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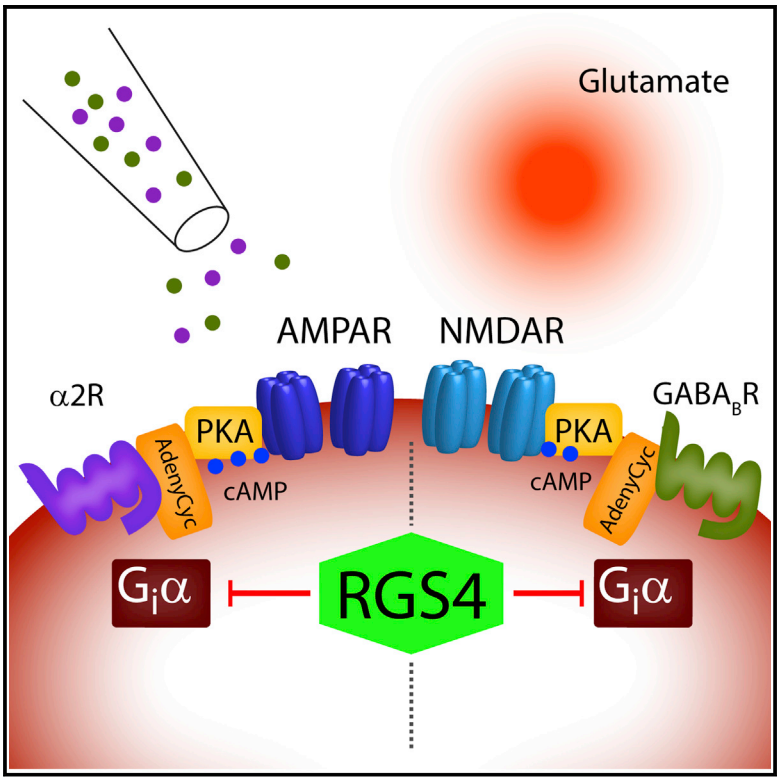
Glutamate Receptor Modulation Is Restricted to Synaptic Microdomains

Graphical Abstract



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In Brief

Lur and Higley demonstrate that norepinephrine and GABA differentially regulate AMPAR-mediated currents and NMDAR-mediated Ca^{2+} influx, respectively. These distinct actions are driven by downregulation of PKA signaling and occur due to the existence of functional microdomains that are maintained by receptor co-localization and the actions of RGS4.

Highlights

- Adrenergic α2Rs reduce AMPAR currents while $GABA_BRs$ reduce NMDAR Ca^{2+} influx
- Adrenergic and GABAergic control of glutamate receptors occurs via inhibition of PKA
- Modulatory microdomains are established by co-localization and the actions of RGS4

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Glutamate Receptor Modulation Is Restricted to Synaptic Microdomains

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SUMMARY

A diverse array of neuromodulators governs cellular function in the prefrontal cortex (PFC) via the activation of G-protein-coupled receptors (GPCRs). However, these functionally diverse signals are carried and amplified by a relatively small assortment of intracellular second messengers. Here, we examine whether two distinct $G\alpha_i$-coupled neuromodulators (norepinephrine and GABA) act as redundant regulators of glutamatergic synaptic transmission. Our results reveal that, within single dendritic spines of layer 5 pyramidal neurons, alpha-2 adrenergic receptors (α2Rs) selectively inhibit excitatory transmission mediated by AMPA-type glutamate receptors, while type B GABA receptors ($GABA_BRs$) inhibit NMDA-type receptors. We show that both modulators act via the downregulation of cAMP and PKA. However, by restricting the lifetime of active $G\alpha_i$, RGS4 promotes the independent control of these two distinct target proteins. Our findings highlight a mechanism by which neuromodulatory microdomains can be established in subcellular compartments such as dendritic spines.

INTRODUCTION

Neuromodulation via G protein-coupled receptors (GPCRs) provides a ubiquitous mechanism for regulating neuronal activity in the mammalian brain. In contrast to classical neurotransmitters that directly excite or inhibit postsynaptic neurons, neuromodulators alter neuronal excitability and modify synaptic transmission (Destexhe et al., 1994; Dismukes, 1979). Interestingly, there is a paradoxical mismatch between the diversity of modulatory ligands and the relative paucity of GPCR-linked second messenger systems such as adenylate cyclase and phospholipase C. The mobility of dissociated G protein subunits and downstream molecules such as calcium (Ca^{2+}), cAMP, and inositol-1,4,5-triphosphate should further reduce the cellular capacity for segregated signaling pathways. Nevertheless, there is evidence for the functional compartmentalization of soluble messengers into independent microdomains, which could contribute to neuromodulatory specificity. For example, rapid intracellular buffering coupled with potent extrusion mechanisms spatially restricts Ca^{2+} within presynaptic terminals and dendritic spines (Higley and Sabatini, 2008; Lisman et al., 2007; Yuste et al., 2000). However, the potential for mobile, non-ionic signaling molecules to be isolated within synaptic microdomains is largely unknown.

In the prefrontal cortex (PFC), neuromodulation by both norepinephrine (NE) and gamma-amino butyric acid (GABA) regulates higher cognitive functions, including attention and short-term "working" memory (Gamo and Arnsten, 2011; Kesner and Churchwell, 2011). Altered levels of NE and GABA are also linked to neuropsychiatric disorders, such as schizophrenia, attention deficit, and addiction (Arnsten, 2011; Stan and Lewis, 2012; Tyacke et al., 2010). Experimental evidence suggests that both type 2 alpha adrenergic receptors (α2Rs) and type B GABA receptors ($GABA_BRs$) modulate excitatory glutamatergic signaling in the PFC (Chalifoux and Carter, 2010; Ji et al., 2008; Liu et al., 2006). Additionally, ultrastructural studies have localized both α2Rs and $GABA_BRs$ to dendritic spines, the location of synaptic glutamate receptors (Kulik et al., 2003; Wang et al., 2007). Both α2Rs and $GABA_BRs$ are GPCRs coupled to the G protein subunit $G\alpha_i$, whose activation leads to the inhibition of adenylate cyclase and decreased production of cAMP (Knight and Bowery, 1996; Summers and McMartin, 1993). The subsequent reduction in cAMP-dependent protein kinase (PKA) activity provides a potential mechanism for the control of both AMPA- and NMDA-type glutamate receptors (AMPARs and NMDARs, respectively) (Chen et al., 2008; Esteban et al., 2003; Raymond et al., 1994). These observations raise the question of whether α2Rs and $GABA_BRs$ act as redundant modulators of prefrontal synaptic transmission.

To test this hypothesis, we combined electrophysiological recordings and two-photon imaging of PFC pyramidal neurons with optical stimulation of excitatory glutamatergic synapses using focal glutamate uncaging (Carter and Sabatini, 2004). Our results reveal the surprising observation that activating α2Rs reduces AMPAR-mediated responses, whereas activating $GABA_BRs$ decreases NMDAR-mediated responses. Notably, both modulatory pathways utilize $G\alpha_i$-mediated downregulation of cAMP and PKA signaling, and this dissociation occurs despite functional evidence that both α2Rs and $GABA_BRs$ are located in the same dendritic spines. We further find that inhibiting the GTPase activating protein RGS4 eliminates the selective compartmentalization of adrenergic and GABAergic actions. Thus, RGS4 promotes the independent control of two distinct