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Activity-Driven Dendritic Remodeling Requires Microtubule-Associated Protein 1A

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Summary

Activity-prompted dendritic remodeling [1, 2] leads to calcium-influx-dependent activation of signaling pathways within minutes and gene transcription within hours [3–6]. However, dendrite growth continues for days [2, 3, 7–9] and requires extension and stabilization of the cytoskeleton in nascent processes [8]. In addition to binding microtubules [10], microtubule-associated proteins (MAPs) associate with the actin cytoskeleton [11–13], anchor ion channels [14–19] and signaling complexes [17, 20], and modulate synaptic growth [21]. MAP2 is predominantly dendritic [22–24]. MAP1B is at postsynaptic densities (PSD) [25] and modulates ion channel activity [26], in addition to affecting axon growth [27, 28]. Less is known about MAP1A [10, 29–32], but it is also enriched in dendrites [29, 32] at input locations [33], including PSDs where MAP1A associates with channel complexes [19, 34] and the calcium sensor caldendrin [35]. MAP1A rescued hearing loss in tubby mice [36]. Here we show that MAP1A becomes enriched in dendrites concurrently with dendritic branching and synapse formation in the developing brain; that synaptic activity is required for establishing mature MAP1A expression levels; and that MAP1A expression is required for activity-dependent growth, branching, and stabilization of the dendritic arbor.

Results and Discussion

In the adult brain, MAP1A is enriched in dendrites, including dendrites of Purkinje cells and pyramidal neurons in the hippocampus and cortex (Figure 1; Figure S1 in the Supplemental Data available with this article online). Dendrites containing both MAP1A and MAP2 are common, but overall patterns are quite different. Typically, MAP2 is more generally distributed than MAP1A (Figure 1A; Figure S1). No MAP1A was detected in mature, myelinated axons beyond the initial segment in major tracts, such as the corpus callosum and cerebellar white matter (Figures 1A–1B). In contrast, prominent staining was seen in white matter of younger brains, and some of this staining was clearly localized in cell bodies and processes within the white matter (Figure S2). Restricted dendritic localization of MAP1A was reported previously [33, 37]. Interestingly, a specific form of MAP1A was enriched in layer IV of the barrel cortex at the terminal field of thalamo-cortical projections of whisker sensory input [33]. Although we do not know whether the antibodies we used are directed to specific MAP1A forms, the MAP1A recognized is low in mature axons and enriched in specific dendritic domains, and expression levels change with neuronal activity (see below).

MAP1A levels rise during the second postnatal week in the brain (Figure S3), when afferent inputs are established, dendrites elongate and branch, and synapses form. This increase is concurrent with a decline in the structurally related MAP1B, found in growing axons, and is delayed relative to the rise in MAP2. Among several markers for neuronal maturation, the pattern of the rise in MAP1A was most similar to that of synaptophysin (Syp), a synaptic marker [38]. MAP1A was first detected in Purkinje soma at P5 (Figure S3B), whereas MAP2 and calbindin were seen earlier (at P1, not shown). Also, in Purkinje cell cultures MAP1A expression lagged behind that of calbindin (Figure 1D). At each age, MAP1A distribution was more restricted than that of calbindin and MAP2 and was not detected in axons beyond the initial segment (Figures 1C–1D; Figure S3). Between P5 and P16, when granule-cell parallel fibers establish synapses on Purkinje cells, MAP1A staining increased in Purkinje cell dendrites. These results suggest a role for MAP1A in a later stage of dendritic maturation rather than in initial outgrowth.

