Activity-Induced Notch Signaling in Neurons Requires *Arc/Arg3.1* and Is Essential for Synaptic Plasticity in Hippocampal Networks

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SUMMARY

Notch signaling in the nervous system has been most studied in the context of cell fate specification. However, numerous studies have suggested that Notch also regulates neuronal morphology, synaptic plasticity, learning, and memory. Here we show that Notch1 and its ligand Jagged1 are present at the synapse, and that Notch signaling in neurons occurs in response to synaptic activity. In addition, neuronal Notch signaling is positively regulated by *Arc/Arg3.1*, an activity-induced gene required for synaptic plasticity. In *Arc/Arg3.1* mutant neurons, the proteolytic activation of Notch1 is disrupted both in vivo and in vitro. Conditional deletion of Notch1 in the postnatal hippocampus disrupted both long-term potentiation (LTP) and long-term depression (LTD), and led to deficits in learning and short-term memory. Thus, Notch signaling is dynamically regulated in response to neuronal activity, *Arc/Arg3.1* is a context-dependent Notch regulator, and Notch1 is required for the synaptic plasticity that contributes to memory formation.

INTRODUCTION

Notch receptors and ligands are highly conserved transmembrane proteins that are expressed in the developing mammalian nervous system and in the adult brain (Givogri et al., 2006; Stump et al., 2002). The function of Notch signaling in the nervous system has been most studied in the context of neural stem/progenitor cell regulation, and neuronal/glial cell fate specification (Louvi and Artavanis-Tsakonas, 2006). However, numerous reports have suggested that Notch also plays a role in neuronal differentiation (Breunig et al., 2007; Eiraku et al., 2005; Redmond et al., 2000; Sestan et al., 1999), neuronal survival (Lütolf et al., 2002; Saura et al., 2004), and neuronal plasticity (Costa et al., 2003; de Bivort et al., 2009; Ge et al., 2004; Matsuno et al., 2009; Presente et al., 2004; Saura et al., 2004; Wang et al., 2004).

While studies in both vertebrates and invertebrates suggest that Notch signaling regulates neuronal plasticity, learning, and memory, it remains unclear where and how Notch is activated in mature neurons, how it affects synaptic plasticity, and whether it interacts with known plasticity genes. Here we provide evidence that Notch signaling is induced in neurons by increased activity, and that this signaling is heavily dependent upon the activity-regulated plasticity gene *Arc/Arg3.1* (*Arc* hereafter) (Chowdhury et al., 2006; Link et al., 1995; Lyford et al., 1995; Shepherd et al., 2006). Furthermore, disruption of Notch1 in CA1 of the postnatal hippocampus reveals that Notch signaling is required to maintain spine density and morphology, as well as to regulate synaptic plasticity and memory formation.

RESULTS

Notch1 Is Present at the Synapse and Is Induced by Neuronal Activity

Using an antibody that recognizes the active form of Notch1 (NICD1, S3 fragment), we found Notch1 present in the cell soma and dendrites of neurons in many regions of the brain, including the cerebral cortex and hippocampus (Figure 1A and data not shown). We also found that NICD1 and the activity-induced protein Arc were present in many of the same cells, suggesting that Notch1 signaling occurs in active neurons. Indeed, most EGFP+ neurons in a transgenic Notch reporter (TNR) mouse line (Mizutani et al., 2007) expressed Arc (e.g., 73% of EGFP+ cells in the cortex; see Figure 3A). In cultured neurons, Notch1 was enriched in the dendrites and cell soma, while the ligand Jagged1 (Jag1) was enriched in axons (Figures 1B and S1A, available online). Notch1, NICD1, and Jag1 colocalized with synaptic proteins (Figures 1C–1E and S1), and NICD1 was enriched in synaptosomal fractions derived from cortical extracts (Figure 1F).

