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RGS14 expression in CA2 hippocampus, amygdala, and basal ganglia: Implications for human brain physiology and disease

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Abstract

RGS14 is a multifunctional scaffolding protein that is highly expressed within postsynaptic spines of pyramidal neurons in hippocampal area CA2. Known roles of RGS14 in CA2 include regulating G protein, H-Ras/ERK and calcium signaling pathways to serve as a natural suppressor of synaptic plasticity and postsynaptic signaling. RGS14 also shows marked postsynaptic expression in major structures of the limbic system and basal ganglia, including the amygdala and both the ventral and dorsal subdivisions of the striatum. In this review, we discuss the signaling functions of RGS14 and its role in postsynaptic strength (long-term potentiation) and spine structural plasticity in CA2 hippocampal neurons, and how RGS14 suppression of plasticity impacts linked behaviors such as spatial learning, object memory and fear conditioning. We also review RGS14 expression in the limbic system and basal ganglia and speculate on its possible roles in regulating plasticity in these regions, with a focus on behaviors related to emotion and motivation. Finally, we explore the functional implications of RGS14 in various brain circuits and speculate on its possible roles in certain disease states such as hippocampal seizures, addiction, and anxiety disorders.

Keywords

RGS14; RGS proteins; hippocampus; synaptic plasticity; basal ganglia; limbic system; striatum; amygdala

Introduction

Regulators of G proteins signaling (RGS) proteins are a large family (20 members) of diverse multifunctional signaling proteins that regulate GPCR-G protein signaling pathways in brain and in all cells/tissues (Alqinyah & Hooks, 2018; O'Brien, Wilkinson, & Roman, 2019; Stewart & Fisher, 2015). The proteins are classified into four subfamilies (A/RZ, B/R4, C/R7, and D/R12) based on sequence homology and the presence of additional non-RGS domains (Hollinger & Hepler, 2002; Ross & Wilkie, 2000; Willars, 2006). All

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RGS protein family members share a conserved functional RGS domain that interacts with specific active Gα subunits, to serve as a GTPase activating protein (GAP) and negatively regulate GPCR-Gα signaling (Tesmer, Berman, Gilman, & Sprang, 1997). While many RGS proteins are small simple proteins that serve as dedicated GAPs, some are larger more complex proteins with other domains and signaling partners that have roles outside of their canonical GAP function (Gerber, Squires, & Hepler, 2016; Hollinger & Hepler, 2002; Sjogren, 2011). In this review we focus on RGS14, a complex multifunctional brain protein member of the D/R12 subfamily that is highly expressed in CA2 hippocampus.

RGS14 is a multifunctional signaling protein that integrates key signaling pathways important for synaptic plasticity

RGS14 is a multifunctional scaffolding protein that integrates G protein, mitogen activated kinase/extracellular regulated kinase (MAPK/ERK), and calcium signaling pathways linked to postsynaptic signaling and plasticity. As such, RGS14 regulates post-synaptic signaling and plasticity in neurons (Harbin, Bramlett, Montanez-Miranda, Terzioglu, & Hepler, 2021) (Figure 1). Like all RGS proteins, RGS14 binds directly to active Gα subunits acting as a GAP to negatively regulate GPCR-G protein signaling. RGS14 is highly selective for active Gαi/o binding through its canonical RGS domain (Cho, Kozasa, Takekoshi, De Gunzburg, & Kehrl, 2000; Hollinger, Taylor, Goldman, & Hepler, 2001; Traver et al., 2000). Serving as a GAP, RGS14 forms a Gαi/o-dependent complex with Gαi-linked GPCRs that is regulated by receptor agonists (Vellano, Maher, Hepler, & Blumer, 2011). The RGS14-Gi/o complex also can be regulated by the unconventional guanine nucleotide exchange factor Ric-8A (resistance to inhibitors of cholinesterase-8A) (Vellano, Shu, et al., 2011), though cellular functions for this interaction are much less clear.

Besides serving as a conventional RGS protein GAP for GPCR-G protein complexes, RGS14 also is a multifunctional protein that binds and regulates other signaling partners (Figure 1). Through the tandem Rap/Ras (R1/R2) binding domain (RBD), RGS14 interacts with the monomeric GTPases Rap1 and Rap2 (Traver et al., 2000), H-Ras (Shu, Ramineni, & Hepler, 2010; Vellano, Brown, Blumer, & Hepler, 2013; Willard et al., 2009), and Raf kinases (Shu et al., 2010). In the heart, RGS14 diminishes myocardial remodeling and attenuates the development of cardiac remodeling through the MEK-ERK1/2 signaling pathway (Li et al., 2016). Within the RBD domain region, RGS14 binds calcium/calmodulin (Ca^{2+}/CaM) and Ca^{2+}/CaM-dependent kinase II (CaMKII) (Evans, Gerber, et al., 2018). The G protein regulatory (GPR)/GoLoco motif binds inactive Gαi1/3-GDP (Hollinger et al., 2001; Kimple et al., 2001; Mittal & Linder, 2004). Binding of RGS14 to inactive Gαi1-GDP or Gαi3-GDP serves to anchor RGS14 at the plasma membrane thereby allowing for the RGS domain to bind and GAP a second active Gαi/o (Figure 1) (Brown et al., 2015; Brown, Lambert, & Hepler, 2016).

Besides acting at postsynaptic spines, RGS14 also is a nucleocytoplasmic shuttling protein (Branch & Hepler, 2017; Cho, Kim, & Kehrl, 2005; Shu, Ramineni, Amyot, & Hepler, 2007). Roles for RGS14 protein in the nucleus so far remain undefined and are the focus of ongoing studies. However, more is known about the regulation of RGS14 nucleocytoplasmic shuttling. RGS14 contains a nuclear localization sequence (NLS) located between the RGS

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and R1 domain and a nuclear export sequence (NES) found within the GPR motif (Figure 1). RGS14's nucleocytoplasmic shuttling and movement in the cell is guided by the NLS, and its nuclear export is guided by binding of exportin (XPO1) to the NES (Gerber, Squires, & Hepler, 2018; Harbin et al., 2021). This shuttling movement of RGS14 has been reported in cultured cell lines and neurons (Branch & Hepler, 2017; Cho et al., 2005; Gerber et al., 2018; Shu et al., 2007; Squires et al., 2021). Studies examining endogenous RGS14 in natural host B35 neuroblastoma cells found that the protein localizes to juxtanuclear membranes encircling the nucleus, at the nuclear pore complexes on both sides of the nuclear envelope, within intranuclear membrane channels, and within both chromatin-poor and chromatin rich-regions of the nucleus in a cell cycle dependent manner (Branch & Hepler, 2017). Recent studies have examined human RGS14 genetic variants and how these affect RGS14 shuttling function in neurons. Human genetic variants L505R and R507Q are located within the nuclear export sequence of RGS14 (Squires et al., 2021). These genetic variants affect binding to Gα1-GDP and XPO1, thus affecting normal nuclear localization. Wild type RGS14 localizes to the soma, dendrites, and spines of neurons while L505R is concentrated in the nucleus, and R507Q exhibits a mixed phenotype (Squires et al., 2021). RGS14 is stabilized in the cytosol by 14-3-3γ binding at Phospho-Ser218 (Gerber et al., 2018) (Figure 1). However, RGS14 movement from the cytoplasm to the nucleus is regulated by phosphorylation-independent 14-3-3γ binding elsewhere on the protein (undefined). This 14-3-3γ binding inhibits RGS14 nuclear import and nucleocytoplasmic shuttling in neurons (Gerber et al., 2018). Studies are ongoing to define RGS14 roles in the nucleus.