In cultured hippocampal neurons, MAP1A levels rose most dramatically between 4 and 7 days in vitro (DIV) (Figure 1E), a time of increased dendrite growth and branching (Figures 1F, 2, and 3; Table S1). This time course of dendritic development in our cultures matches results of previous reports [39] and parallels the time of synapse formation in these cultures. Synaptic development in cultures of embryonic hippocampal neurons has been described in detail. The number of synapses (based on synapsin I puncta) increases from a very few (approximately 0) to approximately 80 per cell in 3–7 DIV E18 hippocampal cultures [40]. The rate of increase in the number of synapses is proportional to cell density in these cultures. Spontaneous Ca^{2+} currents were observed in slices from the hippocampus at E17, and the number of active cells increased approximately 8-fold in about 4 days [41]. Also, synchronous oscillation of Ca^{2+} transients was seen at 7 DIV but not at 3 DIV; synaptic activity further increased between 7 DIV and 14 DIV in cultures of E18 rat cortical neurons. Therefore, neurons in cultures from embryonic brains form functional synapses in parallel with dendritic growth.

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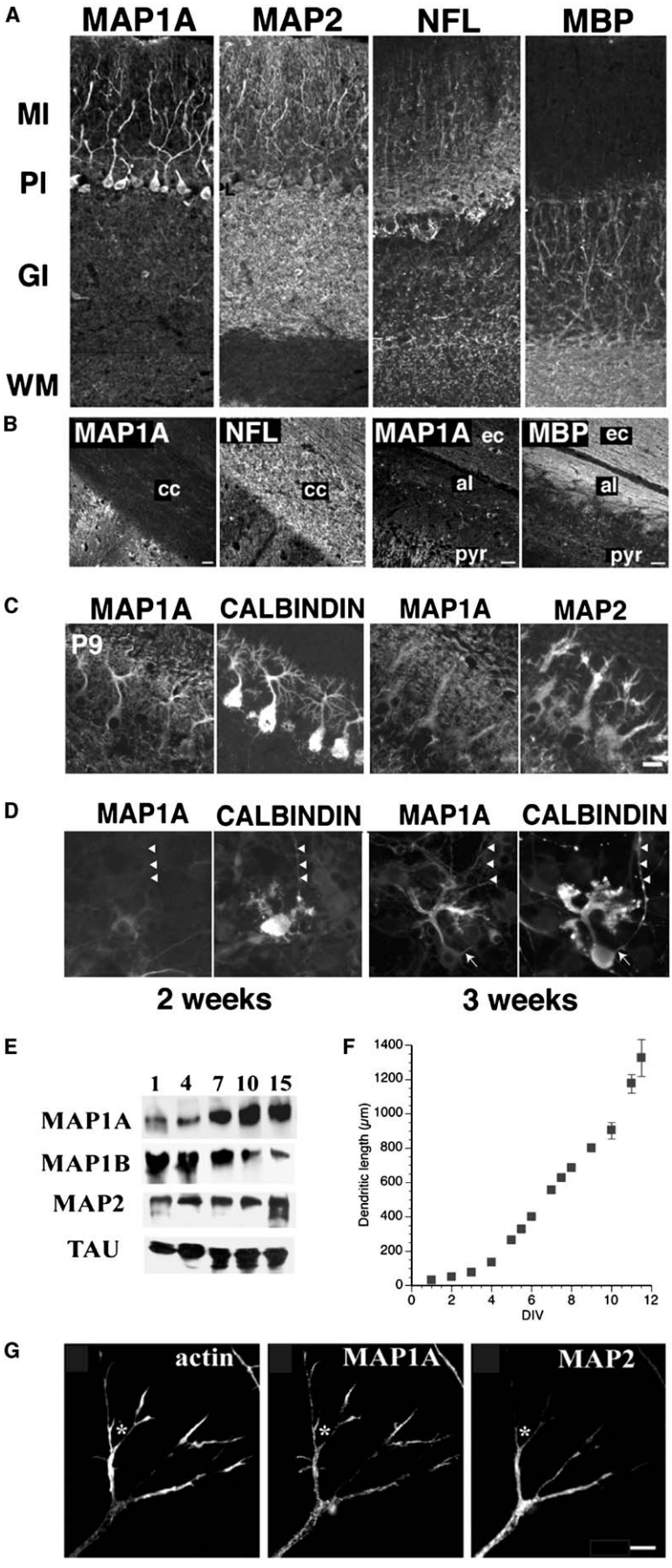

Figure 1. MAP1A Becomes Enriched in Dendrites in Parallel with Dendritic Growth in Both the Brain and Neuronal Cultures

(A) Immunolocalization of MAP1A in relation to MAP2, NF-L, and MBP in a 6-month-old rat brain. The molecular layer (MI), Purkinje layer (PI), granule layer (GI), and white matter (WM) of the cerebellar cortex are indicated.

