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Motor Protein KIF1A Is Essential for Hippocampal Synaptogenesis and Learning Enhancement in an Enriched Environment

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SUMMARY

Environmental enrichment causes a variety of effects on brain structure and function. Brain-derived neurotrophic factor (BDNF) plays an important role in enrichment-induced neuronal changes; however, the precise mechanism underlying these effects remains uncertain. In this study, a specific upregulation of kinesin superfamily motor protein 1A (KIF1A) was observed in the hippocampi of mice kept in an enriched environment and, in hippocampal neurons in vitro, BDNF increased the levels of KIF1A and of KIF1A-mediated cargo transport. Analysis of *Bdnf*$^{+/-}$ and *Kif1a*$^{+/-}$ mice revealed that a lack of KIF1A upregulation resulted in a loss of enrichment-induced hippocampal synaptogenesis and learning enhancement. Meanwhile, KIF1A overexpression promoted synaptogenesis via the formation of presynaptic boutons. These findings demonstrate that KIF1A is indispensable for BDNF-mediated hippocampal synaptogenesis and learning enhancement induced by enrichment. This is a new molecular motor-mediated presynaptic mechanism underlying experience-dependent neuroplasticity.

INTRODUCTION

Environmental enrichment refers to housing conditions, where animals experience higher levels of sensory, motor, social, and cognitive stimuli compared to a normal cage environment (van Praag et al., 2000; Nithianantharajah and Hannan, 2006). Enrichment has a variety of effects on the brains of wild-type mice and rats at many levels, ranging from molecular and cellular to behavioral. At the cellular level, enrichment increases dendritic branching and length, as well as the number of dendritic spines and the size of synapses on some neuronal populations (Moser et al., 1994; Rampon et al., 2000a; Faherty et al., 2003; Leggio et al., 2005). Furthermore, enrichment enhances hippocampal neurogenesis (Kempermann et al., 1997; van Praag et al., 1999) and synaptogenesis (Rampon et al., 2000a; Gogolla et al., 2009; Bednarek and Caroni, 2011). Many of these morphological changes are consistent with enrichment-induced alterations in the expression of genes involved in synaptic function and neuroplasticity (Rampon et al., 2000b). Enrichment can increase levels of neurotrophins, such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), which play integral roles in neuronal signaling (Young et al., 1999; Ickes et al., 2000). Enrichment also increases the expression of synaptic proteins, such as the presynaptic vesicle protein, synaptophysin, and postsynaptic density-95 protein (PSD-95) (Nithianantharajah et al., 2004). At the behavioral level, enrichment induces learning enhancement in various behavioral tests (Kempermann et al., 1997; Rampon et al., 2000a), reduces memory decline in aged animals (Bennett et al., 2006), decreases anxiety, and increases exploratory activity (Benaroya-Milshtein et al., 2004). Furthermore, enrichment has beneficial effects on brain disorders, such as Huntington's disease, Alzheimer's disease, Parkinson's disease, and various forms of brain injury (Nithianantharajah and Hannan, 2006). BDNF plays an important role in these enrichment-induced changes (Falkenberg et al., 1992; Rossi et al., 2006); however, the precise mechanism of this action is not well understood.

The kinesin superfamily proteins (KIFs) are microtubule-based molecular motors that transport membrane organelles, protein complexes, and messenger RNAs (mRNAs) (Hirokawa et al., 2009). KIFs have fundamental roles in neuronal function, plasticity, morphogenesis, and survival by transporting such cargos (Hirokawa et al., 2010). Intriguingly, recent reports have shown that some KIFs (KIF5 and KIF17) are implicated in learning and memory (Puthanveettil et al., 2008; Yin et al., 2011). We therefore sought to examine possible alterations in levels of KIFs after enrichment, and then to investigate the possible relationship of KIFs to enrichment-induced structural and behavioral changes, such as hippocampal synaptogenesis (Rampon et al., 2000a; Gogolla et al., 2009; Bednarek and Caroni, 2011) and learning enhancement (Kempermann et al., 1997; Rampon et al., 2000a).

We found that enrichment causes a specific upregulation of KIF1A in the mouse hippocampus and revealed that KIF1A is indispensable for BDNF-mediated hippocampal synaptogenesis and learning enhancement induced by enrichment. In neurons, KIF1A transports synaptic vesicle precursors containing synaptic vesicle proteins, such as synaptophysin, synaptotagmin, and Rab3A (Okada et al., 1995; Yonekawa et al., 1998). Our findings suggest a new molecular motor-mediated presynaptic mechanism underlying experience-dependent neuroplasticity.

RESULTS

Enrichment Causes BDNF-Dependent KIF1A Upregulation in Mouse Hippocampus

To address the question of whether KIFs might be regulated in response to enrichment, we first examined the protein levels of major KIFs in the hippocampi of wild-type C57BL/6J mice after enrichment (Figure 1A). Quantitative immunoblot analyses revealed that enriched mice exhibited a prominent increase in levels of KIF1A, which peaked after exposure to enrichment for 3 weeks (enriched/nonenriched ratio of 3 weeks: 1.70 ± 0.05, $p < 0.001$, two-tailed t test) (Figure 1B). Moderate increases in the levels of KIF1Bβ, KIF5A, and KIF17 were also observed (enriched/nonenriched ratio of 3 weeks: KIF1Bβ, 1.20 ± 0.04, $p = 0.0098$; KIF5A, 1.24 ± 0.06, $p = 0.0151$; KIF17, 1.22 ± 0.07, $p = 0.0287$, two-tailed t test) (Figure 1B). Levels of *Kif1a* mRNA were also increased (enriched/nonenriched ratio of 3 weeks: 1.89 ± 0.06, $p = 0.0046$, two-tailed t test) (Figure 1C), suggesting that transcriptional regulation is involved in KIF1A upregulation. Interestingly, the protein level of synaptophysin, a cargo of KIF1A, was also upregulated after enrichment (enriched/nonenriched ratio of 3 weeks: 1.23 ± 0.05, $p = 0.01$, two-tailed t test) (Figure 1B), which is consistent with previous reports (Nithianantharajah et al., 2004). The level of BDNF, an increase of which is associated with learning and memory (Cunha et al., 2010), was also elevated in the hippocampi of enriched mice (enriched/nonenriched ratio of 3 weeks: 1.78 ± 0.11, $p = 0.0022$, two-tailed t test) (Figure 1B), as has been previously reported (Rossi et al., 2006). Upregulation of KIF1A and BDNF levels was also observed in the BALB-c mouse hippocampus after enrichment (enriched/nonenriched ratio of 3 weeks: KIF1A, 1.42 ± 0.05, $p = 0.0135$; BDNF, 1.41 ± 0.06, $p = 0.0221$, two-tailed t test) (see Figure S1 available online).

We next examined the functional significance of BDNF and KIF1A in enrichment-related molecular events using *Bdnf* and *Kif1a* mutant mice. For these purposes, $Bdnf^{+/-}$ and $Kif1a^{+/-}$ mice were analyzed, because $Bdnf^{-/-}$ and $Kif1a^{-/-}$ mice die shortly after birth (Ernfors et al., 1994; Yonekawa et al., 1998). We first compared the protein levels of BDNF and KIF1A in $Bdnf^{+/-}$ and $Kif1a^{+/-}$ mouse hippocampi between mice that were and were not exposed to enrichment. Compared with wild-type mice, the increase of BDNF levels in $Bdnf^{+/-}$ mice and that of KIF1A levels in $Kif1a^{+/-}$ mice was attenuated following 3 weeks of enrichment exposure (enriched/nonenriched ratio: $Bdnf^{+/-}$ mice, BDNF, 1.15 ± 0.03, $p = 0.0039$; $Kif1a^{+/-}$ mice, KIF1A, 1.13 ± 0.02, $p = 0.0056$, two-tailed t test) (Figure 1D). Importantly, no significant increase in KIF1A levels was observed in enriched $Bdnf^{+/-}$ mice (1.06 ± 0.03, $p = 0.1501$, two-tailed t test), while enriched $Kif1a^{+/-}$ mice showed a moderate increase of BDNF levels (1.37 ± 0.04, $p < 0.001$, two-tailed t test) (Figure 1D). These results indicate the possibility that BDNF works as an upstream signal in enrichment-induced KIF1A upregulation.

