Published in final edited form as:

Brain Res. 2007 October 3; 1172: 1–9.

Hippocampal dendritic arbor growth *in vitro*: Regulation by Reelin-Disabled-1 signaling

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Abstract

The cytoplasmic adaptor protein Disabled-1 (Dab1), which is a key component of the Reelin-signaling pathway, has been suggested to be required for neuronal dendritic development. However, only data from studies on immature cultures [≤ 6 days *in vitro* (DIV)] and cytoarchitectural analyses of mutant mice have been used to formulate this hypothesis. Therefore, to determine if Reelin-Dab1 signaling is specifically required for neurons to develop mature dendrites in respect to length and complexity we analyzed dendritic development in mature cultures derived from Dab1 knockout (ko) embryos. No significant differences in dendritic length or complexity between Dab1 ko and wt cultures were found at 20 DIV. An examination of dendritic development in maturing cultures found significant differences in dendritic length between mutant and wt cultures at 4 DIV, but detected no differences in complexity. In addition, by 7 DIV all measures were statistically the same between cultures. Therefore, although Reelin-Dab1 signaling promotes hippocampal dendrite development, Dab1 is not required for neurons to reach maturity with respect to dendritic length and complexity. Furthermore, analyses of 4 DIV cultures derived from Dab1 heterozygotes or mice that express only the natural splice form of Dab1 (p45) found that *Dab1*^{p45/-} hemizygote, but not *Dab1*^{p45/p45} and Dab1 heterozygote cultures had significantly shorter dendrites than those in wt cultures. Thus, a substantial attenuation of the Reelin-Dab1 signal is required before dendrite elongation is significantly decreased at 4 DIV. Moreover, experiments that incorporated a Reelin-neutralizing antibody support the hypothesis that the role(s) Reelin-signaling plays in dendritic maturation is different than the one it has in neuronal positioning.

Keywords

Dendritic Maturation; Reelin; Disabled-1; development

1. Introduction

The *reeler* mouse is a naturally occurring mutant that has been an invaluable tool in identifying crucial components of the Reelin-signaling pathway, which is required for the establishment of the normal brain cytoarchitecture [4,9,10,23,25,29]. The *reeler* mutation arises in the reelin gene and disrupts expression of Reelin, a large extracellular matrix protein. In addition to

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Reelin, the main components of the Reelin-signaling pathway are the apolipoprotein E receptor-2 (ApoER2), the very-low-density lipoprotein receptor (VLDLR), and the cytoplasmic adaptor protein disabled-1 (Dab1). Disruption of this pathway via deletion of Reelin, Dab1, or both the lipoprotein receptors results in brain cytoarchitectural abnormalities that are indistinguishable from each other [10,30].

In a recent study by Niu and colleagues [23], Reelin was found to regulate the length and complexity of dendrites *in vitro* through the VLDLR/ApoER2-Dab1 pathway. Their findings that the dendrites of neurons incapable of receiving the Reelin-Dab1 signal were nearly four times shorter than those of wt controls and that they had severe branching defects at 6 days *in vitro* (DIV)] suggest that mutant neurons can not develop fully elongated and highly complexed dendritic arbors. However, since only the dendrites of very immature neurons (≤ 6 DIV) were analyzed in the *in vitro* studies performed by Niu and colleagues, the question remains: Can neurons incapable of receiving the Reelin-Dab1 signal eventually reach maturity with respect to dendritic length and complexity? Here, we have extensively studied the *in vitro* development of neurons derived from mutant mice deficient in Reelin-Dab1 signaling to address this question.

