BRAIN RESEARCH 1415 (2011) 96–102

available at www.sciencedirect.com

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Decreased dendritic spine density and abnormal spine morphology in Fyn knockout mice

Lenard W. Babus[a,b,1], Elizabeth M. Little[a,b,1], Kathleen E. Keenoy[a,b], S. Sakura Minami[a], Eric Chen[a,b], Jung Min Song[a], Juliet Caviness[a,b], So-Yeon Koo[a,b], Daniel T.S. Pak[c], G. William Rebeck[a], R. Scott Turner[b], Hyang-Sook Hoe[a,b,*]

[a]Department of Neuroscience, Georgetown University Medical Center, 3970 Reservoir Road NW, Washington, DC 20057-1464, USA
[b]Department of Neurology, Georgetown University Medical Center, 3970 Reservoir Road NW, Washington, DC 20057-1464, USA
[c]Department of Pharmacology, Georgetown University Medical Center, 3970 Reservoir Road NW, Washington, DC 20057-1464, USA

ARTICLE INFO

Article history:
Accepted 28 July 2011
Available online 5 August 2011

Keywords:
Fyn
Dendritic spine
Synapse
Cortex
Hippocampus

ABSTRACT

Fyn is a Src-family tyrosine kinase that affects long term potentiation (LTP), synapse formation, and learning and memory. Fyn is also implicated in dendritic spine formation both in vitro and in vivo. However, whether Fyn's regulation of dendritic spine formation is brain-region specific and age-dependent is unknown. In the present study, we systematically examined whether Fyn altered dendritic spine density and morphology in the cortex and hippocampus and if these effects were age-dependent. We found that Fyn knockout mice trended toward a decrease in dendritic spine density in cortical layers II/III, but not in the hippocampus, at 1 month of age. Additionally, Fyn knockout mice had significantly decreased dendritic spine density in both the cortex and hippocampus at 3 months and 1 year, and Fyn's effect on dendritic spine density was age-dependent in the hippocampus. Moreover, Fyn knockout mice had wider spines at the three time points (1 month, 3 months, 1 year) in the cortex. These findings suggest that Fyn regulates dendritic spine number and morphology over time and provide further support for Fyn's role in maintaining proper synaptic function in vivo.

1. Introduction

Fyn is a member of the Src family of non-receptor tyrosine kinases and plays important roles in synapse development (Maness, 1992) and synaptic plasticity (Grant and Silva, 1994). For example, Fyn-deficient mice have impaired long-term potentiation (LTP) (Grant et al., 1992; Kojima et al., 1997) as well as impairments in both short- and long-term contextual fear memory (Huerta et al., 1996; Isosaka et al., 2008). Reduced activation of Fyn by infusion of Src kinase inhibitor also correlated with a reduction in spatial memory in the radial arm maze (Mizuno et al., 2003). Fyn may regulate learning and memory through phosphorylation of NMDA receptor subunits, glutamate receptors that play a key role in many forms of synaptic plasticity (Cheung and Gurd, 2001; Jiang et al., 2008; Nakazawa et al., 2001).

In addition to Fyn's effect on LTP and learning and memory, Fyn regulates dendritic spine and synapse formation. Dendritic

* *Corresponding author at:* Department of Neurology, Department of Neuroscience, Georgetown University, 3970 Reservoir Road NW, Washington, DC 20057-1464, USA. Fax: +1 202 687 0617.
E-mail address: hh69@georgetown.edu (H.-S. Hoe).
[1] Contributed equally to this work.

0006-8993/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2011.07.059

spines are small, highly motile protrusions that form the primary sites of excitatory synaptic transmission, and their number and morphology play an important role in synaptic plasticity (Verpelli et al., 2010). Active Fyn inhibits spine and synapse formation through an effect on protein tyrosine phosphate receptor T (PTRPT) (Lim et al., 2009). Moreover, primary cortical neurons from Fyn knockout mice had decreased spine formation compared to wild-type cultures, and Fyn knockout mice also had reduced dendritic spine density of pyramidal neurons in cortical layer V at 3 months of age compared to wild-type mice (Morita et al., 2006). These data suggest that Fyn plays an important role in regulating spinogenesis in vitro and in vivo, as well as in synaptic plasticity and learning and memory. Disruption of these functions may contribute to the synapse and spine loss that strongly correlates with cognitive impairments observed in neurodegenerative disorders such as Alzheimer's disease (AD) (Baloyannis, 2009; Crews and Masliah, 2010; Hoe et al., 2008; Shankar et al., 2008; Shirazi and Wood, 1993; Wei et al., 2010).