(B) MAP1A/NFL double immunostaining in the corpus callosum (CC) at the midline of a 6-month-old brain and MAP1A/MPB in the alveus (al) and adjacent external capsule (ec).

(C) MAP1A/calbindin and MAP1A/MAP2 immunostaining in developing postnatal 9-day-old cerebellar cortex.

(D) MAP1A/calbindin staining in Purkinje cells cultured for 2 and 3 weeks. MAP1A dendritic staining is only seen at 3 weeks, whereas calbindin staining is clear at 2 weeks. Arrowheads trace the calbindin-positive/MAP1A-negative axon. MAP1A was detected only in the initial segment of these axons (indicated by an arrow).

(E) Immunoblots of homogenates from hippocampal cells cultured for 1–15 days. MAP1A increases the most between 4 and 10 days.

(F) Dendritic length in hippocampal neurons cultured for 3–12 days. Length increases are greatest between 4 and 10 days.

(G) Actin (phalloidin) and MAP1A colocalize in distal regions of a dendritic growth cone of a 6 DIV hippocampal neuron. MAP2 is located more proximally. An asterisk indicates the identical position in each panel. Figure S5 shows a color version of these panels. The scale bar represents 5 μm. Error bars represent the standard error of the mean.

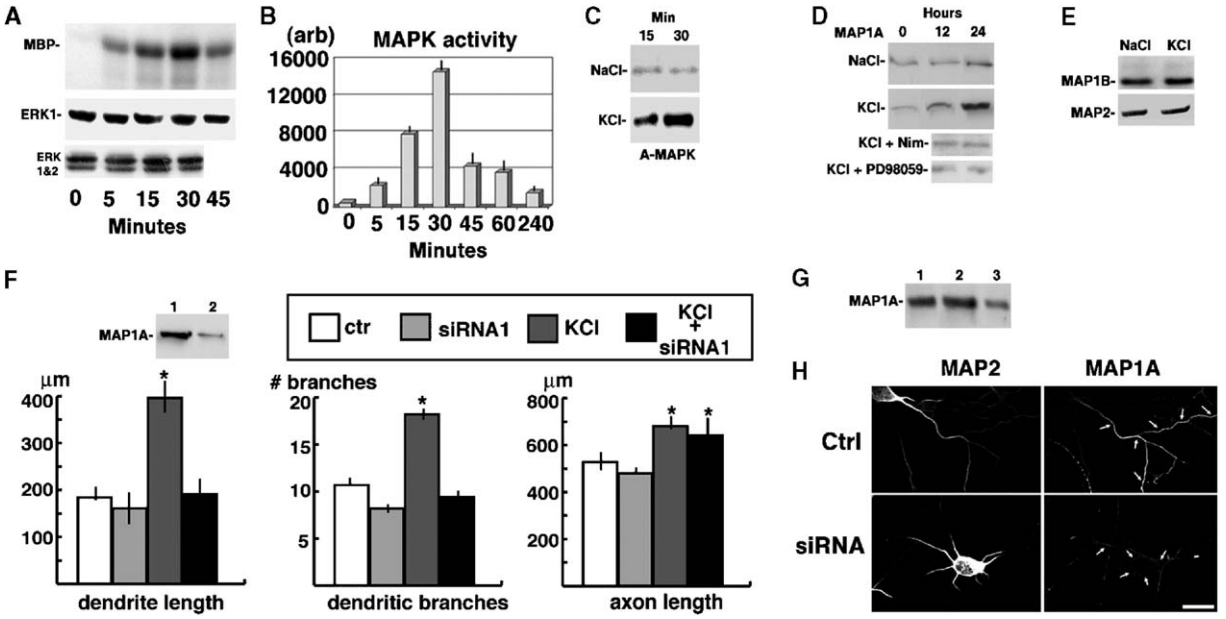

Figure 2. Membrane Depolarization Alters MAP1A Expression via Ca^{2+} Channels and MAPK Activity, and Increased MAP1A Expression Is Required for Activity-Enhanced Dendritic Growth and Branching

(A) (Top) MAPK activity in cell extracts after 0–45 min KCl treatment of 3 DIV hippocampal neurons was assayed by incorporation of ^{32}P into MBP substrate and visualized by Phosphorimager; (middle and bottom) ERK1 and ERK2 protein levels were unchanged in immunoblots.

