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

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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.

Keywords

Fyn; Dendritic spine; Synapse; Cortex; Hippocampus

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

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Appendix A.

Supplementary data

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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 sub-units, 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 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).

Additionally, Fyn knockout mice exhibited a significant shift in the distribution of hippocampal spine head widths and lengths toward narrower and longer spines at 3 months of age (Fig. S2G,H). Furthermore, Fyn knockout mice had a significant shift in distribution to shorter spines in the hippocampus at 1 year (Fig. S3G,H), with no change in spine head width. Taken together, these data suggest that Fyn regulates spine morphology in an age-dependent manner across brain regions.

2.5. Summary over time of averaged spine morphological changes in cortical layers II/III and the hippocampus

A summary of dendritic spine morphology demonstrates that Fyn knockout mice trended toward wider spine heads and longer spines in the cortex at 1 month of age (Fig. 3A, B). Fyn

knockout mice had much wider spine heads compared to wild-type mice at 3 months and 1 year of age. Additionally, 1 year old Fyn knockout mice had significantly wider spine heads compared to 3 months old Fyn knockout mice in cortical layers II/III (Fig. 3A, $p=0.002$). However, 3 months and 1 year old Fyn knockout mice had no change in spine length compared to wild-type mice (Fig. 3B).

In the hippocampus, the average spine head widths show that 1 month old Fyn knockout mice had much wider spine heads compared to wild-type mice (Fig. 3C); however, 3 months old Fyn knockout mice had narrower spine heads compared to wild-type mice and trended toward narrower spine heads at 1 year old. In addition, Fyn knockout mice had shorter average spine lengths compared to wild-type mice at 1 month and 1 year (Fig. 3D). Thus, Fyn is required to complete spine morphogenesis of individual spines in the brain.

3. Discussion

3.1. Reduced spine density in Fyn knockout mice

Accumulating evidence implicates Fyn in synapse regulation that varies over neuronal development. Consistent with this idea, our main finding was that genetic ablation of Fyn in mice led to decreases in dendritic spine density that were in general exacerbated with age, particularly between 1 and 3 months. Notably, perturbation of LTP in Fyn-deficient mice is also age-dependent, showing impairment at 3.5 months of age but not at 2, 6, or 10 weeks (Kojima et al., 1997). These results provide a possible link whereby Fyn could affect cognitive abilities through reduction of excitatory synapses. A question that emerges from these observations is why Fyn deletion has reduced effect on spines of 1 month old animals. Clearly, Fyn is expressed in early development as well as adult neurons (Umemori et al., 1992); it is possible that other related kinases of the Src family can functionally compensate for loss of Fyn in young animals, but not in the adult. Alternatively, in immature neurons Fyn may be involved more in dendrite formation than spine formation (Morita et al., 2006). A final possibility is that Fyn is not necessary for initial spinogenesis, but rather spine maintenance. In this regard, a decrease in spine stability or an increase in spine pruning could lead to the observed reduction in spine number; however, live imaging of spine dynamics will be required to resolve this issue.

Another key finding of our study was that Fyn knockout had distinct effects on dendritic spine density in cortical and hippocampal neurons, as well as in apical vs. basal dendrites. The most pronounced example of this differential regulation was the selective effect of Fyn knockout on BS dendrites of cortical neurons, but not hippocampal neurons, a phenotype that did not change over time. The reason for this specific effect is currently unclear, but presumably reflects the fact that apical and basal dendrites of cortical layer II/III neurons receive distinct afferent inputs (Feldmeyer et al., 2002; Schaefer et al., 2003; Spruston, 2008). Taken together, our findings suggest that Fyn's effect on dendritic spine formation is dependent on age, brain region, and dendrite subcompartment (AO vs. BS). However, it remains unclear whether the loss of Fyn causes a constant or increasing negative effect on dendritic spine density in the Fyn knockout mice beyond 1 year of age. Further studies are required to elucidate these effects.