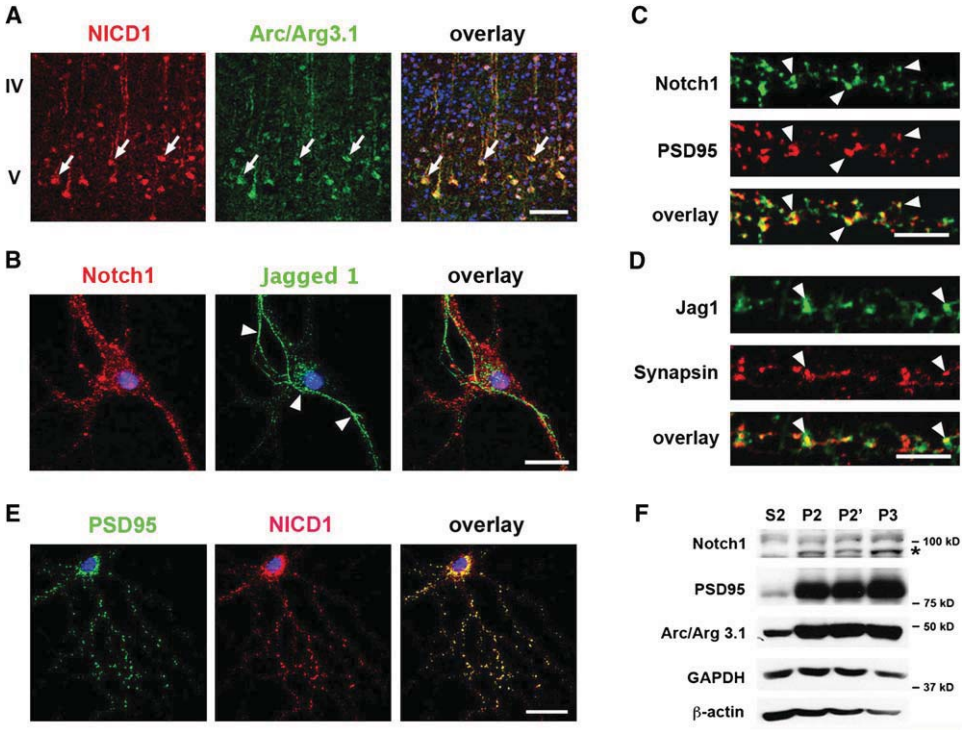

Figure 1. Notch1 Is Present at the Synapse in Mature Neurons

(A) The somatosensory cortex is shown. Arc and NICD1 are both present in the soma (arrows) and apical dendrites of layer V neurons. (B) DIV21 (21 days in vitro) hippocampal neuronal cultures immunostained to detect Notch1 and Jag1. While Notch1 is localized to the cell soma and dendrites, the ligand Jag1 is enriched in axonal processes (arrowheads). (C) Notch1 colocalizes in dendritic spines with the synaptic protein PSD95 (see also Figure S1). (D) Jag1 colocalizes with the synaptic protein Synapsin I. (E) The activated form of Notch-1 (NICD1) colocalizes with PSD95 in DIV21 neurons. (F) Subcellular fractionation of adult mouse cortices reveals that S3 fragment of Notch1 (asterisk) is enriched in synaptosomal fractions (P2, washed P2′, and membranes P3), as compared to the cytoplasmic fraction (S2). Scale bars = 75 μm (A), 25 μm (B and E), and 5 μm (C and D).

Increased neuronal activity after treatment with NMDA (Figures 2A and 2B) or bicuculline (Figure 2C) led to higher NICD1 levels, while treatment with the NMDA receptor blocker AP5 led to reduced NICD1 levels (Figure 2B). Neuronal activity also increased Notch1 protein levels (Figures 2C–2E, see also Figure 3E), including the preprocessed form of the receptor (Figures 2D and 2E), and *Jag1* expression (Figure 2F). Activity-induced Notch1 expression occurred in the presence of the transcriptional inhibitor actinomycin-D, suggesting that pre-existing Notch1 transcript is translated in response to synaptic activity (Figures 2D and 2E).

To test the effect of synaptic activity on Notch expression and processing further, we used acute hippocampal slices. The Schaffer collateral pathway was activated to induce LTP (Figure 2I), and increased Arc expression was observed in both CA3 and CA1 neurons (Figure 2G). In addition, somal Notch1 expression was increased in CA3 and CA1 (6.1-fold by pixel count, $n = 6$, $p < 0.02$) (Figure 2G), as was NICD1 staining (Figure 2H and data not shown). The increase in Notch expression in CA1 could be reduced by AP5 (Figure S2).

Neuronal Notch Signaling Occurs In Vivo in Response to Exploration

We next evaluated Notch expression and signaling in response to neuronal network activity in vivo after exploration of a novel environment. This behavioral paradigm activates specific ensembles of hippocampal pyramidal neurons that can be identified by expression of Arc (Guzowski et al., 1999). TNR mice were allowed to explore a novel environment for 5 min, and were sacrificed 1.5 or 8 hr later. Consistent with prior work (Ramírez-Amaya et al., 2005), the number of Arc+ hippocampal CA1 neurons was increased ~3-fold at 1.5 hr, and ~2-fold at 8 hr (Figure S3A). In addition, the number of Notch1+ CA1 neurons was elevated at both time points (~3-fold, Figures S3A and S3C), as was EGFP expression (indicative of Notch activity) at 8 hr (Figures S3B and S3C). Notably, nearly all (94%–97%) of the Arc+ neurons also had Notch1 signal in the nucleus (e.g., see Figure 3C), indicating that Arc induction and Notch signaling occur in the same neuronal networks in response to exploration. Some Notch1+ neurons did not express Arc (18% in controls and at 1.5 hr, and 29% at 8 hr). Thus, the temporal dynamics of Notch1 and Arc may be different, with Notch1 persisting longer than Arc, or not all neuronal Notch signaling occurs in Arc+ networks.

Neuronal Notch Signaling Is Disrupted in *Arc* Mutants In Vivo

Both Notch signaling (Fortini and Bilder, 2009; Vaccari et al., 2008) and Arc function (Chowdhury et al., 2006; Shepherd et al., 2006) engage Dynamin-mediated endocytosis, raising