Enrichment Does Not Induce Learning Enhancement in $Bdnf^{+/-}$ or $Kif1a^{+/-}$ Mice

Next, to examine the possible role of KIF1A upregulation in spatial learning ability, we performed the Morris water maze test for wild-type, $Bdnf^{+/-}$, and $Kif1a^{+/-}$ mice after enrichment. After enrichment for 3 weeks, wild-type mice performed significantly better in the hidden platform trial (nonenriched versus enriched: latency, $F_{(1,22)} = 13.50$, $p = 0.0013$, two-way repeated-measures ANOVA) (Figure 2A), spent significantly more time in the target quadrant (TQ) in the probe test (nonenriched versus enriched: 31.4% ± 3.5% versus 45.6% ± 3.2%, $p = 0.0069$, two-tailed t test) (Figure 2B), and crossed the targeted area more frequently (nonenriched versus enriched: 3.08 ± 0.36 versus 5.08 ± 0.48, $p = 0.0030$, two-tailed t test) than nonenriched wild-type mice (Figure S2A). These data are consistent with previous reports (Kempermann et al., 1997). Compared with nonenriched littermate control mice, nonenriched $Bdnf^{+/-}$ mice exhibited impaired spatial learning (littermate control versus $Bdnf^{+/-}$: latency, $F_{(1,22)} = 7.724$, $p = 0.0109$, two-way repeated-measures ANOVA; time in TQ in probe test, 42.6% ± 3.6% versus 28.4% ± 3.9%, $p = 0.0134$, two-tailed t test) (Figures S2M–S2O), as previously reported (Linnarsson et al., 1997). Nonenriched $Kif1a^{+/-}$ mice showed an intact spatial learning ability (littermate control versus $Kif1a^{+/-}$: latency, $F_{(1,22)} = 0.3175$, $p = 0.5788$, two-way repeated-measures ANOVA; time in TQ in probe test, 42.9% ± 3.5% versus 41.2% ± 3.8%, $p = 0.7465$, two-tailed t test) (Figures S2T–S2V). Significantly, in contrast to wild-type mice, no enhancement of spatial learning was found in enriched $Bdnf^{+/-}$ or $Kif1a^{+/-}$ mice, compared with respective nonenriched mice (nonenriched versus enriched $Bdnf^{+/-}$: latency, $F_{(1,22)} = 0.2391$, $p = 0.6297$, two-way repeated-measures ANOVA; time in TQ in probe test: 39.1% ± 3.3% versus 40.1% ± 3.4%, $p = 0.8460$, two-tailed t test; number of platform crossings: 3.83 ± 0.51 versus 3.92 ± 0.49, $p = 0.9076$, two-tailed t test; non-enriched versus enriched $Kif1a^{+/-}$: latency, $F_{(1,22)} = 1.103$, $p = 0.3050$, two-way repeated-measures ANOVA; time in TQ in probe test: 42.0% ± 3.1% versus 44.1% ± 3.0%, $p = 0.6424$, two-tailed t test; number of platform crossings: 4.25 ± 0.44 versus 4.42 ± 0.40, $p = 0.7824$, two-tailed t test) (Figures 2D, 2E, 2G, and 2H and Figures S2E and S2I). In the visible platform trial, nonenriched $Bdnf^{+/-}$ and $Kif1a^{+/-}$ mice showed performances comparable to nonenriched littermate control mice (littermate control versus $Bdnf^{+/-}$: latency, $F_{(1,22)} = 0.01681$, $p = 0.8980$; littermate control versus $Kif1a^{+/-}$: latency, $F_{(1,22)} = 0.007734$, $p = 0.9307$, two-way repeated-measures ANOVA) (Figures S2P and S2W), and there were no significant differences between nonenriched and enriched mice (latency of nonenriched versus enriched: wild-type, $F_{(1,22)} = 0.3455$, $p = 0.5626$; $Bdnf^{+/-}$, $F_{(1,22)} = 0.1733$, $p = 0.6812$; $Kif1a^{+/-}$, $F_{(1,22)} = 1.461 \times 10^{-14}$, $p = 1.000$, two-way repeated-measures ANOVA) (Figures S2B, S2F, and S2J). Throughout the experiments, there were no significant differences in the average swim speed between nonenriched and enriched mice (nonenriched versus enriched [cm/s]: wild-type, 23.9 ± 1.0 versus 25.4 ± 0.9, $p = 0.2959$; $Bdnf^{+/-}$, 25.1 ± 0.7 versus 25.3 ± 0.8, $p = 0.8373$; $Kif1a^{+/-}$, 24.0 ± 1.0 versus 25.5 ± 0.9, $p = 0.2622$, two-tailed t test) (Figures 2C, 2F, and 2I and Figures S2C, S2D, S2G, S2H, S2K, and S2L), and between genotypes (littermate control versus $Bdnf^{+/-}$ [cm/s]: 24.5 ± 0.8 versus 24.8 ± 0.8, $p = 0.8605$; littermate control versus $Kif1a^{+/-}$ [cm/s]: 24.6 ± 1.1 versus 23.9 ± 1.0, $p = 0.6275$, two-tailed t test) (Figures S2Q–S2S and S2X–S2Z).

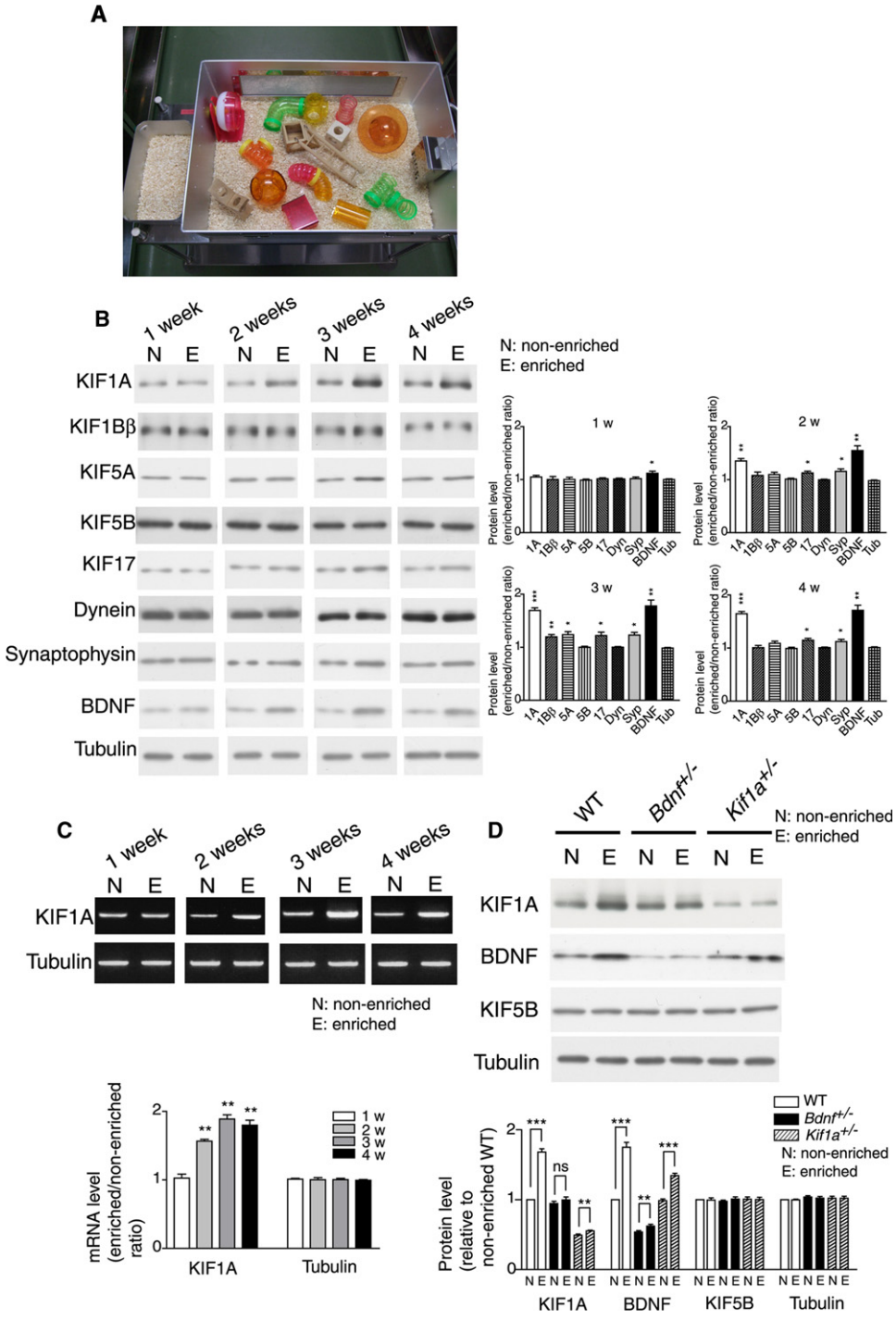

Figure 1. Enrichment Causes BDNF-Dependent KIF1A Upregulation in Mouse Hippocampus

(A) Nonenriched cage (left) versus enriched cage (right).

(B) Quantitative immunoblots of major KIFs and other associated proteins in hippocampal extracts with or without enrichment exposure for 1, 2, 3, or 4 weeks (n = 5 mice).

(C) Semiquantitative RT-PCR of *Kif1a* from total hippocampal RNA (n = 3 mice).

(D) Quantitative immunoblots of KIF1A and BDNF from $Bdnf^{+/-}$ and $Kif1a^{+/-}$ hippocampal extracts with or without enrichment exposure for 3 weeks (n = 5 mice).

Means ± SEM are shown in all histograms. *p < 0.05, **p < 0.01, ***p < 0.001; ns, not significant (two-tailed t test).

See also Figure S1.