(B) MAPK activity after treatment with KCl was determined with an in vitro phosphorylation assay that [46] that was quantified by phosphorimaging; note that the peak of MAPK activity is at 30 min after treatment.

(C) Western blots showing that active MAPK (A-MAPK) levels in cultures stimulated for 15 or 30 min with 50 mM KCl increased in comparision to levels in unstimulated (50 mM NaCl) cells.

(D) Western blots showing MAP1A protein levels at 3 DIV (0 hr) and at different time points (12 and 24 hr) after treatment with KCl or NaCl; KCl treatment led to a substantial increase in MAP1A levels 12–24 hr later. The KCl-mediated increase in MAP1A was completely blocked by cotreatment with nimodipine (a blocker of L-type Ca^{2+} channels) or with PD98059 (an inhibitor of MAPK activity).

(E) KCl treatment had no effect on MAP1B and MAP2 protein levels.

(F) (Insert) Western blot showing that MAP1A siRNA (lane 2) blocked KCl-induced increases in MAP1A (lane 1); siRNA was applied for 36 hr, starting 12 hr prior to KCl (applied at 3 DIV), and samples were collected 24 hr after the addition of KCl. Statistical comparisons between NaCl-treated and KCl-treated samples were performed by ANOVA followed by post-hoc comparisons using the Tuckey test. (***) $p < 0.0001$ (stars in graph). Graphs showing that dendritic length and branching increased after KCl treatment (KCl) compared to NaCl treatment (for controls; ctr). MAP1A siRNA transfection (siRNA1) blocked KCl-induced changes (siRNA1+KCl). Axonal length also increased with activity, but MAP1A siRNA treatment did not affect axonal growth.

(G) Western blot showing that siRNA transfection reduces MAP1A protein levels in older cultures; for this experiment 5 DIV cultures were transfected with siRNA1 for 36 hr. Lane 1, 5 DIV; lane 2, scrambled siRNA, 6.5 DIV; lane 3, MAP1A siRNA1, 6.5 DIV.

(H) MAP2/MAP1A immunostaining in 6 DIV control (ctr) and MAP1A siRNA (siRNA)-treated hippocampal neurons. Arrows outline cells. The scale bar represents 10 µm.

All error bars represent the standard error of the mean.

At the earliest stages of dendritic growth in hippocampal cultures, MAP1A immunofluorescence was low and widespread, including the nascent axon and associated growth cones (Figure S4A). Dendritic growth cones also stained prominently where MAP1A localized distally, and it was especially prominent in actin-rich filopodial extensions, a locale well suited for regulated contact-dependent dendritic remodeling (Figure 1G; Figure S5). Given that MAP1A has both microtubule and actin binding activities [11–13], it could be involved in mediating interactions between these cytoskeletal elements; such interactions are important for growth-cone guidance and neurite extension [42] as well as for synaptic growth [12]. In contrast to distal accumulation of MAP1A, MAP2 staining at this stage was stronger in proximal regions of dendrites.

In high-density cortical cultures, MAP1A became restricted to dendrites of pyramidal neurons after about 2 weeks (Figures S4B–S4C). In younger cultures, MAP1A distributed throughout cells, including the axon, with enrichment in distal neurite regions. This juvenile pattern of MAP1A was retained in cells grown in isolation for 2 weeks (Figure S4C, "LD"), correlating with shorter and less branched dendrites in low-density cultures, as compared to higher-density cultures where cells are in contact with one another. Furthermore, in low-density hippocampal cultures as opposed to high-density cultures, a delay of 4–6 days was observed in MAP1A accumulation in dendrites. Taken together, these observations suggested that cell-cell contact might regulate MAP1A levels and subcellular distribution. These findings prompted us to examine whether MAP1A expression was responsive to neuronal activity and played a role in the branching of dendrites.

