localization and specific function of RGS19 at the synapse is unclear (Jeanneteau et al., 2004). While these RGS proteins are highly expressed in the brain, with some shown to affect signaling specifically at the synapse, further research is necessary to elucidate their exact role in modulating synaptic plasticity.

CONCLUDING REMARKS

Here we have highlighted specific roles for particular RGS proteins (RGS2, RGS4, RGS7, RGS9-2 and RGS14) in the modulation of GPCR and G protein signaling at the synapse. Of these, RGS2 and RGS4 are expressed throughout the brain, suggesting broad and less specific actions, whereas RGS7, RGS9-2 and RGS14 are expressed more discretely within only a few brain regions, suggesting more targeted actions. These actions likely are paired with the GPCRs whose signaling they regulate. One open question centers on how the specificity of these RGS protein actions is achieved within neurons. Several converge upon and regulate the same signaling event within the same brain region and neuron (e.g. GPCR regulation of postsynaptic GIRK channels) (Doupnik, 2015). For example, RGS7 and RGS9-2, two closely related RGS proteins, are both expressed in the same postsynaptic dendritic compartment of striatal neurons (Anderson et al., 2009). The relative level of expression of RGS7 and RGS9-2 in these neurons is tightly controlled by the availability of R7BP (Anderson et al., 2009), suggesting distinct functional roles for these two RGS proteins. In other neurons, two very different RGS proteins, RGS2 (Labouebe et al., 2007) and RGS7 (Fajardo-Serrano et al., 2013; Ostrovskaya et al., 2014), can regulate the $GABA_B$-GIRK signaling complex. In all of these cases, specificity of RGS action is likely dictated by proper localization in space and time by

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preferential coupling between the RGS protein and a specific GPCR/G protein complex (Neitzel and Hepler, 2006). Specific examples of preferential GPCR-RGS coupling have been reported (Bernstein et al., 2004; Zeng et al., 1998), including RGS7 coupling with GPR158 as discussed here (Orlandi et al., 2012). Another area in need of clarification centers on understanding roles for non-RGS domains in the larger, multi-domain RGS proteins (e.g. RGS7, RGS9, RGS14, etc.) and the signaling functions of the multi-protein complexes they form. How these are assembled in time and space, and their functions beyond regulating G protein signaling remain unclear. Clarifying this will be important for understanding specific roles of these RGS proteins at the synapse moving forward.

Due to the importance of RGS proteins in regulating GPCR/G protein signaling in synaptic plasticity and other physiological processes, many RGS proteins (including those highlighted here) have emerged as attractive therapeutic targets (Sjogren et al., 2010; Sjogren and Neubig, 2010). To date, efforts have focused on the identification and development of inhibitors of the RGS domain/Gα protein-protein interactions. This idea has been bolstered by the intriguing phenotypes observed in mice carrying RGS-insensitive Gα mutants, which showed that blocking RGS actions potentiate neurotransmitter actions and linked behaviors in a targeted fashion (Lamberts et al., 2013; Talbot et al., 2010). While initial efforts focused on the identification and development of peptide inhibitors of RGS proteins as proof of concept (Jin et al., 2004; Wang et al., 2008), more recent work has focused on the development of small molecule inhibitors with the goal of treating multiple diseases, including neurological disorders (Blazer et al., 2010; Blazer et al., 2011; Roman and Traynor, 2011; Storaska et al., 2013; Turner et al., 2012). Thus far, RGS4 has been the main focus for the development of small molecule RGS inhibitors. Of reported compounds that act on RGS4, all act by modifying cysteine residues in the RGS domain through an irreversible covalent interaction, thereby preventing association with Gα (Kimple et al., 2007; Roman and Traynor, 2011). More recently, a novel, cell-based high throughput assay with regulated RGS4 expression has identified new RGS4

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small molecule inhibitors that not only have cellular activity, but are also reversible (Storaska et al., 2013), thereby increasing their utility as potential future therapeutics. As outlined above, other RGS proteins including RGS2, RGS7, RGS9 and RGS14 also are attractive drug targets. Similar assays must be used to identify inhibitors/modulators for these and other RGS proteins, not only as experimental pharmacological tools, but also as possible therapeutic agents to treat identified neurological disorders associated with RGS proteins in the CNS.