3.2. Morphological changes associated with Fyn knockout mice

Accompanying the changes in dendritic spine density in Fyn knockout mice, we also observed alterations in spine morphology. In cortical layers II/III, Fyn knockout mice exhibited larger spine heads, an effect that became more pronounced with age. Fyn knockout mice also had greater cortical spine length at 1 month of age, but not in older mice. Small spines have less synaptic strength compared to larger spines (Matsuzaki et al., 2001; Zito et

al., 2004). However, smaller spines are viewed as more dynamic and plastic, acting as “learning spines” (Kasai et al., 2002), whereas larger spines, which correlate with older spines (Hofer et al., 2009; Yasumatsu et al., 2008), are more stable and referred to as “memory spines” (Kasai et al., 2002). We speculate that the abnormal spine morphology observed in Fyn knockout mice may reflect an initial destabilization of spines, leading to longer and more immature spine morphology. Over time as synapse loss becomes more pronounced, other mechanisms may become engaged that homeostatically compensate for the loss of excitatory synapses by increasing head diameter and strength of remaining spines (Turrigiano, 2007). Indeed, mice in later stages of AD-like pathology have reduced number but wider (more mushroom type) spines (Dickstein et al., 2010).

Unexpectedly, Fyn knockout mice had inconsistent morphological changes in the hippocampal CA1 region in the time period examined. For example, Fyn knockout mice had a wider average spine head at 1 month compared to wild-type mice, but a narrow average spine head compared to wild-type mice at 3 months and 1 year old. In addition, Fyn knockout mice had shorter spines compared to wild-type mice at 1 month and 1 year in the hippocampus. These complicated phenotypes suggest that multiple pathways may be affected by Fyn deletion in a complex manner in hippocampal neurons. Regardless, the results demonstrate that Fyn plays important and changing roles in dendritic spine morphogenesis over time, and reinforce the notion that cortical and hippocampal spines are differentially regulated by Fyn. Interestingly, Fyn knockout mice also display cortical layer-specific abnormalities in dendritic morphology and polarity (Sasaki et al., 2002). Further study will be required to investigate the difference in dendritic spine morphology between the cortex (including cortical layers II/III vs. layer V) and hippocampus of Fyn knockout mice.

3.3. Mechanisms of Fyn action on dendritic spines

How does Fyn regulate dendritic spine formation? One possibility is via receptor-type protein tyrosine phosphatase (RPTs), which have been shown to regulate spine formation through interaction with Fyn (Lim et al., 2009). Genetic interaction with the semaphorin class guidance cues could also play a role (Morita et al., 2006). Furthermore, we recently found that Fyn interacts with both APP and ApoEr2, AD-related molecules important for synapse and dendritic spine formation (Lee et al., 2010). Therefore, Fyn may affect spine formation through an interaction with these transmembrane receptors. Lastly, it is also possible that Fyn affects dendritic spines through Reelin signaling, which is known to play an important role in dendritic spine formation (Qiu and Weeber, 2007), by altering the levels of Reelin signaling proteins. However, further study is required to investigate the molecular mechanism by which Fyn regulates dendritic spine formation.

3.4. Conclusion

Fyn knockout mice have reduced spine density in cortical layers II/III and the hippocampus, and we demonstrate for the first time that this deficit increased over time in both brain regions. Additionally, we observed that Fyn knockout mice had abnormal spine morphology in the cortex and hippocampus, an effect that increased with age in the cortex. These findings suggest that Fyn differentially regulates dendritic spine number and morphology in the cortex and hippocampus over time.

4. Experimental procedures

4.1. Animals

We analyzed 1 month, 3 months, and 1 year old Fyn knockout and wild-type mice. Protocols were approved by the Georgetown University Animal Welfare and Use Committee. Fyn

wild-type C57BL6/SV129 mice and Fyn knockout mice (C57BL6/SV129 background) were obtained from Jackson Laboratory (Bar Harbor, ME, USA).