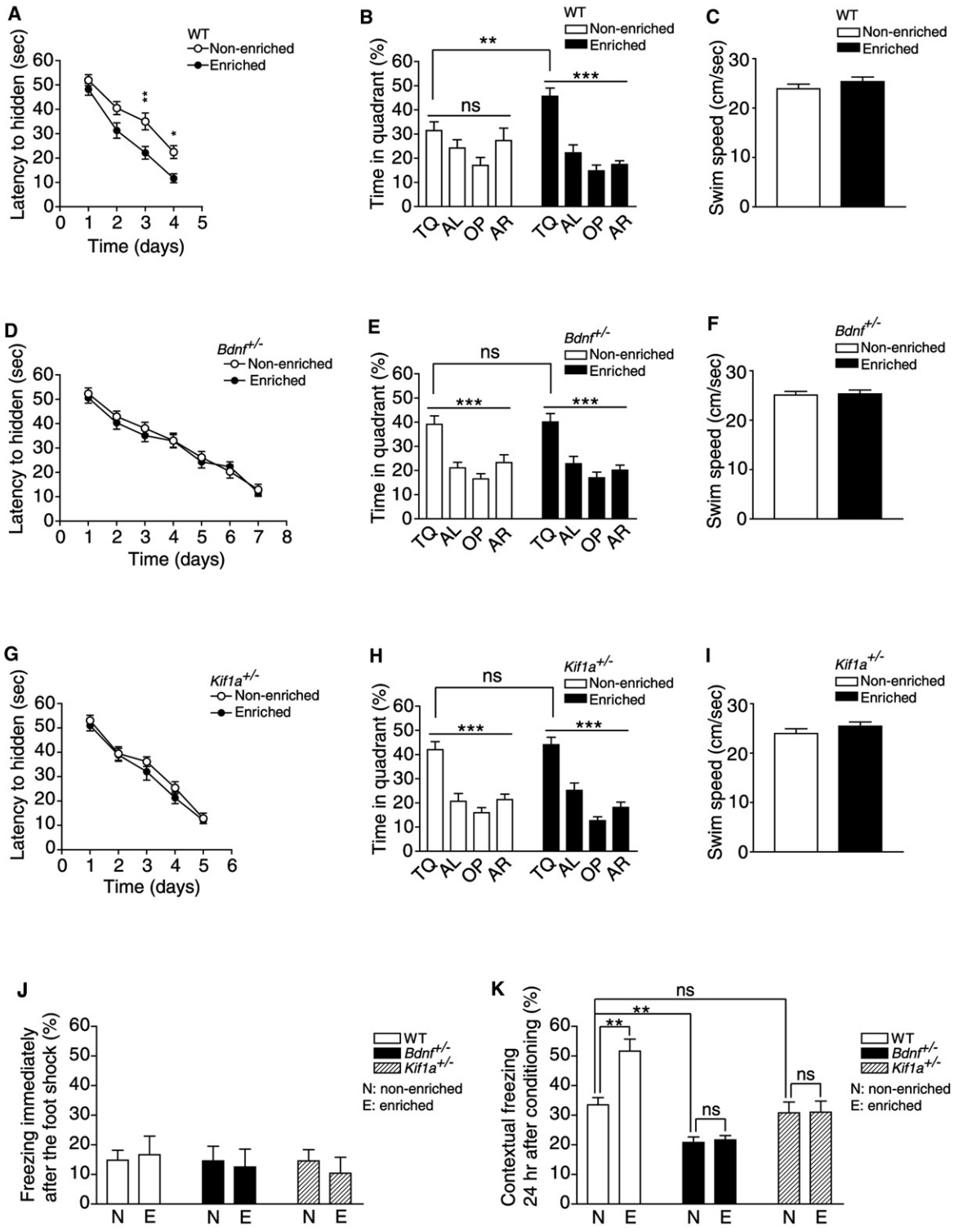

Figure 2. Enrichment Does Not Induce Learning Enhancement in *Bdnf*$^{+/-}$ or *Kif1a*$^{+/-}$ Mice

(A–I) Morris water maze test for spatial learning. Comparison between nonenriched mice and mice exposed to enrichment for 3 weeks. Data for wild-type (A–C), *Bdnf*$^{+/-}$ (D–F), and *Kif1a*$^{+/-}$ (G–I) mice are shown (n = 12 mice in each group). (A, D, and G) Escape latency in the hidden platform trial. *p < 0.05, **p < 0.01 (two-way repeated-measures ANOVA with a post hoc Bonferroni's test). (B, E and H) Swim time spent in each quadrant in the probe test. TQ, target quadrant; AL, adjacent left; OP, opposite; AR, adjacent right. **p < 0.01, ***p < 0.001; ns, not significant (one-way ANOVA or two-tailed t test). (C, F, and I) Average swim speed in the water maze test.

We then examined the possible role of KIF1A upregulation in nonspatial learning ability, using the contextual fear conditioning test. Exposure of wild-type mice to enrichment for 3 weeks significantly enhanced contextual freezing responses 24 hr after conditioning (nonenriched versus enriched: 33.5% ± 2.5% versus 51.7% ± 4.0%, p = 0.0013, two-tailed t test) (Figure 2K), consistent with previous reports (Rampon et al., 2000a). Compared with nonenriched wild-type mice, nonenriched *Bdnf*$^{+/-}$ mice exhibited impaired contextual fear learning (wild-type versus *Bdnf*$^{+/-}$: 33.5% ± 2.5% versus 20.8% ± 1.8%, p < 0.01, post hoc Dunnett's test) (Figure 2K), as previously reported (Liu et al., 2004). Nonenriched *Kif1a*$^{+/-}$ mice showed intact contextual fear learning (wild-type versus *Kif1a*$^{+/-}$: 33.5% ± 2.5% versus 30.8% ± 3.6%, p > 0.05, post hoc Dunnett's test) (Figure 2K). Significantly, in contrast to wild-type mice, no enhancement of contextual fear learning was found in enriched *Bdnf*$^{+/-}$ or *Kif1a*$^{+/-}$ mice, compared with respective nonenriched mice (nonenriched versus enriched: *Bdnf*$^{+/-}$, 20.8% ± 1.8% versus 21.7% ± 1.5%, p = 0.7289; *Kif1a*$^{+/-}$, 30.8% ± 3.6% versus 31.0% ± 3.7%, p = 0.9686, two-tailed t test) (Figure 2K). There were no significant differences in freezing responses immediately after the foot shock between nonenriched and enriched mice (nonenriched versus enriched: wild-type, 14.8% ± 3.3% versus 16.7% ± 6.3%, p = 0.7921; *Bdnf*$^{+/-}$, 14.6% ± 4.9% versus 12.5% ± 6.1%, p = 0.7942; *Kif1a*$^{+/-}$, 14.6% ± 3.8% versus 10.4% ± 5.4%, p = 0.5373, two-tailed t test) (Figure 2J).

Taken together, these behavioral findings indicate that increases in the levels of both BDNF and KIF1A are required for enrichment-induced enhancement of spatial learning and contextual fear memory.

Enrichment Does Not Induce Hippocampal Synaptogenesis in *Bdnf*$^{+/-}$ or *Kif1a*$^{+/-}$ Mice

To examine the possible role of KIF1A upregulation in enrichment-induced structural changes, we used electron microscopy and an unbiased stereological method (Rampon et al., 2000a) to quantitatively analyze synapse densities in the stratum radiatum of the hippocampal CA1 region of wild-type, *Bdnf*$^{+/-}$, and *Kif1a*$^{+/-}$ mice after enrichment (Figures 3A–3F). Exposure to enrichment for 3 weeks significantly increased the spine synapse density in wild-type mice (nonenriched versus enriched [synapses/100 μm^3]: 67.0 ± 2.3 versus 86.1 ± 3.5, p = 0.0106, two-tailed t test) (Figure 3G), consistent with previous reports (Rampon et al., 2000a). Compared with nonenriched wild-type mice, there was no significant difference in synapse density in nonenriched *Bdnf*$^{+/-}$ or *Kif1a*$^{+/-}$ mice (wild-type, *Bdnf*$^{+/-}$, and *Kif1a*$^{+/-}$: spine synapses, p = 0.7378; shaft synapses, p = 0.8175, one-way ANOVA) (Figures 3G and 3H). Significantly, in contrast to wild-type mice, *Bdnf*$^{+/-}$ or *Kif1a*$^{+/-}$ mice did not show any increase in spine synapse density after enrichment (nonenriched versus enriched (synapses/100 μm^3): *Bdnf*$^{+/-}$, 64.0 ± 3.1 versus 66.8 ± 2.6, p = 0.5254; *Kif1a*$^{+/-}$, 64.9 ± 2.7 versus 67.2 ± 2.9, p = 0.5898, two-tailed t test) (Figure 3G). Meanwhile, the shaft synapse densities remained unchanged after enrichment in all three genotypes (nonenriched versus enriched (synapses/100 μm^3): wild-type, 2.4 ± 0.3 versus 2.4 ± 0.3, p = 0.8979; *Bdnf*$^{+/-}$, 2.2 ± 0.1 versus 2.3 ± 0.2, p = 0.8189; *Kif1a*$^{+/-}$, 2.3 ± 0.1 versus 2.3 ± 0.2, p = 0.9590, two-tailed t test) (Figure 3H). For further reliable quantification of synapse densities, we performed immunohistochemical analysis. We examined the densities of synaptophysin/PSD-95-double-positive puncta in the stratum radiatum of the hippocampal CA1 region of wild-type, *Bdnf*$^{+/-}$, or *Kif1a*$^{+/-}$ mice with or without enrichment (Figure S3A). Exposure to enrichment for 3 weeks significantly increased the density of double-positive puncta in wild-type mice (nonenriched versus enriched [normalized to nonenriched wild-type]: 1.00 ± 0.07 versus 1.38 ± 0.08, p = 0.0114, two-tailed t test) (Figure S3B); however, no significant increase in the density of double-positive puncta was observed in enriched *Bdnf*$^{+/-}$ or *Kif1a*$^{+/-}$ mice compared with respective nonenriched mice (nonenriched versus enriched [normalized to nonenriched wild-type]: *Bdnf*$^{+/-}$, 0.98 ± 0.07 versus 1.04 ± 0.11, p = 0.6452; *Kif1a*$^{+/-}$, 0.99 ± 0.07 versus 1.04 ± 0.13, p = 0.7352, two-tailed t test) (Figure S3B). These immunohistochemical data support our electron microscopy results. Taken together, these morphological findings indicate that increases in the levels of both BDNF and KIF1A play important roles in the enrichment-induced increase of spine synapse density in the hippocampus.

