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α5 Integrin Signaling Regulates the Formation of Spines and Synapses in Hippocampal Neurons*

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Abstract

The actin-based dynamics of dendritic spines play a key role in synaptic plasticity, which underlies learning and memory. Although it is becoming increasingly clear that modulation of actin is critical for spine dynamics, the upstream molecular signals that regulate the formation and plasticity of spines are poorly understood. In non-neuronal cells, integrins are critical modulators of the actin cytoskeleton, but their function in the nervous system is not well characterized. Here we show that α5 integrin regulates spine morphogenesis and synapse formation in hippocampal neurons. Knockdown of α5 integrin expression using small interfering RNA decreased the number of dendritic protrusions, spines, and synapses. Expression of constitutively active or dominant negative α5 integrin also resulted in alterations in the number of dendritic protrusions, spines, and synapses. α5 integrin signaling regulates spine morphogenesis and synapse formation by a mechanism that is dependent on Src kinase, Rac, and the signaling adaptor GIT1. Alterations in the activity or localization of these molecules result in a significant decrease in the number of spines and synapses. Thus, our results point to a critical role for integrin signaling in regulating the formation of dendritic spines and synapses in hippocampal neurons.

Integrins are heterodimeric, transmembrane cell surface receptors that mediate cell-cell and cell-matrix interactions. Integrin cytoplasmic domains bind to signaling molecules and other components of the actin cytoskeleton and provide a functional link between the extracellular environment and the interior of the cell. In this way, integrins can initiate and regulate several different signal transduction pathways in both neuronal and non-neuronal cells. A modest, emerging literature implicates integrins in learning and memory in both invertebrate and vertebrate species. A mutation in a synapse-associated integrin α subunit (*vol*) in *Drosophila* impairs short term memory processes (1). Mice with reduced expression of the α3, α5, or α8 integrins are defective in hippocampal long term potentiation and spatial memory (2). In addition, function-blocking antibodies against the α5 integrin significantly reduce long term potentiation stabilization in the rat hippocampus (3). Taken together, these studies suggest that integrins regulate some processes underlying memory formation; however, the mechanism by which integrins do this remains unknown.

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A number of studies associate changes in the number, size, and shape of dendritic spines with synaptic plasticity, which underlies learning and memory, and with neurological disorders, such as mental retardation, epilepsy, schizophrenia, and Alzheimer disease (4-7). Dendritic spines are small, actin-rich protrusions that function as bridges between axons and dendrites and serve as sites of post-synaptic contact and signal integration for most of the excitatory synapses in the central nervous system (8-10). Available data suggest that the morphological plasticity of dendritic spines is due to reorganization of the underlying actin cytoskeleton (9, 11,12). This actin-based dynamic behavior of spines is proposed to maximize the likelihood of connecting with presynaptic axons, although the function of spine motility may not be limited to the initial contact and could serve additional roles, such as altering signaling processes after synapses have formed (13). Although it is becoming increasingly clear that modulation of actin is critical for spine motility, the upstream molecular signals that regulate the formation and plasticity of spines are poorly understood.

In this study, we show that α5 integrin regulates spine morphogenesis and synapse formation in hippocampal neurons. Knockdown of endogenous α5 integrin expression using small interfering RNA (siRNA)[2] decreased the number of dendritic protrusions, spines, and synapses. Expression of an α5 integrin, which has a F1025A mutation that locks the integrin in a constitutive signaling state, results in the formation of multiple dendritic protrusions and a decrease in the number of spines and synapses. Expression of an α5 mutant deficient in integrin signaling produces smooth dendrites with a reduction in the number of protrusions, spines, and synapses. α5 integrin signaling regulates spine morphogenesis and synapse formation by a mechanism that is dependent on Src kinase, Rac, and the signaling adaptor GIT1. Alterations in the activity or localization of these molecules result in a significant decrease in the number of spines and synapses. Thus, our results reveal an important function for integrin signaling in regulating the formation of spines and synapses and identify molecules that are critical for these processes.

EXPERIMENTAL PROCEDURES

Reagents and Plasmids

The synaptic vesicle protein SV2 monoclonal antibody was obtained from the Developmental Studies Hybridoma Bank (University of Iowa, Iowa City, IA) and used at a 1:100 dilution for immunostaining. α5 integrin polyclonal antibody clone 1928 (1:100) was from Chemicon, and GIT1 polyclonal antibody (1:100) was previously described (14). α5 siRNAs were prepared by annealing 75 base pair sense and antisense oligos, which contained the following 19 nucleotides from the rat α5 integrin sequence: 5′-GGCATGCGCTCCACTGTAT-3′ or 5′-CACTAGCCAACCAGGAGTA-3′. The annealed oligos were then subcloned into pSUPER at the BglII and HindIII sites as previously described (15). The GIT1 siRNA has been previously described (15). Human α5 integrin-GFP was prepared as previously described (16). Kinase-active Src (CA-Src) and kinase-defective (KD)-Src were a generous gift from Sally Parsons. CA-Src, which has a tyrosine→phenylalanine substitution at amino acid 527, has been previously described (17). KD-Src has an alanine→valine substitution at residue 430, which renders it deficient in kinase activity and inhibits the phosphorylation of c-Src substrates when over-expressed in cultured cells (18). Myc-tagged constitutively active Rac (RacV12) and dominant negative Rac (RacN17) were kindly provided by Alan Hall.

