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BRAIN RESEARCH

Research Report

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

Sarah A. MacLaurin[a], *Thomas Krucker*[a], *Kenneth N. Fish*[b,*]

[a]*Novartis Institutes for BioMedical Research, Inc. Cambridge, MA 02139, USA*
[b]*Department of Psychiatry, Western Psychiatric Institute and Clinic, University of Pittsburgh School of Medicine, Biomedical Science Tower, Room W1651, Pittsburgh, PA 15213, USA*

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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.

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 (D'Arcangelo et al., 1995; Gallagher et al., 1998; Goldowitz et al., 1997; Niu et al., 2004; Rice et al., 1998; Sweet et al., 1996). The *reeler* mutation arises in the reelin gene and disrupts expression of Reelin, a large extracellular matrix protein. In addition to Reelin, the main components of the Reelin-signaling pathway are the apolipoprotein E receptor-2 (ApoER2), the very-low-density

* *Corresponding author*. Fax: +1 412 624 9910.
E-mail address: knf8@pitt.edu (K.N. Fish).

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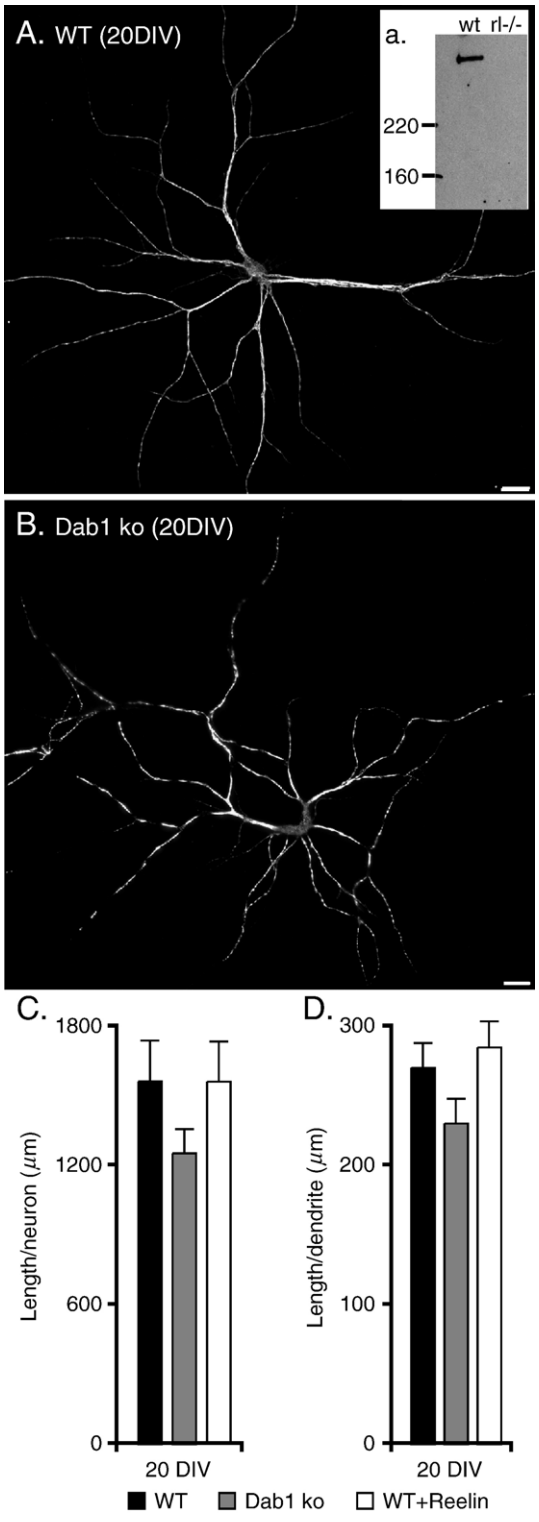
lipoprotein receptor (VLDLR), and the cytoplasmic adaptor protein Disabled-1 (Dab1). Disruption of this pathway via deletion of Reelin, Dab1, or both the lipoprotein receptors results in brain cytoarchitectural abnormalities that are indistinguishable from each other (Goldowitz et al., 1997; Trommsdorff et al., 1999).

In a recent study by Niu et al. (2004), 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 cannot develop fully elongated and highly complex 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 dendritc 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

2.1. *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 Supplementary 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 at 20 DIV between wt and Dab1 ko cultures in the total dendritic length per neuron ($p=0.24$) or average dendrite length ($p=0.16$; Figs. 1C and D, respectively). Since this was an unexpected outcome to our experiments, we

Fig. 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 ± SEM. Scale bars in panels A and B equal 20 μm.