2. Results

A functional Reelin-Dab1 signaling pathway is not required for dendrites to fully elongate *in vitro*

During development, neurons transition through a sequence of 5 morphological stages. First, unpolarized neurons extend lamellipodia that develop into short immature neurites (stages 1-2). One of the neurites, which will eventually become the axon, rapidly elongates producing the first morphological sign of neuronal polarity (stage 3). The remaining neurites slowly elongate to become dendrites (stage 4). In the final stage, the axon and dendrites elongate and become highly branched, and dendritic spines form (stage 5). *In vitro* stage 4 begins between 2-4 DIV and dendritic spine density begins to approach those values found in CA1 of the hippocampus *in vivo* around 14-18 DIV. Therefore, to determine if the Reelin-Dab1 signaling pathway is required for neurons to reach maturity *in vitro* we performed a microscopic examination of dendritic processes in neuronal cultures derived from wt and Dab1 ko mice at 20 DIV. Visual differences in the length or branching of MAP2 positive neuronal processes in wt (Fig. 1A) and Dab1 ko (Fig. 1B and Supplemental Fig. 1A) cultures were not obvious by immunofluorescence microscopy. Therefore, we performed a quantitative analysis of dendritic length using high magnification images of isolated neurons from three or more different cultures from each genotype. There were no statistical differences in the total dendritic length per neuron ($p=0.24$) or average dendrite length ($p=0.16$; Fig. 1C and 1D, respectively) between wt and Dab1 ko cultures at 20 DIV. Since this was an unexpected outcome to our experiments we confirmed that Reelin was present in the culture media (Fig. 1a). In addition, analyses of cultures found that 11% of cells in 7 DIV and 9% in 20 DIV cultures expressed Reelin, which is similar to previously reported findings [26]. Examples of Reelin expressing neurons in 20 DIV cultures are shown in Supplemental Figure 2. Although we found that 9% of the neurons in 20 DIV cultures expressed Reelin and full length Reelin was easily detected by immunoblot, it is possible that some neurons in the wt cultures do not see enough Reelin to elongate their dendrites at wt rates. If true, the inclusion of these cells in our analyses may decrease the determined average dendritic length of wt neurons enough such that there is no statistical difference between wt and Dab1 ko cultures. To rule out this possibility, we included wt cultures in which the media was supplemented with recombinant Reelin (designated wt +Reelin; approximately 250 ng/ml; Supplemental Fig. 1B) in the above experiments. We found no statistical differences in all measures made between wt, Dab1 ko, and wt+Reelin cultures at 20 DIV (Figure 1C&D).

Our findings at 20 DIV challenges the hypothesis that Dab1 is required for hippocampal dendrite development [23]. Therefore, to further investigate the role played by the Reelin-Dab1 signaling pathway in early neuronal development we performed a microscopic examination of MAP2 positive dendritic processes in maturing (4 and 7 DIV) primary neuronal cultures derived from wt (Fig. 2A&C) and Dab1 ko (Fig. 2B&D) embryos. At 4 DIV dendrites in the Dab1 ko cultures were significantly shorter than those in wt cultures, which could be detected at both the average total dendritic length per neuron ($p<0.05$) and the average dendrite length ($p<0.05$; Fig. 2E&F, respectively). In contrast, there were no statistically significant differences in either measurement of dendritic length at 7 DIV. An analyses of Dab1 and wt cultures at 3, 5, and 6 DIV found significant differences in total dendritic length starting at 3 DIV ($p<0.05$) and ending at 5 DIV ($p<0.05$). These results suggest that Reelin-Dab1 signaling sets a rate-varying tempo of dendritic elongation: initial dendrite outgrowth is rapid (2-4 DIV), which is then followed by a slower rate (5-7 DIV). When this signaling event is unavailable, dendrites elongate at a steadier, albeit slower, rate, eventually catching up to wt lengths around 6 DIV.