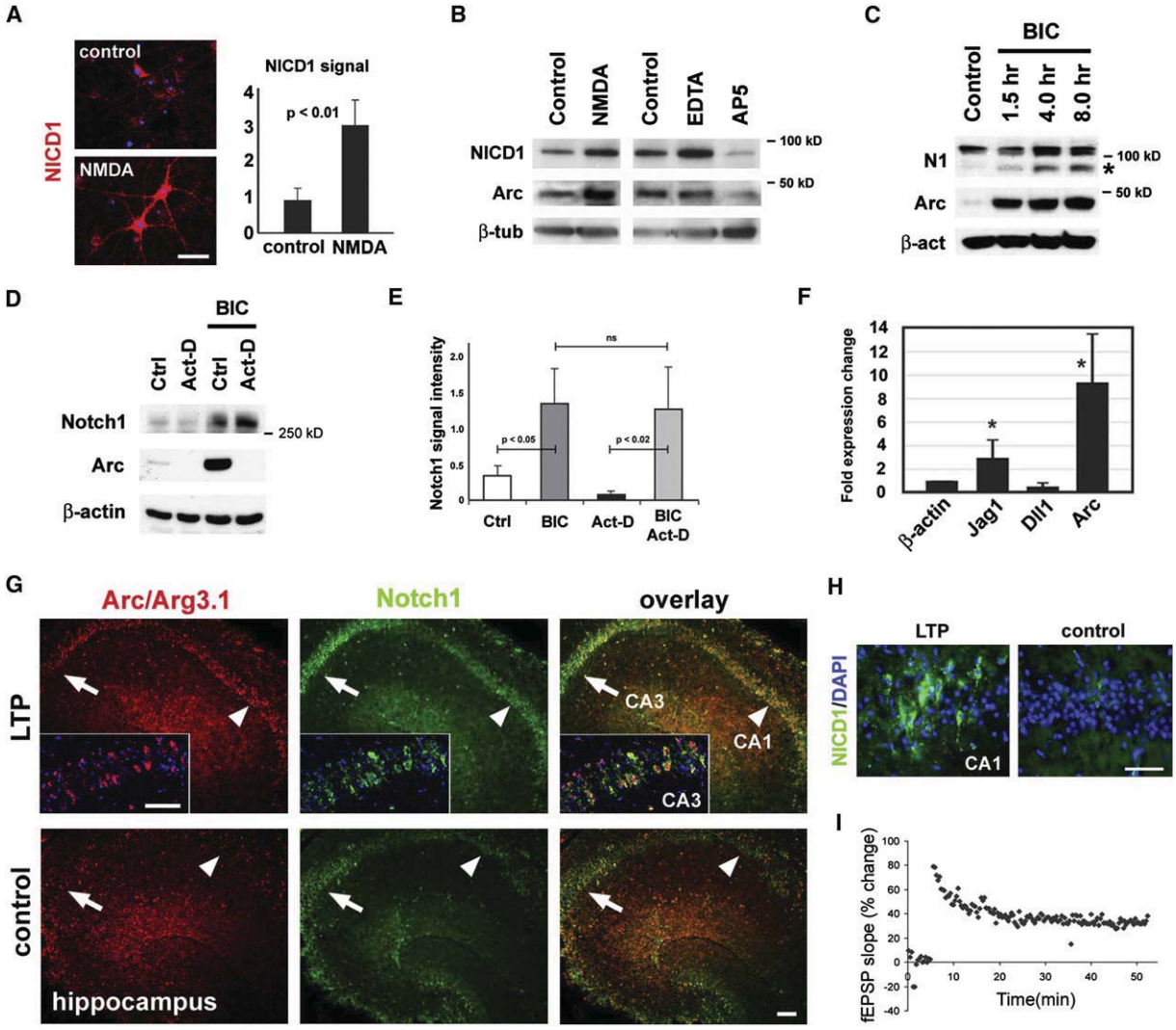

Figure 2. Notch Signaling Occurs in Neurons in Response to Activity

(A) NICD1 is increased in cultured hippocampal neurons after NMDA treatment. Relative NICD1 signal levels with (n = 4) or without (n = 3) treatment (right) are shown. Scale bar = 40 μm. (B) Western blot analysis showing that NMDA increased NICD1 (2.8-fold, n = 3, $p < 0.05$) and Arc (6.6-fold, n = 3, $p < 0.01$) protein levels, while NMDA receptor blockade (AP5) decreased NICD1 (4.3-fold, n = 3, $p < 0.05$) and Arc (10.0-fold, n = 3, $p < 0.01$). EDTA treatment was used a positive control to activate Notch1 (Rand et al., 2000). (C) Treatment of hippocampal neurons with bicuculline increased Arc and Notch1 S3 fragment levels (2.9-fold at 4 hr, n = 5, $p < 0.001$) (asterisk). (D) Western blot (WB) showing that full-length (pre-S1 cleavage) Notch1 protein levels increase in response to bicuculline, even with the transcriptional inhibitor actinomycin-D (Act-D). (E) Quantification of four experiments shows that the expression of full-length Notch1 (normalized to β-actin) is substantially increased after bicuculline treatment, with or without Act-D. ns, not significant. β-tub, β-tubulin; β-act, β-actin. (F) Quantitative RT-PCR of hippocampal cultures treated with bicuculline for 4 hr shows that *Jag1* expression was increased in response to increased neuronal activity (*$p < 0.04$, n = 4). (G) Three and one-half hours after LTP induction in the CA1 region of acute hippocampal slices from adult mice, both Arc and Notch1 protein levels were elevated in the soma of CA3 (arrow) and CA1 (arrowhead) neurons. (H) Immunohistochemistry (IHC) revealed increased NICD1 in CA1 in response to LTP. (I) Plot of field excitatory postsynaptic potential (fEPSP) in hippocampal slices. Mean values from four animals are shown. Scale bars = 50 μm. Standard deviation is shown in (A), (E), and (F).

the possibility that they might interact. Thus, we examined Notch activity in the adult brain of *Arc* mutants using the TNR mouse line. Of 15 *Arc* mutants, 12 (80%) had reduced EGFP expression (Notch activity) throughout the cerebral cortex as compared to 22 nonmutants (Figures 3A and 3B). *Arc* mutants also had reduced NICD1 levels, consistent with less Notch signaling in the absence of Arc (Figure 3B).

