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# Arg Kinase Signaling in Dendrite and Synapse Stabilization Pathways: Memory, Cocaine Sensitivity, and Stress

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## Abstract

The Abl2/Arg nonreceptor tyrosine kinase is enriched in dendritic spines where it is essential for maintaining dendrite and synapse stability in the postnatal mouse brain. Arg is activated downstream of integrin α3β1 receptors and it regulates the neuronal actin cytoskeleton both by directly binding F-actin and via phosphorylation of substrates including p190RhoGAP and cortactin. Neurons in mice lacking Arg or integrin α3β1 develop normally through postnatal day 21 (P21), however by P42 mice exhibit major reductions in dendrite arbor size and complexity, and lose dendritic spines and synapses. As a result, mice with loss of Arg and Arg-dependent signaling pathways have impairments in memory tasks, heightened sensitivity to cocaine, and vulnerability to corticosteroid-induced neuronal remodeling. Therefore, understanding the molecular mechanisms of Arg regulation may lead to therapeutic approaches to treat human psychiatric and neurodegenerative diseases in which neuronal structure is destabilized.

### Keywords

Abl2/Arg; integrin α3β1; actin cytoskeleton; dendritic spine; neuronal stability; cocaine; corticosteroid

## Introduction

Neurons in the developing brain dynamically reorganize their dendritic branches and spines in order to acquire elaborate morphologies and integrate into active signaling networks. Once formed, however, neuronal structure must be maintained for extended periods of time to ensure proper connectivity. In fact, the destabilization of neuronal structure is commonly observed in many psychiatric and neurodegenerative diseases, where it is a contributing



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factor that compromises brain function. Research over the past decade has elucidated distinct signaling cascades that regulate long-term dendrite and dendritic spine stability (Lin and Koleske, 2010). This review will focus on Arg, a central regulator of neuronal stability. Arg (Abl-related gene, or Abl2) and its paralog Abl are the vertebrate members of the Abl nonreceptor tyrosine kinase family that also includes *C. elegans* Abl, and *Drosophila* (D−) Abl. These kinases play fundamental roles in translating extracellular signals from adhesion and growth factor receptors into cytoskeletal rearrangements that power changes in cell shape and movement (Bradley and Koleske, 2009).

Arg is most highly expressed in the brain, where it is enriched in dendritic spines, reaching a concentration of 400–600nM (Koleske et al. , 1998). The Arg N-terminal half has tandem SH3, SH2, and kinase domains. The SH3-SH2 domains form an inhibitory scaffold that engages the back of the kinase domain and holds it in an inactivate state (Nagar et al. , 2003). Engagement of the SH3, SH2, or kinase domains with cell-surface receptors, kinase substrates, and adaptor proteins is believed to release this inhibitory conformation and allow kinase activation. Additionally, following this release, kinase activation can be reinforced by phosphorylation of tyrosine residues in the activation loop between the SH2 and kinase domain (Bradley and Koleske, 2009, Tanis et al. , 2003). Abl family kinases also contain unique C-terminal extensions that interact with cytoskeletal regulators and directly with the actin and microtubule cytoskeletons. In particular, Arg, has at least three tandem SH3 domain binding motifs (P-XX-P), two distinct F-actin binding domains, and a microtubule binding domain (Bradley and Koleske, 2009, MacGrath and Koleske, 2012, Miller et al. , 2004, Wang et al. , 2001).

## Functions

Arg signaling is essential for a variety of diverse physiological roles beyond the scope of this review, including breast cancer invasion and metastasis, viral, bacterial, and parasite infection, T cell development, and neurotransmitter release (Bradley and Koleske, 2009). This review will focus on Arg function in the regulation of neuronal structural stability.

Dendrites and synapses develop normally in the hippocampus and cortex of mice lacking *arg* and are indistinguishable from wild type littermates in size and morphology by weaning at postnatal day 21 (P21). However, these structures are destabilized by early adulthood (P42), leading to significantly smaller dendrite arbors and a 30% loss of synapses, Figure 1 (Gourley et al. , 2012, Moresco et al. , 2005, Sfakianos et al. , 2007). Knockdown of *arg* (*argKD*) in neuronal cultures recapitulates the dendrite morphology and dendritic spine reductions found *in vivo*, indicating that Arg functions cell-autonomously to control morphological stability (Lin et al. , 2013). In mice, the loss of dendrites and synapses correlates temporally with the onset of impairment in novel object recognition, a behavior that is dependent on proper hippocampal and cortical connectivity. For example, $arg^{-/-}$ mice can identify an object as novel at P21, but lose this ability as they age to adulthood.