In order to further analyze dendritic maturation, we compared the average primary, secondary, and tertiary dendritic branch lengths between wt and Dab1 ko cultures at 4 (Fig. 3A), 7 (Fig. 3B), and 20 (Fig. 3C) DIV and found no significant differences. As a measure of dendritic complexity we quantified the total number of dendrites and the number of dendrites that had secondary and tertiary branches at 4, 7, and 20 DIV (Fig. 3D). No significant differences were detected between Dab1 ko and wt cultures in dendritic complexity at all time points analyzed. Taken together, these results suggest that Reelin-Dab1 signaling only moderately promotes dendritic elongation and is not required for neurons to reach maturity with respect to dendritic length and branching *in vitro*. In addition, they suggest that the significant differences in average total dendritic length per neuron and the average dendrite length detected at 4 DIV between wt and Dab1 ko cultures are not the consequence of deficits in complexity or individual branch length, but result from the cumulative effects of non-significant reductions in primary, secondary, and tertiary dendritic branch lengths.

A considerable attenuation of the Reelin signal is required to alter the kinetics of dendritic maturation

In their study, Niu and colleagues proposed that Reelin-Dab1 signaling functions in a dose dependent manner to promote dendritic maturation [23]. To further explore this hypothesis, we analyzed dendritic development in cultures derived from transgenic and mutant Dab1 mice. For these studies we took advantage of a knock-in mutant that expresses the Dab1 p45 splice variant, which was recently shown to be quantitatively less effective at signaling than the full-length Dab1 p80 protein [13]. Presumably, neurons derived from *Dab1*^{p45/-} hemizygotes are less effective at Reelin-signaling than Dab1 het and *Dab1*^{p45/p45} neurons, but more effective than those in Dab1 ko cultures. For these experiments, we cultured neurons from wt, *Dab1*^{p45/p45}, *Dab1*^{p45/-} hemizygotes, Dab1 het, and Dab1 ko mice. A quantitative analyses of dendritic length in these cultures at 4 DIV found significant differences between groups ($F_{4,606}=3.935$, $p<0.005$). Post hoc testing detected no significant differences in dendrite length between neurons derived from wt, Dab1 het, and *Dab1*^{p45/p45} mouse embryos. However, neurons in *Dab1*^{p45/-} hemizygotes and Dab1 ko cultures had significantly shorter dendrites than those in wt cultures ($p<0.05$ and $p<0.05$, respectively; Fig. 4A). These results suggest that a significant loss in Reelin-signaling is required before the rate at which dendritic maturation occurs is affected.

Reelin-signaling over several days is required to establish normal rates of dendritic elongation *in vitro*

In order to determine the timeline of Reelin's role in facilitating the tempo of dendritic elongation we took advantage of the Reelin antibody CR-50 [24]. CR-50 has previously been

shown to neutralize Reelin's ability to signal through the ApoER2 and VLDLR receptors [5, 22-24]. For these studies we exposed cultures derived from het *reeler* mice to CR-50 or to a control mouse immunoglobulin Fc fragment for different lengths of time (Fig. 4B). An analyses of dendritic length in CR-50 exposed cultures, het *reeler* controls, wt cultures, and Dab1 ko cultures found a significant difference between groups ($F_{5,347}=8.389$, $p<0.001$). Post hoc analyses detected no significant differences between *reeler* het controls, which were exposed to control antibody for the entire time course, and wt cultures at 3 DIV. As expected, a significant difference was detected in dendritic length between wt cultures and a Dab1 ko control at this time point ($p<0.05$). No statistical difference in dendritic length was detected between 24-hour CR-50 exposed het *reeler* cultures and control cultures at 3 DIV. In contrast, exposure of cultures to CR-50 for 72 hours post plating or from 24-72 hours resulted in a significant reduction in average dendrite length at 3 DIV compared to wt ($p<0.005$ and $p<0.05$, respectively) and het *reeler* controls ($p<0.005$ and $p<0.05$, respectively). The inability to receive the Reelin signal results in a substantial increase in the expression levels of Dab1 protein [25]. Therefore, we analyzed Dab1 expression levels by quantitative fluorescence microscopy in the cultures exposed to CR-50 for 72 hours to confirm that the CR-50 antibody was neutralizing the effect of Reelin. Our analyses found that Dab1 expression was approximately 2.75-fold higher than that found in control cultures (compare Fig. 4C to 4D and data not shown). Importantly, there was no statistical difference in the average somatic expression of Dab1 in het *reeler* cultures exposed to CR-50 and ApoER2 ko/VLDLR ko neurons (data not shown). Thus, the CR-50 antibody neutralized the effect of Reelin. Taken together, these results suggest that the role(s) Reelin-signaling plays in dendritic maturation is different than the one it has in neuronal positioning.