To test if Arc is required for Notch pathway recruitment in response to network activity in vivo, we compared Notch1 expression in the hippocampus of wild-type and *Arc* mutants after exploration of a novel environment. In controls, we observed elevated expression of both Arc and Notch1, the latter of which was localized to both the cell soma and the nucleus, in CA1 (not shown) and CA3 (Figure 3C). In contrast, no change

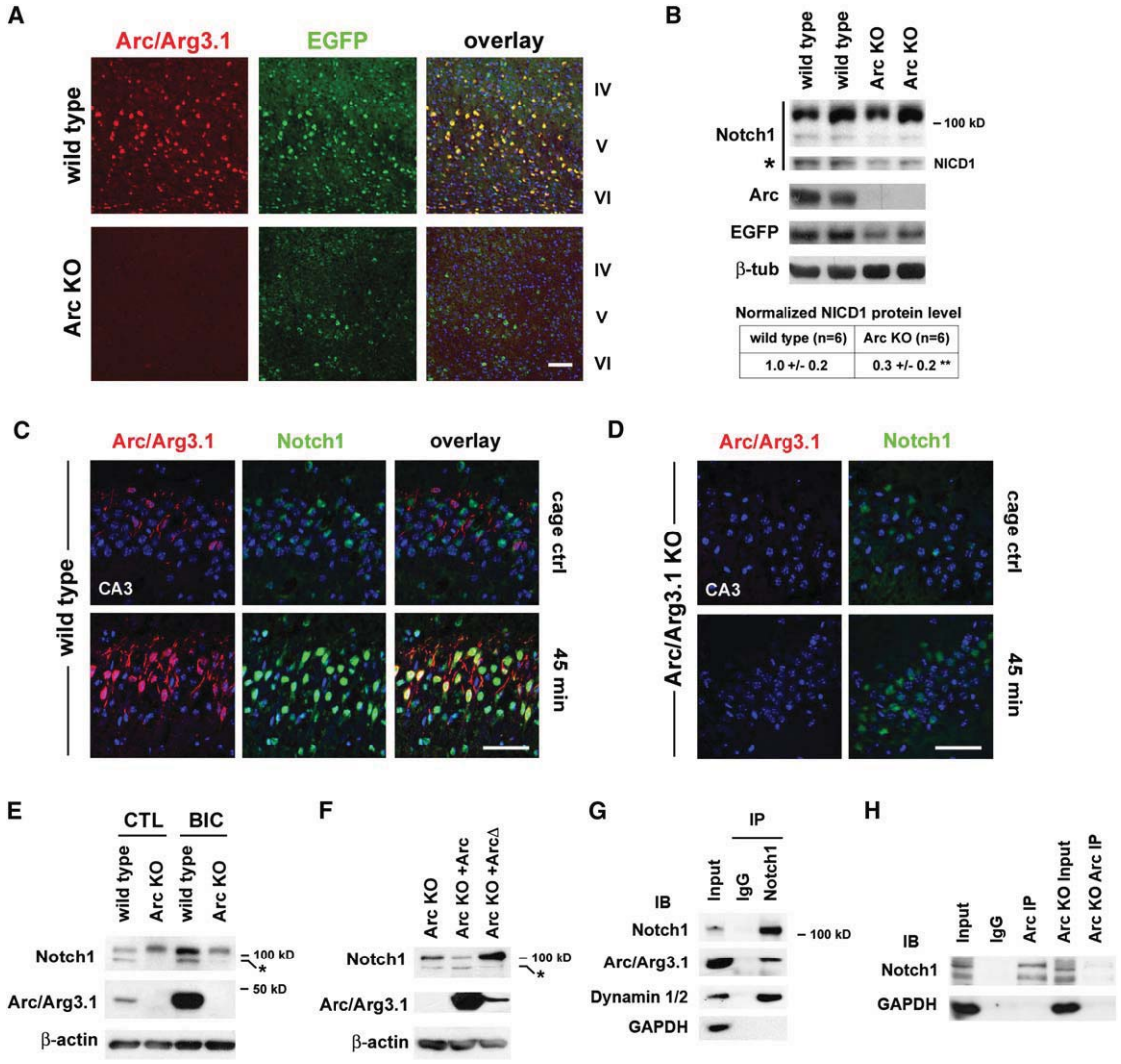

Figure 3. Neuronal Notch Signaling Is Disrupted in *Arc* Mutants In Vivo

(A) Five-week-old *Arc* mutant and wild-type animals containing the TNR transgene were examined to determine the impact of *Arc* disruption on the Notch pathway in vivo. *Arc* mutants had reduced EGFP expression, indicating reduced Notch activation (somatosensory cortex is shown). Scale bar = 70 μm. (B) Western blot of cell lysates derived from the cerebral cortex of *Arc* knockout (KO) animals revealed reduced NICD1 generation (asterisk) and EGFP expression. Two different exposures of the S3 band are shown. NICD1 band intensity (normalized to β-tubulin) was compared between six wild-type and six *Arc* KO animals (**$p < 0.01$). Standard deviation is shown. (C) In 5-week-old wild-type animals, exploration of a novel environment resulted in a rapid increase in Arc and Notch1 expression in CA1 (not shown) and CA3 (Notch1 signal intensity for cage control and 45 min after exploration was 6.8 ± 2.6 arbitrary units [a.u.], and 21.5 ± 5.2 a.u., respectively; $n = 3$ each, $p < 0.01$). Much of the Notch1 protein was in the cell soma and nucleus, consistent with active Notch1 signaling. (D) No increase in Notch1 expression was observed in *Arc* KO animals after exploration (cage control and 45 min after exploration was 11.2 ± 3.3 a.u. and 13.4 ± 4.8 a.u., respectively; $n = 3$ each). (E) Western blot analysis from *Arc* KO and wild-type hippocampal neuronal cultures revealed that, in the absence of Arc, Notch processing is reduced; the S3 band (asterisk) is nearly absent, unlike the S1 band (upper). (F) Western blot of *Arc* mutant hippocampal cultures infected with Sindbis virus expressing either full-length Arc or a nonfunctional form lacking residues 91–100 (Δ) (Chowdhury et al., 2006). (G) Arc and Dynamin coimmunoprecipitate with Notch1 from cortical protein preparations. (H) Notch1 coimmunoprecipitates with Arc from protein lysates generated from neuronal cultures. This interaction was not detected in Arc KO cultures. Scale bars = 50 μm.

in Notch1 expression or subcellular localization was observed in *Arc* mutants (Figure 3D).