Arg has also been implicated in structural changes produced in response to cocaine administration. Chronic exposure to drugs of abuse causes dendritic spine rearrangements, behavioral inflexibility, drug sensitization, and ultimately addiction. For example, repeated cocaine administration reduces dendritic spine density and increases the head size of spines that remain within the prefrontal cortex (Gourley et al., 2012). The dendrites in $arg^{-/-}$ mice already contain fewer spines and the remaining spines do not enlarge in response to cocaine. As a result, Arg-deficient mice have an increased psychomotor response and behavioral sensitivity to cocaine administration (Gourley et al. , 2009, Gourley et al., 2012). Alterations in other key components of the Arg signaling cascade may also contribute to cocaine-induced pathology. For example, integrin β1 receptor, a major Arg regulator in the brain,

shows increased expression following cocaine exposure (Wiggins et al. , 2009) and its loss results in exaggerated psychomotor sensitivity to cocaine (Warren et al. , 2012).

The stress hormone corticosterone contributes to structural remodeling of dendrites and dendritic spines in multiple brain regions including the hippocampus, prefrontal cortex, and amygdala. Repeated stressor exposure induces immediate structural rearrangements (Vyas et al. , 2002), as well as persistent loss of dendritic arbors and spines in distinct brain regions (Gourley et al. , 2013). Interestingly, mice with a reduced gene dosage of the Arg substrate *p190RhoGAP* are vulnerable to behavioral and structural impairments at sub-threshold corticosterone exposure (Gourley et al., 2013), suggesting that Arg-mediated signaling events may contribute to neuronal response to stressor exposure. Future studies should identify the specific biochemical mechanism(s) responsible for this vulnerability.

## Cascades

### p190RhoGAP-RhoA control of dendrite stability

The Rho GTPase inhibitor p190RhoGAP (p190) is a major substrate of Arg. In neurons, active p190 inhibits RhoA GTPase to regulate dendrite arbor size (Hernandez et al. , 2004, Sfakianos et al., 2007). Arg phosphorylates p190 on Y1105, which, along with a second phosphorylation site Y1087, promotes p190 binding to two SH2 domains in p120RasGAP (p120). p120 uses its PH and CaLB domains to recruit the p190:p120 complex to the plasma membrane of cells where it attenuates RhoA GTPase activity at the cell edge (Bradley et al. , 2006). Elevated RhoA activity in neurons destabilizes dendrites via downstream effectors including ROCKII (Sfakianos et al., 2007, Threadgill et al. , 1997). Thus, Arg signaling through p190:p120 complex in neurons acts as a brake on RhoA activation to preserve dendrite stability. Reducing RhoA or ROCKII signaling in $arg^{-/-}$ mice and *argKD* cultures blocks hippocampal dendrite loss. However, this reduction in RhoA activity does not rescue synapse loss, resulting in fully elaborated dendritic arbors that have significantly fewer spines (Lin et al., 2013, Sfakianos et al., 2007). These data suggest Arg acts via additional mechanisms to regulate dendritic spine and synapse stability.

### NMDA receptor control of dendritic spine and synapse stability

Our lab has recently found that the reduction of dendritic spine density in *argKD* cultures can be rescued by blocking NMDA receptor activity via inhibition of the NR2B subunit with ifenprodil (Lin et al., 2013). This treatment does not rescue dendrite destabilization resulting in neurons with diminished dendrite arborization, but normal dendritic spine densities along the remaining branches. Furthermore, *argKD* neurons have altered synaptic transmission with a lower frequency and higher amplitude of mEPSCs consistent with the reduced number of synapses and larger spine head size (Lin et al., 2013). NMDA receptor expression and function is regulated by tyrosine phosphorylation of the intracellular tail of NR2B (Gladding and Raymond, 2011), however, mechanistic details of an interaction between Arg and the NMDA receptor are unknown. Future experiments should elucidate whether and how Arg interacts with the NR2B subunit to control synapse and spine stability.