[2]The abbreviations used are: siRNA, small interfering RNA; GFP, green fluorescent protein; DN, dominant negative; KD, kinase-defective.

Neuronal Cultures and Transfections

Hippocampal neurons were isolated from embryonic day 19 rat embryos as previously described (19). Neurons were plated at an approximate density of 70 cells/mm^2 on glass coverslips coated with 1 mg/ml poly-L-lysine and were transfected by a modified calcium phosphate method as previously described (20).

Immunostaining

Neurons were fixed in phosphate-buffered saline with 4% paraformaldehyde and 4% sucrose for 15 min at room temperature and permeabilized with 0.2% Triton X-100 for 5 min. Non-specific absorption was blocked by incubating the coverslips with 20% goat serum in phosphate-buffered saline for 1 h at room temperature. Coverslips were then incubated with the indicated antibodies in phosphate-buffered saline with 5% goat serum and mounted with Vectashield mounting medium (Vector Laboratories, Burlingame, CA).

Image Analysis

Images were acquired using an Orca II charge-coupled device camera (Hamamatsu, Shizuoka, Japan) attached to an inverted Nikon TE-300 microscope using a 60× objective (numerical aperture = 1.4). Image acquisition was controlled using either iSee (Inovision, Raleigh, NC) or Meta-Morph software (Universal Imaging Corp., Downington, PA). The linear density of dendritic protrusions, spines, and synapses were quantified using NIH Image Software.

RESULTS

α5 Integrin Regulates Spine Morphology and Synapse Formation

Our working hypothesis is that integrin signaling, specifically α5 integrin, contributes to learning and memory by regulating spine morphology and synapse formation through modulation of the actin cytoskeleton. To test this, we first developed a siRNA reagent to knockdown expression of endogenous α5 integrin in mammalian cells. As determined by immunoblot analysis with rat2 fibroblasts, the siRNA decreased expression of endogenous α5 integrin by almost 90% but had no detectable effect on the expression of β1 integrin, indicating its specificity for α5 (Fig. 1*A*). Similarly, the siRNA was very effective in decreasing expression of α5 integrin expression in hippocampal neurons. When neurons were transfected with α5 integrin siRNA or pSUPER empty vector as a control, the α5 siRNA reduced the expression of endogenous α5 integrin by >85% (Fig. 1*B*). In α5 siRNA-expressing neurons, the number of spines and dendritic protrusions decreased significantly as compared with controls (Fig. 1, *C* and *D*). In addition, the number of synapses in the α5 siRNA-expressing neurons decreased by 80% as determined by SV2 immunostaining (Fig. 1, *C* and *D*). To show that the effects of α5 siRNA were due to the loss of endogenous α5 integrin expression, human α5-GFP was co-expressed with α5 siRNA. The α5 siRNA is specific for the rat sequence and has no effect on the expression of human α5 integrin. Expression of human α5 rescued the defects in the siRNA-expressing neurons (Fig. 1*D*). These results show that the defects in spine morphogenesis and synapse formation are due to the loss of endogenous α5 integrin and point to a critical function for this integrin in the regulation of spine and synapse formation.

We then asked whether α5 integrin localizes to synapses in hippocampal neurons. Although we detected α5 integrin in a few synapses, the overall distribution was relatively diffuse (Fig. 2*A*). However, in response to synaptic stimulation with glutamate, the localization of α5 integrin to synapses increased 4-fold (Fig. 2*A*). These observations support and extend previous studies that showed high expression of α5 integrin in pyramidal neurons in the hippocampus and in hippocampal synapses (21,22). Our results suggest that activity-induced changes at

synapses promote α5 targeting to these sites, which is consistent with α5 signaling playing a regulatory role in synaptic function.

