HHS Public Access

Author manuscript

Nat Neurosci. Author manuscript; available in PMC 2012 March 01.

Published in final edited form as:

Nat Neurosci. ; 14(9): 1112–1114. doi:10.1038/nn.2893.

Presenilin 1 Regulates Homeostatic Synaptic Scaling Through Akt Signaling

Kara G. Pratt[1,5], **Eric C. Zimmerman**[1], **David G. Cook**[2,3,4], and **Jane M. Sullivan**[1]

[1]Department of Physiology & Biophysics University of Washington School of Medicine Seattle, WA 98195

[2]Veterans Affairs Medical Center, Geriatric Research Education and Clinical Center (GRECC), Seattle, WA 98108

[3]Department of Medicine University of Washington School of Medicine Seattle, WA 98195

[4]Department of Pharmacology University of Washington School of Medicine Seattle, WA 98195

Abstract

Neurons in vivo and in vitro adapt to long-lasting changes in network activity by adjusting their synaptic strengths to stabilize firing rates. Here we show that homeostatic scaling of excitatory synapses is impaired in mouse hippocampal neurons lacking presenilin 1 ($PS1^{-/-}$) or expressing a familial Alzheimer's disease-linked PS1 mutation ($PS1^{M146V}$). These findings suggest that deficits in synaptic homeostasis may contribute to brain dysfunction in Alzheimer's disease.

Keywords

Alzheimer's disease; γ-secretase; presenilin 1 knock-out; $PS1^{-/-}$; presenilin 1 M146V knockin; CaMKIV; phosphatidylinositol 3-kinase; miniature excitatory postsynaptic currents

Presenilin 1 (PS1) is closely linked to Alzheimer's disease as an integral component of γ-secretase, an enzyme required for the production of amyloid beta[1], and also as the most commonly mutated protein in familial Alzheimer's disease[2]. How mutations in PS1 lead to Alzheimer's disease remains an open question, but a common feature of cells lacking PS1, or expressing familial Alzheimer's disease-linked PS1 mutants is defective calcium signaling[3,4]. Homeostatic synaptic scaling, a compensatory form of synaptic plasticity that maintains action potential firing rates within an optimal range in both young and adult neurons[5,6], is triggered by changes in intracellular calcium levels[7]. To determine whether PS1 is required for homeostatic synaptic scaling, we treated dissociated cultures of mouse

Corresponding author: Jane M. Sullivan, Dept. of Physiology and Biophysics, Box 357290, University of Washington School of Medicine, Seattle, WA 98195-7290, jmsull@u.washington.edu, Ph. (206) 543-4038.

[5]Current address: Dept. of Zoology & Physiology, University of Wyoming, Laramie, WY 82071

Author contributions

K.G.P. and J.M.S. conceived the experiments, and together with E.C.Z. carried them out and analyzed the data. D.G.C. provided critical reagents. All authors contributed to writing the paper.