Arc Regulates the Proteolytic Processing of Notch1 in Neurons

We next examined the status of Notch1 processing in *Arc* mutant neuronal cultures. In the absence of Arc there was a reduction in the S3 cleaved form of Notch1 (NICD1) (Figure 3E), indicating that Arc positively regulates the γ-secretase-mediated cleavage of Notch1 in neurons. Treatment with bicuculline led to elevated Notch1 and NICD1 levels in control neurons, but not in *Arc* mutant neurons (Figure 3E), indicating that Arc is required for the activity-mediated recruitment of neuronal Notch signaling. No change in Jag1 expression was observed in *Arc* mutant

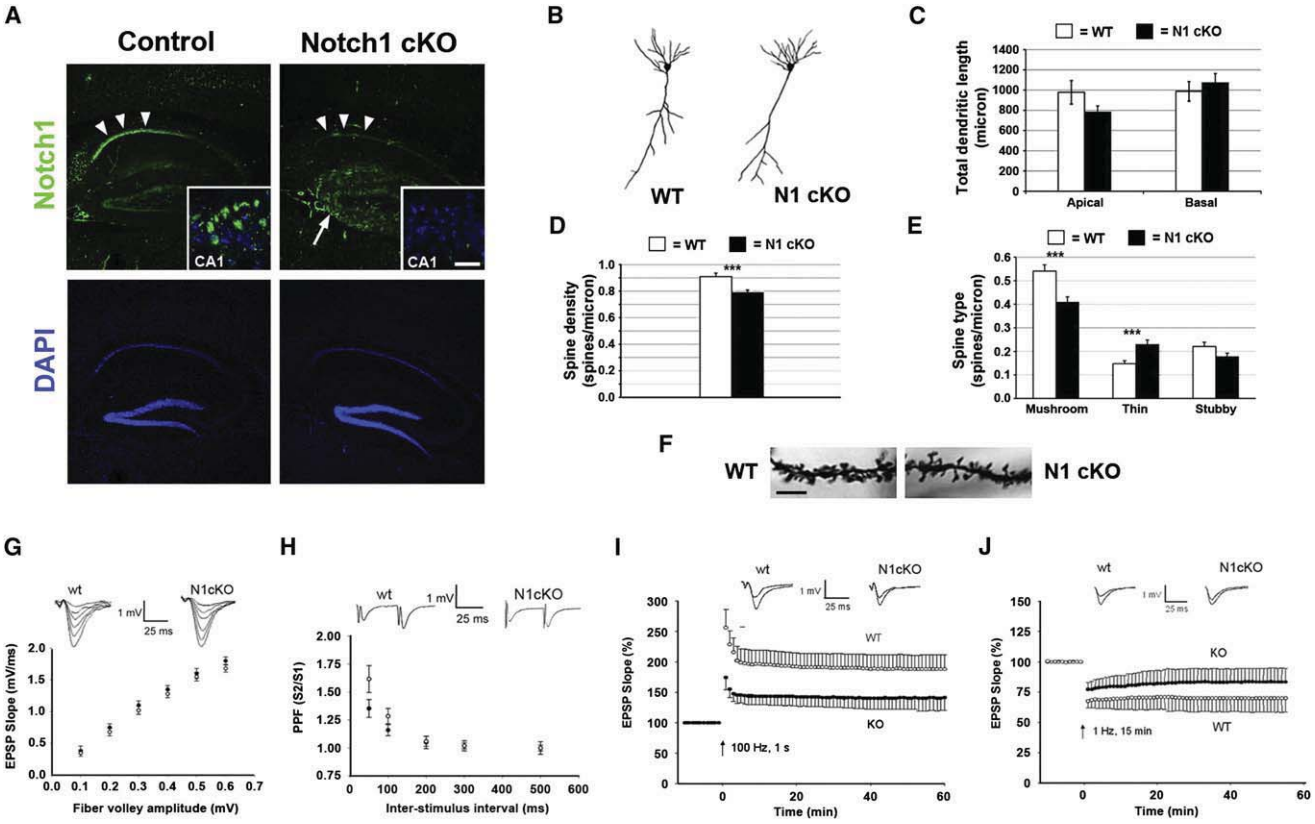

Figure 4. Loss of Notch Function in CA1 Affects Neuronal Morphology and Plasticity

(A) IHC shows that in Notch1 cKO mice, Notch1 expression is reduced in the CA1 region of the hippocampus (arrowheads). cKO animals had increased Notch1 expression in astrocytes (arrow). Inset scale bar = 25 μm. Note that these mice were exposed to a novel environment to increase Notch1 expression. (B) Golgi-impregnated CA1 pyramidal neurons reveal no difference in gross dendritic morphology between Notch1 cKOs and controls. (C) Notch1 cKO CA1 neurons have comparable lengths of apical and basal dendrites. (D) In Notch1 cKOs spine density of CA1 dendrites is reduced 16% ($p < 0.001$). (E) In Notch1 cKOs the number of mushroom spines is 25% reduced on CA1 pyramidal dendrites, and the number of thin spines is 40% increased ($p < 0.001$). (F) Images of Golgi-stained control and Notch1 cKO dendritic spines. Scale bar in (F) = 10 μm. (G) Notch1 cKO (closed circles) has normal basal transmission as compared to controls (open circles). (H) The paired pulse facilitation (PPF) curve is the same in Notch1 cKO and control slices. (I and J) LTP and LTD were reduced in the Notch1 cKO slices ($p < 0.01$ each). Standard deviation is shown in (C)–(E).

cultures (Figure S4), in line with the idea that receptor processing, and not ligand availability, is defective in mutant cells.