### Cortactin control of dendritic spine and synapse stability

Actin is highly enriched in dendritic spines and its proper regulation is essential for spine morphology and dynamics (Fischer et al. , 1998). Cortactin is an actin regulatory protein that promotes the nucleation and stabilization of F-actin via NTA domain interactions with the Arp3-subunit of the Arp2/3 complex (Weaver et al. , 2001). Cortactin was identified as a substrate of Arg in an unbiased proteomic screen (Boyle and Koleske, 2007) and subsequently these two proteins were shown to interact through a series of binding and phosphorylation events. The cortactin SH3 domain binds to a specific P-XX-P motif in Arg,

and integrin-mediated adhesion stimulates Arg to phosphorylate cortactin. This phosphorylation creates a second binding site in cortactin for the SH2 domain of Arg (Lapetina et al. , 2009). Arg binding to actin filaments can also stimulate cortactin recruitment, allowing cortactin to bind to the actin filament at a higher density (MacGrath and Koleske, 2012). These Arg:cortactin interactions are a critical trigger of cell-edge protrusions in non-neuronal cells following simulation by growth factors or adhesion to extracellular matrix (ECM) (Lapetina et al., 2009, Mader et al. , 2011).

In neurons, cortactin localizes to dendritic spines and knockdown of Arg in cultured neurons reduces the amount of cortactin and F-actin in spine heads by 40% (Lin et al., 2013). Fusion of the Arg C-terminal domain to cortactin lacking its SH3 domain mimics an “activated” Arg-bound cortactin. This fusion protein relocalizes to dendritic spine heads and prevents both the loss of F-actin and reduction of dendritic spine density in *argKD* neurons, but does not protect from dendrite loss (Lin et al., 2013). These results support a model in which Arg regulates dendrites and dendritic spines via independent mechanisms. In mature neurons, spine and arbor stability are uncoupled allowing the neuron to simultaneously maintain overall structural stability, while remaining plastic in response to changes in activity. Dendrite stabilization is regulated by an Arg-p190-RhoA signaling axis, while Arg interactions with the NMDA receptor and cortactin control the morphology and dynamics of dendritic spines, Figure 2.

## Key molecules

### Integrins

Integrins are a class of adhesion receptors that serve as physical and functional links between the ECM and cytoskeletal control pathways (Campbell and Humphries, 2011). There are 18 known integrin heterodimers of αβ-subunits and a subset of these are expressed in neurons where they are enriched at synaptic membranes (Kerrisk et al. , 2013, Pinkstaff et al. , 1999). Integrin receptors in the brain function have diverse roles in regulating neuronal migration, synaptic plasticity, and synapse and dendrite morphology (Wu and Reddy, 2012). Arg binds directly and specifically to the intracellular tail of integrin β1 (Warren et al., 2012). Recently integrin α3 has been identified as the major partner for β1 that regulates Arg-mediated dendrite and dendritic spine maintenance (Kerrisk et al., 2013).

### Possible extracellular ligands

The neuronal ECM includes a vast number of molecules that activate cell-surface receptors such as integrin α3β1. ECM components are secreted from both neurons and glial cells in the brain to influence neuronal development, structure, maintenance, plasticity, and regeneration. Laminins are a major class of integrin α3β1 ligands and they regulate neuronal migration and stability *in vitro* (Dityatev et al. , 2010). For example, neurons cultured on laminin-coated plates respond by increasing the number and length of neurite branches. However, neurons cultured from $arg^{-/-}$ mice show no response to laminin, suggesting laminin may influence neuronal morphology via Arg (Moresco et al., 2005). Additional candidate ligands include Reelin (Dulabon et al. , 2000), Netrins (Stanco et al. , 2009), and Semaphorin7A (Moresco et al., 2005), each of which can bind integrin α3β1 and activate downstream signaling. Future studies should identify which of these potential ligands engage integrin α3β1 to regulate Arg kinase signaling and thereby control dendrite maintenance and synapse stability.

## Associated pathologies and therapeutic implications

Reductions in dendrite arbor size and complexity, dendritic spine and synapse densities, and synaptic connectivity are common pathologies in many psychiatric and neurodegenerative

disorders (Lin and Koleske, 2010). Mutations and deletions of genes encoding Arg signaling partners, including integrin α3, integrin β1, Arg, p190, and Rho-family GTPases, are found in human patients with impaired intellectual ability (Kerrisk et al., 2013, Lin and Koleske, 2010, Lin et al., 2013). Furthermore, single-nucleotide polymorphisms in laminin genes potentially upstream of Arg signaling have been identified in cocaine addiction (Mash et al. , 2007), autism spectrum disorders (O'Roak et al. , 2011), intellectual disability (Matejas et al. , 2010), and schizophrenia (van Schijndel et al. , 2009). Continued investigation of these signaling molecules and their biochemical interactions should reveal mechanistic details of their disruption in psychiatric and neurodegenerative diseases and if they can be targeted therapeutically to stabilize neuronal structure and ameliorate disease.