3. Discussion

Using a culture system that supports long-term survival and maturation of neurons we examined the role of the Reelin-Dab1 signaling pathway in dendritic development *in vitro*. Mutant neurons unable to receive the Reelin-Dab1 signal developed mature dendrites with respect to length and complexity. However, their initial tempo of dendritic elongation differed from wt neurons to accomplish this task, which resulted in mutant neurons having significantly shorter dendrites at 3-5 DIV. Taken together, the findings presented here suggest that dendritic abnormalities in Reelin-signaling mutants do not directly result from the inability of neurons to receive the Reelin-Dab1 signal, but are the consequence of alterations in local connectivity and glial cytoarchitecture, and potentially global connectivity.

Our findings at 6 DIV, and to a lesser extent at 3-5 DIV, differ from those of a previous study that found dendritic growth and complexity to be severely reduced in the absence of Reelin at these time points [23]. Potentially, the contrasting findings reflect the substantial differences in culture systems between studies. In our system, we regularly attain a highly pure population of low-density hippocampal pyramidal neurons that developed to maturity. In contrast, in the system used by Niu and colleagues, hippocampal pyramidal neurons end up being mixed amongst other cells (e.g. glial cells, dentate granule cells, cortical neurons). In addition, using their system we found that cultures derived from mutant embryos usually begin to die around 7-9 DIV (unpublished observations). Therefore, the effect a loss in Reelin signaling has on dendritic maturation may have been accentuated in their studies. We hypothesize that the reason why mutant neurons in their system don't reach maturity is because the same E18 embryos that supply the neurons are also used to produce the glial feeder, which produces neurotrophins that are necessary for dendritic growth and long-term neuronal survival *in vitro*. The immediate use of E18 cortices and brain stems derived from animals that have severely disrupted cytoarchitecture most likely results in a glial feeder that is unable to support neuronal differentiation.

The role(s) of Reelin-Dab1 signaling in dendritic maturation

During development, neurons transition through a sequence of 5 morphological stages. Although it is unknown exactly when in this developmental process Reelin-Dab1 signaling is needed to establish normal rates of dendritic elongation, our data suggests that signaling over several days is required. When Reelin inhibits neuronal migration and enables the detachment of neurons from radial glia [7] a substantial amount of actin rearrangements must occur. The stimulation of these rearrangements, whether direct or indirect, may be the earliest involvement of Reelin in neuronal maturation. However, our findings that a block in Reelin-signaling for the first 24 hours *in vitro* has no significant effect on early dendrite elongation rates suggest that the role(s) Reelin plays in neuronal positioning and dendrite maturation are not the same. In support of this hypothesis, minor processes do not begin to elongate and develop the characteristics of dendrites until 2-4 DIV [11]. How long in development Reelin-Dab1 signaling directly participates in promoting dendritic elongation is unknown. We showed that a block in Reelin-signaling from 24-72 hours resulted in dendrites being shorter than controls at 3 DIV, which suggest that Reelin-Dab1 signaling is used to promote dendritic arborization through at least developmental stage 4.

How the Reelin-signal promotes neuronal differentiation at the molecular level is also unknown. The possibility that Reelin-signaling plays a role in modulating the expression of MAP2 has previously been suggested [23]. If such a role exists, it may function during the initial stages of dendritic maturation to increase MAP2 expression because a substantial amount of MAP2 protein is required to build the elaborate dendritic arbors of neurons. Interestingly, a similar function in dendritic maturation has been suggested for the extracellular matrix protein agrin [20]. Alternatively, Reelin-signaling may function similarly to that of BDNF, which has been proposed to destabilize dendrites in order to allow other signals to direct expansion [15].