In the present study, we examined whether the effect of Fyn exerts brain region-specific and age-dependent effects on dendritic spine formation and spine morphology, which is unknown. We found that Fyn knockout mice had significantly decreased spine density in both the cortex and hippocampus at 3 months and 1 year, and that this reduction in dendritic spine density was age-dependent in the hippocampus. In addition, Fyn knockout mice had wider spine heads at the three time points in the cortex, but morphological changes in the hippocampus were more complex. These data indicate that Fyn differentially affects spine density and morphology in the cortex and hippocampus and has age-dependent effects on dendritic spine formation and spine morphology in vivo, which suggests that Fyn is important for normal synaptic function and maintenance over time.

2. Results

2.1. *Fyn knockout mice exhibit age-dependent decrease in spine density in cortical layers II/III*

We first conducted Golgi staining on brains of Fyn knockout and wild-type mice and analyzed dendritic spine density of pyramidal neurons in cortical layers II/III. We quantified spine density at three developmental time points (1 month, 3 months, and 1 year of age) (Fig. 1). Cortical spine density trended toward a decrease in Fyn knockout mice at 1 month old, whereas at 3 months Fyn knockout mice had significantly decreased spine density (12%) and a further decrease at 1 year (17%) compared to wild-type mice (Fig. 1F).

We next subdivided the total spine population into those found on apical oblique (AO) vs. basal shaft (BS) dendrites (Fig. 1A–C) because several studies have demonstrated structural and functional differences between these two dendritic compartments, and their dendritic spines can respond differentially to stimuli or genetic perturbations (Alpar et al., 2006; Henze et al., 1996; Kayser et al., 2011; Lanz et al., 2003; Moser et al., 1997; Perez-Cruz et al., 2011). We found that cortical spine density in AO dendrites mirrored the total spine values, exhibiting progressively decreasing relative spine density with development (Fig. 1D,F). In contrast, Fyn knockout mice had significantly decreased spine density in BS dendrites compared to wild-type mice at all three time points examined, and the extent of the decrease was unvarying over time (Fig. 1E). These data suggest that Fyn may differentially regulate spine density in cortical layer II/III AO and BS dendrites with aging.

2.2. *Age-dependent decrease in hippocampal dendritic spine density in Fyn knockout mice*

Next, we examined whether Fyn differentially regulates dendritic spine formation in the hippocampus. As for the cortical analysis, we conducted Golgi staining on hippocampal CA1 neurons of Fyn knockout and wild-type mice at 3 different time points (1 month, 3 months, and 1 year) and measured spine density on AO, BS, and total (AO+BS) dendrites (Fig. 2). Fyn knockout mice showed unaltered total hippocampal spine density at 1 month of age (Fig. 2F). However, Fyn knockout mice had decreased hippocampal spine density at 3 months of age (10%) and further decreased hippocampal spine density at 1 year old (12%) compared to wild-type mice (Fig. 2F). Moreover, 1 year old Fyn knockout mice had significantly reduced hippocampal spine density compared to 1 month old Fyn knockout mice (Fig. 2F, $p=0.014$). Fyn knockout mice had similarly reduced hippocampal spine density in AO dendrites at 3 months and 1 year old compared to wild-type mice (14% and 15%, respectively, Fig. 2D). However, Fyn knockout mice did not have altered BS spine density compared to wild-type mice at any time frame in the hippocampal CA1 region (Fig. 2E). Overall, these data illustrate that Fyn regulates dendritic spine density in an age-dependent manner and has differential effects on brain regions (cortex and hippocampus) as well as dendritic segments (AO and BS).

2.3. *Fyn knockout mice have longer and wider spines in AO dendrites, but not BS dendrites spine morphology at 1 month old*

To examine whether Fyn regulates spine morphology, we measured spine head widths and spine lengths in cortical layers II/III. The cumulative distribution plots revealed that Fyn knockout mice had wider spine heads compared to wild-type mice in AO dendrites, but not in BS dendrites (Fig. S1A,B). In addition, Fyn knockout mice had longer spines compared to wild-type mice in AO dendrites, but not in BS dendrites (Fig. S1D,E). Moreover, in the hippocampus, Fyn knockout mice trended toward wider spine heads and shorter spines compared to wild-type mice for both AO and BS dendrites (Fig. S1G,H). These results indicate that Fyn knockout mice had abnormal spine morphology during brain development.

2.4. *Fyn knockout mice showed wider spine heads at 3 months and 1 year old, but spine length was unaltered in cortical layer II/III*

Next, we examined whether Fyn knockout mice also have an effect on spine morphology in cortical layers II/III at 3 months and 1 year old. We found that Fyn knockout mice had much wider spine heads in cortical layers II/III compared to wild-type mice at 3 months and 1 year old (Figs. S2A–C, S3A–C). However, Fyn knockout mice did not exhibit a change in spine length at 3 months and 1 year old (Figs. S2D–F, S3D–F).