hippocampal neurons derived from *PS1*$^{-/-}$ embryos and plated onto a feeder layer of wild-type astrocytes with tetrodotoxin (TTX; 1 μM) for 24 – 48 h (see Supplementary Methods). *PS1*$^{-/-}$ neurons failed to scale up synaptic strengths in response to blockade of action potential firing, in contrast to wild-type neurons, which showed a robust increase in spontaneous miniature excitatory postsynaptic current (mEPSC) amplitude (Figs. 1a and b; wild-type = 18.1 ± 1.4 pA, *n* = 11; wild-type + TTX = 34.3 ± 4.0 pA, *n* = 15; *PS1*$^{-/-}$ = 18.4 ± 1.1 pA, *n* = 25; *PS1*$^{-/-}$ + TTX = 19.1 ± 1.4 pA, *n* = 35; ANOVA, P < 0.0001). The similarity between mEPSC amplitudes in untreated *PS1*$^{-/-}$ and wild-type neurons suggests that the inability of *PS1*$^{-/-}$ neurons to scale up was not the result of occlusion (i.e. mEPSC amplitudes were not maximally scaled up prior to network silencing), nor a general defect in mechanisms establishing basal synaptic strength. Treatment of *PS1*$^{-/-}$ neurons with $GABA_A$ receptor antagonist bicuculline (20 μM) for 48 h to enhance network activity produced a significant decrease in mEPSC amplitude (*PS1*$^{-/-}$ = 16.0 ± 0.8 pA, *n* = 22; *PS1*$^{-/-}$ + BIC = 12.8 ± 0.9 pA, *n* = 25; t-test, P < 0.02). Acute (≤ 30 h) virally-mediated expression of wild-type PS1 rescued scaling up in *PS1*$^{-/-}$ neurons treated with TTX (Fig. 1c; *PS1*$^{-/-}$ + GFP = 19.3 ± 2.5 pA, *n* = 5; *PS1*$^{-/-}$ + GFP + TTX = 21.0 ± 1.2 pA, *n* = 17; *PS1*$^{-/-}$ + PS1 = 20.2 ± 1.5 pA, *n* = 13; *PS1*$^{-/-}$ + PS1 + TTX = 31. 9 ± 2.4 pA, *n* = 21; ANOVA, P < 0.01), demonstrating that their inability to scale up was not due to an irreversible developmental defect. Together, these data indicate that PS1 is required for the scaling up of excitatory synaptic strengths in response to suppression of network activity—but is not needed for scaling down in response to enhancement of network activity, or for setting basal mEPSC amplitudes.

Scaling up of synaptic strengths in response to TTX treatment is triggered by a drop in somatic calcium levels and a consequent reduction of CaMKIV activation[7]. Because PS1 is known to play a role in regulating calcium release from internal stores[3,4], we wondered whether the failure of *PS1*$^{-/-}$ neurons to scale up could be due to aberrantly high levels of somatic calcium that might maintain CaMKIV activation in the absence of action potential firing. To test this possibility, we pharmacologically inhibited CaMKIV activity by treating *PS1*$^{-/-}$ neurons with Sto-609 (2 μM) and dantrolene (10 μM) for 3 – 4 h. Sto-069 is a CaMKK inhibitor that has been shown to modulate homeostatic synaptic plasticity through downstream inhibition of CaMKIV[7,8]. Dantrolene is a ryanodine receptor antagonist that suppresses release of calcium from internal stores. This treatment more than doubled the amplitude of mEPSCs in wild-type neurons, but, in spite of bypassing the requirement for a drop in somatic calcium to inactivate CaMKIV, *PS1*$^{-/-}$ neurons still failed to scale up mEPSC amplitudes (Fig. 1d; wild-type = 13.9 ± 1.2 pA, *n* = 7; wild-type + STO = 30.3 ± 4.0 pA, *n* = 9; *PS1*$^{-/-}$ = 14.7 ± 1.3 pA, *n* = 6; *PS1*$^{-/-}$ + STO = 16.8 ± 1.2 pA, *n* = 13; ANOVA, P < 0.0001). These results suggest that an inability to inactivate CaMKIV is not the primary deficit responsible for the failure of *PS1*$^{-/-}$ neurons to scale.

Having established a role for PS1 in synaptic scaling, we decided to test more directly the potential relevance of a homeostatic synaptic scaling deficit in the development of Alzheimer's disease pathology by investigating the effects of TTX on mEPSC amplitudes in cultured hippocampal neurons derived from mice in which a familial Alzheimer's disease-linked PS1 mutation, M146V, is knocked-in[9]. In these *PS1*^{M146V} mice, transcription of the