In an effort to rescue Notch1 processing in *Arc* mutant cells, we used Sindbis virus to introduce functional or nonfunctional Arc into mutant neurons in vitro. Restoration of Arc expression rescued Notch1 processing (2.9-fold increase, n = 3, $p < 0.001$) (Figure 3F), suggesting that the Notch1 cleavage defect in *Arc* mutant neurons is not caused by aberrant neuronal differentiation. A form of Arc lacking the ability to bind Endophilin and participate in endocytic trafficking (Δ91–100) (Chowdhury et al., 2006) was unable to restore Notch1 processing in *Arc* mutant neurons (Figure 3F).

Next, we found that Arc and Dynamin coimmunoprecipitated with Notch1 in protein preparations from adult cortical extracts (Figure 3G). In addition, Notch1 coimmunoprecipitated with Arc in protein extracts from wild-type, but not *Arc* mutant, cortical tissue (Figure 3H). Thus, Arc-mediated Dynamin-driven endocytosis of Notch1 may be important for activity-dependent Notch signaling in neurons. Interestingly, Arc is not required for Notch activation in embryonic forebrain progenitors (Figure S5), indicating that Arc regulates Notch in a context-dependent manner.

Conditional Deletion of Notch1 in the Postnatal Hippocampus

Having shown that Arc-dependent Notch signaling is activated in neuronal ensembles after spatial exploration, we next tested the function of Notch in such ensembles. To conditionally knock out Notch1 in the postnatal hippocampus, we crossed Notch1$^{flox/flox}$ (Radtke et al., 1999) mice with the CamKII-cre (T29-1) driver line (Tsien et al., 1996), and Notch1 deletion was confirmed at both the mRNA and protein levels (Figure S6 and Figure 4A, respectively; n = 6 each). Golgi-Cox staining of CA1 pyramidal neurons revealed that loss of Notch1 postnatally did not affect dendritic length (Figures 4B and 4C). However, spine density on secondary and tertiary dendrites was reduced (Figures 4D and 4F), and spine morphologies were altered (Figures 4E and 4F).

To test the role of Notch in synaptic plasticity, the electrophysiological properties of Notch1 conditional knockout (cKO) animals were tested using hippocampal slices and field