Interestingly, RGS14 in human and non-human primates (but not rodent) contain an extra C-terminal 21 amino acids that code for a class I PDZ-recognition sequence (PDZ motif) (Friedman et al., 2022). Recent studies in kidney show that the human RGS14-PDZ motif binds the scaffolding protein NHERF1 in human kidney proximal tubule cells which, in turn, binds to the sodium phosphate cotransporter 2a (NPT2A) to mediate renal phosphate transport (Friedman et al., 2022). The presence of human RGS14 stabilizes the NPT2A:NHERF1 complex, acting as a regulator of parathyroid hormone (PTH) and FGF23-sensitive phosphate transport (Friedman et al., 2022). Roles for RGS14-PDZ binding partners in brain and specifically within CA2 hippocampal neurons remains an ongoing area of interest.

RGS14 regulation of synaptic plasticity in rodent hippocampal area CA2

RGS14 protein expression has been reported in rodent brain, heart, lung, kidney, and spleen (Agudelo et al., 2018; Kardestuncer, Wu, Lim, & Neer, 1998; Snow, Antonio, Suggs, Gutstein, & Siderovski, 1997). While recent studies have identified roles for RGS14 in heart (Li et al., 2016), adipocytes (Vatner et al., 2018), and kidney (Friedman et al., 2022), most studies of RGS14 have centered on understanding its role in the brain. Within mouse brain, RGS14 is highly expressed in the hippocampus, most notably area CA2 (Figure 3). RGS14 protein is undetectable in mouse brain at birth but is upregulated during early postnatal development, being detected at P7, and reaching highest persistent expression levels in adulthood (Evans, Lee, Smith, & Hepler, 2014). Within rodent hippocampal area CA2, RGS14 has been reported in pyramidal neurons specifically within postsynaptic dendrites

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and spines (Evans et al., 2014; Lee et al., 2010; Traver et al., 2000). Strong but discrete immunoreactivity is observed in soma and neurites of CA2 pyramidal neurons that project to stratum lacunosum moleculare and stratum radiatum of area CA1. RGS14 is also highly expressed within neurons of the fasciola cinerea (FC) of rodents. Within these CA2 neurons, RGS14 is enriched in the soma and within dendritic shafts, spine neck, spine head, and at post synaptic densities (PSD) (Lee et al., 2010).

The hippocampus plays a crucial role in many aspects of learning and memory including episodic, spatial, object recognition, social and contextual memory (Bird & Burgess, 2008; Broadbent, Gaskin, Squire, & Clark, 2010; Broadbent, Squire, & Clark, 2004; Evans, Dudek, & Hepler, 2015; Harbin et al., 2021; Tzakis & Holahan, 2019). Neurons of the hippocampus have a robust capacity to express plasticity. Long-term potentiation (LTP) is the stable increase in synaptic strength in response to brief periods of synaptic stimulation and is very prominent in the hippocampus (Collingridge, Isaac, & Wang, 2004; Kennedy, Beale, Carlisle, & Washburn, 2005; Lynch, Kessler, Arai, & Larson, 1990; Nakazawa, McHugh, Wilson, & Tonegawa, 2004; Neves, Cooke, & Bliss, 2008). LTP is a cellular correlate and underlying mechanism of memory formation and storage (Bliss-Moreau, Bauman, & Amaral, 2011; Collingridge et al., 2004; Evans et al., 2015; Kennedy et al., 2005; Nakazawa et al., 2004; Neves et al., 2008). Unlike area CA1, neurons in area CA2 lack LTP. Specifically, area CA3-derived Schaffer collateral synapses on CA2 neurons lack the capacity to produce LTP (Zhao, Choi, Obrietan, & Dudek, 2007) where RGS14 is expressed (Lee et al., 2010). However, knock-out mice lacking RGS14 in area CA2 gain capacity for high-frequency stimulation (LTP) that was absent before, showing that RGS14 is a natural break on LTP (Evans, Parra-Bueno, et al., 2018; Lee et al., 2010). In tests of RGS14 roles in hippocampal-based learning and memory, mice were subjected to a novel object recognition test (Ennaceur & Delacour, 1988), a measure of declarative memory. RGS14-KO mice spent more time exploring and contacting the novel object than wild type (WT) mice (Lee et al., 2010). In the Morris water maze test of spatial learning (Vorhees & Williams, 2006), both WT and RGS14-KO littermates learned the task, but only RGS14-KO mice showed a significantly (and unexpected) enhanced initial learning rate that was sustained each day (Lee et al., 2010).

Underlying learning and memory is synaptic plasticity, which is often described in terms of changes in synaptic strength but also is associated with structural changes within the spine (Bernardinelli, Nikonenko, & Muller, 2014). Synaptic stimulation leading to LTP induces an enlargement of dendritic spines that results from the trafficking of AMPA receptor-containing vesicles and their insertion into postsynaptic spines (Matsuzaki 2004, Nishiyama and Yasuda 2015). RGS14 is enriched in spines and dendrites and is thus well situated to play a role in structural plasticity. To explore RGS14 roles in structural plasticity, studies examined induced spine plasticity in CA1 and CA2 pyramidal neurons (Evans, Parra-Bueno, et al., 2018). Hippocampal slices from WT and RGS14-KO mice expressing EGFP as a spine marker were subjected to local glutamate uncaging and imaged using two-photon fluorescence microscopy. RGS14 was found to negatively regulate long-term structural plasticity of dendritic spines in CA2 neurons (Evans, Parra-Bueno, et al., 2018). Loss of RGS14 (KO) resulted in a large increase in structural volume change when compared to WT mice (Evans, Parra-Bueno, et al., 2018). CA2 neurons lacking RGS14 also displayed

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significantly enhanced spine calcium transients during plasticity induction compared to WT control mice. Of note, viral delivery of RGS14 to CA2 neurons of KO animals rescued the WT phenotype (no plasticity) and RGS14 delivery to CA1 neurons, where RGS14 is not normally expressed, blocked normal plasticity there. Together, these findings indicate that RGS14 plays a vital role in synaptic plasticity by regulating both synaptic strength and structural plasticity, in part by restricting calcium in CA2 spines (Evans, Parra-Bueno, et al., 2018). Parallel work found that RGS14 directly binds Ca^{++}/CaM and is phosphorylated by CaMKII as possible mechanisms for RGS14 regulation of calcium signaling in postsynaptic spines (Evans, Gerber, et al., 2018) (Figure 1), though further work is needed to define specific mechanisms.

Based on our findings that RGS14 suppresses synaptic plasticity, we speculate that the absence of RGS14 during early postnatal development (until P7) may allow for a period of enhanced learning and linked plasticity that enable pups to develop social recognition. Social recognition memory in animals is essential for social hierarchy, mate and offspring recognition, territorial defense, interspecies recognition, and for the general establishment and maintenance of groups (Ferguson, Young, & Insel, 2002; Tzakis & Holahan, 2019). We speculate that the absence of RGS14 may lead to enhanced plasticity in CA2 neurons, thereby allowing pups to connect with their environment and form strong bonds with their littermates and caregivers, as these forms of memories require CA2 (Laham, Diethorn, & Gould, 2021). After P7, the upregulation of RGS14 and its sustained expression throughout adulthood may serve to filter episodic memories, creating a period to develop new synapses that are structured based on environmental cues. Further studies will be needed to test these ideas.