Next, to examine the possible role of KIF1A in enrichment-induced hippocampal neurogenesis, we performed bromodeoxyuridine (BrdU) labeling of hippocampal dentate gyri of wild-type, *Bdnf*$^{+/-}$, and *Kif1a*$^{+/-}$ mice with or without enrichment (van Praag et al., 1999) (Figure S3C). Exposure to enrichment for 3 weeks significantly increased hippocampal neurogenesis in wild-type mice (nonenriched versus enriched [normalized to nonenriched wild-type]: 1.00 ± 0.08 versus 1.71 ± 0.20, p = 0.0302, two-tailed t test) (Figure S3D), consistent with previous reports (Kempermann et al., 1997; van Praag et al., 1999). Compared with nonenriched wild-type mice, nonenriched *Bdnf*$^{+/-}$ mice showed reduced neurogenesis (wild-type versus *Bdnf*$^{+/-}$ [normalized to nonenriched wild-type]: 1.00 ± 0.08 versus 0.69 ± 0.04, p < 0.05, post hoc Dunnett's test) (Figure S3D), as previously reported (Lee et al., 2002; Sairanen et al., 2005). In contrast to wild-type mice, *Bdnf*$^{+/-}$ mice did not show any increase in neurogenesis after enrichment (nonenriched versus enriched [normalized to nonenriched wild-type]: 0.69 ± 0.04 versus 0.79 ± 0.08, p = 0.3792, two-tailed t test) (Figure S3D). This result is consistent with previous reports (Rossi et al., 2006). Meanwhile, neurogenesis of nonenriched *Kif1a*$^{+/-}$ mice was comparable to that of nonenriched wild-type mice (wild-type versus *Kif1a*$^{+/-}$ [normalized to nonenriched wild-type]: 1.00 ± 0.08 versus 1.04 ± 0.10, p > 0.05, post hoc Dunnett's test) (Figure S3D). Interestingly and consistent with wild-type mice, *Kif1a*$^{+/-}$ mice exhibited enhanced hippocampal neurogenesis after enrichment (nonenriched versus enriched [normalized

(J and K) Contextual fear conditioning test. Comparison between nonenriched mice and mice exposed to enrichment for 3 weeks (nonenriched wild-type, n = 9 mice; other groups, n = 8 mice). (J) Freezing responses immediately after the foot shock. (K) Contextual freezing responses 24 hr after conditioning. **p < 0.01; ns, not significant (two-tailed t test or one-way ANOVA with a post hoc Dunnett's test). Means ± SEM are shown in all histograms.
See also Figure S2.

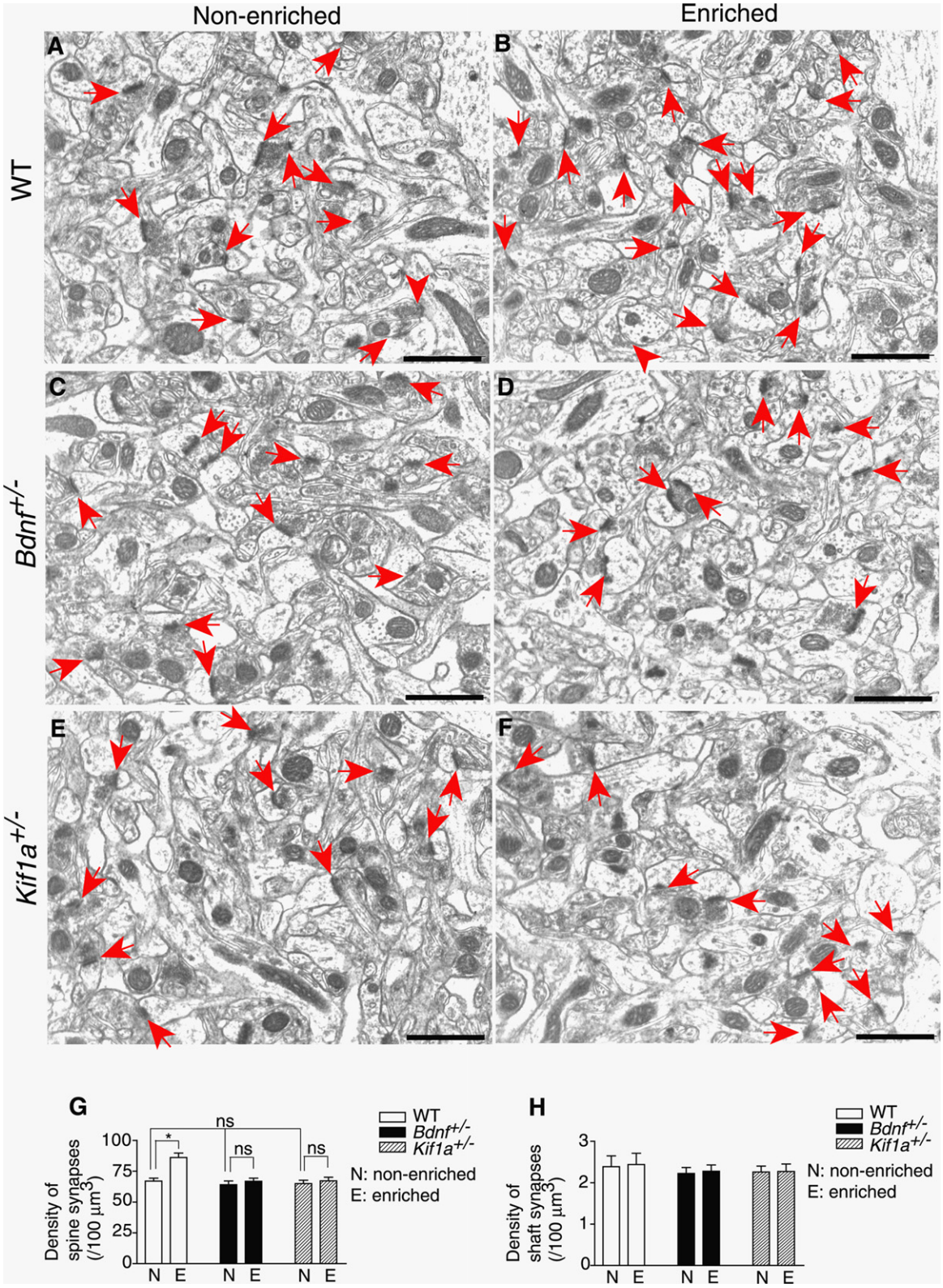

Figure 3. Enrichment Does Not Induce Hippocampal Synaptogenesis in *Bdnf*$^{+/-}$ or *Kif1a*$^{+/-}$ Mice

(A–H) Electron microscopic analysis of synapse density in the hippocampus. (A–F) Representative electron micrographs showing synapses in the stratum radiatum of the hippocampal CA1 region of wild-type (A and B), *Bdnf*$^{+/-}$ (C and D), and *Kif1a*$^{+/-}$ (E and F) mice with or without enrichment exposure for 3 weeks.

to nonenriched wild-type]: 1.04 ± 0.10 versus 1.58 ± 0.16, p = 0.0450, two-tailed t test) (Figure S3D). These results suggest that KIF1A is not required for enhanced hippocampal neurogenesis induced by enrichment.

BDNF Enhances KIF1A Levels and KIF1A-Mediated Axonal Transport

To analyze the possible BDNF-dependent upregulation of KIF1A in more detail, we first examined the effects of BDNF on KIF1A levels in cultured hippocampal neurons. Quantitative immunoblot analyses revealed that there was an increase in KIF1A levels in BDNF-treated neurons in a time-dependent (BDNF-treated/nontreated ratio: 1 day, 1.19 ± 0.07, p = 0.0471; 3 days, 1.62 ± 0.07, p < 0.001; 5 days, 1.67 ± 0.05, p < 0.001, two-tailed t test) (Figure 4A) and dose-dependent manner (BDNF concentration [ng/ml], ratio to 0 ng/ml: 10 ng/ml, 1.40 ± 0.05, p = 0.0016; 50 ng/ml, 1.55 ± 0.05, p < 0.001; 100 ng/ml, 1.64 ± 0.07, p < 0.001; 200 ng/ml, 1.67 ± 0.07, p < 0.001, two-tailed t test) (Figure 4B). This upregulation of KIF1A was completely blocked by K252a, a general inhibitor of Trk tyrosine kinase (BDNF+K252a-treated/non-treated ratio: 1.02 ± 0.06, p = 0.6843, two-tailed t test) (Figure 4C). The level of *Kif1a* mRNA was also increased (BDNF-treated/non-treated ratio: 1 day, 1.22 ± 0.05, p = 0.0487; 3 days, 1.64 ± 0.04, p = 0.0047; 5 days, 1.70 ± 0.07, p = 0.0100, two-tailed t test) (Figure 4D), while the stability of *Kif1a* mRNA was not affected by BDNF treatment (Figure S4A), suggesting that transcriptional regulation is involved in KIF1A upregulation. Furthermore, the protein level of synaptophysin, a cargo of KIF1A, was also increased in BDNF-treated neurons (BDNF-treated/nontreated ratio: 1.20 ± 0.04, p = 0.0077, two-tailed t test) (Figure S4B), consistent with previous reports (Suzuki et al., 2007). In all experiments, there were no changes in KIF5B levels among groups (Figures 4A–4C). In contrast to cultured neurons, no significant increase in KIF1A levels was observed in BDNF-treated astrocytes, compared with nontreated astrocytes (BDNF-treated/nontreated ratio: 1.05 ± 0.07, p = 0.5958, two-tailed t test) (Figure S4C), suggesting that BDNF enhances KIF1A levels in neurons rather than in glial cells.