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## Abbreviations

| | |
|---|---|
| **Arg** | Abl-related gene |
| **F-actin** | filamentous actin |
| **SH2,SH3** | Src Homology Domain 1,2 |
| **P21,P42** | postnatal day 21, 42 |
| ***argKD*** | *arg* knockdown |
| **p190** | p190RhoGAP, 190 kD GTPase activating protein for Rho |
| **p120** | p120RasGAP, 120 kD GTPase activating protein for Ras |
| **PH** | pleckstrin homology domain |
| **CaLB** | $Ca^{2+}$-dependent phospholipid binding domain |
| **NMDA** | *N*-methyl-D-aspartate receptor |
| **mEPSC** | miniature evoked postsynaptic current |
| **NTA** | N-terminal acidic domain |
| **ECM** | extracellular matrix |

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### Signaling Network Facts

- Abl2/Arg is unique among nonreceptor tyrosine kinases because of its ability to directly bind the actin cytoskeleton and microtubules.
- Neurons in mice that lack Arg develop normally, but by late adolescence *arg*$^{-/-}$ mice have significantly smaller dendrite arbors, reduced numbers of dendritic spines and synapses, altered synapse morphology, and impairments in behavior.
- Arg differentially regulates dendrite maintenance and synapse stability via distinct biochemical mechanisms.
- Mutations in Arg signaling networks have been implicated in a variety of human disorders, including autism spectrum disorders, drug addiction, and schizophrenia.

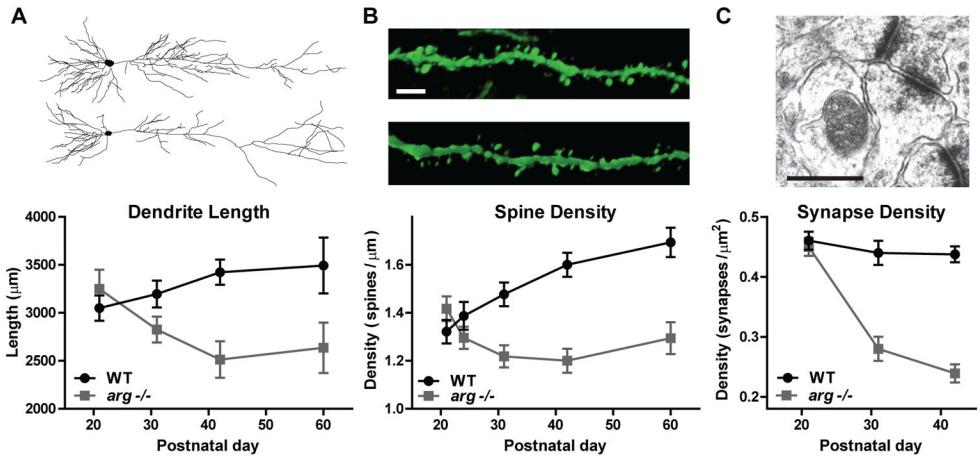


**Figure 1. Phenotype of *arg*$^{-/-}$ hippocampal CA1 neurons**

**A,** Representative reconstructions obtained from dye-filled live neurons. Dendrites develop normally in *arg*$^{-/-}$ mice through postnatal day 21 (P21). However, dendrites begin to regress around P31 and dendrite length (here) and complexity (not shown) are significantly reduced by P42. **B,** Representative confocal micrographs from apical dendrites labeled by thy1-GFP transgene expression. Scale bar 10 μm. Spines densities *arg*$^{-/-}$ neurons in are comparable to WT early in development, but by P31 are significantly reduced. **C,** Representative electron micrograph of a Schaffer-collateral synapse. Scale bar 500nm. Synapses density is normal at P21, but reduced by 30% in *arg*$^{-/-}$ mice at P31 and P42. Data represented in graphs obtained from (Gourley et al., 2012, Moresco et al., 2005, Sfakianos et al., 2007) and representative images modified from (Kerrisk et al., 2013, Warren et al., 2012).