In rodents, RGS14 protein expression is also detected outside of area CA2, specifically in regions that send environmental cues to the hippocampus including the anterior olfactory nucleus (AON), and piriform, orbital, and entorhinal cortex, with transient labeling in the neocortex (Evans et al., 2014). Rodent cortical structures for odor perception and spatial memory are evolutionarily and developmentally linked and, together, form part of the allocortex (Poo, Agarwal, Bonacchi, & Mainen, 2022). The piriform cortex receives input via the olfactory bulb projection neurons, and its circuit architecture shares a functional resemblance to the hippocampus. Both structures exhibit plasticity of broadly distributed and unstructured recurrent connections, suggesting that both brain regions share similar associative learning functions (Poo et al., 2022). In rodents it has been shown that odor codes support episodic memory and social memory (Manns, Howard, & Eichenbaum, 2007; Petrulis, Alvarez, & Eichenbaum, 2005; Strauch & Manahan-Vaughan, 2020). We can speculate that the presence of RGS14 in AON and piriform cortex is well positioned to modulate primary olfactory inputs playing a role in guiding rodent behaviors and processing, which may serve to associate certain odorants with specific events (Evans et al., 2014; Wilson & Sullivan, 2011). The presence of RGS14 in orbital and entorhinal cortical neurons, which receive inputs from the primary olfactory cortical areas, suggests that RGS14 can play an important role in regulating olfactory processes. Further studies are needed to support these ideas.

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RGS14 expression in primate and human hippocampus

RGS14 in primate brain has not been extensively explored, though recent studies have characterized RGS14 protein expression in adult rhesus macaques and humans (Squires, Gerber, et al., 2018). In the macaque brain, RGS14 was found in hippocampus much like rodents, but also was highly expressed in other limbic structures and the basal ganglia. Strong immunoreactivity was observed in the caudate nucleus, putamen, substantia nigra pars reticulata, globus pallidus, and moderate staining in the amygdala and nucleus accumbens (NAc) (Squires, Gerber, et al., 2018). Recent findings show that RGS14 is also expressed in these same limbic structures and basal ganglia of rodents (Foster et al., 2021), and speculation about RGS14 roles in these brain regions and their relationship to the hippocampus will be discussed in depth below.

Within the primate hippocampus, RGS14 is robustly expressed in CA2 and CA1 regions, but absent in CA3 and the dentate gyrus (Squires, Gerber, et al., 2018). Consistent with protein expression in rodent CA2, RGS14 was found in pyramidal cell bodies and dendrites. In contrast to rodent CA1 region which shows a very limited expression of RGS14 in a few neurons (Lee et al., 2010), strong RGS14 expression was found in the neuropil pre- and postsynaptic profiles, pyramidal cell bodies, and proximal dendritic profiles of primate (Squires, Gerber, et al., 2018). In human brain, neurologically healthy post-mortem tissue showed strong RGS14 expression in pyramidal cells of CA2 and CA1 but was absent in area CA3 (Squires, Gerber, et al., 2018). The neuropil staining in human CA1 and CA2 was lighter than the monkey brain (Squires, Gerber, et al., 2018), likely due to protein degradation during post-mortem delay and immersion fixation of the tissue (Squires, Gerber, et al., 2018). Overall, RGS14 expression in human hippocampus is consistent with what is reported for rodent and monkey brain. Monkey and human brain show strong RGS14 expression in pyramidal neurons of area CA2, consistent with that observed in rodents (Squires, Gerber, et al., 2018). Strong immunoreactivity is found in cellular regions and neuropil of primate CA1, specifically in glutamatergic nerve terminals that originate from CA2 axonal projections, cell bodies, dendrites, and spines of pyramidal neurons (Squires, Gerber, et al., 2018). To date, the presynaptic functions of RGS14 in CA1 remain unexplored.

RGS14 protein expression outside of the hippocampus

As mentioned, RGS14 is expressed in brain regions outside of the hippocampus in both primate (Squires et al 2018) and rodent (Foster et al 2021) brain (Figures 2 & 3). In monkey brain (Figure 2), RGS14 was found throughout the rostro-caudal extent of structures of the basal ganglia, including the caudate nucleus and putamen, and in lower density in the NAc (Squires, Gerber, et al., 2018). RGS14 immunoreactivity in monkey striatum was found in postsynaptic dendrites and spines in GABAergic projections, including the nucleus and cytosol of neurons in striatal projections (Squires, Gerber, et al., 2018). Expression of RGS14 was also found in both external and internal segments of the globus pallidus, specifically in presynaptic small unmyelinated axons and axon terminals (Squires, Gerber, et al., 2018). RGS14 also localized to pre-synaptic regions of the substantia nigra pars reticulata (Squires, Gerber, et al., 2018). Within the amygdala, strong RGS14 immunoreactivity was found in the basomedial, basolateral, and centrolateral nuceli, while

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the lateral amygdala displayed lower intensity of RGS14 (Squires, Gerber, et al., 2018). In human brain, besides the hippocampus, RGS14 was also found within the neuropil of the caudate nucleus and putamen, with a woolly fiber like pattern of axonal labeling in pallidal segments (Squires, Gerber, et al., 2018). Speculative roles for RGS14 in limbic structures will be discussed in greater detail below.

Of note, putative RGS14 splice variants were observed in the nuclei of striatal neurons in monkey (Squires, Gerber, et al., 2018). This unexpected finding matches previously reported human splice variants of RGS14 with different molecular weights (Cho et al., 2005; Martin-McCaffrey et al., 2004; Martin-McCaffrey et al., 2005; Squires, Gerber, et al., 2018; P. Zhao, Nunn, Ramineni, Hepler, & Chidiac, 2013). The three reported human variants lack the RGS domain, and two of the variants retain the GPR motif with enhanced binding to inactive Gα (Squires, Gerber, et al., 2018; P. Zhao et al., 2013). These observations suggest that these truncated forms of RGS14 could regulate signaling in the striatum by modulating the MAPK/ERK and/or Ca2+/CaM pathways (i.e. independent of G protein signaling), which are key for modulating synaptic plasticity and gene transcription in striatal neurons (Hutton et al., 2017). Most forms of synaptic plasticity require an increase in intracellular calcium, and RGS14 has been reported to restrict plasticity in hippocampal CA2 by limiting postsynaptic calcium (Evans, Gerber, et al., 2018; Evans, Parra-Bueno, et al., 2018). With this information, we can speculate that RGS14 splice variants lacking the RGS domain in monkey striatum may play role in synaptic plasticity by integrating the MAPK/ERK and calcium signaling pathways. Further studies are needed to test this idea.

RGS14 expression in the primate limbic system and implications for linked behaviors

The limbic system is a neuroanatomical network of discrete structures responsible for emotional processing (Figure 4), and plays a major role in attention, memory, and decision making. The predominant structures of the limbic system are the hippocampus and amygdala, and RGS14 is highly expressed in both structures (Figure 2). The amygdala is an almond-shaped cluster of cortical-adjacent nuclei critical to mediating behavioral and physiological responses to emotionally salient stimuli. Amygdaloid RGS14 in primates is found in the basolateral, basomedial, and centrolateral subregions, as well as the amygdalostriatal transition area (Squires, Gerber, et al., 2018). The anatomical and functional organization of the amygdala has been extensively detailed (Sah, Faber, Lopez De Armentia, & Power, 2003), but we will briefly describe the RGS14-relevant subdivisions here.

The RGS14-containing basomedial and basolateral nuclei are components of the basolateral complex, which, in combination with the lateral nucleus, are collectively referred to as the basolateral amygdala (BLA). The BLA is responsible for the affective valuation of stimuli, as well as the acquisition and expression of both fear- and reward-based learning (Bliss-Moreau et al., 2011; Sun, Gooch, & Sah, 2020; Wassum & Izquierdo, 2015). This brain region is the primary input structure of the amygdala, receiving projections from sensory cortex, prefrontal cortex, hippocampus, and thalamus. In addition to its substantial intra-amygdaloid connections, the BLA sends projections back to the prefrontal cortex, hippocampus, and thalamus. It also extensively innervates the NAc, exerting strong

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influence over motivational drive (Ambroggi, Ishikawa, Fields, & Nicola, 2008; Kochli, Keefer, Gyawali, & Calu, 2020; Stuber et al., 2011).