4.2. Golgi staining

FD Rapid GolgiStain Kit (FD NeuroTechnologies, Elliot City, MD, USA) was used to analyze dendritic spine morphology in brain. Brain slicing was performed using VT1000S Vibratome (Leica, Bannockburn, IL, USA) at 150 μ m thickness. Dissected mouse brains were submerged in Solution A and B for 2 weeks at room temperature and transferred to Solution C for 24 h at 4 °C. Axioplan 2 (Zeiss, Oberkochen, Germany) under bright-field microscopy was used to obtain dendritic images.

4.3. Spine analysis

Dendritic segments (AO or BS dendrites) from areas of cortical layer II/III and hippocampus CA1 region were randomly selected for spine analysis by an observer blind to the experimental conditions. Scion image software (Scion Corporation, Frederick, MD, USA) was used to measure spine length, width, and linear density. Spines from 0.4 to 3.2 μ m in length were analyzed and spine heads from 0.2 to 1.2 μ m in width were analyzed. All analyses of morphology were performed blinded to genotype and experimental condition.

4.4. Statistical analysis

Data in summary charts were normalized by expressing Fyn knockout and wild-type mice data in terms of relative values where the wild-type mice value at each time point is set at 100%. Student's *t*-test was used for the two-group density and average spine head width and length comparisons, while the Kolmogorov–Smirnov test was utilized for two-group spine head width distribution and length distribution comparisons. Asterisks indicate statistically significant differences; **p*<0.05, ***p*<0.01, ****p*<0.001.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Cortical Layers II/III

A. 1 Month

B. 3 Months

C. 1 Year

D. AO Spine Density

E. BS Spine Density

F. Total Spine Density

Fig. 1.

The effect of Fyn on spine density in cortical layers II/III. After Golgi staining, cortical layers II/III were imaged and analyzed (A) Representative dendritic segments of cortical layers II/III pyramidal neurons from 1 month old wild-type and Fyn knockout mice (n=3 brain/group). (B) Representative AO and BS dendrites per genotype from cortical layers II/III (n=4 brain WT, n=5 brain KO) at 3 months old. (C) Representative AO and BS dendrites per genotype from cortical layers II/III (n=4 brain/group) at 1 year old. (D–F) Summary of all averaged spine densities in AO, BS, and total (AO+BS) dendrites for Fyn knockout mice in cortical layers II/III at 1 month, 3 months, and 1 year. Fyn knockout mice have significantly decreased cortical spine density at 3 months (12%) and 1 year (17%) compared to wild-type mice. Asterisks indicate statistically significant differences; *p<0.05, **p<0.01, ***p<0.001.

Hippocampus

Fig. 2.

The effect of Fyn on spine density in hippocampal CA1 region. After Golgi staining, hippocampi were imaged and analyzed (A–C) Representative dendritic segments of hippocampal pyramidal neurons from 1 month (A, n=3 brain/group), 3 months (B, n=4 brain WT, n=5 brain KO), and 1 year old (C, n=4 brain/group) wild-type and Fyn knockout mice. (D–F) Summary of all averaged spine densities in AO, BS, total (AO+BS) dendrites for Fyn knockout mice in the hippocampal CA1 region at 1 month, 3 months, and 1 year. Fyn knockout mice have decreased hippocampal spine density with aging. Asterisks indicate statistically significant differences; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Cortical Layers II/III

Hippocampus

Fig. 3.

The effect of Fyn on spine morphology in cortical layers II/III and hippocampal CA1 region. (A–B) Summary of all the (A) averaged spine head widths and (B) averaged spine lengths for Fyn knockout mice in cortical layers II/III at 1 month, 3 months, and 1 year. Representative dendritic spine images illustrating the method for measuring spine head widths and spine lengths. (C–D) Summary of all the (C) averaged spine head widths and (D) averaged spine lengths for Fyn knockout mice in the hippocampal CA1 region at 1 month, 3 months, and 1 year.