Neurons only need to receive a small amount of the Reelin-Dab1 signal to promote dendritic maturation

Presumably, not all neurons in the hippocampus have the same exposure to Reelin in the extracellular matrix during early stages of pyramidal neuron differentiation. Therefore, it would be beneficial to the neuron if the Reelin-Dab1 signaling pathway had a low threshold. Our findings suggest a considerable attenuation of the Reelin signal is required before dendrites elongate statistically slower *in vitro*. Thus, a relatively small amount of Reelin is capable of promoting normal rates of dendritic maturation, which may be important for synchronizing the overall development of the hippocampus. In addition, our results suggest that the majority of hippocampal pyramidal neurons have the same signaling threshold when it comes to Reelin's role in dendritic maturation. In contrast, recent findings suggest that some neurons are less tolerant than others to reductions in the Reelin-Dab1 signal when it comes to being correctly positioned during development in the stratum pyramidale [13,14]. Taken together, these findings further support the hypothesis that the role(s) Reelin plays in neuronal positioning and dendrite maturation are different.

The development of the hippocampus is a highly ordered process. The two most important steps in this developmental process are the correct positioning of neurons and the establishment of connectivity with specific target cells. Both these steps are disrupted in the development of Reelin-Dab1 signaling mutant hippocampi. While neuronal ectopia is certainly a direct result of a loss in Reelin-Dab1 signaling, there are likely several compounding factors that lead to altered connectivity. The two most obvious factors are the widespread abnormal neuronal orientation and dendritic abnormalities [6,23,27,28]. Proper neuronal orientation, which is established prior to dendritic elongation, is imperative for a neuron to correctly integrate into the local network [17]. Since dendritic arborization is closely coupled to synaptogenesis [3],

the misorientation of a neuron may result in dendritic abnormalities. Indeed, *reeler* hippocampal pyramidal neurons, which are severely misoriented and make poor connections with target cells [2,16], have short and poorly branched dendrites. Why are neurons misoriented in *reeler*? A role for Reelin in axon specification, and thus neuronal orientation, has not been found. However, changes in the glial cytoarchitecture of Reelin-signaling mutants have been reported [8,12,19,32]. The malformation of the glial scaffold in Reelin-Dab1 signaling mutants may affect the expression patterns of extracellular cues that govern axon specification, resulting in pyramidal neuron disorientation and dendritic abnormalities. Future studies are necessary to elucidate why neurons are misoriented during development in mice that have deficits in the Reelin–Dab1 signaling pathway.

4. Experimental Procedure

Antibodies and Recombinant Protein

Rabbit anti-MAP2 antibodies were purchased from Chemicon (Temecula, CA). Fluorescently conjugated phalloidin was purchased from Invitrogen (Carlsbad, CA). The rabbit anti-Dab1 antibody was kindly provided by Dr. Jonathan Cooper (Fred Hutchinson Cancer Research Center, Seattle, WA). Serum-free conditioned cell culture medium containing Reelin, control medium, and CR-50 antibody was obtained from Dr. Gabriella D'Arcangelo (The Cain Foundation Laboratories, Houston, TX). Recombinant Reelin secreted by a stable cell line (CER) was partially purified from serum-free conditioned medium by differential ammonium sulfate precipitation [18]. As a control, medium from another cell line transfected with empty vector (CEP4) was added to the culture media.

Animals

reeler (B6C3Fe *ala-Reln*^{fl/+}) and wt (B6129SF2/J) mice were obtained from The Jackson Laboratory (JAX; Bar Harbor, ME). Dab1 ko and Dab1 p45 knock-in (*Dab1*^{p45/p45}) mice were obtained from Dr. Jonathan Cooper, and have been described elsewhere [13]. Colony maintenance was performed using dietary information provided by JAX. Genotyping was performed by PCR using protocols provided by JAX and Dr Jonathan Cooper. Animal care was in accordance with institutional and NIH guidelines.