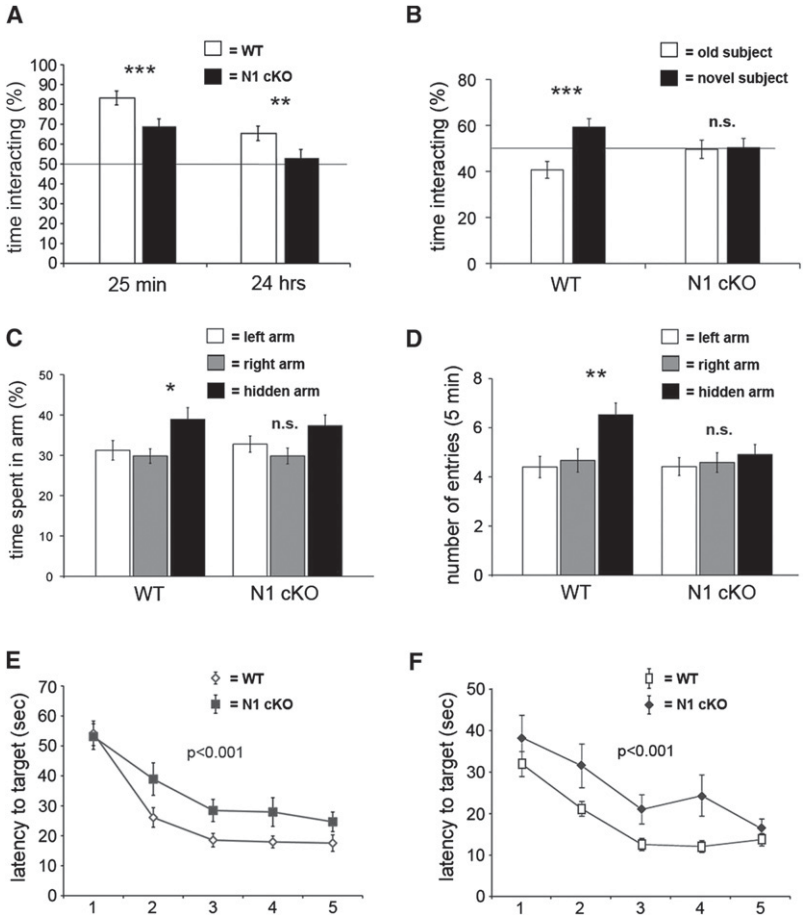

Figure 5. Notch1 Conditional Ablation Causes Deficits in Memory Acquisition

(A) While both control and Notch1 cKO animals spent more time exploring a novel object 25 min after exposure to two identical objects (83.2% ± 3.5% preference versus 68.8% ± 3.9%, respectively, $p < 0.001$), 24 hr later, Notch1 cKOs did not display any preference for the novel object (52.8% ± 4.5%, $p = 0.4$) in contrast to controls (65.4% ± 3.6%, $p < 0.01$) ($n = 12$ for both Notch1 cKO and WT). (B) Notch1 cKOs showed no preference for a novel subject (48.3% ± 4.1%, $p = 0.6$) in contrast to controls (60.2% ± 3.8%, $p < 0.01$) ($n = 13$ Notch1 cKO and $n = 16$ control). (C and D) Despite normal alternation in a Y-maze, Notch1 cKO mice failed to show a robust preference for a previously hidden arm, while controls did ($n = 12$ Notch1 cKO, $p < 0.07$; $n = 15$ control; $p < 0.022$). (E and F) The average time to find the platform in a Morris water maze was higher on day two, three, and four for Notch1 cKO animals ($p < 0.05$ for each time point). Similar results were obtained with reversal learning, where the platform was placed in the opposite quadrant (F). A repeated-measure ANOVA was used to assess statistical significance in (E) and (F). Twenty-four hours after the last of five training sessions for both initial and reversal learning, both Notch1 cKOs and controls spent more time in the target quadrant, indicating comparable memory retrieval after repetitive learning ($n = 14$, Notch1 cKO and $n = 18$, WT) (Figures S6A and S6B). Standard error is shown.

recordings. Basal transmission was the same for mutants and controls (10–11 slices) (Figure 4G), and the paired pulse facilitation (PPF) protocol revealed that Notch1 cKO slices had presynaptic strength comparable to that of controls (Figure 4H). However, when we induced LTP in the Schaffer collateral pathway, the magnitude of LTP in the CA1 region was uniformly higher in controls (188.5 ± 23.1, $n = 6$) than in Notch1 cKO slices (140.9 ± 20.6, $n = 5$, $p < 0.05$) (Figure 4I). Similarly, after low-frequency stimulation, LTD in CA1 was uniformly reduced in Notch1 cKO mice (83.4 ± 11.2, $n = 6$ slices) compared to controls (70.0 ± 11.5, $n = 5$, $p < 0.05$) (Figure 4J). Thus, Notch1 influences the magnitude of both the potentiation and depression of synaptic efficacy.

Notch1 cKO Mice Display Deficits in Learning and Acquisition of New Memory

Next we performed behavioral tests to evaluate the cognitive abilities of Notch1 cKO mice. During novel object recognition testing, mutants initially had a lower novel object preference than controls, and the next day, in contrast to controls, mutants had no preference (Figure 5A). Similarly, in a social interaction test, unlike controls, Notch1 cKO mice did not interact more with a new subject (Figure 5B), although like controls, mutants preferred a subject to an object (not shown). In Y-maze testing, Notch1 cKO mice chose alternating arms at the same frequency as controls (not shown), but showed no preference for a previously hidden arm (Figures 5C and 5D).

Next, spatial reference memory was investigated using the Morris water maze. Performance improved over 5 days of learning in both Notch1 cKO and control mice ($p < 0.0001$), although latency was greater in the mutants (Figure 5E, $p < 0.01$), despite the average swim speed being comparable ($p = 0.4$). A learning deficit was also seen in the Notch1 cKO mice when subjected to reversal learning (Figure 5F). In both cases, 24 hr after the last learning session, mutant and control mice spent more time in the target quadrant (Figures S7A and S7B). Thus, Notch1 cKO mice can learn using spatial cues, although they do so more slowly than wild-types.

In line with the previous report on the *Notch1*$^{+/-}$ mice (Costa et al., 2003), we could not detect any difference between Notch1 cKO and controls in contextual fear-conditioning 24 hr after a shock was delivered (Figure S7C). In addition, Notch1 cKO mutant mice displayed normal motor coordination (rotarod test), motor activity (open field test), and anxiety levels (elevated plus maze) (Figure S7C).

DISCUSSION

Notch1 Is at the Synapse and Can Be Activated in Response to Neuronal Activity

We have shown that Notch1 colocalizes with PSD95 in cultured neurons, and that the transcriptionally active form of the receptor, NICD1, is present at the synapse. In addition, we

have shown that Jag1 is present in axons, localizes to synapses, and is upregulated in response to neuronal activity. Stimulation of neurons in culture, in hippocampal slices, or in vivo after exposure to a novel environment all lead to increased Notch1 expression and signaling. The notion that activity-dependent γ-secretase-mediated Notch receptor activation can occur at the synapse is consistent with recent work showing that synaptic γ-secretase activity cleaves EphA4 in response to neuronal activity (Inoue et al., 2009).

Activity-Induced Neuronal Notch Signaling Requires Arc

The activity-regulated neuronal Notch signaling we have identified both in vitro and in vivo is heavily dependent upon Arc. In *Arc* mutant neurons we observe a drastic reduction in the S3 cleaved form of Notch1, indicating that the γ-secretase-mediated processing is disrupted in the absence of Arc function. Furthermore, our rescue and coimmunoprecipitation experiments indicate that the role of Arc in mediating Notch1 activation requires its association with Endophilin, and that Arc exists in a protein complex with Notch1 and Dynamin. Thus, in addition to its role in AMPA receptor trafficking (Chowdhury et al., 2006; Shepherd et al., 2006), Arc appears to regulate synaptic plasticity through interactions with the Notch pathway.