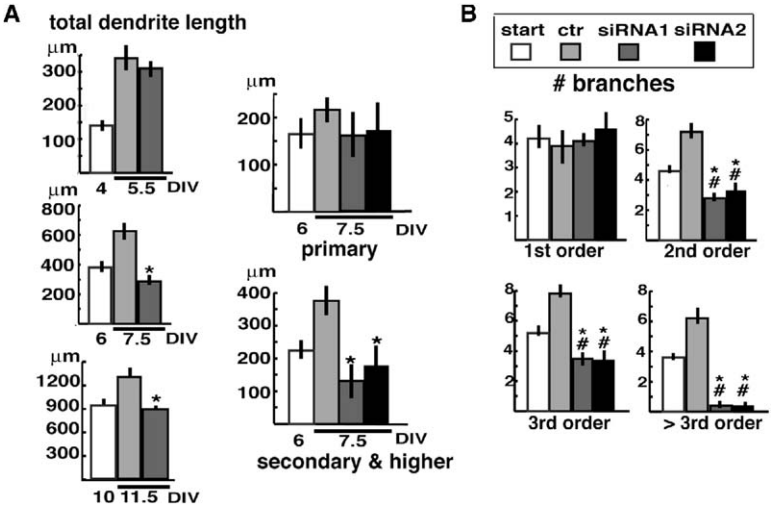

Figure 3. MAP1A Is Required for Elongation and Stabilization of Dendritic Branches

(A) Dendritic length at the start of treatment with siRNA and after 1.5 days of treatment with scrambled (ctr) and MAP1A siRNAs. The initial stage of dendritic growth (4–5.5 DIV) when primary dendrites were forming was not affected significantly by treatment, but as dendrites matured and elongated (6–7.5 DIV or 10–11.5 DIV), growth was blocked by MAP1A siRNA treatment. This effect was most pronounced on the formation of secondary and higher-order dendrites.

(B) Graphs show the number of dendritic branches in control siRNA and MAP1A siRNA-treated cultures. MAP1A expression is required for formation and retention of secondary and higher-order dendritic branches. Bars marked with an asterisk show significant differences from age-matched control, and bars marked with a number sign show significant differences from values at the time of RNAi addition (start). Statistical analysis employed ANOVA post-hoc comparisons performed via the Tuckey test; $p < 0.0001$. In the presence of MAP1A siRNA, higher-order dendrites were significantly shorter and had fewer branches than processes in 36-hr-younger cultures. All error bars represent the standard error of the mean.

To examine whether neuronal activity regulated MAP1A expression, 3 DIV hippocampal cultures were treated with 50 mM KCl. KCl-induced increases in filopodial extensions had been shown to depend on activation of L-type Ca^{2+} channels and the MAPK pathway (reviewed in Wong and Gosh [3]). In our cultures, a 30 min treatment induced a rapid (15–30 min) activation of MAPK, with no significant changes in ERK1 and ERK2 protein levels (Figures 2A–2C). Pretreatment with nimodipine, an L-type calcium-channel blocker, inhibited KCl activation of MAPK by 80% (not shown).

A 30–60 min KCl treatment resulted in a significant increase in MAP1A protein levels (Figure 2D; Table S2) After 12–24 hr, Nimodipine and PD98059, a MEK inhibitor, were applied prior to KCl treatment and blocked increases in MAP1A (Figure 2D). In contrast, no changes in MAP1B and MAP2 were observed after the addition of KCl (Figure 2E). In parallel with the KCl-induced increase in MAP1A levels, total dendritic length increased from 184 ± 28 μm (untreated) to 396 ± 44 μm (KCl), and the number of dendritic branches increased from 10.7 ± 0.3 to 18.2 ±0.4 (Figure 2F). Effects on dendritic length and number were blocked by pretreatment with nimodipine or PD98059; under these circumstances, dendritic lengths after KCl stimulation remained at 178 ± 12 μm and 180 ± 18 μm, respectively (Table S2). Activity also enhanced axonal growth (528 ± 36 μm [untreated] to 680 ± 32 μm [KCl] [Figure 2F]). In summary, neuronal activity triggered by activation of L-type Ca^{2+} channels induced MAP1A expression in 3 DIV neurons to levels normally seen only after about a week. Therefore, we next examined if MAP1A was necessary for activity-induced neurite growth.