mutant PS1 is controlled by the endogenous promoter, and protein is expressed at normal physiological levels[9]. Although they responded more heterogeneously to network silencing than $PS1^{-/-}$ neurons, $PS1^{M146V}$ neurons also failed to show a significant enhancement of mEPSC amplitude with TTX treatment (Fig. 2a; wild-type = 18.7 ± 2.2 pA, n = 15; wild-type + TTX = 30.9 ± 3.6 pA, n = 13; $PS1^{M146V}$ = 19.0 ± 1.9 pA, n = 21; $PS1^{M146V}$ + TTX = 24.8 ± 2.9 pA, n = 26; ANOVA, $P < 0.02$). To determine whether aberrant γ-secretase activity might be playing a role here, we treated wild-type neurons with γ-secretase inhibitor (L685,458; 5 μM) along with TTX for 48 h, and found no significant effect on scaling of mEPSC amplitudes (Fig. 2b; wild-type + L685,458 = 14.8 ± 1.2 pA, n = 12; wild-type + L685,458 + TTX = 25.2 ± 3.1 pA, n = 16; t-test, $P < 0.01$). These results suggest that the failure of $PS1^{-/-}$ and $PS1^{M146V}$ neurons to scale normally is due to a PS1 function that is independent of γ-secretase activity.

Among other deficits, $PS1^{-/-}$ neurons have significantly impaired phosphatidylinositol 3-kinase (PI3K)/Akt signaling that is not rescued with virally-mediated expression of PS1M146V, nor mimicked by treatment of wild-type neurons with γ-secretase inhibitor[10, 11]. PS1 has been proposed to influence PI3K/Akt activity through the promotion of Akt-activating cadherin/PI3K complexes[11], as well as effects on the trafficking of cell surface signaling receptors coupled to PI3K/Akt[12]. A role for PI3K/Akt signaling in synaptic scaling is strongly suggested by the findings that inhibition of PI3K blocks homeostatic AMPA receptor delivery to synapses[13], and that PI3K/Akt-mediated phosphorylation of AMPA receptors enhances their delivery to synapses[14]. To determine whether reduced PI3K/Akt signaling in $PS1^{M146V}$ neurons contributes to scaling deficits, we expressed a constitutively active Akt (CA-Akt) while treating with TTX and found that synaptic scaling was rescued (Fig. 2c; GFP = 18.7 ± 2.2 pA, n = 19; GFP + TTX = 19.2 ± 1.9 pA, n = 21; CA-Akt = 20.1 ± 1.2 pA, n = 19; CA-Akt + TTX = 29.5 ± 2.4 pA, n = 21; ANOVA, $P < 0.0005$). Note that expression of CA-Akt sans TTX did not result in enhanced mEPSC amplitudes, suggesting that Akt activation is necessary but not sufficient to induce scaling. Together, the results presented here raise the possibility that impairments in PI3K/Akt-dependent synaptic homeostasis might contribute to the development of cognitive deficits in familial—and perhaps sporadic[15]— Alzheimer's disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank Mike Ahlquist and Ning Li for excellent technical assistance, and Rachael L. Neve for generously providing the viral constructs. This work was supported by the American Health Assistance Foundation and the US National Institutes of Health/National Institute of Neurological Disorders and Stroke (J.M.S.), and the Veteran's Affairs Office of Research and Development Medical Research Service (D.G.C.).

References

1. De Strooper B. Neuron. 2003; 38:9–12. [PubMed: 12691659]
2. Tanzi RE, Bertram L. Neuron. 2001; 32:181–184. [PubMed: 11683989]
3. LaFerla FM. Nat Rev Neurosci. 2002; 3:862–872. [PubMed: 12415294]