Dissociated Cultures

Neurons were cultured at low density from embryonic day (E) 15 control and mutant mice as described [1,11]. Briefly, hippocampal neurons were plated onto poly-L-lysine-coated glass coverslips that are inverted over a monolayer of glial cells after 2 hr incubation. Cells were plated at a density of 2700 cells per cm² to achieve low-density cultures, which were required in order to accurately measure individual dendritic branch lengths. Importantly, several precautions were taken to ensure that we had highly pure cultures of hippocampal pyramidal neurons for our study. Neurons were always isolated from E15 embryos to decrease the possibility of contaminating cultures with dentate granule cells. Paraffin dots attached to the coverslips were used to keep the neurons separated from the cells making up the glial feeder. Furthermore, since the quality of the glial feeder layer, which supplies neurotrophic substances, determines how well the neurons differentiate, the feeder layer used in all experiments was generated at least one week prior to the day of the experiment from the cortices of P1 wt pups. Using glial cells derived from wt animals assured that neurons in all experiments developed in a similar environment. Most neurons, >90%, developed the characteristic mature morphology of spiny neurons between 16–21 days *in vitro* (DIV). As a measure of culture maturity in experiments using 20 DIV neurons we confirmed that the majority of the cells in the culture had a relatively high density of dendritic spines (0.5–1.5 punctae/μm of dendrite) using rhodamine-phalloidin.

Microscopy

Images were collected on an Olympus FV500 confocal microscope or an Olympus IX-70 microscope (Olympus America Inc., Melville, NY) equipped with a Hamamatsu C4742-98 CCD camera (Hamamatsu Corporation, Bridgewater, NJ) and a Ludl motorized XYZ stage (LEP Ltd., Hawthorne, NY). Data was deconvolved using the Agard/Sadat inverse matrix algorithm.

To quantify dendritic characteristic 5 sequential confocal slices taken 0.1 μm apart through the midplane of the dendrites were collected with fixed laser illumination and pinhole size using a 60X 1.4 NA plan apochromat objective or a 40X 1.3 plan fluorite objective on an Olympus FV500 confocal. Using SlideBook 4.1 Imaging software (Intelligent Imaging Innovations, Inc; Denver, CO) three-dimensional images were constructed of each dendrite. The reconstructed images were then delineated and the threshold set. The number, size, and signal intensity was then measured. No significant difference was detectable in the grayscale values for each voxel within the area of interest in any of the cells or any of the conditions. Using the program ImageJ with the semiautomatic neurite tracing plugin NeuronJ [21] we measured dendritic length based on threshold segmentation. Using these experimental parameters, the dendrites from a minimum of 21 neurons from at least three different experiments per condition were measured.

Statistical Analyses

Unless stated otherwise, one-way analysis of variance with post-hoc comparison via Tukey's Honestly Significant Difference was used to evaluate between group differences. In cases where preliminary analysis of dendritic length revealed this measure to be highly skewed, lengths were first transformed by taking their natural logarithm (ln) prior to statistical analysis. In all cases, diagnostic statistics were used to confirm that the data (actual or ln transformed) were normally distributed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We are extremely grateful to Drs. David Lewis, Athina Markou, and Robert Sweet for their constructive comments, to Drs. Jonathan Cooper, Tom Curran, and Gabriella D'Arcangelo for the reagents and advice they gave us, and to Amy Guzik for technical assistance. This work was supported by a NARSAD Young Investigator Award and NIMH (MH064372) grant to KNF.