Activity modulation of dendritic remodeling was first suggested by a correlation between the timing of afferent growth/activity and dendritic maturation in the developing brain and was subsequently demonstrated directly in neuronal cultures [3]. KCl-induced increases in filopodial extensions were previously shown to require activation of L-type Ca^{2+} channels and the MAPK pathway [43, 44]. To evaluate whether MAP1A was required for activity-induced increases in dendritic length and arborization, we treated 3 DIV cultures with MAP1A siRNA before KCl application. MAP1A expression was reduced 36 hr after transfection with siRNA in 40%–60% of cells (Figure 2F), with no effect on MAP2 or MAP1B levels, (Table S3); actin and tubulin were also unaffected by siRNA treatment. Finally, we confirmed that MAP1A siRNA blocked KCl-induced increases in MAP1A protein levels (Figure 2F; Table S3).

As expected, in young cultures, MAP1A siRNA treatment on its own did not alter process growth because MAP1A protein levels are relatively low at this stage of development. Reducing MAP1A levels had no effect on MAPK activity in 3 DIV cultures (see Table 1). Quantitative Western blots with antibodies against total ERK1/ERK2 in cells treated with scrambled or specific siRNA for MAP1A show that ERK1/ERK2 protein levels are comparable with and without MAP1A or KCl treatment at 3 DIV. Similarly, the extent of MAPK activation by KCl treatment was comparable with and without MAP1A, as shown by immunoreactivity with an antibody against active phospho-MAPK (Table 1).

The mean total length of dendrites was 184 ± 28 μm (untreated) versus 161 ± 43 μm (MAP1A siRNA), and the number of dendritic branches was 10.7 ± 0.3 (untreated) versus 8.2 ± 0.6 (MAP1A siRNA) in 4.5 DIV cultures (Figure 2F). However, suppressing MAP1A expression completely blocked activity-induced increases in dendritic length (KCl at 396 ± 44μm versus KCl + siRNA at 191 ± 33 μm) and branching (KCl at 18.2 ± 0.4 branches versus KCl + siRNA at 9.4 ± 0.6 branches). This effect on neurite growth was specific to dendrites; MAP1A siRNA treatment did not affect KCl-induced enhancement of axon growth (Figure 2F). No differences in axonal length, as revealed by labeling with TAU1 mAb, were detected between MAP1A suppressed neurons and controls. In summary, increases in MAP1A were required for activity-induced remodeling of the dendritic arbor, with no effect on axon growth. The

Table 1. Suppression of MAP1A with siRNA Does Not Affect Basal Levels of MAPK or Activation of MAPK by KCl

Groups	ERK1 and ERK2	A-MAPK
Scrambled + NaCl	642 ± 24	116 ± 8
Scrambled + KCl	645 ± 15	894 ± 36***
SiRNA1+ NaCl	638 ± 28	122 ± 12
SiRNA1 + KCl	630 ± 15	945 ± 27***

Protein levels of Erk1/Erk2 were evaluated by quantitative immunoblot with specific antibodies against total Erk or active MAPK. Densitometry of ERK1/ERK2 or A-MAPK (active MAPK) Western blots were performed with Scion Image software. Quantification was performed in triplicate samples and replicated at least three times. Each value represents the mean ± SEM. Values are expressed in arbitrary units based on pixel intensity normalized to band area after subtraction of backgrounds. Statistical comparisons between NaCl-treated and KCl-treated samples were performed with ANOVA methods followed by post-hoc comparisons using the Tuckey test. Asterisks indicate $p < 0.0001$. The A-MAPK immunoreactivity after KCl stimulation for both Scrambled- and siRNA1-treated cells was significantly different from that in the corresponding unstimulated controls but was not significantly different in either non-control group. For this experiment, 2 DIV cultures were treated with scrambled or siRNA1 oligonucleotides for 24 hours; immediately afterwards NaCl or KCl was added to the culture medium for 60 min. The cells were then scraped, and extracts were obtained and processed for SDS-PAGE and Western blotting.