The central amygdala (CeA) activates physiological and behavioral responses to evocative stimuli through its descending connections to the midbrain and brainstem, as well as subcortical projections to the hypothalamus and bed nucleus of the stria terminalis (BNST). The CeA is critical to behavioral, autonomic, and neuroendocrine stress responses (Callahan, Tschetter, & Ronan, 2013; Hitchcock & Davis, 1986; Kalin, Shelton, & Davidson, 2004; Mogenson & Calaresu, 1973; Stock, Schlor, Heidt, & Buss, 1978). The lateral CeA subregion specifically contains the largest population of corticotropin-releasing factor (CRF)-immunoreactive neurons outside of the hypothalamus (Cassell, Gray, & Kiss, 1986; Cummings, Elde, Ells, & Lindall, 1983). Based on previous findings that RGS14 is a suppressor of LTP in CA2, RGS14 in this region may provide an inherent limit on the capacity of fearful stimuli to activate physiological stress responses. The CeA serves primarily as an output nucleus that is strongly innervated by the BLA, which recruits the CeA for involvement in expression of conditioned fear responses (Sun et al., 2020), though a more nuanced examination of CeA subregions has identified the lateral CeA as important for acquisition of fear conditioning as well (Ciocchi et al., 2010).

In fear conditioning, the lateral amygdaloid nucleus mediates pairing of an unconditioned stimulus with a conditioned stimulus, known as a cue (Romanski, Clugnet, Bordi, & LeDoux, 1993). This region is conspicuously devoid of RGS14, suggesting that RGS14 may not be involved in the initial learning of conditioned-unconditioned stimulus associations. In contrast, while still the subject of debate, the RGS14-containing basal nuclei are thought to be important for maintaining the engram encoding (i.e. storing) learned associations (Davis & Reijmers, 2018). Considering the suppressive effect of RGS14 on hippocampal-based learning and memory, it is reasonable to speculate that its localization within the amygdala may position RGS14 to limit the capacity of emotionally salient stimuli, particularly fearful stimuli, to drive and change behavior. Consistent with this idea, genetic deletion of RGS14 increases freezing in a cued fear memory test in female mice (Alexander et al., 2019). CeA-CRF neuron innervation of the sexually dimorphic BNST is one viable explanation for this female-exclusive effect (Uchida et al., 2019), though further study is required. RGS14 knockout also augments thigmotaxis (movement along the periphery of an open field indicating preference for safety of enclosed spaces) in response to both novelty stress and an anxiogenic dose of cocaine (Foster et al., 2021). Lastly, a non-synonymous coding single nucleotide polymorphism in the RGS14 gene is linked to freezing in response to both cue and context in fear conditioning, though this suggests additional involvement of the hippocampus (Parker, Sokoloff, Cheng, & Palmer, 2012).

The overarching role of the hippocampus involves the mapping of one’s experiences to their environment. This type of cognition fundamentally requires the mechanisms of learning and memory, and as such, learning and memory have historically been attributed to and studied in the hippocampus. As outlined above, the hippocampus is an exceptionally RGS14-rich limbic region. In primates, RGS14 is expressed extensively and selectively in areas CA2 and CA1, with presynaptic terminals of CA2 projection neurons presumed to be the predominant source of RGS14 immunoreactivity in CA1 (Squires, Gerber, et al., 2018). Its highly

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exclusive expression pattern permits the use of RGS14 as an anatomical marker of area CA2.

Social memory is critically dependent on CA2 (Hitti & Siegelbaum, 2014; Meira et al., 2018; Smith, Williams Avram, Cymerblit-Sabba, Song, & Young, 2016; Stevenson & Caldwell, 2014; Young, Li, Wersinger, & Palkovits, 2006), and projections from CA2 onto ventral CA1 are necessary for dynamic aspects of social memory (Meira et al., 2018). CA2 mediates the formation and stabilization of the memory of the mother required for maternal preference in neonates, followed by nonfamilial preference in adulthood (Laham et al., 2021). Age-dependent hippocampal expression of RGS14 has been shown in mice, in which protein levels are absent at birth and then gradually increase throughout development until reaching a sustained plateau upon adulthood (Evans et al., 2014). Considering the importance of maternal bonding for early-life survival, in conjunction with the proclivity for social selectivity demonstrated in mature humans and chimpanzees (Rosati et al., 2020), one may speculate that the gradual increase of RGS14 in CA2 underlies adaptive changes in social behavior that occur over the progression of life stages. An important caveat to this idea stems from the relative complexity of primate social structures compared to rodents. Neurodevelopmental characterization of RGS14 expression in primates would help clarify this relationship. Within the hippocampus, area CA2 is distinctly targeted by the magnocellular projection neurons from the paraventricular nucleus of the hypothalamus (Cui, Gerfen, & Young, 2013; L. Zhang & Hernandez, 2013). The magnocellular neurons produce the neuropeptides oxytocin and vasopressin, which are established effectors of social processing in mammals. In primates and rodents, the brain region with the most abundant expression of *avpr1b,* the gene encoding the vasopressin 1b receptor (V1bR), is area CA2 (Young et al., 2006). Expression of *avpr1b* in CA2 is crucial to intruder recognition in male mice, and V1b receptor signaling permits synaptic plasticity in CA2 (Pagani et al., 2015; Wersinger, Ginns, O'Carroll, Lolait, & Young, 2002). Exploring possible roles for RGS14 in regulating V1bR signaling in CA2 would be of interest.

In addition to social memory, CA2 neuronal ensembles encode temporal information as well as spatial information, particularly spatial information during periods of immobility and sleep (Kay et al., 2016; Mankin, Diehl, Sparks, Leutgeb, & Leutgeb, 2015). CA2 is also capable of driving spatial memory via inputs to CA1, though this is suppressed under normal conditions due to the unique resistance of CA2 neurons to LTP, which is partially imposed by RGS14 (Evans et al., 2015; L. Zhang & Hernandez, 2013). RGS14 roles in CA2-driven spatial memory are demonstrated by improved performance in the Morris water maze and novel object recognition in RGS14-null mice (Lee et al., 2010).

RGS14 expression in primate basal ganglia and implications for linked behaviors

RGS14 also is found in neurons outside of hippocampal and other limbic structures within regions of the basal ganglia (Figures 2 & 4) (Squires, Gerber, et al., 2018). While its molecular/cellular actions have not been examined in these neurons, we speculate that RGS14 is capable of suppressing postsynaptic calcium and ERK signaling to block LTP in these neurons much like it does in area CA2 (Lee et al., 2010; Zhao et al., 2007). The basal ganglia are a set of interconnected subcortical nuclei principally involved in

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selecting actions and coordinating subsequent movements, and include the striatum (ventral and dorsal), globus pallidus (internal and external), ventral pallidum, substantia nigra (pars compacta and pars reticulata), and subthalamic nucleus (Figures 2 & 4). RGS14 is found throughout the primate and rodent basal ganglia, including robust expression in both the ventral striatum (specifically in NAc) and dorsal striatum (caudate nucleus and putamen). The striatum is the primary input node of the basal ganglia, integrating coincident inputs mainly from the hippocampus, amygdala, cortex, and thalamus into a unified output that either drives or inhibits action (Lanciego, Luquin, & Obeso, 2012). The ventral striatum is critical to goal-directed behavior and reward-based learning, while the dorsal striatum processes habitual behavior and habit formation. The signaling that controls behavioral responses to internal and external stimuli flows through broadly hierarchical cortico-striatal-thalamo-cortical loops (Hunnicutt et al., 2016; Kalivas, 2009) that allow for behavioral flexibility in the face of changing contingencies (Cox & Witten, 2019; Schultz, 2016).