Next, to study the possible effects of BDNF on KIF1A-mediated axonal transport, we analyzed the transport of synaptophysin-containing vesicles by live imaging. Time-lapse recordings revealed that the frequency of anterogradely transported vesicles was significantly increased in BDNF-treated neurons (nontreated versus BDNF-treated [vesicles/min]: 3.03 ± 0.34 versus 4.33 ± 0.41, p = 0.0193, two-tailed t test) (Figures 4E and 4F and Movie S1), while the velocity was not affected (nontreated versus BDNF-treated [μm/s]: 0.91 ± 0.06 versus 0.94 ± 0.05, p = 0.7054, two-tailed t test) (Figure 4G). In retrograde transport, there were no significant differences between BDNF-treated and nontreated neurons (nontreated versus BDNF-treated: frequency [vesicles/min], 2.23 ± 0.27 versus 2.40 ± 0.32, p = 0.6929; velocity [μm/s], 0.92 ± 0.07 versus 0.87 ± 0.05, p = 0.5804, two-tailed t test) (Figures 4F and 4G). These results suggest that BDNF augments KIF1A-mediated cargo transport by increasing the levels of KIF1A in neurons.

KIF1A Is Essential for BDNF-Induced Synaptogenesis

BDNF regulates synaptic plasticity and promotes synapse formation in vivo and in vitro (Bramham and Messaoudi, 2005; Bamji et al., 2006; Suzuki et al., 2007); therefore, to directly examine the role of KIF1A in BDNF-induced synaptogenesis, we performed immunocytochemistry using *Kif1a*$^{+/-}$ and *Kif1a*$^{-/-}$ hippocampal neurons, with or without BDNF treatment. We quantified the densities of synaptophysin-positive puncta (Figure 5A), PSD-95-positive puncta (Figure 5B), and synaptophysin/PSD-95-double-positive puncta (Figure 5C) along dendrites. BDNF treatment significantly increased the densities of synaptophysin-positive puncta (nontreated versus BDNF-treated [per 10 μm]: 1.38 ± 0.09 versus 2.32 ± 0.10, p < 0.0001, two-tailed t test) (Figure 5D), PSD-95-positive puncta (0.96 ± 0.06 versus 1.44 ± 0.08, p < 0.0001, two-tailed t test) (Figure 5E), and double-positive puncta (0.90 ± 0.06 versus 1.25 ± 0.09, p = 0.0026, two-tailed t test) (Figure 5F) in wild-type neurons, as previously described (Bamji et al., 2006; Suzuki et al., 2007). In contrast, no significant increases of these densities were observed in BDNF-treated *Kif1a*$^{+/-}$ or *Kif1a*$^{-/-}$ neurons, compared with respective nontreated neurons (nontreated versus BDNF-treated [per 10 μm]: *Kif1a*$^{+/-}$: synaptophysin-positive, 1.25 ± 0.11 versus 1.49 ± 0.12, p = 0.1642; PSD-95-positive, 0.89 ± 0.07 versus 1.01 ± 0.08, p = 0.2809; double-positive, 0.86 ± 0.07 versus 0.93 ± 0.08, p = 0.4911, two-tailed t test; *Kif1a*$^{-/-}$: synaptophysin-positive, 1.03 ± 0.09 versus 1.06 ± 0.10, p = 0.8125; PSD-95-positive, 0.74 ± 0.05 versus 0.75 ± 0.05, p = 0.8378; double-positive, 0.70 ± 0.05 versus 0.71 ± 0.06, p = 0.9163, two-tailed t test) (Figures 5D–5F). These results suggest that KIF1A upregulation is required for BDNF-induced synaptogenesis.

KIF1A Overexpression Promotes Synaptogenesis

Finally, to confirm the role of KIF1A in synaptogenesis, we examined the effects of KIF1A overexpression on cultured hippocampal neurons. Neurons were cotransfected with KIF1A and synaptophysin-green fluorescent protein (syp-GFP), and the density of syp-GFP puncta along dendrites was compared with that of neurons transfected with syp-GFP alone (Figure 6A). Significant increases in the total number and density of syp-GFP puncta along dendrites were observed in cotransfected neurons (syp-GFP alone versus KIF1A+syp-GFP: total number, 55.6 ± 4.6 versus 73.3 ± 5.0, p < 0.05; density [per 10 μm], 0.40 ± 0.02 versus 0.54 ± 0.02, p < 0.0001, post hoc Dunnett's test) (Figures 6C and 6D). Furthermore, there was also a significant increase in

Data for nonenriched (A, C, and E) and enriched (B, D, and F) mice are shown. Arrows indicate spine synapses; arrowheads indicate shaft synapses. Scale bars, 1 μm. (G and H) Quantification of spine (G) and shaft (H) synapse densities (n = 3 mice; four slices per mouse were analyzed in each group). The number of synapses per mouse counted by electron microscopy was as follows: nonenriched versus enriched, number of synapses per mouse: wild-type, 138, 148, 149 versus 167, 186, 191; *Bdnf*$^{+/-}$, 125, 139, 153 versus 134, 138, 153; *Kif1a*$^{+/-}$, 125, 142, 149 versus 130, 148, 151. Means ± SEM are shown in all histograms. *p < 0.05; ns, not significant (two-tailed t test or one-way ANOVA).
See also Figure S3.

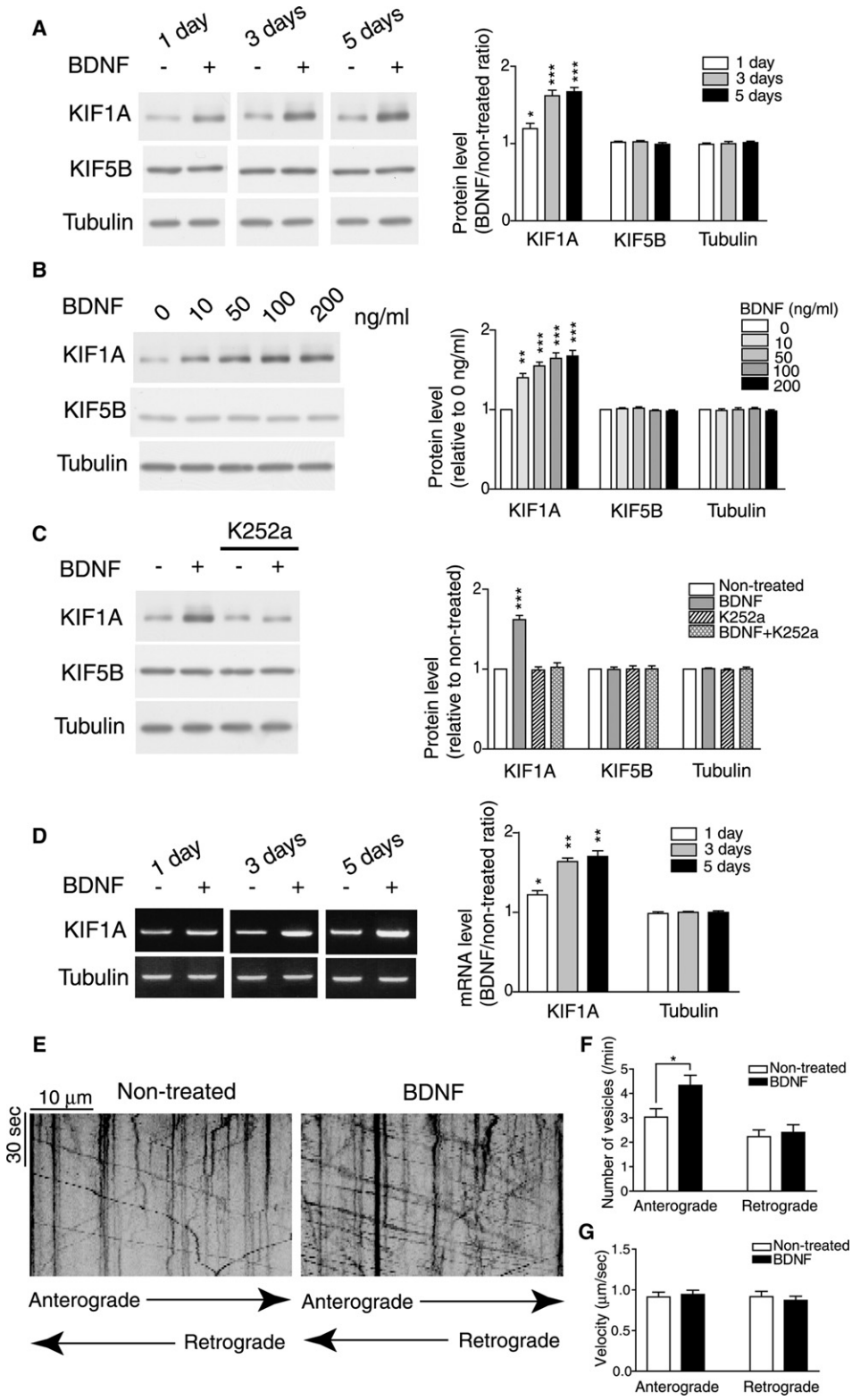

Figure 4. BDNF Enhances KIF1A Levels and KIF1A-Mediated Axonal Transport

(A–C) Quantitative immunoblots of KIF1A in lysates of cultured hippocampal neurons with or without BDNF treatment (100 ng/ml) for 1, 3, and 5 days (A), with BDNF treatment (0, 10, 50, 100, and 200 ng/ml) for 3 days (B), and with or without BDNF treatment (100 ng/ml) for 3 days with or without preincubation with K252a (200 μM) for 1 hr (C) (n = 5 from embryos of five different litters, respectively).