MAP1A siRNA treatment was also effective in blocking MAP1A expression in older cultures (Figures 2G and 2H), so we evaluated whether sustained MAP1A expression was required for the dendrite elongation and arborization that are normally prompted by synaptic activity in high-density cultures.

As noted above, MAP1A siRNA treatment of 3–4 DIV cultures, in which there are no synapses and MAP1A levels are low, had no effect on dendrite or axon length measured 36 hr later (Figures 2F and 3A). In contrast, MAP1A-supressed neurons displayed shorter and less branched dendrites compared to controls in cultures older than 7 DIV. The mean length of dendrites in cultures treated with MAP1A siRNA at 6 DIV was 288 ± 46 μm at 7.5 DIV, whereas it was 625 ± 57 μm in control cells. Similarly, total dendritic length was reduced in 11.5 DIV MAP1A siRNA-treated cultures (from 1326 ± 108 μm to 881 ± 34) (Figure 3A). In control cultures, there is robust growth at this time (Figure 1F; Table S1). In light of the fact that MAP1A preferentially localized to distal dendrites in developing neurons (Figure 1G; Figure S5), effects on primary and higher-order dendrites were evaluated separately for the 6–7.5 DIV treatment. This analysis revealed that MAP1A suppression preferentially affected the length of higher-order branches. Interestingly, MAP1A siRNA did not block dendritic growth stimulated by BDNF (data not shown), a factor that preferentially induces primary dendrites and proximal branches [45].

A comparison of the number of branches before and after MAP1A siRNA treatment revealed a requirement for MAP1A in the stabilization of dendritic arbors (Figure 3B). Although the number of first-order dendrites did not change with MAP1A siRNA, the number of higher-order branches was significantly lower than in age-matched controls; surprisingly, dendrites were even less branched than in younger cultures. Most strikingly, there were only 0.4 ± 0.1 higher-order branches in siRNA-treated cultures at 7.5 DIV as opposed to 3.6 ± 0.2 at the beginning of treatment at 6 DIV. These observations show that MAP1A siRNA not only inhibited dendritic growth but also resulted in the retraction of dendritic processes. In summary, these results indicate that inhibition of MAP1A expression specifically reduces the size of the terminal dendritic arbor. Because MAP1A siRNA blocked dendritic growth at the time when synapses form, we tested whether spontaneous synaptic activity regulated MAP1A expression. Therefore, 6 DIV cultures were treated with tetrodotoxin (TTX, 1 μM) for 24 or 36 hr, and then MAP1A levels and dendritic morphology were analyzed. TTX significantly decreased MAP1A levels but not MAP2 or tubulin protein levels (Figures 4A–4B); it also reduced the number of second- and higher-order dendritic branches (Figure 4C), as well as total dendritic length (Figures 4D and 4E).

Our results implicate MAP1A in activity-stimulated branching and stabilization of the terminal dendritic arbor without affecting axon growth and determination or initial outgrowth and elongation of primary dendrites. Although most studies on synaptic remodeling have focused on channel activity and signal transduction, there are precedents for a cytoskeletal-associated protein's playing a role. For example, β-catenin is another molecule that is a candidate for mediating effects of neuronal activity on dendritic branching by acting locally through the actin cytoskeleton, rather than via gene activation [1]. The effects of MAP1A are more severe than with β-catenin sequestration. Although specific mechanisms for MAP1A effects are not fully understood, MAP1A has the potential to mediate interactions between actin and microtubule cytoskeletons [12, 13] or to interact directly with postsynaptic components such as ion channels [19]. Suppression of MAP1A levels not only inhibited activity-induced dendritic branching but also induced retraction of existing branches. In addition, suppression of MAP1A led to an attenuation of normal activity-dependent dendritic growth and to the retraction of terminal branches. Taken together with observations that in vivo MAP1A levels increase at the time of synaptogenesis and that MAP1A associates with synaptic proteins [19, 34, 35], we conclude that MAP1A may be a critical factor for activity-dependent dendritic modeling in the maturing brain.