Striatal spiny projection neurons are generally subdivided by their participation in either the basal ganglia direct pathway or the opposing indirect pathway, based on whether they innervate populations that project directly to the thalamus or ones that follow a more indirect route through the basal ganglia via an extra stop-off in the pallidum. RGS14 is found in "wooly fiber" axons and axon terminals in the external globus pallidus, which receives indirect pathway input, as well as the internal globus pallidus and substantia nigra pars reticulata, which are subject to both direct and indirect pathway modulation. Direct and indirect pathway neurons are also subdivided by largely exclusive expression of either D1 or D2 dopamine receptors, though this dichotomy is somewhat less reliable in the ventral striatum (Kupchik & Kalivas, 2017). Regardless, this classification is still conceptually useful in that activation of D1- and D2-containing ventral striatal spiny neurons exerts opposing effects on motivated behavior, being approach/appetitive behavior for D1-neurons and avoidance/aversive behavior for D2-neurons (Gore & Zweifel, 2013; Hikida, Kimura, Wada, Funabiki, & Nakanishi, 2010; LeBlanc et al., 2020; Murata et al., 2019). Our group has observed colocalization of RGS14 with both D1 and D2 receptors in murine NAc (unpublished observations, manuscript in progress). The expression pattern of RGS14 throughout both the basal ganglia direct and indirect pathways suggest a complex role in regulating behavioral drive, a topic of ongoing and future studies by our group.

Implications for RGS14 in human neurological disease states

Possible roles for RGS14 in addiction

As outlined above, RGS14 is expressed in brain regions linked to motivated reward behaviors. Addiction is a disordered state in which engaging in an initially rewarding behavior becomes uncontrollable and compulsive in the face of compounding or continued negative consequences. Traditionally, addiction has been considered in terms of the intake of reinforcing psychoactive substances such as cocaine, opiates, and alcohol. More recently, the definition has expanded to include behaviors that are in and of themselves reinforcing, including gambling, eating, and social media use (Sun & Zhang, 2021). The progressive course of addiction can be broken down into four stages based on the distinct

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neurobiological mechanisms at play: acquisition, maintenance, cessation/abstinence, and relapse.

RGS14 is enriched in brain structures linked to reward behavior and addiction, including the ventral and dorsal striatum, the BLA, CeA, and hippocampus (Squires, Gerber, et al., 2018). Based on the known role of RGS14 in suppressing hippocampal-based learning and plasticity, we speculate it could be operating in a similar fashion throughout the limbic system to potentially interfere with the underlying neurobiology of addiction at multiple points. Individuals are initially driven to engage in an addictive behavior because it elicits some form of positive (typically hedonic) response. Reward learning is mediated by dopamine release from the midbrain onto the NAc when a behavior leads to a better-than-predicted outcome. Meanwhile, the BLA and hippocampus record information conveying affect states, cue associations, and context, which are communicated to the NAc and serve to reinforce the behavior (Bliss-Moreau et al., 2011; Paton, Belova, Morrison, & Salzman, 2006; Puaud, Higuera-Matas, Brunault, Everitt, & Belin, 2021).

Repeated engagement in an addictive behavior gradually switches its processing from ventral to dorsal striatum, which is a key mechanism of progression to the maintenance stage of addiction as learned behaviors become habitual (Everitt & Robbins, 2016). It is accompanied by homeostatic adaptations to chronic reward system overactivation, including blunted reward function (e.g., decreased NAc dopamine neurotransmission), a byproduct of which is increased recruitment of stress response systems (e.g., increased CRF release from the CeA) (de Guglielmo et al., 2019; Liu et al., 1989; Volkow et al., 1993; Volkow et al., 1996; Volkow et al., 2007), and results in escalation of the behavior (de Guglielmo et al., 2019; Liu et al., 1989). In the maintenance stage, allostatic brain changes cause the negative affective and interoceptive states associated with withdrawal that drive compulsive drug-taking (Gilpin, Herman, & Roberto, 2015; Koob & Le Moal, 2008). Some brain changes that occur as a consequence of addiction-related plasticity can become remarkably hardened and persistent (Scofield et al., 2016).

Cessation/abstinence from addiction is thought to occur through extinction learning, which overrides but does not erase the original learning (Hermans et al., 2005; Pearce & Bouton, 2001; Vervliet, Vansteenwegen, Baeyens, Hermans, & Eelen, 2005). This results in reward- and extinction-related memories that can be variably recalled (N. Sun et al., 2011). A variety of influences can reinstate extinguished craving leading to relapse, including addiction-associated cues, contexts, and companions (de Wit & Stewart, 1981; LeCocq, Sun, & Chaudhri, 2022; Meng et al., 2021) (“people, places, and things”).

RGS14 suppresses learning and memory in area CA2. While speculative, it may be the case that RGS14 acts as an endogenous defender against addiction by instituting a natural break against “runaway” associative and habitual learning and memory that occurs throughout the limbic system during addiction. Ongoing and planned studies by our group aim to clarify the role of RGS14 in addiction-related learning and memory. Our preliminary results suggest that RGS14-KO mice show heightened vulnerability to the locomotor sensitizing effects of cocaine, a behavioral correlate of cocaine-induced neuroadaptations (Bramlett et al., 2021). The high expression of RGS14 in CA2 hints that RGS14 may be relevant to social

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learning and memory during addiction, given the role of CA2 in social processing (Dudek, Alexander, & Farris, 2016; Tzakis & Holahan, 2019).

Possible roles for RGS14 in anxiety disorders

RGS14 also is expressed in brain regions linked to anxiety, most notably structures of the limbic system including the amygdala and hippocampus. Anxiety is a complex emotion derived from fear, characterized by hypervigilance, sympathetic activation, and feelings of unease that occur in anticipation of dangers perceived to be “just on the horizon”. The contemporary prevalence of anxiety disorders is high, affecting up to one-third of the population during their lifetime (Bandelow & Michaelis, 2015). The amygdala is key to both fear and anxiety (Kalin et al., 2004; S. C. Lee, Amir, Haufler, & Pare, 2017), though the macrostructure of striatal and striatal-like nuclei known as the extended amygdala is thought to play a larger role in anxiety generation due to its processing of less-specific and psychogenic fear responses (Shackman & Fox, 2016). The hippocampus is also important for anxiety processing, with differentiable functions observable along the dorsal-to-ventral axis (posterior-to-anterior in humans). The dorsal hippocampus is important for contextual encoding of anxiogenic stimuli, while the ventral hippocampus mediates avoidance behavior (Barkus et al., 2010). Hippocampal dysfunction is linked to enhanced aversive learning and impaired extinction of avoidance behavior, and reduced hippocampal plasticity is observed in a rat model of anxiety vulnerability (Cominski, Jiao, Catuzzi, Stewart, & Pang, 2014).

Studies done by us and our collaborators suggest an inhibitory effect of RGS14 in fear and anxiety processing in mice, evidenced by an increased thigmotaxis response to novelty stress in RGS14-KO mice and increased cue-conditioned freezing to a fearful stimulus in RGS14-KO females (Alexander et al., 2019; Foster et al., 2021). RGS14 limits spatial memory by suppressing dendritic spine growth in hippocampal neurons and may be doing the same in the amygdala to limit anxiety-like behavior (Evans, Parra-Bueno, et al., 2018). This is supported by the fact that enhanced amygdala size, connectivity, and/or laterality are linked to anxiety disorders in humans, including social anxiety disorder, generalized anxiety disorder, separation anxiety disorder, panic disorder, and specific phobias (Etkin, Prater, Schatzberg, Menon, & Greicius, 2009; Redlich et al., 2015; Roy et al., 2013; Shin & Liberzon, 2010; Suor, Jimmy, Monk, Phan, & Burkhouse, 2020; Wang et al., 2021).