To show that activation of integrin signaling pathways modulates spine morphology, we generated a constitutively active α5 (CA-α5) mutant, which locks the integrin in an activated state and turns on integrin signaling pathways. Previous studies have shown that a point mutation in the cytoplasmic tail of αIIbβ3, which converts the GFFKR sequence to GAFKR, results in constitutive activation of the integrin (23). This constitutively activated αIIbβ3 phosphorylates focal adhesion kinase when cells are in suspension and localizes to adhesions independently of ligand binding, indicating that integrin signaling pathways are activated. Our CA-α5 mutant, which has a F1025A mutation that converts GFFKR to GAFKR, phosphorylates focal adhesion kinase and paxillin when cells are in suspension, confirming that the integrin is in an activated state. Neurons expressing CA-α5-GFP formed multiple dendritic protrusions with a significant decrease in the number of spines (Fig. 2, *B* and *C*). The number of synapses decreased by almost 80% in neurons expressing CA-α5-GFP compared with control cells (Fig. 2, *B* and *C*). We next used an α5 mutant that has previously been shown to be deficient in integrin signaling (24). This α5 mutant significantly decreased the adhesion, spreading, and migration of epithelial cells (24). In addition, epithelial cells expressing the α5 mutant showed significantly reduced tyrosine phosphorylation and inhibited focal adhesion assembly (24). Thus, the α5 mutant functions as a dominant negative (DN) and impairs integrin-mediated signaling. Neurons expressing the dominant negative α5 mutant fused to GFP (DN-α5-GFP) exhibited smooth dendrites with a dramatic reduction in the number of spines and synapses when compared with the control neurons (Fig. 2, *B* and *C*). Taken together, our results show that α5 integrin-mediated signaling regulates spine morphogenesis and synapse formation in hippocampal neurons.

Src Kinase, an Integrin Effector, Regulates Spine Morphology and Synapse Formation

In non-neuronal cells, integrin engagement has been shown to regulate actin dynamics through a signaling pathway that contains both Src family protein tyrosine kinases and Rac (25,26). This raises the interesting question as to whether integrin signaling regulates spine morphology and synapse formation through a similar mechanism. Expression of CA-Src resulted in the formation of multiple dendritic protrusions with a dramatic decrease in the number of spines and synapses (Fig. 3, *A* and *B*). By contrast, neurons expressing Src with a point mutation at residue 430 (Ala→Val), which renders it deficient in kinase activity (KD-Src), exhibited smooth dendrites with a decrease in the number of dendritic protrusions (Fig. 3, *A* and *B*). These neurons also had significantly fewer spines and synapses than control cells (Fig. 3, *A* and *B*). The phenotypic changes observed in neurons expressing CA-Src and KD-Src are reminiscent of those seen in CA-α5- and DN-α5-expressing neurons, suggesting a role for Src in mediating integrin signaling. If the dendritic protrusions induced by CA-α5 integrin are mediated through Src, then inhibiting Src activity should block the formation of these protrusions. Consistent with this, expression of KD-Src with CA-α5 inhibited the formation of the dendritic protrusions that are typically observed with CA-α5 alone (Fig. 3, *C* and *D*). In addition, if Src is an effector of integrin signaling, expression of CA-Src with α5 siRNA should reverse the α5 siRNA-induced phenotype. Expression of CA-Src with α5 siRNA produced multiple protrusions along the dendrites as opposed to the smooth dendrites observed in neurons expressing α5 siRNA alone (Fig. 3, *C* and *D*). Taken together, these results suggest that Src is necessary for the integrin-mediated regulation of spine morphology and synapse formation in hippocampal neurons.

α5 Integrin Regulates Spine Morphogenesis through a Rac-dependent Mechanism

We next addressed the role of Rac in the regulation of spine morphology and synapse formation by integrins. We have previously shown that expression of dominant negative Rac (DN-Rac)

produced smooth dendrites with a reduction in the number of spines and synapses (20). Expression of dominant negative α5 integrin (DN-α5) produced a very similar phenotype (Fig. 4, *A* and *B*). By contrast, CA-α5-expressing neurons formed numerous long, thin dendritic protrusions, which are analogous to those seen in neurons expressing constitutively active Rac (CA-Rac) (Fig. 4, *A* and *B*). If the CA-α5-induced dendritic protrusions are mediated through Rac, then inhibiting Rac activity should block their formation. Expression of DN-Rac with CA-α5 blocked the formation of the long, thin dendritic protrusions and produced smooth dendrites with very few spines (Fig. 4, *C* and *D*). If integrins are signaling through Rac, then expression of CA-Rac should also rescue the α5 siRNA-induced phenotype and result in the formation of multiple dendritic protrusions. As anticipated, neurons expressing CA-Rac and α5 siRNA exhibited numerous protrusions along the dendrites (Fig. 4, *C* and *D*). Our results suggest that integrin signaling regulates spine morphology and synapse formation in the neurons through modulation of Rac activity.

Expression of the Signaling Adaptor GIT1 Is Required for the α5 Integrin-mediated Effects on Spine Morphology

We have previously shown that the signaling adaptor GIT1 regulates spine morphogenesis and synapse formation by organizing a Rac signaling module that locally modulates Rac activity at synapses (20). To determine whether the α5 integrin-mediated effects on spine morphogenesis are GIT1 dependent, we transfected neurons with CA-α5 and GIT1 siRNA. As expected, neurons expressing CA-α5 alone exhibited numerous dendritic protrusions (Fig. 5, *A* and *B*). However, expression of GIT1 siRNA with CA-α5 blocked the formation of these dendritic protrusions and produced smooth dendrites with very few spines, suggesting that the integrin-induced effects on spine morphology are mediated through GIT1 (Fig. 5, *A* and *B*). We then asked whether the increase in α5 integrin localization to synapses is accompanied by a parallel activity-dependent change in GIT1 localization. In neurons stimulated with glutamate, we observed a 3-fold increase in the localization of GIT1 to synapses (Fig. 5*C*). This shows that, as with α5 integrin, synaptic stimulation increases the targeting of GIT1 to synapses.