4. Bezprozvanny I, Mattson MP. Trends Neurosci. 2008; 31:454–463. [PubMed: 18675468]
5. Turrigiano GG. Cell. 2008; 135:422–435. [PubMed: 18984155]
6. Pozo K, Goda Y. Neuron. 2010; 66:337–351. [PubMed: 20471348]
7. Ibata K, Sun Q, Turrigiano GG. Neuron. 2008; 57:819–826. [PubMed: 18367083]
8. Goold CP, Nicoll RA. Neuron. 2010; 68:512–528. [PubMed: 21040851]
9. Guo Q, et al. Nat Med. 1999; 5:101–106. [PubMed: 9883847]
10. Baki L, et al. J Neurosci. 2008; 28:483–490. [PubMed: 18184791]
11. Baki L, et al. Embo J. 2004; 23:2586–2596. [PubMed: 15192701]
12. Kang DE, et al. J Biol Chem. 2005; 280:31537–31547. [PubMed: 16014629]
13. Hou Q, Zhang D, Jarzylo L, Huganir RL, Man HY. Proc Natl Acad Sci U S A. 2008; 105:775–780. [PubMed: 18174334]
14. Qin Y, et al. Genes Dev. 2005; 19:2000–2015. [PubMed: 16107614]
15. Hyman BT. Nat Neurosci. 2011; 14:5–6. [PubMed: 21187847]