RGS14 is known to suppress plasticity in area CA2, a region known to be critical for social processing. A dorsal CA2 → ventral CA1 → NAc shell circuit has been identified to mediate social memory, demonstrated by the inability to distinguish novel and familiar conspecifics upon inactivation(Meira et al., 2018; Raam, McAvoy, Besnard, Veenema, & Sahay, 2017). RGS14 could be a gatekeeper of social memory in either or both the hippocampus and NAc, in which case RGS14 dysfunction is likely to particularly impact social anxiety disorder, though further studies are required to test this idea.

Anxiety and stress response systems are highly interconnected by reciprocal projections between the amygdala and locus coeruleus and innervation of the paraventricular and lateral thalamic nuclei by the extended amygdala (specifically medial CeA and BNST) (Daviu, Bruchas, Moghaddam, Sandi, & Beyeler, 2019). CeA RGS14 expression is restricted to the lateral subregion, which as mentioned previously, contains the largest extrahypothalamic

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population of CRF+ neurons (Cassell et al., 1986; Cummings et al., 1983). Lateral CeA-CRF neurons innervate the BNST as part of a relay system to the hypothalamus that causes sympathetic activation (Lebow & Chen, 2016). Conscious or unconscious interoception of stress response activation (e.g. feeling increased heart rate) causes the BLA to communicate negative affect states (e.g. uneasiness and discomfort) (Knuepfer, Eismann, Schutze, Stumpf, & Stock, 1995). Indirect projections from the BLA to the NAc (by way of ventral hippocampus and extended amygdala) translate negative affect into aversive learning (e.g., fear conditioning) and avoidance behavior (Delgado, Li, Schiller, & Phelps, 2008). Amygdala RGS14 is poised to mitigate stress-induced anxiety by tempering sympathetic activation via its CeA activity, and the development of disordered anxiety-like behavior via its actions in the BLA (also possibly NAc).

Possible roles for RGS14 in other brain disorders

Due to its specific regional expression in area CA2 of the hippocampus, RGS14 may play a part in disorders of social function. Of particular interest is the possible role of RGS14 in autism spectrum disorder, a neurodevelopmental condition characterized by deficits in social behavior and communication that typically emerge in early childhood. Several genes linked to autism are downregulated in CA2 (Shen, Overly, & Jones, 2012). The gradual increase of CA2 RGS14 expression throughout maturation into adulthood may underlie a closing developmental window for social behavior that could explain why early intervention in autism is most effective in improving outcomes (Evans et al., 2014). Consequently, manipulations or mutations that alter RGS14 levels or function may be relevant to autism development. Evidence for this comes from a recent study which showed that genetic knockout of the mineralocorticoid receptor leads to a dramatic loss of RGS14 expression as well as impairment of social discrimination (McCann et al., 2021). The gene for the mineralocorticoid receptor, *Nr3c2*, is listed by the Simons Foundation Autism Research Initiative as being mutated in some syndromic autism (Banerjee-Basu & Packer, 2010).

The gene encoding RGS14 has been identified as a risk gene in schizophrenia (Nguyen et al., 2017). Schizophrenia involves social impairments and is associated with a substantial loss of non-pyramidal neurons in CA2 (Benes, Kwok, Vincent, & Todtenkopf, 1998). Compared to other hippocampal subregions that express much lower amounts of RGS14, CA2 is uniquely resistant to epileptic injury (Haussler, Rinas, Kilias, Egert, & Haas, 2016). A recent study found that RGS14 protects against inflammatory damage in the kidneys, raising the possibility that it also mitigates insults in CA2 to prevent schizophrenia pathogenesis (J. K. Zhang et al., 2022). Parkinson’s disease is a neurodegenerative disease caused the progressive destruction of midbrain dopamine neurons. Reduced levels of RGS14 in the putamen of Parkinson’s disease patients may also be evident of a reduced capacity to regulate neuroinflammation in these patients (Lewis & Cookson, 2012). Further study is required.

Conclusions and Future Directions

The possible implications for RGS14 in human disease, especially psychiatric and cognitive disease, are wide-ranging and multifaceted. Several RGS proteins have been linked to

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neurological diseases including RGS4, RGS9–2, and RGSz (Hooks, Martemyanov, & Zachariou, 2008; Sakloth, Polizu, Bertherat, & Zachariou, 2020). However, to date, RGS14 dysfunction alone has not yet been shown to be causative (i.e., monogenic) of psychiatric and/or cognitive diseases. Nonetheless, impaired RGS14 actions in combination with disruption of other signaling proteins/pathways (polygenic), likely contribute to neurological dysfunction and/or differences in individual traits (Squires, Montanez-Miranda, Pandya, Torres, & Hepler, 2018). For example, in CA2 hippocampus, RGS14 serves as a natural suppressor of synaptic plasticity and may act to minimize neuronal damage following tissue insult or seizure induction. Consistent with this idea, CA2 hippocampus is protected against neuronal injury following seizure (Steve, Jirsch, & Gross, 2014). We speculate that in the other limbic and basal ganglia regions where it has been found, RGS14 operates similarly as a suppressor of synaptic plasticity. Altogether, the brain regions that express RGS14 are associated with processing learning and memory (especially social memory), emotion, motivation, and movement. Though speculative at this point, RGS14 could be regulating any number of behaviors involving these functions or the interplay between them. For example, impaired RGS14 function could increase drug seeking behavior when an addicted person feels the dysphoric effects of withdrawal, or it could impede the development of social relationships in a person with autism.

Despite the conceivable therapeutic relevance of RGS14, investigation of its roles in human disease remains largely unexplored. Current animal studies of RGS14 have predominantly been performed in mice due to availability of an established RGS14-KO mouse line. Studies of RGS14 in mouse brain are valuable, but are limited in human translatability because RGS14 is expressed at different levels in certain brain regions in rodents relative to primates (Figs. 2 and 3, and also see Fig. 2 in: (Harbin et al., 2021)); however note that RGS14 is one of the few proteins highly expressed in CA2 in both mice and humans (Evans et al., 2014; Lein et al., 2007; Squires, Gerber, et al., 2018). In addition, human RGS14 contains a C-terminal PDZ motif that is not present in the rodent homolog that binds PDZ proteins in humans (Friedman et al., 2022). While differential expression patterns are more difficult to address, differences in RGS14 protein homology between primates and rodents could be addressed by performing studies on “humanized” mice manipulated to express human RGS14. Such studies would help to further elucidate roles for RGS14 at postsynaptic spines, in particular the role of RGS14 in regulating the various abundant and important PDZ regulatory proteins at the post-synaptic density (PSD).

Another issue to consider is that primates have more complex social structures and relationships than rodents, which makes studies of RGS14 effects on social behavior particularly difficult to translate from mouse models. While genetic manipulation of RGS14 in laboratory primates is currently not practicable, a more feasible approach would be to examine RGS14 genetic variants and expression levels post-mortem in primate brains that have been previously characterized for social behavior and social memory. It would also be useful to examine post-mortem brain tissue from humans diagnosed with autism, social anxiety disorders, and other social deficits. Lastly, the current gold standard for models of drug addiction is drug self-administration, which is generally not feasible to perform in mice due to the technical difficulty of implanting intravenous catheters at such a small scale. This

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could be addressed by genetically altering RGS14 expression using AAV or CRISPR/Cas9 techniques in rats, which are commonly used in self-administration studies.