DISCUSSION

Taken together, our results suggest a molecular mechanism by which integrin signaling regulates the formation of spines and synapses and provides an explanation, at least in part, for the role of integrins in cognitive processes. Integrins, which are transmembrane receptors, are poised to transmit extracellular cues to the interior of cells. How signals are initiated through these receptors in the dendritic spines is unknown. One possibility is that they are initiated by an unknown ligand, as the synapse is not reported to be rich in fibronectin. It is also possible that integrins are acting in conjugation with other receptors at the membrane, such as neurotransmitter receptors. β integrin-induced actin reorganization in hippocampal neurons is dependent on the *N*-methyl-D-aspartate receptor (27). In hippocampal slices, the rapid outgrowth of the long, thin dendritic protrusions, which are induced by synaptic stimulation in a glutamate receptor-dependent manner (5), is reminiscent of the phenotype that we observe when integrin-signaling pathways are activated. In non-neuronal cells, integrin-signaling pathways and growth factor receptors function coordinately to control cell behavior (28,29), but whether integrin signaling functions in coordination with neurotransmitter receptors to regulate spine morphology is an avenue for future studies.

Our results point to a model in which synaptic stimulation induces the targeting of integrin signaling complexes, which include integrins, Src, and GIT1 to dendritic spines and synapses. GIT1 recruits other molecules, including the Rac activator PIX and the Rac effector PAK, to synapses (15,20). Once assembled, these signaling complexes modulate the formation of spines

and synapses by regulating reorganization of the actin cytoskeleton. The locally regulated activation of Rac, which is a key modulator of actin dynamics, is critical for spine morphogenesis and synapse formation. Mislocalized Rac activity leads to the formation of multiple dendritic protrusions, which are due to aberrant actin organization and an inhibition of synapse formation. Interestingly, this abnormality is a pathology seen in mental retardation (6), and three of the seven recently discovered genes mutated in non-syndromic mental retardation, including αPIX and PAK3, are actin regulators (30,31).

Although it is not presently known whether integrins target the GIT1 complexes to synapses, it is interesting to point out that, in non-neuronal cells, integrin signaling through Src promotes the phosphorylation of GIT1 (32). The majority of the phosphorylation sites detected in GIT1 are found within the domain that localizes it to synapses (33), raising the possibility that phosphorylation in this region could serve a regulatory function.

In summary, we have shown that α5 integrin signaling mediates the formation of dendritic spines and synapses in a Src/GIT1/Rac-dependent manner. Alterations in this pathway lead to a significant decrease in the number of spines and synapses, indicating the importance of integrin signaling in spine and synapse formation.

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REFERENCES

1. Grotewiel MS, Beck CDO, Wu KH, Zhu X-R, Davis RL. Nature 1998;391:455–460. [PubMed: 9461212]
2. Chan CS, Weeber EJ, Kurup S, Sweatt JD, Davis RL. J. Neurosci 2003;23:7107–7116. [PubMed: 12904471]
3. Chun D, Gall C, Bi X, Lynch G. Neuroscience 2001;105:815–829. [PubMed: 11530220]
4. Engert F, Bonhoeffer T. Nature 1999;399:66–70. [PubMed: 10331391]
5. Maletic-Savatic M, Malinow R, Svoboda K. Science 1999;283:1923–1927. [PubMed: 10082466]
6. Fiala JC, Spacek J, Harris KM. Brain Res. Brain Res. Rev 2002;39:29–54. [PubMed: 12086707]
7. Lisman JE, Harris KM. Trends Neurosci 1993;16:141–147. [PubMed: 7682347]
8. Gray EG. Nature 1959;183:1592–1593. [PubMed: 13666826]
9. Fischer M, Kaech S, Knutti D, Matus A. Neuron 1998;20:847–854. [PubMed: 9620690]
10. Yuste R, Denk W. Nature 1995;375:682–684. [PubMed: 7791901]
11. Matus A. Science 2000;290:754–758. [PubMed: 11052932]
12. Dunaevsky A, Tashiro A, Majewska A, Mason C, Yuste R. Proc. Natl. Acad. Sci. U. S. A 1999;96:13438–13443. [PubMed: 10557339]
13. Dunaevsky A, Mason CA. Trends Neurosci 2003;26:155–160. [PubMed: 12591218]
14. Manabe R, Kovalenko M, Webb DJ, Horwitz AR. J. Cell Sci 2002;115:1497–1510. [PubMed: 11896197]
15. Zhang H, Webb DJ, Asmussen H, Niu S, Horwitz AF. J. Neurosci 2005;25:3379–3388. [PubMed: 15800193]
16. Laukaitis CM, Webb DJ, Donais K, Horwitz AF. J. Cell Biol 2001;153:1427–1440. [PubMed: 11425873]
17. Wang K, Hackett JT, Cox ME, Van Hoek M, Lindstrom JM, Parsons SJ. J. Biol. Chem 2004;279:8779–8786. [PubMed: 14679211]
18. Chang JH, Settleman J, Parsons SJ. J. Cell Biol 1995;130:355–368. [PubMed: 7542246]
19. Goslin, K.; Asmussen, H.; Banker, G. Culturing Nerve Cells. Vol. 2nd Ed.. MIT Press; Cambridge, MA: 1998. p. 339-370.