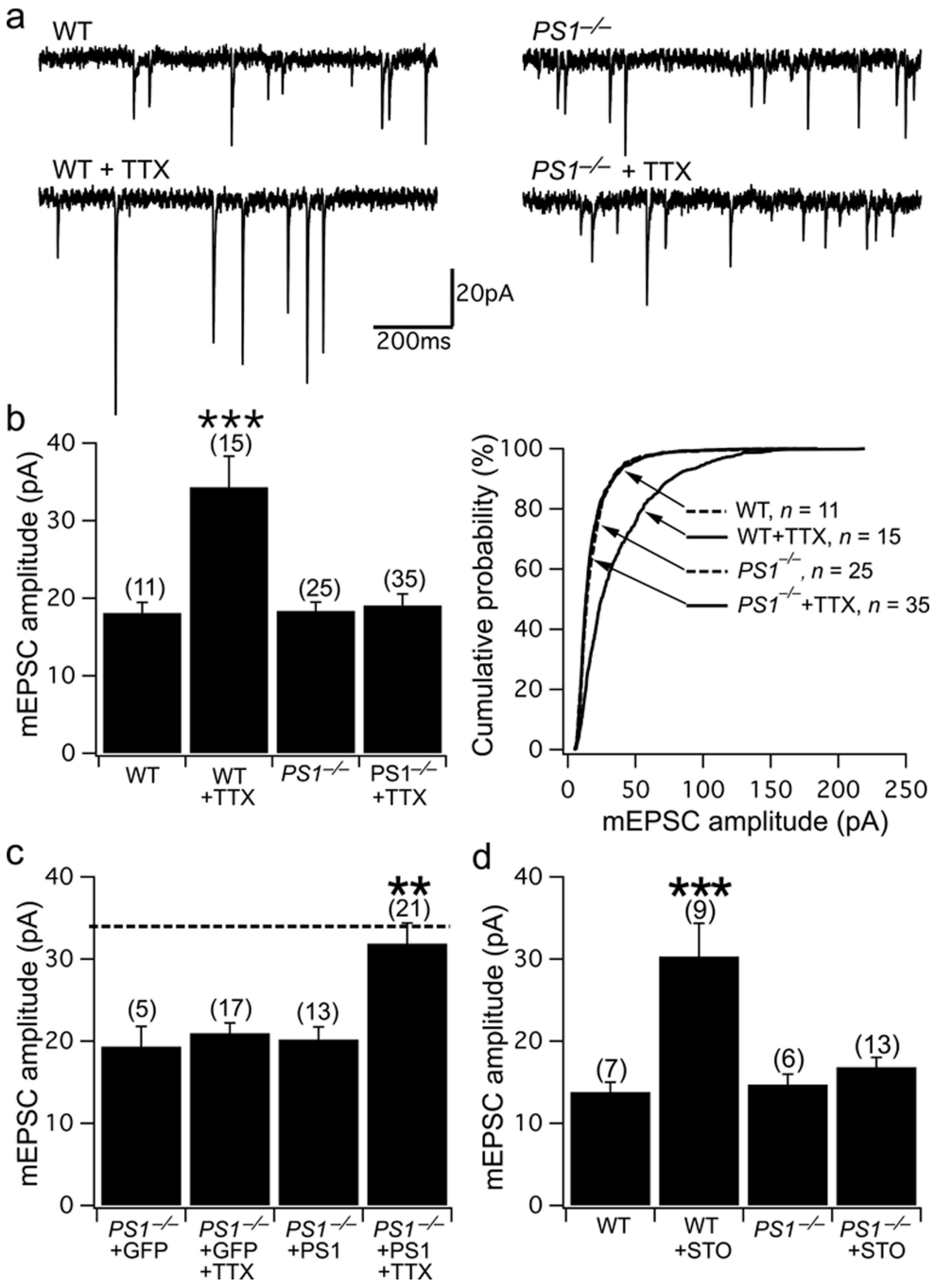

Figure 1. Neurons lacking PS1 do not scale up synaptic strengths in response to chronic activity blockade or inhibition of CaMKIV

(a) Representative traces of mEPSCs from (left) wild-type (WT) control and TTX-treated neurons; (right) *PS1*$^{-/-}$ control and TTX-treated neurons. **(b)** Chronic TTX treatment significantly increased average mEPSC amplitudes in wild-type but not *PS1*$^{-/-}$ neurons, while average mEPSC amplitudes of wild-type and *PS1*$^{-/-}$ controls were similar. Left, average mEPSC amplitudes of control and TTX-treated wild-type and *PS1*$^{-/-}$ neurons (ANOVA, $P < 0.0001$; ***Bonferroni's Multiple Comparison Test, $P < 0.001$). Right,

cumulative probability distribution plot of mEPSC amplitudes from the same cells. **(c)** Virally-mediated expression of wild-type PS1 in *PS1*$^{-/-}$ neurons restored the scaling response elicited by TTX (ANOVA, $P < 0.01$; **Bonferroni's Multiple Comparison Test, $P < 0.01$). Dashed line shows the average mEPSC amplitude of TTX-treated wild-type neurons (reported in Fig. 1b). Notice that expression of wild-type PS1 in the absence of TTX had no effect on mEPSC amplitude. **(d)** Inhibition of CaMKIV scaled up mEPSC amplitudes in wild-type but not *PS1*$^{-/-}$ neurons (ANOVA, $P < 0.0001$; ***Bonferroni's Multiple Comparison Test, $P < 0.001$). All error bars, s.e.m.