As potential therapeutic targets, multiple RGS proteins have been implicated in various neurological disorders. RGS2 has been implicated in panic disorder (Hohoff et al., 2015; Otowa et al., 2011) and PTSD (Amstadter et al., 2009; Koenen et al., 2009), and RGS4 in schizophrenia (Ding and Hegde, 2009; Mirnics et al., 2001; Prasad et al., 2005). The clear roles for certain RGS proteins in synaptic plasticity, as outlined here and supported by findings with RGS-insensitive Gα (Neubig, 2015), allow new insights into how these proteins are regulated, and also the myriad of ways in which G protein signaling can affect synaptic connections. Future studies must not only elucidate the role of RGS proteins in neuronal signaling, but also work towards the application of newly developed RGS inhibitors as therapeutics in the CNS (Blazer et al., 2015; Sjogren et al., 2010). Beyond disrupting the RGS/Gα interface as a drug target, protein binding domains outside of the RGS domain should also be considered (Sjogren and Neubig, 2010) as we begin to better understand the broader multifunctional signaling roles of RGS proteins. In conclusion, RGS proteins regulate multiple forms of synaptic plasticity throughout the brain through regulation of neuronal G protein signaling and represent a compelling new target for the development of therapeutics for the treatment of a variety of neurological disorders.

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Authorship Contributions

Wrote or contributed to the writing of the manuscript: Gerber, K.J. Squires, K.E. Hepler, J.R.

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Footnote

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Figure Legends

Figure 1. RGS Protein Regulation of Synaptic Signaling. RGS proteins regulate many aspects of pre- and post- synaptic signaling. **A)** Activation of presynaptic GPCRs releases Gβγ to inhibit $Ca_V2.2$ (N-Type) Ca^{2+} channels and suppress neurotransmitter (glutamate, Glu and gamma-aminobutyric acid, GABA) release. Stimulated upregulation of RGS2 expression blocks Gβγ inhibition of $Ca_V2.2$ channels. **B)** Presynaptic release of acetylcholine activates M4 muscarinic acetylcholine autoreceptors that release Gβγ, and inhibit $Ca_V2.2$ channels to suppress neurotransmitter release. RGS4 blocks Gβγ inhibition of $Ca_V2.2$ channels. **C)** RGS14 associates with Gαi/o and forms a stable complex with Gαi at the plasma membrane, where it regulates H-Ras/ERK- and possibly calmodulin and CaMKII-dependent signaling events that underlie induction of LTP. Independent of this, RGS4 inhibits mGluR5 and Gαq-mediated suppression of the afterhyperpolarization current following action potential firing. Postsynaptic mGluR5 signaling also stimulates retrograde opioid release, which activates presynaptic mu-opioid receptors (MORs), suppressing presynaptic GABA release. RGS4 blocks mGluR5-mediated retrograde opioid release from parvocellular neuroendocrine cells (PNCs) in the hypothalamus, increasing GABA release onto these neurons. RGS2 and the RGS7-Gβ5 complex both block postsynaptic $GABA_B$ receptor-stimulated GIRK currents by promoting Gβγ deactivation. **D)** RGS4 blocks postsynaptic serotonin 5-HT1A receptor and Gαi/o-mediated inhibition of NMDA receptor currents. Postsynaptic mGluR5 and dopamine D2 receptor signaling stimulates retrograde release of endocannabinoids that stimulate presynaptic cannabinoid CB1 receptors to suppress Glu release and induce long-term depression (LTD) at the synapse. RGS4 suppresses both mGluR5/Gαq and D2DR/Gαi/o-mediated retrograde opioid release to inhibit induction of LTD. The RGS9-2:Gβ5 complex blocks postsynaptic $GABA_B$ receptor-stimulated GIRK currents by enhancing Gβγ deactivation. Finally, the RGS9-2:Gβ5 complex inhibits agonist-induced internalization of MORs and D2DRs. See the text for further details.