20. Zhang H, Webb DJ, Asmussen H, Horwitz AF. J. Cell Biol 2003;161:131–142. [PubMed: 12695502]
21. Bi X, Lynch G, Zhou J, Gall CM. J. Comp. Neurol 2001;435:184–193. [PubMed: 11391640]
22. Bahr BA, Lynch G. Biochem. J 1992;281:137–142. [PubMed: 1731748]
23. Hughes PE, Diaz-Gonzalez F, Leong L, Wu C, McDonald JA, Shattil SJ, Ginsberg MH. J. Biol. Chem 1996;271:6571–6574. [PubMed: 8636068]
24. Cao Z, Huang K, Horwitz AF. J. Biol. Chem 1998;273:31670–31679. [PubMed: 9822628]
25. del Pozo MA, Price LS, Alderson NB, Ren XD, Schwartz MA. EMBO J 2000;19:2008–2014. [PubMed: 10790367]
26. Shattil SJ. Trends Cell Biol 2005;15:399–403. [PubMed: 16005629]
27. Shi Y, Ethell IM. J. Neurosci 2006;26:1813–1822. [PubMed: 16467530]
28. Hynes RO. Cell 2002;110:673–687. [PubMed: 12297042]
29. Sastry SK, Horwitz AF. Dev. Biol 1996;180:455–467. [PubMed: 8954718]
30. Allen KM, Gleeson JG, Bagrodia S, Partington MW, MacMillan JC, Cerione RA, Mulley JC, Walsh CA. Nat. Genet 1998;20:25–30. [PubMed: 9731525]
31. Bienvenu T, des Portes V, McDonell N, Carrie A, Zemni R, Couvert P, Ropers HH, Moraine C, van Bokhoven H, Fryns JP, Allen K, Walsh CA, Boue J, Kahn A, Chelly J, Beldjord C. Am. J. Med. Genet 2000;93:294–298. [PubMed: 10946356]
32. Bagrodia S, Bailey D, Lenard Z, Hart M, Guan JL, Premont RT, Taylor SJ, Cerione RA. J. Biol. Chem 1999;274:22393–22400. [PubMed: 10428811]
33. Webb DJ, Mayhew MW, Kovalenko M, Schroeder M, Jeffery ED, Whitmore L, Shabanowitz J, Hunt DF, Alan Horwitz F. J. Cell Sci 2006;119:2847–2850. [PubMed: 16825424]

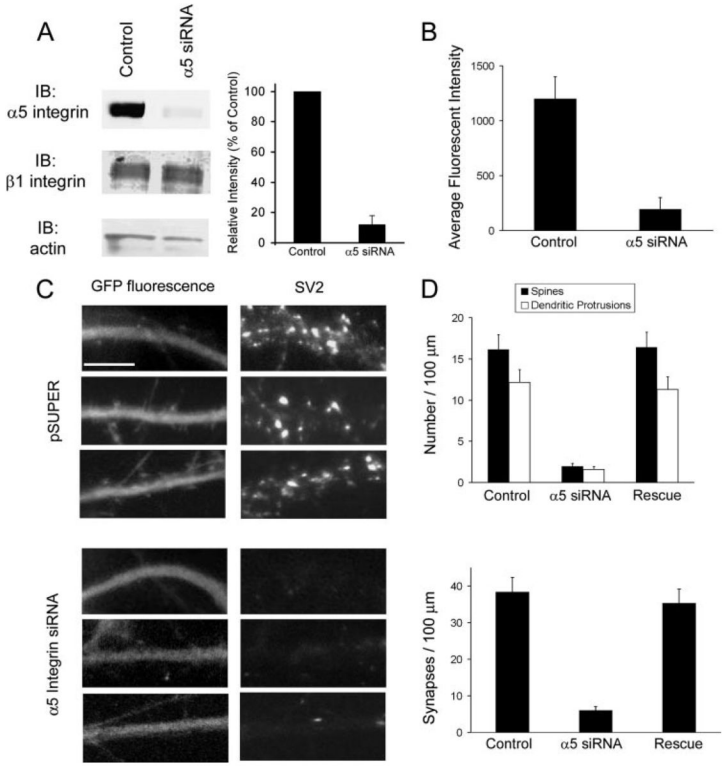

FIGURE 1. Knockdown of endogenous *α*5 integrin expression alters spine and synapse formation