Notch1 Ablation Affects Neuronal Morphology, Plasticity, and Memory Acquisition

We next probed the potential function of activity-induced Notch signaling by conditionally deleting Notch1 in CA1 of the adult hippocampus. This model is an improvement over the *Notch1*$^{+/-}$, *CBF1*$^{+/-}$ (Costa et al., 2003) and Notch1 antisense mice (Wang et al., 2004), because deletion occurs after development is complete. Ablation of Notch1 in pyramidal CA1 neurons affects both spine density and morphology, and the electrophysiological properties of mutants are altered, with both synaptic potentiation and depression reduced. Our LTP result is consistent with reduced potentiation resulting from decreased Notch1 expression (Wang et al., 2004), or conditional γ-secretase disruption (via ablation of Presenilin 1/2) (Saura et al., 2004). However, our LTD result differs from those in previous studies, the former of which found enhanced LTD, and the latter of which found no change in LTD. This incongruence can be explained by the fact that the previous studies were confounded by possible developmental defects (Wang et al., 2004), and by lack of specificity with respect to Notch signaling (Saura et al., 2004).

Finally, to assess the effect of Notch disruption on learning and memory processes in hippocampal networks, we tested the Notch1 cKO mice using numerous behavioral paradigms. In the absence of Notch1, learning and rapid memory retrieval of newly presented cues are affected, whereas memory after repetitive learning is not. A function for Notch in rapid processing is consistent with the increase in Notch activation in hippocampal networks that occurs shortly after sensory input.

In summary, we have shown that Notch signaling is highly dynamic in mature neurons, and that it is induced in response to neuronal activity both in vitro and in vivo. In addition, we have identified the activity-regulated gene *Arc* as a context-dependent regulator of Notch signaling, and have shown that Arc is required for the γ-secretase-mediated activation of Notch1 in response to neuronal activity. Finally, using conditional disruption we have shown that Notch1 is required for normal spine morphology, synaptic plasticity, and memory processing.

EXPERIMENTAL PROCEDURES

Animals

All mice were maintained in accordance with the Institutional Animal Care and Use Committee (IACUC) at Johns Hopkins University School of Medicine. Generation of *Arc* mutant mice has been previously described (Plath et al., 2006). Notch1 cKO and wild-type littermate control (Notch1$^{flox/+}$, Notch1$^{flox/flox}$, and CamKII-Cre) mice were obtained by crossing Notch1$^{flox/flox}$ mice on a CD1 background to the CamKII-Cre (T29-1) mouse line on a C57BL6/129 background (Tsien et al., 1996).

Behavioral Experiments

For novel spatial exploration, cage control mice (t = 0 hr) were killed directly from their home cages, whereas the experimental mice performed a 5 min exploration session, and were returned to their home cage prior to analysis at the given time point. Novel object recognition was done accordingly to a published protocol (Bevins and Besheer, 2006). In the Y-maze mice were videotaped and scored for time spent in each arm and number of entries in each arm using the StopWatch Plus software. The social interaction testing was carried out in three sessions using a three-chambered box with openings between the chambers. The Morris water maze test was done according to a published protocol (Vorhees and Williams, 2006). Details for all behavioral tests are provided in the Supplemental Information.

Cell Culture and In Vitro Manipulation

Neuronal cultures were prepared from the hippocampus of E17.5 embryos and plated on poly-L-lysine-coated 60 mm dishes or 18 mm glass coverslips. Neurons were exposed to pharmacological manipulations after 14 days in vitro (DIV). For Sindbis virus infection, the pSinRep5 vector (Invitrogen) was used to generate viruses expressing either full-length Arc or a nonfunctional form with residues 91–100 deleted (Chowdhury et al., 2006).

Subcellular Fractionation, Immunoprecipitation, and Western Blot

Synaptosomal fractions were prepared as previously described (Blackstone et al., 1992). Standard western blot protocols were used. Details regarding fractionation, immunoprecipitation, and western blot protocols are provided in the Supplemental Information. Quantitation of individual protein bands was done using ImageJ software. Values were averaged between experiments, and Student's t test was used to compare samples.

Antibodies, Immunostaining, and Image analysis

A complete list of the antibodies used can be found in the Supplemental Information. Brain tissue and neuronal cultures were fixed in 4% PFA, and postfixed in ice-cold acetone-methanol (1:1) at –20°C for 10 min. The immunostainings with rabbit anti-Arc and anti-Notch1 antibodies were performed using the TSA fluorescence amplification kit (Perkin Elmer). ImageJ software (NIH) was used to quantify fluorescence intensity of immunostainings with NICD1 (Figure 2A), EGFP (Figure S3B), and Notch1 (see legend for Figures 3C and 3D). Student's t test was used to determine p values.

Golgi-Cox Staining and Spine Imaging and Analysis

Golgi-Cox staining (FD NeuroTechnologies) was performed according to the manufacturer's instructions. Dendrite and spine lengths/widths were measured using Reconstruct software by the Neural Systems Laboratory (http://www.bu.edu/neural/Reconstruct.html). Spine length and width data were analyzed using the Kolmogorov-Smirnov statistical test.

Hippocampal Slice Preparation and Electrophysiology

Transverse hippocampal slices (350 μm) were prepared from Notch1 cKO and control mice, and maintained in artificial cerebrospinal fluid at room temperature. Data were collected using an Axopatch 1D amplifier

(Molecular Device); signals were filtered at 2 kHz, digitized at 10 kHz, and analyzed using pCLAMP 8 software (Molecular Device).

SUPPLEMENTAL INFORMATION

Supplemental Information for this article includes Supplemental Experimental Procedures and seven Supplemental Figures and can be found with this article online at doi:10.1016/j.neuron.2011.01.004.

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