In addition to these possible avenues for future RGS14 research, other studies are underway to examine novel roles for RGS14 in brain. As discussed above, RGS14 contains both a nuclear localization sequence (NLS) and nuclear export sequence (NES) and is a nucleocytoplasmic shuttling protein. Related to this, splice variants of RGS14 have been reported to lack the RGS domain, and RGS14 has been observed in the nuclei of primate striatal neurons suggesting non-canonical roles for RGS14 (Squires, Gerber, et al., 2018). To date though, RGS14 in the nucleus remains largely unexplored. A human genetic variant of RGS14 that disrupts nuclear export (L505R) localizes and accumulates in the nuclei of neurons when introduced to cultured neurons or whole mice (Squires et al., 2021). This mouse model is being investigated to define the impact of constitutive RGS14 nuclear localization on neuronal function and animal behavior. This and other genetic variants of RGS14, as well as other RGS14 splice variants are the subject of ongoing investigation.

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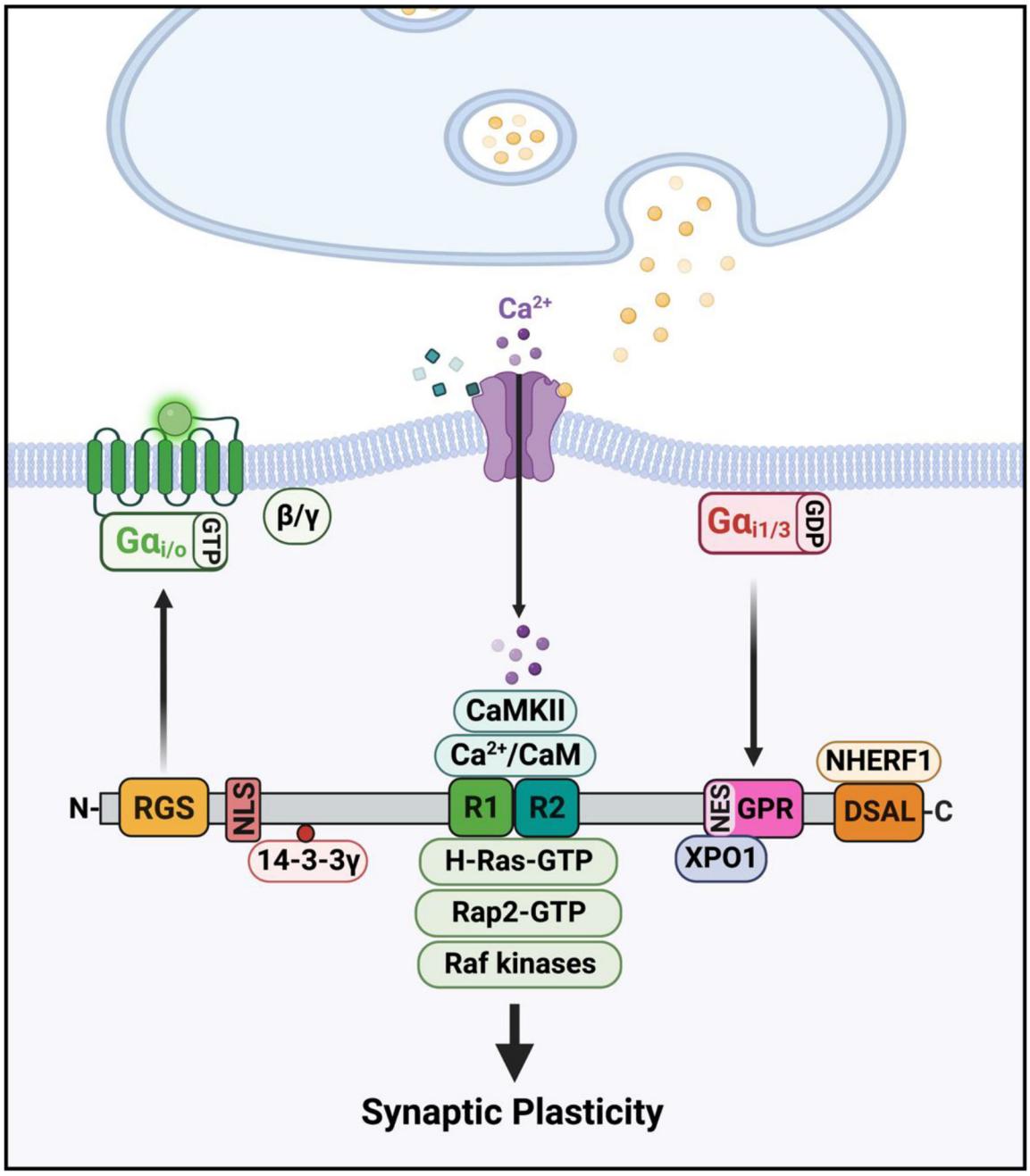

Figure 1: RGS14 binding partners and regulation of postsynaptic plasticity.

Human RGS14 (566 amino acids, ~63 KDa) is a multi-domain scaffolding protein that is highly expressed in postsynaptic dendritic spines of pyramidal neurons in hippocampal area CA2. The protein contains an RGS domain that binds non-selectively to active GTP-bound Gαi and Gαo isoforms to serve as a GTPase activating protein (GAP). RGS14 also contains tandem Ras-binding domains (R1 and R2). R1 binds active GTP-bound H-Ras and Rap2. RGS14 also bind Raf kinases (Raf1 and B-Raf). RGS14 is unusual in that it contains a GPR motif that selectively binds a second Gα subunit, specifically inactive GDP-bound Gαi1 or Gαi3, which anchor the protein complex at the plasma membrane. Human RGS14 also

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contains a C-terminal PDZ binding motif (-DSAL) that binds the PDZ protein NHERF1. RGS14 is phosphorylated at Ser218 which allows binding of 14-3-3γ to stabilize the protein complex in the cytosol. RGS14 is a nucleocytoplasmic shuttling protein that contains a nuclear localization sequence (NLS) and a nuclear export sequence (NES), the latter of which binds exportin (XPO1). RGS14 also binds activate calcium calmodulin (Ca^{2+}/CaM) and Ca^{2+}/CaM-Kinase-II (CaMKII), though the exact binding sites for these proteins are undefined. This image was created with BioRender.com

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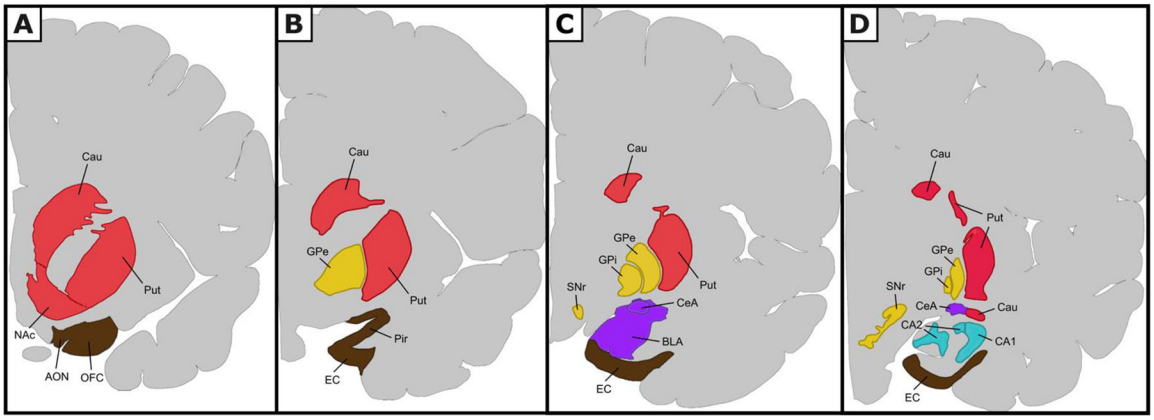

Figure 2: RGS14 protein expression pattern in primate brain.