A, cell lysates from rat2 fibroblasts expressing either pSUPER empty vector (*Control*) or *α*5 integrin siRNA were subjected to SDS-PAGE and immunoblot analysis for *α*5 integrin, *β*1 integrin, and actin as a loading control. Quantification of blots from three separate experiments is shown in the *right panel*. In cells expressing *α*5 siRNA, a nearly 90% decrease in endogenous levels of *α*5 integrin was observed. *α*5 siRNA did not affect expression of *β*1 integrin. *B*, hippocampal neurons were co-transfected with GFP and either *α*5 integrin siRNA or pSUPER empty vector (*Control*) and immunostained for endogenous *α*5 integrin at day 12 in culture. Quantification of the average fluorescence intensity of the immunostaining shows that *α*5 siRNA reduced the expression of endogenous *α*5 integrin by >85% compared with control cultures. *C*, expression of *α*5 integrin siRNA significantly decreased the number of spines and synapses in hippocampal neurons. Images from three separate experiments are shown. Note that the dendrites of *α*5 siRNA-expressing neurons are very smooth with a decrease in the number of spines compared with pSUPER (*Control*)-transfected cultures. Also, the *α*5 siRNA-expressing neuron had significantly fewer synapses, as determined by immunostaining with the synaptic marker SV2. *Scale bar* = 5 *μ*m. *D*, quantification of the number of spines, protrusions, and synapses in neurons transfected with *α*5 siRNA or pSUPER empty vector (*Control*). The defects of *α*5 siRNA on spine morphogenesis and synapse formation were reversed by expression of human *α*5-GFP (*Rescue*). For each condition, 60 – 80 dendrites from 15–20 neurons were analyzed.

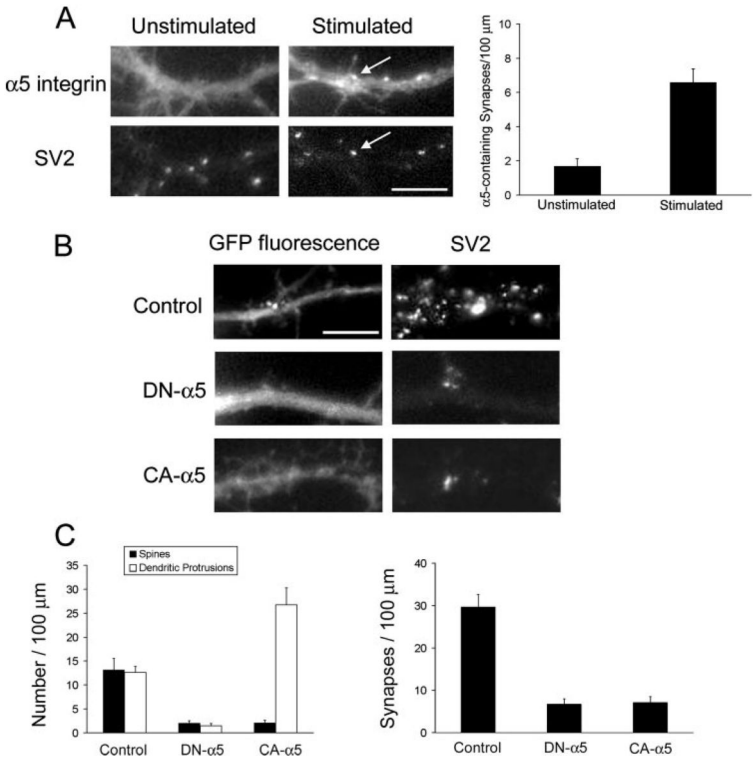

FIGURE 2. *α*5 integrin signaling regulates spine morphology and synapse formation

A, hippocampal neurons at day 12 in culture were incubated in the presence or absence of 100 μM glutamate for 10 min, fixed, and immunostained for *α*5 integrin and SV2. In unstimulated neurons, the distribution of *α*5 integrin was relatively diffuse; however, in response to stimulation with glutamate, the localization of *α*5 integrin to synapses (*arrows*) increased 4-fold. The quantification of *α*5 integrin in synapses is shown in the *right panel*. *Scale bar* = 5 *μ*m. *B*, hippocampal neurons were transfected with wild-type *α*5 integrin with GFP (*Control*) or with GFP-tagged *α*5 integrin mutants, fixed, and immunostained for SV2 at day 10 in culture. Expression of constitutively active *α*5 integrin (*CA-α5*), which turns on integrin signaling pathways, increased the number of dendritic protrusions and decreased the number of spines and synapses compared with control cultures. Neurons expressing dominant negative *α*5 integrin, which impairs integrin-mediated signaling, had smooth dendrites with a reduction in the number of spines and synapses compared with controls. *Scale bar* = 5 *μ*m. *C*, quantification of the number of protrusions, spines, and synapses in neurons transfected with wild-type *α*5 integrin (*Control*) or *α*5 integrin mutants. For each condition, 60–75 dendrites from 15–20 neurons were analyzed.