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Figure 1. Reelin-Dab1 signaling is not required for dendrites to elongate to mature lengths
 (A-B) MAP2 immunofluorescence of 20 DIV primary hippocampal neurons derived from wt (A) and Dab1 ko (B) E15 embryos. (a) Western blot analysis using an antibody directed against Reelin revealed that full-length Reelin protein is present in the media of wt cultures. (C-D) Double-label immunofluorescence using a MAP2 antibody and Hoechst dye was used to quantify dendritic length in wt, Dab1 ko, and wt+Reelin (wt cultures in which the media was supplemented with recombinant Reelin) neuronal cultures at 20 DIV. Values equal mean $\pm$ SEM. Bars in A&B equal 20 μm .

Figure 2. Dendritic length in developing cultures derived from wt and Dab1 ko embryos
 (A-D) MAP2 immunofluorescence of 4 and 7 DIV primary hippocampal neurons derived from wt and Dab1 ko E15 embryos. (E-F) Double-label immunofluorescence using a MAP2 antibody and Hoechst dye was used to quantify the average total dendritic length per neuron and average dendrite length in wt and Dab1 ko neuronal cultures at 4 and 7 DIV. Values equal mean $\pm$ SEM. Asterisks in E and F designate a significant difference from wt ($p < 0.05$ for both). Bars in A-D equal $10\mu\text{m}$.

Figure 3. Dendritic complexity in wt and Dab1 ko cultures

(A-C) Double-label immunofluorescence using a MAP2 antibody and Hoechst dye was used to quantify dendritic branch length in wt and Dab1 ko neuronal cultures at 4, 7, and 20 DIV. Dendritic complexity was determined using a modification of the *centrifugal method* of dendritic branch ordering to describe the density of dendritic arborizations [31]. Our characterization was such that the dendrites emerging from the cell soma are called primary up to the point of bifurcation into second order branches. Therefore, first branches are called secondary and so on, with increasing order until the tips are reached. Values equal mean $\pm$ SEM. (D) Additional analyses of dendritic complexity were performed using the data collected for Figures 1, 2, and 3A-C. The data presented was analyzed using the Fisher Exact test. No statistical differences were found between wt and Dab1 ko cultures.

Figure 4. Dendrite differentiation is dependent on receiving a specific dose of the Reelin signal over several days

(A) Immunofluorescence microscopy using a MAP2 antibody was used to quantify dendritic length in wt, Dab1 het, Dab1 ko, *Dab1^{p45/p45}*, and *Dab1^{p45/-}* neuronal cultures at 4 DIV. The dendrites of neurons derived from Dab1 ko and *Dab1^{p45/-}* hemizygote embryos were significantly shorter than the dendrites of wt neurons at 4 DIV (asterisks; $p < 0.05$ and $p < 0.05$, respectively). (B) Neurons derived from het *reeler* embryos were exposed to CR-50 (a Reelin neutralizing antibody) or to a control mouse immunoglobulin Fc fragment for 72 hours post plating. In addition, het *reeler* cultures were also exposed to CR-50 during the first 24 hours post plating or from 24-72 hours. There were no significant differences between control cultures and wt cultures or the 24-hour CR-50 treated cultures in dendritic length at 3 DIV. In contrast, exposure of cultures to CR-50 for 72 hours post plating or from 24-72 hours resulted in a significant reduction in average dendrite length at 3 DIV compared to wt ($p < 0.005$ and $p < 0.05$, respectively) and het *reeler* controls ($p < 0.005$ and $p < 0.05$, respectively). Neurons in 3 DIV Dab1 ko cultures, which were included as a control, also had significantly shorter dendrites than wt neurons ($p < 0.05$). (C-D) Neurons were exposed to a control antibody or CR-50 for the first 72 hours post plating. After being rinsed the cultures were fixed and used in triple-label immunofluorescence experiments in which Hoechst was used to mark the nuclei and phalloidin the cell bodies. Dab1 (green) expression was found to be approximately 2.75-fold higher in cultures exposed to CR-50 (D) than cultures exposed to control serum (C). In A-B Values equal mean $\pm$ SEM and the asterisks designate a significant difference from wt, while the $\neq$ symbols in B designate a significant difference from the het *reeler* control. The scale bars equal 10 μ m.