Images are presented rostral/front (*A*) to caudal/back (*D*). RGS14 is expressed in structures of the basal ganglia including the striatal (red) regions comprised of the caudate nucleus (Cau), putamen (Put) (*A*-*D*), and nucleus accumbens (NAc) (*A*), and also the non-striatal basal ganglia (yellow) regions that are the external globus pallidus (GPe) and internal globus pallidus (GPi) as well as the substantia nigra pars reticulata (SNr) (*C*-*D*). RGS14 also is found in structures of the limbic system: the olfactory areas (brown) including the entorhinal cortex (EC) (*A*-*D*), anterior olfactory nucleus (AON), (*A*) and piriform cortex (Pir) (*B*), amygdala (purple) regions including the basolateral amygdala (BLA) (*C*) and central amygdala (CeA), and in hippocampal (cyan) areas CA1 and CA2 (*D*).

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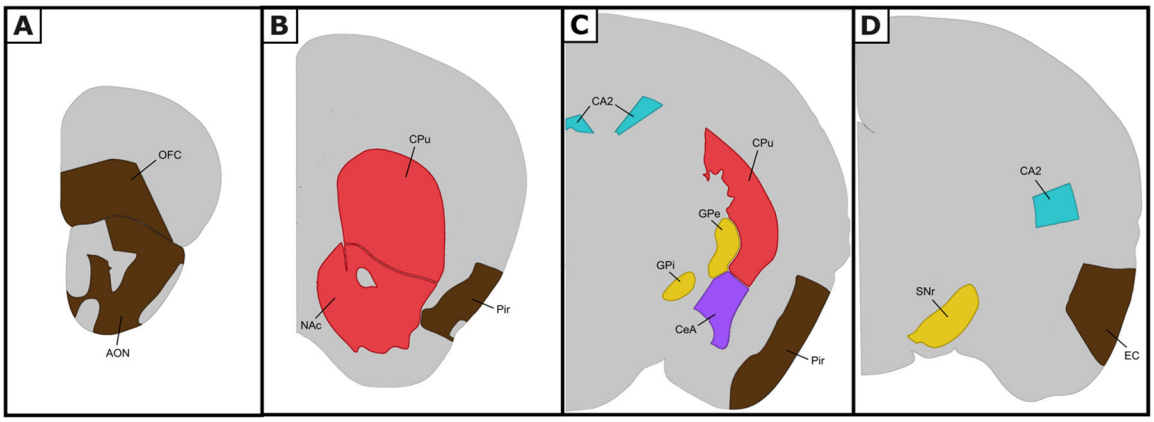

Figure 3: RGS14 protein expression pattern in mouse brain.

Images are presented rostral/front (*A*) to caudal/back (*D*). At the most rostral end of mouse brain (*A*), RGS14 is expressed in olfactory areas (brown) including the orbitofrontal cortex (OFC) and anterior olfactory nucleus (AON) and, moving more caudal, also in the piriform cortex (Pir) (*B-C*) and entorhinal cortex (*D*). RGS14 is expressed in rodent striatal (red) structures of the basal ganglia including the caudoputamen (CPu) and the nucleus accumbens (Nac) (*B and C*). Non-striatal basal ganglia structures where RGS14 is found (yellow) include the external globus pallidus (Gpe) and internal globus pallidus (Gpi) (*C*) as well as the substantia nigra (SNr) (*C and D*). in rodent brain, RGS14 is most highly expressed in limbic structures including hippocampal (cyan) area CA2 (*C and D*) and, to a lesser extent, the central amygdala (purple, CeA) (*D*).

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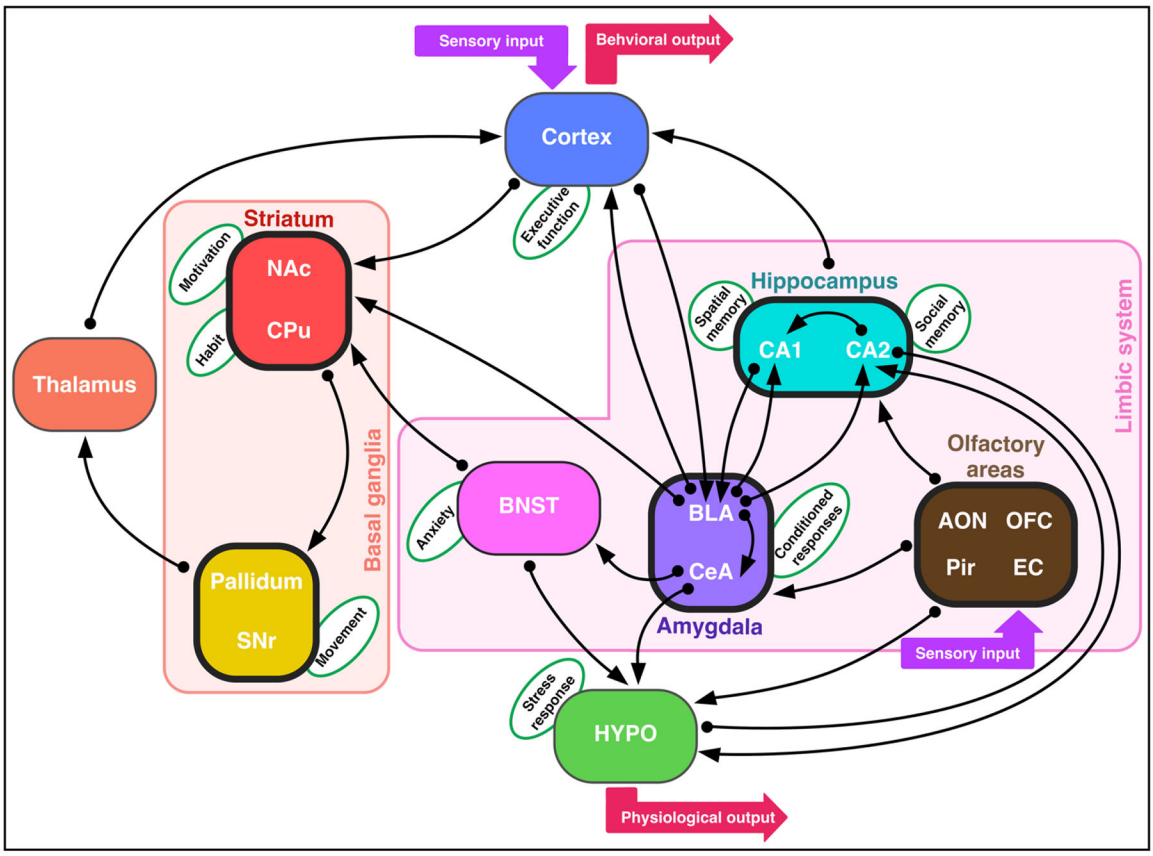

Figure 4: Major neurocircuits affected by RGS14.

Thick box borders indicate RGS14-expressing regions. Behavior is driven by a complex signaling flow between regions including the cortex, limbic system, basal ganglia, thalamus, and hypothalamus. See text for detailed descriptions. As a general overview, external information is first processed cortically (including olfactory cortex), then flows to the limbic system for emotional interpretation, then to the basal ganglia for processing of motivation and physical movement, then to the thalamus and back to the cortex for behavioral execution or inhibition. Limbic regions are also highly interconnected with the hypothalamus, which drives the physiological stress response. RGS14 may affect the synaptic strength of these connections at several points throughout the limbic system and basal ganglia. Abbreviations: AON = anterior olfactory nucleus, BLA = basolateral amygdala, BNST = bed nucleus of the stria terminalis, CeA = central amygdala, CPu = caudoputamen (rodents) or caudate and putamen (primates), EC = entorhinal cortex, HYPO = hypothalamus, NAc = nucleus accumbens, OFC = orbitofrontal cortex, Pir = piriform cortex, SNr = substantial nigra pars reticulata.