the number of syp-GFP puncta colocalized with PSD-95 in cotransfected neurons (syp-GFP alone versus KIF1A+syp-GFP: 37.1 ± 2.6 versus 47.2 ± 3.6, $p < 0.05$, post hoc Dunnett's test) (Figures 6B and 6I). No significant differences were detected in neurons cotransfected with KIF5B and syp-GFP (syp-GFP alone versus KIF5B+syp-GFP: total number, 55.6 ± 4.6 versus 57.2 ± 4.2, $p > 0.05$; density [per 10 μm], 0.40 ± 0.02 versus 0.42 ± 0.02, $p > 0.05$; number of colocalized puncta, 37.1 ± 2.6 versus 37.4 ± 2.9, $p > 0.05$, post hoc Dunnett's test) (Figures 6C, 6D, and 6I). There were no differences in the number or total length of dendrites contacted by axons of transfected neurons among groups (syp-GFP alone, KIF1A+syp-GFP, and KIF5B+syp-GFP: number of dendrites, 18.3 ± 1.3, 19.1 ± 1.2, and 18.5 ± 1.3, respectively, $p = 0.9053$; total length [μm], 1,389 ± 111, 1,411 ± 118, and 1,394 ± 109, respectively, $p = 0.9894$, one-way ANOVA) (Figures 6E and 6F). Also no significant differences were observed in the number of neurons contacted by axons of transfected neurons or in the mean distance from transfected neurons to contacted neurons among groups (syp-GFP alone, KIF1A+syp-GFP, and KIF5B+syp-GFP: number of neurons, 6.95 ± 0.41, 7.10 ± 0.51, and 6.80 ± 0.48, respectively, $p = 0.9034$; mean distance [μm], 245 ± 20, 249 ± 20, and 238 ± 20, respectively, $p = 0.9254$, one-way ANOVA) (Figures 6G and 6H). Collectively, these results suggest that increased KIF1A levels promote synaptogenesis via the formation of presynaptic boutons.

DISCUSSION

In this study, we found that enrichment causes BDNF-dependent KIF1A upregulation in the mouse hippocampus and revealed that a specific upregulation of KIF1A is indispensable for BDNF-mediated hippocampal synaptogenesis and learning enhancement induced by enrichment (summarized schematically in Figure 7).

A New Molecular Motor-Mediated Presynaptic Mechanism Underlying Experience-Dependent Neuroplasticity

Previous studies have shown that enrichment promotes synapse formation and improves learning behavior (van Praag et al., 2000; Nithianantharajah and Hannan, 2006). Although both axonal and dendritic factors could be important for these structural and behavioral changes, attention has mainly been paid to postsynaptic mechanisms, such as altered properties of NMDA (N-methyl-D-aspartate) and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors (Gagné et al., 1998; Rampon et al., 2000a; Tang et al., 2001; Naka et al., 2005). However, enrichment also causes alterations in the expression of presynaptic vesicle proteins (Rampon et al., 2000b; Nithianantharajah et al., 2004); therefore, it has been assumed that presynaptic processes are also involved in enrichment-induced changes. Although several different kinds of synaptic molecules, such as β-neurexin, nectin-1, and SynCAM, are involved in synaptogenesis (McAllister, 2007), presynaptic mechanisms influencing enrichment-induced changes have remained unclear. Recent studies have reported that Wnt signaling (Gogolla et al., 2009) and β-adducin (Bednarek and Caroni, 2011) are required for regulation of synapse numbers under enrichment. However, for the first time, our results demonstrate that enrichment-induced KIF1A upregulation acts presynaptically via the transport of synaptic vesicle proteins in axons of hippocampal neurons, and thus contributes to synaptogenesis. Moreover, we showed that KIF1A upregulation is essential for not only hippocampal synaptogenesis but also for learning enhancement induced by enrichment, indicating the possibility that learning/behavioral changes in an enriched environment could reflect structural synaptic alterations. This involvement of KIF1A in experience-dependent behavioral plasticity suggests that KIF1A upregulation contributes to the fine-tuning of brain function, through the remodeling of neuronal circuits.

Environmental enrichment has been defined as "a combination of complex inanimate and social stimulation" (van Praag et al., 2000). As for social interaction, rodents are highly social, and social contact with conspecifics is their most challenging enrichment factor. With social partners, in contrast to static enrichment objects, animals can perform social behaviors such as mutual grooming, social exploration, vocalizations, and play (Van Loo et al., 2004; Sztainberg and Chen, 2010). Therefore, the enrichment-induced changes observed in our study are likely to be caused by not only an addition of toys but also by a marked increase in social interactions through contact with larger numbers of animals per cage (nonenriched versus enriched: 3 mice versus 15 mice per cage).

BDNF-Dependent Upregulation of KIF1A

We found that the upregulation of BDNF preceded that of KIF1A in the hippocampi of enriched wild-type mice (Figure 1B), and *Bdnf*$^{+/-}$ mice did not show enrichment-induced KIF1A upregulation (Figure 1D). These results suggest that BDNF is an upstream regulator of KIF1A levels in vivo. Why, then, did it take 2 weeks for KIF1A to be upregulated? One possibility is that BDNF levels did not reach a minimal threshold for KIF1A upregulation in the first 2 weeks of enrichment, and another possibility is that there was a time lag between BDNF and KIF1A upregulation.

BDNF plays integral roles in neuronal signaling in various biological processes, such as synaptic plasticity, cell survival, and gene expression (Segal, 2003; Lu et al., 2005). In cultured hippocampal neurons, BDNF was shown to enhance KIF1A levels (Figures 4A–4C) and KIF1A-mediated axonal transport (Figures 4E–4G). Furthermore, our results suggest that transcriptional regulation is involved in BDNF-dependent KIF1A

(D) Semiquantitative RT-PCR of *Kif1a* from total RNA of cultured hippocampal neurons (n = 3, from embryos of three different litters).
(E–G) Dynamics of syp-GFP-containing vesicles in axons of cultured hippocampal neurons with or without BDNF treatment (100 ng/ml) for 3 days. (E) Kymographs of vesicles (left side, proximal; right side, distal). (F) The number of vesicles moving across the middle part of axons (n = 20 neurons, derived from embryos of three different litters). (G) The velocities of vesicles (n = 50 vesicles from 30 neurons, derived from embryos of three different litters). Means ± SEM are shown in all histograms. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (two-tailed t test).
See also Figure S4 and Movie S1.

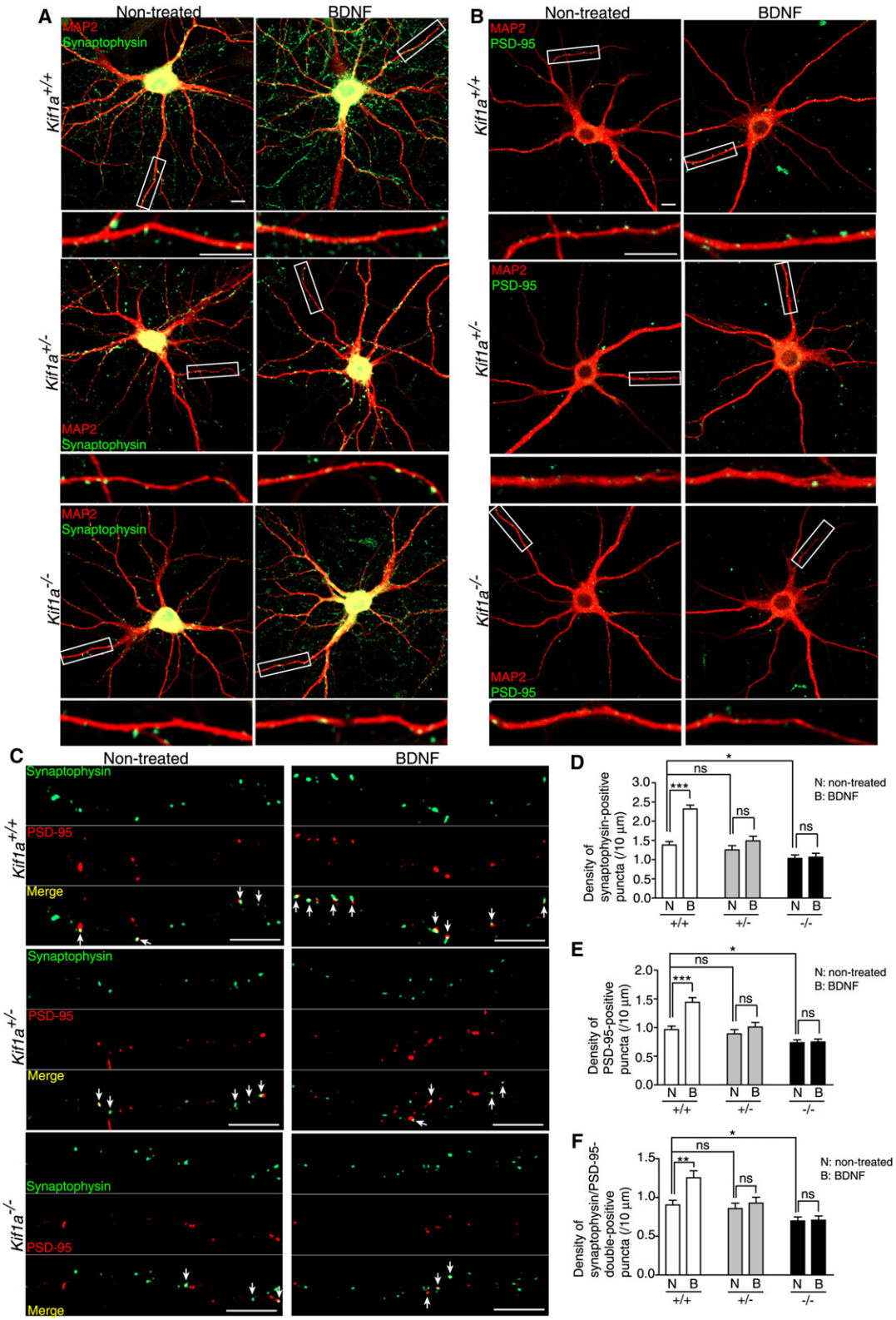

Figure 5. KIF1A Is Essential for BDNF-Induced Synaptogenesis

(A–F) Immunocytochemical analysis of cultured hippocampal neurons with or without BDNF treatment. (A–C) Representative images of *Kif1a*$^{+/+}$, *Kif1a*$^{+/-}$, and *Kif1a*$^{-/-}$ neurons stained for synaptophysin/MAP2 (A), PSD-95/MAP2 (B), and synaptophysin/PSD-95 (C) with or without BDNF treatment (100 ng/ml) for 3 days.

upregulation (Figure 4D). Interestingly, it has recently been shown that KIF1A transports BDNF-containing vesicles in hippocampal neurons (Lo et al., 2011). This raises a possibility that KIF1A-mediated transport might in turn affect the function of BDNF; therefore, a positive feedback loop of BDNF and KIF1A trafficking can be proposed. In our current study, however, *Kif1a* mutation did not affect the level of BDNF (Figure 1D); therefore, this possibility should be carefully examined in future studies.