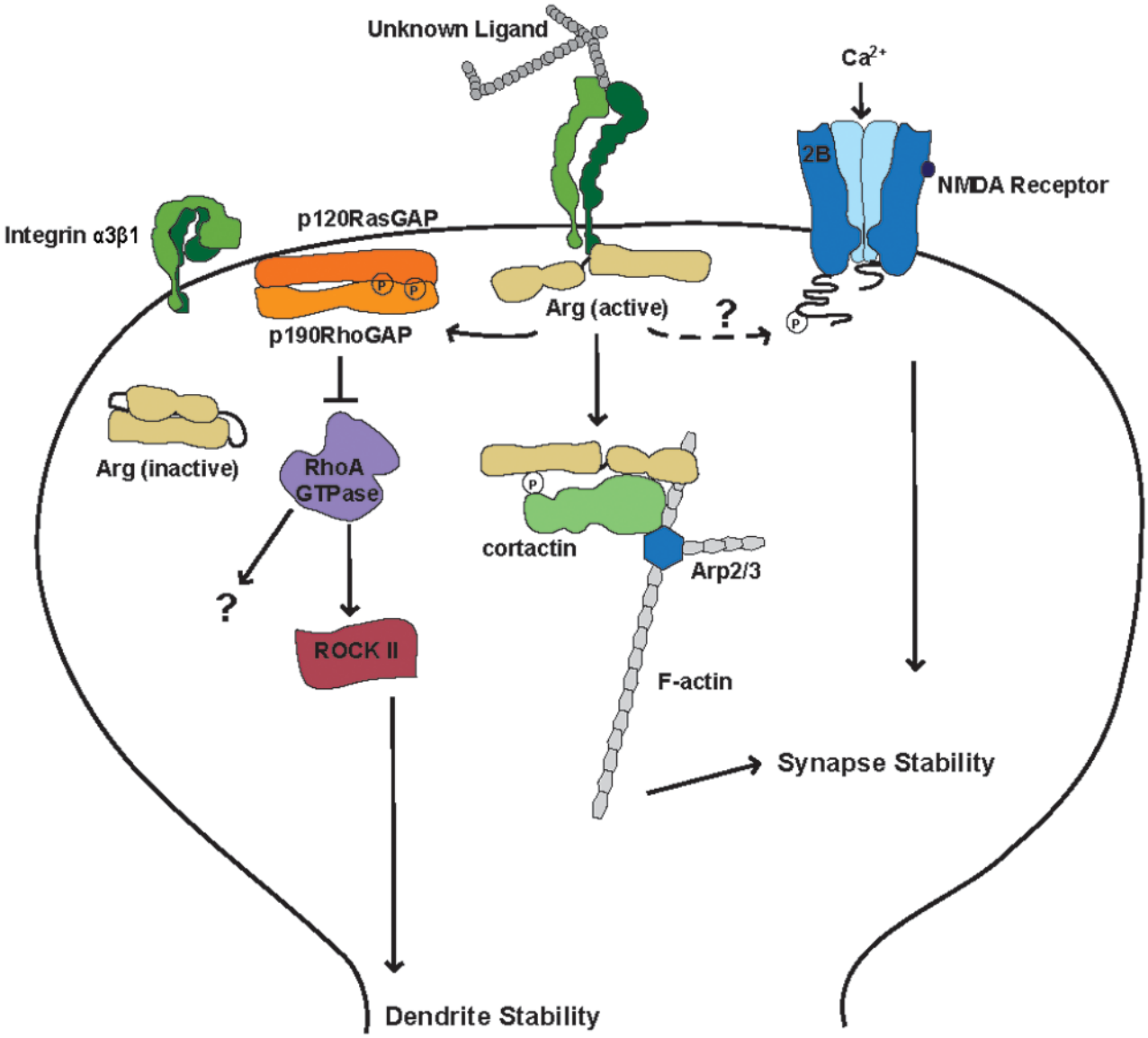


**Figure 2. Model of Arg kinase signaling pathways in hippocampal dendritic spines**

Integrin α3β1 is activated by an unknown upstream extracellular ligand. Integrin activation promotes binding of the intracellular tail of integrin β1 to Arg and activation of Arg kinase activity. Arg phosphorylates downstream substrates including p190RhoGAP (p190) and cortactin. Phosphorylated p190 allows complex formation with p120RasGAP (p120) at the post-synaptic membrane, which inhibits RhoA GTPase activity and promotes dendrite stability. Arg also phosphorylates and binds to cortactin, which promotes the nucleation and stabilization of F-actin by interactions with the Arp2/3 complex. Arg-cortactin interactions promote the stability of dendritic spines. Inhibition of the NR2B subunit of the NMDA receptor can block dendritic spine reductions associated with loss of Arg suggesting that Arg may interact with this receptor, but the mechanism of this interaction is unknown.