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Tables

Table 1. Summary of RGS protein functions at the synapse and possible role in disease.

Here, we list the binding partners, brain distribution, subcellular localization, functional signaling roles, and possible roles in disease of specific RGS proteins (RGS2, RGS4, RGS7, RGS9-2, RGS14) that have been clearly demonstrated to serve critical roles in modulating synaptic signaling and plasticity throughout the brain.

*Additional binding partners for many of these RGS proteins have been identified and shown to have functional roles modulating or mediating RGS protein signaling (Abramow-Newerly et al., 2006; Sethakorn et al., 2010).

RGS	Binding Partners at Defined Domains*	Brain Distribution	Subcellular Localization	Role in Synaptic Plasticity/Signaling	Links to Neurological Disease
RGS2	**$G\alpha_q$ at the RGS domain**(Heximer et al., 1997; Ingi et al., 1998) **$G\alpha_{i/o}$ at the RGS domain** (Han et al., 2006; Ingi et al., 1998)	**Hippocampus** (Han et al., 2006)	**Presynaptic in hippocampal pyramidal neurons** (Han et al., 2006)	**Regulates short term synaptic plasticity in the hippocampus** (Han et al., 2006) **Disinhibits GABA mediated inhibition of dopamine neurons in the VTA** (Labouebe et al., 2007)	**Anxiety** (Doupnik et al., 1997; Hohoff et al., 2015; Lifschytz et al., 2012; Okimoto et al., 2012) **Depression** (Lifschytz et al., 2012; Mandelli and Serretti, 2013) **Post-traumatic stress disorder** (Amstadter et al., 2009) **Suicide** (Cui et al., 2008) **Panic disorder** (Hohoff et al., 2015; Koenen et al., 2009; Otowa et al., 2011)
		Striatum (Labouebe et al., 2007)	**Postsynaptic in dopamine neurons in the VTA** (Labouebe et al., 2007)		
		Amygdala (Okimoto et al., 2012)	NA		
		Thalamus (Ingi and Aoki, 2002), **Neocortex** (Ingi and Aoki, 2002), **Cerebellum** (Ingi and Aoki, 2002)	NA		
RGS4	**$G\alpha_q$ at the RGS domain** (Hepler et al., 1997) **$G\alpha_{i/o}$ at the RGS domain** (Berman et al., 1996)	**Layer V Prefrontal Cortex** (Paspalas et al., 2009)	**Distal Dendrites, Spines, and Axons** (Paspalas et al., 2009)	**Block postsynaptic serotonin-mediated signaling** (Gu et al., 2007) **and prevent signaling crosstalk between multiple GPCRS at the synapse** (Paspalas et al., 2009) **LTD_{GABA}** (Wamsteeker Cusulin et al., 2013) **Increase neuronal excitability** (Saugstad et al., 1998) **Inhibition of M4 autoreceptor signaling** (Ding et al., 2006) **Striatal LTD** (Lerner and Kreitzer, 2012)	**Fragile X Syndrome** (Pacey et al., 2011) **Schizophrenia** (Prasad et al., 2010; Prasad et al., 2005) **Parkinson's Disease** (Ding et al., 2006; Lerner and Kreitzer, 2012)
		Hypothalamus (Gold et al., 1997; Kim et al., 2014; Ni et al., 1999; Wamsteeker Cusulin et al., 2013)	**Parvocellular neuroendocrine cells in the paraventricular nucleus** (Wamsteeker Cusulin et al., 2013)		
		Hippocampus (Gold et al., 1997; Heraud-Farlow et al., 2013; Saugstad et al.,	**Postsynaptic in CA1 pyramidal neurons** (Gold et al., 1997; Heraud-Farlow et al.,		