Neurogenesis and KIF1A in Enrichment-Induced Learning Enhancement

Environmental enrichment has been shown to enhance neurogenesis in the hippocampal dentate gyrus of the adult mouse (Kempermann et al., 1997; van Praag et al., 1999), and enhanced hippocampal neurogenesis is related to improvement in some forms of learning (Bruel-Jungerman et al., 2005; Sahay et al., 2011). However, some studies have reported that enhanced neurogenesis is not required for enrichment-induced improvement in other behavioral tasks (Meshi et al., 2006; Bednarek and Caroni, 2011). Collectively, there are two types of enrichment-induced learning enhancement: one is neurogenesis-dependent, and the other is neurogenesis-independent.

Importantly, $Bdnf^{+/-}$ mice did not show any increase in hippocampal neurogenesis after enrichment; however, $Kif1a^{+/-}$ mice exhibited enhanced neurogenesis (Figures S3C and S3D), suggesting that enrichment-induced hippocampal neurogenesis requires BDNF, but not KIF1A. In other words, hippocampal neurogenesis is regulated under the control of a BDNF-dependent, but KIF1A-independent pathway. On the other hand, neither $Bdnf^{+/-}$ nor $Kif1a^{+/-}$ mice showed any enhancement of spatial learning (Figures 2D, 2E, 2G, and 2H) or contextual fear memory (Figure 2K) after enrichment, suggesting that this enrichment-induced learning enhancement requires both BDNF and KIF1A. Taken together, it is likely that the enrichment-induced enhancement of these learning and memory processes is mediated by the BDNF/KIF1A-dependent pathway, independently of enhanced hippocampal neurogenesis. Furthermore, it is also likely that enhanced synaptogenesis (Figures 3A–3H and Figures S3A and S3B) might underlie the neurogenesis-independent (BDNF/KIF1A-dependent) learning enhancement induced by enrichment.

Conclusions

This is the first report of a motor protein that plays a key role in enrichment-induced structural and behavioral changes. Our data demonstrate a new molecular motor-mediated presynaptic mechanism underlying experience-dependent neuroplasticity. Considering that enrichment is beneficial to ameliorate symptoms of brain disorders (van Praag et al., 2000; Nithianantharajah and Hannan, 2006), KIF1A is a potentially important therapeutic target that merits further investigation.

EXPERIMENTAL PROCEDURES

Environmental Enrichment

Three- to four-week-old male mice were housed in standard (nonenriched) cages without special equipment (3 mice per cage) or in enriched cages (15 mice per cage) equipped with running wheels, tunnels, igloos, huts, retreats, and wooden toys. All mice received standard lab chow and water *ad libitum*.

Mice

Bdnf mutant mice have been previously described (Ernfors et al., 1994) and were obtained from The Jackson Laboratory (Bar Harbor, ME). *Kif1a* mutant mice have been produced and described (Niwa et al., unpublished). $Kif1a^{+/-}$ mice are generally healthy and do not exhibit any sensory or motor neurological abnormalities up to 3 months old. These mutant mice had been backcrossed at least seven generations with C57BL/6J mice. Male mice were used in all experiments. Detailed information is provided in the Supplemental Experimental Procedures.

Immunoblotting

Mouse hippocampi, cultured hippocampal neurons, and cultured astrocytes were lysed in Nonidet P-40 (NP-40) buffer (10 mM HEPES [pH 7.4], 150 mM NaCl, 1% NP-40). The lysates were subjected to SDS-PAGE followed by immunoblotting as previously described (Yin et al., 2011). Quantification analyses were performed using ImageJ (National Institutes of Health) software. The respective protein levels in nonenriched wild-type mice, nontreated cultured hippocampal neurons, or nontreated cultured astrocytes were set as 1 at each time point. Detailed information is provided in the Supplemental Experimental Procedures.

The sources of antibodies used were as follows: anti-KIF1A (rabbit polyclonal, Niwa et al., 2008), anti-KIF1Bβ (rabbit polyclonal, Niwa et al., 2008), anti-KIF5A (rabbit polyclonal, Kanai et al., 2000), anti-KIF5B (rabbit polyclonal, Kanai et al., 2000), anti-KIF17 (rabbit polyclonal, Yin et al., 2011), anti-dynein (mouse monoclonal, Millipore), anti-synaptophysin (mouse monoclonal, Chemicon), anti-BDNF (rabbit polyclonal, Santa Cruz Biotechnology), anti-αTubulin (mouse monoclonal, Sigma), and anti-GAPDH (mouse monoclonal, Abcam).

Semiquantitative RT-PCR and Analysis of mRNA Stability

Total RNA was isolated from mouse hippocampi and cultured hippocampal neurons using ISOGEN II (Nippon gene) according to the manufacturer's instructions, and semiquantitative RT-PCR analysis was performed as previously described (Yin et al., 2011). The respective mRNA levels in nonenriched mice or nontreated cultured hippocampal neurons were set as 1 at each time point. Detailed information is provided in the Supplemental Experimental Procedures.

To determine stability of mRNA, hippocampal neurons at 7 days in vitro (DIV) were incubated with or without BDNF (100 ng/ml) in the presence of actinomycin D (10 μg/ml) to inhibit transcription as previously described (Yin et al., 2011). Total RNA samples were collected at 0, 6, 12, and 24 hr after actinomycin D treatment. Sample preparation and semiquantitative RT-PCR analysis were performed as described above. The respective mRNA levels at 0 hr were set as 100%.

Morris Water Maze Test

The water maze protocol was performed as previously described (Crawley, 2007; Yin et al., 2011) with slight modifications. For the visible platform trial, three sessions were performed. For the hidden platform trial, four to seven sessions were performed (four trials per session per day). Detailed information is provided in the Supplemental Experimental Procedures.

Insets in (A) and (B) show magnified views of boxed areas. Arrows in (C) indicate synaptophysin/PSD-95-double-positive puncta. Scale bars, 10 μm. (D–F) Densities of synaptophysin-positive puncta (D), PSD-95-positive puncta (E), and synaptophysin/PSD-95-double-positive puncta (F) along dendrites in $Kif1a^{+/+}$, $Kif1a^{+/-}$, and $Kif1a^{-/-}$ neurons (n = 30 neurons in D and E, n = 21 neurons in F, derived from embryos of three different litters, respectively). Means ± SEM are shown in all histograms. *p < 0.05, **p < 0.01, ***p < 0.001; ns, not significant (two-tailed t test or one-way ANOVA with a post hoc Dunnett's test).