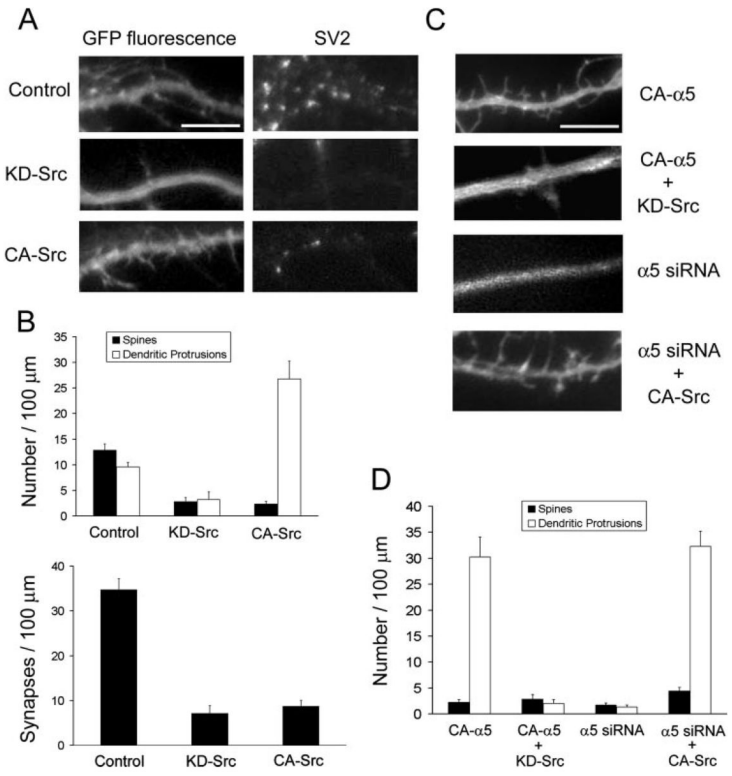

FIGURE 3. Src kinase regulates spine morphology and synapse formation

A, neurons were co-transfected with GFP and the Src mutants or empty vector (*Control*), fixed, and immunostained for SV2 at day 10 in culture. Expression of constitutively active Src (*CA-Src*) resulted in the formation of multiple dendritic protrusions with a dramatic decrease in the number of spines and synapses compared with control cultures. Neurons expressing Src, which is deficient in kinase activity (KD-Src), exhibited smooth dendrites with significantly fewer spines and synapses than control cells. *Scale bar* = 5 μm. *B*, quantification of the number of spines, protrusions, and synapses in neurons transfected with empty vector (*Control*) or the Src mutants is shown. For each condition, 60–75 dendrites from 15–20 neurons were analyzed. *C*, neurons were co-transfected with the indicated α5 integrin and Src constructs, fixed, and viewed in fluorescence at day 10 in culture. Expression of KD-Src with CA-α5 inhibited the formation of the dendritic protrusions, which are typically seen with CA-α5 alone. Expression of CA-Src with α5 siRNA resulted in the formation of multiple dendritic protrusions, which contrasts the smooth dendrites observed in neurons expressing only α5 siRNA. *Scale bar* = 5 μm. *D*, quantification of spines and protrusions for the conditions described in *C* is shown. For each condition, 50–60 dendrites from 15–18 neurons were analyzed.