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		1998)	2013; Saugstad et al., 1998)		
		Striatum (Ding et al., 2006; Geurts et al., 2003; Lerner and Kreitzer, 2012; Schwendt and McGinty, 2007)	**Presynaptic in cholinergic interneurons** (Ding et al., 2006) **Postsynaptic in indirect pathway medium spiny neurons** (Lerner and Kreitzer, 2012)		
RGS7	**$G\alpha_{i/o}$ at the RGS domain** (Hooks et al., 2003; Posner et al., 1999; Rose et al., 2000) **Gβ5 at the GGL domain** (Snow et al., 1999) **R7BP at the DEP domain** (Drenan et al., 2005; Martemyanov et al., 2005)	**Hippocampus** (Fajardo-Serrano et al., 2013; Ingi and Aoki, 2002; Khawaja et al., 1999; Ostrovskaya et al., 2014; Shelat et al., 2006; Xie et al., 2010)	**Extrasynaptic in dendrites** (Fajardo-Serrano et al., 2013)	**Accelerates GIRK deactivation via $GABA_B$ Receptors** (Fajardo-Serrano et al., 2013) **LTD and depotentiation in hippocampus** (Ostrovskaya et al., 2014)	**Hippocampal Ischemia** (Shelat et al., 2006) **Panic disorder** (Hohoff et al., 2009)
		Striatum (Anderson et al., 2010; Anderson et al., 2009; Khawaja et al., 1999; Lopez-Fando et al., 2005)	**Postsynaptic** (Anderson et al., 2010; Anderson et al., 2009)		
		Cerebellum (Ingi and Aoki, 2002; Khawaja et al., 1999) **Thalamus** (Ingi and Aoki, 2002; Khawaja et al., 1999) **Hypothalamus** (Khawaja et al., 1999) **Amygdala** (Khawaja et al., 1999)	NA		
RGS9-2	**$G\alpha_{i/o}$ at the RGS domain** (Hooks et al., 2003) **Gβ5 at the GGL domain** (Makino et al., 1999) **R7BP at the DEP domain** (Martemyanov et al., 2005)	**Striatum** (Gold et al., 1997; Rahman et al., 1999)	**Extrasynaptic in dendrites** (Mancuso et al., 2010)	**Accelerates GIRK deactivation rate via D2 dopamine receptors** (Celver et al., 2010; Kovoor et al., 2005; Rahman et al., 2003)	**Dyskinesias** (Kovoor et al., 2005) **Schizophrenia** (Seeman et al., 2007)
		Periaqueductal grey (Zachariou et al., 2003)	NA		
		Thalamus (Lopez-Fando et al., 2005)	NA		
RGS14	**$G\alpha_{i/o}$ at the RGS domain** (Cho et al., 2000; Hollinger et al., 2001; Traver et al., 2000)	**Hippocampus** (Evans et al., 2014; Lee et al., 2010; Traver et al., 2000)	**Somatodendritic compartment of CA2 pyramidal neurons including the PSD** (Lee et al., 2010)	**Suppresses LTP in CA2 hippocampal neurons** (Lee et al., 2010; Vellano et al., 2011a)	**Anxiety** (Parker et al., 2012)