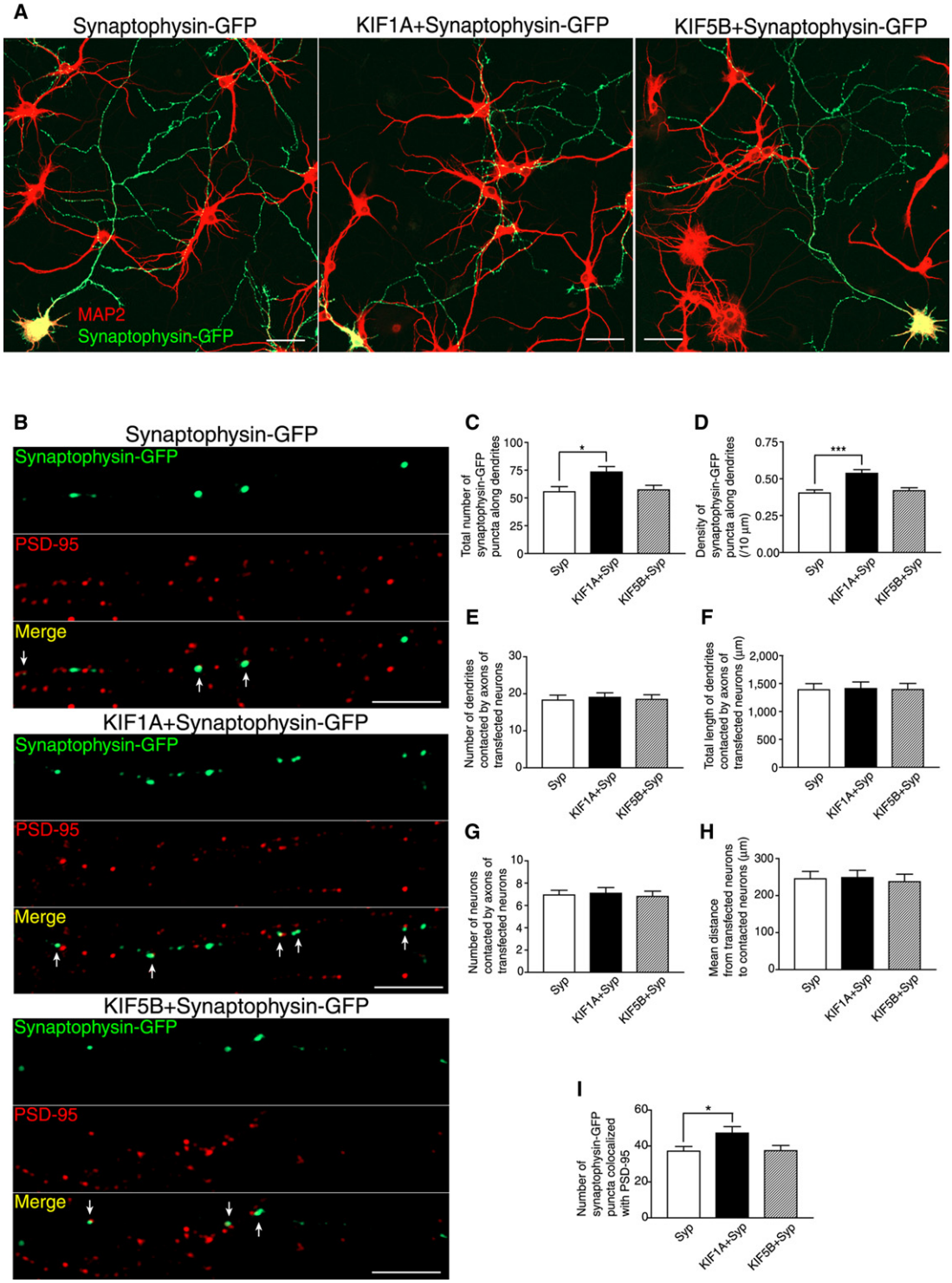

Figure 6. KIF1A Overexpression Promotes Synaptogenesis

(A–I) Overexpression experiment in cultured hippocampal neurons. (A and B) Representative images of neurons 3 days after transfection with the indicated constructs. Arrows indicate syp-GFP puncta colocalized with PSD-95 (B). Scale bars, 50 μm (A) and 10 μm (B). (C and D) Total number (C) and density (D) of syp-GFP puncta along dendrites per transfected neuron. (E and F) Number (E) and total length (F) of dendrites contacted by axons of transfected neurons. (G) Number of neurons contacted by axons of transfected neurons. (H) Mean distance from transfected neurons to contacted neurons. (I) Number of syp-GFP puncta colocalized with PSD-95 per transfected neuron. Means ± SEM are shown in all histograms (n = 20 neurons in C–H, n = 21 neurons in I, derived from embryos of three different litters, respectively). *p < 0.05, ***p < 0.001 (one-way ANOVA with a post hoc Dunnett's test).

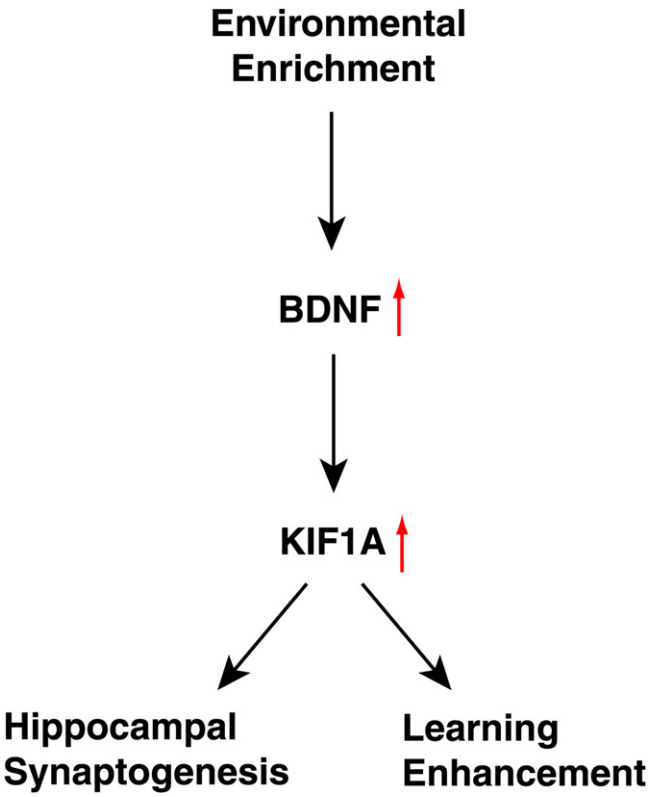

Figure 7. Schematic Summary of the Main Findings of This Study
BDNF-dependent KIF1A upregulation is required for enrichment-induced hippocampal synaptogenesis and learning enhancement.

Contextual Fear Conditioning Test
The contextual fear conditioning protocol was performed as previously described (Yin et al., 2011). Detailed information is provided in the Supplemental Experimental Procedures.

Electron Microscopy Analysis
Mice were anesthetized and perfused with 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). Matching areas from dorsal hippocampus were dissected out, and ultrathin sections were prepared as previously described (Rampon et al., 2000a). Synapse densities were estimated by an unbiased stereological method as previously described (Rampon et al., 2000a). Detailed information is provided in the Supplemental Experimental Procedures.

Immunohistochemistry
Mice were anesthetized and perfused with 4% paraformaldehyde in PBS. Coronal sections (20 μm) of the entire hippocampus were prepared as previously described (Yin et al., 2011). Dorsal hippocampus sections were double-stained for synaptophysin and PSD-95 as previously described (Fukaya and Watanabe, 2000). Synaptophysin/PSD-95-double-positive puncta in the stratum radiatum of the hippocampal CA1 region were counted. The values were normalized to nonenriched wild-type mice. Detailed information is provided in the Supplemental Experimental Procedures.

Neurogenesis Analysis
Mice were designated into a nonenriched group or an enriched group, and BrdU (50 mg/kg body weight) was injected intraperitoneally once a day during the first 7 days as previously described (van Praag et al., 1999). Coronal sections (20 μm) of the entire hippocampus were prepared as described above. The sections were double-stained for BrdU and the neuronal marker NeuN as previously described (van Praag et al., 1999). BrdU/NeuN-double-labeled cells in the granule cell layer of the hippocampal dentate gyrus were counted. The values were normalized to nonenriched wild-type mice. Detailed information is provided in the Supplemental Experimental Procedures.

Neuronal Cultures, Transfection, and Constructs
Dissociated hippocampal neurons were prepared as previously described (Yin et al., 2011). Neurons at 7 DIV were treated with the indicated concentrations of BDNF for 1, 3, and 5 days. Transfection of hippocampal neurons was performed using an improved calcium phosphate protocol at 7 DIV (Jiang and Chen, 2006). A syp-GFP construct (generated from a mouse complementary DNA clone) was provided by Dr. Niwa (our laboratory). KIF1A and KIF5B expression vectors were generated by standard molecular methods. Detailed information is provided in the Supplemental Experimental Procedures.

Astrocyte Cultures
Astrocyte cultures were prepared as previously described (Suzuki et al., 2007). The cultures were or were not treated with BDNF (100 ng/ml) for 3 days. Detailed information is provided in the Supplemental Experimental Procedures.

Live Imaging
Neurons were transfected with syp-GFP at 7 DIV and were incubated with or without BDNF (100 ng/ml) for 3 days. At 10 DIV, time-lapse recordings were performed with an LSM710 confocal laser-scanning microscope (Zeiss). We selected the middle part of axons of transfected neurons for live imaging. Images were acquired every 1 s, and syp-GFP containing vesicles moving across the center line of the imaged area were counted. Images were analyzed using ImageJ software.

Immunocytochemistry
Neurons at 7 DIV were or were not treated with BDNF (100 ng/ml) for 3 days. At 10 DIV, neurons were fixed and immunostained as previously described (Niwa et al., 2008). Cells were fixed with 4% paraformaldehyde in PBS for 10 min, permeabilized with 0.1% Triton X-100 in PBS, and blocked with 5% bovine serum albumin in PBS. Cells were incubated with primary antibodies overnight at 4°C, followed by incubation with the appropriate Alexa-labeled secondary antibodies for 1 hr. Images were acquired using an LSM510 confocal laser-scanning microscope (Zeiss). Immunopositive puncta along MAP2-labeled dendrites and synaptophysin/PSD-95-double-positive puncta were counted. For immunocytochemistry, anti-synaptophysin (mouse monoclonal, Chemicon, 1:1000; rabbit monoclonal, Abcam, 1:2000), anti-PSD-95 (mouse monoclonal, ABR, 1:200), and anti-MAP2 (chicken polyclonal, Abcam, 1:2000) antibodies were used.

Overexpression Experiment
Neurons were transfected with syp-GFP alone or cotransfected with syp-GFP and KIF1A or KIF5B at 7 DIV. At 10 DIV, neurons were fixed and immunostained for MAP2 and PSD-95, and images were acquired as described above. Synaptophysin-GFP puncta along MAP2-labeled dendrites and colocalized with PSD-95 were counted.

Statistical Analysis
Data were analyzed by the two-tailed t test or one-way ANOVA with a post hoc Dunnett's test. For analysis of water maze test data, one-way ANOVA and two-tailed t test were used in the probe test, and two-way repeated-measures ANOVA with a post hoc Bonferroni's test was used to compare differences between groups at several time points.

SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures, Supplemental Experimental Procedures, and one movie and can be found with this article online at doi:10.1016/j.neuron.2011.12.020.

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