Supplemental Data

Supplemental Data including additional figures and tables as well as Experimental Procedures are available with this article online at http://current-biology.com/cgi/content/full/15/20/1820/DC1/.

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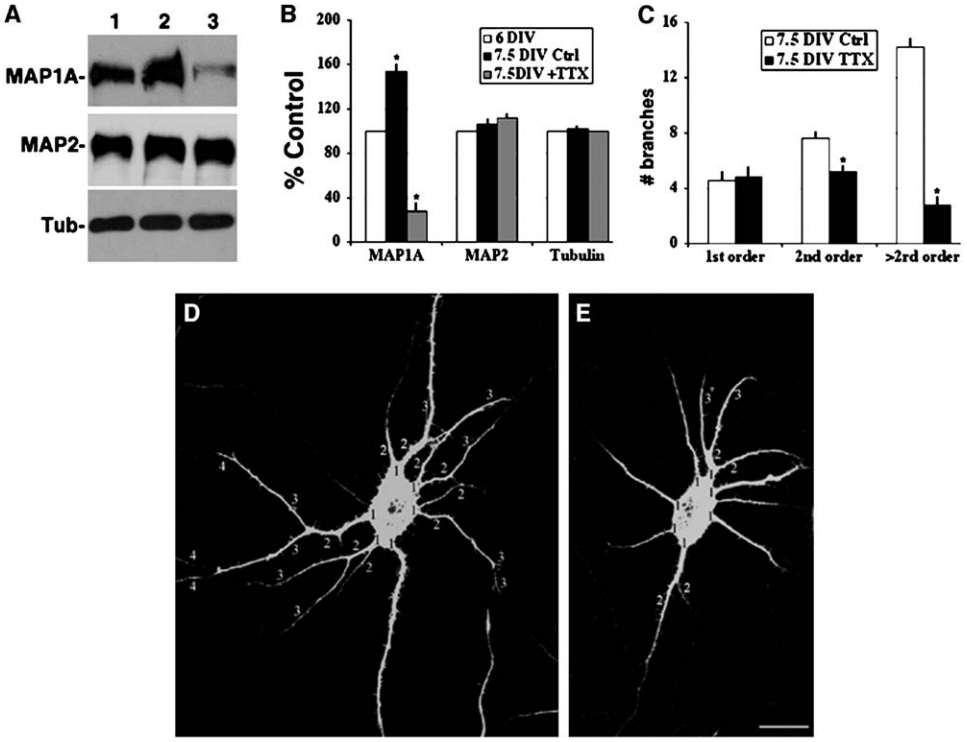

Figure 4. Effects of TTX Treatment on MAP1A and Dendritic Morphology

(A) Western blots showing MAP1A, MAP2, and β-tubulin (Tub) protein levels in control and TTX-treated cultures; lane 1, 6 DIV cultures; lane 2, vehicle-treated 7.5 DIV cultures; lane 3, TTX-treated 7.5 DIV cultures.

(B) Graphs showing changes in MAP1A, MAP2, and β-tubulin protein levels after treatment with TTX; values are expressed as percent of change with respect to the 6 DIV control group (100%).

(C) Graph showing changes in the number of dendritic branches between control and TTX-treated cultures. All measurements were performed at 7.5 DIV, and cultures were treated with TTX for 36 hr.

(D and E) Immunofluorescence micrographs showing dendritic morphology in 7.5 DIV hippocampal neurons from control (D) and TTX-treated (E) cultures. Cells were stained with a MAP2 mAb; numbers indicate branch order. The scale bar represents 10 μm.

All error bars represent the standard error of the mean.

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