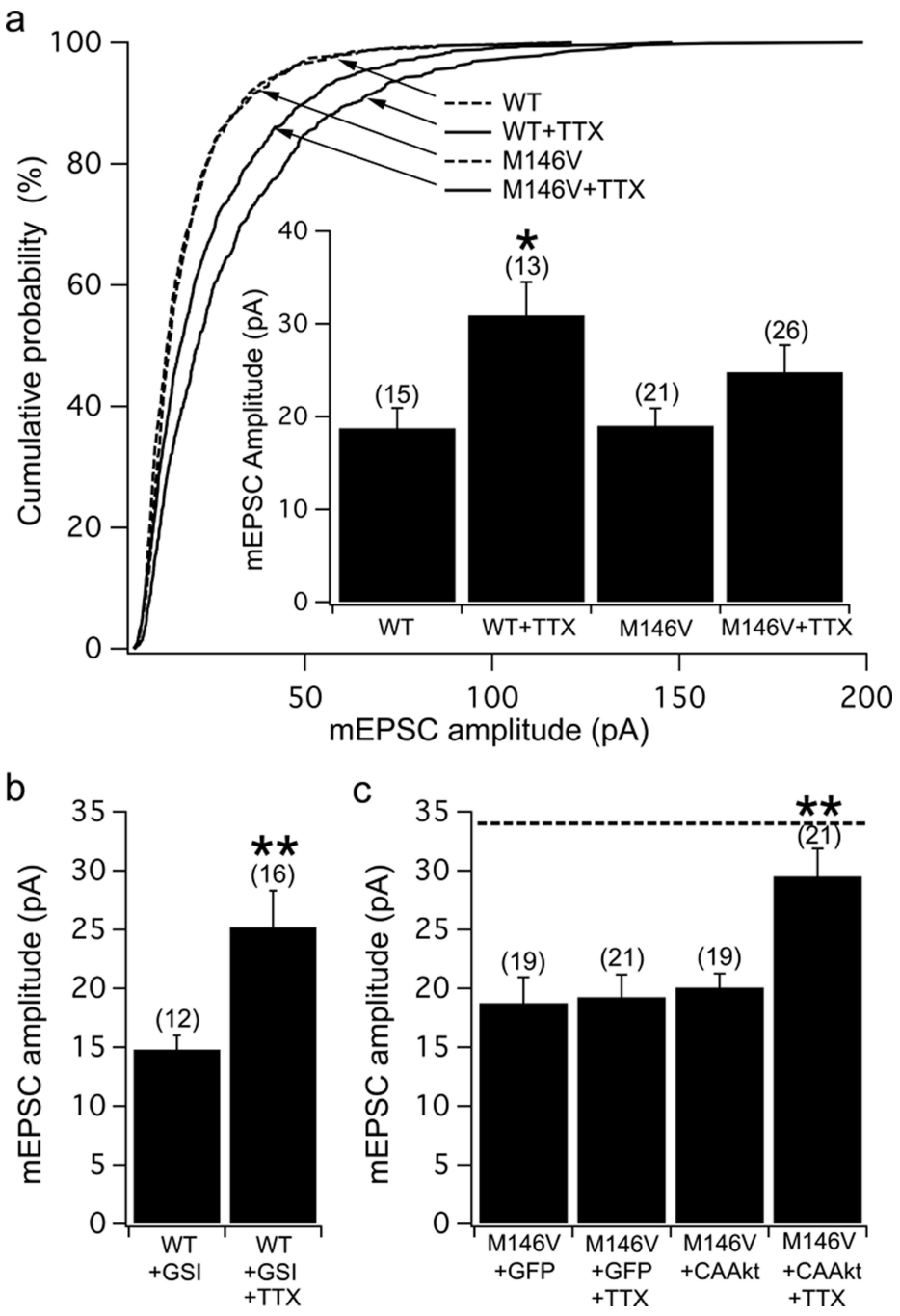

Figure 2. *PS1*^{M146V} neurons display synaptic scaling deficits that can be rescued by expression of constitutively active Akt

(a) Cumulative probability plot showing the distribution of mEPSC amplitudes from wild-type (WT) and *PS1*^{M146V} (M146V) control and TTX-treated neurons; (inset) average mEPSC amplitudes of the same neurons. Notice that TTX treatment failed to induce a significant increase in mEPSC amplitudes in *PS1*^{M146V} neurons (ANOVA, $P < 0.02$; *Bonferroni's Multiple Comparison Test, $P < 0.05$). **(b)** γ-secretase inhibitor (GSI) did not affect scaling up of mEPSC amplitudes in TTX-treated wild-type neurons (**unpaired t-test,

$P < 0.01$). **(c)** Virally-mediated expression of CA-Akt in $PS1^{M146V}$ neurons had no effect on mEPSC amplitudes on its own, but restored the ability to scale up in response to TTX (ANOVA, $P < 0.001$; **Bonferroni's Multiple Comparison Test, $P < 0.01$). Dashed line shows the average mEPSC amplitude of TTX-treated wild-type neurons (reported in Fig. 1b). All error bars, s.e.m.