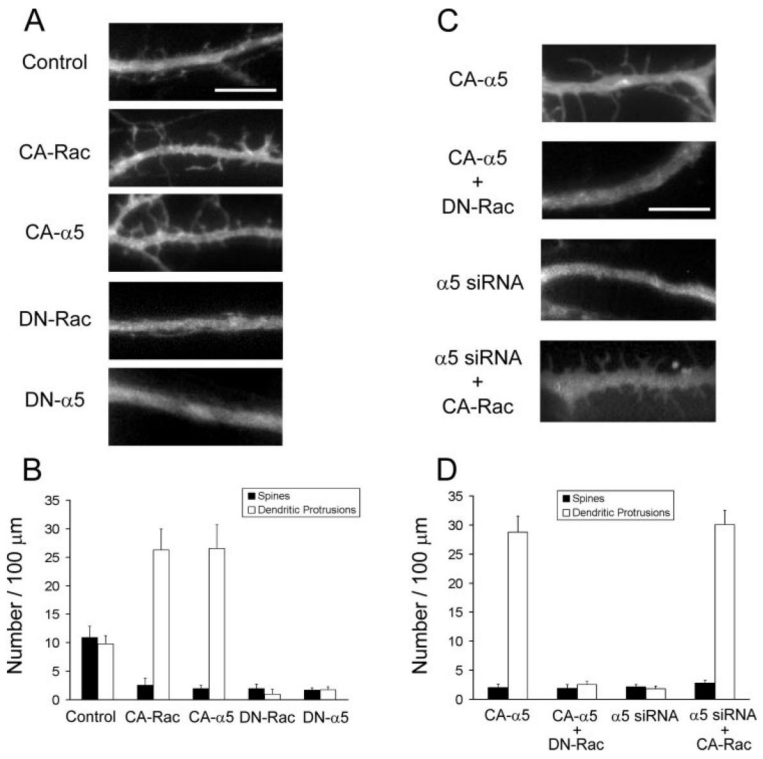

FIGURE 4. *α*5 integrin signaling regulates spine morphogenesis through a Rac-dependent mechanism

A, neurons were transfected with the indicated *α*5 integrin and Rac constructs, fixed, and viewed in fluorescence at day 10 in culture. CA-*α*5-expressing neurons formed multiple dendrite protrusions, which is similar to the phenotype observed with constitutively active Rac (*CA-Rac*). Expression of DN-*α*5 integrin produced smooth dendrites with a decrease in the number of spines, which is analogous to the phenotype observed with dominant negative Rac. *Scale bar* = 5 *μ*m. *B*, quantification of the number of spines and protrusions in neurons expressing the indicated *α*5 integrin and Rac mutants is shown. For each condition, 50–65 dendrites from 12–15 neurons were analyzed. *C*, expression of DN-Rac with CA-*α*5 integrin blocked the formation of the long, thin dendritic protrusions and produced smooth dendrites with very few spines. Neurons expressing CA-Rac and *α*5 siRNA exhibited multiple dendritic protrusions indicating that CA-Rac can rescue the *α*5 siRNA-induced phenotype. *Scale bar* = 5 *μ*m. *D*, quantification of spines and protrusions for the conditions described in *C* is shown. For each condition, 55–65 dendrites from 15–18 neurons were analyzed.

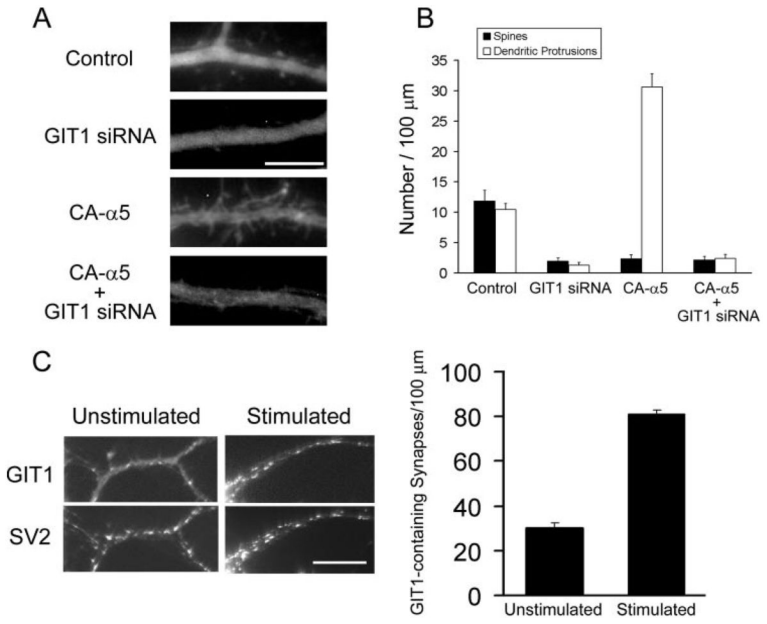

FIGURE 5. α5 integrin regulates spine morphology through a mechanism that is dependent on the signaling adaptor, GIT1

A, hippocampal neurons were transfected with the indicated α5 integrin and GIT1 constructs, fixed, and viewed in fluorescence at day 12. Expression of GIT1 siRNA with CA-α5 blocked the formation of the multiple dendritic protrusions, which are typically observed with CA-α5 alone, and produced smooth dendrites with very few spines. *Scale bar* = 5 μm. *B*, quantification of the number of spines and protrusions in neurons transfected with CA-α5 integrin and GIT1 siRNA is shown. For each condition, 50–65 dendrites from 15–18 neurons were analyzed. *C*, neurons at day 12 in culture were incubated in the presence or absence of 100 μM glutamate for 10 min, fixed, and immunostained for GIT1 and SV2. In neurons stimulated with glutamate, a 3-fold increase in the localization of GIT1 to synapses was observed. The quantification of GIT1 in synapses is shown in the *right panel. Scale bar* = 5 μm.