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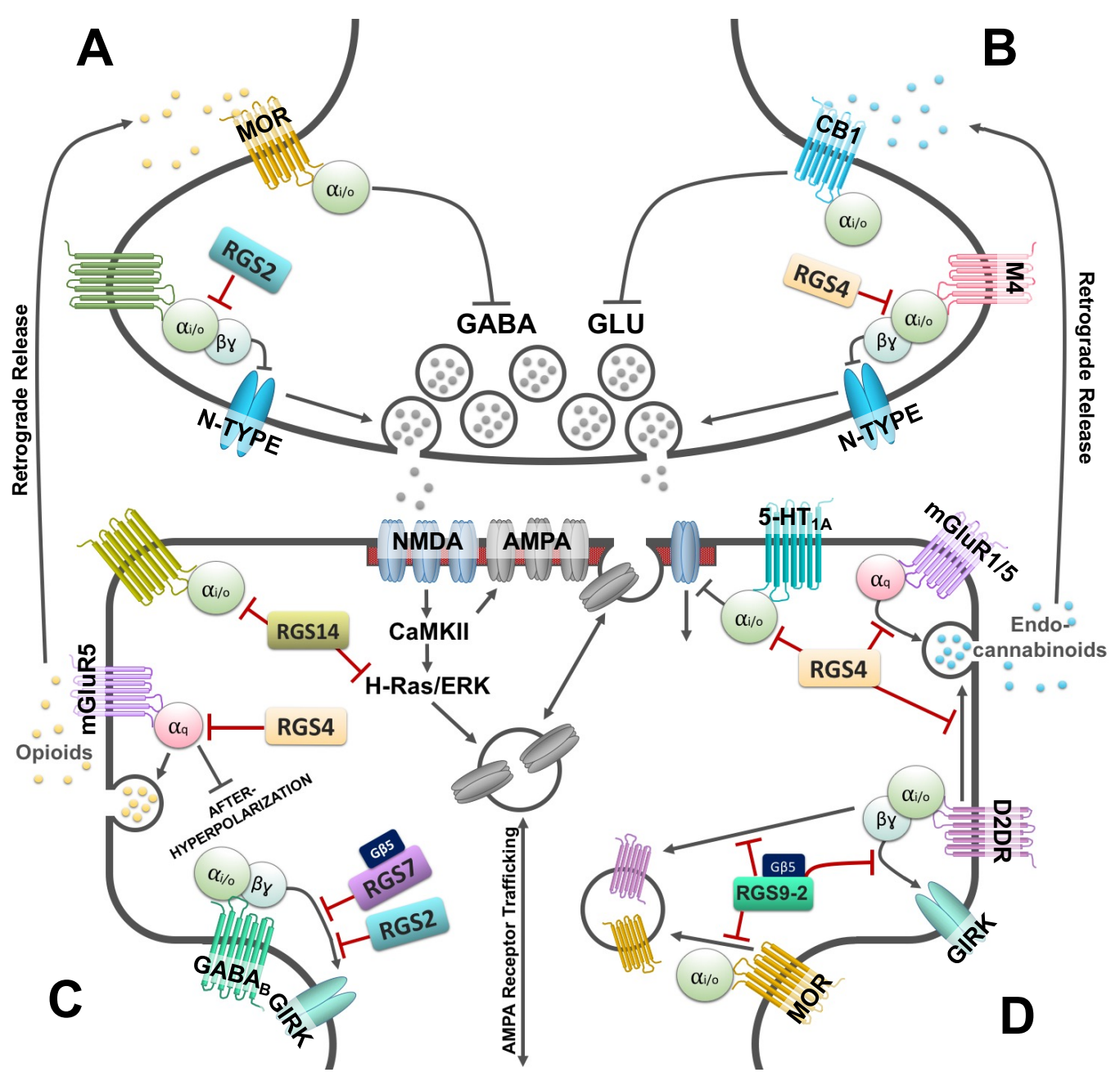
	H-Ras at the R1 Ras binding domain (Vellano et al., 2013; Willard et al., 2009) **Gαi1/3 at the GPR (GoLoco) motif** (Hollinger et al., 2001; Kimple et al., 2001; Mittal and Linder, 2004)	**Piriform cortex** (Evans et al., 2014; Grafstein-Dunn et al., 2001) **Orbital cortex** (Evans et al., 2014) **Striatum** (Lopez-Aranda et al., 2006)	NA		


A
B
C
D
MOR
CB1
M4
RGS2
RGS4
GABA
GLU
N-TYPE
N-TYPE
Retrograde Release
Retrograde Release
NMDA
AMPA
5-HT1A
mGluR1/5
RGS14
CaMKII
H-Ras/ERK
RGS4
Endo-cannabinoids
mGluR5
Opioids
RGS4
AFTER-HYPERPOLARIZATION
Gβ5
RGS7
RGS2
GABAB
GIRK
AMPA Receptor Trafficking
Gβ5
RGS9-2
D2DR
GIRK
MOR